Physical activity, *apolipoprotein*-ε4 and cognition among cognitively healthy older adults: a longitudinal study

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ABSTRACT

Introduction: Alzheimer's disease (AD) is fast becoming a public health priority. In the absence of effective drug treatment, the focus has shifted towards lifestyle change for prevention. Physical activity (PA) engagement is consistently associated with preserved cognition and lower risk of cognitive decline among older adults. Whether PA mitigates enhanced risk for dementia via *APOE*ε4 allele carriage, demographic and/or biomarker risk factors is less understood. This thesis aimed to evaluate the longitudinal relationship between PA and cognition among cognitively healthy older adults, alongside the role of other demographic, genetic and peripheral biomarkers.

Methods: Data of 901 cognitively healthy older adults (60-85 years; mean age = 68.7 ± 3.9) collected as part of a prospective observational cohort study, CHARIOT: PRO, were utilised. The mean follow-up period was 18.5 months (SD 1.7), ranging from baseline to 30-months. Mixed-models regression methods were used to analyse the associations between PA and cognition over time, including effect modification by *APOE*ε4 carrier status, age, and sex. Moderation and mediation by peripheral amyloid-beta and brain-derived-neurotrophic-factor (BDNF) were explored. All analyses were controlled for *a priori* selected health and demographic factors.

Results: Being physically active was positively associated with longitudinal cognitive performance, with the largest effect sizes among those reporting high PA in both mid- and late-life. The positive association between late-life PA and delayed memory trajectories were augmented among *APOE*ε4 carriers, when compared to non-carriers. Conversely, *APOE*ε4 stratified analysis found associations to be consistently augmented among *APOE*ε4 non-carriers for remaining domains. Age and sex did not significantly modify the association between PA and cognition. BDNF was not associated with PA or cognition, nor modified by *APOE*ε4 carrier status. There was an inverse association between amyloid-beta plasma levels and global cognition and attention index scores, which were modified by late-life PA, especially among *APOE*ε4 non-carriers.

Conclusion: This thesis highlights the benefits of PA in mitigating cognitive decline in older age, especially among certain risk groups. Elucidating the complex interrelations between modifiable and non-modifiable risk factors for AD will aid in promoting lifestyle intervention as a viable preventative strategy for public health recommendations.

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DECLARATION OF ORIGINALITY

I declare that the work described in this dissertation to be my own and original. Specification of work I carried out within the CHARIOT: PRO Main Study and collaborators, is outlined at the beginning of the appendix. Any other contributions not mentioned in the appendix will be referenced in text accordingly. This thesis was carried out in accordance with the requirements of Imperial College London's regulation and code of practice for research degree programs.

SIGNED: Catherine Robb DATE: 5th June 2019

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
APOEε4	Apolipoprotein epsilon 4
APP	Amyloid Precursor Protein
ATP	Adenosine-triphosphate
Aβ	Amyloid beta
B	Beta coefficient
BDNF	Brain Derived Neurotrophic Factor
BMI	Body Mass Index
CDR	Clinical Dementia Rating
CHAMPS	Community Health Activity Mentor Program for Seniors
CHARIOT	The Cognitive Health in Ageing Register: Investigational, Observational, and Trial
CI	Confidence Interval
CIND	Cognitive Impairment No Dementia
COWAT	Controlled Word Association Test
CSF	Cerebrospinal Fluid
CVD	Cardiovascular Disease
DASH	Dietary Approaches to Stop Hypertension
DBD	Discovery Bead Diluent
DKEFS	Delis Kaplan Executive Function System
DSM-IV-TR	Diagnostic & Statistical Manual of Disorders, 4th Edition – Text Revision
EDTA	Ethylene-Diamine-Tetra-Acetic
ELISA	Enzyme Linked Immunosorbent Assay
EPA	Everyday Physical Activity
FDG	Fludeoxyglucose
FSIQ	Full Scale IQ
GCP	Good Clinical Practice
GDS	Geriatric Depression Scale
GPP	Good Pharmacoepidemiology Practices
IGC	Individualised Growth Curve
IGF-1	Insulin-like Growth Factor 1
IL	Interleukin
ILQ	Imperial Lifestyle Questionnaire
IQR	Interquartile Range
ISPE	International Society for Pharmaceutical Engineering
IWG	International Working Group
LNS	Letter Number Sequencing task
LLPA	Late-life Physical Activity
LOD	Late Onset Dementia
MCI	Mild Cognitive Impairment
MeDi	Mediterranean diet
MIND	Mediterranean-DASH Intervention for Neurodegenerative Delay
ML / LL PA	Midlife to Late-life physical activity

MLPA	Midlife leisure Physical Activity
MMSE	Mini Mental State Examination
MRI	Magnetic Resonance Imaging
MSD	Meso Scale Discovery
NAB	Neuropsychological Assessment Battery
NART	National Adult Reading Test
NERI	New England Research Institute
NHS	National Health Services
NIA-AA	National Institute on Ageing-Alzheimer's Association
NOS	Newcastle-Ottawa Quality Assessment Scale
OR	Odds Ratio
PAR	Population Attributable Risk
PASE	Physical Activity Measurement for the Elderly
PET	Positron Emission Tomography
pg/mL	Picogram/millilitre
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRO	Prospective Readiness cOhort study
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
RCT	Randomised Controlled Trial
RoB	Risk of Bias
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute
RR	Risk Ratio
SD	Standard Deviation
SEM	Standard Error of Measurement
SIMOA	Single molecular array
SMD	Standardised Mean Difference
SNP	Single Nucleotide Polymorphism
TIA	Transient Ischemic Attack
TMT	Trail Making Test
TNF	Tumour Necrosis Factor
TrkB	Tropomyosin-related Kinase B
T2D	Type 2 Diabetes
VaD	Vascular Alzheimer's disease
VEGF	Vascular Endothelial Growth Factor
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WMA	World Medical Assembly
WMS-R	Wechsler Memory Scale-Revised
WRAT,	Wide Range Achievement Test
YPAS	Yale Physical Activity Survey
µL	Microlitre
EF	Executive Function
VS	Visuospatial abilities
DM	Delayed Memory
IM	Immediate Memory
GC	Global Cognition

OVERVIEW AND THESIS OUTLINE

There is considerable evidence implicating physical activity as a key lifestyle factor contributing towards enhanced cognitive benefits in older age and reduced risk of dementia. The cognitive benefits of physical activity (PA) have most typically been observed in the memory and executive function domains. A growing body of literature now suggests that PA may also moderate the association between possession of a key risk allele for Alzheimer's disease (AD), apolipoprotein epsilon-4 (*APOE*ε4), and cognitive function in older age, however, results remain contentious and incongruent. Other demographic and biomarker indicators may be moderating associations between PA and cognition in an *APOE*ε4 carrier specific manner. Likewise, how these associations play-out may be influenced by timing and duration of maintaining a physically active lifestyle (e.g. being physically active only during midlife or continued/initiated in later-life). Two of the strongest non-modifiable risk factors for AD are age and sex, which also interact with *APOE*ε4 allele carriage to alter risk of cognitive decline and dementia. Yet, how PA influences later-life cognition as a function of these factors remains largely unknown. Furthermore, very little is known of the underlying biological mechanisms relating to the benefits of PA on cognitive health in later-life. Studies suggest that the synthesis and release of trophic factors, especially brain-derived-neurotrophic-factor (BDNF), may play a crucial role in mediating the benefits of exercise on the brain. Furthermore, both animal and human studies have reported a link between PA and reduction of a key AD biomarker, amyloid-beta (Aβ), in the brain. Growing evidence now suggests a less invasive measure of brain/CSF Aβ, as a viable proxy within the periphery. However, this marker has been minimally explored in the context of cognitive progression in older age. The role of BDNF and peripheral Aβ in the context of PA/cognitive associations and as a function of *APOE*ε4 carriage warrants further investigation. The work here presents an in-depth investigation into the relationship between PA during midlife and/or late-life and multi-domain cognition over time among cognitively healthy older adults and whether such demographic, genetic and peripheral biomarker indicators modify these associations.

1.1.1 Primary Aims

Among cognitively healthy older adults, this project addresses the following aims: (i) to investigate the associations between mid- and/or late-life PA on later-life cognitive function and trajectories over 30 months, and whether these associations are modified by *APOE*ε4 carrier status, age and sex, (ii) to evaluate the role of a key biomechanistic factor, BDNF, in mediating or moderating the association between PA and cognition and, (iii) to explore the role of a proposed AD biomarker proxy, peripheral Aβ, on cognition and whether PA modifies the association between Aβ and cognition.

1.1.2 Thesis outline

This thesis is comprised of an introduction chapter, systematic review chapter and methods chapter (Chapters 1-3), followed by three results chapters (Chapters 4-6), each of which will address the first aim (i). A discussion of results presented within these chapters will follow (Chapter 7). Aims (ii) and (iii) will be addressed in Chapters 8 and 9, respectively, each including results and discussion. The final chapter offers an overall discussion of the findings in relation to the proposed aims and wider context followed by concluding remarks and implications for future research. Below is an overview of what to expect within each chapter.

1.1.2.1 Chapter 1 - Introduction

This chapter will provide a background summary relating to the current state of dementia prevention research. It includes an overview of modifiable and non-modifiable risk factors for dementia. A brief review of domain-specific cognitive function in older age and AD related decline is followed by an introduction to PA and cognitive function in older age. A narrative review of previous studies investigating the association between PA and cognition as well as dementia risk will be presented. Next, a focus on existing literature exploring the effect modification of PA/cognitive associations via *APOE*ε4 carrier status, age and sex will be introduced. A review of possible biological mechanisms underlying the benefits of PA on cognition in older age is followed by a detailed introduction to peripheral BDNF and $A\beta_{1\text{-}42.}$ An emphasis is placed on the associations between PA, these biomarkers and cognition in the context of *APOE*ε4 carrier status.

1.1.2.2 Chapter 2 - Physical activity, apolipoprotein-ε4 and risk of cognitive decline and dementia among healthy older adults: a systematic literature review

Chapter 2 presents a systematic review of randomized controlled trials and prospective observation studies exploring the association between PA and cognition plus risk of dementia as a function of *APOE*ε4 carrier status. Inclusion criteria mirrored those of the current study, including cognitively healthy older adults of ≥60 years of age. Results from the review will be synthesised, evaluated and discussed in the context of the work carried out within this thesis.

1.1.2.3 Chapter 3 – Methods

Chapter 3 describes the design and methods of the Cognitive Health in Ageing Register: Investigational, Observational, and Trial studies in dementia research (CHARIOT): Prospective Readiness cOhort (PRO) Main Study from which data in this thesis was obtained. A detailed description of the key tools utilised for PA and cognitive data collection, alongside biomarker analysis methodologies. The last section contains statistical methods from data cleaning, preparation and variable selection to an account of statistical methods per chapter.

1.1.2.4 Chapter 4 – 6 - The association between late-life physical activity (Ch: 4); midlife physical activity (Ch: 5) and the combined role of midlife to late-life physical activity (Ch: 6) on overall cognitive function and trajectories

These three chapters primarily present results pertaining to the association between late-life PA (Chapter 4), midlife PA (Chapter 5), midlife to late-life PA groups (i.e. being physically active only during midlife or continued/initiated in later-life) (Chapter 6) and domain-specific cognitive function and trajectories across 30 months, among cognitively healthy older adults. Whether possession of at least one *APOE*ε4 allele modifies this association is presented, as well as the differential role of age and sex on these associations. Each chapter begins with an overview and summary of the objectives to be addressed followed by the results and completed with a summary of the key findings.

1.1.2.5 Chapter 7 – Physical activity and cognition: discussing the role of PA timing, age, sex and *APOE*ε4 carrier status.

This chapter will provide a discussion of the findings presented in chapters 4, 5, and 6; pertaining to the first aim. Firstly, the independent and combined role of midlife versus late-life leisure aerobic PA and late-life everyday PA (work/household/leisure composite score) (EPA) on overall cognitive function and trajectories will be discussed. A discussion pertaining to observed effect modification by *APOE*ε4 carrier status, age and sex on the association between PA and cognition will follow. Finally, the relevance and consequence of cognitive domain-specific associations with PA in the context of cognitive ageing will be discussed.

1.1.2.6 Chapter 8 - Physical activity, brain derived neurotrophic factor and cognitive function and trajectories over 30 months

This chapter explores the role of baseline BDNF serum levels in relation to PA and *APOE*ε4 carrier status on cognition in older age. Firstly, the cross-sectional association between baseline BDNF serum levels and late-life EPA, late-life leisure PA and midlife leisure PA is presented, and whether *APOE*ε4 carrier status modifies the aforementioned associations. Secondly, this chapter reports whether higher levels of serum BDNF are associated with better overall cognitive function and

trajectories across 30 months, and again, whether *APOE*ε4 carrier status modifies this association. Whether BDNF mediates or modifies the association between midlife and/or late-life PA and cognitive function will be explored. The chapter will end with a discussion of the results.

1.1.2.7 Chapter 9 - Physical activity, plasma amyloid-beta$_{1-42}$ and cognitive function and trajectories over 30 months

This chapter evaluates the association between baseline Aβ_{1-42} plasma levels in relation to PA and *APOE*ε4 carrier status on cognition in older age. Specifically, the cross-sectional association between Aβ_{1-42} plasma levels and late-life EPA, late-life leisure PA, and midlife leisure PA will be presented. Whether *APOE*ε4 carrier status modifies these associations are reported. Secondly, this chapter reports whether baseline Aβ_{1-42} plasma levels are associated with overall cognitive function across 30 months, and again, whether *APOE*ε4 carrier status and midlife plus late-life leisure PA modifies this association. A discussion of results will follow.

1.1.2.8 Chapter 10 – Overall Discussion and Conclusions

This Chapter provides an overall discussion of the findings presented in this thesis and how these findings relate back to the current state of dementia prevention research. Conclusive remarks will be detailed in the context of strengths and limitations, ongoing gaps in the literature, recommendations for future research and perspectives.

Chapter 1: Introduction

1.1 Dementia and Alzheimer's disease

Dementia prevention is becoming a prevailing public health priority. As the world's demographic shifts toward older age, so are the number of people living with dementia. A global estimate of 50 million individuals are currently diagnosed with dementia, producing an economic cost of US$1 trillion [1]. Lifetime risk of developing dementia is calculated to be 32%, or one in three within the UK. Figures are projected to continue rising, in high, middle and low-income countries [2]. For example, the prevalence of dementia is anticipated to reach 74.7 million cases globally [3]; with a global cost estimate predicting to reach US$2 trillion by 2030 [4]. Numbers are set to quadruple by 2050 [5]. Of this cost, nearly 85% relate to family and social, rather than medical, care. Likewise, billions of dollars have been invested in identifying a pharmaceutical treatment for the predominant form of dementia, Alzheimer's dementia [6, 7]. Public health measures could reduce some of this cost, yet little effort has been placed on implementing such change [8].

Despite major efforts to identify disease-modifying treatments, outcomes have proven futile. This may be as a consequence of targeting those at an advanced disease stage whereby presence of neuronal damage and concurrent symptomology are likely beyond repair. Delivering a preventive intervention among an asymptomatic cohort is an alternative strategy possibly yielding more efficacious results [9, 10]. Recognising these challenges, the World Health Organisation has declared dementia prevention as a public health priority [11]. Although a treatment is currently unavailable, delaying symptom onset by just 2-years could have substantial economic, public health, and societal benefits [11, 12]. Consequently, the last decade has bought about multinational efforts to support research aimed at tackling new ways to address human, economic, and social costs of dementia [13]. One such example is the recently proposed and updated National Institute on Ageing-Alzheimer's Association (NIA-AA) framework for research on Alzheimer's disease (AD) [14]. The framework defines AD as a biological process demonstrable via autopsy or in vivo biomarkers. It emphasises the departure from clinical symptomology by stating; "A biological rather than a syndromal definition of AD is a logical step toward greater understanding of the mechanisms underlying its clinical expression" [14]. This framework is distinct from previously published guidelines by the International Work Group (IWG) in 2014, requiring the inclusion of clinical symptomology alongside biomarker pathology for AD diagnosis [15-17]. The aim of the NIA-AA framework is to enhance efforts to understand the pathobiological manifestation of AD and multifactorial aetiology of dementia by providing a common language and framework for researchers to follow.

Although promising, the proposed NIA-AA framework has not been without conjecture [18]. It is beyond the scope of this thesis to broach this debate in detail. However, criticisms have included disregarding the impact of age, a key AD risk factor [2, 19], and that it has overlooked the mechanistic link between biomarker and AD clinical outcome, which could allow biomarkers to be used as surrogates for AD [20]. It has a reduced applicability among differing racial/ethnic minorities, low socioeconomic status groups and those in rural/medically disadvantaged communities, due to the expense of biomarker measurement [21]. Of relevance to this thesis is the separation of AD neuropathology from cognitive outcomes that may differ by age, sex, vascular factors, lifestyle and genetics [21]. These caveats warrant careful consideration when designing studies and interpreting findings, which will be revisited in the discussion chapter. Nonetheless, the research framework proposed by NIA-AA has acknowledged these limitations and promotes its recommendations following robust evidence indicating abnormal tau and amyloid protein deposits being consistently linked to AD as a distinctive neurodegenerative disease, dominating others, also leading to dementia. The framework further recognises a presymptomatic period from which targeted intervention can be implemented for preventative research. It recognises that many co-morbid diseases, such as cardiovascular disease, in addition to AD, contribute toward dementia. They also acknowledge that there can often be a discordance between pathological burden and symptom manifestation at the individual level, whereby, certain protective lifestyle factors promote resilience or reserve capacity [22-25], of particular relevance to the research presented in this thesis.

A key pathological feature of AD is characterised by extracellular depositions of brain Aβ plaques which are composed of Aβ peptides 39-43 amino acids long; measured via positron emission tomography (PET), or cerebrospinal fluid (CSF) $A\beta_{42}$ as the earliest indicator (Alzheimer's pathologic change) [14]. The second pathological feature is intracellular accumulations of neurofibrillary Tau tangles, which are composed of hyperphosphorylated tau protein or CSF phosphorylated (P)-tau, and when combined with pathological levels of Aβ, confirms AD classification, according to the current NIA-AA framework [14]. A third marker, not required for the labelling of Alzheimer's continuum but as a staging of disease severity, is neurodegeneration and injury biomarkers. These include brain atrophy, measured via magnetic resonance imaging (MRI), CSF Total (T)-tau and brain activity, measured via hypometabolism with fludeoxyglocuse (FDG) PET, alongside cognitive symptomology [14].

Dementia is preceded by mild cognitive impairment (MCI) and is typically characterised by an insidious and progressive cognitive and functional decline leading to inevitable senescence [26]. AD is reportedly responsible for 60-80% of age-related dementia diagnosis [2], now a widely used synonym for dementia. However, up to 60% of cognitively normal individuals over the age of 80 years also have neuropathological indication of AD without dementia [27-30]. Vascular dementia is the next most common form, followed by dementia with Lewy bodies. Mixed dementia is also

common and represents the complexity of the syndrome, disease and processes leading to such. Frontotemporal degeneration and dementias associated with alcohol abuse, brain injury or infection are less usual [31]. Risk of developing dementia rapidly increases from the age of 65, when comorbidity is common [26]. Co-occurrences such as diabetes, hypertension and hypercholesterolemia all increase the risk of possible AD dementia and vascular dementia or mixed dementia with more comorbidities leading to higher risk, possibly due to lack of resilience and repair [32]. Likewise, mixed pathologies such as infarctions, atherosclerosis, arteriolosclerosis, white matter changes, Lewy bodies and TAR DNA-binding protein 43 (TDP-43) and hippocampal sclerosis [33] are also strongly linked to dementia and often co-occur with AD in an additive manner [22, 33-36]. Some studies even suggest that mixed pathologies may account for an even higher proportion of dementia cases than AD alone [37]. The role of mixed-pathologies is important when seeking to understand the impact of risk factors in AD dementia, which are said to account for less than half of the person-specific variance in cognitive decline. Such variation may be explained by differences in an individual's ability to withstand the presence of pathology and maintain cognitive health. This phenomenon is often referred to as cognitive reserve, brain reserve and brain maintenance [38-41].

The heuristic theory of *cognitive reserve* refers to the adaptability or enhanced efficiency of cognitive processes, which contribute toward explaining individual differences in response to brain ageing, pathology or insult [39]. Cognitive reserve is related to the brain's anatomical substrate or functional adaptability of brain networks and optimising performance in cognitive tasks. It is often referred to as an active dynamic construct. In contrast, so called *brain reserve* may be defined as passive and refers to fixed neurobiological capital, such as head circumference [42]. *Brain maintenance* refers to reduced accumulation of pathological burden and insult due to, for example, certain lifestyle factors, reflecting the brains ability to be plastic based on experience. These three constructs, although independent, are also intertwined. For example, brain maintenance reflects enhanced hippocampal volume or reduce brain Aβ in response to a reserve enhancing lifestyle factor. Cognitive reserve would be the apparent functional flexibility of brain networks in overcoming existing pathology or insult and successfully maintaining preserved cognitive function. In turn, these processes may be modified by existing brain reserve. Cumulative and dose-response exposure to reserve enhancing factors including, but not limited to, PA, intellectual stimulation or leisure activities have all been linked to a reduced risk of cognitive decline and dementia in later life, even among those with genetic predisposition to dementia [43-45]. A translational perspective surrounding mechanisms underlying such observations, from a reserve standpoint, has been presented with recommendations for future research and translational approaches [38]. Such recommendations and principles will guide the discussion and translation of findings presented within this thesis.

1.2 Cognitive performance in ageing and dementia

Cognitive assessments designed to detect subtle change prior to noticeable clinical symptomology may provide a reliable marker of pathological ageing. Yet, there is little consensus surrounding methods to detect the earliest stage of pre-clinical AD prior to meeting the threshold for MCI diagnosis [46]. This difficulty may be due to the subtlety of such changes [47] alongside the tendency for cognitive tests to implicate multiple aspects of cognition and resultant discrepancies between cross-sectional and longitudinal study methods [48]. Despite such obstacles, certain cognitive domains have consistently indicated signs of early decline as a departure from normal ageing [49], including episodic memory, working memory and attentional processes, executive function, and visuospatial abilities [49]. Variations in memory, executive function, reasoning, spatial abilities, attention and language are all associated with normal brain ageing [50]. Clinical cognitive impairment occurs when increments of cognitive decline exceed that which would be expected based on an individual's age and education level [51].

The cognitive domain receiving most attention with regard to ageing is memory (for a review; see [52]). A common complaint of ageing is memory loss. Many studies attempting to disentangle normal ageing from early signs of pathological ageing have done so via measuring subtleties of change in this domain. However, memory is not a unitary construct: age-related changes often occur differentially, dependent upon the type of memory [53]. Short-term or immediate memory involves the simple maintenance of information over a short period of time and is often tested by immediate recall tasks. Although there is less emphasis on deficits in immediate memory as a clinical indicator of decline, studies are beginning to implicate reduced performance in such, as an indicator of the earliest stages of memory decline [54]. Long-term or delayed memory, on the other hand, requires retrieval of information that is no longer present or being maintained in an active state. There are two main types of long-term memory considered to be cognitively demanding: semantic memory (e.g. memory of facts), which has been shown to continually improve until approximately 60-years of age, and episodic memory (e.g. memory of events) which remains roughly the same until 60-65 years [55]. Changes in episodic memory is now implicated as a key domain indicative of pre-clinical AD [56]. Decline in episodic memory among older adults usually involves deficiencies in encoding, storage and retrieval processes. At the input stage (encoding) older adults may encode information less meaningfully, fail to consider peripheral detail or fail to integrate contextual characteristics of the memory, yielding less distinctive memory traces. These strategies are often referred to as effortful encoding, which depend on pre-frontal brain regions. Successful encoding is followed by storage and consolidation, which rely upon medial temporal lobe structures, particularly the hippocampus. Storage and consolidation are thought to involve the ability to bind the memory to a spatial and temporal context, which may be a process becoming increasingly

impaired in older age. Retrieval of this information is dependent upon the quality of encoding, storage and/or consolidation.

A further element of cognition, falling under multiple cognitive domains, is termed 'working memory'. Working memory is a multi-dimensional cognitive construct and a fundamental source of age-related deficit in a variety of cognitive tasks including long-term memory, language, attention, problem solving and decision making. It is essentially considered a limited capacity system that involves the active manipulation of information currently being retained in focal attention [57].

Executive function (EF) is a key indicator of pre-clinical symptomology; being an umbrella term for higher order cognitive-cortical processes including memory, inhibition, task switching and self-regulation [58]. EF may be more sensitive to pathological decline, when compared to episodic memory alone [59, 60], due to substantial age-related neurodegeneration occurring in the pre-frontal cortices as the earliest pre-cursor stage to MCI and the main hub for EF [61]. In one study, accelerated rates of EF decline was observed up to five years prior to diagnosis in patients who eventually developed AD dementia [62]. A further study reported EF decline to precede memory loss 13 or more years prior to AD diagnosis, based on the Digit Symbol Modalities Test [63], while another posited EF decline 4.5 years prior to AD diagnosis, as measured via the Trail Making test and intrusion error [64]. The Baltimore Longitudinal Study of ageing reported congruent results, showing accelerated decline in EF measured via category fluency, letter fluency and trail making test B prior to AD diagnosis [62].

Elements of attention also show decline as a departure from normal ageing [65]. Divided attention has shown deficits after 60 years, as attention switching tasks tend to slow and can be sensitive to early preclinical changes [66]. Performance in this domain commonly overlaps with others such as working memory, immediate and delayed episodic memory and executive functions and is considered a general indicator of overall cognitive function. Language abilities have been found to show age-related decline, dependent upon components of language processing. Word-finding tasks have identified the linear increase of those reporting tip-of-the-tongue (TOT) effect as age increases [67]. Picture naming tasks have also provided evidence for decline in language productive abilities with increasing age [68, 69]. Older adults also tend to produce simpler sentences and present with a subtle decline in speech speed when compared to younger adults [67]. It is currently unknown, however, whether subtleties in language may act as an earlier indicator of AD.

Visuospatial (VS) cognition, the ability to comprehend spatial relations and orientation between objects, also declines with age [70]. Decline in VS abilities is exacerbated in pathological ageing [71]. One study of healthy older adults at increased risk for AD (40-59 years old), utilised an extensive neuropsychological battery to evaluate cognitive change and found larger deficits in VS and navigation tasks when compared to episodic memory [71]. Another study reported VS abilities

to decline 9-years prior to AD diagnosis; preceding declines in episodic memory [72]. In a further study, VS abilities exhibited a sharp decline 3-years prior to AD diagnosis, whereas individuals who did not develop AD remained relatively stable in this domain [72]. Few studies have explored the association between PA and visuospatial function. However, given previous reports of visuospatial functioning possibly indicating early signs of AD, it will be insightful to investigate the association between PA and this area of cognition within the current study.

1.3 Risk factors and protective factors for Alzheimer's dementia

Conceptually, a risk factor for dementia may either affect AD pathology, vascular or other pathology, modify the relationship between pathology and dementia, or be independent of known pathology. Meta-analyses have sought to identify the attributable risk (percentage of cases attributable to a given factor) of seven potentially modifiable risk factors: diabetes, midlife hypertension, midlife obesity, smoking, depression, low education, and physical inactivity [73-75]. The first study to conduct such a meta-analysis concluded that up to half of AD cases worldwide (17.2 million) and in the USA (2.9 million) are potentially attributable to these factors and that a 10-25% reduction in all seven factors could potentially prevent up to 1.1-3.0 million AD cases worldwide [75]. The calculation of risk factor reduction on AD prevalence was done so via calculating population attributable risks (PARs), which take into account the prevalence of a given risk factor as well as the strength of its association with the outcome of interest. These results were later extended upon by considering risk-factor communality and calculating risk estimates for Europe and the UK in addition to the USA and worldwide [74]. Congruent with the previous study, a further group reported that the highest PAR world-wide was for low education (19.1%, 95% CI 12.3, 25.6). Physical inactivity had the highest-ranking PAR in the USA at 21.0% (95% CI 5.8, 36.6), Europe at 20.3% (95% CI 5.6, 35.6), and the UK at 21.8% (95% CI 6.1, 37.7). They reported the worldwide total PAR, after adjustment for the association between factors, to be 28.2% (95% CI 14.2, 41.5), or 9.6 million attributable cases (95% CI 4.8, 14.1) of 33.9 million cases and 30% for the USA, Europe and the UK. Finally, they predicted that reductions of 10% per decade in the prevalence of all seven risk factors could reduce the prevalence of AD by 8.3% worldwide by 2050 [74]. A comparable meta-analysis also estimating PAR whilst controlling for each risk factor in Australia, reported near synonymous results. They reported that physical inactivity was related to the largest proportion of dementia cases (17.9%; 95% CI 8.2, 27.3) and that if each risk factor could be reduced by 20% per decade, dementia prevalence in Australia would be reduced by 30.7% in 2050 [73]. A further Lancet Commissions report extended available models of risk by including midlife hearing loss, derived from a new review and meta-analysis conducted by the same authors, as well as later-life social isolation. Results suggested that 35% of dementia is attributable to a combination of these nine risk factors [76]. Such findings highlight the importance of prevention

via risk factor reduction and emphasises the importance of further investigating benefits of PA, given the strong weighting of inactivity published in all aforementioned reports. To follow is a review of the most commonly identified modifiable and non-modifiable risk factors for AD and dementia. As PA is the key focus of this thesis, section 1.4 will be solely dedicated to introducing PA.

1.3.1 Non-modifiable risk factors –*APOE,* age and sex

Non-modifiable risk factors play an even larger role in risk of dementia, proposed to contribute up to 65% [76] and include such factors as age (being the highest contributor) [77, 78], sex, and genetic status; especially possession of the *APOE*ε4 allele [79].

1.3.1.1 Genetics – the role of *APOE*

Apolipoprotein E (ApoE) is a 34-kDa lipid binding protein which transports triglycerides and cholesterol in multiple tissues, including the brain [80, 81]. Cholesterol transport is critical for maintenance of myelin and neuronal membranes both in the central and peripheral nervous systems [82]. ApoE is vital for the brain, being the primary lipid carrier molecule instrumental in the coordination of lipid metabolism following injury. It also plays a role in facilitating synaptic turnover, dendritic reorganisation [83, 84], and clearance of cholesterol and toxic Aβ oligomers [85].

The *APOE* gene codes for three specific isoforms of apoE (E4, E3, and E2) which give rise to three common homozygous (E4/4, E3/3 and E2/2) or three heterozygous (E4/3, E4/2, E3/2) phenotypes. Lipoproteins associated with apoE4 are cleared more efficiently than apoE2 or apoE3; hence, *APOE*ε4 carriers have significantly lowered levels of the apoE protein among serum, CSF and brain tissues. One study, among AD patients, found an inverse relationship between increased Aβ and tissue apoE concentrations, suggesting that reduced concentrations of apoE, particularly in ε4 carriers, are associated with higher levels of soluble Aβ and deposition [86]. Studies on transgenic mice have also shown that *APOE*ε4 allele possession is associated with increased brain Aβ deposition and abnormal tau phosphorylation, plus reduced learning and memory [87]. These results, plus others [88, 89] support the role of apoE as a scavenger/modulator of extracellular Aβ whereby efficacy is markedly affected by *APOE*ε4 allele carriage.

Possession of the *APOE*ε4 allele is now considered a major genetic risk factor for AD [81, 90-92], known to play an unfavourable role on not only Aβ deposition and clearance, but also neuronal maintenance and vulnerability, neuronal signalling pathways, cytoskeleton structure/function and influencing concurrence of cerebrovascular events [93] [94, 95]. Likewise, *APOE*ε4 allele possession is associated with multiple clinical outcomes of ageing, from cognitive decline to AD [96-99]. When compared to *APOE*ε4 non-carriers, there is a 2- to 4-fold increased relative risk of

AD via possessing just one ε4 allele [100] and an 8- to 15-fold increased risk for those possessing two ε4 alleles [101]. Additionally, *APOE*ε4 carriage has been associated with an earlier onset of AD dementia along with a higher rate of cognitive decline [98, 102-104]. Among those diagnosed with AD, up to 60% carry one *APOE*ε4 allele [90], with the strongest associations for AD-risk seen among those entering their early 70's, followed by risk reduction after 85 years [105]. The frequency of AD and mean age at clinical onset are 91% at 68 years old among *APOE*ε4 homozygotes, 47% at 76 years old among heterozygotes, and 20% at 84 years old for *APOE*ε4 non-carriers [97].

Due to *APOE's* link with Aβ deposition, studies have begun exploring the interactive role of these two risk factors. A study of combined datasets from three large comparable observational studies found ε4 allele carriers with clinical levels of Aβ (Aβ+/ε4+) to have significantly faster cognitive decline of verbal episodic memory over a 1.5-year period compared to those without these risk factors [106]. Another study (n= 447) using data from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study, reported episodic memory decline over 72 months in Aβ+/ε4- to be exacerbated upon factoring in ε4 carriage, as noted in the Aβ+/ε4+ stratum. However, those individuals who were Aβ-/ε4+ did not exhibit any faster cognitive decline than those who were Aβ-/ε4- (estimate (SE), 0.001 [0.001]; t = 0.526) [107]. This suggests that the *APOE*ε4 allele may only be strongly associated with memory decline in the presence of high Aβ levels. As studies have consistently reported a direct link between possession of the ε4 allele and higher Aβ, possibly those who possess an ε4 allele but who have low Aβ levels may provide an example of those who engage in activities that promote brain maintenance. This concept eludes that lifestyle factors may additionally mitigate risks associated with *APOE*ε4 carriage via mechanisms targeting the reduction of toxic Aβ levels as will be covered in section 1.6.

1.3.1.2 Age

Although modification of risk factors is encouraging and continues to be greatly important in dementia prevention, age is the *greatest* risk factor for dementia. Risk of dementia exponentially increases from the age of 65, with 80% of dementias occurring among those aged 75 or older [108, 109]. Ageing is a complex and progressive process whereby multiple systems driving brain ageing contribute to risk of AD. This includes such processes as glucose hypometabolism and mitochondria dysfunction, innate immune and inflammatory reactions, Aβ processing, dysregulation of cholesterol homeostasis, white matter reduction and decline in regenerative capacity [110, 111]. What's more, the program of senescence is a highly complex process and non-linear in trajectory; taking stepwise functions between transitional states, which can potentially be modified by external factors [112-114]. Brain changes occur with increasing chronological age, however, the rate of change, differences in biological brain age and underlying processes are less

clear. In terms of personal brain ageing, studies suggest that a healthy lifestyle aimed at reducing cardiovascular risk will undoubtedly benefit the brain and promote resilience [115, 116]. How individuals respond to such exposures may also depend upon the age at which they are implemented [78]. As will be highlighted below, certain risk factors are more strongly linked to later life dementia during midlife, whereas, others more so in late-life. Age is important when interpreting the impact of lifestyle on cognitive and brain health, especially if doing so in the context of cognitive/brain reserve and maintenance. For example, one may expect that endorsing an active lifestyle in midlife may promote both brain maintenance and cognitive reserve, whereas if endorsed at a later-life stage, benefits may manifest via cognitive reserve only. This raises questions surround threshold effects – at what age threshold and for whom is lifestyle intervention beneficial.

1.3.1.3 Sex

In medical research, sex refers to biological and physiological differences between females and males. Sex hormones and gonadal hormones contribute to such differences in cellular, organ and at a systems level. Gender refers to a combination of social, cultural and environmental exposures which may also influence biological factors in females and males. These terms; men/women (gender) versus males/females (sex), are unique constructs and should not be used interchangeably. Although differences in treatment outcomes among many diseases are dependent upon both sex and gender [117], the current study will specifically focus on the effect modification of biological sex on PA/cognitive associations.

Sex has been shown to affect the aetiology, presentation and treatment outcomes of many diseases, including AD dementia. [118]. For example, of the 5.3 million people over the age of 65 with AD dementia in the United States, 3.3 million are estimated to be females and 2.0 million are males [1]. However, sex specific prevalence estimates worldwide vary. For example, higher prevalence has been reported among females in Europe when compared to males [119], yet in the UK a lower prevalence was reported among females when compared to males [120], and no differences in prevalence between males and females worldwide have also been reported [121, 122]. Conversely, higher prevalence of MCI among males than females is consistently reported [123, 124]. Despite incongruent reports, differences in males versus females in response to treatments, risk factors and mortality are well known [118, 122, 125].

One such example is the difference in effect of *APOE* allele variant's on risk of cognitive decline and dementia. Specifically, recent studies reported females who carry the *APOE*ε4 allele to have significantly increased risk of developing AD when compared to non-carrier females, whereas males did not present with the same level of risk [79]. For example, a meta-analysis of nearly 58,000 participants [126] reported sex differences in risk of AD by *APOE* genotype, with *APOE*ε4 carrier females between 65-75 years old exhibiting a fourfold increased risk for AD dementia when

compared to males (females, OR, 4.37; 95% CI, 3.82-5.00; males, OR, 3.14; 95% CI, 2.68-3.67). Although the mechanisms underlying the interaction between sex and *APOE* genotype on increased dementia risk remain unclear, evidence has suggested that *APOE*ε4 carrier females may present with greater levels of AD pathology, more compromised brain network integrity, and accelerated decline over time for a given level of AD pathology, when compared to males [127-130]. Several other studies have reported *APOE*ε4 carrier females to show faster cognitive decline than either female non-carriers or males of any genotype [131, 132].

1.3.2 Modifiable risk factors

1.3.2.1 Education and cognitive activity

Education has been posited as a key factor promoting cognitive reserve capacity. It has been consistently associated with cognitive performance [133], independently to AD pathology [134-137]. However, interactions between education and AD pathology on cognition have also been reported [138, 139]. Likewise, education has been directly associated with infarcts [135] as well as modification effects for infarcts [136]. Furthermore, epidemiological studies have consistently reported higher educational attainment to be linked with reduced dementia risk [139-141]. Not just high education in early life, but later-life cognitive activity is linked with maintained cognitive reserve. For example, occupational complexity [133] and engagement in other intellectual activities [142] are all consistently associated with reduced risk of cognitive decline. Therefore, evidence supports that education and the related construct of occupational complexity plus intellectual engagement in later life promotes resilience to pathology and neural reserve capacity.

1.3.2.1 Cardiovascular risk factors

Many cardiovascular risk factors are directly linked to cerebrovascular disease and vascular dementia, and their association with brain health is well established. Type 2 diabetes (T2D) is strongly associated with Alzheimer's dementia, but clinicopathological studies have remained inconclusive. Most studies show no association between T2D and AD pathology [85], although one recent publication did report a relationship [143], while another study reported T2D to be associated with cerebral infarcts but not AD pathology [144]. High blood pressure has also been linked with increasing dementia risk. Evidence indicates that high blood pressure in midlife is associated with dementia [145], but not in later life. Likewise, high body mass index (BMI) in midlife is associated with increased risk of dementia [146], whereas high BMI in late-life is actually linked with reduced risk of dementia [147]. Furthermore, a decline in BMI in older age, regardless of original BMI status, is also linked with increased risk of dementia [148]. Hypercholesterolemia is a common syndrome in Western societies and its link with AD risk remains inconsistent [149]. It induces

vascular disease and dysregulation of brain cholesterol which is associated with brain Aβ deposition. Hypercholesterolemia is also an integral aspect of metabolic syndrome, which in combination with impaired glucose metabolism and high blood pressure, has repeatedly been associated with cognitive decline and dementia [150].

The above cardiovascular risk factors will be adjusted for in analysis throughout this thesis. Likewise, such factors have also been directly associated with PA levels [151], hence, further sanctioning *a priori* decision for adjustment.

1.3.2.2 Other factors

The role of diet and nutrition in reducing risk of dementia and in relation to cognitive function has only recently been studied in depth, owing mostly to the complexity of nutrient intake measurement (and their interactions). Consensus is emerging that consumption of fats and trans-fatty acids may lead to an increased risk of dementia, while consumption of green leafy vegetables alongside other nutrients and vitamins may reduce risk [152]. Arguably, dietary pattern analysis may yield more reliable outcomes when compared to exploring individual nutrients, which may fail to account for the synergistic contribution of certain nutrients upon one another [153]. Indeed, a review provided compelling evidence to indicate dietary patterns being more strongly associated with cognitive outcomes than were individual nutrients or food groups [154]. Such dietary patterns receiving most attention have been a Mediterranean diet (MeDi), the DASH (Dietary Approaches to Stop Hypertension) Diet and the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet [155]. A systematic review of 32 studies (including five RCT's and 27 cohorts) linked the MeDi with improved cognitive function and reduced risk of cognitive decline, dementia and AD [156]. Likewise, higher adherence to the DASH diet has been associated with better cognitive performance [157] and lower cognitive decline [158] in prospective cohort studies, as well as improved cognitive performance in an RCT [159]. The MIND diet, being a hybrid of the Mediterranean and DASH diets; focussing on plant-based foods with an emphasis on green leafy vegetables and berries, has also provided compelling evidence for reducing AD risk. One study compared MeDi, DASH and MIND among 923 participants finding that moderate adherence to the MIND diet may be adequate in reducing risk of AD whilst high adherence to MeDi or DASH may be required to observe the same benefits [160]. In the context of AD pathology, studies have shown that seafood consumption is associated with less AD pathology among *APOE*ε4 carriers [161], the Mediterranean diet is associated with lower Aβ load [162] and that high-intensity PA may modulate the associations between diet and CSF Aβ levels [163].

Both sleep [164] and diurnal circadian rhythm [165] have been linked to dementia Aβ clearance. However, the associations with both are also bi-directional raising question as to whether these observations are a cause or consequence of pathology. One study reported that *APOE*ε4 carrier

status modified the association between better sleep consolidation on incidence of AD and tau tangle pathology, indicating that it may provide both resilience and resistance for those at risk [166]. Although there appears to be a clear link between sleep quality and risk of dementia, further studies are required to establish causality and tease out the complex interrelations between sleep and AD dementia [155].

Hearing loss as a risk factor for dementia is relatively new, however, results from cohort studies have reported that even mild levels of hearing loss are associated with substantially increased long-term risk of cognitive decline and dementia among cognitively healthy individuals [76, 167, 168]. Although two cohort studies did not detect an association [169, 170], a meta-analysis of three studies identified that hearing loss among individuals over the age of 55 resulted in a pooled Risk Ratio (RR) of 1.94 (94% CI 1.38, 2.73), and calculated 9% reduction in new cases if it were eliminated [76].

Whether depression is a risk factor or symptom of AD is debatable. Long follow-up cohort studies show links between number of depression episodes and risk for dementia [171], yet one cohort study, following people over 28 years prior to dementia diagnosis, found the link between dementia incidence and depressive symptoms to be present just 10-years prior, suggesting that midlife depression may not be a risk factor [172]. However, it is biologically plausible that depression may increase dementia risk due to its effect on stress hormones, hippocampal volume and neuronal growth [173].

A reduction in social contact may be a prodrome or a part of the dementia syndrome. A meta-analysis of 19 longitudinal cohort studies reported low social contact (RR: 1.41 [95% CI 1.13, 1.75]), less frequent social contact (RR: 1.57 [95% CI 1.32, 1.85]) and loneliness (RR: 1.58 [95% CI 1.19, 2.09]) to be associated with incident dementia [174]. A further study, calculating PAF scores, utilised data from this systematic review and identified social isolation to contribute similar weightings towards risk of dementia than did hypertension and physical inactivity [76]. Social contact has also been directly linked with risk of hypertension [175], coronary heart disease [176], and depression [177]. All these variables are risk factors for dementia, highlighting the importance in considering social engagement in older people alongside their physical and mental health.

1.4 Physical activity, late-life cognition and dementia prevention

PA has been identified as a key factor implicated in the possible reduction of cognitive decline and AD dementia risk [73-75]. Evidence pertaining to PA and exercise training have all documented modulation and/or maintenance of cognition in cognitively healthy older adults [178-180]. Following a review of terminology, this section will provide a narrative review of prospective observational and experimental studies exploring the role of PA in dementia prevention and

maintained cognitive health in older age. Given the relatively large body of literature, the below review will focus on those studies providing the strongest evidence based on robust methodologies [181]. Evidence will predominantly come from meta-analytic reviews of prospective and experimental studies focussing on cognitively normal older adults.

1.4.1 Fitness, exercise and physical activity: a review of terminology

The terms PA, exercise and cardiorespiratory fitness (CRF) are often used interchangeably but represent distinct constructs. It is prudent to define how this field has conceptualised these terms and used them within the context of cognitive ageing. The first term, physical activity, is defined as "any bodily movement produced by skeletal muscle that results in energy expenditure" [182]. Physical activities are considered habitual in nature and may be aerobic or anaerobic and vary according to the range of energy expenditure, encompassing varying leisure activities (e.g., walking, playing tennis), occupational (e.g. unloading boxes) or household/outdoor (e.g. cleaning, gardening). Exercise is a structured form of PA to improve fitness, but considered to be a distinct sub-category in that it is "planned, structured, repetitive and purposive in the sense of improvement or maintenance of one or more components of physical fitness" [182].

The newly updated CMO 2019 PA guidelines for older people (65+) recommends 150 minutes of moderate-intensity aerobic activity per week or vigorous-intensity aerobic activity for 75 minutes per week, or a combination of both [183]. The guidelines emphasise the value of PA below moderate levels; increasing volume and frequency of light activities and reducing sedentary behaviour. The guidelines also highlight the importance of participating in some strength exercises to improve balance and co-ordination [184-186]. Further investigation around exercise parameters to better determine the optimal recommendations for preservation of cognition and brain health in older adults is warranted [187].

1.4.2 Physical activity, cognition and reducing dementia risk

1.4.2.1 Prospective studies

Prospective studies in which baseline PA measurement is used to assess future cognitive function and trajectories have typically reported a reduced risk of cognitive decline and better over-all cognitive function among those more physically active. Multiple narrative and systematic reviews [178, 179, 188-193] as well as meta-analyses [180, 194] of prospective evidence have concluded that PA positively influences cognitive function in older adults with small to medium effect sizes. A meta-analysis of 15 prospective studies or 12 cohorts (three studies utilised the same cohort but published on males or females separately, N = 33,816), explored the association between PA and risk of cognitive decline among cognitively healthy older adults. They reported that the cumulative analysis for all the studies under a random-effects model showed that high PA participants were

significantly protected (-38%) against cognitive decline during follow-up (hazard ratio (HR) 0.62, 95% CI = 0.54, 0.75) [180]. Another review extended these findings through inclusion of five additional studies, reporting the risk ratio (RR) for cognitive decline in low active individuals, when compared to high active participants, to be 0.65 (95% CI 0.55, 0.77) [194]. Summary statistics appear to indicate that PA reduces risk of cognitive decline by 35-38%. With regard to domain-specific cognitive associations, observational studies have reported positive associations between partaking in higher levels of PA (when compared to low PA individuals) across multiple domains, including global cognition [114, 195-200], processing speed [201, 202], episodic memory [199, 200, 202-204], executive function [201, 204], visuospatial, immediate and delayed memory [199, 200, 205, 206], language and attention [199, 207, 208].

Cognitive decline is a hallmark indicator of AD risk; however, it does not necessarily lead to AD or dementia. Prospective cohort studies exploring the role of PA on risk of AD or dementia provide important insights. Summary statistics indicate the PA is associated with a 28-49% reduced risk of AD and dementia [209, 210]. A meta-analysis including 16 studies with 163,797 cognitively healthy participants found that in the highest PA group compared to the lowest, the RR of dementia was 0.72 (95% CI 0.60, 0.86) and the RR of AD was 0.55 (95% CI 0.36, 0.84) [210]. A later meta-analysis, including nine studies of 20,326 older adults ≥65 years old, found physically active individuals to have a reduced risk of developing AD when compared to non-active individuals, RR=0.61 (95% CI 0.52, 0.73) [209]. Despite the above, evidence implicating higher PA with reduced risk of AD and dementia is not without conjecture. Indeed, some studies have challenged this hypothesis, reporting no association between PA and risk, especially those with longer follow-up periods [211, 212]. For example, some authors have argued that reverse causation cannot be ruled out. One study following 10,308 participants for up to 27 years, reporting no association between PA and dementia risk, HR = 1.00 (95% CI 0.80, 1.24), when follow-up periods exceeded 10 years [211]. However, another review conducted a sensitivity analysis among studies with >10-years follow-up reporting a statistically significant, weak association between higher levels of PA and reduced risk of both dementia and cognitive decline in later life [194]. Experimental evidence may help prove or disprove this notion, as discussed below.

1.4.2.2 Experimental studies

Randomised Controlled Trials (RCT's) involving PA intervention have typically used aerobic activity of moderate intensity or mixed aerobic and strength training to observe effects on cognition pre- and post- training. Due to insufficient follow-up time, RCT's are currently unable to investigate whether PA reduces risk of AD and/or dementia. Furthermore, two meta-analyses failed to identify significant benefits of PA on cognition [213, 214]. Although, authors of both these studies chose to compute effect sizes by cognitive outcome and study design (i.e. control group type and PA modality), rather than reporting an average overall effect across studies and then testing these

variables as possible moderators. The limitation of this decision is that effect sizes are summarised over a small number of studies (1-6 per cognitive outcome and exercise/control group design), limiting statistical power.

By contrast, at least ten other meta-analyses of RCT's exploring the effects of PA on cognitive function among cognitively healthy adults consistently reported positive effects on cognition with small to moderate effect sizes [188, 215-224]. A high quality meta-analysis of 18 RCT's in older adults found that moderate intensity PA resulted in enhanced cognitive function across all available cognitive domains, with the largest effect size for indices of executive function (g=0.426, SE=0.062) [216]. Another meta-analysis, of five studies exploring the effect of PA on sedentary older individuals, found that just walking was enough to exhibit cognitive improvements, with the strongest effect for specific domains of executive function, d=0.36 (95% CI 0.16, 0.55) [223]. A further meta-analysis (n=7 studies) too reported that a single bout of moderate intensity PA benefited sub-domains of executive function, with a moderate mean effect size (g=0.67) [222]. Exploring a range of different PA types, a high-quality meta-analysis of RCT's among healthy older adults (n=39 studies) reported that aerobic, resistance training and Tai Chi benefited general cognition (standardised mean difference (SMD): 0.29; 95% CI 0.17, 0.41), although not yoga. They also found that overall cognitive benefits were observed among trials prescribing 45-60 minutes of moderate intensity PA per week and resistance training improved executive function, memory and working memory [219]. A further review of RCT's (n=15) examined the effect of PA on working memory performance, reporting that sustained PA over time exhibited an effect on working memory, albeit a small effect size, whereas one single bout of PA found no such benefit [220]. A more recent, high quality meta-analysis of 23 RCT's including healthy older adults (n=1,225) with a mean age 70.3 years old (SD=5.32) investigated the dose-response relationship between exercise and cognitive function. They too reported a small positive effect of general PA on executive function, d=0.27 (95% CI 0.15, 0.40), and memory, d=0.24 (95% CI 0.06, 0.41) but not for global cognition and no differences on effect size as a function of PA dose (frequency, duration and intensity) [224]. Other meta-analysis have reported the positive effect of aerobic activity intervention on executive function and memory among cohorts of a wider age-range (18+ years old) [221], again among older adults (55+ years old) [215, 217] and among those with poor mental health [218].

1.4.3 Physical activity timing: midlife versus late-life

In published work on dementia risk, midlife has been defined as 45-65 years and later life as older than 65 years [76]. As previously mentioned, it has been postulated that a follow-up period of <10-years may not preclude reverse causation [211], and that late-life PA may be influenced by residual factors such as sub-clinical dementia [225]. Indeed, impaired mental and physical function is common among the earliest stages of MCI and can also interfere with protective lifestyle factors

such as PA and social activities [226]. Likewise, it has been reported that high BMI and hypertension during midlife exhibit stronger associations with risk of dementia when compared to these same illnesses in later life risk [227, 228]. To explore PA during an earlier life-stage, such as midlife, and how it is related to later life cognition may provide more meaningful outcomes within an observational context than would PA in later-life. This may be especially the case when exploring differences in response to PA as a function of key non-modifiable factors such as age, sex and *APOE*ε4 carrier individuals.

One systematic review of nine cross-sectional and 14 prospective observational studies in healthy adults explored the role of PA in either midlife or late-life (but at least 10-years prior to the final cognitive assessment), on later life domain-specific cognitive performance [229]. They reported that moderate- to vigorous leisure activity was associated with better global cognition, executive function and memory but not attention or working memory when compared to low active individuals. However, an opposing review was also published [211] as summarised above, stating no association between midlife PA and dementia risk, concluding that any association in later life be a likely consequence of reverse causation. No study, however, has explored the role of midlife PA on later-life domain-specific cognitive function and trajectories as a function of *APOE*ε4 carrier status, age or sex. The differing response to activity on cognitive health based on these factors are yet to be clarified, as will be discussed further below. It will be important to not only observe the association between late-life PA and cognitive function but also midlife PA, when answering these vital questions. Likewise, there is a need to prospectively assess the influence of changes in PA behaviour from mid- to late-life in relation to later-life cognition and whether or not increases or decreases in this behaviour may differentially impact those at increased risk for AD when compared to those with lower risk. Just one study has explored this question in the context of *APOE*ε4 carrier status. They strived to determine whether midlife to late-life PA status were associated with risk of MCI and whether this varied according to *APOE*ε4 carrier status (n=1,830). The study observed an increased risk for incident MCI in *APOE*ε4 carriers reporting no moderate or vigorous activity in midlife compared to non-carriers within the same low active group [230]. The study also found that *APOE*ε4 carriers who were low active in mid- and late-life had a higher risk of incident MCI as compared to low-active non-carries. It is unknown if a decrease in PA from previously high levels will be associated with accelerate cognitive decline, or likewise, whether increasing PA later in life is adequate to exhibit a positive association with cognition, and again, whether these associations would depend on genetic risk, sex and/or age. Attempts to explore such questions will be presented in this thesis.

1.4.4 Type and intensity of physical activity

Sport psychology has suggested that the success of PA programs for improved health rely on several factors including duration, intensity, frequency, variety, if combined with cognitive stimulation and whether the activity occurs in a group or individual [231]. Differences in the effect of aerobic and anaerobic activity have also been published with debates over which is most efficacious for cognitive health [224]. Aerobic activity allows the synthesis of adenosine-triphosphate (ATP) by aerobic mechanisms, adjusting in response to intensity, duration and oxygen availability. In contrast, anaerobic activity is high intensity, short in duration and with an absence of oxygen, depleting ATP and/or phosphocreatine reserves in the muscle, shifting the production of ATP to lactacid or alactacid as alternate anaerobic energy mechanisms [231].

The majority of longitudinal studies have focussed on a single exercise training modality, i.e. aerobic exercise [232-234] or anaerobic exercise [235-237]. Nonetheless, aerobic training consistently yields enhanced benefit toward a wider range of cognitive domains [238, 239]. Although still debated, there has been numerous and robust evidence to indicate aerobic activity to be associated with potent structural and functional neuroplastic changes, which corroborate improvements in cognitive functions [240-243]. The effects of a single bout of aerobic activity has been related to improved cognitive function, especially prefrontal cortex-dependent cognition [244-246], however, the effects on cognitive function are generally small when compared to long-term or chronic aerobic activity, which have shown positive effects [247].

Evidence appears to implicate aerobic activity of moderate to vigorous intensity as being most consistently linked with cognitive benefits. However, there has been a growing interest in utilising a measure of PA most representative among older adults encompassing a wider range of activity such as housework and gardening, leisure related sports and work-related activity, often referred to as everyday physical activity (EPA) [248-251]. Longitudinal studies focussing on EPA have found higher participation levels to be associated with better scores and less decline in multiple cognitive domains such as reasoning, episodic memory, verbal fluency and executive functioning [248, 251, 252]. In the current thesis one such measure of EPA will be used, as will be detailed in the methods chapter. Another popular measure of PA is a type focussing specifically on leisure activities that are sport specific and undertaken to improve fitness such as running, swimming, tennis, golf, etc. This type of activity is termed leisure PA in the current thesis and will be the key measure of aerobic activity.

1.4.5 Summary

There is a large volume of literature pertaining to the benefits of different types of PA on maintaining cognitive health and reducing risk of decline, AD and/or dementia among healthy older adults. The strongest evidence for reduced risk of cognitive decline and/or dementia are in

prospective observational studies. Experimental studies have produced mixed results and are yet to produce long enough follow-up times to be able to identify PA as an AD and/or dementia risk reducing factor. Despite limitations, evidence indicates a positive association between higher PA levels and better cognitive function in older age. Aerobic or mixed aerobic/strength training interventions appear to show more consistent benefits when compared to other types of activity. There is still debate as to what dose (frequency/duration/intensity) is necessary to yield most efficacious outcomes. For example, one meta-analysis identified walking as adequate [223], whereas another reported moderate aerobic activity of 45-60 minutes in duration twice weekly be most beneficial [219].

Cognitive domains benefiting most from PA appear to be executive function and memory. Beneficial effects of PA on executive function and memory may be fuelled by increased connectivity, especially noted within cortical and hippocampal regions, up-regulation of neurotrophic factors known to stimulate synaptic growth and connectivity and increases in brain volume, predominantly hippocampal. The associations between PA and late-life cognitive function may differ between individuals as conflicting results in the literature appear to indicate. These differences in outcomes could be due to modifying variables such as demographic, biological and genetic factors, as well as PA timing and duration (i.e. from midlife to late-life) which warrant further evaluation, as covered in this thesis.

1.5 *APOE*ε4 carrier status, sex and age: potential modifiers

The cognitive benefits garnered by engaging in a physically active lifestyle may be reduced by certain increments of age, genetic risk and sex – three non-modifiable factors associated with an increased risk for AD. The interactive influence of these non-modifiable factors on PA/cognitive associations have been poorly explored. However, evidence informed patterns, distinct contributions, timing of relationships and multi-variable interactions between these factors have been reported [77, 78], providing promising new avenues for exploration of PA/cognitive associations. The sections below provide an overview of such findings to date and justification for further exploration of these factors in this thesis.

1.5.1 *Apolipoprotein*-ε4 carrier status and physical activity

An area of interest is whether the association between PA and cognition is modified as a function of an individual's genetic risk. Despite heightened risk among *APOE*ε4 carriers, possession of the allele does not always result in dementia, suggesting interactions with other genes, pathology and/or lifestyle factors. Evidence has indicated such positive PA/cognitive associations be especially augmented among *APOE*ε4 carrier individuals [114, 198-200, 208], non-carriers [248] or irrespective of *APOE*ε4 carrier status [207, 253, 254]. The impact of PA as a preventative lifestyle

factor may be noted, yet few have studied PA/domain-specific cognitive associations whilst exploring differences in these associations as a function of *APOE* genotype status. The role of PA on cognitive health and underlying biomarkers as a function of *APOE*ε4 carrier status (i.e. differences in response to PA in carriers versus non-carriers) will be explored throughout the thesis and is the consistent element drawing each chapter together. Chapter 2 will be dedicated to the introduction of this topic and provides a systematic review and detailed account of all such studies.

1.5.2 Sex and physical activity

Very few exercise studies have considered an individual's sex as a key modifier in the association between PA and cognitive health among older adults, yet, meta-analyses have indicated sex differences in their physiological response to PA, that also manifest cognitively. For example, a meta-analysis of 18 RCT's first suggested females to exhibit greater cognitive benefits of aerobic training than males (Hedges g=0.604 vs 0.150) [216]. A further meta-analysis synthesising data from 41 included studies found that aerobic, anaerobic and mixed interventions all had beneficial effects on executive function, but with greater benefits observed among studies with a higher proportion of females [117]. Although no study has directly explored the interactions between aerobic training and sex, two RCT's in older adults with MCI found aerobic training for six months [255] and one year [256] improved executive functions and memory in females and memory in both females and males. Together, these studies suggest that aerobic exercise may elicit greater cognitive benefits among females when compared to males, especially within the executive domain.

Results from observational studies, however, have been less conclusive. Benefits have also been reportedly augmented among males when compared to females. For example, aerobic PA has been associated with global cognition among males, but not females [257, 258]. Likewise, the association between PA and executive function [258] was stronger in males than females. Male only cohorts have also reported such benefits. The Zutphen Elderly Study [114] reported that males (n=347) partaking in <1 hour a day of PA had a two-fold increased risk (OR 2.0, 95% CI: 0.9-4.8) of cognitive decline when compared to those participating in >1 hour a day of PA, of which this observation was limited to *APOE*ε4 carriers. Likewise, other prospective studies have reported positive associations between higher frequency/duration PA and reduced cognitive decline over 10-years of observation among males [259] as well as reducing risk of dementia among males walking >2 miles per day versus those walking <2 miles per day [260]. Nonetheless, female only cohort studies have too reported positive associations between PA and cognition. The Nurses' Health Study [261] reported that females who walked 1.5 hours per week performed better on cognitive tasks compared to those walking <40 minutes per week. Another prospective study of 5,925 individuals from the Study of Osteoporotic Fractures reported that those in the highest quartile of walking duration were 37% (OR, 0.63; 95% CI, 0.53-0.76) less likely to experience cognitive

decline when compared to the lowest quartile [262]. Finally, other observational studies including both males and females have too reported positive PA/cognitive associations to be limited to females. For example, the prospective Canadian Study of Health and Ageing cohort (n=4,434) [263] reported that females engaging in moderate or vigorous PA had a lower risk of cognitive decline (OR, 0.58; 95% CI, 0.40-0.82), whereas the same benefits were not observed for males. Furthermore, a Chinese cohort study (n=2,030) reported an association between PA and incident cognitive impairment among females but not males [264].

Alongside hormonal differences, how males and females respond to PA on later-life cognition may be dependent upon their *APOE*ε4 genotype, as mentioned above [114]. Possession of just one *APOE*ε4 allele may modify response to PA, but how *APOE*ε4 carrier status affects risk of decline may also depend upon sex. Specifically, studies have shown the association between *APOE*ε4 carriage and risk of cognitive decline and AD are augmented among females when compared to males, yet *APOE*ε4 homozygous males are thought to be at greater risk of MCI and AD than homozygous females [129, 130, 265]. No study has attempted to disentangle the interactions between sex, *APOE*ε4 carrier status and PA on late-life cognitive function. This remains an important gap when exploring efficacy of PA as a feasible method for promoting cognitive health. Possibly a precision preventative approach may yield optimal PA/cognitive outcomes (i.e. PA programs catered for those of specific characteristics such as sex and *APOE*ε4 carrier status). Thus, the sex of an individual is an important modifier to consider, especially in relation to the association between PA and cognitive function. Further research within an observational context and in relation to *APOE*ε4 carrier status is warranted. Pilot findings from observational data, such as those within the current thesis, will advise methods for optimising sample selection for RCT's and may eventually inform public health recommendations.

1.5.3 Age and physical activity

Unlike chronological age, biological ageing does not unfold in a linear fashion but involves a multitude of coordinated systems, transition states and interactive processes [110, 111, 266-268]. Cognitive trajectories may be differentially modified by PA at differing stages of the ageing process. Likewise, cognitive trajectories may be differentially modified by the way age interacts with genetics [269]. There are important differences in the way PA may prevent cognitive decline dependent upon the age at which an individual engages in this lifestyle, both within an observational context or RCT environment, and at the age at which the cognitive benefits of PA are measured. For example, it may be speculated that engaging in a physically active lifestyle in midlife or even early older age, prior to an irreversible stage of disease progression, will alter one's reaction to such [78]. If people start to be physically active at a later disease stage, albeit asymptomatic, the pathological progression may be too advanced to mitigate effects on cognition. Alternatively,

pathology may continue to advance, yet, the lifestyle at this stage could promote the brain's resilience to pathology and instead recruit flexible pathways and maintained cognitive function (i.e. enhanced cognitive reserve) [39, 270]. Likewise, if an individual engages in higher PA at an earlier life stage, possibly prior to considerable pathological build-up, PA may not only preserve cognitive health but may also target the pathology itself by reducing or slowing its progression (i.e. brain maintenance) [39].

The effect of *APOE*ε4 on age-related decline and risk of dementia is pleiotropic, with highest risk being among those in their early 70's, followed by a gradual decline in risk such that by the age of 85, *APOE*ε4 allele carriers exhibit lower risk when compared to non-carriers [77]. The association between PA and cognition among differing age groups within the current study, will be presented, especially when considering how these associations play out as a function of *APOE*ε4 carrier status.

1.6 Possible mechanisms underpinning the benefits of physical activity on cognition

Although the mechanisms by which PA may influence brain pathology and/or cognition remain largely unclear, there may be both direct effects on pathology such as reducing brain Aβ and/or increasing brain volume and indirect effects through influences on neurotrophic factors, neuroinflammation, cerebrovascular functioning, or glucose metabolism [271, 272]. PA is also linked with reducing psychological stress and encouraging cortisol regulation [273], which in turn supports stabilization of mood [274] and improvement of sleep [275]; both factors linked to an increased risk of cognitive decline and AD dementia [155, 275, 276].

Increased cerebral blood flow and oxygen transport as a result of PA may play a mechanistic role in supporting brain health [277]. Brain processes that lead to cognitive decline often stem from atherosclerotic and cerebrovascular conditions which lead to cerebral hypoperfusion [278]. For example, one study found that higher cerebral blood flow velocity was associated with reduced cognitive decline, whereas low flow velocity was linked with increased risk for AD [279]. Enhanced blood flow may mediate the benefits of PA on cognition, while mitigating the negative association between vascular disease and dementia risk.

Angiogenesis, the formation of vasculature by endothelial cells, occurs in the brain during development but gradually declines with age. Animal studies have found that exercise enhances angiogenesis in not just the hippocampus, being highly oxygen-dependent, but also the motor cortex and cerebellum [280, 281]. The increase in maximal oxygen consumption during aerobic activity [280, 281] may explain why aerobic exercise has shown augmented improvements in cognitive function relative to other forms of activity, as aerobic exercise has also been linked with angiogenesis among animals.

Exercise has been shown to exert a powerful anti-inflammatory effect on the brain, especially within animal models [282]. Neuroinflammatory response can be initiated by factors including infection, trauma and ischemia. A chronic neuroinflammatory response is associated with a variety of neurodegenerative diseases, including AD [283], and is a key feature of AD pathology [284]. More recently, the role of PA in reducing inflammation has been increasingly investigated. Indeed, research has demonstrated that the anti-inflammatory effects of exercise on peripheral body organs, may also manifest via reduced risk of cardiovascular and metabolic disease [285]. Importantly, evidence from animal models indicate that the anti-inflammatory properties of PA extend to the brain, reducing risk of such inflammatory neurodegenerative disorders as AD [286]. The complex processes underlying this observation is less understood. However, animal studies have indicated that exercise induced anti-inflammatory cytokine release (e.g. interleukin (IL)-6 and IL-10) may mediate the metabolic changes induced by PA and downregulate inflammatory cytokines (e.g. tumour necrosis factor (TNF)-α and IL-1β) in the brain [287] plus attenuate lipopolysaccharide-induced inflammation [288].

Imaging studies have explored how PA may modify the downstream manifestations of AD, where several reviews of experimental and observational studies have endorsed the claim that PA exerts a positive effect on brain structural and functional health [289]. PA may also promote brain health via upregulation of neurotrophic factors such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF). Animal models have shown exercise induced upregulation of these neurotrophic factors to provoke brain vascularisation (i.e., angiogenesis), neuronal proliferation and growth (i.e., neurogenesis), synaptogenesis and synaptic plasticity [290-292]. Figure 1 depicts mechanistic pathways triggered by engaging in aerobic PA and resultant mitigation of AD related pathology as well as macro-level risk factors leading to increased risk of cognitive decline and dementia.

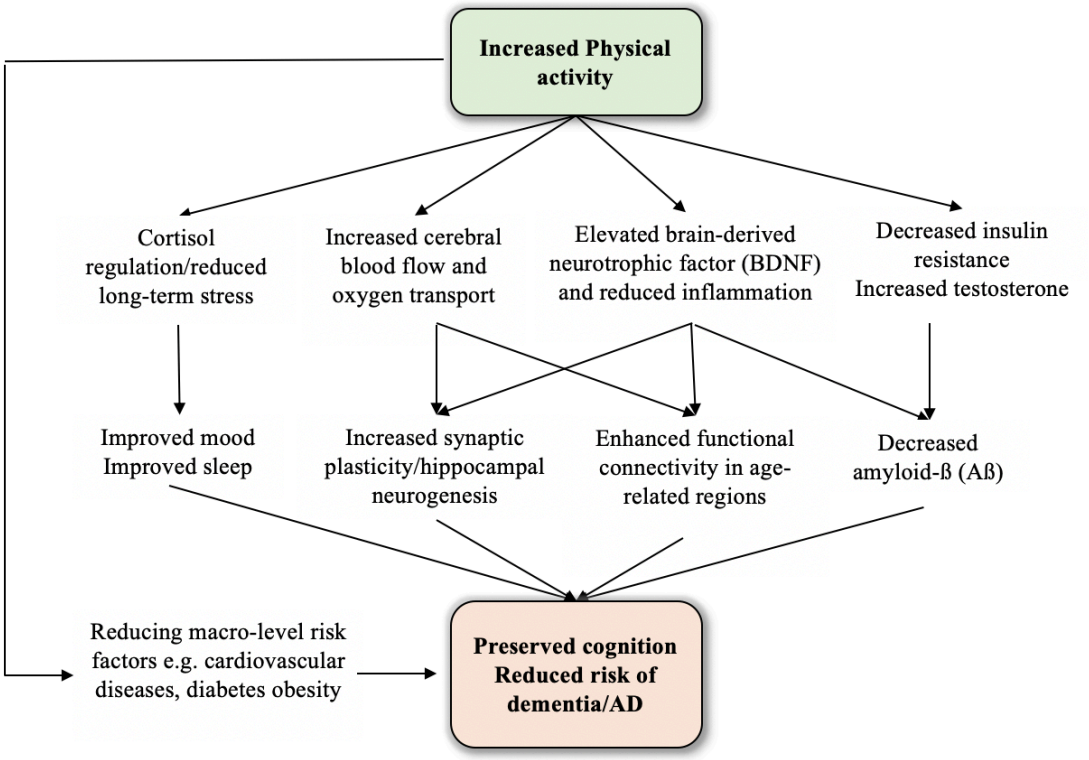


Figure 1. Physical activity and cognitive health: mechanistic pathways

PA elevates brain derived neurotrophic factor (BDNF) (plus IGF-1 and VEGF) [293], testosterone, oestrogen [294], decreased insulin resistance [295] and neuroinflammation [282]. PA increases cerebral blood flow and oxygen transport [277], coupled with upregulated BDNF, promoting synaptic plasticity and brain volume [296, 297], enhancing functional connectivity [289] and preventing Amyloid beta accumulation [298, 299]. The positive effects of PA on cortisol regulation [273] supports stabilization of mood [274] and improvement of sleep [275]; both of which are linked to increased risk of dementia / AD (e.g. depression and poor sleep) [276]. Macro-level risk factors are consequently reduced, such as reduced cardiovascular risk, diabetes and obesity. All these factors combined contribute toward preserved cognitive function, reduced risk of cognitive decline and dementia / AD among older adults.

1.6.1 Brain Derived Neurotrophic Factor

A number of studies suggest that the synthesis and release of trophic factors, especially BDNF, may play a crucial role in mediating the benefits of exercise on the brain [300-302]. BDNF is a member of the growth factor neurotrophic family and promotes brain vascularisation and angiogenesis, neuronal proliferation and growth (i.e., neurogenesis), synaptogenesis and synaptic plasticity through its interaction with its receptor, tyrosine kinase B (TrkB) [290-292, 303]. BDNF is vital for cognitive performance in the short-term and for long-term adaptations in brain morphology [304, 305]. Indeed, higher BDNF levels have been linked to increased hippocampal volume and spatial memory performance in humans [305]. Likewise, growing evidence indicates that lower levels of BDNF leads to an increased risk of AD dementia and is associated with worse cognitive performance in patients with AD [306-308]. Acute bouts of exercise have increased serum BDNF levels, and consequently linked with improvements in cognitive performance among human studies [309]. Likewise, one systematic review identified five RCT's (n=215) of healthy older adults demonstrating upregulated BDNF, as a result of increased aerobic PA, to be linked with improved cognitive scores, with the largest effect size for tests assessing executive functions [310].

Increased circulatory and brain BDNF levels are commonly observed immediately post-exercise as well as maintained at higher levels following longer-term exercise [302, 311]. Meta-analyses including 55 RCT's demonstrated a BDNF dose response from just one episode of aerobic activity, whereby peripheral blood BDNF was higher after exercise (SMD = 0.59, 95% CI: 0.46-0.72), the magnitude of which was enhanced over time by regular exercise [312]. Another meta-analysis of 29 RCT's found resting concentrations of peripheral BDNF to be higher in those receiving exercise intervention specifically for aerobic exercise (SMD = 0.66, 95% CI: 0.33-0.99), but not anaerobic (SMD = 0.07, 95% CI: -0.15-0.30). [302].

The upregulation of BDNF via PA has been well documented. However, conflicting results have also reported either no change or lowered serum BDNF as a result of PA [313, 314]. These inconsistencies remain poorly understood. Possibly other demographic, lifestyle or genetic factors are moderating the benefits of PA on BDNF levels and consequent cognitive function, however further research in this area is required. In healthy adults, possession of at least one *APOE*ε4 allele is associated with lower levels of resting state circulatory BDNF [315, 316]. Furthermore, AD patients and healthy controls who were *APOE*ε4 carriers presented with lower BDNF levels alongside worse global cognition when compared to their non-carrier counterparts [315].

Only one published study has assessed whether *APOE*ε4 carrier status modifies the effect of PA on BDNF levels. An RCT of MCI African American individuals (n=22), completed a 6-month aerobic training program after which they reported that *APOE*ε4 non-carriers only displayed a significant increase in serum BDNF levels, whilst ε4 carriers did not (estimate = -59.4, SE = 21.4, t=-2.8) [317]. No study has yet explored whether resting state serum BDNF mediates or modifies the

association between PA and cognition longitudinally and whether these associations differ as a function of *APOE*ε4 carrier status. Likewise, it is unknown if these associations play out differently among those who report being active during midlife versus late-life. Such questions will be explored in the current thesis.

1.6.2 Peripheral amyloid: a promising new biomarker for dementia

Complementary to the aforementioned findings, in vitro evidence suggests that BDNF may be a direct moderator of Aβ neurotoxicity, as BDNF and TrkB stimulate synaptic excitation and neuroplasticity [318]. This may assist in protecting against Aβ related synaptic death and/or inhibiting the Aβ cascade in both brain and periphery [319, 320]. Indeed, BDNF has been associated with reduced content and/or activity of beta-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), the rate-limiting enzyme in APP processing, leading to toxic Aβ peptide formation [321]. However, the exact molecular process leading to this outcome is unknown and warrants further attention. Supportive of these aforementioned findings, both animal experiments [272, 322-324] and human studies [325-328] have reported an association between PA and lower brain Aβ deposition and/or maintained cognitive performance in the presence of clinically high brain Aβ levels. These findings indicate that PA is able to reduce the accumulation of brain Aβ and/or promote the brains resilience to existing Aβ accumulation.

Plasma Aβ as a biomarker for AD has recently gained traction in the literature and may indeed indicate a less invasive and easily measurable proxy of brain and/or CSF Aβ. The 42 amino acid form of Aβ ($A\beta_{1-42}$) plays a key role in AD pathogenesis, being the toxic form of its counterpart, $A\beta_{1-40}$. AD markers in the blood are difficult to measure due to very low abundance. Consequently, multiple groups have sought to identify ultra-sensitive measurement methodologies, however, leading to incongruent outcomes. One study was able to demonstrate the measurement of high-performance plasma Aβ biomarkers by immunoprecipitation coupled with mass spectrometry across two independent cohorts including healthy subjects, those with MCI and AD. They reported that measurement ratios of plasma $A\beta_{1-42}$ to the reference peptides; APP 669–711 and $A\beta_{1-40}$, strongly correlated with brain Aβ and CSF Aβ [329]. A further study reported immunoassay measured plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ to be lower in AD compared to MCI subjects and correlating with CSF biomarkers (total-tau, phosphorylated-tau and Aβ) in the MCI group alongside cognitive status in both the MCI and AD group [330]. Conversely, two Chinese studies reported increased $A\beta_{1-42}$ plasma levels among AD patients [331, 332], and a further study based on the US also reported analogous results [333]. Likewise, a cross-sectional study including 1,314 participants found higher enzyme-linked immunosorbent assay (ELISA) measured plasma $A\beta_{1-42}$ among a cognitively impaired group; odds ratio (OR) = 1.04 (95% CI 1.00, 1.07) when compared to a healthy group [334]. Yet another group observed a moderately strong inverse association between highly

sensitive immunomagnetic reduction (IMR) measured $A\beta_{1-42}$ plasma and CSF Aβ [335]. Despite some opposing results, a considerable proportion of studies have reported high $A\beta_{1-42}$ plasma levels to be associated with cognitive decline and increased risk of dementia, to be positively associated with PET Aβ and inversely associated with CSF Aβ. Findings lend evidence toward the utility of plasma Aβ measurement as a potential proxy for brain / CSF Aβ, although more research is required via use of homogenous and highly sensitive measurement methodologies, as will be utilised in one of the projects within this thesis.

As summarised above, high PA has been associated with reduced brain Aβ and up upregulated BDNF which, in turn, is linked with BACE1 downregulation triggering the non-amylogenic pathway. One possible molecular pathway may be that PA inhibits the Aβ cascade in both brain *and periphery* [272]. Studies exploring the role of PA in moderating plasma Aβ are limited. One study of healthy older adults reported that higher levels of PA was associated with lower $A\beta_{1-41/1-40}$ ratio as measured by immunoassay and concurrently lower brain Aβ. They also reported that the effect of PA on plasma levels were only evident among *APOE*ε4 non-carriers, whereas, the effect on brain Aβ was only evident among carriers. This study did not, however, explore how these findings influenced cognition [325]. A longitudinal study of cognitively healthy older individuals reported that higher PA levels were associated with lower plasma $A\beta_{1-42}$ levels 9-13 years later, that higher plasma $A\beta_{1-42}$ levels were associated with greater risk of cognitive impairment, and proposed plasma $A\beta_{1-42}$ as a mediator between high PA and reduced risk of cognitive decline [336]. Both studies, however, used a less sensitive double-antibody sandwich ELISA technique. The role of plasma $A\beta_{1-42}$ in relation to cognitive function and *APOE*ε4 carrier status warrants further evaluation. It is likely that this biomarker may indeed be a realistic proxy of brain and CSF Aβ. How his peripheral marker is related to PA and in the context of cognitive performance in older age, is less know. Via utility of a more sensitive measure of $A\beta_{1-42}$, as will be introduced in the methods (Chapter Three, section 3.7.4.1, page 109), research in this thesis will attempt to replicate and extend upon previous findings through exploring the role of PA during both midlife and late-life on cognitive function and whether associations vary among *APOE*ε4 carrier versus non-carrier individuals.

1.7 Conclusion

Despite promising reports of PA's link with preserved cognitive function in older age and reduced risk for dementia, RCTs among healthy older adults have been less successful. Incongruent results hinder translation into realistic public health recommendations. Inter-individual variability in response to PA and exercise on cognition may be a consequence of such conflicting findings. As covered extensively in the literature review to follow (see Chapter 2), there is evidence to indicate

that *APOE*ε4 carrier/non-carrier genotype status may modify associations between PA and risk of cognitive decline. Additional demographic factors may contribute toward these varying results and are yet to be explored. One key factor is the age at which these associations are being observed. The effects of *APOE*ε4 carriage on cognition varies across age. Indeed, studies have found that carriers of a younger age cognitively outperform non-carriers, and only when age-related cognitive decline begins (60 years onward) does the pendulum shift. Additionally, it is known that the effect of *APOE*ε4 carrier status on risk of dementia and cognitive decline in older age reduces in an older-old age group (i.e. 80+) [77], yet how *APOE*ε4 carrier status modifies PA/cognitive associations among different older-age categories is largely unknown. Another factor warranting further evaluation is whether an individual's sex may determine how these associations play out, firstly with regard to PA and domain-specific cognition but also, again as a factor of *APOE*ε4 carrier status. Indeed, we know that the association between both PA and *APOE*ε4 carrier status on cognition in older age varies considerably between females and males, yet together they have not been investigated. These findings may provide relevant insights for future public health recommendations as well as informing targeted intervention trials. Likewise, no study has explored the differential and additive associations between midlife to late-life leisure aerobic PA and later-life cognition and at what stage an active lifestyle may be most beneficial in maintaining cognitive health among certain sub-strata. The benefits of midlife PA and/or late-life PA on cognition might vary as a function of *APOE*ε4 carrier status, age and sex. Work carried out in this thesis aims to contribute insight into these questions.

Finally, substantial gaps remain in the literature with regard to biological mechanisms underpinning PA/cognitive associations, especially as a function of *APOE*ε4 carrier status. The actions of BDNF as a key neurotrophin implicated in such processes has provided promise, yet no study has explored the associations between midlife PA and this neurotrophic factor nor its role in the context of *APOE*ε4 carrier status. Likewise, the emerging evidence that plasma Aβ may indeed be a proxy indicator for brain/CSF Aβ provides the possibility of an invaluable new biomarker for further exploration, being minimally invasive and low cost. This will be the first study to explore the association between mid- to late-life PA and plasma Aβ on resultant cognitive function and trajectories within a healthy cohort and as a function of *APOE*ε4 carrier status. To promote PA as a viable lifestyle factor for dementia prevention it is beneficial to firstly, contribute towards the body of literature exploring the possible up-regulation of circulatory BDNF as a result of PA and whether this up-regulation indeed results in better cognitive outcomes, specifically among *APOE*ε4 carriers. Secondly, to ascertain whether higher PA levels are linked with altered plasma Aβ levels and how this may be reflected cognitively and be dependent upon *APOE*ε4 carrier status.

1.8 Project Objectives and Hypothesis

This thesis is comprised of six research chapters; including a systematic review and five analyses chapters. Chapters 4-6 will investigate the associations between midlife and/or late-life PA on multi-domain cognitive function and trajectories. When referring to late-life PA (Ch: 4), two variables will be explored. To investigate a measure of everyday PA (EPA), a composite score comprised of self-reported work/household/leisure related activity from the previous week will be used. Secondly, to explore the role of aerobic-leisure PA, a binary late-life leisure PA (LLPA) variable will be used. Likewise, midlife PA (MLPA) (Ch: 5) refers to the same variable of aerobic-leisure activity as above, being retrospectively reported during midlife (45-65 years old). Specifically, those reporting ≥2 occasions of moderate/vigorous leisure PA weekly are classed as high PA, those reporting <2 occasions, as low PA. Finally, the temporal role of mid- to late-life leisure PA (Ch: 6) on later-life cognition refers to the following groups comprised of the aforementioned LLPA/MLPA groups: (a) those who report being low-active in both midlife and late-life (lowest PA group), (b) those who increased activity from low in midlife to high in late-life, (c) those who decreased from high in midlife to low in late-life, and (d) those who report high leisure PA in both mid- and late-life (highest PA group). With regard to cognitive terminology: overall cognitive function refers to an individual's average cognitive scores across a 30-month period, whereas cognitive trajectories refer to the change in cognitive scores, or trajectories, across the same 30-month period (see Methods, section 3.9, page 120-122).

Chapters 8-9 will investigate the roles of BDNF (Ch: 8) and peripheral Aβ (Ch:9) on PA/cognitive associations, utilising EPA, MLPA and LLPA in separate analysis in each chapter.

Each sub-heading below is that of a results chapter and will include specific objectives to be address within each of these chapters alongside the proposed hypothesis. Figure 2 provides a depiction of objectives and hypothesis outlined in section 1.8.2 and Figure 3 provides a depiction of the objectives and hypothesis outlined in section 1.8.3 and 1.8.4.

1.8.1 Physical activity, *apolipoprotein* ε4 and risk of cognitive decline and dementia among healthy older adults: a systematic review

Through critical appraisal of included prospective observational and experimental studies investigating the association between PA and cognition and/or dementia risk as a function of *APOE*ε4 carrier status, study results will be synthesised and evaluated. It is hypothesised that PA will benefit both *APOE*ε4 carrier and non-carrier individuals with regard to reduced cognitive decline and/or dementia risk, and that this benefit will be augmented among *APOE*ε4 carriers.

1.8.2 The association between late-life physical activity (Ch: 4); midlife physical activity (Ch: 5) and the combined role of midlife to late-life physical activity (Ch: 6) on overall cognitive function and trajectories across 30 months

(i) *To evaluate the association between physical activity in midlife, late-life and PA from mid- to late-life on overall cognitive function and trajectories across 30 months.* It is hypothesised that:

- Both midlife and late-life leisure PA will be independently associated with better overall cognitive function and trajectories in later life, with the largest effect sizes among those reporting high activity in both mid- and late-life.
- Leisure aerobic PA will be associated with cognitive function and trajectories with a larger effect size, when compared to EPA.
- The association between those reporting higher PA in mid- and/or late-life and higher cognitive function when compared to low PA individuals will be strongest for memory and executive function domains.

(ii) *Investigate whether APOEε4 carrier status modifies associations between midlife and/or late-life physical activity and overall cognitive function and trajectories*: It is hypothesised that the association between PA and cognition will be augmented among carrier individuals such that PA is found to mitigate the negative association between *APOE*ε4 carriage and cognitive function and trajectories in older age.

(iii) *Explore whether the association between midlife and/or late-life physical activity and cognitive function and trajectories differ among males vs females and among those ≥70 years vs <70 years old:* It is hypothesised that females will display with an augmented benefit of PA on cognition when compared to males. The association between PA and cognition will differ among those who are <70 years old when compared to those ≥70 years old.

(iv) *Explore whether APOEε4 carrier status modifies the association between midlife and/or late-life PA and cognition differentially within males vs females and among those ≥70 vs <70 years old:* It is hypothesised that the effect modification of *APOE*ε4 carrier status on PA/cognitive associations will be augmented among females and will differ among those <70 years versus ≥70 years of age.

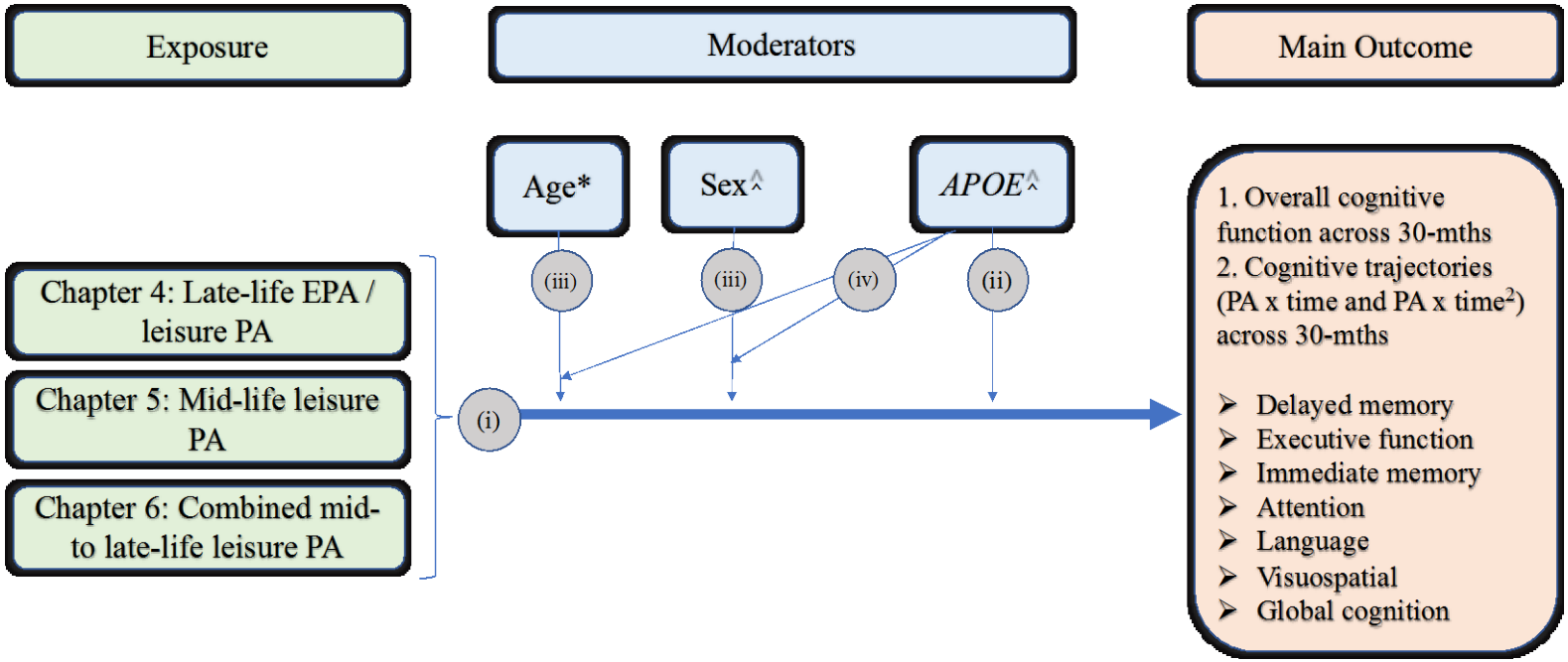


Figure 2. Depiction of objectives outlined in section 1.8.2 pertaining to Chapters 4, 5 and 6.

*Associations between PA and cognition were stratified by age (≥70-yrs versus those <70-yrs old).

^Effect modification by sex and *APOE*ε4 carrier status on the associations between PA and cognition were conducted via running Sex x PA and *APOE* x PA interactions, plus associations between PA and cognition conducted separately among males vs females and carriers vs non-carriers.

Abbreviations: PA, physical activity; *APOE*, apolipoprotein

1.8.3 Serum BDNF, physical activity, *APOE*ε4 carrier status and cognition

1.8.3.1 The association between physical activity, *APOE*ε4 carrier status and serum BDNF

(v) *Whether midlife PA, late-life PA and APOEε4 carrier status is cross-sectionally associated with baseline serum BDNF:* Based on previous reports of PA's association with upregulated BDNF, it is hypothesised that those reporting high PA in mid- and/or late-life will be associated with higher levels of circulatory BDNF serum levels in later-life. Likewise, *APOE*ε4 carrier individuals will have lower BDNF serum levels when compared to non-carriers.

(vi) *Whether APOEε4 carrier status modifies the cross-sectional association between midlife leisure PA and/or late-life leisure PA and baseline serum BDNF levels:* It is hypothesised that higher mid- and/or late-life PA will modify the association between *APOE*ε4 carrier status and BDNF such that carrier individuals who report higher activity will present with significantly higher BDNF serum levels when compared to low active carriers.

1.8.3.2 The association between serum BDNF, physical activity and overall cognitive function and trajectories across 30 months

(vii) *Whether BDNF is associated with overall cognitive function and trajectories across 30 months and if APOEε4 carrier status modifies these associations:* It is hypothesised BDNF will be positively associated with overall cognitive function across 30 months and reduced likelihood of cognitive decline which will be modified by *APOE*ε4 carrier status such that carriers present with lower BDNF and hence worse cognitive scores.

(viii) *If BDNF is associated with both cognition (vii) and physical activity (v); whether BDNF mediates the association between midlife and/or late-life PA will be investigated:* It is hypothesised that BDNF will mediate the associations between higher PA on better overall cognitive function across 30 months.

(ix) *Alternatively, if BDNF is not associated with both PA and cognition (and hence cannot be considered a mediator); whether BDNF modifies the association between PA and cognitive function and trajectories over 30 months will be explored:* It may be hypothesised that BDNF modifies the association between PA and cognition such that those with lower BDNF have an augmented response to PA on cognition. Specifically, those at increased risk of cognitive decline via lower BDNF levels will rely on PA more heavily than those with higher BDNF, in maintaining cognitive health.

(x) *Whether the role of BDNF as a mediator or moderator differs among APOEε4 carrier or non-carrier individuals:* It is hypothesised that the mediation or moderation effect of BDNF

on PA/cognitive associations will be augmented among *APOE*ε4 carriers compared to non-carriers.

1.8.4 Plasma $A\beta_{1-42}$, physical activity, *APOE*ε4 carrier status and cognition

1.8.4.1 The association between physical activity, *APOE*ε4 carrier status and plasma $A\beta_{1-42}$ levels

(xi) *Whether midlife PA, late-life PA and APOEε4 carrier status is cross-sectionally associated with baseline plasma $A\beta_{1-42}$:* It is hypothesised that high PA in midlife and/or late-life will be associated with lower $A\beta_{1-42}$ plasma levels when compared to those reporting low PA. Likewise, *APOE*ε4 carrier individuals will have higher $A\beta_{1-42}$ plasma levels when compared to non-carriers

(xii) *Whether APOEε4 carrier status modifies the association between midlife leisure PA and late-life leisure PA on baseline plasma $A\beta_{1-42}$ levels:* It is hypothesised that *APOE*ε4 carrier status will modify the association between and PA and $A\beta_{1-42}$ levels. Specifically, high active carriers will present with lower $A\beta_{1-42}$ plasma levels when compared to low active carriers who, in turn will have the highest $A\beta_{1-42}$ plasma levels when compared to all other groups.

1.8.4.2 The association between plasma $A\beta_{1-42}$ levels, physical activity and cognition

(xiii) *Whether plasma $A\beta_{1-42}$ is associated with overall late-life cognitive function and trajectories across 30 months and modified by APOEε4 carrier status:* It is hypothesized that $A\beta_{1-42}$ plasma levels will be inversely associated with cognitive function over 30 months and modified by *APOE*ε4 carrier status, such that carriers with high $A\beta_{1-42}$ plasma levels will have significantly worse cognitive function when compared to carriers with low $A\beta_{1-42}$ plasma levels as well as non-carriers regardless of $A\beta_{1-42}$ plasma levels.

(xiv) *Whether midlife PA, and late-life PA modifies the association between $A\beta_{1-42}$ plasma levels and overall cognitive function:* It is hypothesised that midlife and/or late-life PA will modify the association between $A\beta_{1-42}$ plasma levels and cognition such that individuals who report low PA and who have high $A\beta_{1-42}$ plasma levels will exhibit worse cognitive function compared to those with high $A\beta_{1-42}$ plasma levels who are high active and when compared to those with low $A\beta_{1-42}$ plasma levels regardless of activity status.

(xv) *If the effect modification of PA on the associations between $A\beta_{1-42}$ plasma levels and cognition vary among APOEε4 carriers versus non-carriers:* It is hypothesised that those reporting high midlife and/or late-life PA will mitigate the negative impact of possessing

high $A\beta_{1-42}$ plasma levels among both carriers and non-carriers, with associations being augmented among *APOE*ε4 carrier individuals. Likewise, carriers who report low PA and who have high $A\beta_{1-42}$ plasma levels will exhibit worse cognitive function when compared to all other groups.

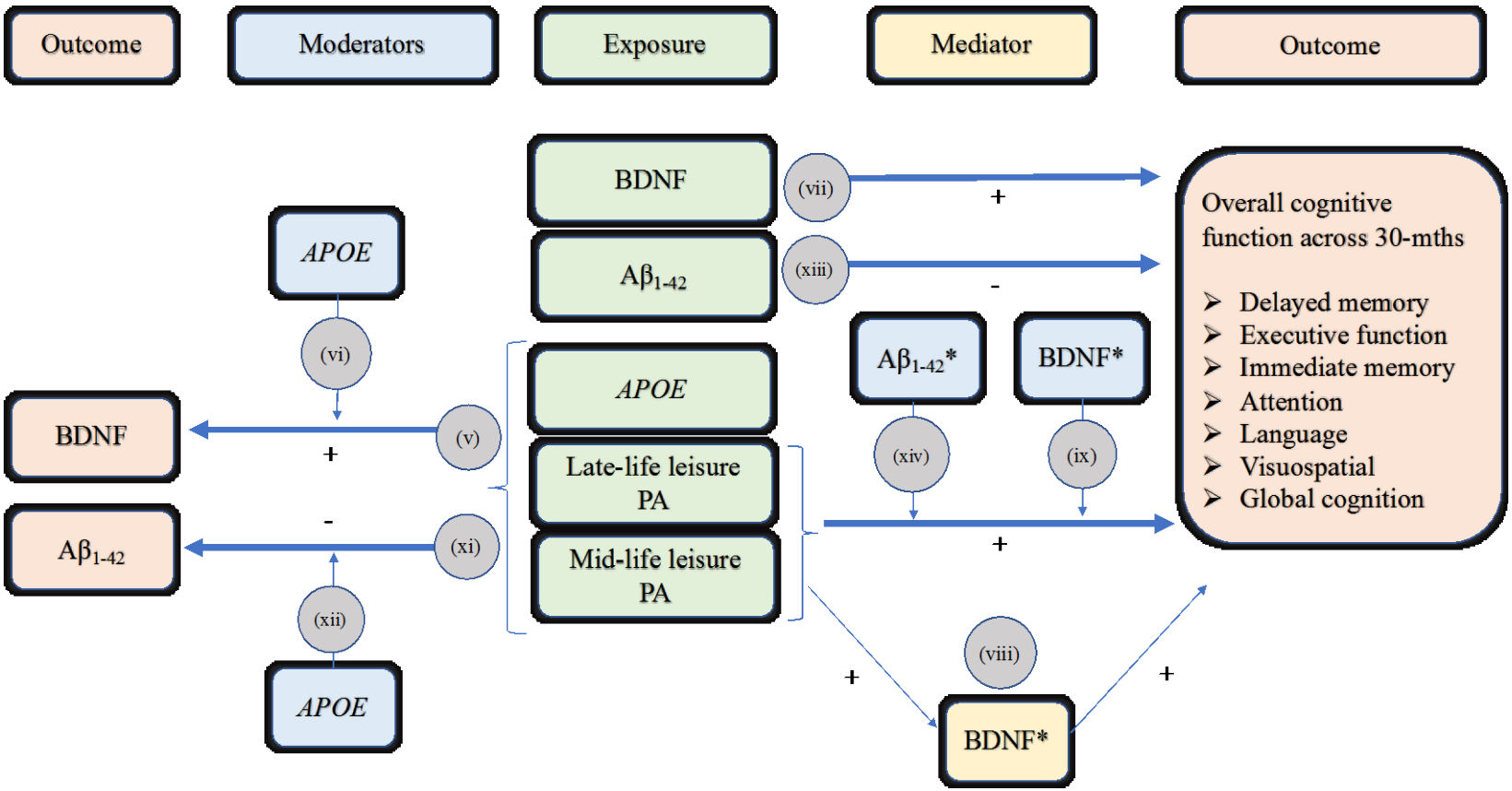


Figure 3. Depiction of objectives outlined in section 1.8.3 and 1.8.4 pertaining to Chapters 8 and 9.

*re-run same analyses within *APOEε4* carriers and non-carriers for objectives **(x) and (xv)**

Abbreviations: PA, physical activity; BDNF, brain derived neurotrophic factor; $A\beta_{1-42}$, peripheral amyloid-beta; *APOE*, apolipoprotein; mths, months

Chapter 2: Physical activity, *apolipoprotein* ε4 and risk of cognitive decline and dementia among healthy older adults: a systematic literature review

2.1 Introduction

Despite heightened risk among *APOE*ε4 carriers, possession of the allele does not always result in dementia, suggesting interactions with other genes, pathology, demographic characteristics and/or lifestyle factors. A handful of observational and experimental studies have explored this question in the context of PA, more commonly as a secondary outcome, yet results have remained inconsistent. Some studies report evidence to indicate that *APOE*ε4 carrier status may modify associations between PA and risk of cognitive decline whereby such positive PA/cognitive correlations are especially augmented among *APOE*ε4 carriers vs non-carriers [114, 198-200, 208]. However, incongruent results have also been reported, whereby PA benefits on executive function [248] or incidence of dementia [337, 338] are augmented in non-carriers when compared to carriers. To corroborate the notion that PA intervention and/or adopting a more physically active lifestyle may too benefit cognitively healthy individuals at increased risk of developing dementia via possession of at least one *APOE*ε4 allele, a critical review of the literature is warranted. This systematic review aims to elucidate reasoning behind conflicting results, comparing methodologies across studies and considering potential bias.

2.2 Methods

Detailed eligibility criteria are provided in Appendix A (section A.1, page 284-285), following the PICOS framework [339], including study design, participants, exposure and outcome. A protocol was written prior to commencing this systematic review and was conducted in concordance with the PRISMA guidelines [340].

2.2.1 Eligibility criteria: study design, participants, exposure and outcome

Included in this review were RCT's, and prospective (cohort) and retrospective (case-control) observational studies investigating whether *APOE*ε4 carrier status modifies the effect or association of PA on improved/maintained cognitive function or delaying dementia/AD among cognitively healthy older adults. Any other type of study design, including cross-sectional and case-control

studies not nested in a cohort study were excluded. No limitation on publication status or date of publication, sample size or duration of intervention/follow-up period were instilled. Participants were cognitively healthy older adults, with an available outcome measure anytime from 60 years old. Those with conditions that would preclude the performance of the PA intervention e.g. with functional limitation or any condition known to impair cognition, such as mild cognitive impairment (MCI) or dementia diagnosis (unless included in a case-control study) were excluded. Conversely, trials containing participants with age-related illness or disorders (e.g. cardiovascular disease) known not to impair cognition or mobility, were included. No further restriction was placed on selection of study participants, including ethnicity or sex. Included in the review were RCT's evaluating any form of aerobic i.e. walking or running, resistance (i.e. weights) or other exercise known to improve fitness (e.g. Tai Chi, dance). Acceptable comparator conditions included no-treatment control (e.g. on a waiting list), non-active educational control (e.g. health advice) or stretching/balance control. PA assessment in observational studies were not restricted and could include measures via subjective reporting (e.g. questionnaire), or objective measurement (e.g. wearing a pedometer). Studies whereby multiple interventions were conducted (e.g. nutrition and physical activity) were only included if they contained an independent PA intervention subgroup.

The primary outcome concerned incidence of dementia (all cause or AD), global cognitive functioning, and/or domain-specific cognitive functioning. Subdomains included, but were not limited to, executive function, attention, language, visuospatial functioning, immediate memory and delayed memory. Other outcomes considered as secondary may be MCI, cognitive impairment no dementia (CIND) or vascular AD (VaD). Cognition must have been assessed via validated measures sensitive to cognitive decline. Where studies include measures of relevant Imaging or fluid biomarkers (e.g. Amyloid-beta (Aß), brain morphology or blood-based markers), or interactions with age and sex; this data was extracted as secondary.

2.2.2 Search strategy

On the 3rd April 2018, Medline (1946 to April 2018), PsychINFO (Ovid SP 1806 to April 2018), EMBASE (Ovid SP 1947 to April 2018), Cochrane Controlled Trials Register (CENTRAL), ClinicalTrials.gov (https://clinicaltrials.gov) and the International Clinical Trials Registry Platform (ICTRP) (http://apps.who.int/trialsearch) were searched to identify eligible studies. A combination of subject heading MeSH terms and free text terms were used to create command groups relating to the intervention (PA), the moderator (*APOEε4* carrier status) and the outcome (cognition/incidence of dementia). Database searches were supplemented with reference lists in relevant review papers. Full details can be found under Search Strategy (Section A.2, page 286-290) within Appendix A.

2.2.3 Study selection, data extraction and bias assessment

Once duplicates were removed, two independent reviewers (Imperial College London Global Health undergraduate student: Grace Grimsey (GG) and, myself: CR) screened titles and abstracts. Full text of remaining studies were screened by the same independent reviewers and any disagreements resolved by a third review author (PhD supervisors: PW or RP). Details of the study selection process can be viewed in Figure 4. Details pertaining to extracted data can be viewed in Appendix A under Data Extraction Template (A.3, page 289).

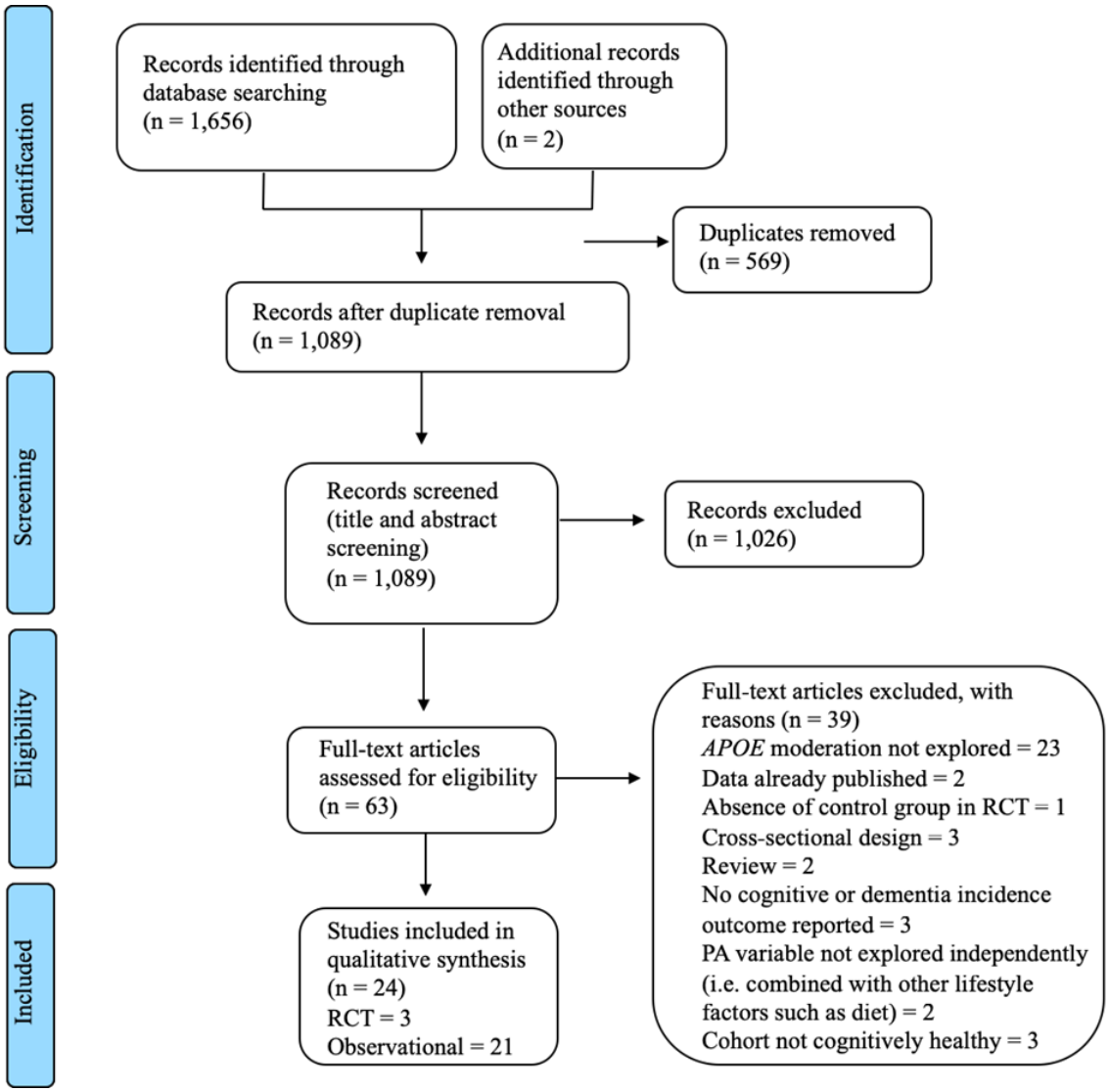


Figure 4. PRISMA study selection

The quality of included RCTs were assessed using the Cochrane Risk of Bias (RoB) Tool, whereby the risk of selection, performance, detection, attrition and reporting bias was judged via criteria derived from the Cochrane Handbook as low, unclear or high risk. Observational study quality was assessed via the Newcastle-Ottawa Quality Assessment Scale for prospective (cohort) and retrospective (case-control) studies. The assessment has three categories; selection of study groups,

comparability of groups and ascertainment of exposure for case-control studies, or outcome for cohort studies. A maximum of one star for each item within the selection and outcome/exposure categories and a maximum of two stars for the comparability category, such that the highest quality studies are awarded nine stars. Two review authors (GG and CR) undertook the assessment independently, with any discordance in assessment being resolved through a third assessor (PW or RP) (see Appendix A, Quality and Bias Assessment tools, section A.4, page 292-294)

2.3 Results

The electronic search identified 1,656 records including an additional two records identified through screening reference lists of relevant papers. After removing duplicates, 1,026 studies were excluded based on review of title and abstract. Sixty-three full-text articles were assessed for eligibility and 39 additional studies were consequently excluded (Figure 4; Appendix A, Table A.5, page 295). Majority of excluded studies were due to the absence of *APOEε*4 sub-group analyses and/or exploring the moderation of *APOEε*4 carrier status on association between exposure and outcome (n=23). Other reasons included participant characteristics (e.g. clinical population) (n=3), being a review article (n=2), not containing an appropriate outcome measure or exposure (n=5), being cross-sectional in design (n=3), containing data already published (n=2) and one experimental study did not include a control group. Twenty-four studies were chosen for inclusion; three RCTs [253, 341-343] and 21 observational studies (Figure 4). Of the 21 observational studies, seven assessed cognition as the outcome measure [114, 198-200, 207, 208, 248] and 14 assessed incidences of all cause dementia/AD/VaD, CIND or MCI as the outcome [113, 230, 337, 344-353].

2.3.1 Design of included studies

2.3.1.1 RCT's

Study details are listed for each trial in the characteristic of included RCT's table (Table 1). The three included trials were all parallel in design and were conducted using individual randomization. Ages ranged between 55 – 85 years old; one RCT included females only [342], another had approximately equal male/female ratio although a smaller proportion of females in the control group [343] and a third study did not report this information [341]. Due to a small number of RCT's identified, one study of individuals aged <60 (i.e. 55+) was included. Proportion of *APOEε*4 carriers were only reported in one of the three studies, being 14% in the control group and 25% in the exercise group [343]. Intervention duration ranged from four months [341, 343] to six months [342], and were aerobic in nature. One study reported 120 minutes weekly of Zumba with a home-based active control not deemed to improve fitness [342] and the other reporting approximately 120 minutes per week of walking with a health education control [343]. The third study did not specify bout, duration or frequency of an unspecified aerobic intervention and included a no-treatment control [341]. The primary outcome for two of the RCTs was change in cognitive function from

pre- to post-intervention [342, 343]. Although the third RCT examined global cognitive change, the study's primary outcome was change in serum BDNF levels [341]. The cognitive domains included visuospatial working memory [342], executive function, episodic memory composite scores [343] and global cognition (MMSE) [341].

Table 1 Characteristics and results of included RCT's

Study	Age	% Female %*APOEε4+*	Intervention	Outcomes measure	Results	Summary and Quality
[a]Stonnington *et al*. (2013, USA) [342] n = 53	Mean age (SD) M^{age} NR Range: 55 – 80	Female 100% ε4+ % NR	**EG**: Zumba, 60 mins 2x/week for 6 months **CG:** Home-based exercises not expected to have a risk modifying effect	**Visuospatial working memory** (GMLT)	**ε4 status x PA** intervention on GMLT performance = NSS -- **TC:** EG ↑ GMLT performance vs CG*	Summary: No significant interaction between PA, cognitive outcomes *and* *APOE*ε4 status **RoB:** Unknown
Legault *et al.* (2011, USA) [343] EG: n = 18 CG: n = 18	Mean age (SD) EG: 77.5 (4.8) CG: 75.4 (4.8) Range: 70 - 85	% Female EG: 56% CG: 38% % *APOEε4 +* EG: 14% CG: 25%	**EG:** AT and flexibility Training, average of 123.2 (16.8) mins/week for 4 months **CG**: Healthy ageing education	**Executive Function** (SOPT, N-back, Eriksen Flanker test, Task switching test, TMT A and B) **Episodic memory** (HVLT, Logical memory I and II)	**ε4 status x PA** intervention on EF or EM performance = NSS **TC** Secondary Result Age x PA on Executive function*	Summary: No significant interaction between PA, cognitive outcomes *and* ε4 status PA lead to improved executive function as a factor of age with increased benefit in older age **RoB**: Low
[a]Pereira *et al.* (2014, Brazil) [341] EG: n = 30 CG: n = 17	Mean age (SD) EG: 70 CG: 68 Range: NR	% Female EG: NR CG: NR % *APOEε4 +* EG: NR CG: NR	**EG:** 16-week AT (bout duration and frequency NR) **CG:** Waiting list	**Global Cognition** (MMSE) Biomarker outcome BDNF serum levels	**ε4 +:** NSS change in cognition from baseline in EG or CG ($p>0.05$) **ε4 - :** not reported Interaction not reported Secondary results **EG ε4 -:** improvement in BDNF levels* **CG ε4 -:** impairments in BDNF levels*	Summary: **ε4 carriers** did not benefit from PA intervention **ε4 non-carriers** benefited from PA intervention with regard to ↑ BDNF. Carriers did not show such benefit. **RoB**: Unknown

NR = not reported; n = number of participants; *APOEε4* = apolipoprotein epsilon-4; TC = total cohort; CG = control group; EG = exercise group; RoB = Risk of Bias; SD = standard deviation; F = female; AT = aerobic training; MMSE = Mini-Mental State Examination; GMLT = Groton Maze Learning Test; SOPT = Self-ordered Pointing Test; HVLT = Hopkins Verbal Learning Test; BDNF = brain derived neurotropic factor; PA = physical activity; NSS = non-statistically significant; [a]Conference abstract
*$p<0.05$, **$p<0.00$

2.3.1.2 Observational studies – physical activity and cognition

Details of included studies pertaining to design, demographics, exposure, outcome, results and quality are listed in Table 2. All seven studies were prospective in design, with a following-up period ranging from 1.5-9 years (average follow-up = 3.7 years). General sample size ranged from 78-483, with one study including a sample of 1,635 [198]. Participant's baseline age ranged between 45-93 years (mean age range 64.9-75.6 years). There was a slightly higher percentage of females when compared to males across studies, ranging from 62-70%, and one male only study [114]. Proportion of *APOE*ε4 carriers across studies ranged from 18%-33.3%. All included studies utilized self-reporting questionnaires to define baseline PA levels and were highly heterogeneous in terms of measurement and PA groupings (i.e. high versus low leisure PA, PA as a continuous variable, domain-specific PA, etc.). Five studies utilized questionnaires collecting information on frequency, duration and intensity of activity from either the previous week [199], previous 2-weeks [198, 207] or previous year [114, 200]. Another study collected frequency and intensity of different sport and leisure activities from the previous two-weeks [248], or frequency and duration from the previous year [208]. Two of these studies followed health guidelines of 150 minutes/week of moderate to vigorous activity (high vs low) via leisure-related activities from the previous week [199] or leisure and household activities averaging over the previous year [208]. The third study created a continuous metabolic equivalent of task (MET) variable pertaining to household, leisure and garden-related PA domains from the previous 2-weeks [207]. Other studies used such metrics to define 'high PA' as > one hour/day of any activity [114], ≥ one activity of any intensity per week [198], ≥ three instances of moderate/high activity per week [200] or a continuous variable calculated by intensity and frequency [248]. Included studies assessed either one or a combination of domains including global cognition [114, 198-200, 207], memory [199, 200, 208], visuospatial abilities [207, 208], executive function/attention [199, 207, 208, 248] and language [207, 208].

Table 2. Characteristics and results of included observational studies in which the outcome is cognition

Study	Design	Participants	Physical activity measurement	Outcomes measure	Results [a]	Summary and Quality
Schuit *et al.* (2001, Netherlands) [114] n = 347	The Zutphan Elderly Study 3 years	Mean age (SD) 74.6 (4.3) Range: 65 - 84 Female: 0% ε4+: 24% (n = 83)	Validated (SR-Q) **Leisure composite** (F/D) walking/bicycling hobbies/gardening (weekly/seasonal), 'odd jobs'/sport (monthly) >1hr/day ≤ 1hr/day	Measure **Global cognition** (MMSE) Outcome Decline of >3 points in MMSE between 1990-1993	**ε4 +** <1hr/day: 29.7% Cognitive decline; OR 3.7* (1.1-12.6) >1hr/day: 10.6% cog decline. OR = 1.0 (ref) **ε4 –** <1hr/day: 6.1% cognitive decline; OR = 0.8 (0.2-3.2) >1hr/day: 3.4% cog decline OR = 1.0 (ref) *No interaction conducted.*	Males who partake in <1hr/day have an increased risk of cognitive decline compared to those who are active. Results were highly amplified and only significant *APOEε4* carriers. **Quality:** 5 out of 9 stars
Niti *et al.* (2008, Singapore) [198] n = 1,635	Singapore Longitudinal Ageing Study 1.5 years (median)	Mean age (SD) 66 (7.3) Range: 55 - 93 Female 65% ε4+: 18% (n = 249)	Minnesota Leisure Time Activities (SR-Q) **Leisure composite** (F/I) walking, active sports, Tai Chi, swimming (< month - weekly), ≥ one activity/week < one activity/week	Measure **Global cognition** (MMSE) Cognitive Outcome Decline of ≥1 points in MMSE between baseline and follow-up assessments	**ε4 +** <1 activity/wk: OR 1.0 (ref) ≥1 activity/wk: OR .34 (.17 - .68)** **ε4 -** <1 activity/wk: OR 1.0 (ref) ≥1 activity/wk: OR .88 (.65 – 1.18) ***APOE* x PA***	Association between PA and risk of cognitive decline is only significant among *APOE*ε4 carriers. **Quality**: 4 out of 9 stars
Woodard *et al.* (2012, USA) [200] n = 78	Unspecified cohort 1.5-years	Mean age (SD) 73.1 (4.9) Range: NR Female 73% ε4+: 33.3% (n = 26)	Stanford Brief Activities Survey (SR) (F/D/I) **Low intensity PA** e.g. *walking, golf* **Moderate intensity PA** e.g. *brisk walking* **High intensity PA** e.g. *jogging* ≤ 2 times low PA/wk ≥ 3 times mod/high PA/wk	Measure **Global Cognition** (DRS-2 Total) **Immediate memory** (RAVLT Trials 1-5) **Delayed memory** (RAVLT Recall) Cognitive Outcome ≥ 1 SD decline in at least one domain Biomarker outcome Hippocampus (fMRI & MRI)	**ε4 +** Probability of decline in all three cognitive measures among Low PA group was significantly higher than the High PA group* *(OR NR)* **ε4 -** Probability of decline was not affected by PA level ***APOE* x PA***	Engagement in PA is associated with a reduced probability of cognitive decline in *APOE*ε4 carriers. Hippocampal vol. and activity Benefits of PA among carriers diminished with reduced Hippocampal volume and activity **Quality:** 4 out of 9 stars

Table 2. ***(Continued)*** **Characteristics and results of included observational studies in which the outcome is cognition**

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Pizzie *et al.* (2014, USA) [208] n = 91	Adult Children Study Cohort 6-years	Mean age (SD) 64.9 (7.4) Range: 45 - 74 Female: 62.6% ε4+: 30.8% (n = 28)	Nurse's Health Study Exercise (SR-Q) (F/D) **Leisure activities** e.g. *jogging* **Home activities** e.g. *mowing the lawn* METS ≥150 mins/wk mod+ < 150 mins/wk	**Verbal memory** (*FCSR)* **Speeded visuospatial function** (TMT A) **Working memory/ Executive Function / Att.** *(TMT B, category fluency test and LNS)* Cognitive outcome Association between PA and cognitive function over time	**ε4 +** ≥150 mins/wk mod+ = improved TMTB* and LNS* (β-coefficient NR) **ε4 -** NSS improvement on TMTB or LNS based on PA levels. **PA x *APOE* x time****	A positive association between PA and EF plus attention trajectories only in *APOE*ε4 carriers **Quality:** 6 out of 9 stars
[b] Tsai *et al.* (2016, China) [199] n = 483	Unspecified Cohort 2-years	M^{age} NR Range: 65 + Female: NR ε4+: NR	International PAQ (SR-Q) **Leisure** (F/D/I) Walking / moderate PA / vigorous PA METS ≥150 mins/wk walk+ < 150 mins/wk PA	**Global cognition** *(Montreal Cognitive Assessment)* **Logical memory and attention** *(Wechsler Memory Scale Third Edition)* **Executive Function** (*TMT-B and verbal fluency test)* Cognitive outcome Change	**ε4 +** ≥150 mins/wk of walking = protected against decline in global cognition. (AOR = .22)* **ε4 -** NSS improvement on any domain based on PA levels. *No interaction conducted.*	Benefits of PA was observed in overall cohort for all domains and remained in *APOE*ε4 carriers only for global cognition. Sex and age moderation Females and younger old (65-74) benefited from PA on global cognition and memory **Quality**: 6 out of 9 stars
de Souto Barreto *et al.* (2018, France) [207] n = 326	Multi-domain Alzheimer's Prevention Trial (MAPT) (secondary analysis of the placebo group) 3-years	Mean age (SD) 75.6 (4.4) Range: 70 + Female: 66.4% ε4+: 23.6% (n = 77)	Minnesota Leisure Time Activities Q (Prev. 2-wks) (F/D/I) **Mod- to vig.** (MVPA) **Leisure (e.g. walking to running)** (LTPA) **Household** (HPA) **Gardening** (GPA) **All Light** (LiPA) Continuous MET/min variable per domain	**Global cognition** *(composite Z-score of below*) **Verbal fluency** *(COWAT)* **Categorical fluency** *(CNT)* **Att. and EF** *(Digit symbol)* **Motor activity and selective attention** *(TMT A & B)* **Orientation** (*MMSE)* Cognitive outcome Change from baseline to follow-up	**ε4 +** NSS association between any PA domain and cognition across all assessments **ε4 –** NSS association *No interaction conducted.*	No statistically significant association within carriers or non-carriers. Time adjusted analyses found associations augmented among carriers (not reported here) **Quality:** 7 out of 9 stars

Table 2. ***(Continued)*** **Characteristics and results of included observational studies in which the outcome is cognition**

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Thibeau *et al.* (2017, Canada) [248] n = 514	Victorian Longitudinal Study 9-years	Mean age (SD) 70.5 (8.5) Range: 53.3 – 95.4 Female: 66.5% ε4+: 25.7% (n = 132)	VLS-Activity Lifestyle Q (VLS-ALQ) (4-item PA subscale) (F/I) **PA composite:** *Gardening indoors out outdoors, exercise activities. and recreational sports* (frequency) zero (never) - eight (daily) Scores range 0 - 32	**Executive Function composite** *(Hayling sentence completion test, Stroop test, Brixton spatial anticipation test, Colour Trails Test part II)* Cognitive outcome Change from baseline to follow-up	**ε4 +** NSS association between EPA and level of EF performance. **ε4 -** Baseline EPA predicted level of EF performance (β = 0.407**) and 9-year EF change (β = 0.019**) *Results were enhanced for <65 and 65-75 y/o, but lost for 75+ y/o* **PA x *APOE* x time**** **Quality:** 4 out of 9 stars	Baseline EPA predicted level of EF performance and 9-year EF change in *APOE*ε4 non-carriers, but not in carriers. Benefits were observed in the overall cohort. Age moderation EPA predicted EF performance and 9-year change in young old group (<65) only. 75+ / *ε4+*/Low PA = sig decline compared to 75+ / ε4- / Low PA*

[a] Statistically significant results of the most adjusted model reported; [b] Conference abstract; F/D/I = frequency / duration / intensity; M^{age} = mean age; N = number of participants; NR = Not Reported; SD = standard deviation; SR = Self-report; Q = questionnaire; PA = physical activity; WHO = World Health Organisation; MET = metabolic equivalent; EPA = everyday physical activity; LTPA = leisure-time physical activity; MMSE = Mini-Mental State Examination; Att. = Attention; TMT: Trail Making Test; FCSRT = Free and Cued Selective Reminder test; LNS = Letter Number Sequencing task; DRS = dementia rating scale; EF = executive function; RAVLT=Rey Auditory Verbal Learning Test; COWAT = Controlled Word Association Test; CNT category naming test; FCRST = free recall + total recall; OR = odds ratio; AOR = adjusted odds ratio; NSS = non-statistically significant.. *p<0.05, **p<0.005

2.3.1.3 Observational studies – physical activity and dementia/AD

The characteristics of included studies in which incidence of dementia (all-cause, AD and VaD), AD, CIND or MCI was the outcome measure are shown in Table 3. Out of the 14 studies, 11 were prospective [113, 230, 337, 345-352], two were retrospective case-control [344, 353] and one was a retrospective nested case-control study [354]. Majority of included studies assessed all cause dementia and/or AD dementia as the outcome variable. However, one study assessed incidence of dementia and CIND [349], another restricted their outcome to MCI diagnosis [230], and a third conducted a case-control study evaluating the association between PA and dementia-related mortality [354]. All studies utilized a method of diagnosis aligned with validated criteria, except for one in which the criteria for AD cases were not reported [344]. Three studies had an extended follow-up period ranging from 21-28 years in which they evaluated the association between midlife PA and late-life dementia/AD incidence [347, 348, 352], with a sample size ranging between 1,251 to 1,432. The remaining studies had follow-up periods ranging from 3.2-10 years and with a sample size from 732 to 3,714, all assessing later life PA on risk of clinical onset, with one study exploring retrospective mid- and late-life PA [230]. PA assessment were all via questionnaires collecting information on either leisure related PA (i.e. sports) [230, 337, 344, 346, 347, 349, 351-354] or a composite of leisure household/gardening related activity [113, 345, 349, 350]. One study also explored the role of work-related and commuting midlife activity [348].

Table 3. Characteristics and results of included observational studies in which dementia / AD is the outcome

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Podewils *et al.* (2005, USA) [113] n = 3, 375	Cardiovascular Health Cognition Study (CHCS) Average follow-up: 5.4 years	Mean age (SD) 74.8 (4.9) Range: 65+ Female: 59.1% *ε*4+: 24.1%	Modified Minnesota LTPA Q. **Habitual PA composite** *(walking, household chores, mowing, golfing etc.)* (F/D/I) **Energy Expenditure:** Quartile 1: <248 kcal/wk Q2: 248-742 kcal/wk Q3: 243-1,1657 kcal/wk Q4: >1657 kcal/wk **Activity Index:** (Q 1-4 respectively) 0-1, 2, 3 or ≥4 (no. of different activities)	**Global cognition** *(MMSE 3MS, administered annually)* Outcome AD diagnosis (NINCSD-ADRDA criteria) VaD diagnosis (ADDTC criteria)	**ε4 +** *Q1 vs Q4 energy* HR= 1.29 (0.75-2.22) *Q1 vs Q4 activity* HR = 1.20 (0.63-2.29) **ε4 -** *Q1 vs Q4 energy* HR= 0.68* (0.44-1.04) *Q1 vs Q4 activity* HR = 0.44** (0.28-0.69) **PA x *APOE* *** Results similar for all-cause dementia, AD and VaD	Inverse association between PA and dementia risk for *APOE*ε4 non-carriers, but no association found for *APOE*ε4 carriers. Similar pattern observed for AD and VaD **Quality:** 8 out of 9 stars
Rovio *et al.* (2005, Finland) [347] n = 1,251	Cardiovascular risk factors, Ageing and Incidence of Dementia study (CAIDE) Average follow-up: 21 years	Mean age (SD) 50.15 (5.9) Range: 65 - 79 Female: 60% *ε*4+: 35%	**Leisure composite:** (F/D/I) Midlife PA Q: "How often do you participate in LTPA that lasts at least 20-30 mins and causes breathlessness and sweating?" **Active in midlife:** ≥ 2x/week **Sedentary in midlife**: < 2x/week	**Global cognition** *(MMSE)* Outcome DSM-IV criteria for dementia NINCDS-SADRDA criteria for AD dementia	**ε4 +** For active vs. sedentary groups: *OR for dementia* = 0.41 (0.16-1.06) *OR for AD* = 0.24* (0.07-0.79) **ε4 –** For active vs. sedentary groups: *OR for dementia* = 0.60 (0.25-1.47) *OR for AD* = 0.61 (0.21-1.80) Additive interaction **Dementia:** RERI = 0.45 **AD**: RERI = 0.73* PA x APOE NSS PA x sex NSS	Association between midlife PA and reduced risk of AD was more pronounced in *APOE*ε4 carriers. There may be an additive interaction between PA and *APOE*ε4 for AD risk. **Quality**: 5 out of 9 stars

Table 3. (Continued) Characteristics and results of included observational studies in which dementia / AD is the outcome

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Rovio *et al.* (2007, Finland) [348] n = 1,158	Cardiovascular risk factors, Ageing and Incidence of Dementia study (CAIDE) Average follow-up: 21 years	<u>Mean age (SD)</u> 50.4 (6.0) Range: NR Female: 62% ε4+: 35%	**Occupational PA: Q:** "How physically active is your work?" **Commuting PA: Q:** "How many minutes do you walk, bicycle or have some other PA when going to and from work?" **Occupational PA:** "active" or "sedentary" **Commuting PA:** (1) active: ≥60 mins (2) moderate: ≤59 mins (3) sedentary: not at all	**Global cognition** *(MMSE)* <u>Outcome</u> DSM-IV criteria for dementia NINCDS-SADRDA criteria for AD dementia	**<u>ε4 +</u>** **Occupational:** NSS associations **Commuting:** NSS associations (results NR) **<u>ε4 –</u>** **Occupational:** NSS associations **Commuting**: NSS associations (results NR) O/C PA x APOE NSS O/C PA x sex NSS O/C PA x Leisure PA NSS	There is no significant association between occupational or commuting PA in overall cohort or based on *APOE*ε4 carrier status on risk of dementia or AD <u>Sex moderation</u> Sex and leisure PA did not modify associations **Quality:** 5 out of 9 stars
Taaffe *et al.* (2008, USA) [350] n = 2, 263	Honolulu-Asia Ageing Study (HAAS) Average follow-up: 6.1 years	<u>Mean age (SD)</u> 77.7 (4.2) Range: 71 - 92 Female: 0% ε4+: 22.3%	**SR-Q about average no. hrs/day** Basal (*sleeping)* Sedentary (*sitting),* Slight (*walking on level ground)* Moderate (*gardening)* Heavy (*lifting/shovelling)* (F/D/I) **PA index** (1.0=basal, 1.1=sedentary, 1.5=slight, 2.4=moderate and 5.0=heavy activity). **Continuous measure**: **LPA: ≤28.7** **MPA: 28.8-32.4** **HPA: ≥32.5**	**CASI and IQCODE** for dementia screening **DSM-IIIR** criteria for dementia **NINCDS-ADRDA** criteria for AD dementia **CADTC** criteria for VaD	**<u>Stratified analysis not conducted</u>** No apparent interaction between *APOE*ε4 status and PA on overall dementia, AD or VaD risk PA x *APOE* NSS for any dementia	No significant interaction between *APOE*ε4 and PA in relation to dementia risk <u>Secondary</u> Those with low physical function benefited from high PA in reducing risk of AD and dementia. **Quality:** 6 out of 9 stars

Table 3. (Continued) Characteristics and results of included observational studies in which dementia / AD is the outcome

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Piallard-Borg *et al* (2009, Sweden) [346] n = 732	Kungsholmen Project Median follow-up: 7.8 years	Mean age (SD) 81.1 (4.9) Range: 75 + Female: 74.2% ε4+: 33.3 %	**Fitness activities:** (frequency) *Swimming, walking, gymnastics.* Personal interview by trained nurses Continuous score and tertiles	**Global cognition** *(MMSE)* **DSM-IIIR** criteria for dementia diagnosis	**Stratified analysis not conducted** No apparent interaction between *APOE*ε4 status and PA on dementia risk PA x *APOE* NSS for dementia	No significant interaction between *APOE*ε4 and PA in relation to dementia risk. Protective trend observed in overall cohort **Quality:** 7 out of 9 stars
[b] Chen *et al.* (2012, China) [344] Cases n = 292 (AD) Controls n = 506	Unspecified cohort Case-control Follow-up duration NR	Mean age (SD) NR Range: 60 + Female: NR ε4+: NR	SR-Q regarding recreational, physical and social activities (F/D) High frequency and low frequency groups (cut-off point of freq. was based on the medium frequency in controls)	AD Measurement tool not reported	**ε4 +** High freq. PA significantly lowered AD risk, OR: 0.30* (0.13-0.70) **ε4 -** High freq. PA significantly lowered AD risk OR: 0.51* (95% CI NR) *Interaction not reported*	PA significantly reduces AD risk in both *APOE*ε4 carriers and non-carriers **Quality:** 2 out of 9 stars

Table 3. (Continued) Characteristics and results of included observational studies in which dementia / AD is the outcome

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Luck *et al.* (2014, Germany) [345] n = 2,492	German study on Ageing, Cognition and Dementia in Primary Care Patients (AgeCoDe) 4.5-years	<u>Mean age (SD)</u> 81.1 (3.5) Range: 75 + Female: 64.7% ε4+: 20.6%%	**Household and leisure PA:** (frequency) Interview regarding frequency of participation in seven physical activities **Low frequency of activities (LPA)** = ≤1/week **High frequency of activities (HPA)** = ≥2/week	All-cause dementia diagnosis *(SIDAM and MMSE)* **NINCDS-ADRDA** criteria for AD dementia diagnosis **DSM-IV** criteria for dementia diagnosis	**<u>ε4 +</u> / LPA:** *Any dementia:* RR=3.02* (2.07-4.42), *AD dementia:* RR=3.73* (2.4-5.8) **ε4+ / HPA:** *Any dementia*: RR=1.76* (1.19-2.6); *AD dementia*: RR=1.80* (1.12-2.89) **<u>ε4 -</u> / LPA:** *Any dementia:* RR=1.73* (1.29-2.32); *AD dementia*: RR=1.51* (1.04-2.19) **ε4 - / HPA: (overall ref)** PA x *APOE* NSS AD dementia RERI = .38 (.07 - .69)* all dementia RERI = NSS	The risk of developing any dementia and AD dementia were significantly higher in low active non-carriers and low & high active carriers when compared to high active non-carriers. An additive interaction between LPA and *APOE*ε4 allele carriage for highest dementia / AD risk was present. **Quality:** 9 out of 9 stars
Strand *et al.* (2015, Norway) [354] Cases: n = 561 Controls n = 584	The Cohort of Norway (CONOR) (nested case-control) Retrospect average 9-years	<u>Mean age (SD)</u> 75.7 (4.7) Range: 65 + Female: 60.5% ε4+: 32.8%	<u>Leisure composite</u> *Light walking, intermediate exercise activities, intensive exercise* Physically inactive (watching TV mostly) Physically active (any exercise)	Dementia cases identified through the Cause of Death Register Assessing dementia-related mortality	**<u>ε4 +</u>** Active: ref Physically inactive*:* AOR 1.63 (0.86-3.08) **<u>ε4 –</u>** Active: ref Physically inactive*:* AOR 2.88* (1.70-4.89) PA x *APOE* NSS	Association between physical inactivity and dementia-related mortality is significant in *APOE*ε4 non-carriers only. Low active ε4 carriers had highest risk of dementia-related mortality. **Quality**: 5 out of 9 stars

Table 3. (Continued) Characteristics and results of included observational studies in which dementia / AD is the outcome

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Yang *et al.* (2015, China) [353] **AD:** n=292 **VaD:** n=144 **HCs:** n=506	Unspecified cohort Case-control Retrospect NR	**Mean age (SD)** AD: 79.1 (7) VaD: 79.6 (7.2) HCs: 72.9 (6) **Female:** AD: 64% VaD: 56% HCs: 52% **ε4+:** AD: 38% VaD: 18% HCs: 14%	**Leisure composite (frequency)** SR-Q (PA 1 year prior to recruitment) **High frequency PA (HPA):** ≥ 3x/week **Low frequency PA (LPA):** < 3x/week	CDR and MMSE for dementia diagnosis. NINDS-AIREN for VaD diagnosis. Short Portable Mental Status Q for HCs assessment	**ε4 +** HPA associated with decreased risk of: AD: AOR = 0.3* (0.13-0.70) VaD: AOR = 0.26* (0.07-0.99) **ε4 –** HPA associated with decreased risk of: AD: AOR = 0.51* (0.32-0.82) VaD: AOR = 0.41* (0.23-0.72) PA x *APOE* NSS	High PA significantly reduces AD and VaD risk in both *APOE*ε4 carriers and non-carriers. Sex moderation PA remained protective from AD in males but not females. **Quality**: 6 out of 9 stars
Tolppanen *et al.* (2015, Finland) [352] n = 1,432	Cardiovascular risk factors, Ageing and Incidence of Dementia study (CAIDE) Mean follow-up: 28.3 years	Mean age (SD) 50.6 (6.0) Female: 62.3% ε4+: 32.4%	**Leisure composite** (F/D/I) Q: "How often do you participate in LTPA that lasts 20-30 mins and causes breathlessness and sweating?" **High level** ≥2 x/weekly **Moderate level** 1x/wk – 2x/mth **Low level** Never – few per/yr	**Global cognition** *(MMSE)* **Recall** *(CERAD)* DSM criteria for dementia; NINCDS-ARDRDA criteria for AD dementia	**ε4 +** High vs moderate: HR=1.47 (0.94-2.28) High vs low: HR=1.24 (0.73-2.08) **ε4 –** High vs mod HR=1.60 (0.99-2.59) High vs low: HR=1.85* (1.12-3.06) PA x *APOE* ** PA x sex **	Low MLPA significantly increased dementia/AD risk in *APOE*ε4 non-carriers. *APOE*ε4 carriers with low or modify MLPA had highest dementia/AD risk. Sex moderation Low PA males exhibited higher dementia risk. **Quality:** 5 out of 9 stars

Table 3. (Continued) Characteristics and results of included observational studies in which dementia / AD is the outcome

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Krell-Roesch *et al*. (2016, USA) [230] n = 1,830	Mayo Clinic Study of Ageing cohort Median follow-up: 3.2 years Assessing midlife (50-65 years) and late-life PA	Median age (IQR) 78 (74-83) Range: 70-93 Female: 50.2% ε4+: 25.9%	1985 National Healthy Interview Study and the Minnesota Heart Survey **Leisure composite:** (F/I) Light (*walking, slow dancing)* Moderate *(brisk walking)* Vigorous *(jogging, tennis (vigorous)* **Midlife PA (MLPA) and Late-life PA (LLPA)** **Light, moderate or vigorous PA** (≥2x/month) vs. **no PA** (≤1x/month)	Mayo Clinic Criteria for MCI [355] Outcome assessed: MCI risk	**ε4 +** -No Vig. PA in mid- and late-life (ref) vs Vig. PA in mid- and late-life: HR .46 (.22 - .95)* **ε4 -** -No light activity in mid- to late-life (ref) vs Light activity in mid- to late-life: HR .58 (.34 - .98)* -No Vig activity in mid- to late-life (ref) vs activity in late-life but not midlife: HR 1.86 (1.05, 3.29)* *Low PA ε4+ had increased risk of MCI compared to low PA ε4- for both mid- and late-life PA independently** Additive Interaction ** PA x *APOE* NSS	Low active carries had significantly higher risk for MCI than high active carriers. *APOEε4* carriers who did not exercise in mid- and late-life had lowest predicted median time to MCI. *APOE*ε4 non-carriers who engaged in both mid- and late-life PA had highest predicted median time to onset (although NSS) **Quality**: 6 out of 9 stars
Tan *et al.* (2017, USA) [351] n = 3,714	The Framingham Study Original and Offspring Cohorts 10 years	Mean age (SD) 70.5 (7.0) Range: 70 + Female: 54% ε4+: 21.5%	SR-Q requesting information on number of hours in a typical day of different activities (F/D/I) A continuous variable was created and quintiles calculated. Quintile 1 (lowest PA)	DSM-IV criteria and CDR for dementia NINDS-ADRDA criteria for AD diagnosis	**ε4 +** Q1 vs Q2-5: HR 1.19 incident dementia Q1 vs Q2-5: HR 1.09 incident AD **ε4 -** Q1 vs Q2-5: HR 1.58* (1.08-2.32) incident dementia Q1 vs Q2-5: HR 1.52 (0.98-2.36) incident AD PA x *APOE* NSS PA x age NSS	Association b/w PA and dementia incidence (not AD) is significant in *APOE*ε4 non-carriers only plus those who were older than 75 years. Brain volume High PA also related to increased brain and HC volume. **Quality:** 6 out of 9 stars

Table 3. (Continued) Characteristics and results of included observational studies in which dementia / AD is the outcome

Study	Design	Participants	Physical activity measurement	Outcomes measure	[a] Results	Summary and Quality
Fenesi *et al.* (2017, Canada) [337] n = 1,646	Canadian Study of Health and Ageing (CSHA) Mean follow-up: 5 years	Mean age (SD) 76.4 (6.8) Range: ≥65 Female: 59.1% ε4+: 22.8%	SR-Q: "Do you engage in regular exercise?" Yes/no response. What type and frequency. Non-exercisers = no exercise reported Exercises = at least some exercise reported	CSHA-1: DSM-III criteria for dementia, NINCDS-ADRDA criteria for AD CSHA-2: same as CSHA-1 + DSM-IV criteria for AD	**ε4 +** Non-exercisers vs exercisers: OR 0.77 (0.46-1.31) **ε4 -** Non-exercisers vs exercisers: OR 1.98* (1.44-2.71) PA x *APOE** **Quality:** 7 out of 9 stars	Significant association between PA and dementia protection among *APOE*ε4 non-carriers only.
Shih *et al.* (2018, USA) [349] n = 1,438	Sacramento Area Latino Study on Ageing (SALSA) 9-years	Mean age (SD) 70.5 (7.0) Range: 60-101 Female: 54% ε4+: 21.5%	**SR-Q Leisure composite**: (F/D/I) *18 types common to adults e.g. dancing, swimming, golf, yard work* MET-hour/week: **Low PA** (LPA) **High PA** (8+ activities per week at ≥3 METS each)	IQCODE and DSM-IV for dementia diagnosis NINCDS-ARDA for AD diagnosis CADDTC for VaD diagnosis Outcome assessed: dementia/CIND	**ε4 +** *High PA:* HR 2.20* (1.29-3.74) *Low PA:* HR 3.44* (1.85-6.39) **ε4 -** *High PA:* HR 1.00 (ref) *Low PA*: HR 1.39 (0.94-2.07) PA x *APOE* NSS **Quality**: 6 out of 9 stars	Association between PA and protection against the onset of dementia/CIND is significant in *APOE*ε4 carriers only. This effect is stronger among low PA / ε4 + diabetics.

F/D/I= frequency and duration and intensity; N = number of participants; SD = standard deviation; F = female; IQR = interquartile range; Q = questionnaire; HCs = healthy controls; PA = physical activity; O/C = Occupation / Commuting; WHO = World Health Organisation; VaD = vascular dementia; AD = Alzheimer's dementia; MET = metabolic equivalent; LTPA = leisure-time physical activity; CIND = cognitive impairment no dementia; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; MMSE 3MS = modified MMSE; DSM = diagnostic and statistical manual of mental disorders; SEVLT = Spanish and English Verbal Learning Test; EF = executive function; RAVLT=Rey Auditory Verbal Learning Test; CDR = clinical dementia rating; TMT = Trail Making Test; DSST: digit symbol-substitution test; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; ADDTC: Alzheimer's Disease Diagnostic and Treatment Centers criteria NINDS-AIREN = National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences; NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association; SIDAM = Structure Interview for the Diagnosis of Dementia of the Alzheimer's Type; CASI = cognitive abilities screening instrument; IQCODE = Information Questionnaire on Cognitive Decline in the Elderly; CADTC = California Alzheimer's Disease and Treatment Centres; TCBV = total cerebral brain volume; HPV = hippocampal volume; AOR = adjusted odds ratio; OR = odds ratio; HR = hazard ratio; RR = relative risk; NSS = non-statistically significant; PCA = principal components analysis. [a] Results from the most covariate-adjusted models; [b] Conference abstract. *p<0.05, **p<0.01

2.3.2 Results

2.3.2.1 Physical activity intervention on cognition among *APOE*ε4 carriers and non-carriers

Two studies reported a non-significant interaction between PA intervention and *APOE*ε4 carrier status on visuospatial working memory [342], executive function and episodic memory [343], however, the former study found a significant positive intervention effect within the total cohort on visuospatial working memory. The third study reported, following stratified analysis, that carriers did not display a significant difference in global cognition pre- and post-intervention, however, an interaction was not reportedly conducted [341]. The same study failed to report whether benefits in cognition were observed among non-carriers or the total cohort. As secondary, this same study reported an *APOE*ε4 specific difference such that non-carriers in the intervention group displayed an increase in serum BDNF compared to non-carriers in the control group. Likewise, one study reported an age dependent effect on the association between PA and executive function in the total cohort such that the older-old individuals appeared to display enhanced benefit compared to younger-old participants [343].

2.3.2.2 The association between self-reported physical activity and multi-domain cognition

Table 4 summarizes the association between PA and different cognitive domains, among *APOEε4* carriers and non-carriers.

Global Cognition

A total of five studies examined the association between physical activity and global cognition using the Mini-Mental State Examination (MMSE) [114, 198, 207], Dementia rating score-2 (DRS-2) Total [200], the Montreal Cognitive Assessment (MOCA) [199] and a composite z-score comprised of tests assessing memory, executive function and language [207] (Table 2). Four studies reported a positive association between PA and global cognitive trajectories, all limited to *APOE*ε4 carriers [114, 198-200] with one study reporting no association PA and global cognition among both carriers and non-carriers [207]. Two studies, utilizing MMSE as the measure of global cognition, found a positive association between higher leisure activity and protection against cognitive decline over 3-years and 1.5-years compared to the low PA group among carriers [114, 198]. Only one of these two studies reported a significant PA × *APOE* interaction, confirming a significant group difference [198]. Two additional studies, utilizing an arguably more sensitive measure of global cognition via the MOCA [199] and DRS-2 [200], reported that engaging in ≥150-

mintutes or ≥3 instances of moderate/vigorous activity per week reduced the probability of global cognitive decline over 2-years and 1.5-years respectively, among *APOEε4* carriers only. However, again just one of these two studies reported a significant PA × *APOE* interaction [200]. The final study did not report an association between PA and global cognition among carriers or non-carriers in the fully adjusted multivariate model, although in a basic time-adjusted model, effect sizes were augmented among carriers for leisure-time PA [207].

Executive Function

A total of four studies examined the association between PA and executive function (Table 2; Table 4) [199, 207, 208, 248]. One study reported a small positive association between partaking in ≥150-minutes of moderate/vigorous PA per week and better performance in individual tests (Trail-Making-Test, TMT-B, category fluency and letter-number sequence) after 6-years, compared to low-active carriers [208]. This same positive association did not reach statistical significance among non-carriers. This study also reported a significant PA × *APOE* × time interaction (p<.001). Contrary to the aforementioned finding, one study reported that partaking in ≥150-minutes per week of light to vigorous activity were not associated with tests of EF (TMT-B and verbal fluency) over 2-years in carriers or non-carriers, finding a significant association only among the overall cohort [199]. Another study reported, after 9-years, a significant positive association between a continuous leisure composite score and overall EF composite scores and trajectories among *APOE*ε4 non-carriers only (PA × *APOE* × time p<.001) [248]. Yet another study found no association between any domain of PA and an EF composite score after 3-years of follow-up, among carriers, non-carriers and the overall cohort [207].

Memory

Two studies evaluated the role of PA on immediate memory, delayed memory [200] and logical memory [199]. One study reported partaking in ≥3 instances of moderate/vigorous activity per week lead to a reduced probability of both immediate and delayed memory decline among *APOEε4* carriers, over 1.5 years [200]. This association did not reach statistical significance among non-carriers (PA × *APOE,* p<.05). The same group also reported that as hippocampal volume and functional connectivity declined so did the positive association of PA on reduced likelihood of cognitive decline, possibly indicating the combined risk of being an *APOE*ε4 carrier and exhibiting hippocampal dysfunction outweighing the associated benefits of PA on cognition. One other study found that partaking in 150-minutes of any leisure activity from low to vigorous intensity was associated with a reduced probability of memory decline at 2-years within the overall cohort [199]. However, stratified analysis found no significant association between PA and memory within carriers or non-carriers.

Language, attention and visuospatial abilities

Three studies reported the association between PA and tests assessing attention and language abilities [199, 207, 208] and two studies examine the association between PA and visuospatial abilities [207, 208]. Attention was mentioned as having been measured concurrently with memory via the Wechsler Memory scale (WMS) [199] and may also be considered a skill required in other tests such as TMT-A and -B [199, 207, 208] number-letter sequencing (LNS) and digit symbol [207] which also assess executive function and visuospatial/motor speed (TMT-A). One cannot report attention abilities as measured independently, however, there is an element of attentional performance being measured concurrently in some included assessments. As mentioned above, one study reported a positive association between LNS and TMT-B among high active *APOE*ε4 carriers [208], however, two further studies did not report a positive association between PA assessment and the WMS [199], TMT-A and –B [199, 207] or digit symbol [207] among carriers, non-carriers and the overall cohort. Likewise, language abilities can fall under a number of assessments concurrently measuring other cognitive domains. For example, verbal and category fluency are both language assessments as well as tests of executive function which were explored among three included studies. The studies reported no association between category verbal fluency [207, 208] and letter verbal fluency [199, 207] among carriers and non-carriers. Two of these studies, however, did report a significant association between high levels of leisure PA and letter plus verbal fluency in the total cohort [199, 207]. Only two studies included tests assessing visuospatial abilities via the TMT-A, both reporting no statistically significant associations between PA and performance in this task among carriers, non-carriers and the total cohort [207, 208].

Table 4. The association between physical activity and cognitive domains among ε4 carriers, non-carriers and the total cohort

Study	Global Cognition	Executive function	Memory	Attention	Language	Visuospatial
Schuit *et al.* (2001)[n]	+ **ε4+** [h]					
Niti *et al.* (2008)*	+ **ε4+** [h]					
Woodard *et al.* (2012)*	+ **ε4+** [i]		+ **ε4+** [a]			
Pizzie *et al.* (2014)**		+ **ε4+** [b, c]	0 [m]	+ **ε4+** [b, c]	0 [d]	0 [e]
Tsai *et al.* (2016)[n]	+ **ε4+** [j]	+ TC [b, f]	+ TC [l]	+ TC [b]	+ TC [f]	
de Souto Barreto *et al.* (2018)[n]	0 [k]	0 [b, g, d]		0 [b, e, g]	+ TC [d, f]	0 [e]
Thibeau *et al.* (2017)**		+ **ε4 -** [k]				

+ = Improvement in cognition based on higher PA; 0 = no association between PA and cognition; ε4+ = *APOEε4* carriers; ε4 - = *APOEε*4 non-carriers; TC = Total Cohort;
[a] Delayed and immediate memory; [b] Trail making B test (TMT-B); [c] letter-number sequence (LNS) test; [d] Category fluency; [e] TMT-A; [f] Verbal letter fluency; [g] digit/symbol; [h] MMSE; [i] DRS-2 Total;
[j] Montreal cognitive assessment; [k] sub-test composite z-score; [l] Weschler memory scale; [m] FCRS verbal memory; [n] No PA x *APOE* interaction conducted.
**p*-interaction (PA x *APOE)* .05; ***p*-interaction .005

2.3.2.3 Association between physical activity and risk of dementia/AD

Table 5 outlines key findings relating to *APOE*ε4 carrier status within each included study. Three studies reported the association between midlife leisure PA [347] or midlife household/leisure/gardening PA [345, 349] and reduced risk of dementia/AD [345, 347] and dementia/CIND [349] to reach statistical significance among *APOEε*4 carrier individuals and the overall cohort. Two of these studies conducted a multiplicative interaction term, PA × *APOE,* finding that although significance was observed among *APOE*ε4 carriers only, the interaction did not reach significance [345, 347]. They both also investigated an additive interaction between low PA levels and *APOE*ε4 allele carriage by assessing relative excess risk from interaction (RERI) [356], which was significant. Contrary to the aforementioned results, four studies reported the association between midlife [230, 352] or late-life [230, 351, 352] leisure PA and dementia/AD [351, 352], MCI [230] and dementia-related mortality [354] to only reach statistical significance in *APOE*ε4 non-carriers as well as in the overall cohort, however, interactions between PA and *APOE* only reached significance for one of these studies [352]. Of these, two reported that although the inverse association between high PA and reduced risk was present only among non-carriers, there appeared to be an additive effect among *APOE*ε4 carriers whereby the combined risk of reporting low PA and possessing an *APOE*ε4 allele deemed an individual at highest risk for earlier MCI diagnosis [230] and mortality due to dementia [354]. Two further studies reported the association

between household/gardening/leisure [113] or leisure PA [337] and dementia [113, 337], AD and VaD [113] to be limited to *APOE*ε4 non-carriers only, with interaction terms of PA and *APOE*ε4 carrier status being statistically significant. Finally, five studies reported no significant moderation by *APOE*ε4 carrier status on the association between PA and dementia/AD/VaD incidence. In this case, two [344, 353] studies reported significant associations between leisure PA and reduced risk of AD and VaD in both carriers and non-carriers, two other studies reported no association between work-related activity [348] or leisure PA [346] and reduced risk of AD/dementia among carriers, non-carriers or the overall cohort. One additional study reported only a sub-group with reduced physical functioning to reveal a statistically significant association between higher levels of household/leisure/gardening related activity and reduced risk of AD/VaD/dementia [350].

Only one study concurrently explored the associations between a biomarker measure alongside PA with incident dementia/AD within the context of *APOE*ε4, reporting those who partake in higher PA presented with increased brain and hippocampal volume, despite *APOE*ε4 carrier status [351].

Table 5. Physical activity and dementia/AD/VaD/MCI risk as a function of *APOE*ε4 carrier status

Nature of association	Study
Association *augmented in carriers* compared to non-carriers and total cohort	Shih *et al* (2018) [a (all dementias & CIND)] Rovio *et al* (2005) [a (all dementia & AD)] Luck *et al* (2014) [a, b (all dementias & AD)]
Association *augmented in non-carriers* compared to carriers and total cohort	Tolppanen *et al* (2015)[a (all dementias & AD)*] Tan *et al* (2017) [(all dementias)] Strand *et al* (2015) [a (mortality due to all dementias)] Podewils *et al* (2005) [(all dementias, AD, VaD)*] Fenesi *et al* (2017) [(all dementias)*]
Association *present in carriers and non-carriers*	Yang *et al* (2015) [b (all dementias, AD, VaD)] Chen *et al* (2012) [b, c (AD)] Krell-Roesch *et al* (2016) [a (MCI)]
No association within carriers, non-carriers and total cohort	Taaffe *et al* (2008) [d (all dementias, AD, VaD)] Paillard-Borg *et al* (2009) [e (all dementia)] Rovio *et al* (2007) [e (all dementias & AD)]

[a] Additive effect: inactive ε4 carriers exhibited highest odds of all cause dementia / AD / CIND;
[b] Statistically significant positive association within carriers and non-carriers;
[c] No PA x *APOE* interaction assessed;
[d] Statistically significant positive association among those with reduced physical functioning;
[e] No statistically significant association in either ε4 carrier / non-carrier groups or overall cohort
*Significant PA x *APOE* interaction (all others NSS)

2.3.3 Risk of bias and quality assessment

2.3.3.1 Risk of bias - RCT's

A detailed quality assessment for each RCT via the RoB tool can be viewed in the Appendix A, section A.6. The primary source of bias in all included RCTs was absence of personnel blinding, introducing risk of performance bias. It should be noted that participants were also not blinded, however, the outcome was unlikely to be influenced as a result. Information regarding random sequence generation and allocation concealment was not reported within two of the studies, thus risk of selection bias remained unclear [341, 342]. A third study reported conducting randomization sequence generation via the equal probability method [343]. An absence of information regarding selective reporting of findings in all three studies resulted in an uncertain bias in this category, however, as each RCT reported non-significant differences between intervention and control groups, it is unlikely that reporting bias is present. One study was allocated a low risk of detection and attrition bias due to sufficient detail regarding the blinding of outcome assessors and the reporting of participant attrition/exclusions [343]. No information regarding these domains were

reported for the remaining included RCTs due to being available only via conference abstract [341, 342]. A summary of the methodological quality of included RCTs can be found in Figure 5.

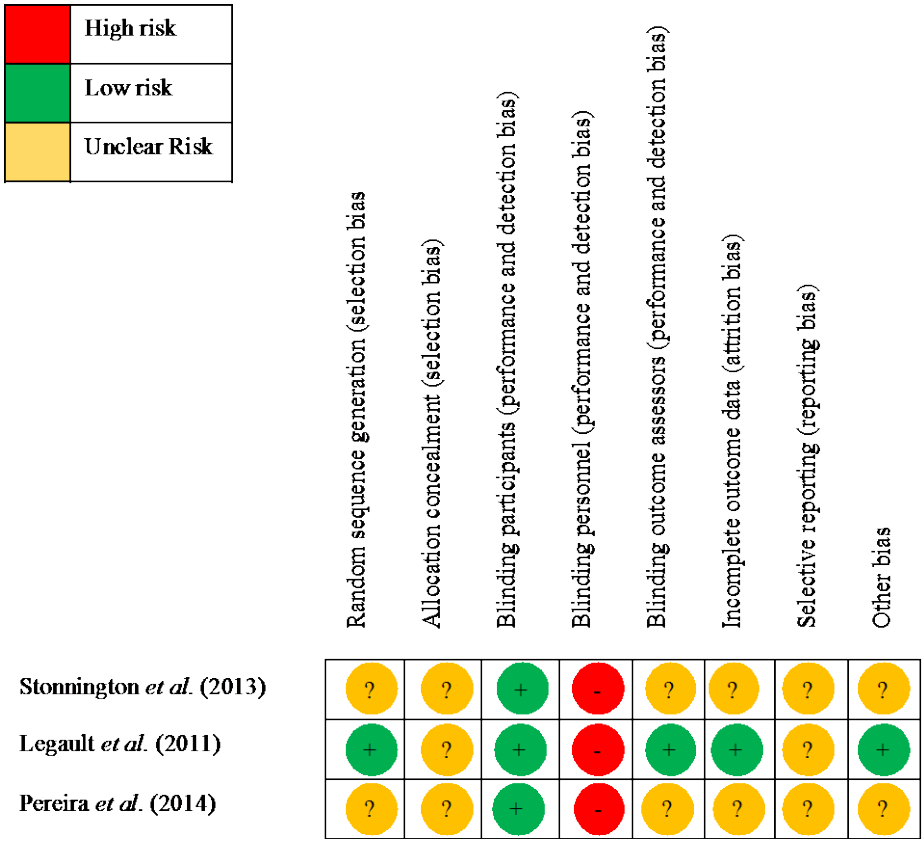


Figure 5. Methodological quality of included RCT's

2.3.3.2 Quality assessment – observational studies

Methodological quality for observational studies assessing cognition and incidence are summarized in Tables 2 and 3 respectively, indicating the number of stars awarded to each study. Four studies were deemed to be of high quality (7, 8 or 9 stars) [113, 337, 345, 346], sixteen were deemed to be of fair quality (4, 5 or 6 stars) [114, 198-200, 207, 208, 230, 248, 347-354] and one study, being an abstract, resulted in a low quality score (1, 2 or 3 stars) due to limited information pertaining to the methodology [344]. The most common element in which prospective studies were denied stars was ascertainment of exposure, with nineteen studies using self-reported questionnaires which may be prone to reporting bias. Three [113, 345, 346] studies used a more favourable method of structured interview. Similarly, two [353, 354] retrospective studies used self-report questionnaires to ascertain PA exposure over a period of retrospect whereby memory of activities may be limited (i.e. the previous year), increasing the risk of recall bias. Ten prospective study cohorts were not deemed to be representative of the average older person, therefore at risk of selection bias [114,

198-200, 230, 248, 346, 349, 350]. None of the retrospective studies were deemed to have representative cases; two [344, 353] did not provide enough information regarding the recruitment of cases, and one [354] study drew cases from the Cause of Death Register, so participants were not representative of those living with dementia. For prospective observational studies, previous reports have indicated that risk individuals 65+ years of age, may exhibit clinically meaningful decline within 18 months [357, 358]. A more prudent cut-off point of 2-years was considered a sufficient minimum length of follow-up. Two studies had a follow-up period of <2 years [198, 200]. In addition, the cut-off point for a sufficient follow-up rate was set at ≥80% [359]. Seven studies had an inadequate follow-up rate, increasing the risk of loss to follow-up bias in these studies, posing threat to validity [114, 198, 208, 347, 348, 352]. Two observational studies did not control for age, which may act as a source of confounding bias in these studies [200, 248]. None of the included observational studies used a non-exposed cohort for comparison and therefore the quality of such a cohort could not be commented on.

2.4 Discussion

This systematic review examined whether *APOE*ε4 carrier status modifies the association between PA, cognitive function and dementia/AD risk among cognitively healthy older adults by assessing findings from relevant RCTs and observational studies. Twenty-four studies meeting pre-defined eligibility criteria were identified; three RCT's, seven prospective observational studies with cognition as the outcome and 14 prospective observational studies with incident dementia (all cause, AD, VaD), MCI or CIND as the main outcome. All three RCT's found no significant association between PA intervention and improved cognition based on *APOE*ε4 carrier status [341-343]. Conversely, five observational studies found a significant association between PA and cognition being limited to carriers [114, 198-200, 208], with one study reporting associations limited to non-carriers [248] and yet another reporting no difference between carriers vs non-carriers [207]. Regarding the associations between PA and risk of dementia; studies reported the association to be augmented among carriers [345, 347, 349] or augmented among non-carriers [113, 337, 351, 352, 354], with three studies reporting the associations between PA and risk of incident dementia/AD/VaD [344, 353] and MCI [230] to be present among both carriers and non-carriers, whereas three other studies reported no association between PA and risk of all cause dementia, AD and VaD within carriers, non-carriers or the overall cohort [346, 348, 350].

2.4.1 Physical activity and cognition

Due to a number of methodological limitations, results from the three included RCT's warrant cautious interpretation. Details of two studies were available via abstract publication only, hence, limiting ability to assess study specifications [341, 342]. A short intervention time; ranging from four to six months, coupled with small sample sizes, may not yield adequate power to detect a true

effect within *APOE*ε4 carrier/non-carrier sub-strata. Likewise, experimental evidence suggests an intervention including both aerobic and anaerobic forms of exercise may be more efficacious in yielding beneficial results among healthy older adults [117, 216]. One may speculate this be particularly the case in those who are at increased genetic risk of dementia.

Whilst non-significant findings were reported for cognitive outcomes, effects on serum BDNF among non-carriers indicate that the *APOE*ε4 allele may preclude the biological responses which mediate the effects of PA on cognitive outcomes [341]. There is a clear lack of experimental studies exploring the effect of PA intervention among cognitively healthy individuals as a function of *APOE*ε4 carrier status. Multiple meta-analyses have reported inconsistent findings with regard to the effect of PA intervention on improved cognitive health among healthy older adults [179, 188, 213, 216, 221]. Such heterogeneity may be explained by a variety of factors including, but not limited to, *APOEε*4 carrier status.

Findings from included observational studies exploring the association between PA on cognition indicated augmented positive PA/cognitive associations among *APOEε*4 carriers when compared to non-carriers. Five studies reported the positive association between PA and global cognition [114, 198-200] executive function [208], memory [200] and attention [208] to be either augmented in or simply limited to *APOEε4* carriers. Conversely, one study found PA to be positively associated with executive function among *APOE*ε4 non-carriers [248], where another study found no significant difference in associations between PA and multi-domain cognition among carriers and non-carriers [207]. This study reported within a basic time-adjusted model, however, that positive associations across cognitive domains were augmented among carriers (Table 3). One possible explanation for the incongruent result reported by one study [248], whereby PA benefits were limited to non-carriers, may have been as a result of selection bias within the cohort, resulting in a higher functioning *APOE*ε4 carrier group. As the author pointed out, the exclusionary criteria of <24 MMSE and without mobility impairment may have excluded those carriers at higher risk of cognitive decline. However, the same study did identify Low PA/ε4 carriers to perform significantly worse that high PA/ε4 carriers. Interactions with age and *APOE*ε4 carrier status also found that those in the low-active/ε4 carrier/+75 age group to exhibit the highest risk of cognitive decline, being significantly steeper than individuals who were high-active/ε4 carrier/+75, indicating an additive negative association, possibly attenuated by PA. It must be noted that three of the aforementioned studies did not conduct an *APOE* × PA interaction analysis [114, 199, 207]. One cannot confer true effect modification via stratified analysis alone, hence, whether an association between PA and cognition truly differs as a function of *APOE*ε4 carrier status within these studies can only be speculative.

Results largely varied across studies in relation to global cognition and domain-specific associations and measurement. Only two of the three studies investigating global cognition also explored sub-

domains. Within a healthy cohort, one may expect that early signs of cognitive change only be evident among specific sub-domains of cognition. Likewise, meta-analysis have reported PA to be associated with enhanced cognitive function across all available cognitive domains, with the largest effect size for indices of executive function [216]. This emphasizes the importance of robust, domain-specific measurement. Majority of included studies utilized raw sub-test scores as measurement of unique cognitive domains. However, domain-specific composite scores comprised of multiple sub-tests would possess superior sensitivity as a preferable alternative. Utility of one test to represent performance within a specific domain may not possess adequate sensitivity for a convincing outcome. Likewise, a measure of global cognition, although often a composite of many tests, could lack sensitivity where PA effects be domain-specific. Future studies should endeavour to include robust measures across key cognitive domains, such as memory and executive function, via multiple sub-tests.

Stronger PA/cognitive associations reported within carriers when compared to non-carriers, speculates that positive associations observed among a healthy cohort will only become evident at the beginning of clinically relevant cognitive decline. Parenthetically, *APOE*ε4 carrier individuals may indeed be exhibiting the earliest stages of cognitive decline prior to non-carriers, hence, exhibiting augmented PA benefits at a shorter follow-up when compared to non-carriers. A longer follow-up duration may detect the same positive PA/cognitive associations among non-carriers.

Only one study explored *APOE*ε4 moderated PA/cognitive associations alongside biomarker data [200]. They reported that the positive associations between PA and cognition among *APOE*ε4 carriers remained consistent even when controlling for hippocampal volume and activation, although the interaction was no longer significant when one or the other of these were extremely high or low. This finding could indicate that *APOE*ε4 carriers with either high brain reserve (robust hippocampal volume and activation) or with degradation beyond repair, may not exhibit cognitive benefit from PA. Of specific importance, is that among those whose disease stage has progressed to a certain irreversible trajectory, PA may no longer be an effective preventative strategy, further emphasizing the need for early intervention, i.e., among a cognitively healthy or pre-symptomatic cohort. Likewise, those with intact and highly functioning hippocampi may exhibit lower risk of cognitive decline regardless of PA levels or *APOE*ε4 carrier status. This is an important insight when considering the theory of threshold effects and cognitive reserve [39], supporting the notion that intervention may be most efficacious at a younger age, at least for *APOE*ε4 carrier individuals.

2.4.2 Physical activity and incident dementia/AD

Results from included observational studies exploring the association between PA and risk of developing all cause dementia, AD, MCI or VaD were inconsistent. Of the 14 studies included, three studies reported benefits to be augmented among carriers, however, PA x *APOE* interactions

did not reach statistical significance. An additional two studies reported PA to reduce risk of AD [344, 353], all cause dementia and VaD [353] among both carriers and non-carriers. Additive effects were noted within four studies, whereby the added risk of possessing an *APOE*ε4 allele, coupled with low PA yielded highest risk for all cause dementia [345], AD [347], MCI [230] and mortality due to dementia [354]. In chronic disease epidemiology it is commonly regarded that risk factors acting independently have an additive rather than synergistic effect [356]. These additive findings imply that the total effect of each risk factor is larger than their independent effects; in this case, highlighting the possibility that *APOE*ε4 carriers may exhibit enhanced benefit from adopting an active lifestyle toward reducing risk of dementia, even later in life. Other included studies did not explore such an additive interaction, and hence, cannot be commented upon.

It is well established that *APOE*ε4 carriers possess sub-optimal neural protection and repair mechanisms, and hence these findings, along with those relating to maintained cognitive health, may lend evidence toward carriers being more dependent upon lifestyle-related factors as a means of protection against cognitive decline and dementia [360]. Indeed, carriers display earlier cognitive change prior to appearance of AD symptomology, as well as earlier loss of brain volume and cortical thickness [361, 362], higher Aβ-burden [363] and increased functional abnormalities [364, 365]. As a result, the benefits of PA on healthy ageing may be most evident within the pre-symptomatic or prodromal stages of AD and/or dementia.

It would be interesting to explore in more detail the cognitive benefits of PA in the context of dementia risk and whether it supports maintenance of cognitive health for a longer duration prior to development of dementia. For example, PA may prolong quality of life via extending cognitive health without reducing the risk of eventual senesce, especially among *APOE*ε4 carrier individuals. This notion is aligned with the cognitive reserve theory [39], whereby studies have shown those with higher cognitive reserve engender maintained cognitive function within the presence of pathology, whilst possibly exhibiting a steeper decline at a later stage of the AD physiological continuum, than would be expected [366].

2.4.3 Midlife physical activity

It has been postulated that a follow-up period of <10-years may not preclude reverse causation [211], and that late-life PA may be influenced by such residual factors as sub-clinical dementia [225]. To explore PA during an earlier life-stage, such as midlife and how it is related to later life cognition and risk of dementia may provide more meaningful outcomes within an observational context, especially among a higher risk population. Four identified studies explored the role of midlife PA on dementia/AD [347, 348, 352] and MCI [230] in the context of *APOE*ε4 carrier status, and no study has yet to explore midlife PA in relation to later life cognitive trajectories as a function of *APOE*ε4 status. One study reported that those who partook in vigorous exercise for at least 20-

minutes twice weekly found a reduced risk for AD 20-years later, but not all cause dementia, among *APOE*ε4 carriers only [347] with a significant additive interaction, although midlife work-related activity was not associated with later-life risk [348]. Conversely, a later study utilizing the same cohort found high PA to reduce risk among non-carriers but at a 28-year follow-up [352]. This same study, however, also reported a low active/*APOE*ε4 carrier additive association on increased risk for AD dementia when compared to high active/*APOE*ε4 carriers and non-carriers. Congruently, another study reported an increased risk for incident MCI in *APOE*ε4 carriers reporting no moderate or vigorous activity in midlife compared to non-carriers within the same low active group [230]. They also found that *APOE*ε4 carriers who were low active in mid- and late-life had a higher risk of incident MCI as compared to non-carries. Considerable heterogeneity between studies pose difficulties in drawing comparison. However, findings lend support that PA at an earlier life stage may also contribute toward reduced risk of incident dementia and / or AD within this sub-group.

2.4.4 Age, sex and *APOE*

Three studies explored the effect modification of age on PA/cognitive or PA/dementia risk associations. Two reported that the benefits of PA were no longer observed among an older-old population (i.e. 75+) [199, 248]. However, the augmented benefits of PA among older *APOE*ε4 carriers was noted, indicating that the additive negative effect of older age and *APOE*ε4 carriage be mitigated by PA [248]. A third study with a much larger sample size (n=3,714) found no effect modification of age [351]. A contentious issue often raised among studies of older adults is that those 75+ years of age who are willing and able to participate may be of superior health, and hence, not representative of the wider population [367]. Alternatively, those over the age of 75 may have reached a threshold whereby the benefits of PA are no longer relevant. Possibly, the positive effects of PA among 75+ individuals are attenuated, and a larger sample size is required to detect a difference. Both studies had sample sizes ranging from 483-514 and did not report what proportion of the sample are among those considered ‘older-old’. These findings emphasize the need for intervention at an earlier age and methodological considerations when selecting a cohort for inclusion in such investigations.

Of the five included studies exploring sex specific effects, two reported that an individual’s sex did not modify associations between midlife leisure PA [347] or work-related PA [348] with risk of dementia/AD at 20-years follow-up, whereas the third study reported low PA males to exhibit highest risk for dementia at 30-years follow-up, all within the CAIDE cohort. One study reported the benefits of higher frequency PA on reduced risk of AD to be restricted to males, although this study did not assess PA duration or intensity which may influence the result [353]. An additional study utilized a male only cohort and found that *APOE*ε4 carriers only benefitted from leisure PA on global cognition [114]. Conversely, one other study reported that the positive association

between leisure PA and global cognition and memory were be restricted to females [199]. Evidence indicates sex differences with regard to risk of cognitive decline and dementia/AD being higher in females [265] and response to PA in reducing such risk has been typically augmented among females [117, 216]. Parenthetically, studies have shown the effect of *APOE*ε4 carriage on risk of cognitive decline and AD may be augmented among females when compared to males, yet *APOE*ε4 homozygous males are reported to be at greater risk of MCI and AD [129, 130, 265]. Results reported in this systematic review, coupled with those previously reported, highlights the complexity of sex interactions with physical activity on risk of cognitive decline and/or dementia.

2.4.5 Strengths and limitations of the review

Strengths of this systematic review include the depth of literature search conducted across six different databases and including a comprehensive list of search terms. Participant eligibility criteria was kept relatively broad, date of publication was not restricted, outcomes were not limited to one type of dementia or cognitive domain and exposure type was not restricted to a specific PA specification (e.g. duration, intensity, frequency, type, etc). Finally, inclusion of both experimental plus prospective observational study designs allowed for broader review across studies.

Despite the originality and strengths of the current review, several limitations warrant discussion. Due to considerable methodological heterogeneity across included studies, a meta-analysis was not conducted. Although four studies utilised the same PA assessment tool, the metric varied between them [113, 198, 207, 230]. No study included a comparable PA measure with cognition as the outcome. Only two studies [337, 354] were found to use the same measure of PA (none versus some), yet, the outcome varied from AD diagnosis to mortality due to dementia. In the absence of a meta-analysis, conclusions drawn within this review are not without conjecture; yet heterogeneity across studies would yield questionable reliability of pooled data. Noteworthy differences existed for PA intervention and measurement. The assessment of PA type varied considerably across studies, from sport related exercise to a 'PA composite' comprising exercise, household and gardening activity to work-related activity. Likewise, some studies collected information pertaining to frequency, intensity and duration (most desirable), whereas others only measured one or two of the aforementioned. Questionnaires varied in level of retrospect, ranging from the previous week to the previous year and on some occasions midlife activity was collected. Quantification of physical activity metric (e.g. what equates 'high' or 'low' PA) also varied considerably across studies. Future studies should assess frequency, duration and intensity of activities commonly undertaken within an elderly population including leisure and household activity which can be combined or examined independently. This will allow for easier comparability across studies and facilitate investigation into intensity, duration and frequency threshold as well as ability to explore activity types within the same cohort. No included study utilized an objective measure of PA which

would increase accuracy, promote comparability and remove risk of reporting bias. To optimize PA measurement in observational studies and assess reliability of adherence to PA in experimental RCT's, future studies should endeavour to adopt a validated form of objective PA measurement, such as accelerometery, alongside a self-reporting questionnaire.

A variety of differing tests were applied to assess global and domain-specific cognition. For example, some studies chose to measure global cognition via the MMSE which provides a crude representation and limited sensitivity in detecting mild cognitive change [368]. Conversely, more sensitive alternatives were also employed, such as the MOCA, comprised of an array of domain-specific assessments more sensitive to pre-clinical cognitive disturbance. Ultimately, researchers should endeavour to reach a consensus on a homogenous battery of cognitive tests to be used across similar studies.

Only three studies included additional biomarker measurement alongside cognitive [200, 341] or incidence dementia/AD outcome [351]. Future studies should endeavour to include biomarker measurement alongside robust cognitive and incident dementia/AD outcomes. This information will supplement our understanding of the underlying biological mechanisms relating to PA and reduced risk of cognitive decline/dementia diagnosis in an *APOE* specific manner. Indeed, *APOE*ε4 specific augmentations have been documented in studies reporting an inverse association between PA and brain Aβ deposition [327]. Two cross-sectional studies presented congruent results using Aβ PET imaging as an indicator of pathology, each showing that high active *APOE*ε4 allele carriers displayed with significantly lower brain Aβ deposition when compared to low active carriers and with levels comparable to non-carriers [369, 370]. The benefits of PA were not apparent among *APOE*ε4 non-carriers, thus suggesting that individuals with increased risk of amyloidoisis may rely more heavily on PA than lower risk individuals. These studies did not, however, explore whether these associations were linked with a reduced risk of cognitive decline and/or dementia.

Finally, all included studies categorized participants as either being an *APOE*ε4 carrier / non-carrier to optimize sub-group sample size. However, putative evidence indicates a dose-response relationship between number of *APOE*ε4 alleles and cognitive outcomes plus risk for AD/dementia [366]. Granted a low frequency of *APOE*ε4 homozygotes in western populations (~2%), future epidemiological studies should strive to explore the associations between PA and cognition and risk of AD/dementia among those who are homozygous as well as heterozygous for the *APOE*ε4 allele.

2.4.6 Conclusions

There is now a large body of literature indicating a positive association between PA and cognitive health and reducing risk of dementia in older adults. Multiple reviews of both cross-sectional and longitudinal observational studies and meta-analyses of experimental studies have indicated a generally positive effect of PA on cognitive and brain health [178, 179, 210, 213, 214, 221, 371,

372]. Yet, this is the first review to systematically explore the impact of PA on cognition and dementia/AD risk among cognitively healthy older individuals as a factor of *APOE*ε4 carrier status. This systematic review lends evidence toward PA as a viable lifestyle factor supporting improvement and/or maintained cognitive health among cognitively healthy older *APOE*ε4 carriers. Whether physical activity reduces risk of dementia and/or AD differentially among carriers and non-carriers remains uncertain, although, evidence points toward PA as a viable approach to incident risk reduction among both carriers and non-carriers.

Sex differences were noted in some studies, however, contrary to widely reported PA benefits among females, just one of the six studies reported such benefits to be augmented among females. However, this study was the only to explore sex differences in response to PA on cognition. The association between PA and incident dementia were reported to be augmented among males in two studies, with no sex differences among two further studies. Possibly other factors may be influencing response as a function of sex, such as genetic risk, age or other lifestyle factors or exposures. Likewise, no study has explored how PA is associated with cognition as a function of sex and *APOE*ε4 carrier status – such question will be addressed in the current thesis. Only two studies explored the effect of age on PA/cognitive associations both reporting that the positive associations of PA on cognition were no longer evident among those >75 years of age. Of these two studies, one also included *APOE*ε4 carrier status with age reporting that relative to low active 75+ carriers, high active matched individuals performed significantly better in tests assessing executive function. This thesis will extend upon these results via exploring a wider array of cognitive domains while investigating the impact of not only late-life PA but also midlife PA.

This review highlights the lack of data exploring the additive and independent associations of midlife to late-life PA on multi-domain cognition as a function of *APOE*ε4 carrier status within the same cohort. Indeed, no study has explored such question as will be covered in the current thesis. Likewise, this review has revealed an additional gap in the literature pertaining to the biomechanistic benefits of PA on cognition and as a function of *APOE*ε4 carrier status. Such questions will be explored via investigating the mediator or moderator role of BDNF as well as the role of peripheral Aβ in PA/cognitive associations as a function of *APOE*ε4 carrier status.

Collectively, findings indicate a complex interplay between genetic risk, age and sex as well as timing, intensity, type and duration of physical activity. Indeed, there appears to be *APOE*ε4 related differences in response to PA on cognitive health and risk of dementia, yet also variability which may be explained by additional confounders such as sex and age. While investigating the interplay between these variables across multiple transitional states of ageing will present challenges, future research should endeavour to unravel these factors with the aim for a precision preventative

approach. It is hoped that findings from the research conducted within this thesis will contribute toward developing this approach.

Chapter 3: Methods

3.1 Introduction

The following chapter provides an overview of methodologies employed for the work described in this thesis, including a detailed description of The Cognitive Health in Ageing Register: Investigational, Observational, and Trial studies in dementia research (CHARIOT): Prospective Readiness cOhort Study (PRO) Main Study (ClinicalTrials.gov Identifier NCT02114372) from which all data in this thesis was utilised. Refer to the Appendix, for a summary of tasks and collaborators. The CHARIOT: PRO Main Study was conducted by Imperial College London and Janssen Research and Development, LLC (Janssen R&D, LLC is a pharmaceutical company of Johnson & Johnson), the Sponsor that funded this study.

Below will describe the ethics, research design, study population, health and lifestyle data, with an emphasis on physical activity data collection and neuropsychological assessment tools. A detailed description of biological sample collection and analysis of blood biomarkers will follow. Although the full CHARIOT: PRO Main study schedule is outlined in Table 6, only data used within this thesis will be described in more detail. Data preparation and statistical analyses methodology will be covered. Note, CHARIOT: PRO 'Main Study' is to be distinguish from a follow-on study termed CHARIOT: PRO Sub-Study. The CHARIOT: PRO Main study from which this thesis is derived will simply be referred to as CHARIOT: PRO.

3.2 Ethics

To ensure the quality and integrity of research, this study was conducted in accordance with Good Clinical Practice (GCP) Guidelines, Good Pharmacoepidemiology Practices (GPPs) issued by the International Society for pharmaceutical Engineering (ISPE), applicable national guidelines, and to the Declaration of Helsinki 2013, as modified by the 52nd World Medical Assembly, Edinburgh, Scotland, 2000, and clarified by the World Medical Assembly (WMA) General Assembly, Washington 2002 and Tokyo 2004. The CHARIOT: PRO study has received National Research Ethics Services approval (15/L0/0711) and internal Imperial College London (Imperial) Research Ethics, Joint Research Compliance Office approval (JRCO:15/1C/2791). Prior to consenting onto the CHARIOT: PRO study, participants were provided with a detailed study information sheet outlining study procedures, risks and benefits associated with participation. Participants were provided with a minimum of 48-hours to consider these factors and fully understand the study requirements prior to discussing further with study staff, where necessary; after which signed informed consent was obtained (see Appendix A.1).

3.3 Design

CHARIOT: PRO is a prospective, single centre, non-interventional study involving six-monthly visits for up to three years. Although a four-year follow-up time was originally planned, the study was terminated early due to the sponsor re-allocating funds to an enhanced study design involving imaging and a more intensive visit schedule (CHARIOT: PRO Sub-Study), due to complete in 2020. This resulted in a mean follow-up period of 18.5 (1.7 SD) months, with the longest period of participation reaching 36 months (range = baseline – 36-months). The study aimed to firstly, characterise key factors and markers of cognitive change in healthy older adults leading to MCI-AD, preceding AD related clinical symptomology. Secondly, to further qualify and validate neuropsychological assessment techniques for earlier indicators of cognitive decline, especially among persons at increased risk of developing MCI-AD.

3.3.1 Population

CHARIOT: PRO is comprised of cognitively healthy older adults (N = 987) between the ages of 60–85 years. Study Participants were recruited from a primary care-based recruitment register, the CHARIOT register (JRCO:12/EM/0234) based at Imperial. To be recruited onto the CHARIOT register, individuals were approached via Primary Care Practices in West London and consequently consented to being contacted for participation in studies relating to dementia prevention (currently ~30,000 individuals). Register participants are community dwelling and considered to be reasonably representative of the wider London population. Specifically, the age, gender and ethnicity of individuals recruited onto the CHARIOT:PRO register, considerably match that of the larger eligible population [373]. Majority of CHARIOT: PRO study participants were recruited directly from the CHARIOT register; however, a small number of participants were also recruited via self-referral (n=9). Participant self-referral were via existing study participants, including such individuals as friends and family.

3.3.2 Screening and Enrolment

CHARIOT register individuals or those self-referred were contacted by Imperial study staff via mail and/or phone to determine interest in partaking in the CHARIOT: PRO study. They undertook a scripted over-the-phone screening assessment in order to confirm eligibility. Upon confirmation of initial eligibility, basic demographics were recorded, and participants were mailed a detailed study information sheet and consent form to be read prior to attending their first screening visit (Appendix B.1). More detailed eligibly-screening as defined by the study screening criteria was covered by a member of the medical team (see Appendix B.2) prior to participants signing the informed consent form.

Cognitive status was determined via performance on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) [374]. If a participant's age and education adjusted RBANS

z-score reached 1.5 SD below normal, the participant was excluded or, if borderline, adjudicated by the study Principle Investigator to confirm inclusion or exclusion. Specifically, if any non-memory domain contained a z-score >1.5 SD below average, the participants overall health and cognitive function on other sub-tests were reviewed and if there were no clear indication of cognitive dysfunction, the participant was included.

Study participants were invited to attend a baseline visit and then 6-monthly visits for up to three years. Figure 6 depicts the CHARIOT: PRO screening and enrolment process, including all those excluded at varying stages of study involvement, as well as those included and randomised to sub-tests based on clinical risk for AD allocation. The original study aimed to reach a minimum of 700 enrolled study participants. This number was selected to provide sufficient likelihood of detecting a rare event (e.g., progression to MCI-AD). These calculations assumed 630 participants to be followed for at least one year, with a 10% drop-out rate. A prospective estimation of required recruitment contacts was not conducted, however, in order to reach targets a total of 2,003 individuals were approached for participation in the study, with 51% deemed unsuitable at phone screening. Reason for participant exclusion following screening were poorly recorded in the CHARIOT: PRO Main Study. However, of the 987 individuals who partook study screening, it is known that 21.5% were excluded due to performing >1.5 SD below normal on at least one age- and education-adjusted RBANS index score, 3% due to reporting prohibited medication or illness (not identified at phone screening) and 3% withdrew or were excluded for reasons not well recorded. Post-screening, 14% of study participants discontinued study participation for reasons not well recorded, with approximately 12% being as a result of withdrawal. Under protocol version 1 (31 October 2013) screening (V0) and baseline (V1) visits were combined as one visit (approximately 312 subjects screened under this protocol), hence, there will be a higher proportion of participants having completed data collected at V1 than reported below at 'baseline'. The following protocol Version 4 (22nd October 2014) included the introduction of V0 for the purpose of reducing participant burden at screening for those excluded.

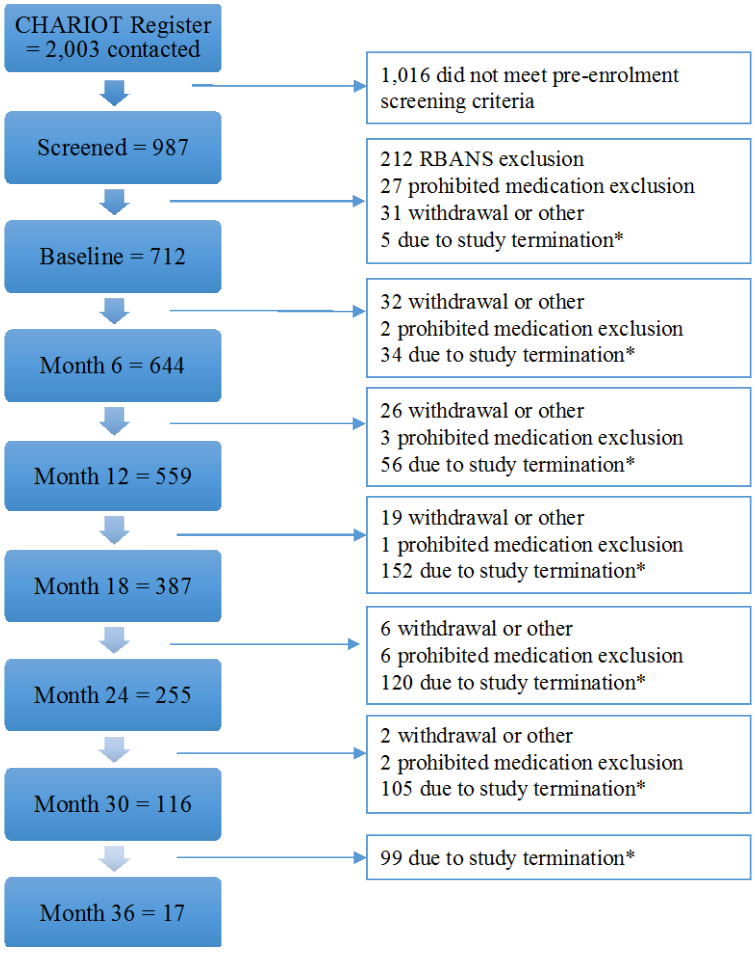


Figure 6. CHARIOT: PRO Study recruitment flow

*The CHARIOT: PRO study was terminated early. Recruitment and screening continued to take place up until termination date, hence, not all included participants reached end-point of 36-months. The average follow-up period was 18.5 (1.7 SD) months.

3.4 Study schedule

A full schedule of CHARIOT: PRO study assessments across 6-monthly time-points are presented in Table 6. All data collected in this study were recorded routinely on ethically approved source paperwork, including updated medical recording alongside medical history.

From protocol amendment 4, the baseline screening visit, based at Charing Cross Hospital, Imperial, was divided into two stages, V0 and V1. At V0, and following confirmation of eligibility, informed consent was first completed. Medication history and concomitant medications (name, dose and frequency) were next recorded followed by biological sample collection. A total of 40 mL of fasting blood was collected in the morning, including three 9-mL EDTA plasma tubes (DNA, buffy coat, and plasma aliquots); one 9-mL serum tube (serum aliquot); and one 2.5-mL RNA tube (RNA and micro-RNA). A mid-stream urine sample was collected in a 30-mL urine pot and aliquoted. Approximately 5-mL of saliva was collected in a 50-mL sterile pot. Blood pressure was measured whilst in the sitting position and prior to neuropsychological testing. Both systolic and diastolic blood pressure readings were obtained through utility of an Osciliometric (automated) device. The participant's height (cm) and weight (kg), head, hip and waist circumference were obtained. Body Mass Index (BMI) was calculated as weight (kg) divided by height (cm) squared (weight/height^2). If the participant was not excluded following V0, they were invited to attend V1 up to 10-days later, whereby additional neuropsychological assessments were conducted, as well as self-reported memory complaint, and self-reporting questionnaires pertaining to health, sleep, functional capacity and lifestyle factors (see Appendix A, A.2).

All enrolled participants were genotyped for apolipoprotein (*APOE)* allele status, with both participants and study investigators blinded to outcome. Six-month follow-up visits collected serial evaluations of neurocognitive, lifestyle and health data, alongside repeated anthropometrics and sample collection, as detailed in Table 6. Participants were reviewed per existing guidelines at each follow-up visit for the management of AD, however, in this study no participant reached AD clinical status.

Table 6. Schedule of assessments for CHARIOT: PRO Main Study

Note: 6-month visit windows are +/- 2 weeks from baseline. V0 and V1 w/in 30-days from each other	Baseline / V0	Baseline / V1	M6	M12	M18	M24	M30	M36
Informed Consent	x							
Randomisation		x						
Demography	x							
Medical History	x							
Blood sample for genetics	x		x		x		x	
Blood, urine and saliva sample	x			x		x		x
Comorbid conditions	x	x	x	x	x	x	x	x
Concomitant medications	x	x	x	x	x	x	x	x
AD history, procedures, treatments	x	x	x	x	x	x	x	x
Major health events		x	x	x	x	x	x	x
Safety reporting	x	x	x	x	x	x	x	x
Anthropometrics	x	x	x	x	x	x	x	x
Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)	x		x	x	x	x	x	x
Mini Mental State Examination (MMSE)		x		x		x		x
Neuropsychological Assessment Battery (NAB) Memory & Executive Functions modules		x		x		x		x
Clinical Dementia Rating (CDR)		x	x	x	x	x	x	x
CogState		x	x	x	x	x	x	x
Delis Kaplan Executive Function System (D-KEFS)		x	x	x	x	x	x	x
National Adult Reading Test (NART)	x							
Geriatric Depression Scale	x		x	x	x	x	x	x
State-Trait Anxiety Inventory	x		x	x	x	x	x	x
Imperial Lifestyle Questionnaire (Baseline Version)	x							
Imperial Lifestyle Questionnaire (Follow-up Version)			x	x	x	x	x	x
International Physical Activity Questionnaire (baseline version)[1]	x		x	x	x	x	x	x
Scottish Collaborative Group Food Frequency Questionnaire	x		x	x	x	x	x	x
Perceived Deficits Questionnaire	x		x	x	x	x	x	x
Work Productivity and Activity Impairment	x		x	x	x	x	x	x
Health Utilities Index Mark 3		x	x	x	x	x	x	x
Berlin Sleep Questionnaire		x						
Pittsburgh Sleep Quality Index		x	x	x	x	x	x	x

[1]Introduced per protocol version 7 (10th August 2015). Abbreviations: M, Month; V, Visit; x, indicates visit occurrence

3.5 Neuropsychological assessment

Study participants underwent administration of a comprehensive array of neuropsychological assessment batteries at baseline and during follow-up visits. Tests selected for use in this thesis were the RBANS [375] and Executive model of the Neuropsychological Assessment Battery (NAB) [376]. Domain-specific composite index scores obtained from RBANS and NAB were age-adjusted and utilised to aid interpretability. Table 7 provides a summary of sub-tests within RBANS and NAB, and the index scores they covered.

One third of participants were allocated to either the DKEFS, CogState or computerised CDR (Bracket) test administration, hence, due to reduced sample sizes, utility of these measures in the current project was decided against. Likewise, the MMSE and NART often report ceiling effects in cognitively healthy cohorts and lack adequate sensitivity in detecting early stages of clinical cognitive change, hence, these assessments were excluded as outcome measures in analyses throughout this thesis.

Appropriately qualified staff undertook psychometric training based on a standardised protocol developed for CHARIOT: PRO. Training and certification were conducted through utility of in-person training sessions, demonstration videos, and reference to psychometric battery manuals detailing standardised administration and scoring methods for each instrument. Psychometric assessors were re-trained regularly to prevent deviation from testing protocol.

Table 7. Description of cognitive assessments

Cognitive domains (sub-test score range)	Test duration (approx. mins.)	Cognitive Domains
RBANS Test Battery	25-30	-
Attention Index	-	-
Digit Span (0 - 16)	3	Attention, Working Memory
Coding (0 - 89)	3	Attention, Processing Speed, Executive
Language Index	-	-
Picture name (0 - 10)	2	Language
Semantic fluency (0 - 40)	1	Language, Executive Function
Visuospatial Index	-	-
Figure copy (0 - 20)	1	Visuospatial / Constructional, Attention
Line orientation (0 - 20)	3	Visuospatial
Immediate Memory Index		-
List learning (0 - 40)	4	Auditory Verbal Immediate Memory
Story memory (0 - 24)	4	Auditory Verbal Immediate / Episodic Memory
Delayed Memory Index		-
List recall (0 - 10)	.5	Auditory Verbal Delayed Memory
List recognition (0 - 20)	2	Auditory Verbal Cued Delayed Memory
Story recall (0 - 12)	1	Auditory Verbal / Episodic Delayed Memory
Figure recall (0 - 20)	1	Visual Delayed Memory, Visuospatial / Constructional
NAB Test Battery Exec.	25-30	-
Executive Index	-	-
Categories (0 – 128)	6	Sustained Attention, Cognitive Flexibility, Generativity
Judgement (0-20)	5	Reasoning, Attention, Language
Mazes (0 – 26)	12	Sustained Attention, Visuospatial, planning and foresight
Word Generation (0 – 36)	2	Language, Generativity

Note: Higher scores indicate better performance for all tests score variables.

3.5.1 Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

RBANS [377] background, methodology and validity as a sensitive assessment for detecting early stage, multi-domain cognitive decline in older adults are discussed in detail below.

The RBANS was created for three main purposes: a) as a stand-alone "core" battery for the early detection and characterization of dementia; b) as a neuropsychological screening battery for use in scenarios where lengthier batteries are either impractical or inappropriate; and c) for repeat evaluations where alternative forms are required to reduce likelihood of practice effects. In congruence with the objectives of RBANS development, the battery offers the following features:

1. The overall administration time is typically no longer than 30 minutes, suitable for sustained corporation and reduced likelihood of testing fatigue.
2. The level of difficulty is appropriate for a wide range of age groups and clinical populations. Specifically, RBANS is sensitive enough to pick up on MCI, hence bridging the gap between healthy to MCI to dementia. Many dementia screeners are either too insensitive to reliably indicate MCI, or too difficult to administer to moderately severe dementia patients [377]
3. The battery measures discrete neuropsychological domains, producing scaled score profiles. The utility of domain-specific cognitive scores is essential when characterising disease specific neuropsychological functioning.
4. Finally, the battery is amenable to production of multiple alternative yet equivalent forms, limiting practice effects in serial evaluation and enhances measurement of disease progression or screening for symptomatic improvements in response to therapeutic intervention.

3.5.1.1 Battery components

Study participants were individually administered the RBANS (Form A and B alternating at each 6-monthly visit). This face-to-face assessment comprises 12 subtests, most of which are analogous to independently developed neuropsychological measures, used to calculate five age-adjusted index scores and a total global cognitive performance score. The test indices are Attention (Digit Span and Coding), Language (Picture Naming and Semantic Fluency), Visuospatial/Construction (Figure Copy and Line Orientation), Immediate Memory (List Learning and Story Memory subtests), and Delayed Memory (List Recall, List Recognition, Story Recall, and Figure Recall subtests).

Immediate memory refers to an individual's ability to recall information immediately after it has been presented. List learning and Story Memory contribute towards this index. List Learning involves the immediate recall of 10 unrelated words verbally presented by the examiner. This task is repeated over four learning trials to create an overall immediate word recall score (rang 0–40).

Story Memory involves the recall of a short story verbally presented by the examiner, which is repeated twice to create a score ranging 0–24.

Visuospatial/Constructional performance refers to the examinee's ability to perceive spatial relations and to construct a spatially accurate copy of a drawing. Figure Copy and Line Orientation sub-tests contribute towards this index. Figure Copy involves the examinee being instructed to copy a multi-part geometric drawing with detailed accuracy (score range: 0 – 20). Line orientation is comprised of 10 different drawings of 13 (number labelled) lines radiating out from a central point in a fan-shaped pattern. Below are two lines radiating out at varying angles from one point. The examinee is asked to identify which two lines on the fan-shape match the orientation of the two lines below (total score range: 0-20).

Language measures an individual's ability to verbally name objects or retrieve learned verbal material. Picture naming and semantic fluency sub-tests make up this index. Picture naming involves presenting a series of recognisable objects to the examinee and asking them to name each one. A semantic cue is provided only in cases where the object is clearly misperceived (score range: 0–10). Semantic fluency involves the examinee verbally listing as many exemplars possible belonging to a semantic category within one minute (e.g. fruits and vegetables, total score range: 0–40).

Attention is the examinee's ability to remember and manipulate both orally and visually presented information in short-term memory. Scores from Digit span and Coding sub-tests contribute towards the Attention index. Digit span involves the examinee immediately repeating increasingly larger strings of numbers in the order an examiner has just verbally presented (score range: 0–16). This test also measures working memory. Coding is a timed task whereby the examinee is asked to match the corresponding number to a symbol based on a provided key. The symbols are presented in grid-like rows with empty spaces below, whereby the numbers are to be recorded for as many boxes as the examinee can complete within 90-seconds (score ranging: 0–89). Coding can also be considered an assessment of executive function involving sustained attention and processing speed.

Delayed Memory is a measure of an individual's anterograde memory capacity. Scores from List Recall, List Recognition, Story Memory, and Figure Recall contribute towards the Delayed Memory index. List Recall involves the examinee reciting remembered words from the 10-word List Learning sub-test presented earlier (score range: 0–10). List recognition involves the examiner reciting 20 words, whereby the examinee must state 'yes' or 'no' if the word was on the previous List Recall list (total score: 0–20). Story recall requires the examinee to recall the previous story read from the Story Memory task as accurately as possible (score range: 0–12). Figure recall requires the examinee to draw, from memory, the figure shown and copied earlier (score range: 0–20).

Total Scale score is calculated by summing the above five Index scores. Once the sum of raw scores are obtained for all RBANS sub-tests, they are converted to Index scores using a table available in the Appendix A of the RBANS Stimulus Booklet.

Domain-specific, age-adjusted index scores will be the outcome of interest in the current thesis.

3.5.1.2 Derivation of Index Standard Scores

Each index represents an age-adjusted standard score with an approximate mean of 100 and a standard deviation of 15 based on a normative study group of 540 healthy subjects, ranging from 20-89 years; US Census matched on sex, ethnicity, years of education and geographic region (U.S. Bureau of the Census, 1995). The standardised samples were divided into six age groups: 20-39, 40-49, 50, 59, 60-69, 70-79 and 80-89, hence, representing age-adjustments via these groups as opposed to age as a continuous variable. Details of Index standard score derivation is thoroughly detailed in the RBANS manual [375], page 37. Briefly, the raw index score was first created, which is simply the sum of sub-test z-scores within that index. For ease of interpretation this score was centred at 100 and adjusted from a value obtained within an age normative curve produced by the aforementioned normative population, with all values available within a look-up table found in the RBANS manual; allowing calculation of an age-adjusted index score. For utility in the CHARIOT: PRO study, scoring was automated via an electronic RBANS score sheet in which raw sub-test scores were entered and the age range specified (in this case either 60-69, 70-79 or 80-89). The age-adjusted index scores were automatically calculated via an automatic look-up command, produced within excel.

3.5.1.3 Standardisation, reliability and score differences

As documented in the manual [375], the internal reliability of each sub-test total score (except Figure Recall Total Score, Coding Total Score, and List Recall Total Score where re-test correlations were used due to no multi-item format or being a timed test) was estimated from the subtest scores from a single administration. The average internal consistency (i.e. split-half) reliability coefficients of the RBANS Indexes across all ages were as follows: Immediate Memory, r=.88; Delayed Memory, r=.83; Attention, r=.85; Visuospatial/Constructional, r=.80; Language, r=.82; and Total Scale, r=.94, as documented in the manual, page 42, table 3.6. [375]. The documented consistency of the RBANS Index scores over time and across alternative Form A and Form B were assessed in two separate studies as are reported in the manual. The standard error of measurement (SEM) estimates the error of an observed test score when taking into consideration the surrounding confidence interval. The greater the reliability, the smaller the SEM, indicating higher confidence in the precision of the test score. Average SEMs for the RBANS were calculated by averaging the sum of the squared SEMs for each age group and obtaining the square root. The average SEMs for each index as reported in the manual were: Immediate Memory, 5.36; Delayed

Memory, 6.04; Attention, 6.06; Visuospatial/Constructional, 7.63; Language, 6.71; and Total Scale, 4.06. These average scores include all those between ages 12-89.

3.5.1.4 Validation

To evaluate the degree to which theoretical rationales and empirical evidence is able to support the adequacy and appropriateness of test score interpretations and consequential decisions based on these scores, is a key aspect of test validation [378], as will be discussed below.

RBANS index scores were assessed for correlation in order to confirm adequate differences in cognitive domain measurement or construct validity. Intercorrelations among RBANS index scores within the sample cohort (n=690, age range 12 – 89) have been reported in the manual [375]. The highest correlation was reported between Immediate Memory and Delayed Memory indexes, r=.634. The correlation coefficients between the Memory indexes and other Indexes ranged between r=.29 to r=.47. Intercorrelations between the Visuospatial/Constructional, Language and Attention were moderate ranging between r=.29 to .34. The overall pattern of the correlation scores indicate that each index score measure relatively distinct cognitive constructs, although there appears to be greater similarity between the Immediate Memory and Delayed Memory index scores, as expected. Correlating baseline index scores among the CHARIOT: PRO participants yielded comparable coefficients, albeit smaller, further strengthening confidence in construct validity of the RBANS (Table 8).

Table 8. RBANS baseline index score correlation coefficients for CHARIOT: PRO data

	Immediate Memory	**Visuospatial**	**Language**	**Attention**	**Delayed Memory**
Immediate Memory	1	.22	.40	.40	.58
Visuospatial		1	.15	.27	.32
Language			1	.31	.30
Attention				1	.25
Delayed Memory					1

The RBANS has been found to display high correlation with an extensive range of well-established neuropsychological assessments, and were reported in the RBANS manual to display convergent and divergent validity of the Indexes (see Table 9) [375, 379]. Of note; data from the external measures were from a range of patient groups with small sample sizes, thus, these findings must be interpreted with caution and seen as preliminary rather than conclusive. Analysis conducted via Pearson's correlation.

Table 9. Correlation coefficients between RBANS Index Scores and comparison assessments

RBANS Indexes and selected sub-tests	Comparison Assessments
Immediate Memory	WMR-R Verbal Memory Index, 0.61; WAIS-R Arithmetic test, 0.58
List Recall	Rey Auditory Verbal Learning Test, 0.65
Delayed Memory	FSIQ, 0.46; WRM-R Verbal memory Index, 0.69
Figure recall	Rey Complex Figure Delay Test, 0.65
Attention	WAIS-R Attention/Concentration, 0.82; WAIS-R Digit Symbol, 0.57
Visuospatial/Constructional	WAIS-R Line orientation, 0.62; WAIS-R Rey complex figure test, 0.79; WAIS-R Digit symbol, 0.62
Figure copy	Rey complex figure copy, 0.76
Language	COWAT, 0.59
Picture name	Boston Naming Test, 0.75
Total Scale	FSIQ, 0.78

WMS-R, Wechsler Memory Scale-Revised; WAIS-R, Wechsler Adult Intelligence Scale-Revised; FSIQ, Full Scale IQ; WRAT, Wide Range Achievement Test; COWAT, Controlled Word Association Test. (RBANS Manual – Randolph; Gontovsky, S.T., Hillary, F.G., & Scott, J.G. (2002). Cross-validation and test sensitivity of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Journal of Cognitive Rehabilitation, 26-31.). Analysis conducted via Pearson's correlation.

With regards to clinical validity, Duff and colleagues (2008) found that RBANS was able to differentiate between clinical groups and normal controls [380]. A further study compared scores among an age-, education- and sex-matched healthy and AD cohort. They reported a balance between sensitivity and specificity, particularly for the immediate and delayed memory indices, indicating optimal AD diagnostic accuracy [374]. Likewise, a previous study [381] compared a group of 60 AD patients with a group of 32 Vascular dementia patients finding that AD patients performed significantly better in visuospatial/constructional and attention indices and significantly worse in immediate memory, delayed memory and language. These findings led to an additional study validating RBANS as a tool able to distinguish between cortical dementia (e.g. Alzheimer's disease related) and subcortical dementia (e.g. Huntington's disease) at 90% or greater accuracy levels. The algorithm used to discern cortical from subcortical dementia was further validated in a study comparing those with Alzheimer's disease versus Parkinson's disease [382]. Likewise, patterns in cognitive performance have also been reported as a means to differentiate AD patients from stroke patients [383], effects of concussion [384], and schizophrenia [385].

Overall, results indicate that the RBANS test battery is a reliable, valid and standardised tool for measuring domain-specific, and global cognitive performance among individuals of varying demographic profiles, and indeed evidence lends this tool particularly sensitive for assessment of cognitive performance in older age and relating to AD type decline.

3.5.2 Neuropsychological Assessment Battery (NAB)

The Executive module of the Neuropsychological Assessment Battery (NAB) [376]; was utilised in this thesis as an outcome measures for the purposes of providing an additional indicator of executive function (EF). The need to explore, in more detail, the association between physical activity and multiple assessment of EF spurred the decision to include this battery as an additional outcome variable. The NAB is a widely used and clinically validated battery comprised of six modules developed to assess an array of cognitive skills and functions in adults aged 18 to 97 years old [376]. The specific cognitive domains measured within the NAB include attention/concentration, language, memory, visuospatial and executive functions. The NAB executive and memory modules were selected for use in the CHARIOT: PRO study. Comparable tests to those in the memory module are already administered in the RBANS; hence, on the EF module was selected. The executive module is administered face-to-face and takes approximately 30 minutes to complete. Sub-tests include maze completion, judgement, problem solving and word generation. The Executive score used in this study was an age adjusted standardised index measure based on published normative data [376], with methodologies congruent to those described above. The index scores were calculated, however, by a software provided with the battery for input of raw sub-test scores. Age adjustment used the same categories as those for the RBANS.

Mazes provide a robust measure of planning and foresight, sustained attention and an element of visuospatial ability. The test involved timed completion of seven mazes of increasing difficulty. The start and end points for each maze are divided between right and left, centre and side (scores range: 0 – 26).

Judgement is a central aspect of mental status testing an individual's general competence. A minimum of 10 questions were asked by the examiner, covering six major categories: home safety, personal hygiene, medication safety, motor vehicle driving, medical decision making, and general judgement. The participant was instructed to provide a verbal answer to each question in detail (scores ranging 0 – 20).

Word generation is often most highly correlated with general executive function tests [386], and this altered version was designed to rule out effect of education and language related impairments associated with reduced lexical performance. The timed task involved presenting the examinee with a list of eight letters; two vowels 'a' and 'o', and six consonants, where they were to verbally generate as many three letter words as they can within 120 seconds (score range: 0 - 36).

The Categories task measures concept formation, cognitive flexibility, generativity, sustained attention and novel problem solving which are all major functions subsumed under the executive function umbrella. The participant was presented with a page containing six boxes within each, a headshot of a person and information pertaining to the pictured person's job, marital status, place and date of birth. Three boxes each are matching in colour and border thickness, with an equal number of males and females – all with varying physical features designed to be categorised (e.g. hair colour, clothing colour, etc). The participant was instructed to create two-group categories based on any information they see on the page, such that all boxes must belong to one group or the other with a minimum of two boxes per group. The groups must be created based on commonalities; e.g. "three people are three males and three females"; "two people work in education, four people work in horticulture", etc. The 4-minute test is repeated twice with two different forms containing new information (score range: 0 - 128).

3.6 Physical activity measurement

3.6.1 Imperial Lifestyle Questionnaire (ILQ)

The Imperial Lifestyle Questionnaire (ILQ) was developed by Imperial to capture self-reporting current and midlife health and lifestyle data such as physical activity, alcohol consumption, smoking, current functional abilities, cognitive and social leisure activities, health and family health history and female reproductive history. The baseline ILQ consists of 106 items, and the follow-up ILQ consists of 69 items; subsequently collected at each 6-montly follow-up visit. Aspects of data from this questionnaire were used in this thesis as will be covered in more detail below.

3.6.2 Physical Activity Measurement for the Elderly (PASE)

Current (late-life) everyday physical activity (EPA) levels were measured via the 12-item self-reporting PASE questionnaire, designed to capture physically active behaviours over the previous seven days in adults aged 65 years and older [387]. Participation in walking outside the home, plus duration/frequency/intensity of leisure activities including sport and recreation, and muscle strengthening exercises were collected. The participants were instructed to record intensity (low/moderate/vigorous); frequency as never, seldom (1-2 days/week), sometimes (3-4 days/week), and often (5-7 days/week-); and duration as less than 1 hour, 1-2 hours, 2-4 hours, or more than 4 hours. Paid or unpaid work was recorded in total hours per week and scored based on number of hours (retired and sitting-related work received a score of zero). Light and heavy housework, home repair, outdoor gardening, and caring for others were recorded as yes/no, with duration and frequency not specified. The continuous PASE score (range 0 – 400), representing EPA, was

explored for linearity and due to a non-linear shape among some sub-analyses plus to enhance ease of interpretability, the data were collapsed into low, moderate and high tertiles for all analysis.

The PASE is designed to assess activities commonly engaged in by older individuals. Most age-neutral questionnaires focus on participation in sport and recreation which may not be appropriate for older people. The initial PASE validation study [388] found, for example, considerably larger amounts of time spent on activities involving household chores, gardening and lawn work when compared to sport-related leisure activities.

As will be discussed in detail below, weights were derived empirically, for individual activity areas, to reflect each activity's contribution to three independent criterion measures. This approach provides an important advantage as it avoids questionable assumptions pertaining to caloric expenditure and metabolic equivalents (METS) based on recall of frequency, intensity and duration of reported activities. A composite score developed via the PASE algorithm is designed to be used as an overall measure of the individual's activity levels (see Appendix B.4 for detail on validity and applicability).

3.6.2.1 Administration

The PASE was administered as part of the ILQ at baseline and at each 6-monthly follow-up visit. The first page of the questionnaire provides general instructions on and examples of activities that may be considered high, moderate or low in intensity. The leisure activities section also contains a space for recording exactly what activities the examinee engaged in for the purposes of aiding correct intensity allocation and scoring. Trained study staff were available to assist study participants if requested.

3.6.2.2 PASE scoring

The PASE composite score it calculated by multiplying the amount of time spent in each activity (hours/week) or participation (yes/no) by empirically derived item weights and summed over all activities (See Appendix B.4). The PASE scores may range from zero to 400. The baseline and 6-month PASE questionnaire data were entered into the online survey tool, Qualtrics (https://www.qualtrics.com) and exported into Excel. As specified in the PASE protocol, cleaning the PASE data involved a review of leisure time activities recorded by the respondent to ensure sports and recreational activities were correctly classified as light, moderate or strenuous, along with specific instructions for more complex coding scenarios (Appendix B.4). Study participants who did not complete the PASE questionnaire in its entirety were excluded from the analyses (N = 62). Cleaned and un-cleaned PASE scores in the current sample were found to be strongly significantly correlated at baseline and six months (r=.85, p<0.001 and r=.98, p<0.001, 2-tailed Pearson's correlation).

3.6.2.3 Preliminary Norms and Validation in CHARIOT: PRO

The validity and reliability of the PASE were first established in a random cognitively healthy sample of 277 older adults aged 65-100 years old (mean age = 74, SD not reported) [389] (see Appendix A.3 for details). Preliminary norms for the PASE in the aforementioned validation study were produced, with scores ranging from 0 to 361, a mean of 102.9 (SD = 64.1); and median of 90 [389]. The CHARIOT: PRO cohort mean baseline score was 120.5 (SD = 55); with a median of 115 and scores ranging from 2 to 413. It appears in the current cohort, subjects were slightly more physically active compared to those in the validation study. The mean age was slightly higher in the validation study which may account for level of activity. Table 10 provides a summary of mean scores obtained within the Washburn (1993) validation study, compared to the current CHARIOT: PRO cohort, stratified by age. Mean scores are approximately congruent between studies, such that increased age found a decrease in PASE scores and females were less physically active than males.

Convergent validity of PASE in the current CHARIOT: PRO cohort was explored through associating the PASE scores with key demographic, health and lifestyle factors reported to effect physical activity levels (see Table 11). As expected, the coefficients between the PASE scores and health status measures were all in the hypothesised direction, with a significant association between PASE scores and age (p<.001). CHARIOT: PRO does not have data on fitness levels (e.g. strength and balance, V02-max, etc), however reported coefficients on other health factors are convincing, along with the mean PASE scores across age and gender strata, congruent with those reported by Washburn (1992) (see Table 10). Test re-test reliability of the PASE within the CHARIOT: PRO study was evaluated via comparing PASE scores at baseline and the following 6-month time point. PASE scores were found to be significantly correlated (r=.50, p < 0.001, 2-tailed Pearson), despite presence statistically significant seasonal influence. Individuals had statistically significantly higher PASE scores in summer, B=12.3 (95% CI 2.21, 22.4), relative to scores during winter.

Table 10. Mean PASE scores for Washburn et al. (1993)'s study cohort versus CHARIOT: PRO cohort, stratified by age and sex

	Washburn - Age group (years)			**CHARIOT: PRO - Age group (year)**				**Total CH:P**
	65-69	**70-75**	**76-100**	**60-64**	**65-69**	**70-75**	**76-85**	**60-85**
Males	144.3 (58.6)	102.4 (53.7)	101.8 (45.7)	125 (71.3)	127.1 (65.1)	116 (53.8)	90.4 (29.4)	120.8 (61.3)
Females	112.7 (64.2)	89.1 (55.5)	62.3 (50.7)	128.3 (54.4)	120.5 (46.2)	120.9 (51.8)	92.8 (44)	119.7 (49)

CH:P=CHARIOT:PRO

Table 11. The association between PASE scores and sociodemographic, health and lifestyle factors in the CHARIOT: PRO cohort

Variable	Unstandardized Coefficients [95% CI]
Age[a]	-2.2 [-3.1, -1.3]**
Sex (1 = female, 0 = male)	-1.8 [-9.0, 5.4]
Education (1 = ≤ 13 years; 0 = > 13 years)	.09 [-7.1, 7.3]
BMI kg/m^2 (1 = > 25; 0 = ≤ 25)	-1.8 [-9.2, 5.6]
Hypertension	-4.7 [-12.1, 2.8]
Hypercholesterolemia	-9.6 [-20, .87]
Diabetes	-5.0 [-18.3, 8.2]
Smoking (1 = past / current; 0 = never)	-.74 [-7.9, 6.5]
Alcohol[a]	.04 [-.25, .26]

[a]Alcohol and age were treated as continuous. Explanatory variables were coded 1 = yes, 0 = no, unless otherwise specified. BMI, body mass index. CI, Confidence Interval. *p<0.05 (2-tailed); **p<0.001 (2-tailed)

3.6.3 Midlife and Late-Life Leisure Physical Activity

Midlife physical activity (MLPA) levels were measured within the ILQ pertaining to aerobic leisure PA in midlife – between the ages of 40 to 60 years old. The physical activity questions related to leisure activity only, comprised of three questions requesting information on general frequency and intensity of PA. Specifically, the questions asked, "how often would you take part in sports or activities that are mildly energetic", "…moderately energetic" and "… vigorous". The response options delineate frequency per activity intensity: never, less than monthly, monthly, fortnightly, weekly or daily. Participants were dichotomised into low- and high- active groups whereby individuals who reported being moderately and/or vigorously active at least twice weekly were classed as 'high active' (N = 310). Individuals would need to specify the frequency for both moderate and vigorous activity as 'weekly' or either moderate and/or vigorous daily to be considered 'high active'. Individuals who reported being moderately / vigorously active less than twice weekly were classed as 'low active' (N = 568). The cut-off for this dichotomisation was based on previous [390, 391] and more conservative [392, 393] recommendations for health promoting physical activity levels. Dichotomisation has been utilised in numerous studies on physical activity and dementia / AD risk and cognitive decline [200, 230, 345, 347, 354, 394, 395]. Preliminary analyses showed some convergent validity; logistic and linear regression found those who reported being moderately/vigorously active less than twice weekly were more likely to be a smoker (past or current), *B*=.34 (95% confidence interval (CI) I 1.1, 1.9), female, *B*=.29 (95% CI 1.0, 1.7), and less physically active in later-life *B*=16.7 (95% CI 9.2, 24.3). Midlife activity based on the current

dichotomisation was, however, not associated with later life vascular factors (i.e. hypertension, diabetes, hypercholesterolemia, BMI).

Late-life leisure activity, as a departure from late-life household/work/leisure activity composite, was an additional variable of interest and was created from the leisure component of the PASE questionnaire. As with midlife activity, individuals who reported being moderately / vigorously active at least twice weekly within the leisure activity section, were classed as 'high active' (N = 227). Likewise, those who reported being moderately / vigorously active less than twice weekly were classed as 'low active' (N = 674). Once again, if a participant reported seldom for moderate or vigorous activity they would need to state 'seldom' for both moderate and vigorous to be classed as having undertaken a minimum of 2 occasions of activity in one week. This classification assumes an individual who reports partaking in moderate activity 1-2 days per week and vigorous activity 1-2 days per week are on separate occasions. In the current dataset, this assumption was made for 26 individuals.

Midlife to late-life PA groups were created as a new variable for use in Chapter 6; aiming to compare individuals who reported low leisure activity at midlife and late-life, with those who were high active in midlife but became less active in late-life, those who became more active in late-life and those who maintained being high active from mid- to late-life. Specifically, four mid- to late-life activity groups were created based on the explained dichotomisation above: those who reported Low midlife PA/Low late-life PA, Low midlife PA/High late-life PA, High midlife PA/Low Late-life PA and High midlife PA/High late-life PA.

3.7 Blood biomarkers

3.7.1 Blood collection

Fasting blood was collected in the morning following a standardised protocol by trained medical staff, utilising the vacutainer system of venepuncture via the cephalic vein. Blood was first aspirated to flush all surfaces and remove initial traces of contact induced coagulation. Five tubes of blood were collected: 2.5mL in one Fisher Scientific Ltd ribonucleic acid (RNA) tube, three 9ml bottles in an Ethylene-Diamine-Tetra-Acetic acid (EDTA) plasma tube and one 9ml bottle Greiner bio-one red serum blood collection tube with a clot activator.

3.7.2 Fractioning plasma and serum from whole blood

For plasma extraction, one EDTA vacutainer was inverted several times to mix the additive with the collected blood, followed by centrifugation at 2800 revolutions per minute (RPM) for 20 minutes at room temperature. The top 75% of supernatant (platelet rich) was transferred into a 15ml falcon tube Greiner bio-one without touching the buffy layer. The buffy layer was aliquot into two

2ml yellow and bottom layer into three 2ml red top Greiner bio-one flat bottom pre-labelled cryovial. The 15ml falcon tube with supernatant (platelet rich plasma) was then centrifuged at 2500 RPM for 20 minutes at room temperature. The top 75% layer of the resultant plasma (now platelet poor) was aliquot into 2ml natural colour Greiner bio-one pre-labelled cryovials.

One 9ml Greiner bio-one red serum blood collection tube with clot activator was collected for the purpose of serum extraction. Directly after collection, the tube was inverted 8 times for proper mixing. The tube was left to clot for at least 30-minutes in an upright position at room temperature and then spun in a cooled swing-out bucket centrifuge at 2200 RPM for 20 minutes. The top serum layer was aliquot into three 2ml top Greiner bio-one pre-labelled cryovials. Serum and platelet poor plasma were then immediately frozen at approximately -20°C until shipment over dry ice in batches to be stored in a -80°C freezer

3.7.3 DNA extraction and *APOE* genotyping

Quest Pharmaceutical Services validated pyrosequencing genotyping assays for *APOE* codon 112T>C and codon 158C>T polymorphic variants. These were then used to genotype clinical samples and identify *APOE*ε4 carrier/non-carrier status. By interrogating these two polymorphic variants the three major *APOE* alleles were identified: ApoE2 (TGC 112, TGC 158), ApoE3 (TGC 112, CGC 158), and ApoE4 (CGC 112, CGC 158). Fragment size determined the following genotypes: *APOE* ε2/ε2: 2 fragments (91bp, 83bp); *APOE* ε3/ε3: 3 fragments (91bp, 48bp, 35bp); *APOE* ε4/ε4: 3 fragments (72bp, 48bp, 35bp); *APOE* ε2/ε3: 4 fragments (91bp, 83bp, 48bp, 35bp); *APOE* ε2/ε4: 5 fragments (91bp, 83bp, 72bp, 48bp, 35bp) and; *APOE* ε3/ε4: 4 fragments (91bp, 72bp, 48bp, 35bp).

3.7.4 Plasma BDNF and amyloid

A sub-sample of participants were analysed for serum BDNF (n = 150) and plasma $A\beta_{1-42}$ (n = 258). Detailed methodology utilising the ultra-sensitive Single Molecular Array (SIMOA) technique for measurement of these analytes is outlined below.

3.7.4.1 Single molecular array (SIMOA)

Serum BDNF and plasma $A\beta_{1-42}$ were analysed using the SIMOA immunoassay technology (Quanterix, Lexington, MA, USA). The SIMOA assay uses digital counting technology to detect the presence of antigen at a single molecular level [396, 397]. The relevant antibody is attached to 2.7-µm paramagnetic microbeads each containing 250,000 antibody attachment sites. The procedure involves formation of antigen-antibody immune complexes across the surface of each microbead; placed in wells containing fluorogenic enzyme substrate (see Figure 7). Once the antigen-antibody formation has occurred, interaction with a biotinylated-detection antibody and then the streptavidin-beta galactosidase allows the generation of fluorescence signals, counted by a

fluorescence analyser. Sample antigen concentration is calculated via the ratio of wells containing an enzyme labelled bead to the total number of bead-containing wells. Quanterix has developed a high capacity fully automated SIMOA HD-1 Analyser able to conduct triplex analysis (assays for multiple analytes), details of which are available on the company's website (www.quanterix.com). The highly efficient instrument can assay 68 samples per hour at steady-state usage, with a throughput of 96-wells within 2 hours [398].

Quanterix has validated use of SIMOA for assaying of plasma, serum and CSF $A\beta_{1-42}$ and BDNF with median values well above the sensitivity limit as can be found on their website (www.quanterix.com).

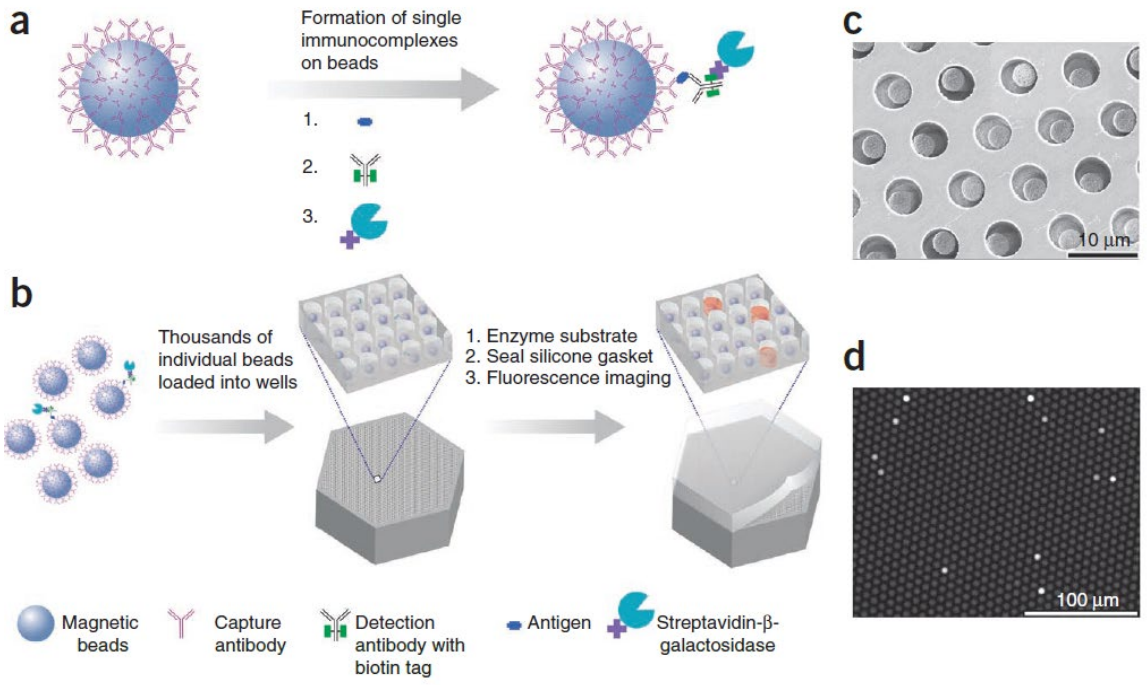


Figure 7 The digital SIMOA ELISA

(a, b) single protein molecules are captured and labelled on beads using standard ELISA reagents (a), immunoconjugate labelled beads are loaded into femtoliter-volume well arrays for fluorescence Imaging (b). (c) Scanning electron micrograph of a femtoliter-volume well array after bead loading. (d) Fluorescence Imaging of the femtoliter-volume well array after signals from single enzymes are generated. Use of image by permission from Springer Nature (see Appendix B.6 for details of permission) [397].

3.7.4.2 SIMOA sensitivity and specificity

Traditional sandwich ELISA, such as the commonly applied meso scale discovery (MSD), for use of complex matrices bring about issues with spike recovery and lack of linearity in dilution (i.e. matrix interferences) [399]. The ultra-sensitivity of the SIMOA assays minimises this matrix effect through allowing dilution of the plasma/serum samples at 1:4, allowing better capacity to detect minute differences in levels of investigated analyte. The enzymatic amplification of analyte binding on single beads in single wells also yields low background signals, and can increase assay sensitivities up to several orders of magnitude over conventional assays [397]. In combination with the markedly heightened sensitivity, SIMOA's ability to digitally measure the target analyte at low concentrations (femtoliter) and analogue measurement at higher analyte concentrations ensures accuracy and precision across a wide dynamic range (>4 logs), hence, minimising sample volume requirements and the need for multiple dilutions [397]. Likewise, as bead-based technology allows for multiplexing, the amount of sample required to run the assays is minimised.

Using the SIMOA technology, recent studies were able to reliably measure the notably difficult plasma tau [400, 401], Aβ [402, 403] and neurofilament light (NFL) [404, 405], which was previously less feasible using standard ELISA. Furthermore, numerous reviews have supported SIMOA as markedly more sensitive than the standard ELISA technique as well as being a suitably sensitive measure of other key plasma biomarkers [401, 406, 407]. The overall benefits of the SIMOA assays are not only its high sensitivity and precision but also the elimination of matrix interferences, hence, justifying this technique as one of the most accurate and suitable technique currently available for the assaying of serum BDNF and plasma $A\beta_{1-42}$.

3.7.4.3 Serum BDNF preparation and assay procedure

The following procedure was undertaken as per the SIMOATM BDNF Discovery Kit instructions for the HD-1 Analyser. The SIMOA BDNF Discovery assay is a 2-step digital immunoassay to measure quantity of total BDNF in the serum using the SIMOA HD-1 Analyser and SIMOA technology.

Frozen serum samples were thawed to room temperature and mixed thoroughly by low-speed vortex until specimens were visually homogenous. Specimens were then centrifuged for approximately 5 minutes at 20,000 g to remove any particulate matter and clarify. Samples were diluted via a two-step serial dilution to obtain 640x dilution. First, 10µL of the serum sample was transferred into 190µL sample diluent to create a 20x diluted intermediate solution. Then 12µL of the 20x diluted solution was transferred into a 327µL diluent to achieve a 640x dilution.

3.7.4.4 Preparing the Calibrators

For maximum accuracy in preparing calibrators, the BDNF calibrator Concentrate and BDNF Calibrator Diluent are bought to room temperature and mixed by vortexing. Eight calibrators were prepared by 3x serial dilutions: 950 microlitres (µL) of BDNF Calibrator Diluent was transferred into a 100 picograms per millilitre (pg/mL) tube. Next, 600µL of the diluent was transferred into each remaining tube. 50µL of the BDNF stock solution was transferred into the 100 pg/mL tube and vortexed. 300µL of the 100 pg/mL was then transferred into a 33.3 pg/mL tube and vortexed. This process was repeated across all tubes, mixing each thoroughly before the next transfer. The BDNF Calibrator Diluent was used as the zero calibrator.

3.7.4.5 Preparing quality controls

Quality controls were prepared using the BDNF Calibrator Concentrate and the BDNF Calibrator Diluent. The controls were placed at 1 and 30 pg/mL to monitor the digital and analogue ranges of the assay (run neat). QC controls were assayed to assess calibration curve validity (Figure 8).

3.7.4.6 SIMOA BDNF discovery reagents

To prepare the bead reagent; beads were washed prior to running by adding 1mL of Discovery Bead Diluent (DBD) to one vial of bead stock, mixed by gentle inversion, placing the vial against the magnet for 1-minute and carefully removing the diluent. Beads were then re-suspended in 1mL of DBD; vortexed to mix. Next, 4mL of DBD was added to a Nalgene Reagent bottle, along with 1 mL of resuspended bead stock and vortexed to mix. The bead reagent was immediately transferred to a new Conical Bottom Reagent bottle and loaded onto a shaking reagent rack to ensure that re-suspended beads did not settle. To prepare the Detector Reagent; 3.96 mL of Discovery Detector Diluent was added to a Nalgene Reagent bottle along with 40µL of BDNF Detector stock, then vortexed to mix. The detector reagent was transferred to a Conical Bottom Reagent bottle prior to loading on the instrument. To prepare the streptavidin ß–D–galactosidase (SBG) Reagent; the following calculation was used to determine the volume of Discovery SBG Concentrate to add to 12 mL of Discovery SBG Diluent:

$$12\ \text{mL (reagent volume} \times 0.050\ \text{nM (final concentration)} = \text{Vol. of SBG Conc mL}$$

$$\text{SBG concentrate nM (from CoA)}$$

12 mL of Discovery SBG Diluent was added to a Nalgene Reagent bottle along with the calculated volume (4.69 µl) of SBG concentrate then vortexed to mix. The SBG reagent was then transferred to a Conical Bottom Reagent bottle and loaded onto the instrument.

<u>SIMOA HD-1 analyser assay run</u>

The SIMOA BDNF Discovery reagents were loaded into the reagent bay and the samples, calibrators, controls and RGP were loaded into the sample bay. The assay run lasted approximately

2 hours. Details on specific automated functions performed within the SIMOA HD-1 analyser can be found elsewhere (www.quanterix.com).

The SIMOA BDNF Discovery assay uses a 4 Parametric Logistic Curve fit data reduction method (4PLC, $1/y^2$ weighted) to generate a calibration curve; plotting average enzyme per bead (AEB) with concentration calibrators overlain with current assay data for confirmation of accuracy, which was confirmed as can be viewed below (Figure 8).

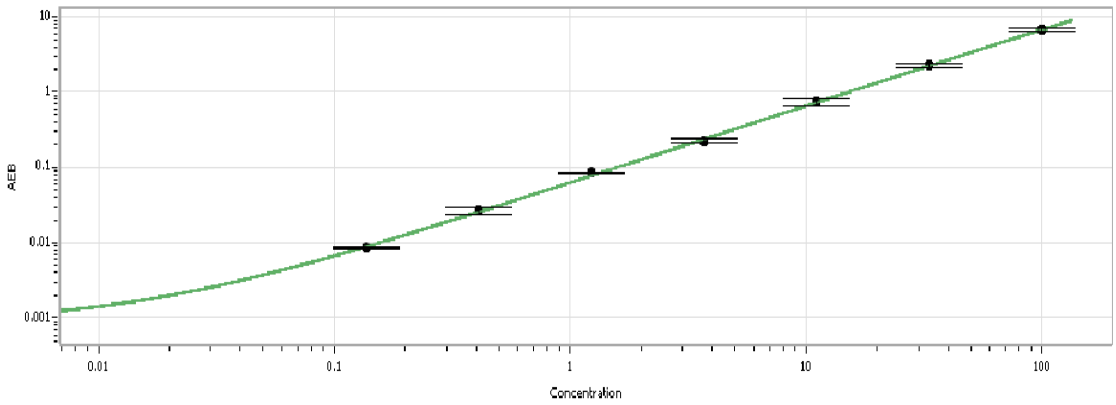


Figure 8. BDNF SIMOA calibration curve of AEB against known BDNF concentration for CHARIOT: PRO sample analysis

To determine protein concentrations, SIMOA is used to generate a calibration curve of AEB versus concentration. Calibration curves evaluate calibration accuracy and run validity. SIMOA is then used to calculate AEB for unknown samples that are compared to the calibration curve to determine concentration

3.7.4.7 Plasma $A\beta_{1-42}$ preparation and assay procedure

The following procedure was undertaken as per the SIMOA™ $A\beta_{1-42}$ Advantage Kit instructions for HD-1. The Advantage Assay Kits contain ready-to-use reagent for running 96 tests of target agent (In this case plasma $A\beta_{1-42}$) on the SIMOA HD-1 Analyser using SIMOA technology. Plasma samples were thawed from frozen to room temperature and vortexed until visually homogenised. Specimens were then centrifuged for 5 minutes at 10,000 g to remove particulate matter and to clarify the sample. Next, sample dilution was completed within the instrument at 4x dilution µL. 200µL of a 1000 pg/mL $A\beta_{1-42}$ stock solution was prepared in Calibrator Diluent using the following equation:

1. Required Calibrator Concentrate µL = (1000pg/mL / Certificate of Analysis conc. Pg/mL) x 200 µL
2. Required Calibration Diluent µL = 200 µL – Required Cal. Concentrate µL

Eight calibrator points and two control levels (analogue and digital) were prepared according to Table 12 and Table 13. Note control samples were prepared via the same dilution protocol as the actual plasma samples. All calibrators and controls were run in replicates of two.

Table 12. Calibration points for Plasma $A\beta_{1-42}$ SIMOA analysis using Neat samples

Calibrator Level	$A\beta_{1-42}$ Calibrator Value (pg/mL)	Calibrator Diluent Volume (µL)	$A\beta_{1-42}$ Calibrator Volume to Transfer (µL)
H	100	900	100 of 1000 pg/mL stock
G	33.3	600	300 of Cal. H
F	11.1	600	300 of Cal. G
E	3.70	600	300 of Cal. F
D	1.23	600	300 of Cal. E
C	0.412	600	300 of Cal. D
B	0.137	600	300 of Cal. C
A	0	600	0

Table 13. Control level preparation for Plasma $A\beta_{1-42}$ SIMOA analysis

Control level	$A\beta_{1-42}$ Control Value (pg/mL)	Calibrator Diluent Volume (µL)	$A\beta_{1-42}$ Calibrator Volume to Transfer (µL)
Analog	60.0	376	24 of 1000 pg/mL Stock
Digital	1.95	387	13 of analog control

On the SIMOA HD-1 analyser, the $A\beta_{1-42}$ assay definition is imported under Custom Assay. The five assay samples, all at room temperature, were then prepared by pipetting the appropriate volume of prepared calibrators, controls and samples into the wells. The bead reagent bottle was vortexed for a minimum of 30 seconds to re-suspend the beads, prior to loading on the HD-1 Analyser. The prepared reagents (Bead Reagent, Detector Reagent, SBG Reagent, sample diluent) were first bought to room temperate and then loaded into the reagent bay and RGP into the sample bay. Digital determination of protein concentrations were assessed via production of a calibration curve as shown in Figure 9. Two samples presented with undetectable levels of $A\beta_{1-42}$ with the dilution, thus they were re-run as neat with new values included in the data.

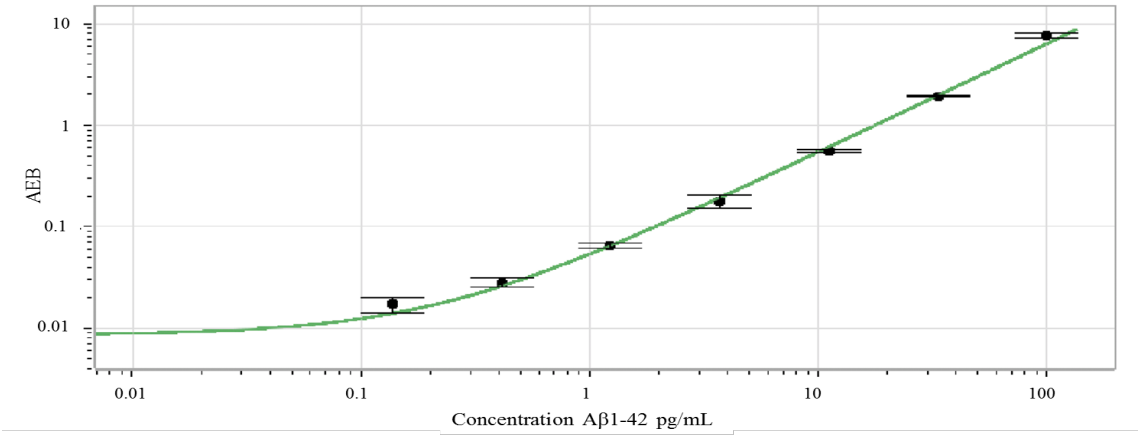


Figure 9 $A\beta_{1-42}$ SIMOA calibration curve of AEB against known $A\beta_{1-42}$ concentration for CHARIOT: PRO sample analysis

To determine protein concentrations, SIMOA is used to generate a calibration curve of AEB versus concentration. Calibration curves evaluate calibration accuracy and run validity. SIMOA is then used to calculate AEB for unknown samples that are compared to the calibration curve to determine concentration, as described in detail elsewhere [408].

3.8 Data preparation for statistical analyses

3.8.1 Sample size

Of the 987 participants who attended an initial screening visit, 20 were excluded due to not having completed the PASE questionnaire, 62 additional participants were excluded due to completing the PASE questionnaire incorrectly or incompletely and of these remaining subjects, five were excluded due to not having completed baseline RBANS. A sub-sample of 901 participants, whereby a full complement of late-life PA data (i.e. PASE), RBANS cognitive data and essential demographic data (age, sex and education) were available for inclusion in this thesis. There were an additional 23 individuals who did not complete the three midlife questions accurately, hence, these were excluded from all analyses involving midlife physical activity. A sub-set of participants (N = 261) did not complete the NAB battery due to a protocol amendment whereby screening visits were split in half (V0 and V1). Any subject screened after this date who were excluded at V0 were not exposed to the NAB battery being administered at V1. Finally, due to limited funds, a sub-sample (N = 258) of the cohort with full complement of baseline and follow-up data were utilised for peripheral biomarker analyses. The total number of variables utilised within analyses for this project are summarised in Table 14.

Table 14. Key variables utilised for analyses within CHARIOT: PRO sub-set

Variable	Number available (% missing)
Demographics (Age, Sex, Education)	901 (0%)
RBANS Cognitive Data	901 (0%)
Late-life Activity (PASE)	901 (0%)
Midlife Activity	878 (2.6%)
APOEε4 carrier status	824 (8.5%)
NAB Executive Function	676 (25%)
Smoking status	896 (0.6%)
Alcohol consumption	805 (10.7%)
Hypertension, Diabetes, Hypercholesterolemia	901 (0%)
Body Mass Index	867 (3.8%)
Serum BDNF	170 (83%)
Plasma $A\beta_{1-42}$	258 (71.4%)

3.8.2 Data cleaning during collection

The CHARIOT: PRO data was subjected to multiple stages of cleaning. All study data were collected onsite by medical, neuropsychological or other appropriately trained staff. Next, the data management team would audit each participant file. In cases of missing and/or incorrectly recorded data, the study participant would either be phoned to obtain such missing information or, if necessary, asked to attend the study site as an unscheduled visit to collect the remaining data. In cases where the participant was no longer partaking in the study, this data would be deemed permanently missing and marked as such. Following this process, the data were entered onto the electronic Clinical Management System, Medidata; a purpose-built platform reflecting study source documentation. Study data were routinely audited by the sponsor both onsite and remotely. Prior to study completion, a final period of intensive re-monitoring of study data was undertaken with study site and sponsor in collaboration to confirm data accuracy.

3.8.3 Data cleaning post study completion for use in this project

Following study completion, data were made available in analysis ready format. An initial review was undertaken to identify any erroneous data points. Data points outside the expected range were reviewed by referring to source documentation and data updated correctly or labelled as missing if confirmed incorrect on source. Strategies such as multiple imputation for missing baseline data were decided against. General consensus indicates that if <5% of data is missing, the potential risk of bias on overall result is negligible [409]. Missing baseline covariate data in the current study included midlife PA (2.6%), smoking status (0.6%), BMI (3.8%), *APOE*ε4 carrier status (8.5%) and alcohol consumption (10.7%). Despite *APOE* and alcohol consumption containing missing

data >5%, multiple imputation was still decided against, and instead, maximum likelihood selection within the mixed models analysis was considered adequate into accounting for this missingness [410, 411].

Binary data were coded 0, 1, and categorical data coded from 1, except for time which was coded from 0 (being baseline). Correct baseline age was re-confirmed for all subjects by subtracting participant's date of birth from baseline study visit date. As date of birth and sex were recorded across multiple documents; screening of such identified some discrepancies, again being resolved via examining source documentation or in some cases, reviewing consented data obtained directly from the UK National Health Services (NHS) records. A congruent strategy was employed to ensure correct recording of baseline study visit date. Educational status was reviewed for all subjects, firstly by a neuropsychologist and secondly by the candidate, whilst conducting final cleaning. Level of education was collected as such: did not complete upper secondary education or high school; completed upper secondary education or high school; some post upper secondary education (i.e. tertiary education) after completing upper secondary or high school; completed bachelor's degree or equivalent; or completed Masters degree, equivalent or higher. Highest awarded education level / degree as well as occupational history were recorded. Multiple screening strategies were employed to ensure correct recorded education. In cases of discrepancy, source file was reviewed, and data updated accordingly.

Comprehensive cleaning of the PASE questionnaire was undertaken to ensure correct self-reporting. As specified in the New England Research Institute (NERI) protocol (Appendix B.4), a review of leisure activities recorded by the respondent was conducted to ensure sports and recreational activities were correctly classified. Furthermore, the NERI protocol provides guidelines for the correct allocation of household and work-related activities, also reviewed. For example, if a participant placed a household activity under a leisure category (e.g. moderate sport due to gardening) it was simply moved to the household section under 'outdoor gardening' and scores re-allocated. In cases where the participant allocated duration and intensity under leisure activities without stating the activity (e.g. gym, tennis, walking the dog, et.), this score was left as recorded. Total cleaned and un-cleaned scores were significantly correlated.

3.8.4 Statistical Variables

Baseline cognition and cognitive change over time was based on age-adjusted normative composite index scores from the RBANS and NAB test battery. Analysis within this thesis did not seek to predict outcomes, but to ascertain associations between the exposure and outcome; hence, all covariates were selected *a priori* based on the literature, as is covered in the introductory chapter. Briefly, the complex relationship between physical activity, cognitive performance and *APOE* genotype imply the presence of multiple mechanisms which interact and influence each other to

varying degrees [412]. Evident confounding variables have included sex [413], age [414] and education [415, 416], which were chosen *a priori* as covariates in the primary analyses. Other possible confounders also included in subsequent adjusted models were BMI and CVD risk factors [352], smoking status and alcohol consumption [417] [325, 326, 328]. Existence of any current serious psychiatric illness were exclusionary in the CHARIOT: PRO study, with only a small number of individuals reporting a previous history of depression and/or anxiety (N = 81, 9%); hence these variables were not included in the analyses. As already discussed, selected effect modifiers included *APOE*ε4 genotype status, sex and age.

The selection of potential confounders was based on existing literature and were explored here in multiple linear regression models. They were investigated in mixed models regression via forward step-wise inclusion of selected covariates alongside interactions with LLPA, MLPA and EPA. This method aimed to explore their independent and additive impact on the association between PA and cognition. Age, sex and education exhibited small differences in the coefficients. Specifically, using global cognition as the outcome, percent difference between the crude effect estimate of LLPA and once controlled for age, sex and education were 1.7%, 1.3%, 2.2%; and for MLPA they were 2%, 9% and 1.5%; respectively, with no differences found among EPA models. Addition of all other covariates exerted insubstantial alterations. All interactions were not statistically significant.

As described above, MLPA and LLPA were utilised as binary variables. The continuous PASE score, representing EPA, was explored for linearity and due to a non-linear shape among some sub-analyses plus to enhance ease of interpretability, the data was collapsed into low, moderate and high tertiles for all analysis. Table 15 summarises the format and coding of variables chosen for inclusion in the various statistical models throughout this thesis.

Table 15. Variable description, format and coding

Variable (baseline, n)	Variable Type	Format	Coding
Everyday physical activity (EPA) n=901	Independent	tertiles	1 = Low 2 = Moderate 3 = High
Midlife physical activity (MLPA) n=878	Independent	Binary	0: Low = < 2 bouts of moderate / vigorous activity per week 1: High = ≥ 2 bouts of moderate / vigorous activity per week
Late-life leisure physical activity (LLPA) n=901	Independent	Binary	0: Low = < 2 bouts of moderate / vigorous activity per week 1: High = ≥ 2 bouts of moderate / vigorous activity per week
Midlife PA/Late-life PA change n=878	Independent	Categorical	1: Always low PA (L-ML/L-LL PA) 2: Increased PA in late-life (L-ML/H-LL PA)_ 3: Decreased PA in late-life (H-ML/L-LL PA) 4: Always high PA (H-ML/H-LL PA)
Age n=901	Covariate Moderator	Categorical Continuous	0: 60-69 1: ≥70 Range: 60 – 85 years old
Sex n=901	Covariate Moderator	Binary	0: Female 1: Male
Education n=901	Covariate	Binary	0: ≤13 years 1: >13 years
*APOEε*4 genotype status n=824	Covariate Moderator	Binary	0: Non-carrier 1: Carrier

Abbreviations: n= baseline sample size; EPA, everyday physical activity; MLPA, midlife physical activity; LLPA, late-life physical activity; *APOE*, apolipoprotein; L-ML, low midlife; H-ML, high midlife; L-LL, low-late-life; H-LL, high late-life; PA, physical activity

Table 15. (Continued) Variable description, format and coding

Variable (baseline, n)	Variable Type	Format	Coding
Body Mass Index (BMI) n=867	Covariate	Binary	0: <25 1: ≥25
Hypertension n=901	Covariate	Binary	0: Healthy 1: Current diagnosis
Hypercholesterolemia n=901	Covariate	Binary	0: Healthy 1: Current diagnosis
Diabetes Mellitus n=901	Covariate	Binary	0: Healthy 1: Current diagnosis
Alcohol (Grams per/week) n=805	Covariate	Continuous	Range: 0 - 80
Smoking n=896	Covariate	Binary	0: Never smoked 1: Current or past smoker
Serum BDNF n=173	Dependant Independent Mediator Moderator	Binary Continuous	0: Low, 1: High Range: 3.44 to 77.85 pg/mL
Plasma $A\beta_{1-42}$ n=258	Dependant Independent Moderator	Binary (median cut-off) Continuous	0: Low, 1: High, Range: 1.05 – 21.80 pg/mL
Cognitive Index scores n=901	Dependant	Continuous	Refer to Table 7
Time	Covariate	Continuous	Range: 0 – 5 (6-monthly units from baseline to 30 months)

Abbreviations: n= baseline sample size; BMI, body mass index; BDNF, brain derived neurotrophic factor; Aβ, amyloid beta

3.9 Statistical modelling – background and justification

Descriptive analyses enhanced understanding of the qualitative and quantitative nature of the data collected and the characteristics of the sample studied. Continuous variables were reported as mean, standard deviation (SD), median and 25th and 75th quartiles, and range where appropriate. Categorical variables were summarised as number and proportion of participants within the selected reference category. The associations between the exposure variable (e.g. physical activity) and the outcome variable (e.g. cognition) were analysed in the overall cohort as well as within age, sex and

APOEε4 carrier strata, where applicable. All analyses presented in this thesis were conducted using mixed-effects models regression for longitudinal analyses [418], and multiple linear regression for cross-sectional analyses.

For mixed models regression, maximum likelihood estimation and an unstructured covariance matrix were the chosen models in all analyses. Maximum likelihood estimation was selected over the alternative option of utilizing the restricted maximum likelihood due the limitation of this method requiring the same fixed effects specification across compared models. That is, to compare models with different fixed effects with a likelihood ratio test, maximum likelihood was used [418-421]. Multiple covariance models were tested using the -2 log likelihood ratio test (-2 LL), the Akaike information criteria (AIC) and the Bayesian information criteria (BIC), whereby smaller statistical values generally indicate a better model fit [422]. Where appropriate, individualised growth curve (IGC) modelling was conducted to determine rate of cognitive change over time [423]. This technique allows the accountability of repeated measures of cognition and intra-individual variability. The term for time in the mixed models analysis refers to the period between completing the baseline physical activity assessment and psychometric assessments at each follow-up. Follow-up time points were treated as within-participants factor (effect over time) and the differences between physical activity groups were treated as between participant's factor.

The benefits of utilising a mixed models approach for longitudinal analysis over commonly observed repeated measures analysis of variance (ANOVA) or covariance (ANCOVA) are well justified [418, 419]. Due to the need for a balanced, repeated-measures design (e.g. equal group differences) in ANOVA and ANCOVA methodologies, the use of the traditional univariate and multivariate test statistics may increase the chance of a type I error under the condition of an unbalanced repeated measures design [420, 421, 423]. Additional assumptions such as sphericity and the requirement of no missing data yield this technique unsuitable for use in real-world data. Mixed models regression provides the ability to model the variance-covariance structure (i.e. not needing to meet sphericity assumptions) [422], rather than imposing a certain type of structure as is common in univariate and multivariate approaches. For example, the error covariance structure of the repeated measurement can be specified via model hypothesis testing, allowing observation of true change. The most flexible option in mixed models is a general 'unstructured' variance-covariance assumption whereby every variance and covariance can be independently estimated, with more restrictive options including autocorrelated structures. Mixed-models regression allows for individual growth modelling, whereby intercepts and slopes can be treated as random or fixed [424], as well as the use of both time invariant and time-varying covariates for a more sensitive and realistic assessment of covariate effects over time. Finally, mixed-models allows modelling of trajectories over time across different polynomial trends. For example, most real-world data may not exhibit perfectly linear trajectories, hence, individual growth curve modelling within mixed-

models regression analysis allows for testing best model fit and selecting which trajectory is most appropriate (e.g. linear vs non-linear).

A small proportion of missing baseline data justified the decision to employ listwise deletion; a strategy often recommended where the objective is to obtain consistent and unbiased estimates of population parameters, provided the researcher still believes there is adequate available data to be representative of the target population [425, 426], as is the case in the current dataset. Furthermore, majority of missing baseline data in this study were deemed to be missing due to collection error, based on source file review. Majority of missing follow-up cognitive data were due to early study termination; hence, this information was never provided with the opportunity for collection. However, it must be acknowledged that a small proportion of participants withdrew or were excluded, during follow-up, due to reasons poorly reported (n=99). Attrition analysis on this sub-sample was not conducted as individual ID's relating to those who withdrew or were otherwise excluded during follow-up (n=99) were not available in the dataset. The dataset was provided via dummy coding due to the candidate being required to remain blinded to *APOE*ε4 carrier status. Hence, those missing due to study termination and those missing due to attrition were not distinguished. Nonetheless, to investigate loss to follow-up among all those either terminated early or early withdrawal, a sensitivity analysis was conducted. Specifically, multiple linear regression compared baseline global cognitive scores among those reaching 24-months of follow-up (n=240) versus those not (n=661). However, there was no statistically significant difference between the aforementioned strata baseline global cognitive scores (not reported), sanctioning a low likelihood of attrition bias. Statistical two-sided significance level was set at 5% ($p<0.05$). All analyses were conducted using IBM SPSS 23 for windows. A detailed analyses plan for each chapter are specified below.

Results tabulation as presented in this these were facilitated via Perl 5 (Practical Extraction and Report Language: www.perl.org). Specifically, a script was written (see Appendix B.5) to parse the SPSS raw output files into an Excel compatible filetype and automatically tabulated into the desired format. This approach reduced likelihood of transcription error.

3.9.1 Descriptive analyses

Differences among participants by *APOE*ε4 carrier status, sex and age were first conducted, with additional descriptive variables by PASE composite score tertiles (or EPA tertiles) and late-life leisure activity groups (Chapter 4), midlife physical activity (Chapter 5), mid- to late-life activity groups (Chapter 6), serum BDNF (Chapter 8) and plasma $A\beta_{1\text{-}42}$ (Chapter 9). Differences across groups were analysed with chi-squared for binary variables and Student's t-test for continuous variables when compared across two groups (with Mann-Whitney U test used for median comparison), one-way analysis of variance (ANOVA). For comparisons across more than two

groups, only when the global p-value was statistically significant would Tukey's post-hoc analysis to compare means and Kruskal-Wallis 1-way ANOVA with Mann-Whitney post hoc test to compare medians across more than two categories. Descriptive figures were reported as median (interquartile range, IQR), mean (standard deviation, SD) or frequencies as appropriate.

3.9.2 The association between physical activity and cognition

Table 16 provides a summary of statistical models fitted within Chapter 4, 5 and 6.

To evaluate associations between physical activity (Chapter 4: late-life EPA tertiles and late-life leisure PA; Chapter 5: midlife leisure PA; Chapter 6: mid- to late-life leisure PA) and cognitive index scores over 30 months, mixed-models regression were utilized, allowing for the accountability of repeated measures of cognition and intra-individual variability (i.e. mixed models). Maximum likelihood estimation and an unstructured covariance matrix were selected, as discussed above.

The basic assumption of growth curve modelling is that the functional form of each individual trajectory is similar (e.g. growth over time in the total sample), hence, it is hypothesised that only the intercept be left as a random factor with all other variables be kept fixed. To test this assumption, model validity was assessed -2LL, AIC and BIC tests comparing the models with fixed effects to the null models with only random effects. Covariates in this model testing included age, sex and time with PA (late-life tertiles / midlife binary / mid-to late-life groups) as the exposure variable and global cognition as the outcome variable. The random model was rejected when results from the fixed effects model were not significantly different to the random effects null model. To test a nonlinear individual growth trajectory over time, linear, quadratic and cubic slopes (i.e. time, $time^2$ and $time^3$) were included for -2LL, AIC and BIC model testing, all adjusted for age, sex and education. Likelihood ratio tests comparing an age, sex and education adjusted fixed effects models to a null model with only random effects found no difference across models, hence, slopes were retained as fixed with the intercept left as random for all analyses. Furthermore, comparing a linear, quadratic and cubic growth trajectory over time for global and domain-specific cognition, results found the quadratic time model (e.g. global cognition: AIC = 20069) to fit most accurately when compared to linear time (AIC = 20105) and cubic time (AIC = 20071) models; hence, time was treated as non-linear in all proceeding analyses within this thesis. To account for non-linearity, time was included as a polynomial of second degree (time and $time^2$) when assessing the association between PA and overall cognitive performance and trajectories across 30 months.

For the primary analyses (association between PA and cognition with effect modification by *APOE*ε4 carrier status), three main models were fitted using *a priori* selected covariates. Model 1 included terms for age (continuous), sex and education (≥13 years, <13 years). Model 2 included

all variables within model 1 plus cardiovascular factors; hypertension, hypercholesterolemia, diabetes (all current yes/no) and BMI (continuous). Model 3 included all terms from model 1 and model 2, plus the lifestyle factors; smoking (ever vs never) and alcohol consumption (continuous). In each model, time was first included as a covariate to determine effect of baseline EPA tertiles, LLPA, MLPA or ML/LL PA groups on overall cognition across the 30-month period, resulting in estimated mean differences at 30 months, or marginal mean estimation. The lowest PA group was the reference category within all analysis. The beta-coefficient, hence, represented difference in index score points relative to the lowest PA group. For example, a beta-coefficient of 2 would indicate that relative to low PA individuals, those reporting high PA would, on average, have higher cognitive scores by an average of 2-points by 30-months. Growth curve modelling was undertaken whereby 2-way interactions by time and PA (PA x time / PA x time^2) were included in the model to determine estimates of cognitive trajectories relative to PA groups over 30 months. A statistically significant interaction with time would indicate that the rate of change of the outcome variable (e.g. cognition) would differ as a function of the predictor variable (e.g. physical activity levels). If physical activity was statistically significantly associated with cognition over time, but the interaction with time was not, it would indicate that the rate of change in cognition are parallel but shifted according to the value of the predictor. The interpretation of beta-values in quadratic time interactions are more complicated that would be linear time interactions. Quadratic time interactions produce a beta-coefficient for the linear component of the model (PA x time) and the quadratic component of the model (PA x time^2). These two components are not one on their own but combined and must be interpreted as such. An informative method of presenting this result is graphically. A step-by-step account of how to calculate the quadratic rate of change based on these beta-values, with an example, can be viewed in Appendix B (section B.7, page 331-333)

If results did not differ substantially across the three covariate adjusted models, results from model 3 will be presented only with those from model 1 and 2 available in the appendix.

Effect modification by *APOE* and sex on the association between PA and cognition were explored in chapter 4, 5, and 6 via inclusion of additional interaction terms of these factors with PA and with time (e.g. PA x *APOE* and PA x *APOE* x time/ PA x *APOE* x time^2), where applicable. Effect modification by *APOE*ε4 carrier status was conducted across the three covariate adjusted models, however, effect modification by sex was explored within the most covariate adjusted model (model 3) only. Data were also stratified by *APOE*ε4 carrier status (carrier vs non-carrier), sex and age (≥70 vs <70 years old) with mixed-models analysis re-run to further explore the within group associations (time and/or non-time dependant), as secondary and only in the most adjusted model. Interactions with age were not conducted due to cognitive index scores already being adjusted for age. Alternatively, to explore whether age may alter the association between PA and cognition, as

stated, data were stratified by those ≥70 versus those <70 years of age and mixed-models analyses re-run within groups, as the most adjusted model only. This age cut-off was chosen due to the fact that the age-adjusted index scores were already adjusted based on these specified age categories. Furthermore, age groups below or above these categories (e.g. 60-64 or 75+ years) had very small sample sizes (see Chapter 4, Table 17). Statistical likelihood of cognitive change within the age-range of 60-69 years as well as within the range of 70-85 years still justified the decision to additionally adjust for age within age-stratified sub-group analyses. To address objective (iv), *APOE* x PA interactions were included in age and sex stratified analyses; if the interaction was statistically significant in any specific sub-group, it would indicate that *APOE*ε4 carrier status modifies the association between PA and cognition more strongly in that sub-group. Reference groups will be the lowest PA group (lowest EPA tertile, low LLPA, low MLPA), *APOE*ε4 carriers and males. In Chapter 6, the primary reference group will be the lowest activity group. However, post-hoc comparisons will be conducted to explore the differential associations of activity groups on cognition relative to each other in analyses pertaining to objective (i). Sub-group analyses within Chapter 6 will only be modelled up to 24 months due to smaller sample sizes within ML/LL PA, *APOE*ε4, sex and age sub-strata.

All analyses were repeated to explore global cognition, executive function, attention, language, visuospatial abilities, delayed and immediate memory as separate outcome variables. Analysis were conducted using SPSS for Windows, version 25 (IBM Corp., Armonk, NY).

Table 16. Summary of statistical models to be repeated within Chapter 4, 5 and 6

Exposure	Outcome	Covariates
Objective (i)	Global Cognition	Model 1:
PA [a]	Delayed Memory	Age, sex, education, time, time^2
PA x time	Immediate Memory	Model 2:
Objective (ii)	Language	Model 1, BMI, hypertension, hypercholesterolemia, diabetes
PA x *APOE*	Attention	
PA x *APOE* x time [b]	Visuospatial	
APOE stratified analyses	Executive function	Model 3:
Objective (iii) [b]		Model 1, model 2, smoking and alcohol intake
PA x Sex		
PA x Sex x time		
Sex stratified analyses		
Age stratified analyses		
Objective (iv)[b]		
Sex stratified and repeat (ii)		
Age stratified and repeat (ii)		

[a] PA refers to either PASE tertiles and late-life leisure PA (binary) (Chapter 4); midlife leisure PA (binary) (Chapter 5), and midlife to late-life leisure PA categories (four groups) (Chapter 6). [b] Only the most covariate adjusted models will be run (Model 3). Time interactions are treated at non-linear in all analyses (i.e. PA x time/PA x time^2

3.9.3 Physical activity, peripheral biomarkers and cognition

Two subsets of the CHARIOT: PRO Main Study data were considered for further analyses. The first subset consisted of (n=173) with available baseline serum BDNF (pg/mL) and a second subset (n=258) with available plasma $A\beta_{1\text{-}42}$ (pg/mL). Included data were chosen based on those with the longest follow-up period.

3.9.3.1 The cross-sectional association between midlife leisure PA, late-life leisure PA, EPA and *APOE*ε4 carrier status on peripheral biomarkers

To assess whether *APOE*ε4 carrier status and physical activity were associated with serum BDNF and plasma $A\beta_{1\text{-}42}$ levels, cross-sectional multivariable linear regression was conducted. Two covariate adjusted models were run step-wise via inclusion of *a priori* selected covariates. Model 1 included terms for age (continuous) and sex. Due to the finding that vascular factors have reportedly been associated with plasma $A\beta_{1\text{-}42}$ levels [403], serum BDNF levels [427], a second model was run including additional adjustment for BMI, hypertension, hypercholesterolemia and type II diabetes.

To examine whether the association between midlife and/or late-life leisure PA on serum BDNF and plasma $A\beta_{1\text{-}42}$ varied as a function of *APOE*ε4 status, an interaction term of PA x *APOE* were included in both models. Exercise group and *APOE* were also included as main effects terms to determine whether these factors independently predicted serum BDNF and plasma $A\beta_{1\text{-}42}$.

Evaluation of the assumptions of linearity, multicollinearity and homoscedasticity of the linear regression models ensured there were no violation of the following. The distribution of both BDNF and $A\beta_{1\text{-}42}$ residuals were normal hence no transformation was required. Upon identification and inspection of outliers, analyses were re-run with and without such data points to determine any change of overall result. These instances are noted, with results presented from the original analyses. Specifically, four outliers of >2 SD's below were identified for BNDF (3.44, 3.94, 4.35 and 6.95). Likewise, two outliers >3 SD's below (1.05 and 1.96) below the range were identified for $A\beta_{1\text{-}42}$.

3.9.3.2 The association between baseline peripheral biomarkers, physical activity and cognition

To determine if serum BDNF and plasma $A\beta_{1\text{-}42}$ levels were associated with overall cognitive function and cognitive trajectories across 30 months; age, sex, education, time and time^2 adjusted mixed models regression analyses were run for global cognition, delayed memory, immediate memory, attention, language, visuospatial, and executive function. A second model, as above, was also run including additional adjustment for vascular factors (hypertension, hypercholesterolaemia, type II diabetes and BMI). BDNF and Aβ variables were primarily treated as continuous. *APOE*

sub-group analyses were re-run with variables also as binary. Based on a median cut-off dichotomisation, Aβ groups were defined as Low = ≤14.70 pg/ml and High = >14.70 pg/ml. BDNF groups were defined as Low = ≤42.49 pg/ml and High = >42.49 pg/ml. Once again, maximum likelihood estimation and an unstructured covariance matrix were selected, while including the intercept as random and all other variables as fixed. A random slope was not deemed necessary and a quadratic time element was included as best fit for growth modelling, both determined after likelihood ratio model testing. Analysis were first run with time as a covariate and then interacting with serum BDNF and plasma $A\beta_{1\text{-}42}$ levels for IGC models.

Mixed-models analyses on the association between midlife PA / late-life PA and cognition were re-run in the BDNF data sub-set (n=173). If BDNF was found to be associated with both MLPA and/or LLPA and cognition, mediation analysis would have been conducted to test the assumption that BDNF may be mediating the association between LLPA, MLPA and cognition over 30 months. Mediation is said to have occurred if the strength of the relationship between the predictor (i.e. PA) and outcome (i.e. cognition) is reduced when including the mediator (i.e. BDNF) in the model. Alternatively, failing mediation; to determine if midlife leisure PA, late-life leisure PA and *APOE*ε4 carrier status modified the association between serum BDNF and plasma $A\beta_{1\text{-}42}$ levels on cognition, two-way interaction terms were included for each and mixed-models analyses re-run for all cognitive domains. All aforementioned analyses were repeated after stratifying the data into *APOE*ε4 carriers and non-carriers, with the exception of IGC modelling due to limited sample size.

Chapter 4: Late-life physical activity and cognitive function and trajectories over 30 months

This chapter presents the association between late-life PA and global plus domain-specific cognitive function and trajectories over 30 months among cognitively healthy older adults from the CHARIOT: PRO cohort. The objectives were:

(i) To evaluate if late-life leisure PA was associated with late-life overall cognitive function and trajectories across 30 months,

(ii) Whether *APOE*ε4 carrier status modifies these associations.

(iii) Whether these associations differ among females versus males and among those ≥70 years/old versus those <70 years/old.

(iv) If the association between late-life PA and cognition was modified by *APOE*ε4 carrier status differentially as a function of sex and/or age-categories.

Two measures of late-life PA were explored. Firstly, to explore the role of every-day physical activity (EPA); a composite score comprised of work/household/leisure related activity from the PASE questionnaire was used. Secondly, to explore the role of aerobic leisure related activity, a late-life leisure PA (LLPA) variable was utilised whereby individuals reporting ≥2 days per/week of moderate/vigorous activity are classed as high-active and those reporting <2 days per/week of moderate/vigorous activity classed as low-active

This chapter will additionally provide demographic characteristics of the CHARIOT: PRO population. The associations between time, *APOE*ε4 carrier status, age and sex on overall cognition function and trajectories over 30 months, prior to taking into account physical activity, will also be summarised.

4.1 Population characteristics in the overall cohort and stratified by *APOE*ε4 carrier status, age and sex

Population characteristics in the overall cohort and stratified by age, sex and *APOE*ε4 carrier status are detailed in Table 17. Details of sample sizes across follow-up time-points and within sub-strata can be view in Appendix C, Table C. In brief, data from a total of 901 cognitively healthy older individuals were available for analysis. Of this sample 55% were female and 23% *APOE*ε4 carriers, with a mean age of 68.7 years old (standard deviation [SD] 3.9). The median BMI was borderline within the normal range of 25 (kg/m^2), 8% reported a type II diabetes diagnosis, 13% with

hypercholesterolemia and 36% with hypertension. Current or previous smokers comprised 47% of the sample, current alcohol intake was averaging at 9 grams per/day or 1.1 units (8 grams = 1 unit) and 45% of individuals reported having undertaken some form of tertiary or university education following completion of high school. Majority of the population were white (92.2%), with 0.8% being black, 4.6% Asian, and 2.4% of mixed/unspecified. The mean PASE score was 115 (IQR = 83-148). With regard to late-life leisure activity, 25% of individuals reported partaking in moderate/vigorous activity ≥2 days per/week, with 36% having undertaken muscle strengthening activity. Majority reported having undertaken light housework within the previous week at 93%, 72% heavy housework, 23% partook in home repairs, 53% outdoor gardening and 35% reported participating in work related activity. Midlife activity found 34% of participants partaking in moderate/strenuous aerobic leisure activity ≥2 days per/week. Based on leisure aerobic activity in midlife and late-life combined; 54% of individuals reported always being inactive during both mid- and late-life, 10% increased their activity in late-life when compared to midlife, whereas 19% reduced their activity in late-life when compared to midlife and 14% reported maintaining a high active lifestyle in both mid- and late-life (i.e. maintaining ≥ 2 days per/week of moderate/strenuous activity).

*APOE*ε4 carriers had significantly lower scores than non-carriers in baseline global cognition, delayed memory, immediate memory, and attention. No differences in demographic factors based on *APOE*ε4 carrier status were evident. With regard to sex; males performed significantly worse than females in baseline global cognition, as well as tests assessing delayed memory, immediate memory, and language. Females, however, performed worse than males in tests assessing visuospatial abilities. There were no differences in cognitive performance when comparing those aged 60 to 69 years versus 70 to 85 years old at baseline. Sex and age differences were also noted with regard to vascular factors. Specifically, males had a higher BMI, and a higher frequency of type II diabetes, hypercholesterolemia, and hypertension diagnosis, compared to females. Likewise, those aged between 70 to 85 years had a higher BMI, and a higher frequency of hypertension diagnosis, when compared to 60 to 69-year old individuals. With regard to lifestyle characteristics, a higher frequency of males reported being a current or previous smoker, a tertiary or university education and being active in midlife, compared to females. Finally, those aged between 60 and 69 were more active in late-life based on the overall PASE score compared to those 70 to 85 years old.

Table 17. Demographic, health and lifestyle characteristics in total cohort and stratified by age, sex and *APOE*ε4 carrier status

	Total Cohort (n=901)	***APOE*ε4 -ve (n=628)**	***APOE*ε4 +ve (n=213)**	**Female (n=495)**	**Male (n=406)**	**60-69 y/o (n=579)**	**70-85 y/o (n=322)**
Age (yrs), mean (SD)	68.7 (3.9)	68.4 (3.8)	69.0 (4.1)	68.7 (4.0)	68.8 (3.9)	66.6 (1.9)	72.6 (3.7)***
Age groups, N (% ≥ 70 yrs/old)	322 (36)	227 (36)	72 (34)	176 (36)	146 (36)	-	-
60-64, N, (%)	82 (9)	49 (66.2)	25 (33.8)	52 (10.1)	35 (8.3)	-	-
65-69, N (%)	497 (55)	342 (75.8)	109 (24.2)	278 (54.1)	237 (56.2)	-	-
70-74, N (%)	263 (29)	181 (75.4)	59 (24.6)	144 (29)	119 (29)	-	-
75+, N (%)	59 (7)	46 (78)	13 (22)	32 (6.5)	27 (6.7)	-	-
Sex, N of females (%)	495 (55)	348 (56)	110 (53)	-	-	319 (55)	176 (55)
APOEε4, N carriers (%)[a]	206 (23)	-	-	110 (22)	97 (24)	134 (23)	73 (23)
Serum BDNF (pg/mL), median (IQR)[g]	42.4 (34.0, 52.1)	40.8 (33.7, 53.7)	44.3 (34.1, 51.4)	44.9 (36.2, 53.3)	40.5 (31.9, 50.2)*	42.5 (34.2, 52.7)	42.2 (33.1, 51.7)
Plasma $A\beta_{1-42}$ (pg/mL), median (IQR)[h]	14.8 (12.9, 16.4)	14.6 (12.9, 16.7)	14.9 (12.9, 16.2)	15.1 (13.0, 16.6)	14.5 (12.7, 16.1)	14.7 (12.8, 16.6)	14.8 (13.0, 16.1)
Cognition, median (IQR)							
Global Cognition	103 (94, 111)	104 (94, 113)	102 (92, 109)*	104 (95, 113)	101 (92, 109)***	103 (93, 111)	103 (93, 110)
Delayed Memory	102 (95, 107)	102 (98, 107)	100 (92, 107)	102 (98, 110)	100 (92, 106)***	102 (98, 106)	101 (93, 110)
Immediate Memory	106 (94, 112)	106 (94, 114)	103 (90, 109)	106 (97, 114)	100 (90, 109)***	106 (94, 112)	106 (94, 114)
Attention	103 (95, 115)	106 (94, 115)	100 (91, 110)*	106 (94, 115)	103 (91, 112)	103 (115, 91)	106 (94, 115)
Language	104 (96, 112)	104 (96, 112)	103 (94, 111)	108 (101, 117)	101 (92, 105)***	104 (96, 116)	105 (96, 112)
Visuospatial Abilities	100 (89, 109)	100 (89, 109)	100 (87, 112)	96 (87, 109)	102 (89, 112)***	100 (89, 112)	96 (87, 109)
Executive Function [b]	115 (103, 125)	114 (103, 125)	114 (102, 123)	115 (103 125)	114 (103, 123)	115 (103, 125)	114 (101, 124)
Vascular Factors							
BMI, median (IQR)[c]	25 (23, 29)	26 (23, 29)	25 (23, 28)	25 (22, 28)	26 (24, 29)***	25 (23, 28)	26 (23, 29)*
Normal weight (<25 kg/m^2)	356 (38)	232 (37)	90 (42)	225 (44)	131 (31)***	230 (40)	121 (38)
Overweight (25-29.9 kg/m^2)	366 (39)	249 (40)	86 (40)	175 (34)	191 (45)***	231 (40)	124 (39)
Obese (≥30 kg/m^2)	163 (17)	120 (19)	31 (15)	82 (16)	81 (19)	96 (17)	65 (20)
Type II Diabetes, N (%)	72 (8)	47 (8)	17 (8)	27 (5)	45 (11)	43 (7)	29 (9)
Hypercholesterolemia, N (%)	124 (13.2)	83 (13.2)	33 (16)	44 (9)	80 (19)	73 (13)	50 (16)
Hypertension, N (%)	325 (36)	214 (35)	84 (41)	142 (29)	183 (45)	192 (33)	133 (41)*

Table 17. (Continued) Demographic, health and lifestyle characteristics in total cohort and stratified by age, sex and *APOE*ε4 carrier status

	Total Cohort (n=901)	*APOE*ε4 -ve (n=628)	*APOE*ε4 +ve (n=213)	Female (n=495)	Male (n=406)	60-69 y/o (n=579)	70 + y/o (n=322)
Lifestyle characteristics							
Ever smoked, N (%)[d]	427 (47)	288 (47)	108 (52)	204 (41)	223 (55)***	266 (46)	161 (50)
Alcohol median (g day)[e]	9 (2, 18)	9 (2, 18)	7 (2, 16)	8 (2, 18)	9 (2, 17)	9 (2, 18)	8 (2, 17)
Education, N<12-yrs (%)	409 (45)	274 (44)	106 (51)	247 (50)	162 (40)***	254 (44)	155 (48)
Total EPA score	115 (83, 148)	112 (83, 148)	119 (85, 152)	117 (95, 145)	113 (78, 156)	116 (85, 153)	111 (77, 141)*
EPA N low (%)	304 (34)	210 (34)	63 (31)	163 (31.7)	135 (32)	190 (33)	114 (35)
EPA N Moderate (%)	296 (33)	204 (33)	73 (35)	168 (32.7)	136 (32.2)	178 (31)	118 (37)
EPA N High (%)	301 (33)	204 (33)	70 (34)	166 (32.3)	136 (32.2)	211 (36)	90 (28)**
MLPA, N low (%) [f]	568 (63)	387 (63)	129 (63)	326 (67)	242 (60)*	365 (63)	203 (63)
Low-MLPA / Low-LLPA, N (%)	483 (54)	329 (53)	107 (52)	282 (57)	201 (50)	299 (52)	184 (57)
Low-MLPA / High-LLPA, N (%)	93 (10)	65 (11)	23 (11)	49 (10)	44 (11)	70 (12)	23 (7)
High-MLPA / Low-MLPA, N (%)	174 (19)	119 (19)	41 (20)	90 (18)	84 (21)	109 (19)	65 (20)
High-MLPA / High-LLPA, N (%)	128 (14)	90 (15)	27 (13)	61 (12)	67 (17)	86 (15)	42 (13)

SD, Standard deviation; B, regression beta-coefficient; CI, confidence interval; IQR, Interquartile Range; PA, physical activity; LLPA, late-life leisure PA; MLPA, midlife leisure PA; EPA, Everyday PA; BDNF; Brain Derived Neurotrophic Factor; Aβ, Amyloid beta; -ve, negative; +ve, positive. [a] 77 subjects missing *APOE* status; [b] 225 Executive function scores missing; [c] 34 cases missing BMI with 11 cases being underweight (BMI<18.5 kg/m^2); [d] five cases missing smoking history (ever vs never); [e] 96 cases missing alcohol consumption; [f] 23 cases missing MLPA data; [g] BDNF sub-sample n=173; [h] $A\beta_{1\text{-}42}$ sub-sample n=258. *p*-values determined by independent samples t-test (or Mann-Whitney U test for median comparison) for comparison of two continuous variable groups and ANOVA for variables of >2-groups (with Tukey's post-hoc analysis to compare means and Kruskal-Wallis 1-way ANOVA with Mann-Whitney post hoc test to compare medians), and chi-squared (χ^2) for categorical variables. *$p\leq$05; **$p\leq$.01; ***$p\leq$.001

4.2 Population characteristics stratified by everyday physical activity tertiles

Population characteristics stratified by late-life EPA tertiles (low, moderate, high) based on the PASE questionnaire are presented in Table 18. There was a difference in age across the EPA tertiles with a larger proportion of individuals <70 years old showing higher EPA than older individuals. There were fewer people with obesity among those who were highly physically active than among those with low physical activity levels, and there were fewer people reporting low midlife PA (MLPA) among those with high activity levels than among those with lower levels of activity. No other differences in characteristics across EPA levels were noted.

Table 18. Population characteristics by late-life everyday physical activity tertiles

	Low EPA (n=304)	Moderate EPA (n=296)	High EPA (n=301)
Age (yrs), mean (SD)	69.3 (4.6)** [j]	69.0 (3.8)** [j]	68.0 (3.1)
Age groups, N ≥70 yrs/old (%)	114 (37.5)	118 (39.9)	90 (29.9)
60-64 N, (%)	26 (31.7)	21 (25.6)	36 (42.7)
65-69 N (%)	164 (33)	157 (31.6)	176 (35.4)
70-74 N (%)	82 (31.2)	97 (36.9)	84 (31.9)
75+ N (%)	32 (54.2)** [j]	21 (35.6)	6 (10.2)
Sex, N female (%)	158 (52)	173 (58)	164 (55)
APOEε4, N carrier (%)[a]	63 (21)	73 (25)	70 (23)
Serum BDNF (pg/mL), median (IQR)[g]	40.0 (30.9, 49.6)* [j]	43.9 (37.6, 52.6)	44.0 (33.1, 54.0)
Plasma $A\beta^{1\text{-}42}$ (pg/mL), median (IQR)[h]	14.6 (12.6, 16.7)	14.6 (12.9, 16.1)	15.0 (12.9, 16.5)
Cognition, median (IQR)			
Global Cognition	103 (92, 111)	103 (93, 112)	103 (94, 111)
Delayed Memory	102 (98, 107)	102 (95, 107)	100 (105, 97)
Immediate Memory	106 (94, 114)	106 (94, 112)	106 (94, 112)
Attention	103 (92, 115)	103 (94, 115)	106 (94, 115)
Language	104 (96, 111)	104 (96, 116)	104 (96, 112)
Visuospatial	100 (89, 109)	100 (89, 112)	100 (87, 109)
Executive Function [b]	114 (103, 125)	117 (104, 125)	113 (102, 124)
Vascular Factors			
BMI, median (IQR)[c]	25 (23, 29)	26 (23, 29)	25 (23, 28)
Under-/normal weight (<25 kg/m^2)	118 (34)	116 (33)	117 (33)
Overweight (25-29.9 kg/m^2)	107 (30)	118 (33.2)	130 (37)
Obese (≥30 kg/m^2)	68 (42)* [k]	49 (30)	44 (27)
Type II Diabetes, N (%)	27 (9)	21 (7)	24 (8)
Hypercholesterolemia, N (%)	44 (15)	48 (16)	31 (10)
Hypertension, N (%)	111 (37)	109 (37)	105 (35)
Lifestyle characteristics			
Ever smoking, N (%)[d]	160 (53)	131 (44)	136 (45)
Alcohol, median (g day) (IQR)[e, f]	9 (2, 22)	8 (2, 18)	9 (3, 17)
Education, N < 12 years (%)	130 (43)	142 (48)	137 (46)
Low MLPA, N (%)	214 (70)*** [j]	190 (64)*** [j]	164 (55)

SD, Standard deviation; B, regression beta-coefficient; CI, confidence interval; IQR, Interquartile Range; PA, physical activity; LLPA, late-life PA; MLPA, midlife PA; BDNF, Brain Derived Neurotrophic Factor; Aβ, Amyloid beta. [a] 77 subjects missing *APOE* status; [b] 225 Executive function scores missing; [c] 34 cases missing BMI with 11 cases being underweight (BMI<18.5 kg/m^2); [d] five cases missing smoking history; [e] 96 cases missing of alcohol consumption, [f] grams per day; [g] BDNF sub-sample n=173; [h] $A\beta_{1\text{-}42}$ sub-sample n=258 [i] 23 cases missing MLPA data; [j] relative to high EPA; [k] relative to moderate EPA and high EPA; *p*-values determined by independent samples t-test and ANOVA for continuous variables, and chi-squared (χ^2) for categorical variables. *$p \leq .05$; **$p \leq .01$

4.3 Late-life physical activity and overall cognitive function and trajectories across 30 months

Time was statistically significantly associated with global cognition, immediate memory, delayed memory and attention index scores over 30 months, but not with language, visuospatial and executive function in the most adjusted models (Appendix C, Figure C.1).

4.3.1 Everyday physical activity and cognition over time

Relative to the lowest EPA terile, scores in global cognition, memory, attention, language and executive function did not differ among those in the moderate or high EPA tertiles, across all covariate-adjusted models. However, visuospatial index scores were lower among those in the highest EPA tertile compared to those in the lowest: model 1, *Beta-coefficient (B)* =-2.02 (95% CI -4.03, -0.02), and model 2, *B*=-2.21 (95% CI -4.22, -0.21), yet, did not reach statistical significance after also adjusting for smoking and alcohol consumption (Appendix C, Table C.3). Likewise, the interaction terms between EPA tertiles and time were non-significant for global cognition and all sub-domains (Appendix C, Table C.3). All other results remained congruent across the three covariate-adjusted models.

4.3.2 Aerobic leisure physical activity and cognition over time

Mixed-models regression analyses found that relative to low LLPA individuals, those in the high LLPA group had significantly higher scores in attention and language over 30 months. After additional adjustment for vascular factors, smoking and alcohol, the statistically significant associations remained for language, *B*=2.33 (95% CI 0.64, 4.01), but became non-significant for attention, *B*=2.28 (95% CI -0.00, 4.57), however, effect estimates were largely unaltered (Appendix C, Table C.3). A further model was run also adjusted for MLPA finding no effect on the results, hence indicating that the association between LLPA and language be independent from MLPA levels.

Global cognition, immediate memory and delayed memory trajectories were modified by LLPA (Appendix C, Table C.5). Both low and high LLPA groups showed increasing cognitive scores up to month-18 after which high LLPA individuals continue increasing whereas those reporting low LLPA indicate lower cognitive scores over time (Figure 10). Results remained unchanged across the three covariate adjusted models, as well as after adjustment for MLPA (not further shown).

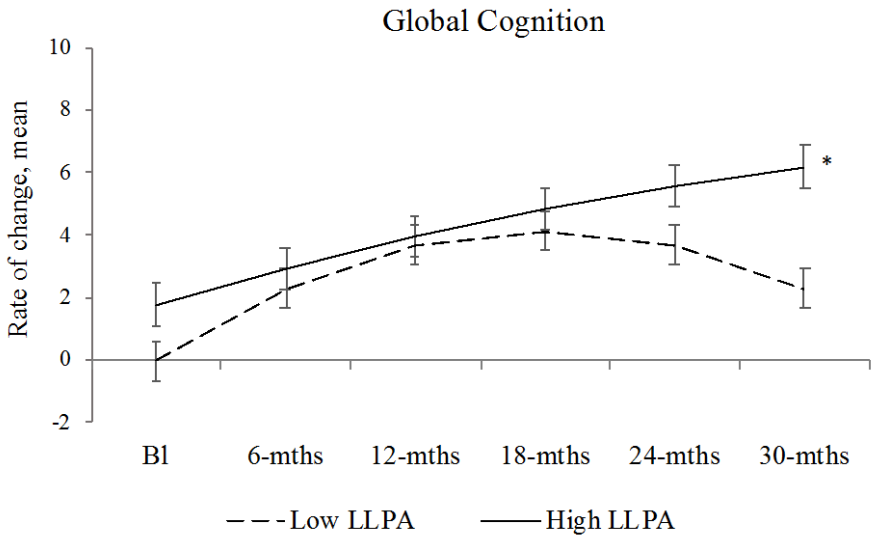


Figure 10. Trajectories of change in global cognition over 30 months among those in the low LLPA versus high LLPA groups.

Figure shows estimated mean change in global cognition index scores from baseline to 30 months (higher scores suggested better performance. Mixed-model regression was used to assess between group differences (LLPA x time interaction) in changes from baseline to 30 months in the overall cohort. The above graph indicates a significant difference between groups in linear, *B*=-1.53 [95% CI -2.87, -0.19] and quadratic, *B*=0.39 [95% CI 0.08, 0.70] global cognitive trajectories over 30-months. Predicted mean index scores at 30-months were 3.9 index points higher among high LLPA versus low LLPA individuals. Inspection of the slopes indicate those reporting high LLPA exhibit continued improvements in global cognitive scores over 30-months, whereas those reporting low LLPA initially show cognitive improvement until 18-months, after which they begin declining. Error bars are SEs. Abbreviation: LLPA, late-life physical activity; mths, months; Bl, baseline. Models adjusted for time, time2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. * $p \leq .05$

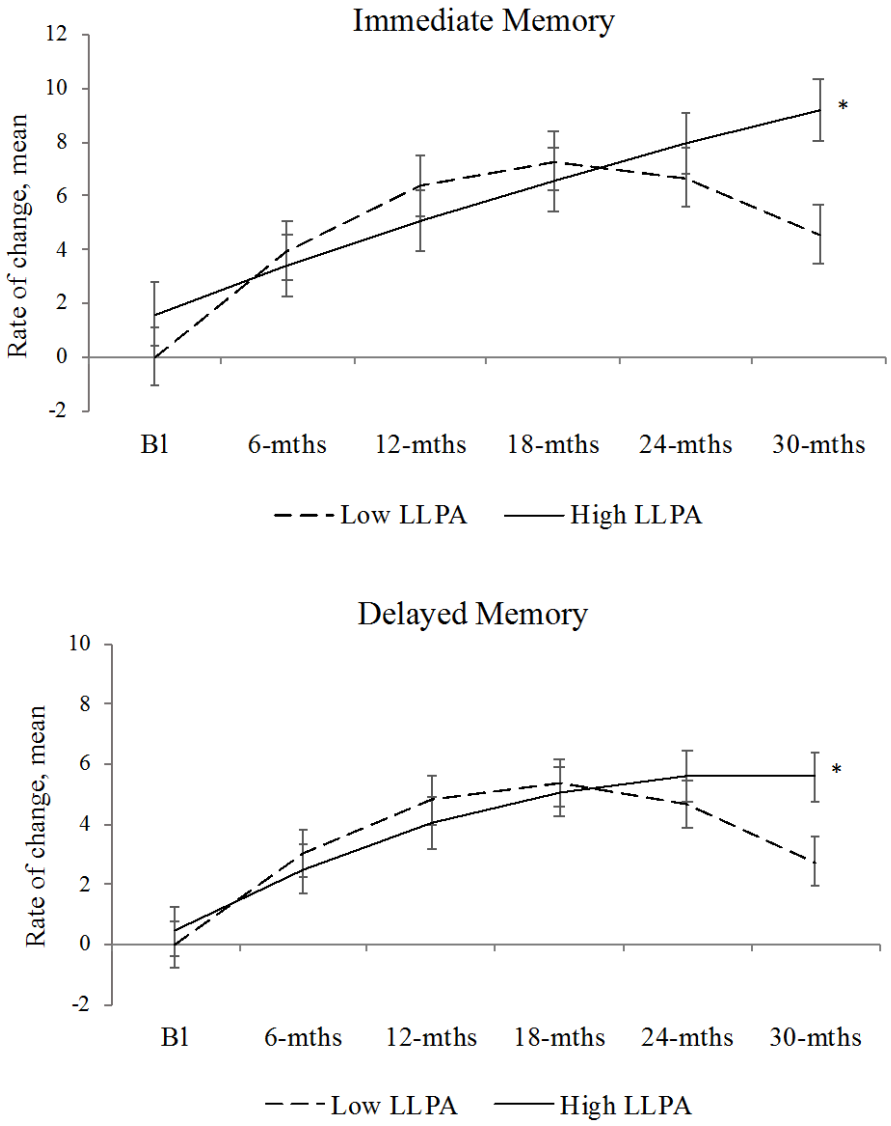


Figure 10. (continued) Trajectories of change in immediate memory and delayed memory performance over 30 months among those in the low LLPA versus high LLPA groups.

Figure shows estimated mean change in delayed memory and immediate memory index scores from baseline to 30 months (higher scores suggested better performance). The above graph indicates a significant difference between groups in linear, *B*=-2.84 [95% CI -4.58, -1.10]; and quadratic, *B*=0.69 [95% CI 0.28, 1.09] immediate memory and linear, *B*=-1.32 [95% CI -2.69, 0.06], plus quadratic, *B*=0.36 [95% CI 0.04, 0.68], delayed memory trajectories over 30-months. Predicted mean index scores at 30-months were 4.7 and 2.8 index points higher for immediate memory and delayed memory, respectively, among high LLPA individuals relative to low LLPA individuals. Inspection of the slopes indicate those reporting high LLPA exhibit continued improvements in cognitive scores over 30-months, whereas those reporting low LLPA initially show cognitive improvement until 18-months, after which they begin declining. Error bars are SEs. Abbreviation: LLPA, late-life physical activity; mths, months; Bl, baseline. Models adjusted for time, time^2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. * $p \leq .05$

4.4 Late-life physical activity, *APOEε*4 carrier status, and overall cognitive function and trajectories across 30 months

This section addresses objective (ii) specifically in relation to late-life EPA and late-life leisure activity. Section 4.4.1 presents background analysis regarding the direct association between *APOE*ε4 carrier status and cognition function and trajectories, followed by section 4.4.2 (EPA) and section 4.4.3 (LLPA) which address the primary objective.

4.4.1 *APOE*ε4 carrier status and cognition over time

A significant change in cognitive trajectories over time were congruent within carriers and non-carriers when compared to the overall cohort, with the exception of delayed memory among non-carriers becoming non-significant (Appendix C, Table C.1).

In the most adjusted model (model 3), relative to *APOE*ε4 carriers, non-carriers had higher overall global cognitive performance, *B*=3.02 (95% CI 1.13, 5.04), attention, *B*=2.72 (95% CI 0.33, 5.12), delayed memory, *B*=2.41 (95% CI 0.66, 4.12), immediate memory, *B*=2.12 (95% CI 0.15, 4.01) and language, *B*=2.01 (95% CI 0.24, 3.83) index scores averaged across 30 months (Appendix C, Figure C.2, Table C.1). Results remained consistent for the three covariate adjusted models (model 1 and 2 not further reported). There were no differences in average visuospatial abilities or executive function performance over 30 months between carriers or non-carriers across all models. Likewise, time did not significantly modify cognitive trajectories as a function of *APOE*ε4 carrier status (Appendix C, Table C.1).

4.4.2 *APOE*ε4 carrier status, everyday physical activity and cognition over time

*APOE*ε4 carrier status did not modify the association between EPA tertiles and overall multi-domain cognition function or trajectories across 30 months (Appendix C, Table C.4; Appendix C, table C.5). Associations between EPA tertiles and overall cognitive function and trajectories remained nonsignificant when repeating such analyses among *APOE*ε4 carrier and non-carrier strata.

Data were stratified by EPA tertiles, and the association between *APOE*ε4 carrier status and multi-domain cognition over 30 months re-run in the most adjusted model. The difference in global cognition, immediate memory, delayed memory, attention and language, as a function of *APOE*ε4 carrier status, remained statistically significant among those reporting low and/or moderate EPA, with larger effect sizes relative to the overall cohort. The associations between *APOE*ε4 carrier status and cognition were no longer significant within the highest EPA group, as supported by the considerably lower coefficients (Table 19). Figure 11 depicts these group differences for selected cognitive domains.

Table 19. Mixed-effects models evaluating the association between *APOEε4* carrier status and multi-domain cognition over time, stratified by everyday physical activity tertiles

Cognition domain	Coefficient [95% CI]			
	Overall cohort	**Low EPA (n=304)**	**Moderate EPA (n=296)**	**High EPA (n=301)**
Global Cognition	3.10 [1.11, 5.13]***	4.12 [0.38, 7.82]**	3.93 [0.56, 7.31]*	1.12 [-2.23, 4.44]
Attention	2.72 [0.35, 5.12]*	3.82 [-0.44, 8.01]	4.62 [0.79, 8.54]*	0.32 [-3.51, 4.23]
Delayed memory	2.40 [0.70, 4.12]**	3.23 [0.01, 6.40]*	2.53 [-0.34, 5.42]†	1.74 [-1.12, 4.45]
Immediate Memory	2.22 [0.19, 4.22]*	4.53 [0.85, 8.11]*	2.12 [-0.92, 5.34]	-0.14 [-3.15, 2.83]
Language	2.33 [0.54, 3.81]**	2.34 [-0.84, 5.52]	3.6 [0.57, 6.73]*	-0.35 [-3.12, 2.46]
Visuospatial	1.23 [-0.86, 3.44]	2.81 [-1.04, 6.62]	-0.81 [-4.12, 2.56]	1.53 [-1.82, 4.74]
Executive function	1.81 [-0.80, 4.32]	1.24 [-1.62, 3.90]	0.59 [-3.56, 4.84]	1.76 [-2.62, 5.93]

†$p \leq .08$, *$p \leq 0.05$, **$p \leq .01$, ***$p \leq .001$. Coefficients represent estimated marginal mean cognitive index score differences among *APOEε4* non-carriers relative to carriers. Magnitude of difference in marginal mean cognitive index scores between carriers/non-carriers were reduced among high EPA individuals indicating that higher PA may mitigate the negative impact of *APOEε4* carriage on cognitive function. Higher scores indicate better performance. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol consumption. *APOEε4* carrier group is the reference category; PA, late-life everyday physical activity. Abbreviations: *APOEε4*, Apolipoprotein epsilon 4; CI, Confidence Interval; EPA, everyday physical activity.

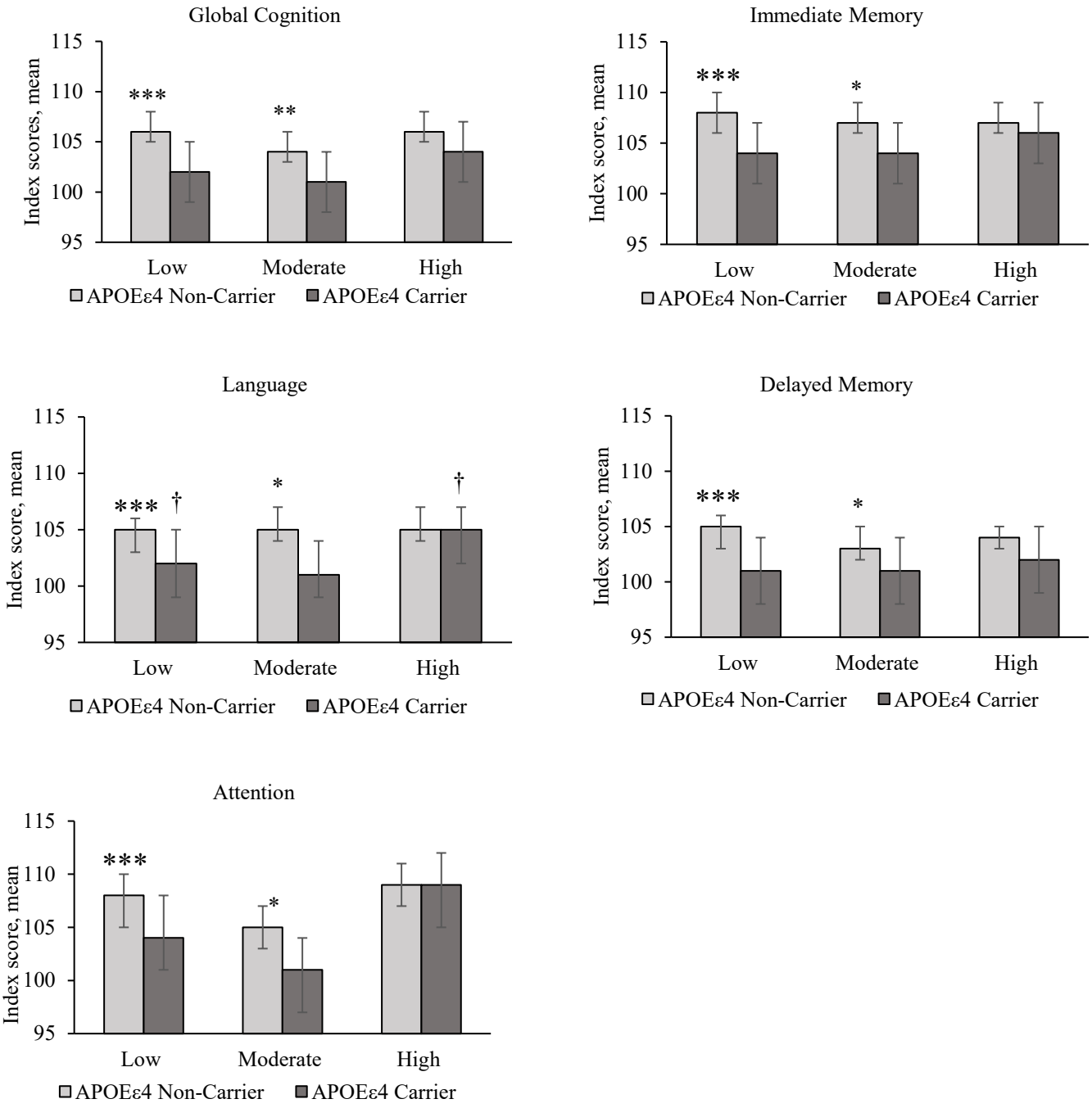


Figure 11. Marginal mean cognitive scores at 30 months for carriers versus non-carriers, and by physical activity tertiles.

*$p \leq .05$, **$p \leq .01$, indicating statistically significant differences between carriers and non-carriers within each cognitive domain and physical activity tertile. †$p \leq .08$, indicates high active carriers performing marginally better than low active carriers in predicted attention scores at 30-months. Coefficients represent estimated marginal mean cognitive index score differences among *APOE*ε4 non-carriers relative to carriers. Magnitude of difference in marginal mean cognitive index scores between carriers/non-carriers were reduced among high EPA individuals indicating that higher PA may mitigate the negative impact of *APOE*ε4 carriage on cognitive function. Higher scores indicate better performance. Error bars represent 95% CI's. Adjusted for time, time^2, age, sex, education, hypertension, hypercholesterolemia, BMI, type II diabetes, smoking and alcohol intake.

4.4.3 *APOEε4* carrier status, late-life leisure PA and cognitive over time

*APOE*ε4 carrier status did not modify the associations between LLPA and global cognition, attention, language, visuospatial, executive function, delayed memory or immediate memory across all three models (nonsignificant LLPA × *APOE* interactions) (Appendix C, Table C.4). However, when including time for three-way interactions, differences between LLPA groups on delayed memory trajectories as a function of *APOE*ε4 carrier status were revealed; LLPA × *APOE* × time *B*=-3.83 (95% CI -7.12, -0.54), LLPA × *APOE* × time^2 *B*=1.0 (95% CI 0.23, 1.76) (Appendix C, table C.5). There was also a statistically significant LLPA × *APOE* × time interaction for visuospatial trajectories, *B*=4.98 (95% CI 0.44, 9.53), however, interactions with time^2 did not reach statistical significance. *APOE*ε4 stratified analyses found significant differences in delayed memory trajectories to be augmented among and limited to ε4 carriers, such that high LLPA individuals show continually increased scores, whereas low active carriers display with some score increase, followed by decline from 18 months. Conversely, visuospatial trajectories were augmented in and remained significant among ε4 non-carriers, such that low LLPA individuals have reducing scores from baseline whereas high LLPA individuals exhibit reducing scores from 12 months. Significant differences in immediate memory trajectories remain for both carriers and non-carriers, and differences in global cognitive trajectories remained significant among non-carriers only (Figure 12).

With regard to overall cognitive function among carriers and non-carries: relative to low LLPA, better scores among high LLPA individuals were augmented among non-carriers for overall language, *B*=2.00 (95% CI 0.04, 3.96), and attention, *B*=3.06 (95% CI 0.35, 5.78) when compared to *APOE*ε4 carriers; language, *B*=1.5 (95% CI -2.31, 5.34), attention, *B*=-1.05 (95% CI -6.01, 3.90), in the most adjusted model (Appendix C, Table C.4).

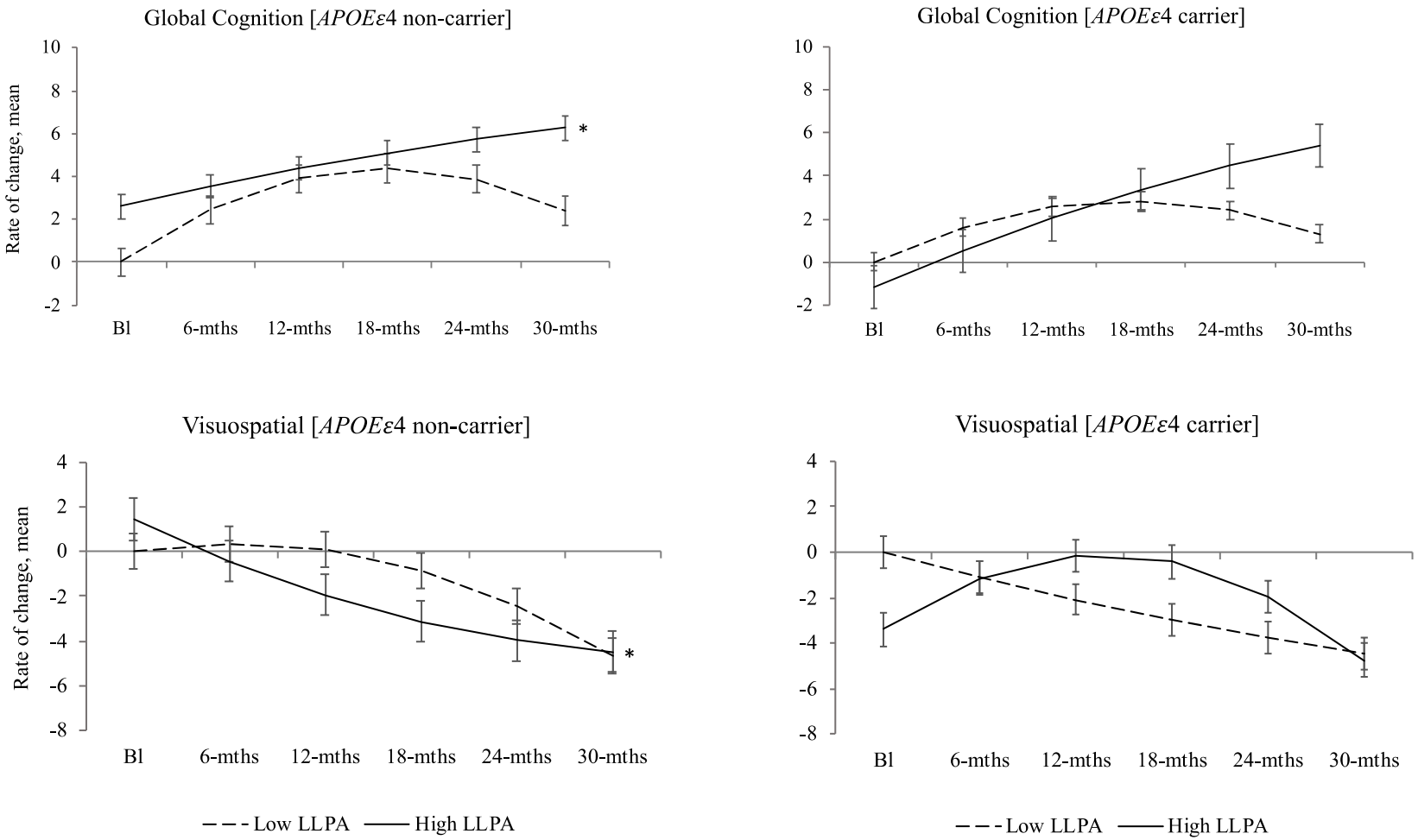


Figure 12. Trajectories of change in global cognition and visuospatial abilities among low versus high LLPA groups within *APOE*ε4 carriers and non-carriers.

Mixed-models regression were used to assess between group differences (LLPA x time interaction) in changes from baseline to 30 months in the overall cohort. Visuospatial trajectories were augmented in and remained significant among ε4 non-carriers, such that low LLPA individuals have reducing scores from baseline whereas high LLPA individuals exhibit reducing scores from 12 months. Differences in global cognitive trajectories remained significant among non-carriers only, with scores being 3.86 points higher at 30-months relative to low active non-carriers. Higher scores indicate better performance. Error bars are SEs. Abbreviation: LLPA, late-life physical activity; mths, months; bl, baseline. Models adjusted for time, time2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. *p≤0.05, **p≤.01.

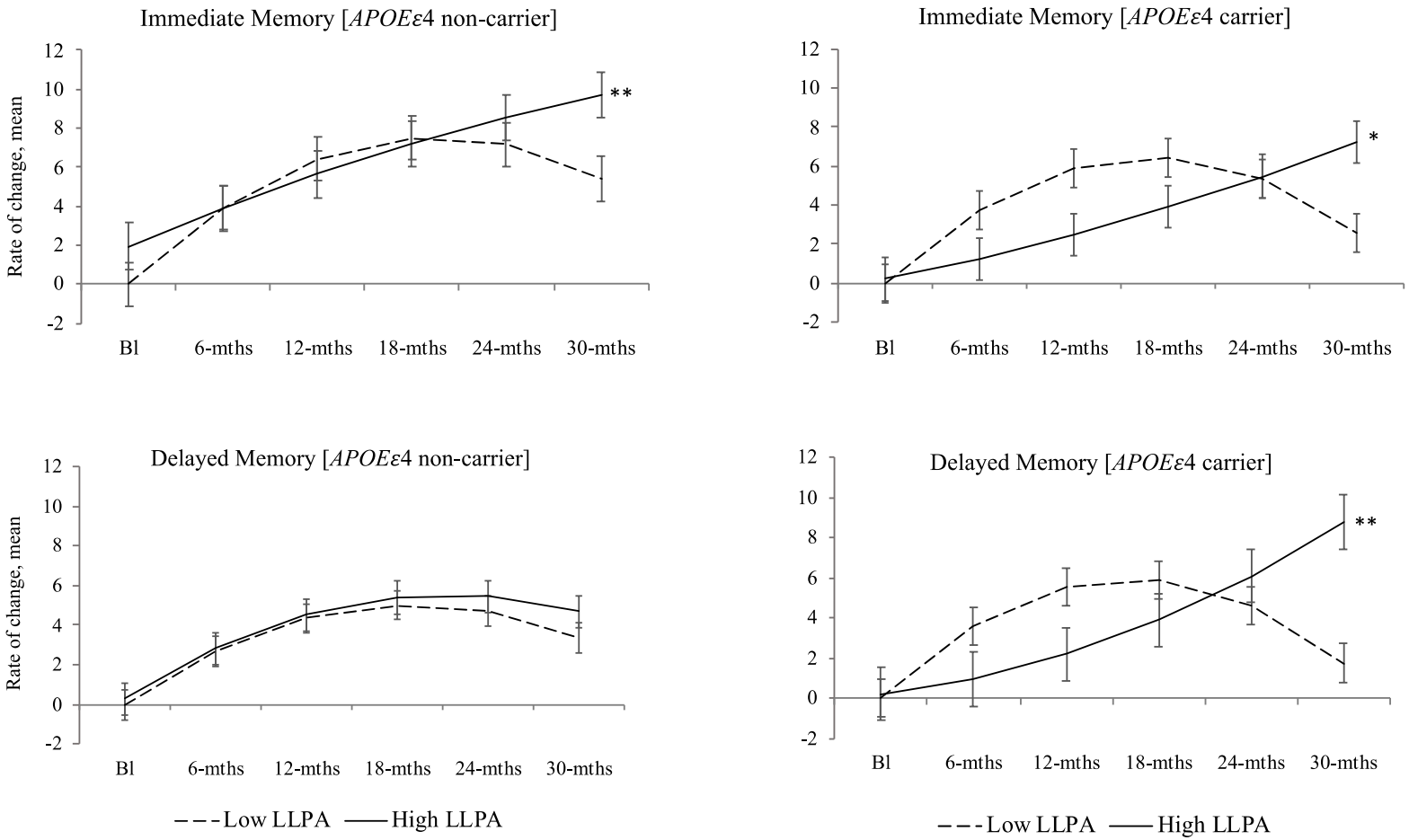


Figure 12. (continued) Trajectories of change in delayed and immediate memory among low versus high LLPA groups within *APOE*ε4 carriers and non-carriers.

Mixed-models regression was used to assess between group differences (LLPA x time interaction) in changes from baseline to 30 months among *APOE*ε4 carriers and non-carriers. *APOE*ε4 stratified analyses found significant differences in delayed memory and immediate memory trajectories which were augmented among ε4 carriers, such that high LLPA individuals show continually increased scores, whereas low active carriers display with some score increase, followed by decline from 18 months. Mean difference in delayed memory index scores at 30-months were 7.0 points among high active versus low active carriers compared to a mean difference in delayed memory scores of 1.4 among their non-carrier counterparts. Mean difference in immediate memory index scores at 30-months were 4.7 points among high active versus low active carriers compared to a mean difference of 4.3 among their non-carrier counterparts. Higher scores indicate better performance. Error bars are SEs. Abbreviation: LLPA, late-life physical activity; mths, months; bl, baseline. Models adjusted for time, time^2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. *$p\leq0.05$, **$p\leq.01$, ***$p\leq.001$

4.5 The association between physical activity and overall cognitive function and trajectories across 30 months among females vs males and those ≥70-yrs vs <70-yrs

This section addresses objective (iii) specifically in relation to late-life EPA and late-life leisure activity (LLPA). Section 4.5.1 presents results pertaining to the direct association between sex and cognition function and trajectories, followed by section 4.5.2 (EPA) and section 4.5.3 (LLPA) which addresses differences in PA/cognitive associations among females versus males. Finally, section 4.5.4 (EPA) and 4.5.5 (LLPA) addresses differences in associations among those aged ≥70 years versus those aged <70 years.

4.5.1 Cognitive function and trajectories over time among females versus males

The association between time and cognition remained consistent within males and females for global cognition, immediate memory, delayed memory and attention, however, males also had a significant interaction with time for language (Appendix C, Table C.2).

In the most adjusted model (Appendix C, Table C.1), females performed significantly better than males over 30 months in overall global cognition, *B*=4.43 (95% CI 2.75, 6.11), delayed memory, *B*=4.54 (95% CI 3.06, 6.01), immediate memory, *B*=7.22 (95% CI 5.52, 8.93) and language, *B*=5.51 (95% CI 3.98, 7.04). Males, on the other hand, performed significantly better than females in visuospatial abilities over 30 months, *B*=3.64 (95% CI 5.46, 1.82). There were no differences in overall performance between males and females in attention scores or executive function index scores. There was a statistically significant linear (but not quadratic) sex difference in trajectories (sex × time) for global cognition, immediate memory, visuospatial, executive function and a quadratic plus linear time interaction association for language scores (Appendix C, table C.1).

4.5.2 Late-life everyday physical activity and cognition over time among females versus males and those ≥70 versus <70 years old

Sex did not modify the association between EPA tertiles and global cognition or any sub-domain (EPA × sex interactions were not statistically significant). Likewise, inclusion of time for three-way interactions identified no significant difference in cognitive trajectories as a function of EPA and sex (Appendix C, Table C.6). In sex stratified analyses, the associations between EPA tertiles and cognition as well as cognitive trajectories remained non-significant among both males and females (Appendix C, Table C.5a and C.5b).

4.5.3 Late-life leisure physical activity and cognitive over time among females versus males

Sex stratified analyses identified that the main association between LLPA and overall language was reduced to a non-significant trend among females, *B*=2.36 (95% CI -0.08, 4.80), while remaining statistically significant among males, *B*=2.29 (95% CI 0.01, 4.58), although effect estimates are similar. The main association between LLPA and all other domains were no longer statistically significant among both males and females (Appendix C, Table C.7a and Table C.7b).

Statistically significant differences in global cognition, delayed memory and immediate memory trajectories in the high LLPA versus low LLPA group (LLPA × time and LLPA × time^2) among females only were identified, with notably larger effect estimates when compared to males (Figure 13). Inspection of the trajectories show that females in the high LLPA groups indicate increasing global cognition, delayed memory and immediate memory scores over 30 months, whereas low LLPA females display with an initial increase until 18 months, after which scores indicate decline. Males, on the other hand, show slight non-significant differences with higher scores among those reporting high LLPA relative to low LLPA individuals. However, both high and low LLPA individuals display decline after 18 months for delayed memory and global cognition, with the exception of immediate memory where high LLPA individuals exhibit continual improvement. Yet again, sex × LLPA as well as sex × LLPA × time (linear and quadratic) interactions were not statistically significant for global cognition or any sub-domain (Appendix C, table C.6).

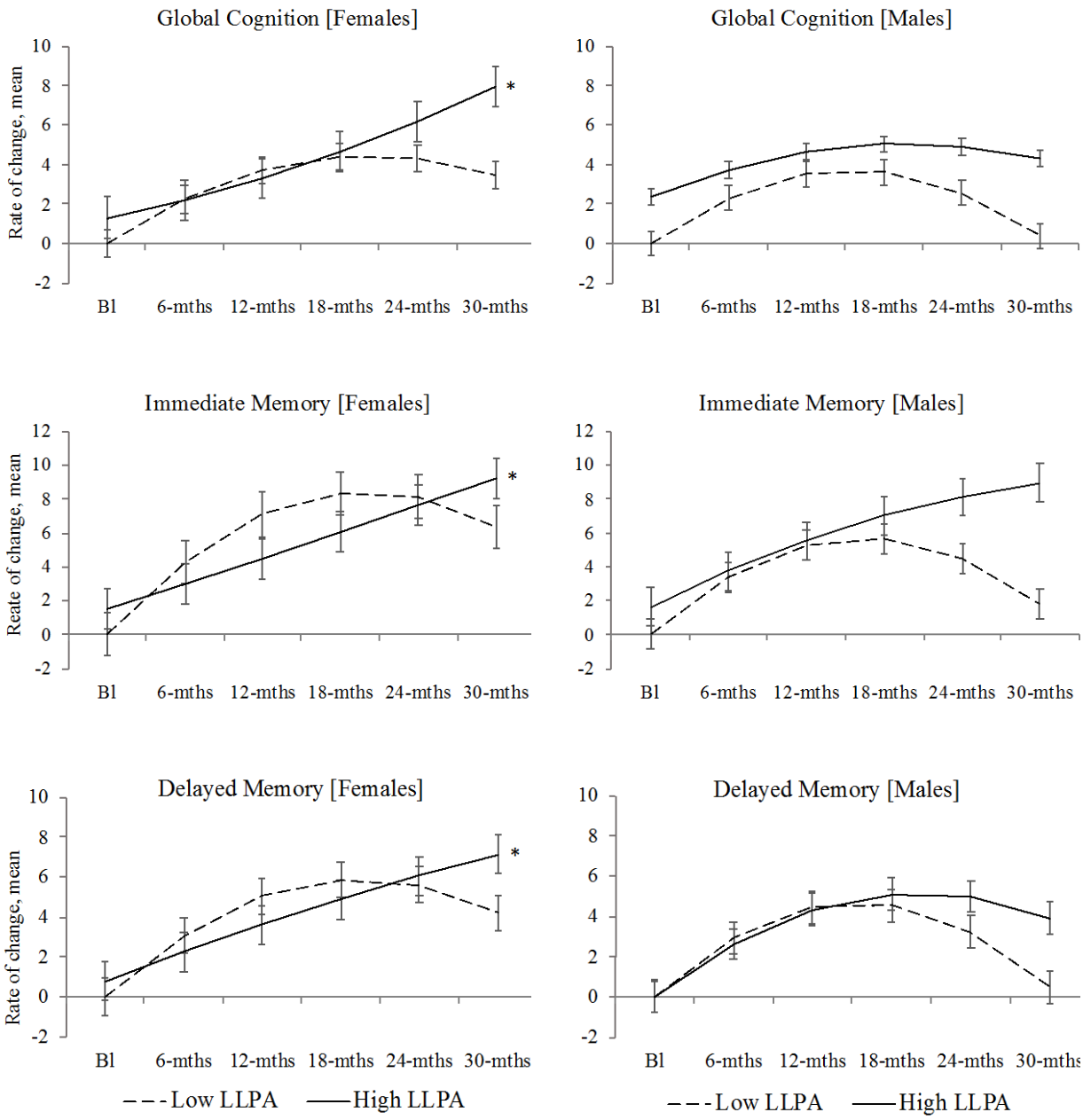


Figure 13. Trajectories of change in global cognition, delayed memory and immediate memory among those low versus high LLPA groups within males and females.

Mixed-models regression was used to assess between LLPA group differences (LLPA x time interaction) in cognitive index score change from baseline to 30 months among males and females. Inspection of the trajectories indicate that females in the high LLPA groups show increasing global cognition, delayed memory and immediate memory scores over 30 months, whereas low LLPA females display with an initial increase until 18 months, after which scores indicate decline. Mean difference in global cognitive, delayed memory and immediate memory index scores at 30-months were 4.5, 2.9 and 2.9 points higher, consecutively, among high active versus low active females. Mean difference in Males, on the other hand, show slight non-significant differences in those reporting high LLPA having higher index scores than low LLPA individuals. Error bars are SEs. Abbreviation: LLPA, late-life physical activity; mths, months; Bl, baseline. Models adjusted for time, time^2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. *$p \leq 0.05$

4.5.4 Late-life everyday physical activity and cognition over time among those ≥70 and <70 years old

The influence of time on cognitive trajectories over 30 months remained statistically significant for global cognition, immediate memory and delayed memory among those ≥ 70 years of age and were significant for global cognition, immediate memory, delayed memory and also attention among those <70.

Among the ≥70 age group only, there was a significant association between EPA tertiles and global cognition and attention scores such that those in the moderate PA tertile performed significantly worse than those in the low PA tertile, *B*=-3.34 (95% CI -6.51, -0.17) and *B*=4.71 (95% CI -8.50, -0.93), consecutively. No significant association between baseline EPA tertiles and all other domains over 30 months were evident within either age category (Appendix C, Table C.8a and C.8b). Interactions of EPA tertiles by time (EPA × time and EPA × time^2), were not statistically significant for any domain of cognition within both age categories.

4.5.5 Late-life leisure physical activity and cognition over time among those ≥70 and <70 years old

The significant association between LLPA and overall language remained statistically significant among those <70 years of age, *B*=2.36 (95% CI 0.32, 4.40), but no longer significant and with reduced effect size for those ≥7 years old, *B*=1.78 (95% CI -1.21, 4.76). The association between LLPA and all other cognitive domains were not statistically significant within both age-categories.

Inclusion of interaction terms of LLPA by time and time^2 found statistically significant differences in immediate memory trajectories as a function of LLPA groups within those <70 years old, and a significant difference in delayed memory and visuospatial trajectories as a function of LLPA groups within those ≥70 years of age (

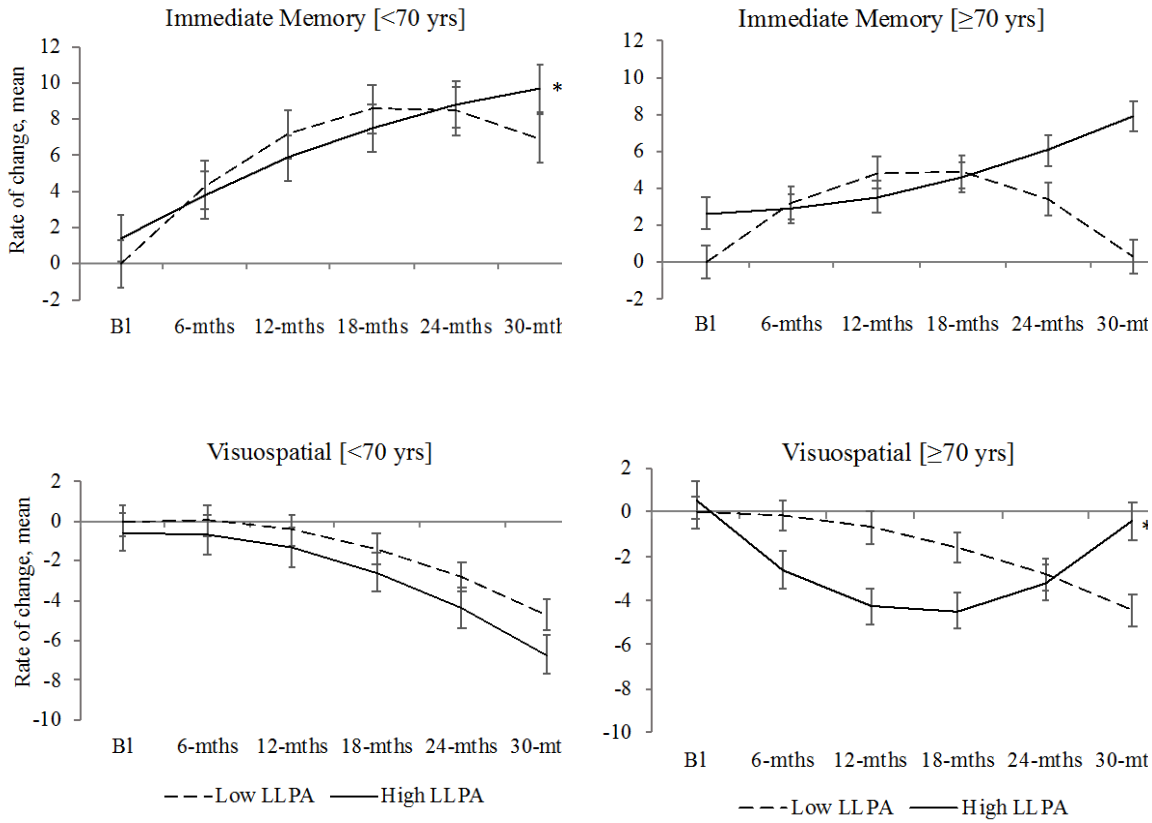


Figure 14, Appendix C, Table C.8a and C.8b). Inspection of the graphed trajectories suggest that those in the high LLPA group indicate increasing immediate memory scores within both age categories, whereas those in the low LLPA group initially display with increased scores up to 12 months in the ≥70 age group and 18 months in the <70 age group, after which predicted scores indicate decline. Immediate memory decline is steepest among low active, ≥70 year/old individuals. With regard to visuospatial index scores, both high and low LLPA individuals display decline in the <70 age group, albeit non-significant. Those in the ≥70 age group who reported low LLPA show increasing decline over 30 months, where those reporting high LLPA initially display with decreased scores up until 18 months, after which they exhibit some improvements.

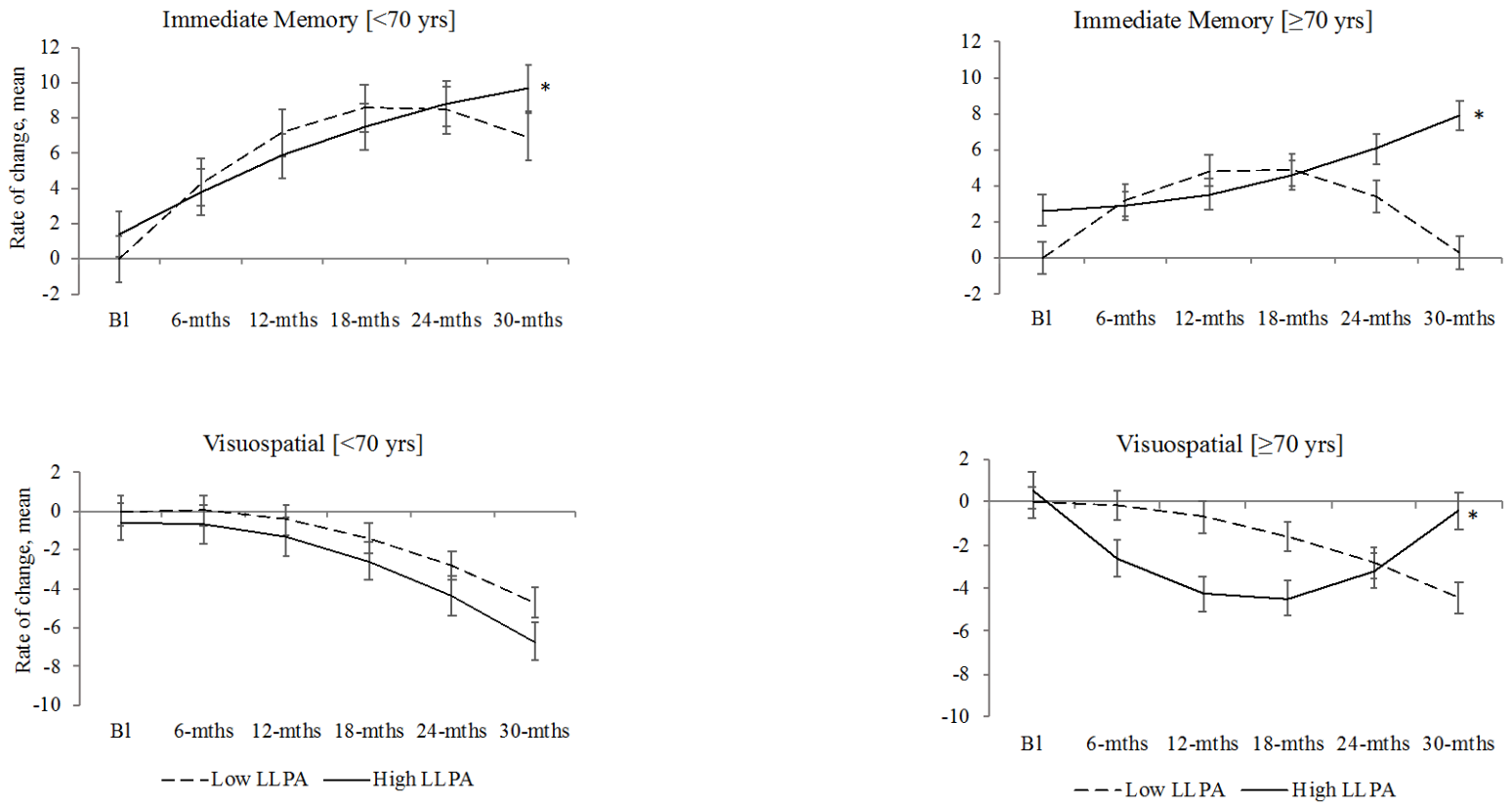


Figure 14. Trajectories of change in immediate memory and visuospatial index scores among low versus high LLPA groups within ≥70- and <70-year/old individuals.

Mixed-models regression was used to assess between LLPA group differences (LLPA x time interaction) in changes from baseline to 30 months age categories. Inspection of the graphed trajectories suggest that those in the high LLPA group indicate increasing immediate memory scores within both age categories, whereas those in the low LLPA group initially display with increased scores up to 12 months in the ≥70 age group and 18 months in the <70 age group, after which predicted scores indicate decline. Mean difference in immediate memory index scores at 30-months were 4.5 points higher in high LLPA versus low LLPA individuals who were ≥70 years old and 2.8 points higher in high versus low LLPA among <70-year olds. With regard to visuospatial index scores; the ≥70 age group who reported low LLPA show increasing decline over 30 months, where those reporting high LLPA initially display with decreased scores up until 18 months, after which they exhibit increased score. Score differences at 30-months were 4.0 points higher in the high LLPA versus slow LLPA groups. Error bars are SEs. Abbreviation: LLPA, late-life physical activity; mths, months; Bl, baseline. Models adjusted for time, time^2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. *$p \leq 0.05$

4.6 Effect modification of *APOE*ε4 carrier status on the association between late-life physical activity and cognition over time among males versus female and those ≥70 versus those <70 years old

While stratifying the data by sex (male versus female) and age (≥70 versus <70), *APOE* × EPA and *APOE* × LLPA interaction terms were included to explore whether the effect modification of *APOE*ε4 carrier status on PA/cognitive associations varied as a function of sex and/or age (Appendix C, Tables C.6a/b – C.7a/b).

In sex stratified analyses, there was a significant association between *APOE*ε4 carrier status and global cognition, delayed memory and language among females only, whereby non-carriers had higher scores over 30 months when compared to carriers (appendix C, Table C.2). *APOE*ε4 carrier status did not statistically modify associations between EPA tertiles or LLPA on any cognitive domain over 30 months within both males and females (Appendix C, Table C.7a/b).

The association between *APOE*ε4 carrier status and cognition within those <70 years of age were statistically significant for global cognition, language and attention index scores over 30 months, whereby non-carriers performed better than carriers (Appendix C, Table C.2). Among those ≥70 years of age, there was a significant association between *APOE*ε4 carrier status and global cognition, delayed memory and immediate memory index scores over 30 months. A significant *APOE* × high EPA tertile interaction, *B*=9.98 (95% CI 1.29, 18.66), suggested that the association between the highest PA tertile, relative to the lowest PA tertile, on immediate memory scores may differ as a function of *APOE*ε4 carrier status among ≥70 years old (Appendix C, Table C.8a/b), albeit a very wide confidence interval explained by a reduced sample size over time within the LLPA/*APOE* strata. Further stratified analyses found a non-significant trend suggesting that 70+/*APOE*ε4 carrier individuals in the highest EPA tertile to have better immediate memory scores at 30 months when compared to 70+/*APOE*ε4 carriers in the lowest EPA tertile. *APOE*ε4 carrier status did not statistically modify associations between EPA tertiles or LLPA on any other cognitive domain over 30 months within both age groups (Appendix C, Table C.8a/b).

4.7 Summary of results

- Time was significantly associated with global cognition, immediate memory, delayed memory and attention scores over 30 months.
- Being an *APOE*ε4 carrier and male resulted in worse global cognitive performance as well as lower scores in immediate memory, delayed memory, language and attention over 30 months. Only males performed significantly better than females in visuospatial abilities. The negative impact of *APOE*ε4 carrier status on cognitive function was only present among females.
- There were no statistically significant associations between late-life EPA tertiles and cognitive function or trajectories. *APOE*ε4 carrier status alone and in conjunction with time did not significantly moderate the association between EPA tertiles or LLPA and global cognition or any cognitive sub-domain. However, it was identified that both carriers and non-carriers in the highest EPA tertile performed equally well in global cognition, attention, delayed memory, immediate memory and language, whereas carriers in the low and moderate EPA tertiles performed significantly worse in global cognition and across these four cognitive domains when compared to non-carriers.
- Conversely, relative to low LLPA, those reporting ≥2-days per/week of moderate/vigorous aerobic leisure activity had better language and attention index scores, and appeared augmented among *APOE*ε4 carriers, and for language, among those <70 years of age.
- A statistically significant difference in global cognitive, immediate and delayed memory trajectories as a function of late-life leisure PA was identified, such that both groups initially showed cognitive improvements followed by a deviation in trajectories in the low PA group who exhibit decline after 18 months whereas the high PA group continue showing improved scores. Associations were augmented and remained statistically significant among females and non-carriers for global cognition, and among carriers and females for delayed memory.
- 70+/*APOE*ε4 carrier individuals in the highest LLPA tertile had better immediate memory scores at 30 months when compared to 70+/*APOE*ε4 carriers in the lowest PA tertile. *APOE*ε4 carrier status did not significantly moderate the association between EPA tertiles or LLPA and global plus domain-specific overall cognition or cognitive trajectory when explored within males and females separately, and within those ≥70 versus those <70 years of age for all other domains.

Chapter 5: Midlife physical activity and late-life cognitive function and trajectories over 30 months

This chapter reports the association between midlife aerobic-leisure PA and global plus domain-specific cognitive function and trajectories over 30 months among cognitively healthy older adults from the CHARIOT: PRO cohort. Whether possession of at least one *APOE*ε4 allele modifies this association was investigated, as well as the differential effects of age and sex on these associations. A midlife leisure PA (MLPA) variable was utilised, whereby individuals reporting ≥2-days per/week of moderate/vigorous activity were classed as high-active and those reporting <2-days per/week of moderate/vigorous activity classed as low-active. The following aims will be addressed: (i) to evaluate if midlife leisure-aerobic PA is associated with late-life cognitive function and trajectories, (ii) whether *APOEε4* carrier status modify these associations, (iii) if these associations differ among males versus females and among those ≥70 years/old versus those <70 years/old, and (iv) if the association between midlife PA and cognition is modified by *APOE*ε4 carrier status differentially as a function of sex and/or age-categories.

5.1 Population characteristics stratified by midlife physical activity

Table 20 presents population characteristics stratified by low (n=568) and high (n=310) MLPA. Independent samples t-test for mean comparison or Mann-Whitney U test for median comparison of continuous variables and chi-squared analysis identified a higher proportion of females, those with a higher BMI and lower EPA scores within the low MLPA group when compared to high MLPA group. Conversely, the high MLPA group had a higher proportion of past and/or current smokers, when compared to those reporting low MLPA. All other group differences were not statistically significant.

Table 20. Population characteristics stratified by midlife activity

	Low MLPA (n = 568)	High MLPA (n = 310)
Age (yrs), mean (SD)	68.9 (4.2)	68.6 (3.5)
≥70yrs N (%)	165 (36)	111 (36)
60-64 N, (%)	55 (10)	25 (8)
65-69 N (%)	310 (55)	174 (56)
70-74 N (%)	157 (28)	99 (32)
75+ N (%)	46 (8)	12 (4)
Female, N (% Female)	326 (57)	156 (50)*
APOEε4 carrier, N (% carrier) [a]	130 (23)	69 (22)
Serum BDNF (pg/mL), median (IQR) [g]	42.6 (34.5, 53.4)	42.1 (32.3, 50.6)
Plasma $A\beta_{1-42}$ (pg/mL), median (IQR) [h]	14.7 (12.7, 16.7)	14.8 (13.1, 16.1)
Cognition, median (IQR)		
Global Cognition	102, (92, 111)	105 (95, 113)**
Delayed Memory	102 (92, 110)	103 (98, 112)*
Immediate Memory	105 (94, 112)	106 (94, 114)*
Attention	103 (92, 112)	103 (94, 118)
Language	104 (96, 112)	104 (96, 116)
Visuospatial Abilities	100 (87, 109)	102 (89, 112)**
Executive Function [b]	114 (102, 124)	117 (105, 127)*
Vascular Factors, N (%)		
BMI, median (IQR)[c]	26 (23, 29)	25 (23, 28)*
Under-/normal weight (<25 kg/m2)	211 (37)	132 (43)
Overweight (25-29.9 kg/m2)	223 (39)	125 (40)
Obese (≥30 kg/m2)	111 (20)	45 (15)
Type II Diabetes	52 (9)	19 (6)
Hypercholesterolaemia	86 (15)	36 (12)
Hypertension	220 (39)	101 (33)
Lifestyle characteristics		
Smoking (ever vs never), N (% ever) [d]	253 (45)	165 (53)*
Alcohol (grams p/d), median (IQR) [e,f]	8 (2, 17)	9 (2, 21)
Education, N (% < 12 years)	258 (45)	140 (45)
Total LLPA score, median (IQR)	111 (77, 141)	122 (92, 162)***

*$p \leq .05$; **$p \leq .01$; ***$p \leq .001$ Abbreviations: SD, Standard deviation; B, beta-coefficient; CI, confidence interval; IQR, Interquartile Range; PA, physical activity; LLPA, late-life PA; MLPA, midlife PA; BDNF; Brain Derived Neurotrophic Factor; Aβ, Amyloid beta. [a] 77 subjects missing *APOE*ε4 carrier status; [b] 225 Executive function scores missing; [c] 34 cases missing BMI with 11 cases being underweight (BMI<18.5); [d] five cases missing smoking history; [e] 96 cases missing alcohol consumption, [f] grams per day; [g] BDNF sub-sample n=173; [h] $A\beta_{1-42}$ sub-sample n=258. *p*-values analysed by Student's t-test for mean comparison, Mann-Whitney U test for median comparison of continuous variables and chi-squared (χ^2) for categorical variables. Higher cognitive index scores indicate better performance.

5.2 Midlife physical activity and late-life cognition over time

In the most adjusted model, those reporting high MLPA had significantly higher index scores in global cognition, *B*=2.67 (95% CI 0.98, 4.36), delayed memory, *B*=1.58 (0.09, 3.06), language, *B*=1.82 (95% CI 0.27, 3.37), attention, *B*=2.52 (95% CI 0.44, 4.60) and executive function, *B*=3.0 (95% CI 0.58, 5.41), over 30 months, relative to those reporting low MLPA. Further adjustment for LLPA made no impact on the associations except for language, which reduced in effect size and become non-significant (not reported). Age, sex and education adjusted analyses also found statistically significant associations between high MLPA and immediate memory plus visuospatial abilities which became non-significant after adjustment for vascular factors, smoking and alcohol consumption, *B*=1.65 (95% CI -0.09, 3.39) and *B*=1.83 (95% CI -0.01, 3.66), $p<.08$, respectively (Figure 15; Appendix D, Table D.1).

Inclusion of MLPA × time and MLPA × time^2 interactions revealed a significant difference between MLPA groups in immediate memory and delayed memory trajectories over 30 months. Results remained statistically significant for immediate memory in all models, with delayed memory no longer being significant by model 3 (Figure 16; Appendix D, Table D.1). Adjustment of LLPA did not alter the associations (not reported). Inspection of the slopes indicate those reporting high MLPA exhibit continued improvements in immediate memory scores over 30 months, whereas those reporting low MLPA initially show cognitive improvement until 18 months, after which they present with decreasing scores.

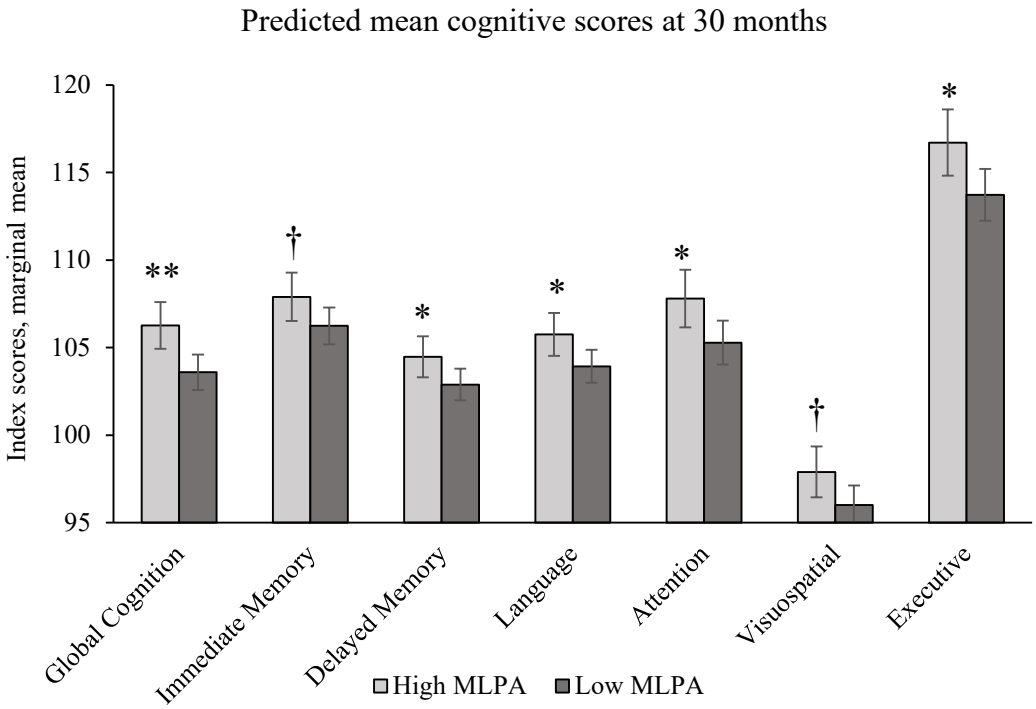


Figure 15. Marginal mean cognitive scores at 30 months within the low versus high MLPA groups.

†$p \leq .08$, *$p \leq .05$, **$p \leq .01$, indicating significant differences in marginal cognitive index scores at 30 months among high MLPA versus low MLPA groups. Higher cognitive index scores indicate better performance. Error bars represent 95% CI's. Adjusted for time, time^2, age, sex, education, hypertension, hypercholesterolemia, BMI, type II diabetes, smoking and alcohol intake.

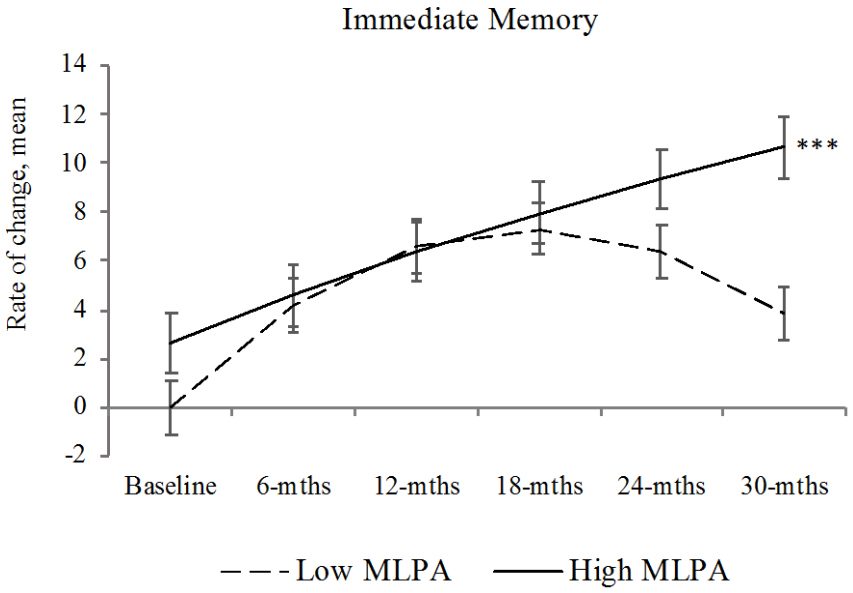


Figure 16. Trajectories of change in immediate memory over 30 months among those in the low versus high MLPA groups.

Figure shows significantly different estimated mean change in immediate memory index scores from baseline to 30 months (higher scores suggested better performance) among high MLPA versus low MLPA individuals (MLPA x time). Inspection of the slopes indicate those reporting high MLPA to exhibit continued improvements in immediate memory scores over 30 months, whereas those reporting low MLPA initially show cognitive improvement until 18 months, after which scores indicate decline. Predicted mean index scores at 30 months were 6.9 index points higher for immediate memory among high LLPA individuals relative to low LLPA individuals. Abbreviation: MLPA, midlife physical activity; mths, months. Models adjusted for time, time^2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. ***$p \leq 0.001$.

5.3 Midlife physical activity and *APOE*ε4 carrier status on late-life cognition over time

Inclusion of interactions (MLPA × *APOE*ε4) found that *APOE*ε4 carrier status did not modify the association between MLPA and overall global cognition or any sub-domain of cognition across all three covariate adjusted models (Appendix D, Table D.2). Three-way interactions (i.e. MLPA × *APOE* × time) with time found that differences in cognitive trajectories based on MLPA were also not modified by *APOE*ε4 carrier status in the most adjusted model (Appendix D, Table D.2).

Nonetheless, *APOE*ε4 carrier versus non-carrier stratified analyses found a statistically significant association between high MLPA, relative to low MLPA, and overall global cognition, *B*=3.54 (95% CI 1.51, 5.56), immediate memory, *B*=2.32 (95% CI 0.25, 4.39), delayed memory, *B*=2.10 (95% CI 0.41, 3.79), visuospatial, *B*=2.53 (95% CI 0.40, 4.66), language, *B*=1.95 (95% CI 0.14, 3.76), attention, *B*=3.17 (95% CI 0.68, 5.67) and executive function, *B*=4.58 (95% CI 1.75, 7.42), over 30 months to be limited within *APOE*ε4 non-carriers only, with considerably larger effect sizes when compared to ε4 carriers. (Figure 17; Appendix D, Table D.2).

Within both carriers and non-carriers MLPA × time and MLPA × time^2 interactions revealed a significant difference between MLPA groups for immediate memory trajectories over 30 months (Figure 18). Results remained unchanged across all three covariate adjusted models (Appendix D, Table D.2). Inspection of the trajectories show gradual improvements in immediate memory scores over 30 months within the high MLPA group, whereas low MLPA individuals initially display improvements up until 18 months, after which score decrease. Low MLPA carriers display steeper decline and high MLPA carriers display with steeper improvements when compared to non-carrier individuals.

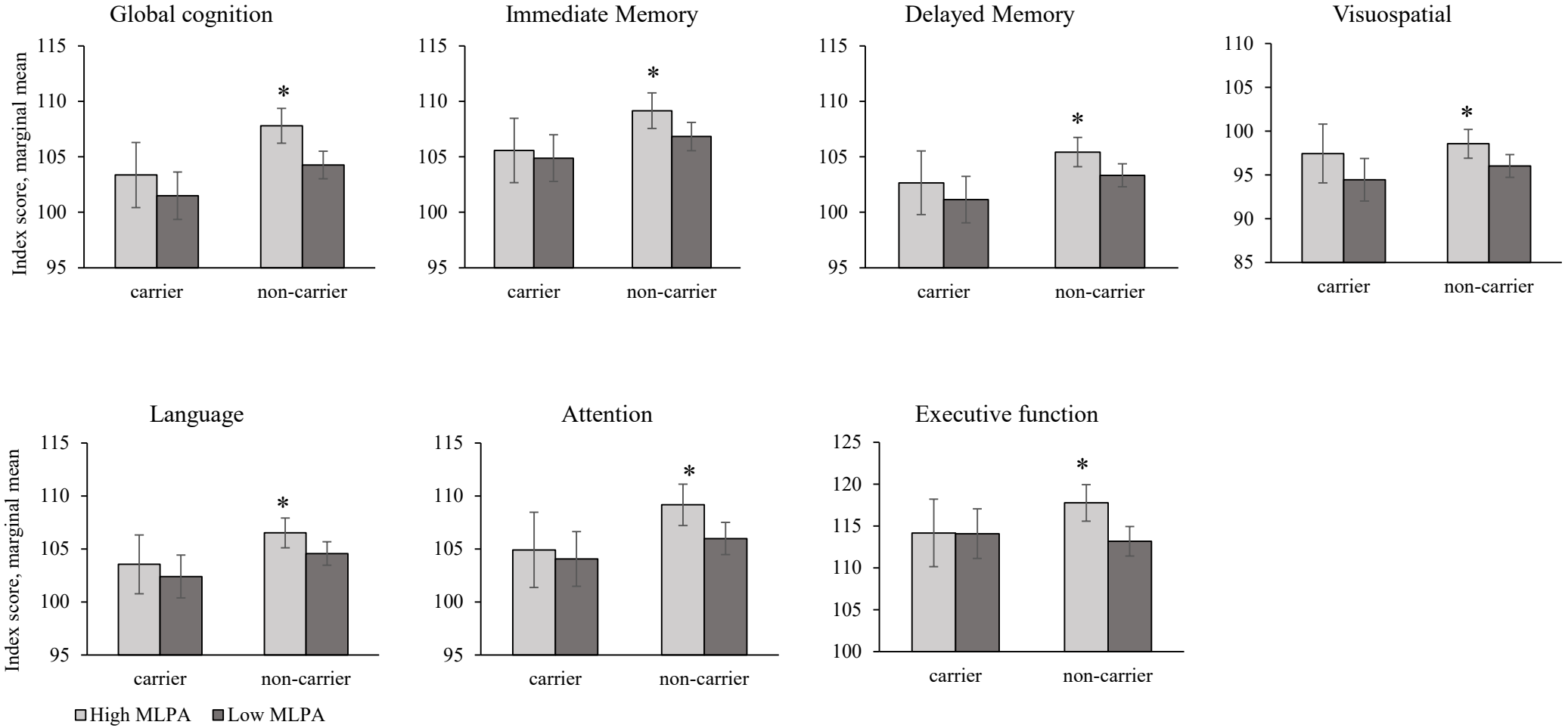


Figure 17. Marginal mean cognitive scores at 30 months for low versus high MLPA groups within *APOE*ε4 carriers and non-carriers.

Figure shows estimated mean cognitive index scores at 30-months within high MLPA versus low MLPA groups and among carrier and non-carrier sub-strata. *APOE*ε4 non-carriers reporting high MLPA have significantly better cognitive scores, whereas, the same MLPA group differences do not reach statistical significance among carriers. Higher scores indicate better performance. *$p \leq .05$, Error bars represent 95% CI's. Vertical axis represents cognitive index scores. Adjusted for time, time^2, age, sex, education, hypertension, hypercholesterolemia, BMI, type II diabetes, smoking and alcohol intake.

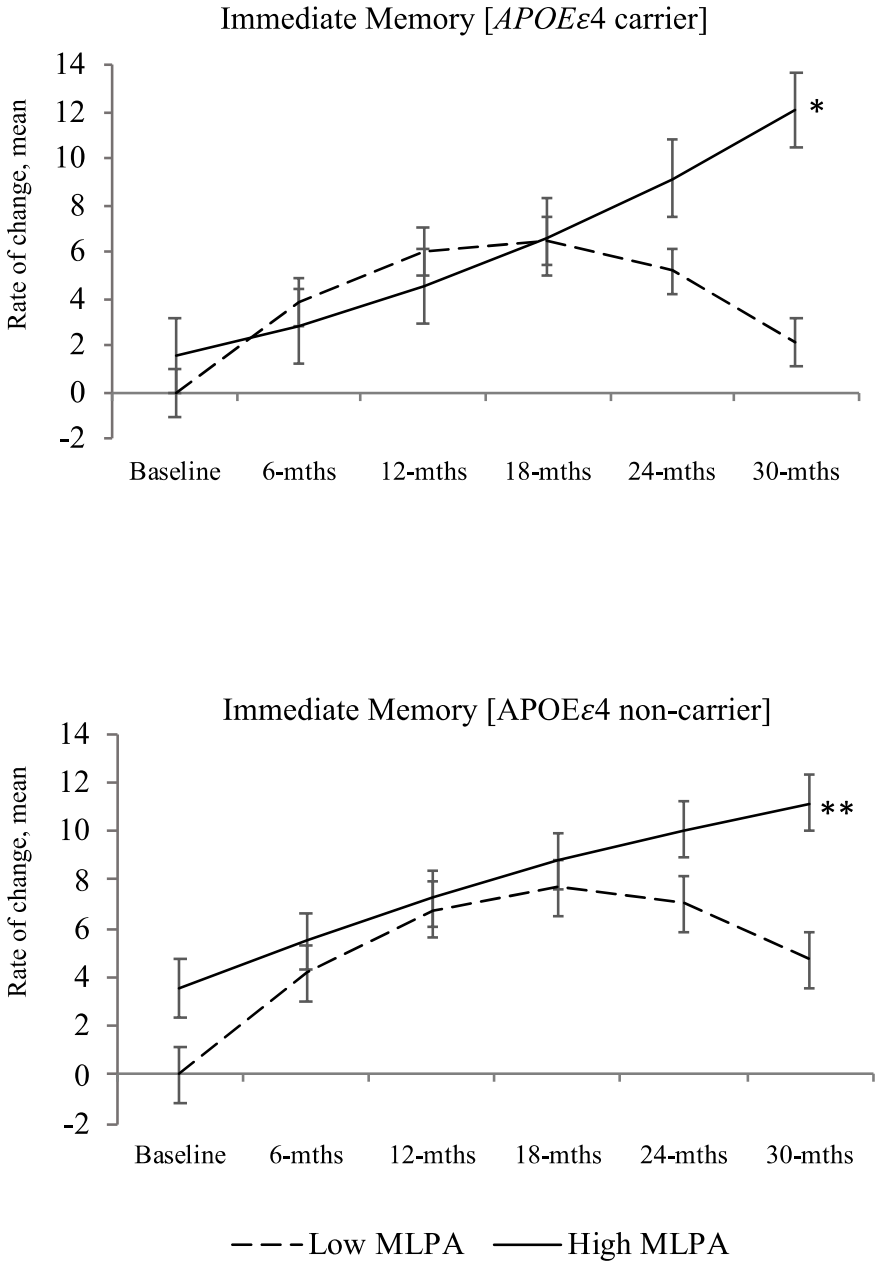


Figure 18. Trajectories of change in immediate memory over 30 months among high versus low MLPA carriers and non-carriers.

Figure shows estimated mean change in immediate memory index scores from baseline to 30 months. Within both carriers and non-carriers MLPA × time and MLPA × time^2 interactions revealed a significant difference between MLPA groups for immediate memory trajectories over 30 months. Trajectories indicate gradual improvements in immediate memory scores over 30 months within the high MLPA group, whereas low MLPA individuals initially display improvements up until 18 months, after which scores indicate decline. Mean difference in immediate memory index scores at 30-months were 9.9 points among high active versus low active carriers compared to a mean difference in immediate memory scores of 6.4 among their non-carrier counterparts. Higher scores indicate better performance. Error bars are SEs. Abbreviation: MLPA, midlife physical activity; mths, months; *APOE*ε4, apolipoprotein epsilon-4. Models adjusted for time, time^2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. * $p \leq .05$; ** $p \leq .001$

5.4 Midlife physical activity and cognition over time among females vs males and those ≥70-yrs vs <70-yrs

5.4.1 Midlife physical activity and cognition over time among females versus males

In the most covariate adjusted model (model 3), sex did not statistically modify associations between MLPA and overall cognition plus cognitive trajectories across all domains (Appendix D, Table D.3). Nonetheless, sex stratified analyses revealed that the positive association between MLPA and executive function index scores were augmented in and remained statistically significant among females only, *B*=3.56 (95% CI -.26, 6.85), when compared to males. Conversely, the positive associations between MLPA and overall global cognition, *B*=3.78 (95% CI 1.34, 6.23), visuospatial, *B*=2.61 (95% CI .03, 5.20), language, *B*=2.75 (95% CI .59, 4.90) and attention, *B*=3.26 (95% CI .39, 6.20) index scores over 30 months were augmented in effect sizes and remained statistically significant among males only, with reduced effect sizes notable among females. Differences in immediate memory trajectories as a function of MLPA remained statistically significant among both males and females, however, inspection of the coefficients and trajectories indicate an augmented association among females (Figure 19, **Error! Reference source not found.**). Differences in cognitive trajectories as a function of MLPA among both males and females were not statistically significant for all other cognitive domains (Appendix D, Table D.3). The association between MLPA and cognition within both males and females were not statistically modified by *APOE*ε4 carrier status (i.e. MLPA × *APOE* interactions were not statistically significant) (Appendix D, Table D.3).

5.4.2 Midlife physical activity and cognition over time among those ≥70 versus <70 years old

Mixed-models regression analyses stratified by those <70 years and ≥70 years of age found some group differences with regard to the association between MLPA and later life cognition. Specifically, the positive association between MLPA and overall global cognition, *B*=2.54 (95% CI .48, 4.61), delayed memory, *B*=2.14 (95% CI .40, 3.87), language, *B*=2.28 (95% CI .34, 4.23) and executive function, *B*=3.12 (95% CI .21, 6.03) index scores were augmented in and remained statistically significant among those <70 years old, with reduced effect sizes notable among the older age group (see Appendix D, Table D.4). Conversely, those ≥70 years of age retained an augmented positive association between MLPA and attention scores, *B* =3.90 (95% CI .48, 7.32), when compared to those <70 years old, *B*=1.26 (95% CI -1.35, 3.87).

Once again, differences in immediate memory trajectories as a function of MLPA remained statistically significant among both age-groups, although effect sizes varied slightly. Inspection of the trajectories found immediate memory scores among those <70 years old reporting high MLPA

indicate a continual increase, whereas, those reporting low MLPA show some increasing scores up until 18 months, followed by decline. However, among those ≥70 years of age, those reporting high MLPA show minimal increased scores, likewise, those reporting low MLPA display with some improvement, followed by decline after 18 months (Figure 19). Interactions of time by MLPA were not significant among the remaining cognitive domains within both age groups (Appendix D, Table D.4).

The association between MLPA and cognition within both age groups were not statistically modified by *APOE*ε4 carrier status (i.e. MLPA × *APOE* interactions were not statistically significant) (Appendix D, Table D.4).

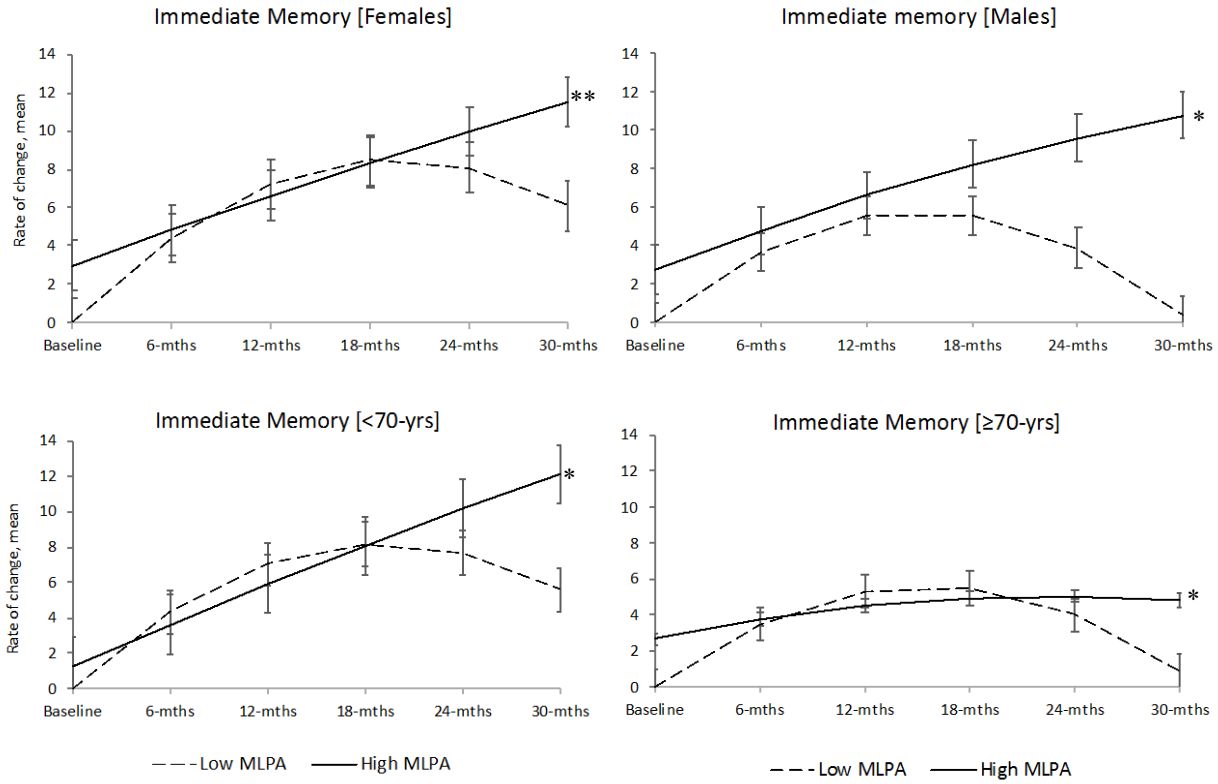


Figure 19. Trajectories of change in immediate memory among low versus high MLPA individuals, stratified by sex and age-categories.

Figure shows estimated mean change in immediate memory index scores over 30 months. Differences in immediate memory trajectories as a function of MLPA remained statistically significant among both age-groups, although effect sizes varied slightly. Inspection of the trajectories found immediate memory scores among those <70 years old reporting high MLPA indicate a continual increase, whereas, those reporting low MLPA show some increasing scores up until 18 months, followed by decline. However, among those ≥70 years of age, those reporting high MLPA show minimal increased scores, likewise, those reporting low MLPA display with some improvement, followed by decline after 18 months. Error bars are SEs. Abbreviation: MLPA, midlife physical activity; mths, months; *APOE*ε4, apolipoprotein epsilon-4. Models adjusted for time, time^2, age, sex education, type II diabetes, hypertension, hypercholesterolemia, BMI, smoking and alcohol intake. * $p \leq .05$; ** $p \leq .001$

5.5 Summary of results

- Relative to low MLPA individuals, those reporting high MLPA had better overall scores in global cognition, delayed memory, attention, language and executive function averaged across 30 months. For executive function, associations remained statistically significant and augmented among non-carriers and females; for all other domains they were significant and augmented among non-carriers and males. Likewise, for attention, associations were significant and augmented among those ≥70 years old; for all other domain's associations were augmented among those <70 years old.
- There was a statistically significant difference in immediate memory index score trajectories as a function of midlife leisure PA, such that those reporting high midlife leisure PA show continually increasing scores over 30 months, whereas those reporting low midlife leisure PA initially display with increasing scores however, begin declining from 18 months. Differences in immediate memory trajectories as a function of MLPA remained statistically significant among both age-groups, among males and females, and among *APOE*ε4 carriers versus non-carriers.
- The association between MLPA and overall cognition plus immediate memory trajectories within sex and age strata were not statistically modified by *APOE*ε4 carrier status (i.e. MLPA × *APOE* interactions were not statistically significant)

Chapter 6: Physical activity from midlife to late-life and late-life cognitive function and trajectories over 30 months

This chapter reports the association between four midlife to late-life leisure-aerobic PA groups on late-life global and domain-specific cognitive function and trajectories over 30 months among cognitively healthy older adults (65-80 years) from the CHARIOT: PRO cohort. Four mid- to late-life (ML/LL) PA groups were classified as the following: those who report being low-active in both midlife and late-life (lowest PA group; Low-LL/Low-ML PA), those who increased activity from low in midlife to high in late-life (Low-ML/High-LL PA), those who decreased from high in midlife to low in late-life (High-ML/Low-LL PA), and those who report high in both mid- and late-life (highest PA group; High-ML/High-LL PA). As previously described, high leisure activity was defined by those reporting ≥2-days per/week of moderate/vigorous aerobic activity and low as those reporting <2-days per/week of moderate/vigorous aerobic activity. This chapter will address the following aims: (i) to evaluate the differential associations of ML/LL activity groups on late-life cognitive performance and trajectories, (ii) whether *APOE*ε4 carrier status modify these associations, and (iii) if these associations differ among males versus females and among those ≥70 years/old versus those <70 years/old.

6.1 Population characteristics stratified by midlife to late-life leisure physical activity status

Table 21 presents population characteristics stratified by the four ML/LL activity groups. BMI was higher in the lowest PA group when compared to the three other groups, and there was a higher proportion of those with hypertension diagnosis in the lowest PA group and the High-ML/Low-LL PA group when compared to the highest PA group. All other characteristics did not differ across the four ML/LL PA groups.

Table 21. Population characteristics stratified by midlife to late-life physical activity groups

	Always Low PA (n=483)	Low midlife/ High late-life (n=93)	High midlife/ Low late-life (n=174)	Always High PA (n=128)
Age (yrs), mean (SD)	68.6 (2.7)	68.6 (2.0)	68.5 (2.3)	67.9 (2.3)
Age group N (% ≥70 yrs)	184 (38.1)	23 (24.7)	65 (13.2)	42 (32.8)
Sex, N (% Female)	282 (58)	49 (53)	90 (52)	61 (48)
APOEε4, N (% carrier) [a]	108 (22)	23 (25)	41 (23.6)	27 (21)
Serum BDNF (pg/mL), median (IQR) [h]	42.1 (32.4, 52.6)	46.2 (39.4, 55.4)	42.1 (35.1, 51.5)	43.3 (32.5, 50.4)
Plasma $A\beta^{1-42}$ (pg/mL), median (IQR) [i]	14.7 (12.7, 16.7)	15.0 (12.7, 16.8)	14.8 (13.1, 16.6)	14.5 (12.9, 15.5)
Cognition, median (IQR)				
Global Cognition	103 (92, 111)	101 (93, 112)	103 (95, 114)	105 (97, 114)
Delayed Memory	102 (95, 107)	102 (97, 107)	102 (95, 110)	102 (98, 110)
Immediate Memory	106 (94, 112)	100 (90, 109)	106 (94, 112)	106 (97, 114)
Attention	103 (91, 115)	103 (94, 115)	103 (94, 111)	106 (94, 118)
Language	104 (96, 112)	101 (96, 112)	104 (96, 111)	105 (101, 116)
Visuospatial	100 (87, 109)	100 (89, 112)	102 (89, 112)	101 (89, 112)
Executive Function [b]	114 (102, 123)	113 (100, 127)	117 (105, 125)	118 (105, 128)
Vascular Factors, N (%)				
BMI, median (IQR) [c]	26 (23, 29)	25 (22, 28)	25 (23, 28)	25 (23, 27)
Normal weight (<25 kg/m^2)	171 (35)	43 (46)	67 (39)	62 (48)
Overweight (25-29.9 kg/m^2)	196 (41)	31 (33)	68 (39)	53 (41)
Obese (≥30 kg/m^2)	97 (20)	15 (16)	32 (18)	12 (9)
Type II Diabetes	47 (10)	5 (5)	15 (9)	4 (3)
Hypercholesterolemia	75 (16)	12 (13)	19 (11)	131 (16)
Hypertension	193 (40)* [g]	29 (31)	71 (41)* [g]	28 (22)
Lifestyle characteristics				
Smoking, N (% ever) [d]	218 (45)	38 (41)	96 (55)	66 (52)
Alcohol, median (IQR)[e, f]	8 (2, 17)	10 (2, 19)	8 (2, 17)	10 (2, 23)
Education, N (% < 12 years)	224 (46)	37 (40)	84 (48)	53 (41)

SD, Standard deviation; IQR, Interquartile Range; PA, physical activity; LLPA, late-life PA; MLPA, midlife PA; BDNF; Brain Derived Neurotrophic Factor; Aβ, Amyloid beta. [a] 77 subjects missing *APOE* status; [b] 225 Executive function scores missing; [c] 34 cases missing BMI with 11 cases being underweight (BMI<18.5); [d] five cases missing smoking history; [e] 96 cases missing of alcohol consumption, [f] grams per day; [g] when compared to always high PA; [h] BDNF sub-sample n=173; [i] $A\beta_{1-42}$ sub-sample n=258. *p*-values determined by ANOVA with Tukey's post-hoc analysis to compare means and Kruskal-Wallis 1-way ANOVA with Mann-Whitney post hoc test to compare medians. Higher scores indicate better cognitive function. *$p \leq .05$; **$p \leq .01$

6.2 Physical activity during mid- to late-life and cognition over time

Relative to the lowest PA group, those in the highest PA group had significantly better scores in global cognition, language, attention and executive function over 30 months. Likewise, relative to those in the lowest PA group, those reporting High-MLPA/Low-LLPA had significantly better visuospatial index scores over 30 months. Results remained unchanged across the three covariate adjusted models (Table 22; Appendix E, Table E.1). Post-hoc comparisons found, relative to those reporting Low-MLPA/High-LLPA, those in the highest PA group had better global cognition and language scores, and those in the High-MLPA/Low-LLPA group had better visuospatial scores over 30 months. It was also found, that relative to those reporting Low-MLPA/High-LLPA, the highest PA group had better language scores over 30 months (Table 22).

There were statistically significant differences between the highest PA group relative to the lowest in global cognition, immediate memory and delayed memory trajectories over 30 months (i.e. ML/LL PA × time and ML/LL PA × time^2 interactions). Likewise, a significant difference in immediate memory trajectories over 30 months was evident among all PA groups relative to the lowest PA group (Table 22; Figure 20). Once again, results remained unchanged across the three covariate adjusted models (model 1 and 2: Appendix E, Table E.1). Inspection of the slopes indicated that those in the highest PA group to exhibit increasing global cognition, immediate memory and delayed memory scores continually over 30 months relative to the lowest PA group, who showed improvements up to 18 months, after which they began to decline. Likewise, those reporting high activity in either mid or late-life showed increasing immediate memory scores and global cognition but exhibited decline from 18 months in delayed memory index scores. Post-hoc comparisons between those reporting Low-MLPA/High-LLPA and High-MLPA/Low-LLPA relative to each other and relative to High-MLPA/High-LLPA did not detect differences in cognitive trajectories across any domain (result not reported). No other ML/LL PA group differences were identified with regard to cognitive function or trajectories.

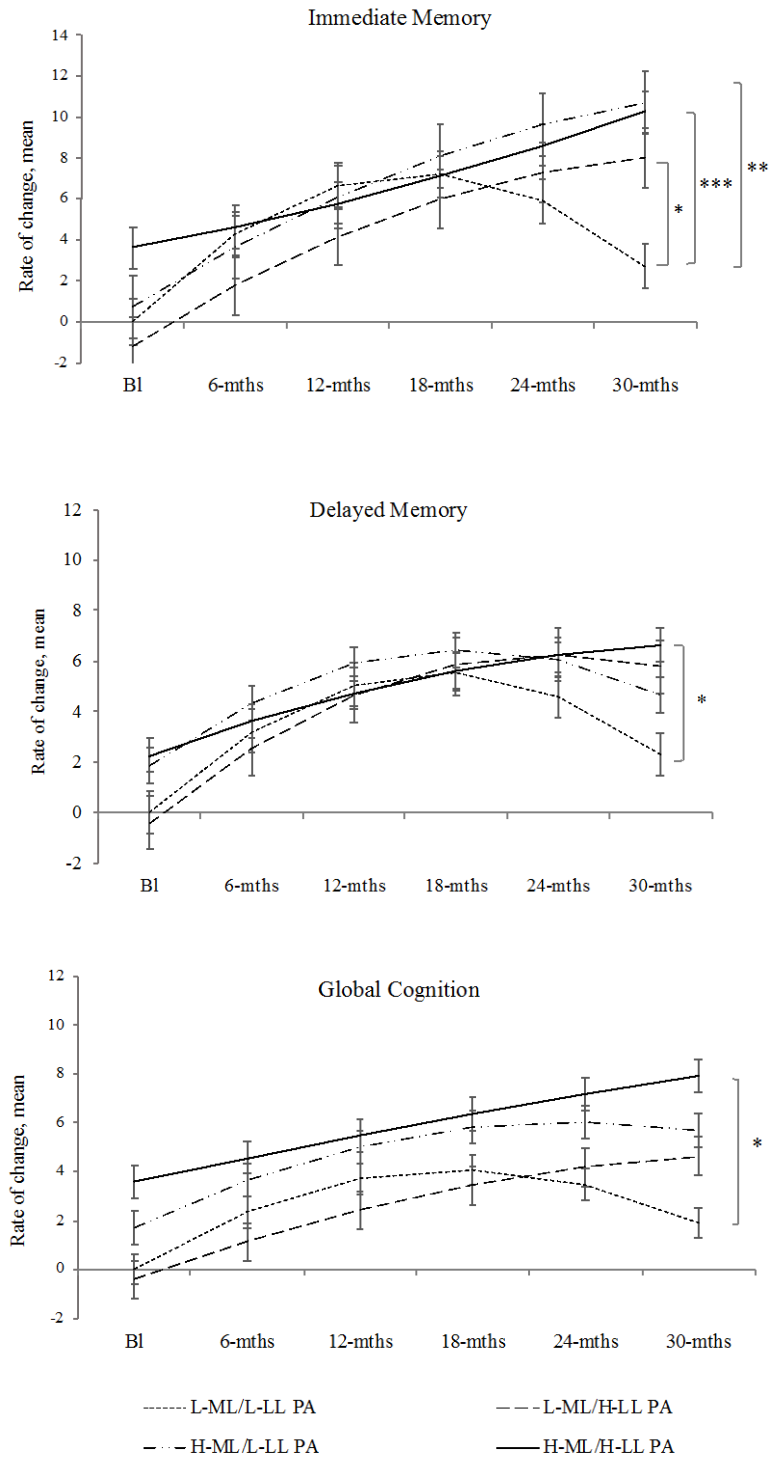


Figure 20. Trajectories of change in global cognition and immediate memory among ML/LL PA groups.

Estimated mean change in global cognition, immediate memory and delayed memory index scores from baseline to 30 months (higher scores suggested better performance). Mean difference in scores at 30-months for the highest active group versus lowest were 6.02 points for global cognition, 7.56 for immediate memory and 4.36 points for delayed memory. *$p \leq .05$, **$p \leq .01$, indicates difference in rate of change relative to the lowest PA group. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; L-ML/L-LL PA= comparator.

Table 22. Mixed effects models evaluating the association between mid- to late-life physical activity groups on late-life cognitive function and trajectories across 30 months

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-0.17 [-2.77, 2.44]	-1.18 [-3.79, 1.43]	0.12 [-2.13, 2.36]	-0.84 [-3.58, 1.90]	-0.02 [-2.31, 2.27]	0.93 [-2.22, 4.07]	-1.03 [-4.55, 2.49]
H-ML/L-LL PA	1.95 [-0.08, 3.99] †	0.89 [-1.16, 2.93]	1.65 [-0.11, 3.40] †	2.27 [0.12, 4.42]*	0.21 [-1.59, 2.01]	1.50 [-0.96, 3.95]	1.95 [-0.92, 4.81]
H-ML/H-LL PA	3.13 [0.84, 5.42]**	1.72 [-0.58, 4.02]	1.26 [-0.71, 3.23]	1.16 [-1.26, 3.57]	3.25 [1.23, 5.27]**	3.72 [0.95, 6.49]**	3.37 [0.20, 6.54]*
L-ML/H-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
H-ML/L-LL PA	2.25 [-0.81, 5.31]	1.98 [-1.17, 5.12]	2.08 [-0.61, 4.77]	3.60 [0.28, 6.91]*	-0.37 [-3.16, 2.42]	0.81 [-2.95, 4.57]	2.48 [-1.82, 6.78]
H-ML/H-LL PA	3.50 [0.31, 6.68]*	3.16 [-0.11, 6.43]	1.61 [-1.19, 4.41]	2.30 [-1.14, 5.74]	3.29 [0.39, 6.19]*	2.39 [-1.53, 6.30]	4.00 [-0.45, 8.45]
H-ML/L-L PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
H-ML/H-LL PA	1.25 [-1.50, 3.99]	1.18 [-1.64, 4.00]	-0.47 [-2.88, 1.94]	-1.30 [-4.27, 1.67]	3.66 [1.16, 6.16]**	1.58 [-1.80, 4.95]	1.52 [-2.38, 5.42]

Table presents results from a mixed-effects models regression. Relative to the lowest PA group (L-ML/L-LL PA), those in the highest PA group had significantly better scores in global cognition, language, attention and executive function over 30 months. Likewise, relative to those in the lowest PA group, those reporting High-MLPA/Low-LLPA had significantly better visuospatial index scores over 30 months. Beta-coefficients represent difference in index score points at 30-months (marginal mean difference), when compared to the reference [Ref] group. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, T2 diabetes, smoking and alcohol. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; CI, Confidence Interval. L-ML/L-LL PA= comparator. †$p \leq .08$, *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$.

6.3 *APOE*ε4 carrier status, physical activity from mid- to late-life and cognition over time

*APOE*ε4 carrier status did not significantly modify the associations between ML/LL PA groups and global cognition or any sub-domain across all three covariate adjusted models (i.e. ML/LL PA × *APOE* interactions were not statistically significant) (Appendix E, Table E.3). ML/LL PA × *APOE* × time and ML/LL PA × *APOE* × time^2 interactions were statistically significant for delayed memory, *B*=-5.53 (95% CI -10.17, -.89), *B*=1.40 (95% CI .36, 2.44), respectively, indicating significantly different trajectories with regard to Low-MLPA/High-LLPA relative to the lowest PA group being modified by *APOE*ε4 carrier status. Results remained congruent across the three covariate adjusted models (Appendix E, Table E.3).

*APOE*ε4 stratified analyses found, relative to the lowest PA group, those in the highest PA group performed significantly better in global cognition, language, attention and executive function index score over 30 months, with such associations to be limited to *APOE*ε4 non-carriers. Likewise, relative to the lowest PA group, those reporting High-MLPA/Low-LLPA had significantly better index scores in global cognition, visuospatial abilities, and executive function over 30 months, again limited to non-carriers. Differences in overall domain-specific cognitive index scores as a function of ML/LL PA groups were not statistically significant among *APOE*ε4 carriers. Results remained congruent across the three covariate adjusted models (Table 23; Appendix E, Table E.2).

Table 23. Mixed-effects models evaluating the association between mid- to late-life physical activity groups and cognition over 24 months within *APOE*ε4 carriers and non-carriers

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
***APOE4* carrier**							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-3.79 [-9.51, 1.93]	-3.50 [-9.16, 2.17]	-1.69 [-7.30, 3.92]	-7.27 [-13.71, 0.84]	1.25 [-4.20, 6.71]	-2.97 [-9.92, 3.98]	-3.67 [-11.38, 4.05]
H-ML/L-LL PA	1.66 [-2.85, 6.16]	0.56 [-3.89, 5.02]	2.19 [-2.23, 6.61]	0.90 [-4.15, 5.96]	1.04 [-3.25, 5.33]	1.53 [-3.95, 7.00]	-1.55 [-7.69, 4.58]
H-ML/H-LL PA	1.01 [-4.31, 6.32]	-0.08 [-5.32, 5.16]	-0.64 [-5.85, 4.57]	3.59 [-2.36, 9.54]	1.48 [-3.57, 6.53]	-0.09 [-6.54, 6.37]	1.74 [-5.79, 9.26]
***APOE4* non-carrier**							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	0.28 [-2.96, 3.52]	-0.80 [-4.10, 2.50]	-0.52 [-3.22, 2.18]	0.41 [-2.98, 3.80]	-0.46 [-3.33, 2.41]	2.11 [-1.86, 6.09]	0.93 [-3.53, 5.39]
H-ML/L-LL PA	2.50 [-0.05, 5.05]	1.26 [-1.34, 3.86]	1.79 [-0.34, 3.91]	3.25 [0.57, 5.93]*	-0.19 [-2.45, 2.08]	2.22 [-0.91, 5.35]	4.33 [0.72, 7.94]*
H-ML/H-LL PA	3.80 [0.99, 6.61]**	2.45 [-0.41, 5.31]	1.80 [-0.54, 4.14]	0.70 [-2.24, 3.63]	3.74 [1.25, 6.22]**	4.43 [0.98, 7.88]*	4.97 [1.10, 8.83]*

*APOE*ε4 stratified mixed-effects models regression analyses found, relative to the lowest PA group, those in the highest PA group performed significantly better in global cognition, language, attention and executive function index score over 30 months, with such associations to be limited to *APOE*ε4 non-carriers. Likewise, relative to the lowest PA group, those reporting High-MLPA/Low-LLPA had significantly better index scores in global cognition, visuospatial abilities, and executive function over 30 months, again limited to non-carriers. Differences in overall domain-specific cognitive index scores as a function of ML/LL PA groups were not statistically significant among *APOE*ε4 carriers. Beta-coefficients represent difference in index score points at 30-months (marginal mean difference), when compared to the reference [Ref] group. Model 3: Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, T2 diabetes, smoking and alcohol. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; *APOEε4*, Apolipoprotein epsilon 4; CI, Confidence Interval. L-ML/L-LL PA= comparison. *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$.

A significant difference was noted in global cognitive trajectories among those in highest PA group, when compared to the lowest PA group, being augmented in and remaining statistically significant among *APOE*ε4 non-carriers only (Figure 21; Appendix E, Table E.2). Inspection of the trajectories found *APOE*ε4 non-carriers in the highest PA group to exhibit increasing global cognitive scores when compared to the three other groups, followed shortly by those reporting High-MLPA/Low-LLPA. Whereas, those in the lowest PA group displayed with some initial improvement until 18 months, after which scores indicated decline. Differences in global cognitive trajectories across ML/LL PA groups within carriers were not statistically significant.

A statistically significant difference in immediate memory trajectories in the highest PA group and those reporting high-MLPA/Low-LLPA, both displaying with increasing scores over time, relative to those in the lowest PA group who again show initial improvement followed by decline. Group differences were augmented in and only statistically significant among *APOE*ε4 non-carriers, with the exception of a significant difference in the High-MLPA/Low-MLPA group quadratic trajectory among carriers, showing steepest improvements in immediate memory from approximately 12 months when compared to the lowest PA group who exhibit decline from 18 months (Figure 22; Appendix E, Table E.2). Within ε4 carriers, those in the highest PA group and those reporting Low-MLPA/High-LLPA also showed continually increased immediate memory scores, but at a slower rate.

A statistically significant difference in delayed memory trajectories was identified, when comparing those in the lowest PA group to those reporting low midlife/high late-life PA, among *APOE*ε4 carriers only. Upon inspection of the trajectories it appeared that those in the lowest PA group showed increasing scores followed by decline from 18 months. Whereas those in the high midlife/low late-life group, although have lower scores at baseline, show continual improvement (Figure 23; Appendix E, Table E.2).

Differences between ML/LL PA groups on trajectories within all other cognitive domains were non-significant within carriers and non-carriers in the most adjusted model.

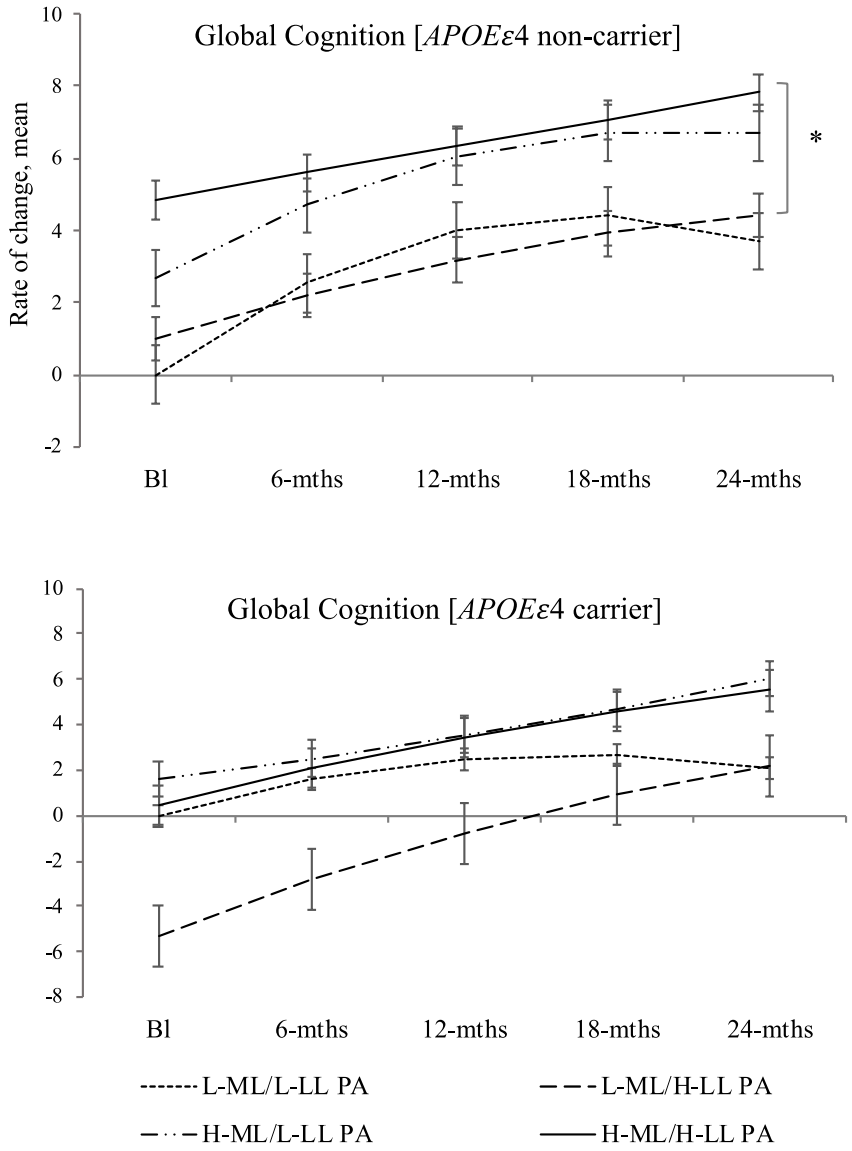


Figure 21. Trajectories of change in global cognition across ML/LL PA groups within *APOE*ε4 carriers versus non-carriers.

Figure shows estimated mean change in global cognitive index scores from baseline to 24 months (higher scores suggested better performance). There is a significant difference in global cognitive trajectories among those in highest PA group, when compared to the lowest PA group, being augmented in and remaining statistically significant among *APOE*ε4 non-carriers only. *$p \leq .05$, indicates difference in rate of change relative to the lowest PA group. The highest active group's mean cognitive index score was 4.1 points higher than those in the lowest PA group at 24 months. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; L-ML/L-LL PA= comparator

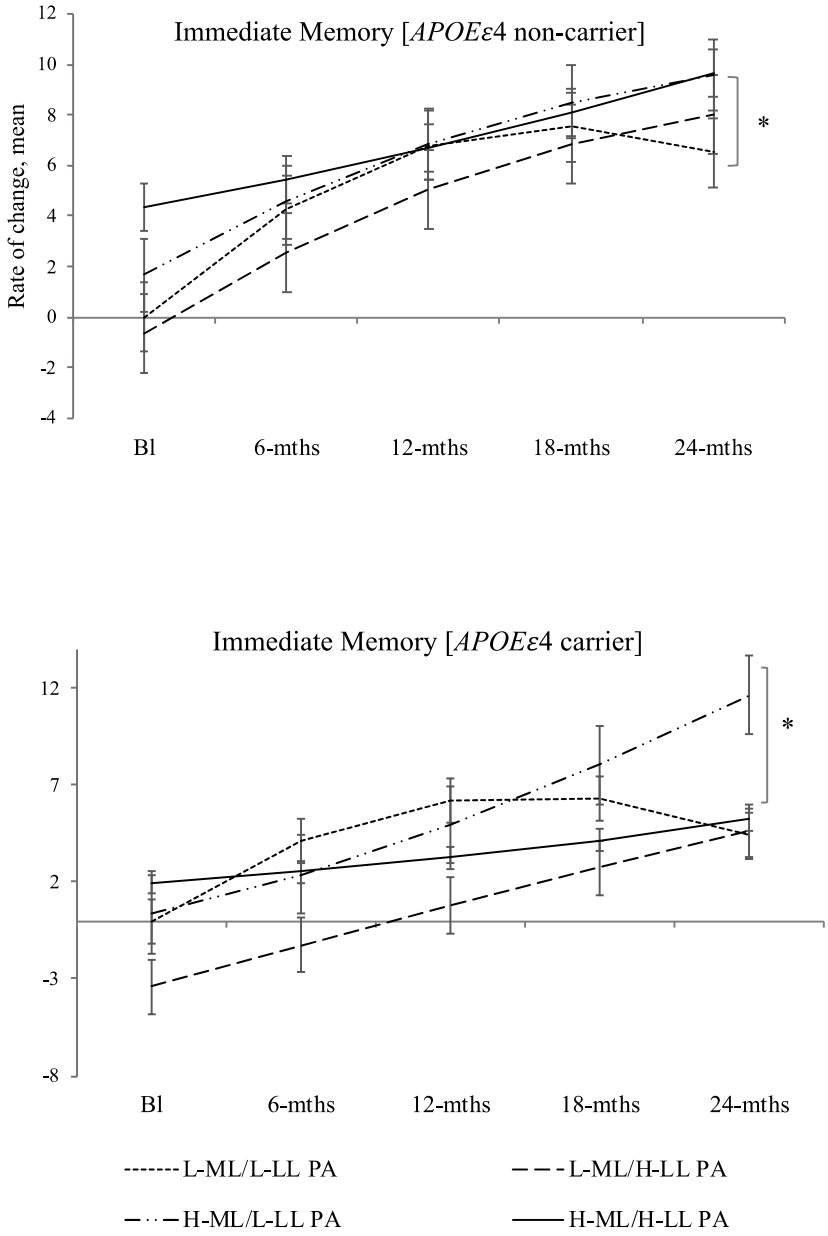


Figure 22. Trajectories of change in immediate memory across ML/LL PA groups within *APOE*ε4 carriers versus non-carriers.

Figure shows estimated mean change in immediate memory index scores from baseline to 24 months (higher scores suggested better performance). Graph depicts a significant difference in immediate memory trajectories among the highest active group (H-ML/H-LL PA) when compared to the lowest active group, among non-carriers, with high PA individuals having a score of 3.2 index points higher than low-active individuals at 24 months. Among carriers, differences in trajectories were statistically significant among those reporting high midlife PA / low late-life PA (H-ML/L-LL PA) versus the lowest PA group (L-ML/L-LL PA), with the former group presenting with a mean score 7.2 point higher than the latter, at 24 months. *$p \leq .05$, indicates difference in rate of change relative to the lowest PA group. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; L-ML/L-LL PA= comparator

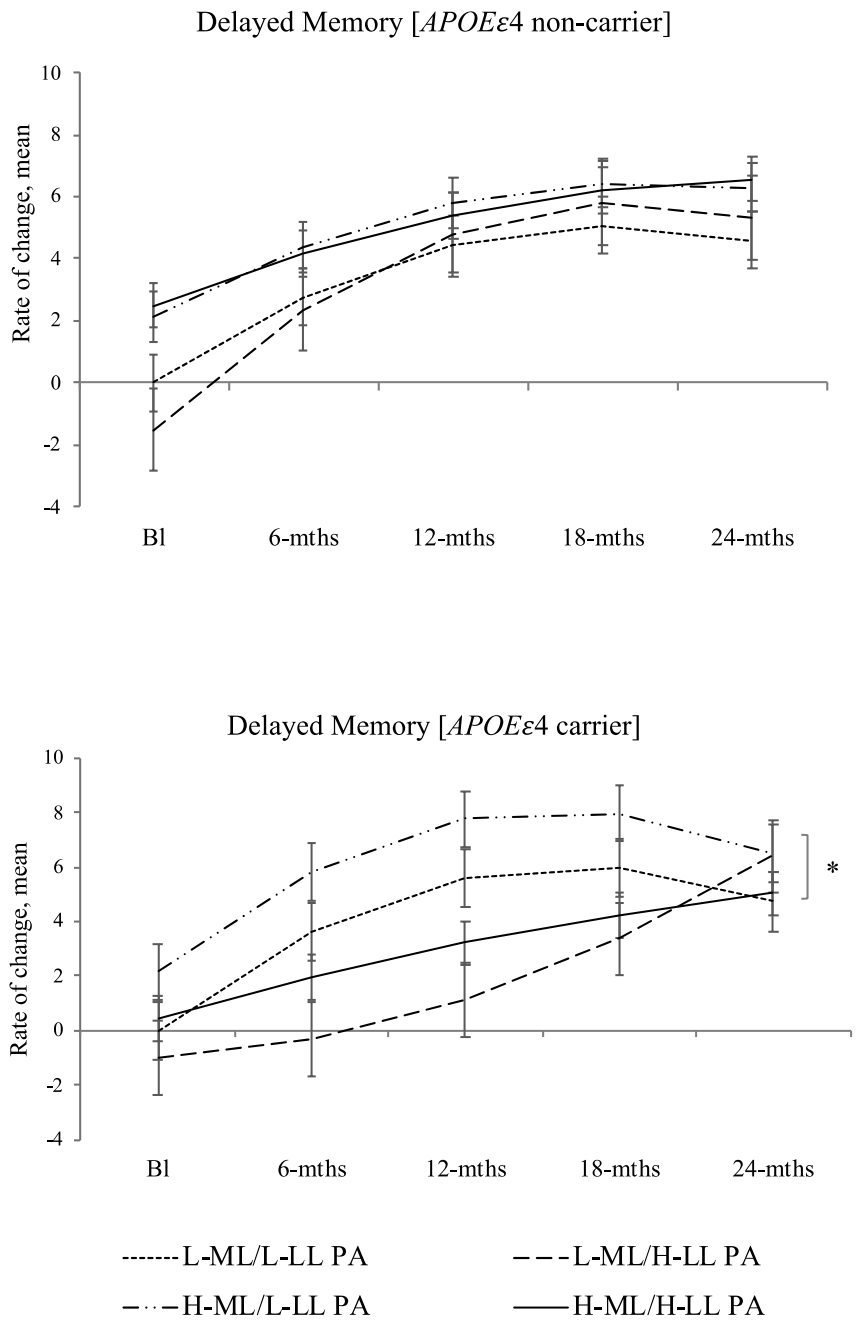


Figure 23. Trajectories of change in delayed memory across ML/LL PA groups within *APOE*ε4 carriers versus non-carriers.

Figure shows estimated mean change in delayed memory index scores from baseline to 24 months (higher scores suggested better performance). A statistically significant difference in delayed memory trajectories was identified, when comparing those in the lowest PA group to those reporting low midlife/high late-life PA, among *APOE*ε4 carriers only, with the former group finishing with an estimated mean score 1.8 points higher than the lowest PA group. *$p \leq .05$, indicates difference in rate of change relative to the lowest PA group. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; L-ML/L-LL PA= comparator

6.4 Physical activity from mid- to late-life and cognition over time among females vs males and those ≥70 years versus <70 years old

6.4.1 Physical activity from mid- to late-life and cognition over time among females versus males

Sex did not statistically modify the associations between ML/LL PA groups and overall multi-domain cognitive function and trajectories (Appendix E, Table E.3). Conversely, sex stratified analyses found some differences within males versus females. Within females, relative to the lowest PA group, language and executive function scores were significantly better within the highest PA group, *B*=3.61 (95% CI 0.50, 6.73) $p \leq .05$ and *B* =4.92 (95% CI .20, 9.63), $p \leq .05$, respectively, over 24 months. Within males, relative to the lowest PA group, those reporting High-MLPA/Low-LLPA had better scores in global cognition, *B*=4.30 (95% CI .95, 7.65), $p \leq .05$, language, *B* =3.94 (95% CI 1.00, 6.89), $p \leq .01$, and attention, *B* =4.42 (95% CI .45, 8.39), $p \leq .05$, over 24 months (Appendix E, Table E.4).

Relative to the lowest PA group, there was a significant difference in trajectories for those reporting Low-MLPA/High-LLPA for global cognition, immediate memory and delayed memory within females only, as well as those reporting High-MLPA/Low-LLPA for immediate memory within both males and females. Likewise, relative to the lowest PA group, there was a significant difference in immediate memory trajectories in the highest PA group over 24 months within both males and females. And lastly, within males, there was a significantly different delayed memory trajectory in the highest PA group, relative to the lowest (Figure 24; Appendix E, Table E.4). Inspection of the trajectories show that, within both males and females, the highest PA group exhibit the highest cognitive scores and steepest rate of improvement in global cognition when compared to the other PA groups. Highest active females, however, show steeper global cognitive improvements when compared to males. Conversely, males in the highest PA group show a steeper rate of improvement in immediate memory scores when compared to females.

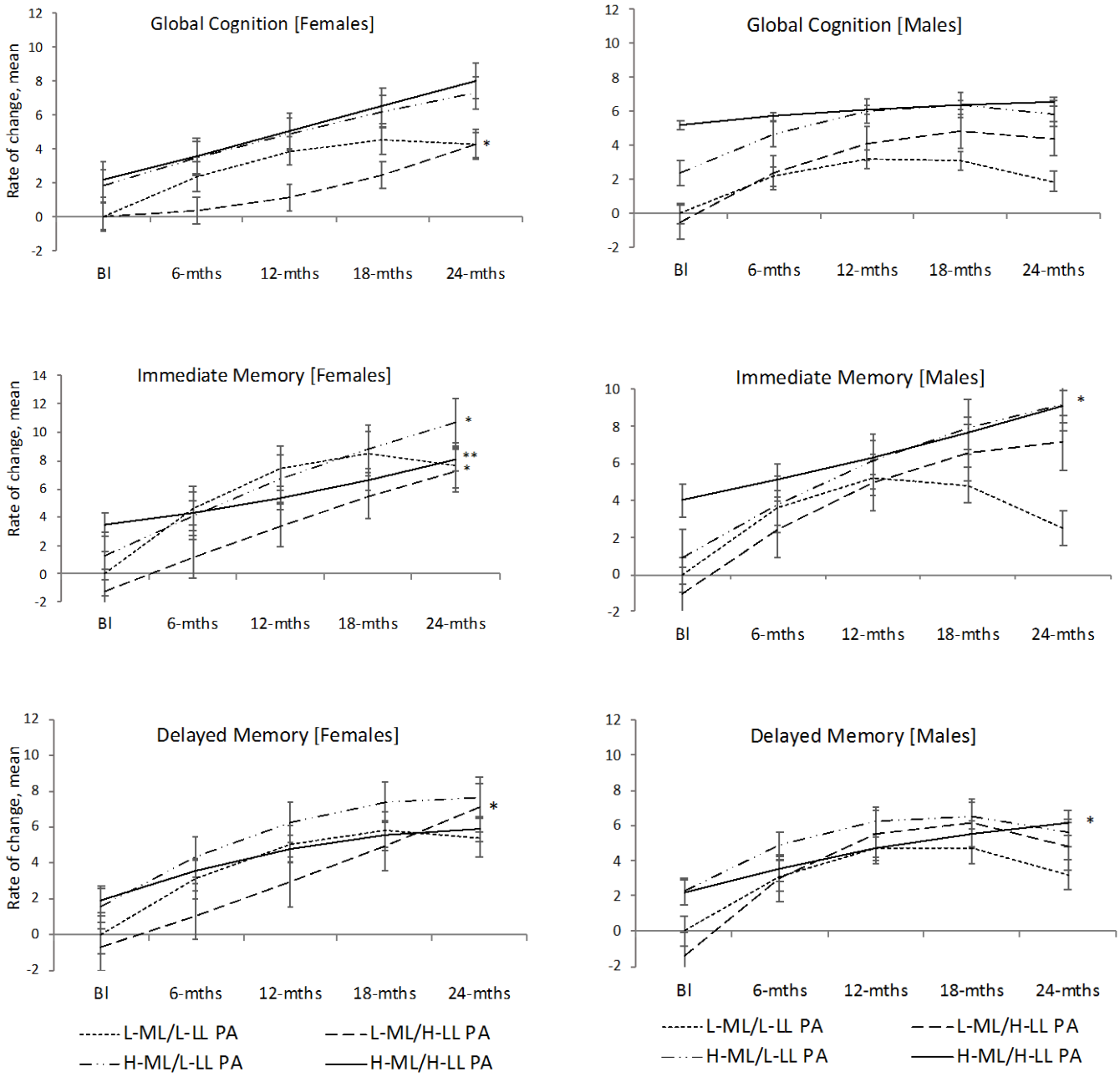


Figure 24. Trajectories of change in immediate memory, delayed memory and global cognition across ML/LL PA groups within males versus females.

Figure shows estimated mean change in immediate memory, delayed memory and global cognitive index scores from baseline to 24 months (higher scores suggested better performance). *$p \leq .05$, indicates difference in rate of change relative to the lowest PA group. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; L-ML/L-LL PA= comparator

6.4.2 Physical activity from mid- to late-life and cognition over time among those ≥70 years versus those <70 years old

Mixed-models regression analyses stratified by those ≥70- versus those <70 years found some group differences. Among those <70 years old, relative to the lowest PA group, those in the highest PA group had better global cognition, B=3.50 (95% CI 0.67, 6.33), $p \leq .05$, language, B=4.20 (95% CI 1.55, 6.85), $p \leq .01$, and executive function index scores, B=5.17 (95% CI 1.24, 9.11) over 24 months. Conversely, there was no significant difference between ML/LL PA groups on global cognition or any sub-domain among individuals ≥ 70 years of age, and effect sizes were notably lower (Appendix E, Table E.5).

Inclusion of ML/LL PA × time and ML/LL PA× time^2 interactions found; relative to the lowest PA group, those in the highest PA group had significantly different immediate memory trajectories among both those ≥70- and <70 years of age, and the High-MLPA/Low-LLPA group had significantly different immediate memory trajectories among those <70 years old, again relative to the lowest PA group. Likewise, relative to the lowest PA group those in the highest PA group had significantly different delayed memory trajectories among those <70 years old only. Finally, relative to the lowest PA group there was a significant difference in visuospatial trajectories among those ≥70 years. All other ML/LL PA by time interactions were non-significant for global cognition or any sub-domain (Appendix E, Table E.5). Inspection of trajectories show that within those <70 years of age, the highest PA group indicate better scores and steeper improvement in immediate memory and delayed memory plus milder decline in visuospatial abilities, whereas the lowest PA group show initial improvement followed by decline in delayed and immediate memory, as well as steeper continual decline for visuospatial abilities relative to the highest PA group.

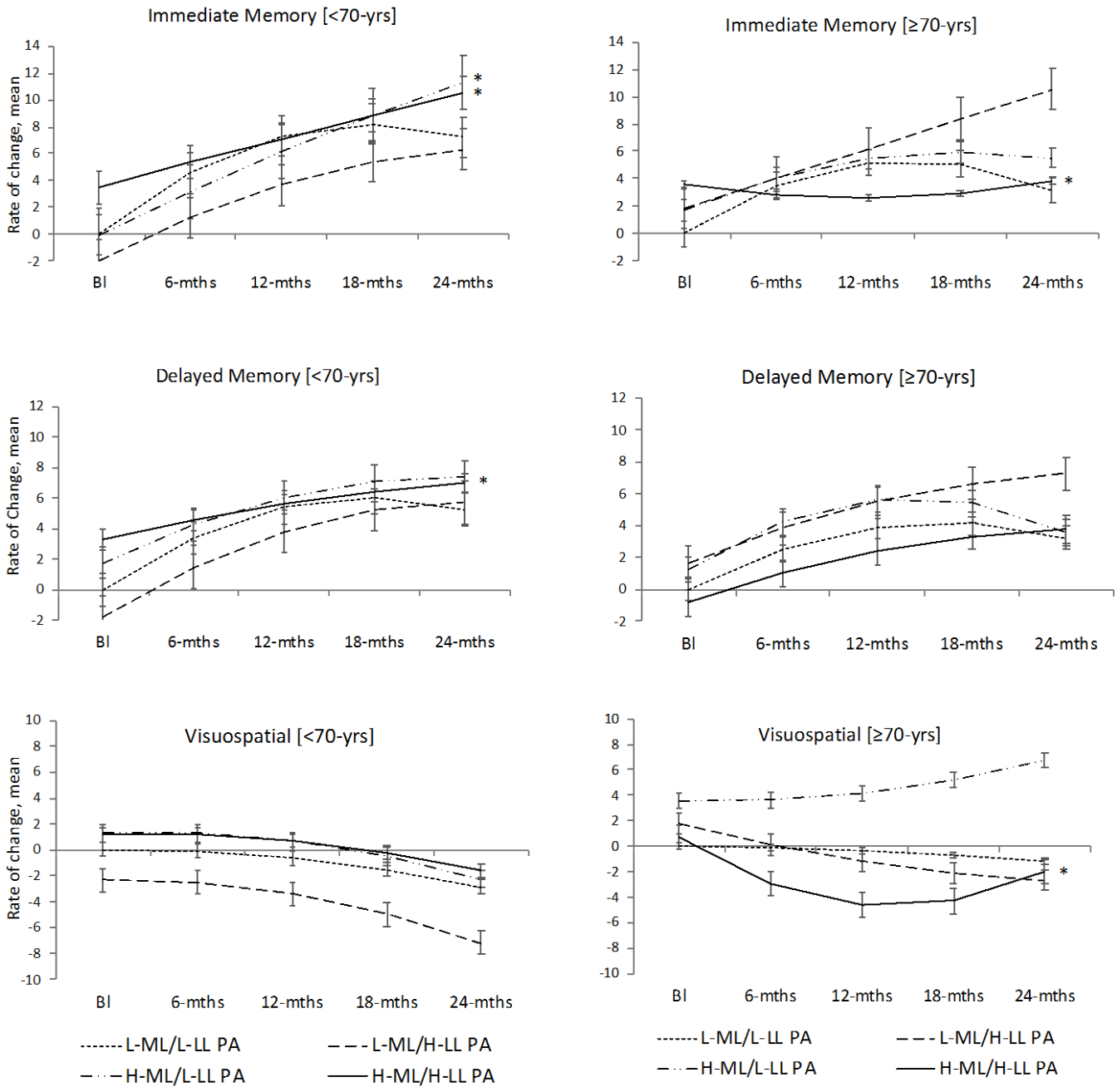


Figure 25. Trajectories of change in immediate memory, delayed memory and visuospatial scores across ML/LL PA groups within those <70-yrs versus ≥70-yrs of age.

Figure shows estimated mean change in immediate memory, delayed memory and visuospatial index scores from baseline to 24 months (higher scores suggested better performance). *$p \leq .05$, indicates difference in rate of change relative to the lowest PA group. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; L-ML/L-LL PA= comparator

6.5 Summary of results

- A statistically significant association between those reporting maintained high leisure PA during both mid- and late-life and overall better scores at 30-months in global cognition, attention, language and executive function relative to those reporting low leisure PA in both mid- and late-life or just one of mid- or late-life, was identified. Associations remained statistically significant and were augmented among non-carriers for global cognition, attention, language and executive function; among males for global cognition, language and attention; and among females for language and executive function, and among those <70 for global cognition, language and executive function.
- Also reported was a statistically significant difference in global cognition, immediate memory and delayed memory index score trajectories such that those reporting low PA in both mid- and late-life show improvements in immediate memory followed by decline from 18 months, relative to the other PA groups and relative to the highest PA group for global cognition and delayed memory who all exhibit with continual improvements over 30 months.
- For global cognition, aforementioned differences in trajectories remained statistically significant and augmented among non-carriers and females. For immediate memory differences in trajectories were augmented among females, ε4 non-carriers and those <70 years of age and for delayed memory associations were augmented among males and those <70 years old.

Chapter 7: Physical activity and cognition over time: discussing the role of PA timing, age, sex and *APOE*ε4 carrier status

Extensive evidence posits physical activity as a promoter of cognitive function and brain health in older age [188, 189, 214, 222, 428-430]. However, very few studies have explored this question in the context of three key non-modifiable factors existent in man: *APOE*ε4 carrier status, age and sex. Whether these factors modify associations between midlife and/or late-life PA and multi-domain cognition over time is largely unknown. The purpose of the results presented in Chapter 4, 5 and 6 were to address these questions, via the following objectives; (i) to evaluate the association between everyday PA (EPA) in late-life, leisure aerobic PA in late-life and midlife independently, and the temporal benefits of PA from mid- to late-life (combined) on overall cognitive function and trajectories across 30 months, (ii) whether *APOE*ε4 carrier status modifies these associations, (iii) if these associations differ among females versus males and among those ≥70 years/old versus those <70 years/old, and (iv) If *APOE*ε4 carrier status modifies the association between midlife and/or late-life PA and cognition differentially within males vs females and among those ≥70 vs <70 years old.

To follow will be a discussion of the findings, in order of objectives (i) to (iv). Table 24 provides a summary of the key findings reported within these Chapters.

Table 24. Summary of significant associations between midlife and/or late-life leisure PA and cognitive function and trajectories over 30 months in the overall cohort and stratified by age, sex and *APOE*ε4 carrier status

PA variable [a]	Global cognition	Immediate memory	Delayed memory	Attention	Language	Visuospatial	Executive function
Differences in overall cognition							
LLPA [b]	-	-	-	X^{NC}	X$^{NC, M/F, <70}$	-	-
MLPA [c]	X$^{NC, M, <70}$	-	X$^{NC, <70}$	X$^{NC, M, ≥70}$	X$^{NC, M, <70}$	M/NC	X$^{NC, F, <70}$
L-ML/H-LL PA [d]	-	-	-	-	-	-	-
H-ML/L-LL PA [d]	-	-	-	-	-	X	-
H-ML/H-LLPA [d]	X$^{NC, M, <70}$	-	-	X$^{NC, M}$	X$^{NC, M/F, <70}$	-	X$^{NC, F, <70}$
Differences in cognitive trajectory							
LL-LPA	X$^{NC, F}$	X^{Δ}	X$^{C*, F}$	-	-	NC*, ≥70	-
MLPA	-	X^{Δ}		-	-	-	-
L-ML/H-LL PA	F	X^{F}	F/C	-	-	-	-
H-ML/L-LL PA	-	X$^{F, <70}$	-	-	-	-	-
H-ML/H-LLPA	X^{NC}	X$^{NC, M/F}$	X^{M}	-	-	≥70	-

[a] Low PA = <2-days per/week of moderate/vigorous aerobic activity; High PA = ≥2-days per/week of moderate/vigorous aerobic activity.
[b] Represents statistically significantly better scores in the high LL-LPA group when compared to the low LL-LPA group.
[c] Represents statistically significantly better scores in the high MLPA group when compared to the low MLPA group.
[d] Represents statistically significantly better scores relative to the L-ML/L-LL PA group.
*LLPA x time x *APOE* = statistically significant for delayed memory and executive function. All other 3-way interactions were not statistically significant (p<.05).
PA x *APOE,* PA x sex all not statistically significant.
Δ Indicates that significant associations remained within all strata (i.e. carriers vs non-carriers, male vs female, ≥70yr vs <70yr old)
Abbreviations: **X**, Indicates significant among entire cohort, **NC**; significant among *APOE*ε4 non-carriers; **C**, significant among *APOE*ε4 carriers; **M**, significant among males; **F**, significant among females; **≥70**, significant among those ≥70 years/old at baseline; **<70**; significant among those <70 years/old at baseline; LLPA, late-life leisure physical activity; MLPA; midlife PA; L-ML/H-LL PA; low midlife PA/high late-life PA; H-ML/L-LL PA; high midlife PA/low late-life PA; H-ML/H-LL PA; high midlife PA/high late-life PA;
p≤.05 = statistically significant.

7.1 Physical activity in midlife versus late-life on cognitive function and trajectories across 30 months

After the most covariate adjustment, individuals reporting high and/or moderate EPA did not differ in overall cognitive function or trajectories when compared to low EPA individuals, within this cohort. Conversely, those reporting high leisure PA in either midlife or late-life were independently associated with higher overall cognitive function scores as well as differences in cognitive trajectories when compared to low PA. Specifically, relative to low PA individuals, those reporting ≥2 occasions per week of moderate/vigorous aerobic leisure PA in late-life had higher overall language and attention index scores. Likewise, those reporting high activity in late-life presented with increasing global cognitive, immediate and delayed memory scores over 30 months whereas those reporting low PA in late-life initially showed some improvement, however, scores eventually exhibited decline from around 18 months.

Regarding midlife PA, those reporting ≥2 occasions per week of moderate/vigorous aerobic leisure PA presented with better overall global cognition, delayed memory, attention, language and executive function scores relative to low MLPA individuals. Differences in trajectories, however, were only observed for immediate memory, again showing that those reporting high MLPA exhibited continual improvements over 30 months, whereas those reporting low MLPA show some improvements before eventually declining in scores after 18 months.

In chronic disease epidemiology it is commonly recognised that risk factors acting independently have an additive rather than synergistic role [356]. It was expected that being active in both midlife and late-life would yield strongest benefits on later-life cognition. Congruently, individuals reporting ≥2 occasions per week of moderate/vigorous aerobic leisure PA in both midlife *and* late-life had higher overall attention, language, executive function and global cognitive scores when compared to the other PA groups (i.e. those reporting low PA in both midlife and late-life or one of either). With regard to cognitive trajectories; individuals reporting high PA in both midlife and late-life presented with continual improvements in immediate memory, delayed memory and global cognition, whereas those in the lowest PA group initially showed an increase in scores, but then decline from around 18 months. Additionally, for immediate memory only, trajectory differences were apparent relative to the lowest PA group when comparing to all other PA groups. These additive findings imply that the combined association of those reporting 'high PA' in both mid- and late-life is larger than their independent associations; hence, highlighting the possibility that being active in both mid- and late-life exhibits enhanced benefit toward reducing risk of cognitive decline. This will be further discussed in the following sections pertaining to modifications via risk groups relating to *APOE*ε4 carriage, sex and age.

In summary, engaging in higher midlife PA seemed to be positively associated with performance in a wider range of cognitive domains, when compared to late-life PA. There is a consistent trend indicating that those who report high midlife/low late-life PA to have overall higher scores and have steeper improvements in global cognition, immediate and delayed memory when compared to those reporting low midlife PA/high late-life PA. Self-reported midlife PA may be a more accurate indicator of activity, not altered by disease (i.e. reduced risk of reverse causality), and in this cohort can be seen to exert positive influences on overall cognitive function across multiple domains. Midlife PA as a viable means of promoting cognitive health in later life is supported by the known link between other midlife risk factors on later-life dementia risk. For example, higher BMI and hypertension during midlife are both linked with an increased risk of dementia, whereas these factors in late-life are, in fact, inversely associated with risk [227, 228]. It may be postulated that being highly active in midlife will also be associated with lower BMI and an absence of hypertension which, in turn, mediate the positive association between PA and cognition. Whether midlife PA benefits later-life cognition regardless of these factors is yet to be elucidated. A published review of cross-sectional and prospective observational studies exploring the role of PA from midlife to late-life reported positive associations between higher midlife PA and later life cognition to be consistently observed for global cognition, executive function and memory and being less commonly significant among tests assessing attention or working memory [229]. A further study followed 10,705 cognitively healthy participants for 14 years and evaluated the role of leisure PA in midlife through to late-life and its associations with cognitive change and risk of dementia [431]. This study reported that relative to low midlife leisure PA, those reporting high PA in midlife were associated with a lower incidence of dementia and lower decline in late-life global cognitive performance, with the same benefits not observed for late-life PA. These findings are not without conjecture; with opposing findings having also been reported [211].

There are important distinctions between the interpretation of results pertaining to overall cognitive function averaged across a time-period versus cognitive trajectories. Overall cognitive function implies a representation of general static cognitive performance during the 30-month period of measurement. However, this information does not capture whether an individual's cognition is beginning to alter across the time course, for example, whether early stages of decline are occurring. This is important when seeking to determine any early indicators of change and whether certain exposures can modify this change. The presence of significant interactions between both midlife and late-life PA and time on memory and global cognition, indicate that change is indeed occurring within these domains. Conversely, midlife and/or late-life PA appear to be associated with non-memory domains when exploring overall cognition. Differences in trajectories based on LLPA, being limited to both delayed and immediate memory domains, leads one to speculate that reverse causation be a contributor [211]. Indeed, PA levels measured ≤10-years prior to dementia diagnosis

may be confounded by early disease symptom, such that those in the earliest prodromal stage could in fact present with reduced PA [211]. Typically, the earliest signs of AD related decline are observed among memory related domains [52], whereas the beneficial effects of PA are often reported among domains of executive function [216]. It may therefore be postulated that those exhibiting the earliest signs of cognitive decline, may have reduced their activity level. An alternative explanation is that in this cohort, LLPA and MLPA are indeed mitigating the earliest stages of age-related decline. Yet, whether late-life PA versus midlife PA is more suitably poised in mitigating clinical cognitive decline in later life can only truly be established via well designed intervention trials. No trial has achieved a follow-up period long enough to observe temporal PA/cognitive associations across decades (i.e. from mid adulthood to later adulthood).

Differences in cognitive function across EPA tertiles were largely insubstantial. This may be due to the sub-components contributing toward the overall EPA score differentially, being a composite of household, occupational and leisure related activity. For example, higher work-related activity has been associated with worse cognitive function [432] and increased risk for dementia [348]. An occupation requiring higher PA is often linked with a lower education (e.g. labourer) and less cognitive demand. One study (also included in the systematic review, Chapter 2), reported that those with high occupational physical activity alongside low education had a higher risk for dementia, whereas their highly educated counterparts did not [348]. Other studies utilising EPA composite scores have not typically included work-related activity, possibly for this reason. For example, all measures used among studies included in the current projects systematic review did not include questions surrounding occupational activity (see Table 2 and 3, pages 63 – 73; Appendix A.8, page 313), despite being comparable in age to the CHARIOT: PRO cohort. Indeed, in the current CHARIOT: PRO cohort, cross-sectional linear regression controlled for age, sex and education found an inverse association between work-related activity and cognitive function, albeit not statistically significant (not reported). Exploring the impact of other PA domains, such as work-related PA, was beyond the scope of this project. However, such an observation may explain the apparent null association between EPA and cognition. Notwithstanding, one study was able to demonstrate that a higher PASE score was positively associated with executive function over time among 600 cognitively healthy older adults [433], with congruent results reported elsewhere [434]. Both studies also included a cohort with a mean age of >70 years, which may result in a higher proportion of those retired and hence less residual confounding as a result of self-reported work PA. As reported in the CHARIOT: PRO cohort, there appears to be minimal difference in cognitive scores as a result of EPA and instead, aerobic leisure PA yields stronger and possibly more meaningful benefits, as hypothesised (see section 1.4.4 of the Introduction Chapter, page 38). Certainly, distinguishing the differential and additive role of PA types would be informative, especially in an older population where habitual PA may be more representative. One should be

cautious when exploring the role of work-related PA, a variable perhaps more suitably considered a confounder as opposed to an exposure.

Overall, engaging in ≥2 occasions of moderate/vigorous leisure PA during midlife and late-life was associated with better overall cognition function and trajectories. In comparison to late-life PA, midlife PA appears to act independently, though may have an additive positive association with later-life cognition. Whether the benefits of endorsing an active lifestyle in midlife surpasses that in later-life is still speculative.

7.2 Physical activity in midlife and/or late-life: the role of *APOE*ε4

*APOE*ε4 stratified analyses found that the positive association between high PA in either midlife and/or late-life and better overall cognitive function averaged across 30 months were consistently augmented in non-carrier individuals. A prudent interpretation would be that, although benefits were observed to be limited to non-carriers, *APOE* x PA interactions were not statistically significant, hence, one cannot conclude with certainty that these are not chance findings. Associations remaining significant among non-carriers may reflect the larger sample size among the non-carrier group (non-carrier n=628 versus carrier n=213), yielding stronger power to detect a statistically significant difference. Indeed, in the CHARIOT: PRO cohort, there is still a consistent trend among high active carriers having higher cognitive scores averaged over 30 months across all cognitive domains when compared to low active carriers. Furthermore, differences in overall cognitive scores among carriers versus non-carriers were no longer evident among those in the highest EPA tertile, indicating that EPA may be mitigating the impact of *APOE*ε4 carriage on lower cognitive function, even among cognitively healthy individuals (see Figure 11, page 140). Comparable studies with smaller sample sizes have detected *APOE*ε4 carrier group differences on PA/cognition associations, as confirmed via statistically significant PA x *APOE* interactions [198, 200, 208, 248]. Of these four studies, three reported the benefits of PA to be augmented among carrier individuals for global cognition, immediate and delayed memory plus executive function [198, 200, 208], whereas one reported PA benefits limited to non-carriers for executive function [248] (see Table 2, page 63-65).

A significant *APOE* x LLPA x time indicated that *APOE*ε4 carrier status significantly modified the positive association between late-life PA and delayed memory trajectories. Specifically, differences in delayed memory trajectories were augmented in and remained statistically significant among the *APOE*ε4 carrier group only, being contradictory to those reported above. This finding may indicate that *APOE*ε4 carriers are, in fact, exhibiting an enhanced benefit of PA on delayed memory trajectories. Alternatively, as delayed memory is a key domain indicative of early signs of clinical decline, this finding may be indicative of reverse causation. Incongruently, differences in

visuospatial trajectories based on high versus low late-life PA were found to be statistically different among non-carriers only. Likewise, although a non-significant interaction, stratified analyses identified differences in global cognitive trajectories based in both late-life PA independently or in combination with high midlife PA to also be augmented among non-carriers. These results highlight the importance of exploring PA benefits among sub-strata, especially risk individuals. Re-visiting the notion of reverse causation, one may expect that if this were true in the current study, the differences in high versus low active individuals on cognitive function would be stronger among those who are at increased risk of dementia, i.e., *APOE*ε4 carriers. With the exception of delayed memory trajectories, this data largely reports the contrary, *possibly* providing a case against this theory within the current cohort.

One other prospective longitudinal study exploring the effect modification of *APOE*ε4 carrier status on late-life PA/cognitive associations reported PA/executive function associations to be augmented among ε4 non-carriers [248]. Utilising data from the Victorian Longitudinal Study (n=532; mean age = 70.4, SD 8.5), executive function trajectories over 9-years were found to be positively associated with EPA (gardening and sport related leisure PA) in the overall cohort. They also reported that differences in trajectories were significantly modified by *APOE*ε4 carrier status whereby stratified analyses found effect sizes to be augmented among non-carriers. A further study demonstrated that partaking in leisure activity ≥3 times a week (of any intensity) resulted in lower risk of delayed memory decline, being augmented among ε4 carriers [200]; congruent to findings reporting here. Results reported within Chapters 4-6 have extended upon findings from these studies by exploring a larger array of cognitive domains, investigating PA timing (i.e. midlife versus late-life) and utilising two different PA measurements (EPA versus leisure aerobic PA).

No study has explored the effect modification by *APOE*ε4 carrier status on associations between midlife PA and later-life cognitive function. A few studies, however, have investigated the effect modification of *APOE*ε4 carrier status on the association between midlife PA and dementia [347, 348, 352] and MCI risk [230]. As already summarised in the systematic review chapter (Table 3, page 67-73), three of these studies utilised the CAIDE cohort to explore differing PA types and follow-up periods yielding a heterogeneous mix of outcomes. One study reported that partaking in two occasions of vigorous PA per week in midlife resulted in a reduced risk of AD 20 years later, associations of which were limited to *APOE*ε4 carriers [347]. However, when extending the follow-up period to 28-years, risk reduction became limited to *APOE*ε4 non-carriers [352]. They also failed to identify any association between work-related PA and risk of AD 20 years later within the overall cohort or among carrier/non-carrier strata [348]. A further study, utilising data from the Mayo Clinic Study of Ageing explored the role of both midlife and late-life PA on MCI risk and whether these associations varied among carriers versus non-carrier. They reported an increased risk for

incident MCI in *APOE*ε4 carriers who reported no moderate or vigorous activity in midlife compared to non-carriers within the same low active group [230]. They also found that *APOE*ε4 carriers who were low active in mid- and late-life had a higher risk of incident MCI as compared to non-carries and that non-carriers who were active had the lowest risk of MCI when compared to both carriers and non-carriers.

Further RCT's are required to determine whether there are any differences in response to PA as a function of *APOE*ε4 carrier status. As previously summarised in Chapter 2 (Table 1, page 61), only three RCT's have explored effect modification of PA by *APOE*ε4 carrier status on cognitive function, all reporting non-significant associations between PA intervention and improved cognitive function based on *APOE*ε4 carrier status [341-343]. A short intervention time; ranging from four to six months, coupled with small sample sizes, may not yield adequate power to detect a true effect within *APOE*ε4 carrier/non-carrier sub-strata. The Physical Activity and Alzheimer's Disease (PAAD) study, an experimental study without a control group, explored the effect of PA intervention on cognition as a function of *APOE*ε4 carrier status [253]. Participants with a family history of dementia (age 50-65 years, n=54) attended three sessions per week of moderate aerobic PA/strength training for eight months Significant improvement in immediate and delayed memory, but not attention and executive function were reported. They also found that *APOE*ε4 carrier status did not modify the effect of PA intervention on any cognitive domain. It is important to note, however, that due to a lack of control group, this study is unable to confirm absence of practice effects or causality. Regarding the association between PA and risk of dementia as a function of *APOE*ε4 carrier status, results were reasonably heterogeneous. Three studies reported the association to be augmented among carriers [345, 347, 349]; five studies among non-carriers [113, 337, 351, 352, 354], with three studies reporting the association between PA and risk of incident dementia/AD/VaD [344, 353] and MCI [230] to be present among both carriers and non-carriers. Three other studies reported no association between PA and risk of all cause dementia, AD and VaD within carriers, non-carriers or the overall cohort [346, 348, 350].

Overall, there is a highly heterogeneous mix of results indicating that the benefits of PA may be seen among both carriers and non-carriers. Clearly, more RCT's of an enhanced design are required to fully elucidate the differential impact of PA on cognition among carriers versus non-carriers. Results reported in this thesis may indicate that the benefits of PA in both midlife and late life on multi-domain cognition are observed among both groups, although possibly with a larger effect size among non-carrier individuals. Nonetheless, in an observational context, a larger sample size and ideally an objective measure of PA is required to further sanction this statement.

7.3 Physical activity in midlife and/or late-life: the role of sex

Females were found to perform significantly better than males in global cognition, delayed and immediate memory, and language. Whereas males performed significantly better than females in visuospatial abilities. Females also had significantly steeper cognitive improvements in global cognition, language and executive function, and males had significantly steeper improvements in immediate memory and visuospatial abilities, relative to one another. These findings mirror those previously reported, whereby females have been shown to outperform males on tests of episodic memory, executive functions and language [435-437]. Whereas, in both rodent and human studies, males perform better in tasks of spatial learning and spatial memory compared to females [438-440]. The expectation that females would exhibit an augmented beneficial association between PA and cognition was partly met. In the CHARIOT: PRO cohort, females appeared to display an augmented response to PA on cognitive trajectories, but less so for overall cognitive function averaged across 30 months (see Table 24, page 179). Specifically, significant differences in global cognitive and delayed memory trajectories as a function of LLPA were augmented and remained significant among females only. Differences in global cognitive trajectories based on LLPA and based on the highest versus lowest ML/LL PA groups became augmented in and remained significant among females only. Regarding overall cognitive function averaged across 30 months, differences in executive function as a result of both MLPA and LLPA plus ML/LL PA groups (highest vs lowest), remained significant among females. All other differences were limited to males.

Although sex stratified analyses identified sex differences in PA/cognitive associations, no PA x sex interaction or PA x sex x time interactions were statistically significant. Whether the association with midlife and/or late-life PA on multi-domain cognitive function and trajectories differed as a function of sex in the CHARIOT: PRO cohort is speculative. However, previous research consistently indicates sex differences in response to PA on cognition, whereby females typically present with augmented benefits, especially for executive function [117, 441]. Females consistently show an augmented PA association on global cognitive and immediate memory trajectories based on both LLPA and MLPA plus delayed memory trajectories based on LLPA. Differences in cognitive trajectories, as opposed to overall cognitive function, across 30 months, provides a fuller picture of an individual or sub-group cognitive profile in the context of change over time. Change over time is also a more sensitive indicator of whether decline may be occurring; hence, the fact females are showing augmented PA benefits specifically within trajectory analyses may be indicating exactly that, as congruently reported extensively elsewhere that females are at higher risk of dementia (see Introduction section 1.3.1.3).

Differences in cognitive function and trajectories as a function of *APOE*ε4 carrier status were non-significant among males, whereas they remained significant among females. This finding further

supports the widely reported finding that ε4 allele carriage is not associated with cognitive decline among males but remains so among females [126, 131, 442]. Based on these reports, it was hypothesised that there would be augmented PA/cognitive associations among ε4 carrier females. However, contrary to this hypothesis, *APOE* x sex interactions among males and females were all non-significant, indicating that *APOE*ε4 carrier status did not modify PA/cognitive associations among both sexes. Just one other study has examined the *APOE*ε4 carrier moderation on sex differences in PA/cognitive associations. This study is a follow-up from that previously described [248], who reported that the associations between EPA and executive function change over 9-years were limited to non-carriers within the Victorian Longitudinal Study (n=514, mean age [SD] = 70.5 [4.4]). They further explored whether such associations varied among males versus females and if *APOE*ε4 carrier status modified these associations [443]. Firstly, they identified that the beneficial association between EPA and EF change were limited to females, with a statistically significant EPA x sex interaction. Secondly, they observed a significant sex x *APOE* interaction effect such that the combination of lower genetic risk and higher EPA benefitted females but not males. Significant sex x *APOE* interactions were not replicated in the CHARIOT: PRO cohort. However, it was congruently identified that the positive association between PA and overall executive function were present among females only.

There is now considerable biological evidence supporting claims of sex specific responses to PA, specifically relating to sex steroid hormones. An extensive body of literature support the roles of estradiol (estrogen) and androgen (testosterone) in neuroplasticity and preservation of cognitive function [439, 444, 445]. Females with higher levels of remaining estradiol in menopause present with better performance in tasks measuring episodic and semantic memory, executive function, and verbal memory relative to menopausal females with lower estradiol [446]. The same associations for androgen among both males and females were mixed, with evidence finding little to no association between androgen and cognitive function in older males [447, 448]. Indeed, PA has been shown to upregulate both estradiol and androgen in not just the periphery but also in muscles and the brain [449, 450], possibly in a sex-dependant manner. Furthermore, sex differences in response to earlier life hormones on brain structure and function, as well as peripheral organs such as muscles, adipose tissue, liver and kidneys [451, 452] bring about differing response to PA later in life. For example, females have higher rates of cerebral blood flow, males have higher glucose metabolism in limbic regions plus an average higher brain volume, likewise, males and females differ in grey matter volumes of several brain regions [438, 453].

The female advantage in response to PA may be owing to sex differences in BDNF signalling. Although BDNF levels decline with age, the decline in relation to cognitive impairment is only reported among older females [454], suggesting a greater link between BDNF in females versus males. As high PA is commonly linked with upregulated BDNF, possibly females exhibit enhanced

PA benefit via this upregulation and cascade of events. Estradiol directly upregulates BDNF levels via an estrogen response-like element within the promoter region of the BDNF gene which is related to increased BDNF expression in the cortex and hippocampus of females [455]. The complex relationship between BDNF and testosterone, however, has been less studied and may differ to that which is seen with estradiol.

Positive associations between both midlife/late-life PA and overall cognitive function appear to be augmented among males, with the exception of executive function whereby females display enhanced benefit. Whereas the differences in memory and global cognitive trajectories as a function of LLPA were limited to females. These findings, again, raise important questions surrounding the differences in observation of *overall cognitive function* averaged across a certain time-frame versus the exploration of *cognitive trajectories* across time. One may argue a more meaningful indicator would be change or trajectories. Results reported here indeed indicate that females exhibit an enhanced benefit of PA on memory and global cognitive trajectory whereby PA may be seen to mitigate age-related declines. It must be noted, however, that all sex x PA x time interactions did not reach statistical significance, hence, this conclusion is only speculative and driven by congruently reported previous research.

7.4 Physical activity in midlife and/or late-life: the role of age

Cognitive performance was generally lower among those ≥70 years when compared to those <70 years old. It was expected that PA/cognitive associations would differ among individuals ≥70 years of age at baseline versus those <70 years old. As hypothesised, age stratified analyses identified an augmented and statistically significant association between LLPA and overall language scores, MLPA and overall delayed memory, language, and executive function index scores, and ML/LL PA groups and overall global cognitive, language and executive function scores averaged across 30 months to be limited to those <70 years old. Conversely, those ≥70 years had an augmented and retained significant association between MLPA and attention scores. Significant differences in immediate memory trajectories as a function of midlife and late-life PA were retained for both age groups. Differences in visuospatial trajectories as a function of LLPA were augmented in and become significant among those ≥70 years old. Furthermore, differences in trajectories among the lowest ML/LL PA group, when compared to the highest, remained significant for immediate memory and also became significant for visuospatial abilities among those ≥70 years old only.

The effect of *APOE*ε4 carrier status on risk of decline reportedly varies as a function of an individual's age (see introduction, section 1.5.3); hence, it was expected that PA x *APOE* interactions would vary among age groups. Contrary to this hypothesis, there was no effect modification of *APOE*ε4 carrier status on PA/cognitive associations as a function of age. Only one

other study has investigated whether the effect of *APOE*ε4 carrier status on the association between PA and cognition differs as a function of older age categories [248]. This study (n=514) reported the highest risk for decline in EF to be among low active *APOE*ε4 carriers over the age of 75 years, when compared to non-carrier, low active 75+ individuals. They also reported the benefits of PA on EF to be present only among non-carriers aged between 53-65 and 65-75, but to be no longer present among those ≥75 years of age. Although the age categories between CHARIOT: PRO and the above study differ by five years, similar results are reported here. Possibly the risk of being in an older-age category on cognitive decline may nullify the cognitive benefits of high PA, even if active in both mid- and late-life.

Very few studies have explicitly investigated the differential association between PA and cognitive function and trajectories among healthy older adults divided into young old / older old age categories. Of those who have, outcomes have predominantly indicated PA/cognitive benefits to be augmented among a younger old age category (e.g. ≤70 years) [199, 248]. One study, however, reported the opposite. This prospective longitudinal study (n=3,714), including combined cohorts from the Framingham Study Original and the Offspring cohort (mean age [SD] = 70 [7]), reported that PA was significantly associated with decreased dementia risk, and that this observation was augmented among those who were 75 years and older [351]. However, owing to non-significant PA x age interaction, these findings only indicate a trend difference in response to PA based on these age groups. One may also question if they were due to reverse causation (especially as the results were augmented among the older age group), however, as the follow-up period was over a decade this is unlikely.

It may be suggested when exploring the role of PA on key markers of age-related decline, that earlier PA (e.g. midlife) be more beneficial among individuals above a certain age threshold (e.g. 70 years). One explanation is that the ability of PA to attenuate such factors as Aβ and promote cognitive reserve is most beneficial within a specific window of time, prior to or during an earlier stage of senescence and/or before pathology is beyond amelioration [82, 83]. For example, the association between brain Aβ levels and PA in midlife may indeed reflect a time-sensitive neural process in which midlife practices have a greater influence on AD pathology, and hence cognitive health, than later-life practices. Sanctioning these findings, one study demonstrated significant age x PA interactions on Aβ burden, glucose metabolism and hippocampal volume such that, with advancing age, physically active individuals exhibited a reduced degree of biomarker alterations when compared to inactive individuals [328]. Furthermore, they reported the same interactions for cognition such that the reduced impact of PA on AD biomarkers with older age were found to reflect reduced PA/cognitive associations for immediate memory and visuospatial abilities. It has also been speculate that those >70-75 years of age who are willing and able to participate may be of superior health, and may not respond as hypothesised to preventative strategies [367]. Once

again, RCT's are required to confirm such speculations. Capturing multi-domain cognitive data alongside biomarker data would elucidate the impact of cognitive reserve among an older-old population demonstrating sustained cognitive health despite exposure to the largest AD risk factor: age.

7.5 Associations between PA and cognitive sub-domains

An emphasis and strength of the current project was to determine whether PA/cognitive associations varied across specific domains of cognition. The temporal decline of cognition as a departure from normal ageing is domain-specific. Furthermore, the associations and effects of PA on specific domains of cognition among cognitively healthy individuals have most commonly been reported among tests assessing aspects of executive function and episodic memory [219]. As defined by the RBANS, domains examined were language, attention, visuospatial abilities, immediate memory and delayed memory. Global cognition was also included as an outcome variable and is simply a composite score of all sub-domains within the RBANS. Likewise, to include a measure of executive function via an assessment designed specifically to measure such, the executive function module of the NAB was used.

Although the composite index scores propose to assess these cognitive domains independently from one another and have been consistently referred to as such throughout this thesis, this labelling is not definitive. Indeed, it is naïve to assume that cognitive tests are highly specific to a given function. In fact, most cognitive tests implicate multiple cognitive processes. Consequently, throughout published studies, the same test has often been used to justify dysfunction in diverse cognitive domains. Neuropsychological testing can only assume that an individual with a given cognitive deficit will perform poorly on a test claiming to assess such domain; however, whether a person performing poorly on such an assessment indicates a deficit limited to such, is erroneous. The below discussion will be divided among sub-headings alluding to each domain as named within this thesis based on RBANS and NAB classification, however, the discussion will also consider how tests used to define these domains may also be implicating other aspects of cognition.

7.5.1 Immediate and Delayed Memory

Leisure aerobic PA in both midlife and late-life were found to be consistently associated with delayed and immediate memory trajectories. These results are consistent with the hypothesis that PA exhibits associations with memory domains (as well as executive function domains) with the largest effect size when compared to other domains. With regard to overall cognitive function averaged across 30 months, only MLPA was associated with overall higher delayed memory scores. Overall delayed memory index scores were significantly higher among those reporting high MLPA versus low. Likewise, differences in delayed memory trajectories were noted among those reporting

high LLPA as well as those in the highest ML/LL PA group, relative to the lowest PA groups. No differences in trajectories were observed as a function of MLPA. There were no differences in overall immediate memory scores across 30 months based on LLPA, MLPA or ML/LL PA groups. However, there were strong and consistently significant group differences in immediate memory trajectories. Differences with the largest effect sizes were observed for those reporting high PA in both mid- and late-life exhibiting continual improvements.

Episodic memory consistently appears to be a robust predictor of dementia in prospective studies, with changes being detectable some 7 to 15 years prior to diagnosis as supported by meta-analyses [51, 456]. Consequently, differences in trajectories among memory domains as a function of PA was anticipated. In a cognitively healthy, reasonably young cohort, it may be expected that any differences in cognitive trajectories be present only among domains known to show early stages of decline. Indeed, no other cognitive domain found differences in trajectories based on PA levels except for global cognition: 50% of which is comprised of sub-tests contributing toward delayed memory and immediate memory (see Table 7, page 97).

Although delayed memory is more typically linked with earlier decline, associations within this cohort appeared to be stronger and more consistent for immediate memory. Researchers have begun focussing on disentangling the differences in immediate and delayed memory in their ability to indicate early stages of decline. Numerous studies emphasise the importance of decline in learning with repeated trials (as reflected in a total learning score across immediate recall trials). Short and long-term recall performance in tests of verbal episodic memory are early indicators of AD, although there is still no consensus as to which measure is affected earlier [457-459]. One prominent study, utilising data from the Baltimore Longitudinal Study (n=895, mean age 70.1 (8.4)), found that performance in immediate versus delayed episodic memory recall varied according to the temporal stage of disease progression [54]. Contrary to the common view that delayed memory is more predictive of dementia, they found that immediate verbal recall measures were the first to decline in pre-clinical AD followed by delayed recall on the same test closer to MCI diagnosis [54]. Congruent results have been reported elsewhere [460]. These findings suggest that immediate recall is more informative during earlier preclinical stages of disease for risk assessment and monitoring, whereas delayed memory are better indicators later in the disease stage. Results presented in this thesis may be interpreted with regard to this theory. Among other studies, however, there is no consensus with regard to the earliest indicator or predictive ability of immediate versus delayed memory. One study found verbal delayed recall to be the most significant predictor at 5- to 10- years prior to diagnosis [461], whereas another study reported the short-delayed cued recall score on a verbal memory task to more optimally discriminate between stable and declining prodromal individuals than a delayed memory task [462]. Yet another study reported both total learning and delayed recall of the RAVLT (a word list-learning task) to predict

progression to AD and better classify between MCI and normal ageing [463]. However, unlike the Baltimore Longitudinal Study, these other studies did not consider the possibility that individuals in the same cohort may be at differing stages of disease progression. Group-wise comparisons often do not take the temporal disease stage into account and treat individuals as the same. Such heterogeneities within the clinical diagnostic groups may bias results. In the CHARIOT: PRO cohort, all individuals were previously screened to be cognitively healthy. Based on the observed cognitive trajectories (i.e. very few scores at 30 months were below the initial score at baseline), it is likely that no individual in the 30-month follow-up converted to MCI. Although differences in immediate memory trajectories provided a stronger association with both mid- and late-life PA. Delayed memory also found significant differences regarding LLPA and overall PA groups, albeit smaller effect sizes and becoming non-significant in age stratified analysis.

7.5.2 Executive function, attention and language

Individuals reporting high MLPA had significantly higher executive function (EF) scores averaged across 30 months when compared to low MLPA individuals. Likewise, when compared to the lowest PA group (those reporting low mid- and late-life PA), the highest mid- to late-life PA group also had significantly better EF scores. There were no differences in overall EF scores based on LLPA or trajectories based on any PA group.

Emerging evidence from prospective cohort studies implicates executive dysfunction during preclinical phases [464-467]. In fact, one study of African and European Americans (n=2,000) reported that a reduction in EF abilities, as measured by the symbols digits modalities test (also in the RBANS under attention domain) was detected 18 years prior to diagnosis [464]. Likewise, meta-analyses have reported PA to exhibit strongest effects on executive function as previously summarised (see section 1.4.2.2). Ageing research suggests that physical activity seems to exert benefits on higher order cognitive-executive processes [216]. Executive function is an umbrella term comprising an array of cognitive functions including verbal reasoning, problem-solving, planning, sequencing, the ability to sustain attention, resistance to interference, multitasking and cognitive flexibility [468]. Tests designed for executive functioning are primarily based on theories or models and appear to be sensitive to prefrontal cortex activity. However, there is still no gold standard for assessment of executive function [468]. Sub-tests used to create the EF index score from NAB included mazes – being a measure of planning, foresight, sustained attention, motor visuospatial abilities; Judgement – testing the judgment of appropriate behaviour in everyday activities; Word generation – a measure of lexical fluency (producing three-letter words from a list of letters); and Categories – measuring attention, concept formation, cognitive flexibility and generativity. A wide range of cognitive abilities are being assessed and combined into one broad EF domain. The strength of this indicator is that EF is indeed a broad measure of cognitive performance, and hence, speculating that this domain allows the capturing of overall and

generalised EF performance. A limitation, however, is that it will most certainly overlap with performance across other tests targeting different cognitive domains within the RBANS. This may not be unexpected or problematic but must be acknowledged and interpreted as such.

Attentional performance may also be regarded as a form of executive function where both component sub-tests are measures of such. Digit symbol substitution (referred to as coding in RBANS) is often labelled as a measure of executive function. It also measures attention switching and working memory. Number generation, the second sub-test within the attention domain, has been referred to as an assessment of attention, working memory and again, elements of executive function [468]. As with language, attention scores averaged across 30 months were significantly better in the high active LLPA, MLPA and ML/LL PA groups, when compared to the low active groups, once more, with the largest effect size for those reporting high PA in both mid- and late-life. Differences in trajectories were not significant. There is evidence for age deficits in attentional tasks, especially those involving executive attention, and that they can be reduced by aerobic activity [469]. Congruent with current findings, a Cochrane review of eleven RCTs examining the effectiveness of physical activity on cognitive function found large effects of aerobic exercise on motor function and auditory attention, and moderate effects on cognitive speed and visual attention [215].

Relative to low PA in midlife, late-life and the lowest mid- to late-life PA group, language scores averaged across 30 months were significantly better in the high active groups, with the largest effect size for those reporting high PA in both mid- and late-life, as expected. There were no differences in language trajectories, however, as a function of different PA categories. Sub-tests used to assess the language domain included verbal semantic fluency and picture naming. Both tests are also typically used to examine certain aspects of semantic memory. Verbal category fluency measures both verbal fluency and knowledge of different categories (i.e. fruit is a category and apples, pears, etc. are the words to be listed within the category), including how quickly and readily the participant is able to produce words from a semantic category. Semantic memory also appears to be an early indicator of future MCI, according to cohort studies [47]. Both episodic and semantic forms of memory are processed in sub-regions of the medial temporal cortex. These regions are affected at difference stages of neurofibrillary degeneration, which may affect the timing of memory impairments. The starting point is the transentorhinal cortex (perirhinal cortex), being responsible for semantic processing, followed by the entorhinal cortex and hippocampus, involved in episodic memory. Theoretically, changes in semantic memory may precede episodic memory as previously reported [470, 471]. However, majority of studies still support episodic memory as the earliest indicator. Assessment of semantic memory is often poor, and typically implicating multiple cognitive functions. For example, tests used within the RBANS are appropriately considered more indicative of language performance whilst capturing aspects of semantic memory. Semantic verbal

fluency has also been considered to encompass an aspect of executive functioning. Prospective studies of healthy older adults have also reported PA to be associated with verbal fluency. One study reported that older participants with mild cognitive impairment who partook in an aerobic exercise intervention found a beneficial effect on semantic fluency when compared to a contact controlled cognitive intervention group [472]. A further study also demonstrated improved semantic verbal fluency among previously sedentary older adults partaking in just 12-weeks of aerobic activity when compared to a non-intervention control group [473]. The same group undertook a follow-up study to identify the neural underpinnings in semantic fluency via fMRI, examining right inferior frontal gyrus activity during language tasks [474]. As expected, inferior frontal gyrus activity was reduced in the active group indicating improved semantic verbal fluency performance. Overall, results reported here and within other studies indicate that PA can indeed improve or preserve verbal semantic fluency performance.

7.5.3 Visuospatial abilities

Differences in visuospatial abilities as a function of EPA tertiles were in the opposite direction to that hypothesised; those in the lowest EPA tertile had better overall scores across 30 months when compared to those in the highest EPA tertile. Differences in overall VS scores and trajectories as a function of midlife and/or late-life PA only became significant within certain sub-strata. Specifically, associations between MLPA and overall VS function became significant among males and *APOE*ε4 non-carriers. Differences between cognitive trajectories were only significant among individuals ≥70 years old and among *APOE*ε4 non-carriers, such that those reporting low LLPA initially display with decline, followed by some improvements after 18 months, whereas low LLPA ≥70-year olds show continual decline.

Visuospatial abilities were the only scores found to exhibit very little improvement and generally, marginal mean index scores at 30 months were considerably lower when compared to index scores across other domains (e.g. see Figure 15, page 154). Incongruently, test-retest stability between alternate RBANS forms A and B indicated most practice effects among visuospatial index score outcomes (coefficient 0.80) in a validation study among individuals 20-89 years of age (RBANS manual). Why, in the current cohort, an absence of practice effects appeared to be evident may only be speculative. The two sub-tests contributing toward this index score is a task involving the drawn copy of a complex design (figure copy), assessing VS and attentional abilities. The other assessment, line orientation, tests the ability to visually determine orientation; difficulty in this task indicates more severe visuospatial impairment unlikely to be observed in a healthy cohort. The scoring of the figure copy test requires judgement, possibly yielding variability and deviation of such, dependent upon the assessor. No other scoring methodology within the RBANS is open to such variability; all other sub-tests simply requires a clear correct/incorrect score. Another

explanation may be that, in fact, this measure of visuospatial/construction ability is indicating very early stages of age-related cognitive decline, as has been reported previously (see Introduction, section 1.2). Why differences in overall scores and trajectories based on physical activity groups are observed among just some sub-strata raise question as whether these findings are by chance. This speculation is sanctioned by the unexpected link with physical activity – on some occasions finding those in the highest PA group to exhibit worse VS scores, whereas on other occasions exhibiting better VS scores. Very few PA studies have included measures of visuospatial/construction and those including such have used differing assessments to those in the current study. Two studies [207, 208] in the systematic review included a measure of speeded VS ability both via the trail making test form A – whereby numbered circles randomly placed across a page must be connected in order. No associations were identified between performance on this task and PA.

Overall, the association between PA and visuospatial abilities, as measured via the figure copy and line orientation assessment, remain inconclusive and warrant cautious interpretation. Spatial abilities, specifically the ability to comprehend spatial relations between objects or images [475, 476], may indeed be an early indication of pre-clinical decline. Given this evidence, further studies should investigate the association between PA and a wider range of VS measures to determine if PA is able to attenuate such decline.

7.5.4 Global cognition

The global cognition index score is an additive indicator of overall cognitive function as assessed via the RBANS. One may expect it only be significantly associated with PA when the combined or additive associations between its sub-domains are large enough to exhibit a 'global cognitive' association.

Late-life EPA and LLPA were both not associated with overall global cognitive function averaged across 30 months. Conversely, individuals reporting high MLPA as well as those reporting high PA in both midlife and late-life had significantly better overall global cognitive scores across 30 months when compared to the lowest PA groups. Differences in global cognitive trajectories over 30 months found that those reporting high late-life PA as well as those reporting high PA in both mid- and late-life show increasing global cognitive index scores over 30 months. The lowest PA groups initially display with some improvements up to around 18 months, after which scores indicate decline.

It was expected that differences in overall global cognitive scores as a function of midlife PA or ML/LL PA groups were observed, as significant differences were also observed among majority of the sub-domains making up this global cognitive composite measure. Likewise, differences in global cognitive trajectories for late-life leisure PA and those reporting high midlife and late-life

leisure PA combined is synonymous with the fact that differences based on these PA variables were also observed for delayed memory and immediate memory. A positive association between PA and global cognition only strengthens the conclusion that PA indeed exhibits beneficial effects on overall cognitive health and vitality in later life. Numerous observational studies have reported congruent results, as highlighted in the systematic review within this thesis (see section 2.3.2.2).

7.5.5 Practice effects in longitudinal cognitive change

Repeated testing among a cognitively healthy cohort can be susceptible to practice effects [477, 478]; typically defined as improvements in performance due to prior test exposure as opposed to actual cognitive improvements [478, 479]. The absence of practice effects or reduced practice effect over time may signal cognitive decline, whereas improvements may indicate cognitive stability [480]. Individuals within the CHARIOT: PRO study, regardless of PA group, *APOE*ε4 carrier status, age or sex, exhibited increased cognitive scores from baseline, with some individuals continually improving whereas others indicating a plateau in scores followed by the possible beginnings of cognitive decline. These observations are a typical example of practice effects and have been interpreted as such. Specifically, in this cohort, low PA appears to mitigate practice effects, usually appearing to plateau at approximately 12 months and shifting to decline from 18 months. Whereas high PA individuals present with persistent practice effects, with a slight reduction in rate of improvement becoming evident again from 18 months, although not in all cases. There are several explanations for practice effects including reduced anxiety, episodic memory for test content or greater procedural memory for test structure and rules. Several other trials have observed this phenomenon [481-483]. One study of cognitively healthy older adults examined whether presence or absence of brain Aβ and/or neurodegeneration (N) would influence observed practice effects over time [484]. They reported that all individuals exhibited practice effects, but that those with high brain amyloid and neurodegeneration (Aβ+N+) or low amyloid with neurodegeneration (Aβ-N+) exhibited some initial practice effect, followed by decline from approximately 15 months. Despite these differences, 93% of participants remained cognitively normal by month 30, however, the author concluded that the lack of practice effects among those with neurodegeneration may indicate future dementia. Similar trends are also presented in results throughout Chapters 4-6.

An alternative hypothesis surrounding the observed increase in scores over time (i.e. as participants aged) may be relating to attrition. Specifically, 99 (10.99%) CHARIOT: PRO participants either withdrew or were excluded due to alternative reasons poorly recorded (most likely health-related) after screening. Possibly, these participants cognitive scores were lower. However, a sensitivity analysis failed to identify any statistically significant difference in cognitive scores among those

who did not reach at least 24 months of follow-up versus those who did. Furthermore, such a small sub-sample would unlikely influence the overall result.

When examining cognitive ageing, practice effects are of intrinsic interest and warrant careful consideration. In this case, it appears that a reduction or absence of practice effect is of most interest, as can be seen in this cognitively healthy cohort among individuals with a higher risk profile. Although practice effects followed by decline were observed among suspect risk groups, inspection of the trajectories show that, even though decline appeared to be occurring, no score was reduced to that of their baseline score – i.e. all subjects appeared to remain cognitively normal. Given a relatively healthy, well-educated and motivated cohort; such a short follow-up period may not expect to reveal any clinically significant change in cognition. In fact, according to the RBANS score interpretation guidelines as specified in the manual (Chapter five) [375], a decline of 1-1.5 standard deviations (i.e. 15-22.5 index points) below the norm may be considered 'clinically significant', but the relevance of this clinical outcome should be interpreted alongside a multitude of factors. For example, in the context of AD, specific sub-domains should be evaluated (i.e. memory domains), alongside decline in functional activities of daily living and imaging data. This information was not available for evaluation in the current dataset, and in order to ascertain the clinical relevance of these findings, longer follow-up data is also required. This fact does not warrant disregard of reported group differences in cognitive function and trajectories within the CHARIOT PRO cohort. Based on previous reports of PA's beneficial influence on cognitive function among healthy older adults, and with a longer follow-up period, it is anticipated that the low PA group may continue declining below the baseline score, albeit at a slower rate. A deviation in trajectories among the low PA group, after a longer follow-up period, may begin to reveal an enhanced risk group on the route to MCI and eventual dementia. Due to an absence of RCT's, whether high PA mitigates this risk after longer follow-up periods is yet to be elucidated.

Chapter 8: Physical activity, brain derived neurotrophic factor and cognitive function and trajectories over 30 months

8.1 Introduction

This chapter explores the role of baseline BDNF serum levels in relation to PA and *APOE*ε4 carrier status on cognition in older age. It will present and discuss results pertaining to the following objectives:

(v) Whether midlife leisure PA, late-life leisure PA, and EPA tertiles are cross-sectionally associated with baseline BDNF serum levels;

(vi) Whether *APOE*ε4 carrier status modifies these associations;

(vii) If BDNF is associated with overall cognitive function and trajectories across 30 months and modified by *APOE*ε4 carrier status;

(viii) Whether BDNF mediates the association between midlife and/or late-life PA;

(ix) Alternatively, if mediation is not identified, whether BDNF modifies the association between PA and cognitive function and trajectories over 30 months;

(x) Whether the role of BDNF as a mediator or moderator differs among *APOE*ε4 carrier or non-carrier individuals.

These findings identified if, in this cohort, physical activity (either during midlife and/or late-life) indeed upregulates resting-state BDNF serum levels and whether *APOE*ε4 carrier status modifies this proposed association. Secondly, it was expected that BDNF be positively associated with cognition and mediate the positive association between PA and cognition. Alternatively, if mediation was not present, it was hypothesised that BDNF modifies the association between PA and cognition such that those with lower BDNF present with an augmented response to PA on cognition. Finally, it was expected that those at increased risk of cognitive decline via lower BDNF levels (possibly as a function of *APOE*ε4 carriage), was more reliant upon PA than those with higher BDNF, in maintaining cognitive health.

This Chapter systematically presents results generated from the above objectives. The results will be followed by a summary and discussion.

8.2 Results

8.2.1 Descriptive statistics stratified by BDNF serum levels

Table 25 presents population characteristics stratified by BDNF median cut-off groups (high/low). BDNF serum levels ranged from 3.4 to 77.8 pg/mL with a median of 42.4 (IQR = 33.9, 52.1). There were significantly more females in the high BDNF group when compared to males. Likewise, overall PASE scores were markedly higher in the high BDNF group, median=121.4 (IQR 96.7, 150.5), compared to the low BDNF group, median=108.6 (IQR 83.2, 153.4). No other characteristics were noticeably different.

Table 25. Population characteristics stratified by BDNF median-cut groups

	Low BDNF (<42.4 pg/mL) (n=88)	**High BDNF (≥42.4 pg/mL) (n=85)**
Age (yrs), mean (SD)	68.4 (2.4)	68.8 (2.1)
Age groups, N ≥70 yrs/old (%)	33 (37.5)	31 (36.5)
Sex, N of females (%)	44 (50)	56 (65.9)*
APOEε4, N carriers (%)[a]	31 (35.2)	23 (27.1)
$A\beta^{1-42}$ (pg/mL), median (IQR)[h]	15.2 (13.0, 16.8)	15.00 (13.1, 16.0)
Cognition, median (IQR)		
Global Cognition	107 (100, 114)	106 (99, 115)
Delayed Memory	102 (98, 110)	102 (98, 110)
Immediate Memory	109 (100, 116)	103 (94, 112)
Attention	109 (100, 118)	103 (91, 115)
Language	108 (101, 111)	105 (100, 116)
Visuospatial	98 (89, 112)	100 (87, 112)
Executive Function [b]	111 (102, 126)	118 (110, 129)*
Vascular Factors, N (% diagnosed)		
BMI, median (IQR)[c]	25 (22, 28)	26 (24, 29)
Type II Diabetes	11 (12.5)	7 (8.2)
Hypercholesterolemia	14 (15.9)	8 (10.6)
Hypertension	34 (38.6)	28 (32.9)
Lifestyle characteristics		
Ever smoked, N (%)[d]	41 (46.6)	40 (47.1)
Alcohol, median (g day^{-1}) (IQR)[e,f]	11.71 (4.02, 27.5)	11.26 (2.9, 20.6)
Education, N < 12 years (%)	41 (46.6)	38 (44.7)
MLPA, N low (%)	52 (59)	52 (61)
LLPA, N low (%)	73 (83)	61 (72)
PASE, median (IQR)	108.56 (83.2, 153.4)	121.45 (96.7, 150.5)

Low/high BDNF serum levels dichotomised via median cut-off. Abbreviations: SD, Standard deviation; IQR, Interquartile Range; PA, physical activity; LLPA, late-life PA; MLPA, midlife PA; BDNF; Brain Derived Neurotrophic Factor; Aβ, Amyloid beta. [a] five subjects missing APOEε4 carrier status; [b] one case missing executive function scores; [c] eight cases missing BMI with one case being underweight (BMI<18.5); [d] two cases missing smoking history; [e] 20 cases missing alcohol consumption, [f] grams per day; p-values determined by Student's t-test for mean comparison, Mann-Whitney U test for median comparison of continuous variables and chi-squared ($\chi 2$) for categorical variables. *p ≤ .05; **p ≤ .01; ***p≤.001

8.2.2 The cross-sectional association between physical activity, *APOE*ε4 carrier status and BDNF

Multiple linear regression found a positive trend between BDNF and PA, with the largest effect sizes for late-life EPA and LLPA when compared to MLPA. However, associations did not reach statistical significance (Table 26). A positive trend also indicated higher BDNF among *APOE*ε4 carriers relative to non-carriers, albeit not statistically significant (Table 26). *APOE*ε4 carrier status did not modify the associations between PA and BDNF as indicated by non-significant interactions between *APOE* × PA tertiles, *APOE* × LLPA and *APOE* × MLPA (Table 26). *APOE*ε4 stratified re-analysis indicated a positive trend, suggesting higher BDNF among the high LLPA group relative to the low LLPA group, with differences between activity groups being larger among *APOE*ε4 carriers (Figure 26, Table 27). Results did not differ when removing the four outliers of >2 SD's below the range (3.44, 3.94, 4.35 and 6.95), hence, all results are presented with these data points included. Assumptions of linearity, normality, multicollinearity and homoscedasticity were all tested and met in the current analyses.

Table 26. The cross-sectional association between physical activity, *APOE*ε4 carrier status and BDNF

	Beta-Coefficients [95% CI]	
	Model 1	**Model 2**
Late-life EPA		
APOE	2.04 [-6.51, 2.43]	1.15 [-6.03, 3.73]
Mod EPA	1.97 [-3.55, 7.49]	3.37 [-2.31, 9.06]
High EPA	2.29 [-3.20, 7.77]	4.05 [-1.80, 9.90]
Mod EPA x *APOE*	-2.30 [-13.64, 9.05]	-2.72 [-14.43, 9.00]
High EPA x *APOE*	4.97 [-6.82, 16.76]	4.95 [-7.04, 16.93]
Late-life leisure PA		
APOE	1.80 [-6.23, 2.64]	1.73 [-6.27, 2.81]
LLPA	3.06 [-1.88, 7.99]	3.69 [-1.35, 8.73]
LLPA x *APOE*	2.89 [-13.17, 7.39]	4.01 [14.45, 6.43]
Midlife leisure PA		
APOE	0.45 [-0.48, 1.38]	0.42 [-0.55, 1.39]
MLPA	0.63 [-4.99, 3.73]	0.77 [-5.41, 3.87]
MLPA x *APOE*	1.65 [-2.94, 6.23]	1.51 [-3.18, 6.19]

Table presents results from multiple linear regression models investigating the independent associations between late-life EPA, late-life leisure PA, midlife leisure PA, *APOE*ε4 carrier status and continuous BDNF serum levels. Beta-coefficient represents point difference in BDNF serum levels (pg/mL) relative to the reference category. Refence categories *APOE*ε4 carrier, Low EPA, Low MLPA, Low LLPA. All analysis were not statistically significant ($p \leq 0.05$) Abbreviations: CI, Confidence interval; *APOE*, apolipoprotein; Mod LLPA; moderate late-life PA; High LLPA, High late-life physical activity; LLPA, late-life leisure physical activity; MLPA, midlife leisure physical activity. Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, hypertension, hypercholesterolemia, type II diabetes and BMI.

Table 27. The cross-sectional association between physical activity and BDNF stratified by *APOE*ε4 carrier status

	Beta-Coefficients [95% CI]			
	***APOE*ε4 non-carrier (n=114)**		***APOE*ε4 carrier (n=53)**	
	Model 1	**Model 2**	**Model 1**	**Model 2**
Late-life EPA				
Mod EPA	2.35 [-3.71, 8.42]	4.19 [-1.97, 10.35]	0.11 [-9.95, 10.16]	2.16 [-7.71, 12.03]
High EPA	1.18 [-4.77, 7.13]	5.05 [-1.37, 11.48]	5.75 [-4.59, 16.09]	6.89 [-2.83, 16.62]
Late-life leisure PA				
LLPA	2.33 [-3.52, 8.18]	2.04 [-3.97, 8.05]	4.68 [-4.75, 14.11]	7.59 [-1.31, 16.48]
Midlife leisure PA				
MLPA	-0.55 [-5.75, 4.65]	-0.21 [-5.44, 5.04]	0.08 [-9.50, 9.67]	0.41 [-9.70, 10.52]

Table presents results from multiple linear regression models investigating the independent associations between late-life EPA, late-life leisure PA, midlife leisure PA and continuous BDNF serum levels among carriers versus non-carriers. Beta-coefficient represents point difference in BDNF serum levels (pg/mL) relative to the reference category. Reference categories: lowest EPA tertile, low MLPA; Low LLPA; *APOE*ε4 carrier. All analysis were not statistically significant ($p \leq 0.05$). Abbreviations: CI, Confidence interval; *APOE*, apolipoprotein; Mod LLPA; moderate late-life PA; High LLPA, High late-life physical activity; LLPA, late-life leisure physical activity; MLPA, midlife leisure physical activity. Model 1: adjusted for age and sex; Model 2: adjusted for model 1 and hypertension, hypercholesterolemia, type II diabetes and BMI.

8.2.3 BDNF and cognitive function and trajectories over 30 months

Mixed-effects non-linear regression analyses revealed a statistically significant negative association between baseline BDNF serum levels and scores in global cognition, delayed memory and attention. Conversely, there was a positive association between BDNF and overall executive function index scores in model one, becoming nonsignificant in model two. The association between BDNF and all other cognitive domains were non-significant (Table 28). Neither time nor *APOE*ε4 carrier status modified the association between BDNF and multi-domain cognition (Table 28). Results remained congruent across the two covariate adjusted models (Model 1 in Appendix F, Table F.1).

Due to the reduced sample size, associations between MLPA and LLPA were weakened. Specifically, a statistically significant association between high MLPA and better scores in delayed memory, relative to those reporting low MLPA were retained. Coefficients were in the expected direction for all other cognitive domains (with the exception of language), but no longer reaching statistical significance. Conversely, relative to those reporting low LLPA, the high LLPA group performed significantly worse in attention scores which is contradictory to results reported in Chapter 4. Incongruent results may be as a consequence of considerably reduced sample size, and hence power, in the current analyses.

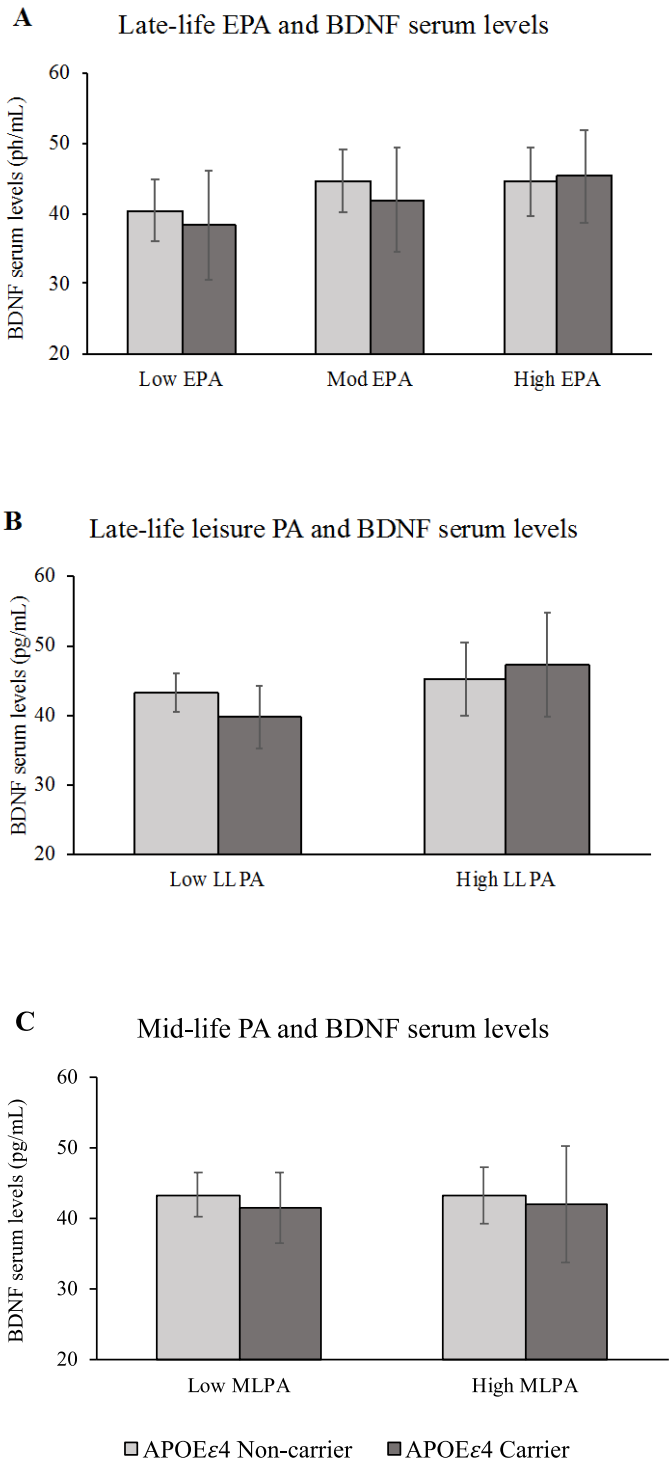


Figure 26. The association between EPA, LLPA, MLPA and BDNF serum levels among *APOE*ε4 carriers and non-carriers.

*APOE*ε4 carriers reporting high late-life EPA (A) and late-life leisure PA (B) show a positive trend indicating higher BDNF serum levels when compared to low-active carries. Minimal differences in BDNF serum levels can be seen among individuals reporting high versus low MLPA (C). Error bars represent 95% CI's. Adjusted for age, sex, hypertension, hypercholesterolemia, type II diabetes and BMI. Abbreviations: EPA, everyday physical activity; PA, physical activity; LLPA, late-life physical activity; MLPA, midlife physical activity; BDNF, brain derived neurotrophic factor; APOE, apolipoprotein

8.2.4 BDNF as a mediator or modifier

For mediation to be an appropriate technique, it is required that the mediator (e.g. BDNF) be associated with the exposure variable (i.e. PA) and the outcome variable (i.e. cognition). It is assumed that mediation has occurred when, upon addition of the mediator (i.e. BDNF) within a model, the statistically significant association between primary exposure variable (PA) and outcome (cognition) is reduced or removed [485]. Due to a non-insignificant association between BDNF and PA, mediation was not tested.

Inclusion of interaction terms between LLPA, MLPA and BDNF (PA × BDNF) identified that BDNF also did not statistically modify the association between MLPA or LLPA with any cognitive domain (Table 28; Appendix F, Table F.1).

8.2.5 BDNF, midlife and late-life leisure aerobic activity and cognition among *APOE*ε4 carriers versus non-carriers

A significant positive association between BDNF and immediate memory, plus executive function index scores over 30 months were observed to be limited to *APOE*ε4 carrier individuals only. BDNF was not significantly associated with any other cognitive domain among both carriers and non-carriers (Table 29).

LLPA did not modify the association between BDNF and any cognitive domain within carriers and non-carriers. However, BDNF × MLPA interaction was significantly associated with immediate memory index scores over 30 months, among *APOE*ε4 carriers only. Further inspection revealed that *APOE*ε4 carriers who report high MLPA and with high BDNF levels to have significantly better scores than those reporting high MLPA with low BDNF levels (not reported). However, due to very wide confidence intervals and small sample sizes within sub-group analyses, any conclusions drawn from these observations are tentative. MLPA did not modify the association between BDNF and any other cognitive domain among both carrier and non-carrier individuals (Table 29). All aforementioned analyses were adjusted for age, sex, education, vascular factors, time and time^2

Table 28. Mixed-models regression on the association and interactions between BDNF, MLPA and LLPA on multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Main associations [a, b]							
BDNF [c]	-0.15 [-0.27, -0.02]*	-0.12 [-0.24, -0.00]	-0.09 [-0.17, -0.01]*	-0.08 [-0.21, 0.04]	-0.08 [-0.17, 0.02]	-0.16 [-0.29, -0.02]*	0.15 [-0.01, 0.30]
MLPA	0.52 [-3.09, 4.14]	0.39 [-3.07, 3.84]	2.71 [0.34, 5.08]*	3.21 [-0.42, 6.84]	-0.04 [-2.80, 2.72]	0.27 [-3.89, 4.43]	2.19 [-2.33, 6.70]
LLPA	0.61 [-3.18, 4.40]	0.66 [-2.97, 4.28]	-0.18 [-2.72, 2.36]	-0.79 [-4.65, 3.06]	0.81 [-2.07, 3.70]	-1.02 [-5.30, 3.26]	-0.29 [-5.25, 4.67]
Interactions [c, f]							
BDNF × Time	0.03 [-0.05, 0.11]	0.05 [-0.05, 0.15]	-0.01 [-0.08, 0.06]	0.05 [-0.07, 0.16]	0.05 [-0.03, 0.14]	0.02 [-0.06, 0.09]	-0.05 [-0.34, 0.23]
BDNF × Time^2	-0.00 [-0.02, 0.01]	-0.01 [-0.03, 0.01]	0.00 [-0.01, 0.02]	-0.01 [-0.04, 0.01]	-0.01 [-0.03, 0.01]	-0.00 [-0.02, 0.01]	0.01 [-0.05, 0.06]
BDNF × *APOE*	-0.11 [-0.38, 0.16]	-0.17 [-0.41, 0.07]	0.07 [-0.11, 0.26]	-0.00 [-0.29, 0.28]	-0.08 [-0.28, 0.12]	-0.09 [-0.41, 0.23]	-0.11 [-0.44, 0.21]
BDNF × MLPA	0.26 [-0.03, 0.56]	0.26 [-0.02, 0.55]	0.01 [-0.18, 0.20]	-0.19 [-0.49, 0.10]	0.01 [-0.22, 0.24]	0.15 [-0.19, 0.49]	0.13 [-0.25, 0.51]
BDNF × LLPA	0.17 [-0.15, 0.50]	0.17 [-0.13, 0.48]	0.03 [-0.18, 0.25]	0.17 [-0.15, 0.49]	0.11 [-0.13, 0.35]	0.23 [-0.15, 0.61]	0.22 [-0.16, 0.59]

Table presents results from mixed-models regression investigating the independent and interactive associations between late-life leisure PA (LLPA), midlife leisure PA (MLPA), BDNF median-cut groups and cognitive function and trajectories across 30 months. For main associations, Beta-coefficient represents point difference in marginal mean cognitive index scores at 30 months relative to each independent variable reference category. Higher scores indicate better cognitive performance. [a] BDNF and MLPA / LL-LPA in separate models; [b] BDNF and MLPA / LL-LPA in the same model (i.e. associations are independent from each another). [c] BDNF as a continuous variable in all analyses. [d] Interactions in separate models controlled for age and sex. Controlled for age, sex, education, time, time^2, hypertension, hypercholesterolemia, type II diabetes and BMI. Abbreviations: BDNF, brain derived neurotrophic factor; *APOE*, apolipoprotein epsilon 4 carrier / non-carrier; MLPA, midlife leisure physical activity; LLPA, late-life leisure physical activity; CI, confidence interval. Comparators: Low MLPA, Low LLPA, *APOE*ε4 carrier. †$p \leq .08$; *$p \leq .05$; **$p \leq .01$.

Table 29. Mixed-models regression exploring the association and interactions between BDNF, MLPA and LLPA on multi-domain cognition among *APOE*ε4 carrier and non-carrier groups

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
***APOE*ε4 Carrier**							
Main associations [a]							
BDNF	0.15 [-0.07, 0.38]	0.23 [0.02, 0.43]*	-0.02 [-0.16, 0.12]	0.10 [-0.15, 0.36]	0.10 [-0.09, 0.29]	0.06 [-0.21, 0.33]	0.29 [0.01, 0.56]*
MLPA	0.70 [-7.21, 8.61]	2.63 [-4.41, 9.68]	3.79 [-0.80, 8.38]	0.31 [-8.32, 8.93]	-0.50 [-7.00, 6.00]	0.29 [-8.85, 9.42]	-1.37 [-10.90, 8.16]
LLPA	2.05 [-5.05, 9.14]	1.48 [-5.10, 8.06]	0.40 [-3.87, 4.66]	-1.39 [-9.15, 6.38]	3.22 [-2.54, 8.98]	1.87 [-6.24, 9.99]	0.65 [-8.27, 9.57]
Interactions [b]							
BDNF × MLPA	0.41 [-0.29, 1.12]	0.71 [0.12, 1.30]*	0.05 [-0.37, 0.47]	0.08 [-0.71, 0.86]	-0.15 [-0.74, 0.43]	0.13 [-0.70, 0.97]	0.22 [-0.65, 1.09]
BDNF × LLPA	0.29 [-0.14, 0.73]	0.10 [-0.31, 0.50]	0.03 [-0.24, 0.30]	0.40 [-0.08, 0.88]	0.15 [-0.22, 0.51]	0.40 [-0.11, 0.91]	0.32 [-0.21, 0.85]
***APOE*ε4 Non-Carrier**							
Main associations [a]							
BDNF	-0.05 [-0.21, 0.12]	-0.02 [-0.17, 0.12]	0.02 [-0.10, 0.14]	-0.02 [-0.19, 0.15]	-0.03 [-0.15, 0.08]	-0.06 [-0.26, 0.14]	0.12 [-0.08, 0.32]
MLPA	4.18 [-0.07, 8.44] †	2.90 [-0.92, 6.73]	3.12 [-0.04, 6.28] †	1.88 [-2.68, 6.45]	0.97 [-2.25, 4.20]	4.99 [-0.46, 10.44] †	2.86 [-2.68, 8.40]
LLPA	5.35 [0.66, 10.04]*	5.10 [0.99, 9.21]*	2.15 [-1.32, 5.62]	1.92 [-3.12, 6.95]	1.91 [-1.54, 5.37]	4.80 [-0.90, 10.50]	-1.19 [-7.44, 5.07]
Interactions [b]							
BDNF × MLPA	-0.15 [-0.49, 0.19]	-0.19 [-0.50, 0.12]	0.00 [-0.25, 0.26]	-0.11 [-0.48, 0.26]	-0.02 [-0.28, 0.24]	-0.06 [-0.49, 0.37]	0.17 [-0.29, 0.62]
BDNF × LLPA	-0.20 [-0.63, 0.23]	-0.17 [-0.55, 0.21]	-0.08 [-0.40, 0.24]	-0.15 [-0.62, 0.31]	-0.04 [-0.36, 0.28]	-0.04 [-0.56, 0.48]	0.04 [-0.53, 0.61]

Table presents results from mixed-models regression investigating the independent and interactive associations between late-life leisure PA (LLPA), midlife leisure PA (MLPA), BDNF median-cut groups and cognitive function and trajectories across 30 months among *APOE*ε4 carrier / non-carrier strata. For main associations, Beta-coefficient represents point difference in marginal mean cognitive index scores at 30 months relative to each independent variable reference category. Higher scores indicate better cognitive performance. [a] BDNF and MLPA / LL-LPA in separate models; [b] Interactions in separate models. Comparators: Low BDNF, Low MLPA, Low LLPA, *APOE*ε4 carrier. Controlled for age, sex, education, time, time^2, hypertension, hypercholesterolemia, type II diabetes and BMI. Abbreviations: BDNF, brain derived neurotrophic factor; *APOE*, apolipoprotein epsilon 4 carrier / non-carrier; MLPA, midlife leisure physical activity; LLPA, late-life leisure physical activity; CI, confidence interval. †$p \leq .08$; $^*p \leq .05$.

8.3 Discussion

Figure 27 provides a depiction of results reported within this chapter. *APOE*ε4 carrier status, midlife and late-life aerobic leisure activity and late-life EPA were all not associated with baseline BDNF serum levels (v). Likewise, *APOE*ε4 carrier status did not modify the associations between PA and BDNF serum levels (vi). Prospective observational studies and RCT's have typically indicated a positive association between PA and resting-state circulatory BDNF serum and/or plasma levels [293, 302, 310]; hence, these results are unexpected (see hypothesis v and vi). Although differences were not significant in the CHARIOT: PRO cohort, trends in the hypothesised direction were consistently reported such that non-carriers and high active individuals presented with higher BDNF levels. Based on the strength of consistent evidence implicating PA as a feasible means of BDNF upregulation, if the sample size were larger in this study, these findings may become statistically significant. The hypothesis that higher BDNF serum levels would be positively associated with better cognitive function (vii) was not met. Findings indicate an inverse association, such that higher BDNF was, in fact, associated with lower overall global cognition, delayed memory and attention index scores averaged across 30 months. *APOE*ε4 carrier status did not significantly modify the associations between BDNF and cognition (i.e. *APOE*ε4 x BDNF interaction was non-significant), however, stratified analyses indicated differential BDNF/cognitive associations among carriers versus non-carriers (see Figure 27, part B). Specifically, within carriers, BDNF was positively associated with global cognition, delayed memory and attention. There were no significant associations between BDNF and cognition among non-carriers. These findings allude that the positive associations between BDNF and cognition appear to only be apparent among carriers in this cohort. BDNF did not appear to mediate (viii) nor modify (ix) the associations between midlife or late-life leisure PA and EPA on overall cognitive function or trajectories. It must be noted, however, that statistically significant associations between high LLPA and better overall scores in language and attention, as well as high MLPA and better overall scores in global cognition, attention, language and executive function (see Chapters three and four) were also no longer statistically significant. This alludes that the reduced sample size (n=173) may be yielding insufficient power to detect previously reported differences based on PA. Nonetheless, within *APOE*ε4 stratified analyses, interactions between MLPA and BDNF were significant for immediate memory, such that carriers who report high MLPA and high BDNF serum levels had significantly better immediate memory scores relative to those reporting high MLPA with low BDNF (x) (see Figure 27, Part B). These results may indicate a positive additive effect of high MLPA alongside high BDNF levels mitigating *APOE*ε4 carrier risk on cognitive function. Likewise, seeing that BDNF was positively associated with cognition only among the ε4 carrier strata, possibly, the benefits of BDNF on cognition is only observed among individuals at increased

risk of AD. However, in this sub-analyses, sample sizes were reduced, resulting in wide confidence intervals, hence, conclusions drawn from these observations are tentative.

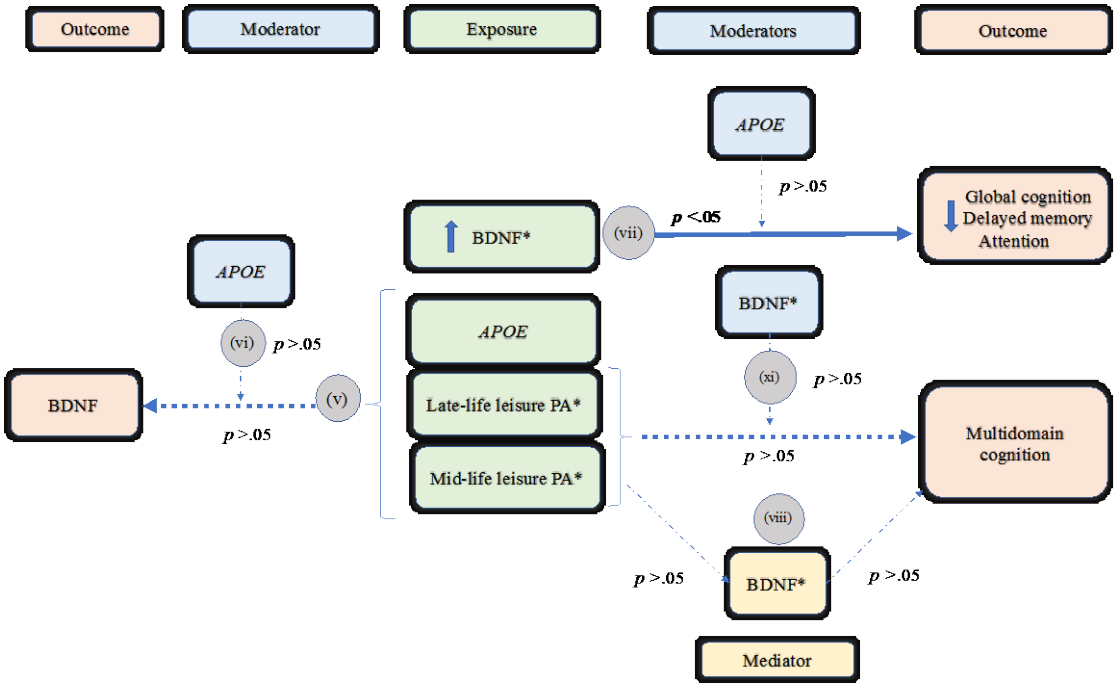
Findings reported here indicate that the positive associations between PA and cognition were not mediated by BDNF, and/or inadequate statistical power prevented further in-depth exploration. The alternative hypothesis; that BDNF acts as a modifier in such PA/cognitive associations implies that BDNF could indeed be acting independently from PA. Possibly other genetic or lifestyle factors are influencing BDNF levels, as found in the *APOE*ε4 carrier groups within this cohort.

Existing evidence also implicates the *BDNF* Val66Met single-nucleotide polymorphism as a strong genetic candidate for the modulation of Aβ-related cognitive decline, influencing neuronal processes linked to neurogenesis [486, 487]. Reduced levels of circulatory BDNF is a notable manifestation of the BDNF Val66Met single nucleotide polymorphism [488], and specifically, reports link the Met allele with lower BDNF levels [489]. On the other hand, high PA has reportedly attenuated AD-genetic risk-mediated progression of cognitive deficits in Met homozygotes [490]. Likewise, it has been reported that possession of the *APOE*ε4 allele alongside the Met allele yields higher risk for cognitive decline [491]. This may explain why higher BDNF is positively associated with cognition among ε4 carriers only, and why high PA alongside higher BDNF levels appeared to mitigate *APOE*ε4 carrier risk of decline, in this case, for immediate memory and executive function when compared to low PA/low BDNF individuals. Due to funding limitation, *BDNF* genotyping was not conducted in this cohort, but the *BDNF* Val66Met single-nucleotide polymorphism would be important to consider for future analyses. This theory still may not explain why, in the overall cohort, BDNF was inversely associated with cognition. Indeed, previous studies have investigated the cognitive and clinical significance of low serum BDNF, with inconsistent results. One study found higher levels of serum BDNF in MCI and AD patients [492], while other studies have found the associations in the opposite direct [493] or failed to identify an association between BDNF serum levels and risk of cognitive decline [494] or AD [307]. Nonetheless, as covered in the introduction (see section 1.6.1, page 45), a large volume of studies reported a positive association between BDNF serum levels and cognitive function [310]. Due to a small sample size in the current study, other residual confounding may also be influencing the direction of an association, yielding biased results. For example, studies indicate sex differences in response to BDNF signalling, whereby BDNF levels appear to be associated with cognitive performance among females only [454]. Likewise, age has been found to modify the associations between BDNF and cognition as well as BDNF mediated effects of exercise intervention. A walking intervention study (n=90, mean age 66.82) found that, even after controlling for *BDNF* genotype, serum BDNF was found to mediate task switching performance among those >70 years old, whereas younger individuals did not find the same mediation effect [495]. It would be informative to repeat such sex and age modifying analyses in the current study. However, a limited sample size raised concern

around sub-analyses by sex and age (as is observed with *APOE*ε4 stratified analyses) hence, these questions were not further explored in the current study. With larger sample sizes, however, it would be vital to investigate the effect of such factors on the association between BDNF and cognition as well as in response to PA.

Results reported here are inconclusive. Trends indicate that both high midlife and late-life leisure aerobic PA plus late-life EPA may be linked with higher BDNF serum levels. Likewise, it appears that *APOE*ε4 allele carriers have an enhanced benefit of PA on increasing BDNF serum levels, and improvements in cognition owing to higher BDNF serum levels. Finally, results indicate that the positive additive effect of possessing higher BDNF serum levels alongside reporting high MLPA has an augmented positive association with immediate memory and executive function among *APOE*ε4 carriers only. It is proposed that these findings are likely beyond that of chance, owing to previous RCT's consistently linking both acute and chronic PA with sustained upregulated BDNF serum levels [302, 309]. Possibly, high PA upregulates BDNF levels, although this upregulation may be augmented among certain risk groups such as ε4 carriers. Higher BDNF serum levels, if able to cross the blood brain barrier, may encourage neurogenesis and synaptic plasticity, increased hippocampal volume, reduction of BASE1 and reduced brain Aβ accumulation. However, the benefits of higher BDNF serum levels likely depend on other mediating factors, such as lifestyle, genetics and demographic characteristics. Likewise, how the benefits of enhanced BDNF on pathological features manifests cognitively may depend on such factors including, but not limited to: PA timing and type, age, sex, *APOE*ε4 carrier status and *Met* carrier status. Future research should attempt to disentangle these complex interrelations through this multi-stage process. This report, and those previously published, highlight the complexity of measuring, evaluating and interpreting hypothesised biomechanistic pathways mediating the benefits of PA on cognitive health in older age.

(A) Overall Cohort



(B) Within *APOE*ε4 carrier individuals

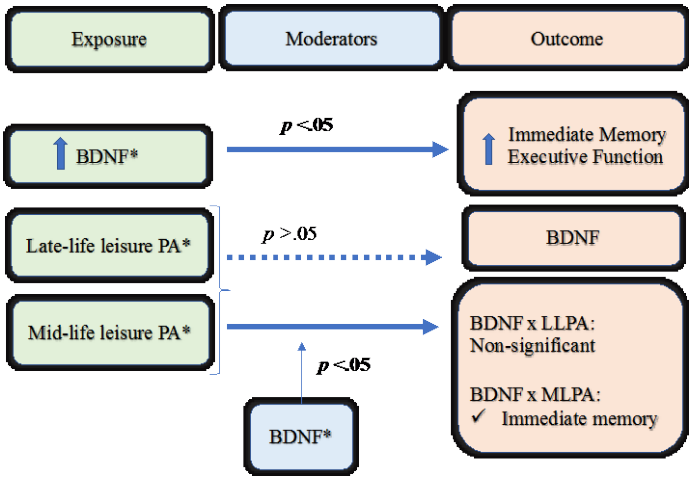


Figure 27. Depiction of results pertaining to the role of BDNF on the association between physical activity, *APOE*ε4 carrier status and cognition

**APOE*ε4 stratified re-analyses; results remained non-significant among the *APOE*ε4 non-carrier strata

Chapter 9: Physical activity, plasma amyloid-beta$_{1-42}$ and cognitive function and trajectories over 30 months

9.1 Introduction

This chapter presents the association between baseline $A\beta_{1-42}$ plasma levels in relation to physical activity and *APOE*ε4 carrier status with cognitive function and trajectories in older age.

Objectives to be addressed are;

(xi) whether *APOE*ε4 carrier status, midlife leisure PA, late-life leisure PA and EPA tertiles are associated with baseline $A\beta_{1-42}$ plasma levels;

(xii) whether *APOE*ε4 carrier status modifies the associations between PA and $A\beta_{1-42}$ plasma levels;

(xiii) if baseline $A\beta_{1-42}$ plasma levels are associated with cognitive function and trajectories over 30 months and whether *APOE*ε4 carrier status modifies these associations;

(xiv) whether the association between $A\beta_{1-42}$ plasma levels and cognition are modified by midlife and/or late-life leisure physical activity;

(xv) If PA modified $A\beta_{1-42}$ /cognition associations differ among *APOE*ε4 carriers versus non-carriers.

It was expected that physical activity will be associated with $A\beta_{1-42}$ plasma levels and that *APOE*ε4 carrier status will modify this association. Likewise, it was expected that $A\beta_{1-42}$ plasma levels will be associated with cognitive function and trajectories over 30-months and moderated by *APOE*ε4 carrier status and physical activity levels.

Results will be presented below in section 9.2. To follow, section 9.3 will be a discussion of these results in light of previously introduced literature (Chapter 1, section 1.6.2, page 46-47)

9.2 Results

9.2.1 Descriptive statistics stratified by $A\beta_{1-42}$ groups

Table 30 presents sample characteristics stratified by median cut-off $A\beta_{1-42}$ groups. $A\beta_{1-42}$ plasma levels ranged from 1.05 to 21.8 pg/mL with a median of 14.8 (IQR = 12.9, 16.4). There was a lower

proportion of individuals reporting a hypertension diagnosis and current or previous history of smoking in the high $A\beta_{1\text{-}42}$ group when compared to the low $A\beta_{1\text{-}42}$ group. No other group differences were noted among high/low median split plasma $A\beta_{1\text{-}42}$ groups.

Table 30. Population characteristics stratified by $A\beta_{1\text{-}42}$ plasma levels

	Low $A\beta_{1\text{-}42}$ (<14.8 pg/mL) (n=129)	**High $A\beta_{1\text{-}42}$ (≥14.8 pg/mL) (n=129)**
Age (yrs), mean (SD)	68.57 (2.1)	68.27 (2.4)
Age groups, N (% ≥70 yrs/old)	42 (32.6)	43 (33.3)
Sex, N (% Female)	65 (50)	76 (59)
APOEε4, N (% carrier)[a]	38 (29.5)	34 (26.4)
Serum BDNF (pg/mL), median (IQR)[g]	42.59 (32.9, 52.8)	42.19 (34.2, 51.8)
Cognition, median (IQR)		
Global Cognition	107 (100, 116)	105 (97, 114)
Delayed Memory	102 (99, 110)	102 (98, 110)
Immediate Memory	109 (100, 114)	109 (100, 117)
Attention	106 (99, 108)	109 (97, 118)
Language	105 (99, 112)	105 (99, 112)
Visuospatial	102 (94, 112)	92 (87, 111)**
Executive Function [b]	114 (105, 127)	115 (103, 127)
Vascular Factors		
BMI, median (IQR)[c]	25 (23, 28)	25 (22, 28)
Type II Diabetes, N (% diagnosed)	12 (9.3)	10 (7.8)
Hypercholesterolemia, N (% diagnosed)	19 (14.7)	16 (12.4)
Hypertension, N (% diagnosed)	50 (38.8)	35 (27.1)*
Lifestyle characteristics		
Smoking, N (% ever)[d]	69 (53.5)	51 (39.5)*
Alcohol, Median (IQR)[e,f]	9.57 (1.6, 18.7)	11.15 (4.4, 22.7)
Education, N (% < 12 years)	56 (43.4)	61 (47.3)
Low MLPA, N (%)	77 (59.7)	73 (56.6)
Low LLPA, N (%)	92 (71.3)	95 (73.6)
PASE (continuous)	113.87 (87.5, 149.4)	121.88 (85.4, 149.4)

Low/high Aβ plasma levels dichotomised via median cut-off. Abbreviations: SD, Standard deviation; IQR, Interquartile Range; PA, physical activity; LLPA, late-life PA; MLPA, midlife PA; BDNF; Brain Derived Neurotrophic Factor; Aβ, Amyloid beta. [a] nine subjects missing *APOE*ε4 carrier status; [b] 16 Executive function scores missing; [c] 10 cases missing BMI with one case being underweight (BMI<18.5 kg/^{2}); [d] three cases missing smoking history; [e] 25 cases missing alcohol consumption, [f] grams per day, [g] 85 BDNF cases missing; *p*-values determined by Student's t-test for mean comparison, Mann-Whitney U test for median comparison of continuous variables and chi-squared (χ^2) for categorical variables. *$p \leq .05$; **$p \leq .01$;

9.2.2 The cross-sectional association between physical activity, *APOE*ε4 carrier status and $A\beta_{1-42}$ plasma levels

Multiple linear regression controlled for age and sex (model 1); and vascular factors (model 2) found no statistically significant associations between $A\beta_{1-42}$ plasma levels and *APOE*ε4 carrier status, late life EPA tertiles, LLPA or MLPA (Table 31). Likewise, interactions between *APOE*ε4 carrier status and EPA tertiles, LLPA and MLPA were all non-significantly associated with $A\beta_{1-42}$ plasma levels. *APOE*ε4 stratified repeated analyses again found no differences in associations among carriers and non-carriers (Table 32). Results did not differ when removing two outliers being ≥3 SD's below the range, hence, all results are presented with these data points included. As mentioned, assumptions of linearity, normality, multicollinearity and homoscedasticity were all tested and met in the current analyses.

Table 31. The cross-sectional association between physical activity, *APOE*ε4 carrier status and $A\beta_{1-42}$ plasma levels

	Beta-Coefficients [95% CI]	
	Model 1	**Model 2**
***APOE* (ref: carrier)**	-0.14 [-0.90, 0.62]	-0.01 [-0.75, 0.74]
Late-life EPA		
Mod EPA	-0.05 [-0.97, 0.87]	0.12 [-0.65, 1.03]
High EPA	0.31 [-0.59, 1.21]	0.40 [-0.44, 1.25]
Mod EPA × *APOE*	-0.44 [-2.40, 1.52]	-0.18 [-2.04, 1.68]
High EPA × *APOE*	-0.82 [-2.81, 1.18]	-0.81 [-2.66, 1.04]
Late-life leisure PA		
LLPA	0.02 [-0.75, 0.80]	0.09 [-0.70, 0.86]
LLPA × *APOE*	-0.39 [-2.06, 1.28]	-0.44 [-2.07, 1.18]
Midlife leisure PA		
MLPA	-0.02 [-0.77, 0.72]	-0.17 [-0.90, 0.56]
MLPA × *APOE*	-0.11 [-1.12, 0.90]	-0.20 [-1.90, 1.50]

Table presents results from multiple linear regression models investigating the independent associations between late-life EPA, late-life leisure PA, midlife leisure PA, *APOE*ε4 carrier status and continuous $A\beta_{1-42}$ plasma levels. Beta-coefficient represents point difference in $A\beta_{1-42}$ plasma levels (pg/mL) relative to the reference category. Abbreviations: CI, Confidence interval; *APOE*, apolipoprotein; Mod LLPA; moderate late-life PA; High LLPA, High late-life physical activity; LLPA, late-life leisure physical activity; MLPA, midlife leisure physical activity. Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, hypertension, hypercholesterolemia, type II diabetes and BMI. Reference categories: Low LLPA (tertile); low MLPA; Low late-life leisure PA; *APOE*ε4 carrier.

Table 32. The cross-sectional association between physical activity, *APOE*ε4 carrier status and Aβ1-42 plasma levels among *APOE*ε4 carriers and non-carriers

	Beta-Coefficients [95% CI]			
	***APOE*ε4 non-carrier (n=163)**		***APOE*ε4 carrier (n=65)**	
	Model 1	**Model 2**	**Model 1**	**Model 2**
Late-life EPA				
Mod EPA	-0.01 [-1.07, 1.05]	0.31 [-0.72, 1.24]	0.40 [-1.10, 1.91]	0.31 [-1.27, 1.89]
High EPA	0.07 [-0.97, 1.10]	0.22 [-0.80, 1.24]	0.98 [-0.54, 2.50]	0.99 [-0.59, 2.57]
Late-life leisure PA				
LLPA	-0.04 [-0.99, 0.92] [	-0.17 [-1.11, 0.76]	0.47 [-0.85, 1.78]	0.29 [-1.08, 1.67]
Midlife leisure PA				
MLPA	-0.10 [-1.01, 0.80]	-0.08 [-1.68, 1.51]	-0.13 [-1.55, 1.28]	-0.82 [-0.41, 0.25]

Table presents results from multiple linear regression models investigating the independent associations between late-life EPA, late-life leisure PA, midlife leisure PA and continuous Aβ1-42 plasma levels among *APOE*ε4 carrier / non-carrier strata. Beta-coefficient represents point difference in Aβ1-42 plasma levels (pg/mL) relative to the reference category. Abbreviations: CI, Confidence interval; *APOE*, apolipoprotein; Mod EPA; moderate everyday physical activity; High EPA, High everyday physical activity; LLPA, late-life leisure physical activity; MLPA, midlife leisure physical activity. Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, hypertension, hypercholesterolemia, type II diabetes and BMI. Reference categories: Low EPA (tertile); low MLPA; Low LLPAPA; *APOE*ε4 carrier.

9.2.3 The association between Aβ1-42 and cognitive function and trajectories with effect modification by LLPA, MLPA and *APOE*ε4 carrier status

9.2.3.1 The association between Aβ1-42 plasma levels and cognition over time among *APOE*ε4 carriers versus non-carriers

Mixed-models regression analyses controlled for age, sex, education and non-linear time revealed a statistically significant inverse association between Aβ1-42 plasma levels and global cognition plus attention index scores over 30 months. Associations remained statistically significant upon additional adjustment for vascular factors (Table 33). Neither *APOE*ε4 carrier status nor time significantly modified the association between Aβ1-42 plasma levels and global cognition or any sub-domain, across both covariate adjusted models. However, *APOE*ε4 carrier/non-carrier stratified re-analyses identified that carrier individuals with high Aβ1-42 plasma levels (via median split) had significantly worse global cognition, immediate memory and executive function index scores over 30 months, when compared to non-carriers with either high or low Aβ1-42 plasma levels (Figure 28). Specifically, relative to the low Aβ1-42 group, immediate memory scores over 30 months were

statistically significantly lower among those with high $A\beta_{1\text{-}42}$ plasma levels, *B*=-5.12 (95% CI -9.91, -0.33), among carriers only. Likewise, data stratified by high versus low $A\beta_{1\text{-}42}$ found *APOE*ε4 carriers to have statistically significantly worse global cognition, *B*=-4.62 (95% CI -8.53, -0.74), immediate memory, *B*=-6.07 (95% CI -9.97, -2.16), and executive function, *B*=-6.59 (95% CI -11.87, 1.31), index scores over 30 months, relative to non-carriers, being present only among those with high $A\beta_{1\text{-}42}$ plasma levels (Figure 28). No differences in cognition as a function of $A\beta_{1\text{-}42}$ / *APOE*ε4 carrier status was observed across the remaining cognitive domains (results not further reported).

9.2.3.2 MLPA and LLPA modify the association between $A\beta_{1\text{-}42}$ plasma levels and cognitive function

MLPA significantly modified the association between $A\beta_{1\text{-}42}$ plasma levels and overall attention and delayed memory index scores across 30 months in the age, sex and time adjusted model ($A\beta_{1\text{-}42}$ × MLPA). However, following adjustment for vascular factors the effect modification was reduced for attention but augmented for delayed memory (Table 33). To explore these interactions further, MLPA and $A\beta_{1\text{-}42}$ stratified analyses found significant differences in delayed memory only, as a function of $A\beta_{1\text{-}42}$ plasma levels and MLPA status. Specifically, those reporting low MLPA with high $A\beta_{1\text{-}42}$ plasma levels presented with lowest scores in delayed memory when compared to those reporting low MLPA with low $A\beta_{1\text{-}42,}$ *B*=-2.74 (95% CI -5.23, -0.24), as well as when compared to those reporting high MLPA with high $A\beta_{1\text{-}42}$ plasma levels, *B*=3.06 (95% CI 0.49, 5.63) (Figure 29).

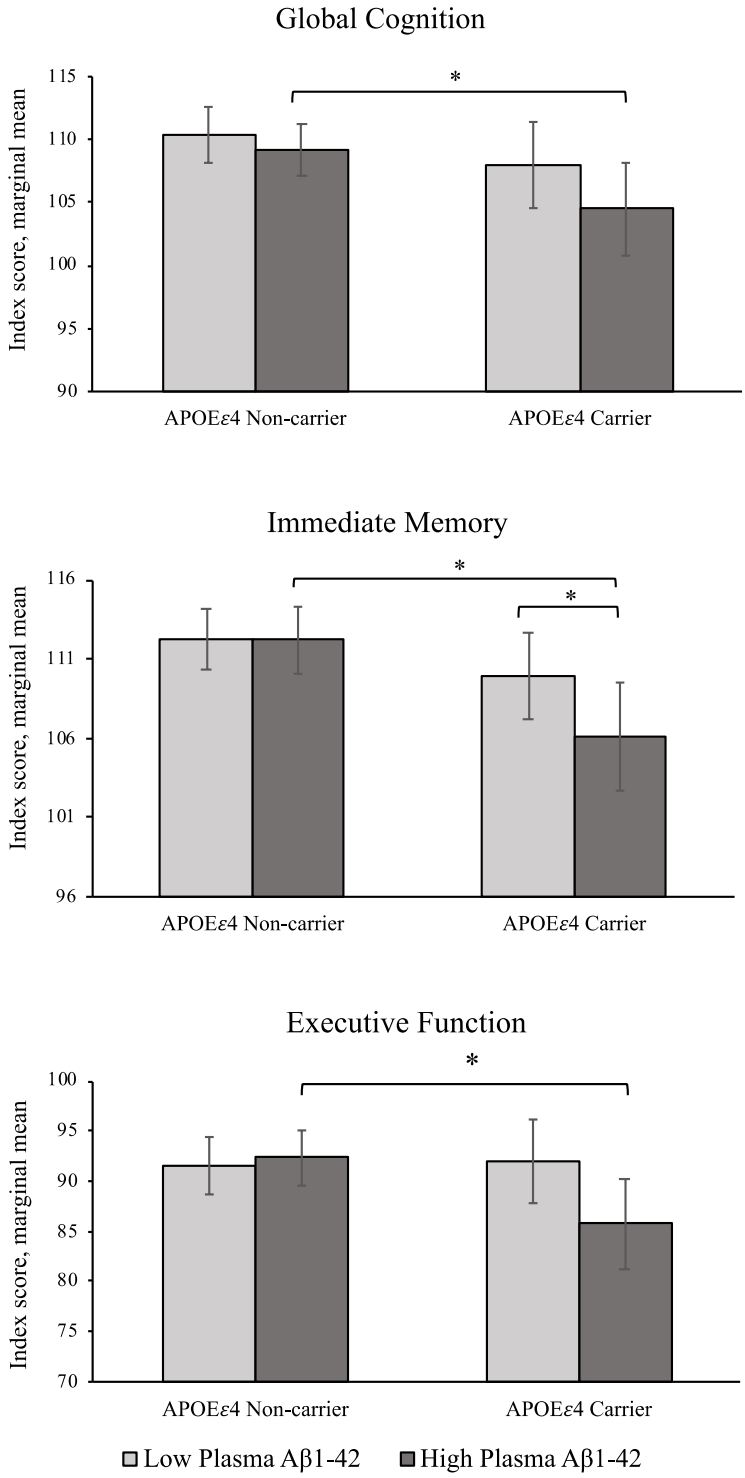


Figure 28. The association between $A\beta_{1\text{-}42}$ plasma levels and cognitive function among *APOE*ε4 carriers and non-carriers.

Graphs depict a significant difference in the association between $A\beta_{1\text{-}42}$ plasma levels and cognition as a function of *APOE*ε4 carrier status. Higher scores indicate better cognitive performance. Adjusted for age, sex, education, hypertension, hypercholesterolemia, type II diabetes, BMI, time and time^2. Error bars represent 95% CI's. Abbreviations: *APOE*ε4, apolipoprotein epsilon 4; Aβ, amyloid beta 1-42 (plasma). *$p \leq .05$

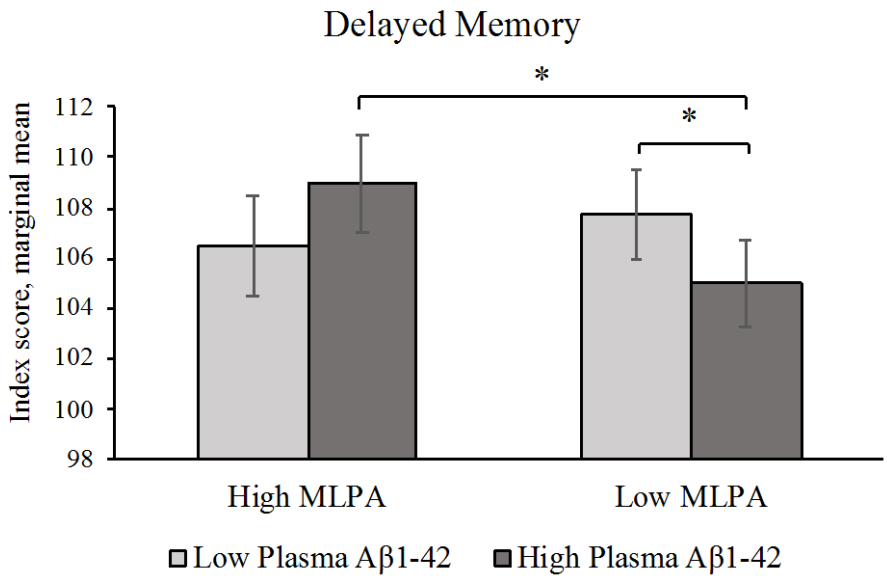


Figure 29. Midlife leisure PA modifies the association between $A\beta_{1-42}$ plasma levels and delayed memory.

Graph depicts a significant difference in the association between $A\beta_{1-42}$ plasma levels and delayed memory as a function of MLPA. Higher scores indicate better cognitive performance. Adjusted for age, sex, education, hypertension, hypercholesterolemia, type II diabetes, BMI, time and time^2. Error bars represent 95% CI's. Abbreviations: MLPA, midlife physical activity; Aβ, amyloid beta 1-42 (plasma). *$p \leq .05$

LLPA statistically significantly modified the associations between $A\beta_{1-42}$ plasma levels and global cognition, visuospatial and attention index scores over 30 months ($A\beta_{1-42}$ × LLPA interaction). After additional adjustment for vascular factors, the interaction remained statistically significant for attention scores only (Table 33). To further visualise the aforementioned interactions, LLPA and high/low $A\beta_{1-42}$ stratified analyses revealed those with high $A\beta_{1-42}$ plasma levels who reported low LLPA to have significantly worse global cognitive scores, *B*=-4.27 (95% CI -7.40, -1.14), and attention scores, *B*=-4.94 (95% CI -9.28, -0.60) relative to those with high $A\beta_{1-42}$ plasma levels reporting high LLPA. Likewise, those with high $A\beta_{1-42}$ plasma levels reporting low LLPA also had significantly worse global cognitive performance, *B*=-4.70 (95% CI -8.63, -0.77), when compared to those reporting low LLPA with low $A\beta_{1-42}$ plasma levels (Figure 30).

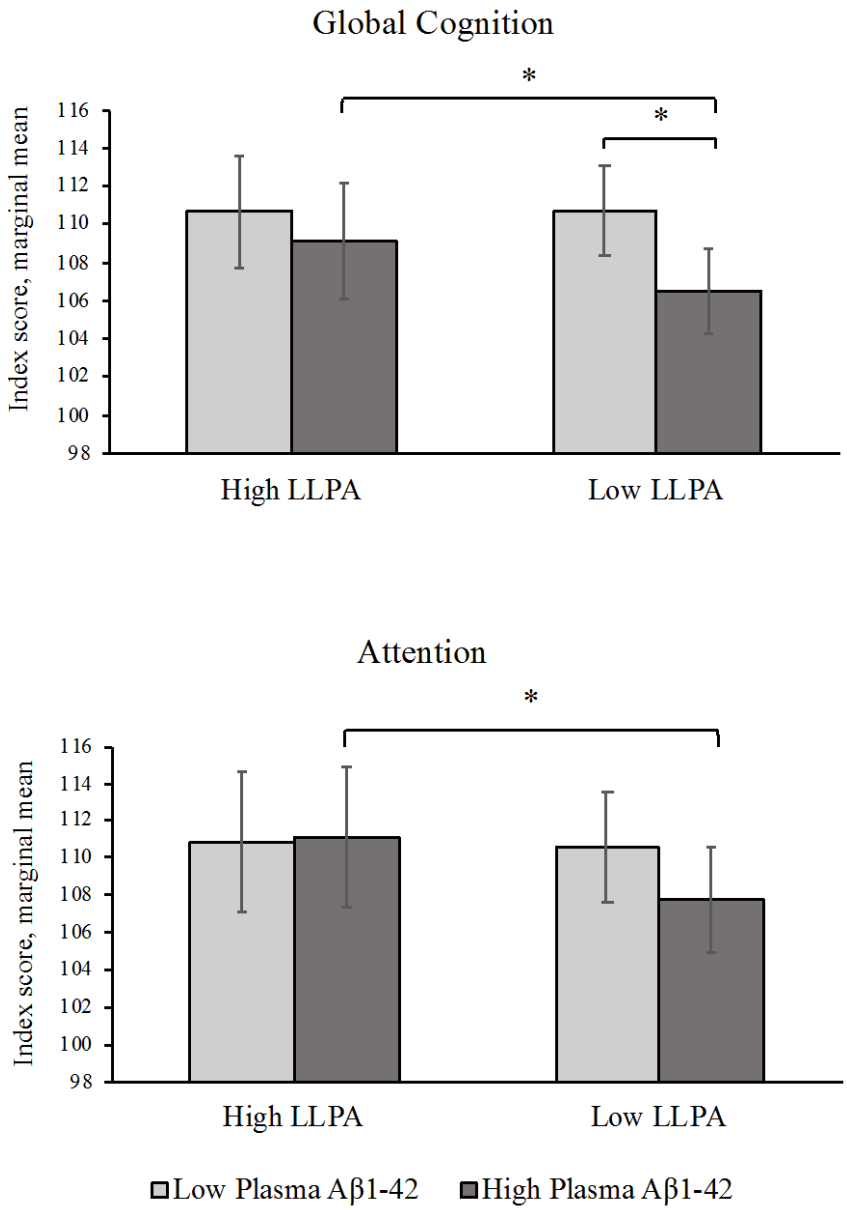


Figure 30. Late-life leisure PA modifies the association between Aβ$_{1-42}$ plasma levels and attention plus global cognitive function

Graphs depict a significant difference in the association between Aβ$_{1-42}$ plasma levels and attention plus global cognition as a function of LLPA. Higher index scores indicate better cognitive performance. Adjusted for age, sex, education, hypertension, hypercholesterolemia, type II diabetes, BMI, time and time^2. Error bars represent 95% CI's. Abbreviations: MLPA, midlife physical activity; Aβ, amyloid beta 1-42 (plasma). *$p \leq .05$

Table 33. Mixed-models regression exploring the association and interactions between Aβ1-42, *APOE*ε4 carrier status MLPA and LLPA on multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 1 [a, b]							
$A\beta_{1-42}$	-0.52 [-0.96, -0.07]*	-0.13 [-0.54, 0.29]	-0.10 [-0.42, 0.21]	-0.18 [-0.64, 0.27]	-0.21 [-0.58, 0.15]	-0.67 [-1.22, -0.13]*	-0.40 [-0.98, 0.18]
$A\beta_{1-42}$ × Time	0.15 [-0.17, 0.47]	-0.06 [-0.47, 0.35]	-0.04 [-0.32, 0.24]	0.30 [-0.16, 0.75]	0.12 [-0.24, 0.48]	0.13 [-0.20, 0.46]	-0.43 [-1.56, 0.71]
$A\beta_{1-42}$ × Time^2	-0.04 [-0.10, 0.03]	0.00 [-0.09, 0.09]	0.00 [-0.06, 0.06]	-0.04 [-0.13, 0.06]	-0.02 [-0.10, 0.05]	-0.03 [-0.10, 0.04]	0.07 [-0.15, 0.30]
$A\beta_{1-42}$ × *APOE*	-0.23 [-1.13, 0.85]	0.20 [-0.78, 1.17]	-0.21 [-0.98, 0.56]	-0.42 [-1.53, 0.70]	0.14 [-0.74, 1.03]	-0.90 [-2.27, 0.48]	1.13 [-0.23, 2.50]
$A\beta_{1-42}$ × MLPA	-0.51 [-1.42, 0.39]	-0.47 [-1.31, 0.37]	0.44 [-0.19, 1.07]	0.02 [-0.91, 0.96]	0.09 [-0.67, 0.85]	-1.38 [-2.50, -0.27]*	0.21 [-1.00, 1.42]
$A\beta_{1-42}$ × LLPA	-1.23 [-2.29, -0.18]*	-0.21 [-1.23, 0.81]	-0.01 [-0.77, 0.76]	-1.33 [-2.45, -0.20]*	-0.73 [-1.63, 0.17]	-1.91 [-3.18, -0.65]**	-0.78 [-2.32, 0.76]
Model 2 [a, b]							
$A\beta_{1-42}$ [c]	-0.75 [-1.23, -0.28]**	-0.24 [-0.68, 0.21]	-0.17 [-0.51, 0.17]	-0.38 [-0.86, 0.10]	-0.31 [-0.70, 0.07]	-1.02 [-1.61, -0.43]**	-0.50 [-1.13, 0.13]
$A\beta_{1-42}$ × Time	0.10 [-0.23, 0.44]	-0.09 [-0.53, 0.34]	-0.10 [-0.39, 0.20]	0.27 [-0.21, 0.75]	0.14 [-0.24, 0.52]	0.09 [-0.24, 0.43]	-0.33 [-1.54, 0.87]
$A\beta_{1-42}$ × Time^2	-0.03 [-0.10, 0.04]	0.01 [-0.08, 0.10]	0.01 [-0.05, 0.07]	-0.03 [-0.13, 0.07]	-0.03 [-0.11, 0.05]	-0.03 [-0.10, 0.04]	0.06 [-0.18, 0.30]
$A\beta_{1-42}$ × *APOE*	0.42 [-0.69, 1.53]	-0.02 [-1.04, 1.00]	0.37 [-0.43, 1.17]	0.68 [-0.46, 1.82]	-0.34 [-1.24, 0.57]	1.05 [-0.36, 2.46]	-0.97 [-2.39, 0.44]
$A\beta_{1-42}$ × MLPA	0.14 [-0.84, 1.12]	-0.11 [-1.03, 0.81]	0.80 [0.13, 1.48]*	0.51 [-0.46, 1.48]	0.23 [-0.57, 1.02]	-0.63 [-1.87, 0.61]	0.65 [-0.64, 1.94]
$A\beta_{1-42}$ × LLPA	-0.84 [-1.94, 0.27]	-0.11 [-1.18, 0.97]	0.17 [-0.64, 0.98]	-1.01 [-2.16, 0.15]	-0.58 [-1.51, 0.35]	-1.44 [-2.79, -0.09]*	-0.85 [-2.46, 0.76]

Table presents results from mixed-models regression investigating the independent and interactive associations between late-life leisure PA (LLPA), midlife leisure PA (MLPA), BDNF median-cut groups and cognitive function and trajectories across 30 months. For main associations, Beta-coefficient represents point difference in marginal mean cognitive index scores at 30 months relative to each independent variable reference category. Higher scores indicate better cognitive performance[a] $A\beta_{1-42}$ as a continuous variable in all analyses. [b] Interactions in separate models. Model 1 controlled for age, sex, education, time and time^2; Model 2 controlled for age, sex, education, hypertension, hypercholesterolemia, type II diabetes, BMI, time and time^2. Abbreviations: $A\beta_{1-42}$, plasma amyloid-beta$_{1-42}$; *APOE*, apolipoprotein epsilon 4 carrier/non-carrier; MLPA, midlife leisure physical activity; LLPA, late-life leisure physical activity; CI, confidence interval. Comparators: Low MLPA, Low LLPA, *APOE*ε4 carrier. †$p \leq .08$; *$p \leq .05$; **$p \leq .01$.

9.2.4 $A\beta_{1\text{-}42}$ plasma levels, leisure aerobic activity and cognition over time among *APOE*ε4 carriers versus non-carriers

There were no significant interactions between $A\beta_{1\text{-}42}$ and LLPA on any cognitive domain within carriers and non-carriers. However, there was a significant $A\beta_{1\text{-}42}$ × MLPA interaction for global cognition, *B*=1.27 (95% CI 0.17, 2.38), delayed memory, *B*=1.21 (95% CI 0.37, 2.06), and visuospatial, *B*=1.26 (95% CI 0.14, 2.38), index scores over 30 months among *APOE*ε4 non-carrier individuals only (Appendix F, Table F.2). These results revealed that, among non-carriers with high $A\beta_{1\text{-}42}$ plasma levels, those reporting high MLPA had higher index scores when compared to their low MLPA counterparts (Figure 31). Likewise, among non-carriers with low $A\beta_{1\text{-}42}$ plasma levels, those reporting low PA had significantly higher delayed memory and cognitive index scores averaged over 30 months when compared to low PA non-carriers with high $A\beta_{1\text{-}42}$ plasma levels. Delayed memory scores were also higher among high PA ε4 carrier individuals with low $A\beta_{1\text{-}42}$ plasma levels when compared to high PA ε4 carriers with high $A\beta_{1\text{-}42}$ plasma levels. There were, however, no group differences for visuospatial abilities among carriers or non-carriers despite a significant $A\beta_{1\text{-}42}$ × MLPA interaction. (Figure 31). No other $A\beta_{1\text{-}42}$ × MLPA interactions reached significance across remaining cognitive domains among both carrier and non-carrier individuals.

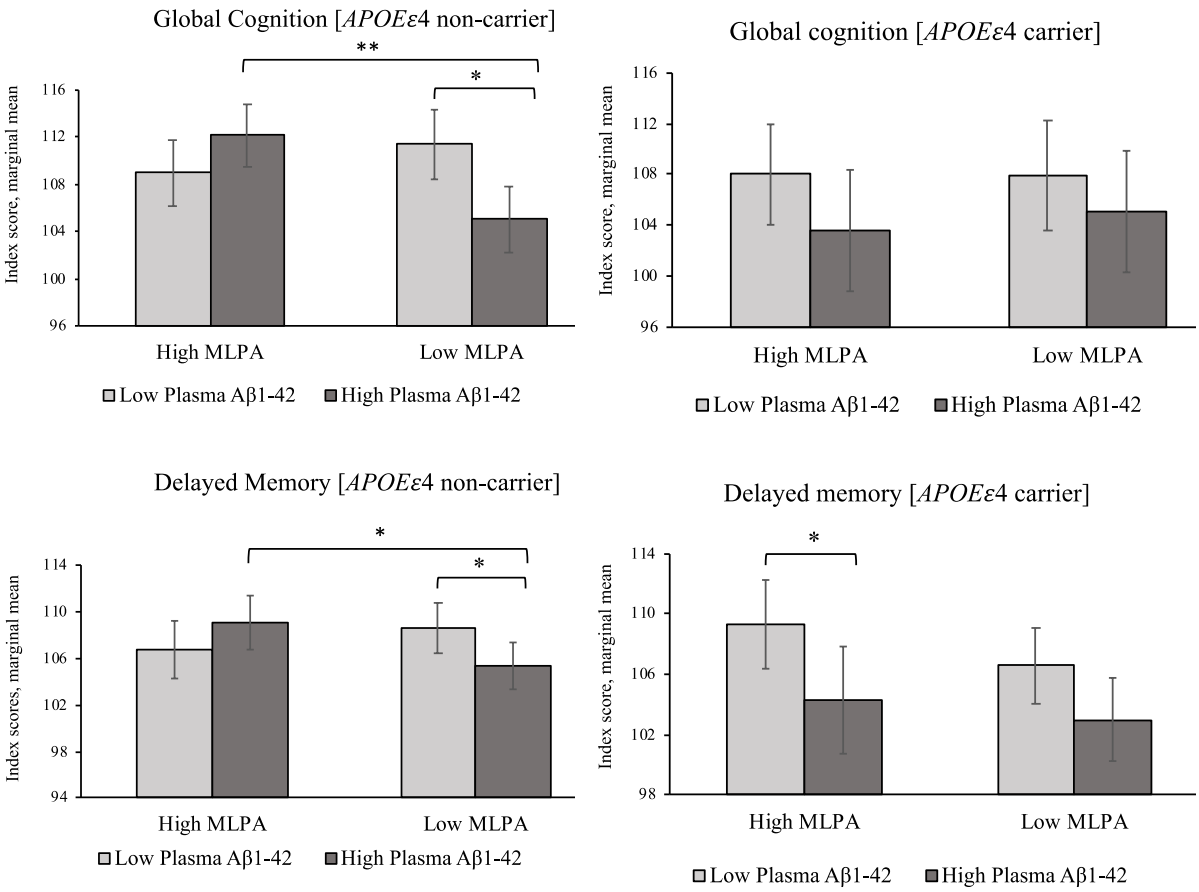


Figure 31. Midlife leisure PA modifies the association between $A\beta_{1\text{-}42}$ plasma levels and global cognition and delayed memory among *APOE*ε4 carriers versus non-carriers.

Graphs shows that the association between $A\beta_{1\text{-}42}$ plasma levels and cognition vary according to MLPA and *APOE*ε4 carrier status. Higher index scores indicate better cognitive performance. Adjusted for age, sex, education, hypertension, hypercholesterolemia, type II diabetes, BMI, time and time^2. Error bars represent 95% CI's. Abbreviations: MLPA, midlife physical activity; Aβ, amyloid beta 1-42 (plasma). *$p \leq .05$

9.3 Discussion

Figure 32 provides a depiction of results reported within this chapter. Contrary to that which was hypothesised (xi), late-late EPA, late- and midlife leisure aerobic PA and *APOE*ε4 carrier status were all not associated with $A\beta_{1\text{-}42}$ plasma levels. Likewise, *APOE*ε4 carrier status did not modify associations between PA and $A\beta_{1\text{-}42}$ plasma levels (xii). The prediction that $A\beta_{1\text{-}42}$ plasma levels would be significantly inversely associated with cognition was met (xiii); specifically, lower $A\beta_{1\text{-}42}$ plasma levels were associated with better overall global cognitive and attention index scores averaged across 30 months. $A\beta_{1\text{-}42}$ plasma levels did not, however, modify cognitive trajectories for any domain. Likewise, although Aβ x *APOE* interactions were not significantly associated with any cognitive domain, *APOE*ε4 stratified analyses found that carriers with high Aβ had significantly worse global cognition, immediate memory and executive function index scores over 30 months, when compared to non-carriers with high $A\beta_{1\text{-}42}$ plasma levels. Likewise, *APOE*ε4 carriers with high $A\beta_{1\text{-}42}$ plasma levels also performed significantly worse in overall immediate memory scores across 30 months when compared to ε4 carriers with low $A\beta_{1\text{-}42}$ plasma levels. These findings may indicate an independent additive effect whereby being an ε4 carrier alongside also possessing higher $A\beta_{1\text{-}42}$ plasma levels yield worse cognitive function, than possession of one or other alone. Indeed, confidence in this observation is further supported by congruent findings via utility of brain Aβ measures elsewhere. For example, studies have reported that among cognitively healthy cohorts, those who are Aβ+/ε4+ had significantly steeper decline on tests assessing memory, e.g. [106] (see section 1.3.1.1, page 28-29).

Congruent with hypothesis (xiv), high LLPA appeared to mitigate the negative association between those with higher $A\beta_{1\text{-}42}$ plasma levels and worse scores in global cognition and attention. Specifically, it was found that those with high $A\beta_{1\text{-}42}$ plasma levels who also reported high LLPA had significantly better global cognitive and attention index scores when compared to low active individuals with high $A\beta_{1\text{-}42}$ plasma levels. Likewise, it was also observed that those who reported low LLPA and had low $A\beta_{1\text{-}42}$ plasma levels had significantly better global cognitive scores compared to low LLPA individuals with high $A\beta_{1\text{-}42}$ plasma levels. The same associations were observed with MLPA regarding delayed memory. Those with low MLPA and high $A\beta_{1\text{-}42}$ plasma levels had worse performance relative to those with high $A\beta_{1\text{-}42}$ plasma levels reporting high MLPA or those with low $A\beta_{1\text{-}42}$ plasma levels and low MLPA. Finally, the ability of MLPA to mitigate the negative effects of higher $A\beta_{1\text{-}42}$ plasma levels on cognitive function remained significant for delayed memory and became significant for global cognition and attention among *APOE*ε4 non-carrier individuals only. Associations were no longer significant among *APOE*ε4 carriers, which

presented with notably lower effect sizes when compared to non-carriers. This finding is incongruent with hypothesis (xv), whereby it was expected that the ability of PA to mitigate the negative impact of high $A\beta_{1\text{-}42}$ plasma levels on cognitive function to be augmented among *APOE*ε4 carriers. These results, however, are partly in alignment with those reported in Chapters 4–6 whereby the association between PA and overall cognitive function were, too, augmented among non-carriers, with the exception of delayed memory.

Both midlife and late-life aerobic leisure PA did not modify $A\beta_{1\text{-}42}$ plasma levels directly, but instead moderate the impact of higher $A\beta_{1\text{-}42}$ plasma levels on worse cognitive function. This observation may provide an efficacious example of cognitive reserve. Although, the benefits of PA in promoting cognitive reserve is most evident among *APOE*ε4 non-carrier individuals. If it were observed among the enhanced risk group (i.e. ε4+/Aβ+), this would have provided yet stronger evidence of cognitive reserve. This same question has yet to be investigated among studies utilising either CSF or brain Aβ. These findings elude that partaking in ≥2 occasions of moderate to vigorous aerobic activity in midlife or late-life does not mitigate the risk of possessing the *APOE*ε4 allele on future cognitive function and decline. However, it may be effective in attenuating risk of cognitive decline among those with higher $A\beta_{1\text{-}42}$ plasma levels, especially among non-carrier individuals. It is prudent to acknowledge, however, that differences in trajectories were not statistically significant in this data sub-set, hence, it cannot be postulated that these same effects are being observed in relation to cognitive change; arguable a more robust indicator of the benefits observed on later-life cognition as a function of PA. The finding that PA does not directly attenuate $A\beta_{1\text{-}42}$ plasma levels, yet still mitigates the impact of high Aβ on lower cognitive scores have been reported elsewhere, specifically among studies investigating the role of PA on brain Aβ levels and resultant cognitive function. One cross-sectional study (n = 118, mean age [SD] 63.5 [5.93]) reported that the significant association between higher fitness and improved immediate and delayed memory to be augmented among those with higher brain and/or lower CSF Aβ load, versus those with lower brain or higher CSF Aβ load. These results also may indicate that PA attenuates the effects of Aβ induced cognitive impairment, and hence, promotion of cognitive reserve [496]. Another cross-sectional study, utilising data from the Berkley Ageing Cohort (n=92, mean age [SD] = 75.2 [5.6]), found high leisure aerobic PA to be associated with improved global cognitive function, despite no association between both PA and brain Aβ and/or cognition and Aβ [497]. This study, however, did not determine whether PA directly modified associations between Aβ and cognition, only reporting Aβ modified associations between neural integrity and cognition without conducting main effects analysis between Aβ and cognition.

Just three studies have explored the association between PA and peripheral Aβ on cognitive outcomes among humans, and no study has explored this question whilst taking into account all three variables (i.e. *APOE*ε4 carrier status, PA and cognition) [325, 336, 498]. The observation that PA was not associated with $A\beta_{1\text{-}42}$ plasma levels in the CHARIOT: PRO cohort is not in alignment with those previously reported. However, of the two studies also exploring the direct and interactive associations of PA and plasma Aβ on cognition, results are congruent. A small RCT (n=25) was able to demonstrate that just 12-weeks of a cognitive-motor dual task (mean age [SD] = 74.2 [4.3]) or single task (mean age [SD] = 74.2 [3.4]) exercise intervention [498] resulted in improved executive function as well as decreased plasma $A\beta_{1\text{-}42/1\text{-}40}$ ratio. They also reported that the enhanced cognitive benefits observed in the dual-tasks training when compared to single-task did not reflect reductions in plasma $A\beta_{1\text{-}42/1\text{-}40}$ ratio, being equivalent over both exercise groups. This finding eludes that benefits in cognition may not be entirely due to differences in plasma $A\beta_{1\text{-}42/1\text{-}40}$ ratio levels, as also reported among the CHARIOT: PRO cohort. A further study was able to successfully implicate plasma $A\beta_{1\text{-}42}$ as a possible mediator in the benefits of PA on cognition [336]. Utilising data from the Cardiovascular Health Study (n=149, mean age [SD] = 79.53 [3.15]), they reported that lower baseline self-reported PA levels predicted higher plasma $A\beta_{1\text{-}42}$ at 9-13 years later. They also found that higher plasma Aβ levels at year 9 predicted greater risk for cognitive impairment at year 13, and that lower levels of Aβ by year 9 mediated the relationship between PA and cognitive impairment. A further cross-sectional study investigated whether plasma $A\beta_{1\text{-}42/1\text{-}40}$ ratio was associated with EPA levels and if this association was modified by *APOE*ε4 carrier status [325]. Utilising data of cognitively healthy individuals from the Australian Imaging and Biomarkers Lifestyle study (AIBL) cohort (n=546, mean age [SD] = 89.6 [6.8]), they reported that higher self-reported EPA were associated with lower plasma $A\beta_{1\text{-}42/1\text{-}40}$ ratio and that this association was augmented and limited to *APOE*ε4 non-carrier individuals. They did not, however, explore the impact of these findings on cognitive function.

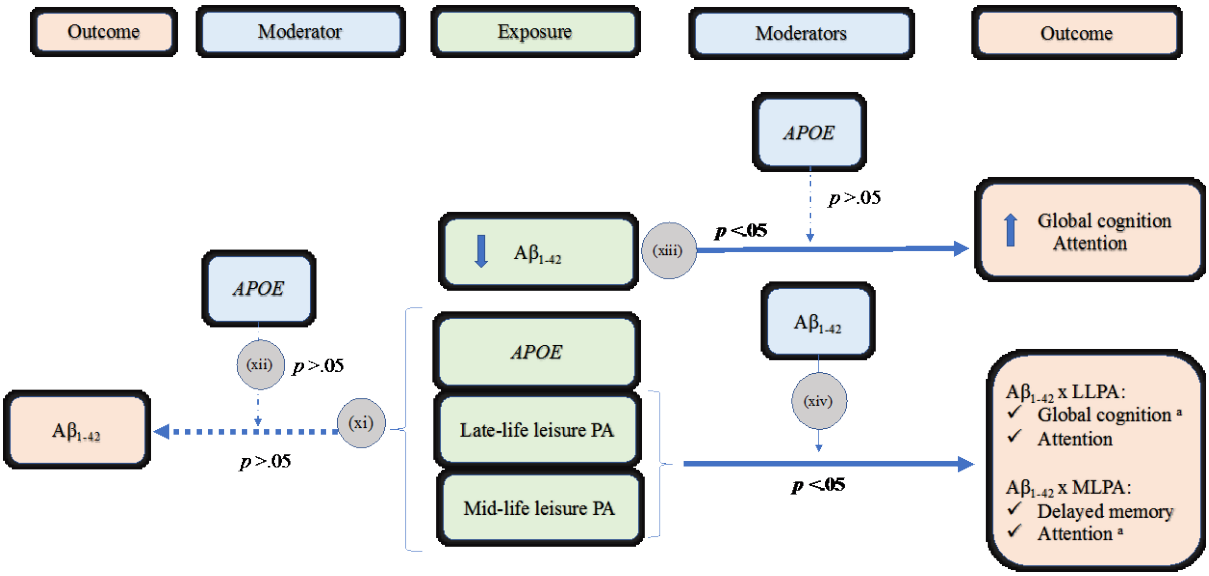
Meaningful interpretation of results presented in this chapter relies upon previous literature implicating plasma Aβ as a reliable proxy of CSF and/or brain Aβ. However, standardised measurement techniques of plasma Aβ are limited, hence, applicable interpretation of data and previous literature is challenging. Mixed methodologies may have resulted in conflicting findings – with levels reportedly either decreased [331, 332] or increased [329, 403] in AD populations when compared to normal controls. Likewise, plasma Aβ tends to be within the lower limits of detection when measured via traditional ELISA assays, resulting in a loss of sensitivity for detecting narrow differences between samples. ELISA's are also less sensitive to the interference of other factors

within serum and plasma, known to bind to Aβ proteins, hence, affecting accurate Aβ readings [401]. To overcome these challenges, ultra-sensitive techniques have emerged – one of which is the SIMOA technique, as utilised in the current study. Indeed, this is the first study to explore the role of SIMOA measured plasma $A\beta_{1-42}$ in association with PA and cognition measured longitudinally. Although studies utilising a comparably sensitive measure, immunomagnetic reduction (IMR), have consistently reported increased plasma Aβ with cognitive decline and AD, studies utilising the SIMOA technique have typically reported associations in the opposite direction [335, 403]. However, one study utilising the SIMOA technique, reported that although lower $A\beta_{1-42}$ levels were associated with an increased risk of conversion from MCI to AD, higher levels were associated with vascular risk factors (i.e. those with white matter lesions, cerebral microbleeds, hypertension, diabetes and heart disease), among the cognitively healthy control group [403]. They also reported, in the pre-clinical cohort, a slight reduction of plasma $A\beta_{1-42}$. It may be postulated that higher $A\beta_{1-42}$ plasma levels at a younger age be linked with worse cognition and that only at a later disease stage does the pendulum shift. This notion may reflect alterations in clearance processes across the disease course [499]. Possibly, higher Aβ plasma levels are observed at an earlier stage, before clinical symptomology, indicating advanced clearance. As the disease progresses, clearance processes may be inhibited; hence, manifesting reduced Aβ plasma levels during a later disease stage. Sanctioning this speculation, one study exploring the temporal role of plasma Aβ on cognitive function, found that healthy older adults with higher baseline $A\beta_{1-42}$ plasma levels were consistently associated with faster memory decline than those with lower $A\beta_{1-42}$ plasma levels [500]. However, they also reported that change in $A\beta_{1-42}$ plasma levels predicted change in memory and visuospatial scores, with decreasing $A\beta_{1-42}$, over time, predicting cognitive decline. It will be important for future work to determine more definitively the specificity of Aβ profiles for predicting dementia, versus their significance for cognitive ageing more generally. Certainly, further studies utilising the SIMOA technique to explore the specificity of Aβ plasma profiles from an earlier age and considering cognitive function and trajectories, alongside brain and/or CSF Aβ measurement is warranted.

Overall, previous findings support $A\beta_{1-42}$ plasma levels as a relevant biomarker of increasing risk of cognitive decline. This study was able to demonstrate that both midlife and late-life leisure aerobic PA played a possible role in mitigating the negative effects of higher baseline $A\beta_{1-42}$ plasma levels on multi-domain cognition. Mechanisms by which exercise may influence Aβ deposition and/or cognition remain unclear, although there may be both relatively direct effects on APP metabolism [271, 272, 501] and indirect effects through influences on neurotrophic factors,

neuroinflammation, cerebrovascular functioning, or glucose metabolism [271, 501, 502], all of which have not yet been thoroughly explored in such context. In addition, *APOE-ε4* carriage is considered a key risk factor associated with increased Aβ deposition [88, 89], yet studies exploring PA-Aβ-cognitive associations have not included this key variable in their analyses.

(A) Overall cohort



(B) *APOE*ε4 stratified

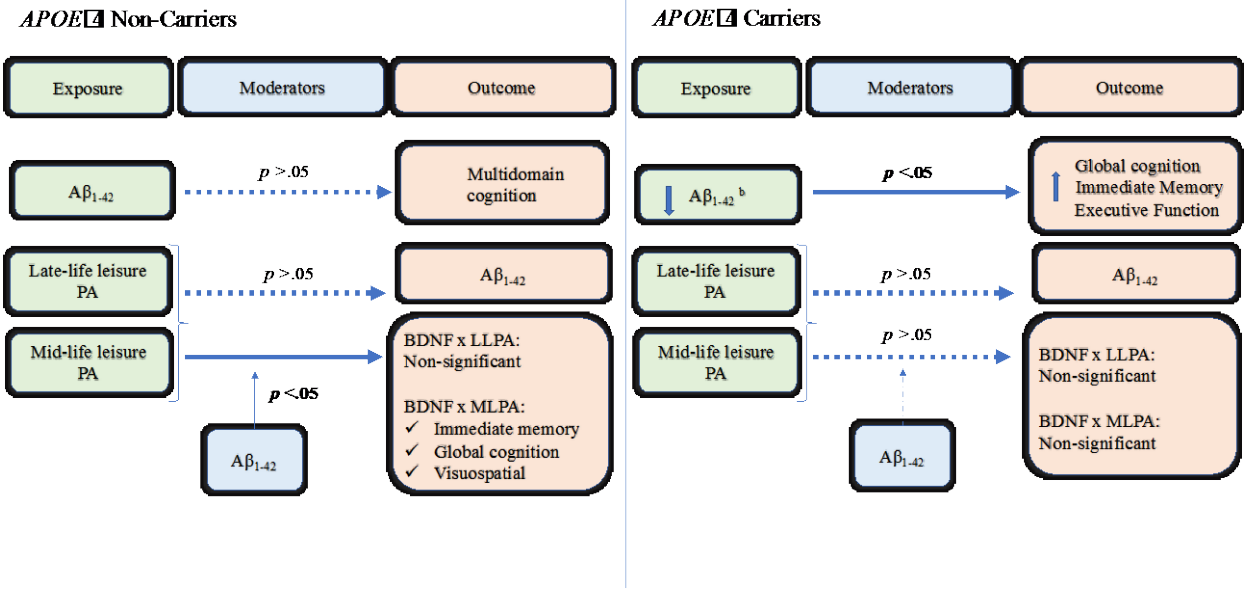


Figure 32. Depiction of results pertaining to the role of Aβ-42 plasma levels on the association between physical activity, *APOE*ε4 carrier status and cognition

[a] Became non-significant after adjustment for vascular factors; [b] median split Aβ variable

Chapter 10: Overall Discussion and Conclusions

This thesis has investigated the role of physical activity in mitigating cognitive decline among older adults, and if this lifestyle factor maintains a positive association with cognitive performance among those at increased risk of dementia via possession of certain genetic, demographic and biomarker indicators. Utilising a cognitively healthy older cohort, the role of PA during midlife, during late-life and also temporally across both midlife to late-life on multi-domain cognitive function and trajectories over a 30-month time period was investigated. Whether these associations were modified by *APOE*ε4 carrier status and sex and if they differed among those ≥70 and those <70 years of age was explored. The role of a proposed biomechanistic marker, BDNF, was further evaluated to determine if it was implicated in the causal pathway between higher levels of PA and better cognitive outcomes. Furthermore, this is one of the few studies to explore the role of $A\beta_{1\text{-}42}$ plasma levels measured via the highly sensitive SIMOA technology on multi-domain cognitive function and the only study to explore whether physical activity in midlife or late-life as well as *APOE*ε4 carrier status modifies these associations.

To follow is a conclusive discussion of the findings presented in this thesis. The major findings from Chapters 4-6 are summarised in Table 24 and findings from Chapters 8 and 9 summarised in Figure 27 and Figure 32. The overall discussion and concluding remarks will be detailed in the context of strengths and limitations, ongoing gaps in the literature, recommendations for future research and perspectives.

10.1 Strengths, limitations and scope for future research

There are a number of points to address in light of strengths and limitations. Awareness of both elucidates the need for future research on this topic, and recommendations for improvement whilst highlighting ongoing gaps in the literature.

10.1.1 Physical activity measurement

A limitation in the current study was the method of assessing physical activity. The PASE questionnaire captures the previous 7-days of EPA without taking into account any source of intra-individual variation (e.g. seasonal variation) of PA levels longitudinally and thus may not represent a full picture of each participants' average PA in general [503]. Nonetheless, the PASE questionnaire has been shown to be valid for use in an older population [504]. As covered in the methods (section 3.6.2.3, page 106), PASE scores within the CHARIOT: PRO cohort correlated

significantly with expected health variables, indicating reasonable accuracy. Test re-test reliability of the PASE within the CHARIOT: PRO study was evaluated via comparing PASE scores at baseline and the following 6-month time point. PASE scores were found to be significantly correlated (r=.50, $p < 0.001$, 2-tailed Pearson), despite presence of statistically significant seasonal influence. These findings indicate that each individual's PA levels in the current cohort appeared to remain reasonably consistent from the week period at baseline to the week period at six months. Certainly, an optimal methodology would be to include PA data at each follow-up time-point to determine whether change in cognition also related to change in PA over time. Due to the lack of resource to enter such data, it was not possible to explore this question in the current study.

More importantly, the very use of self-reporting has its short-comings; the misinterpretation of questions, inaccurate recall of activity frequency, duration or intensity, and the resulting inaccurate under- and over-estimation of overall PA has been reported [388]. Physical activity questionnaires have also been shown to exhibit better accuracy in measuring high-intensity than light or moderate-intensity activity as a result of self-reporting [503]. PA captured during midlife in the current study also possesses limitations. The 'midlife' age period overlaps with the youngest age of inclusion from which point an individual's self-reported PA via PASE was considered 'late-life', although only a small proportion of included participants were below the age of 65 years (n=82, 9%). The questions utilised for midlife PA attainment were limiting in that they did not collect frequency per week; the response was simply either 'daily' or 'weekly'. Despite these limitations, self-reporting methodologies are widely utilised and also possesses some strengths. Administration of self-reporting is less demanding for both researcher and participant, especially when exploring large cohorts over repeated time-points, being low-cost and possessing the capacity to capture a large range of physical activities, from hobbies to structured exercise. The majority of observational studies exploring the role of physical activity on cognition and risk of AD and/or dementia have utilised self-reporting.

A growing number of studies are utilising objectively measured PA via accelerometer devices, all showing positive PA/cognitive associations and reduced incidence of AD [249, 505, 506]. Despite the arguably higher validity and reliability when compared to self-reporting, objective measures of PA have been found to corroborate PA measured via self-reporting within the same dataset [507]. Results reported via objectively measured PA also echo those reported subjectively; hence, one may argue that PA levels, as measured in this thesis and widely elsewhere, is a comparably reliable and representative indicator of an individual's physical activity levels. Nonetheless, these results also highlight the need to extend findings by utilising a measure of both subjective and objective PA.

10.1.2 Cognitive measurement

A strength of this study is the detailed temporal cognitive profiling of study participants via utility of a variety of sub-tests for the development of composite cognitive index scores. As covered extensively in the methods, the RBANS and NAB were both developed and validated as batteries designed to be sensitive in identifying the earliest clinical cognitive change. However, one must be cautious when interpreting results produced from utility of the RBANS domain index scores, and likewise those from the NAB. Assessments designed to measure specific domains of cognition should not be definitively labelled as such, due to the known overlap between domains alongside the tests that assesses them. Also, one cannot ignore previous recommendations in exploring RBANS sub-test results in order to ascertain whether any variation in sub-tests are unjustifiably effecting index scores and resulting in misleading outcomes. The psychometric stability of the composite indices have been well reported [375, 508], however, the RBANS' author later produced a supplement of normative subtest data, indicating that certain index scores may be affected by minor changes on subtests, recommending the use of sub-tests in further exploratory analyses [509]. It will be important to follow up the current findings produced in this thesis with re-analyses of the sub-test scores. This will also aid in further comparability across studies, seeing most fail to utilise a composite index score and instead select performance on a specific sub-test as a measure of any one domain. Seasonal influence on self-reported PA was noted in the current cohort. Cognition may also be influenced by season, and in fact, some exploratory age and sex adjusted linear regression analyses within the CHARIOT: PRO cohort found between-subject scores to be higher in spring and summer when compared to winter. Certainly, within-subject changes over time would be more informative, yet to explore such is beyond the scope of the current study. Nonetheless, future studies should consider seasonal influence on PA/cognitive associations.

10.1.3 The population, sample size and follow-up duration

The CHARIOT: PRO cohort, although recruited from an age, sex and ethnicity matched population [373], it still may be considered an underrepresentation of the wider population, hence, results cannot be generalised beyond those within the current study. People who are eligible for and often volunteer to participate in such studies are often well educated, healthy, higher socio-economic representatives [510]. Certainly, including only those who volunteered for participation will create some selection bias. Being a predominantly Caucasian demographic in the current study, results cannot be generalised toward a wider range of ethnic groups. It will be important to promote inclusion of commonly underrepresented demographics such as ethnic minority groups and/or those of a lower socioeconomic status.

The CHARIOT: PRO Main Study was terminated prior to completion, consequently leading to a reduced sample size over time and hence, reduced statistical power in cognitive trajectory analyses (see Figure 6). Despite such limitations, the cohort is a reasonably sized and well-characterised longitudinal cohort. As can be seen in Table 17, characteristics of this community-dwelling sample were similar across *APOE*ε4 carriers and non-carriers, males versus females and those ≥70 years versus those <70 years.

A follow-up time of 30 months, especially among a reasonably young and healthy older cohort is limiting. Longer follow-up duration will shed light on earliest cognitive indicators of dementia risk as well as the impact of PA on delaying onset, especially among risk individuals. For example, is dementia onset itself delayed or just cognitive decline? Studies exploring the role of cognitive reserve have found that those with higher cognitive reserve, appear to maintain cognitive and functional health for a longer period, yet present with a steeper rate of decline in later stages of life [511]. Nonetheless, despite a relatively short follow-up within the CHARIOT: PRO cohort, previous studies have reported the benefits of PA on cognition with an even smaller time-frame, e.g. [198-200, 481, 512]. A 30-month follow-up period does not preclude meaningful interpretation given previous reports. However, it would enhance existing findings if the same cohort were to be re-evaluated for progression to dementia at a later date.

10.1.4 Statistical modelling

As highlighted in the Methods Chapter (section 3.9, page 120-128), the benefits of a mixed-effects regression approach for longitudinal analysis surpasses more traditional approaches such as repeated measures ANOVA or ANCOVA methodologies. The decision to explore both overall cognitive function averaged across 30 months as well as cognitive trajectories, or growth curve modelling, allowed a deeper investigation of the data. The influence of practice effects and possibilities of reverse causation could also be examined. Trajectories in cognition among healthy individuals will realistically be non-linear, especially when considering practice effects over time. Despite this fact, most studies investigating the association between PA and cognitive trajectories only ever consider a linear trend, which is a limitation among many published studies.

The choice to include *a priori* selected covariates based on existing literature, for the purpose of conservatively estimating association, raises the potential for model over-correction. To overcome this limitation, model building was conducted. Associations from a basic age, sex and education adjusted model was followed by adjustment for vascular factors, and within Chapters 4-6, a third model including alcohol and smoking, within all primary analyses. Effects size remained largely unchanged across models, removing any suspicion of overcorrection.

With regard to the exploratory nature of this study, it is prudent to recognise the need for cautious interpretation of statistically significant outcomes, especially with regard to risk of Type I error. Notwithstanding, observed exploratory correlations may indicate true correlations, whereby confidence in such inferences are strengthened by congruent findings elsewhere as discussed in this thesis [513]. Whether p-values be adjusted for multiple comparisons is controversial [514-517]. However, results reported in this study are consistently within the expected direction. This finding lends toward reinforcing the results, rather than detracting from a genuine finding as one may argue methods such as Bonferroni adjustment encounters. Nonetheless, owing to the large volume of repeated analyses conducted, there is a risk that several false positive results have occurred; such instances were evaluated in light of effect sizes as well as associations going against that previously reported. Certainly, there is value in running a confirmatory study, where outcomes would be sufficiently informative whether or not statistically significant. For example, owing to small sample sizes in the BDNF analyses, risk of Type II error should be acknowledged, whereby observed non-significant trends might have been statistically significant if the sub-sample size had been larger.

It must be acknowledged that the sample size was relatively small, especially among some sub-analysis, and follow-up duration short, also raising concern around validity of results within some interactions. Nonetheless, a retrospective post-hoc power calculation was chosen against in this study. The research conducted here was designed retrospectively and used existing data resources. When designing a study with *a priori* selected hypothesis a power calculation maybe more relevant [518]. Indeed, the disadvantage of post-hoc power calculation features heavily in the literature [516, 519]. Arguably, pre-defined primary and secondary hypothesis alongside the use of estimate effects and confidence intervals more clearly and directly addresses uncertainty than would a post-hoc power calculation. As mentioned in the paragraph above, this method has aided interpretation throughout this thesis and is justifiably sufficient. However, once again, within exploratory sub-analysis, sample sizes were small which do not discredit the results but rather emphasise the need for future confirmatory exploration.

10.1.5 Biomarker assessment: serum BDNF, plasma $A\beta_{1-42}$ and *APOE* genotype

Analyses and interpretation of the results presented in Chapter 8 (from page 198) are based on serum BDNF levels, rather than BDNF directly from brain tissue. BDNF as a possible biomechanistic factor responsible for the cognitive benefits of PA depends on how readily it can bi-directionally cross the blood-brain barrier and hence stimulate central neurotrophin increases, especially in the hippocampus. A couple of human studies suggest an association between peripheral and central BDNF concentrations [520, 521], however, the evidence in humans is limited. It has been reported that BDNF can cross the blood-brain barrier in mice and rats [520,

522], yet again, evidence among animals is also inconsistent [523, 524]. Furthermore, the human blood brain barrier is structurally and functionally different from those in animal models. This fact may partly explain why BDNF was not statistically significantly associated with cognition nor was it substantially modified by PA in the current study.

BDNF serum levels and $A\beta_{1-42}$ plasma levels were only assessed at one time-point (baseline). It would be more informative to characterise how PA is associated with temporal BDNF and Aβ in the periphery and how these factors relate to cognitive change over time. This approach would reduce causational bias and is particularly important given the uncertainty of plasma Aβ as a biomarker of AD and other neuropathology. For example, studies have reported mixed results as to whether plasma Aβ is associated with central Aβ and if plasma Aβ is able to predict cognitive decline and future AD [407]. Such discrepancies suggest unwarranted focus on peripheral Aβ as a causal factor in the development of AD. Another interpretation, however, is that other factors may be modifying such observations (i.e. genetics, demographics, measurement timing and type). In fact, utility of SIMOA in the current study is a strength, due to its added sensitivity in measuring differences in minute concentrations [398] as well as reducing the risk of human error, given that the assay process is largely automated. It is well recognized that current biomarkers of AD research are expensive or invasive, hence, validation of peripheral alternatives are vital. Blood based biomarker tests along with genetic, clinical and demographic information will likely play a screening role for selecting individuals for more invasive and expensive biomarker testing.

*APOE*ε4 carriage is suggested to exhibit a gene-dose effect on dementia risk, with homozygotes at 68 years compared to heterozygotes at 76 years. It would be informative to have observed the effect modification of homozygote ε4 individuals alongside heterozygotes. However, a very small sample of homozygotes in the current cohort (n = 8), resulted in inadequate power. This study did not take into account *APOE*ε2 carriage in the context of ε4 carriage, as this allele has been associated with reduced risk of cognitive decline and incident dementia [525]. It may be argued by some that, among ε2/ε4 heterozygotes, the ε2 allele mitigates ε4 allele carrier risk. Nevertheless, existing findings coupled with reports from the Rotterdam Study indicate that this effect only exists in the absence of an ε4 allele [526]. Yet again, due to a small sample of ε2/ε4 participants in the present cohort (n=29), there was inadequate power to explore such a question.

10.1.6 A multi-domain lifestyle approach

Future studies should endeavour to concurrently measure other classes of lifestyle alongside PA. For example, previous studies have reported cognitive activity to effectively mitigate the increased risk of inactivity [527-529]. A compelling review highlights the importance of a multi-domain

lifestyle approach whereby a combination of lifestyle interventions, namely PA, diet and cognitive activity, may be more effective in targeting a larger array of risk factors and mechanisms [530]. Just three large multi-domain lifestyle interventions have been completed (FINGER, MAPT and PreDIVA) [481, 482, 531] however, only one of the three observed a benefit on reducing cognitive decline, especially among those at increased risk [481]. A key difference between these RCT's were that MAPT (n=1,680) [532] and PreDIVA (n=3,526) [531] had a population of an older mean age (75 years) when compared to the FINGER study (mean age (SD) = 69.3 (4.7) years) [481]. As discussed extensively in this thesis, this finding again highlights the importance of intervention timing. Targeting individuals at a younger and healthier stage of life and where brain pathological processes have not begun, may lead to better outcomes. The FINGER study (n=1,109) not only reported post-intervention cognitive improvements at 24 months, but sub-analysis identified that the difference between the control and intervention group on global cognitive change was 0.037 (95% CI 0.001-0.073) among carriers and 0.014 (95% CI -0.011-0.039) among non-carriers, indicating an augmented effect among carrier individuals [533]. These findings highlight the importance of identifying the right treatment for the right group. The FINGER trial selected a cohort known to be at an increased risk for dementia based on the CAIDE risk score [534]. They also only included individuals whose cognitive function were average or below that expected for their age. Recruitment strategy may be an important factor to consider. PreDIVA recruited an unselected population. MAPT recruited via several strategies including advertisement, which would most likely attract more highly educated volunteers and those who are most likely aware of the benefits of healthy behaviour. It has been postulated that those who are within the prodromal stages of AD may benefit most from lifestyle intervention, identification of which can now be possible via the new diagnostic criteria for AD. Although this 'targeted' cohort approach reduces ability to generalise results to a wider population, a targeted approach will allow for the development of treatments designed toward targeting those with higher risk profiles. Indeed, post-hoc analysis identified an enhanced benefit of intervention on dementia outcomes among those with a CAIDE scores ≥6 or evidence of brain AD pathology within the MAPT trial, and among those with untreated hypertension within the PreDIVA trial. Differences in intervention intensity may have also resulted in varying outcomes across these three trials, with FINGER being more intensive when compared to PreDIVA and MAPT.

Certainly, there is growing evidence that participation in both physically and cognitively challenging activities, as well endorsing certain dietary patterns, may provide stronger protection against cognitive decline than PA alone [535] and may individually influence age-related cognitive decline via different mechanisms and pathways [146]. Likewise, exercise intensity may mitigate

age related cognitive decline more effectively than duration, warranting further investigation [259, 536].

10.1.7 Summary

To refine our existing knowledge and further investigate the role of PA in cognition, future studies can be strengthened by several methodologies. These include a control group (in the case of RCT's) to guard against reverse causality and other such factors as practice effects and to guard against any potential effect of environmental enrichment (i.e. cognitive activity, social or other leisure activities); the use of a standardised and sensitive neuropsychological assessment battery able to assess multi-domain cognition; collection and statistical control of possible residual confounding via such factors as socioeconomic status, intelligence quotient, occupation level, mental well-being, diet/nutrition, midlife vascular health factors, baseline fitness and prior PA levels; a large enough sample to investigate the effect modification of PA/cognitive associations based on different age groups, sex and/or gender and genetics; incorporating varying exercise types, intensities and duration to assess dose-response relationships; and addition of key biomarker data including known peripheral biomarker indicators (i.e. Aβ, tau and neurofilament light), structural and functional imaging data and brain/CSF Aβ and tau data. Ideally, a follow-up time spanning ≥10 years whilst repeatedly collecting exposure, outcome and confounding data would be necessary to elucidate the long-term temporal relationship of such variables and, optimally, within the context of a PA RCT. Achieving these methodologies within an experimental design would require a hugely coordinated effort and rely upon vast levels of resource as well as identifying a sustainable lifestyle intervention whereby attrition were kept to a minimum.

10.2 Perspectives and Concluding remarks

Overall, epidemiological data suggest an association between higher PA levels and lower risk of cognitive impairment and AD later in life, even after confounder adjustment. However, due to the large clustering of AD risk factors also associated with reduced PA, its independent link with reduced risk of cognitive decline remains equivocal. This does not preclude PA as a feasible means to reduce risk of cognitive decline and dementia but indeed supports this lifestyle factor as key to risk reduction. The alignment of PA with other risk factors such as obesity, smoking, alcohol consumption, and diseases such as diabetes and cardiovascular disease, only further strengthens the need to promote PA as a key lifestyle factor for not just dementia prevention but reduction of multiple and often co-morbid diseases.

RCT's have implicated exercise as a means to promote cognitive and brain plasticity. Behavioural and mechanistic outcomes have converged to support a cohesive picture. Several reviews have endorsed the claim that PA exhibits a potent effect on brain structural and functional health [289, 325, 537], which are expressed symptomatically via preserved cognitive health. There is now a growing body of literature exploring the role of PA on pathology as a function of *APOE*ε4 carrier status. For example, one study found that highly active ε4 carriers had higher levels of semantic memory activation across several brain regions compared to non-carriers [538, 539]. Another study reported *APOE*ε4 carriers with high aerobic fitness to have greater glucose uptake in the temporal lobe following working memory tasks compared to low-active carriers [540]. Likewise, similar observations have reported associations between higher PA and reduced brain Aβ to be augmented among *APOE*ε4 carrier individuals [325, 326], although the same associations have also been reported to be augmented among non-carriers [327]. No study, however, has compared *APOE*ε4 x PA interactions on pathology whilst also considering cognitive outcomes, particularly at the preclinical stage.

Although the evidence is leaning strongly toward PA as a feasible means for dementia prevention (see Chapter 1, section 1.4.2, page 34-35), clear gaps in our knowledge remain. Nonetheless, such gaps do not warrant continued disregard of the existing evidence. In fact, exercise should be promoted as an essential component of healthy ageing given that reducing physical inactivity by 25% could prevent as many as one million cases of dementia worldwide [73-75, 541]. Offering healthcare providers, seniors and even the wider public with specific and individualised recommendation based on their health profile and demographic characteristics will promote exercise as a treatment for cognitive decline. Furthermore, individually catered specifications detailing type, intensity and frequency of exercise for optimal benefit will be necessary. Failing to consider individual differences in response to PA on cognitive benefits, including, but not limited to age, sex, and *APOE*ε4 carrier status may have resulted in mixed findings.

Outcomes reported in this thesis, and elsewhere, highlight the importance of endorsing an active lifestyle in not just late-life but also midlife. Although this study explores the role of PA across mid- to late-life, how this lifestyle contributes toward later-life AD risk may also depend on earlier life factors. Indeed, an individual's propensity toward endorsing an active lifestyle may stem from factors as early as infantile development. For example, studies have found that earlier infantile motor milestone achievement is positively associated with PA levels in adodolence [541] and among adults [542]. Another study investigated the role of certain bio-psychosocial factors across the life-span on glycaemic health and risk of type 2 diabetes [543]. They reported that a combination of metabolic and psychosocial factors was significantly linked to metabolic health in midlife. This

is relevant in the context of AD prevention research as T2D in midlife is a known and key risk factor, and again supports the importance of endorsing an active lifestyle in earlier life. Identification of developmental factors linked to health behaviours in later-life may be an efficacious method of disease risk reduction in later-life. In fact, very few studies have investigated the role of a healthy lifestyle from a life course epidemiological perspective [544]. A life course approach provides an interdisciplinary framework for application in health, human development and ageing. This framework warrants further attention and scope for future research as larger datasets, with longer follow-up periods become available.

Ability to translate evidence implicating PA as a feasible means for maintained cognitive health and dementia also depends on an in-depth understanding of the science behind the phenomenon, especially seeing the public may not appreciate that PA has the ability to alter the structure [296, 545] and function [297, 546-548] of the brain itself. Furthermore, the reliability of dementia diagnosis, as defined by the NIA-AA guidelines, profoundly affects prospects for development of preventative strategies. There has been an ongoing debate surrounding methods to synergise the NIA-AA guidelines and the IWG guidelines. One comparable factor is that they both aim to facilitate an earlier and more accurate diagnosis than preceding frameworks, which is important when seeking preventative strategies. Moreover, guidelines to standardise the aetiology and clinical spectrum of cognitive impairment due to CVD are currently being devised [549] which will greatly benefit research among cognitively healthy cohorts where identification of early indicators of cognitive decline and dementia is vital. In addition, focussing on dementia and AD prevention as defined by current criteria is limiting in the sense that risk factors for AD dementia and the neuropathological hallmarks of AD might not necessarily be comparable. Cognitive impairment is substantially more clinically important than pathological manifestation (whereby a discordance between such is often reported) and should remain the focus of prevention.

Together, the literature appears to indicate that the positive associations between PA and later-life cognitive health exists among *APOE*ε4 carrier individuals as well as non-carriers. Indeed, five out of the seven prospective observational studies included in the systematic review reported PA/cognitive associations to be augmented among *APOE*ε4 carrier individuals [114, 198-200, 208]. Findings in the current thesis indicate domain-specific benefits among both carriers and non-carriers. Stratified analyses found augmented benefits among non-carriers and as a function of LLPA on language and attention and MLPA on global cognition, delayed memory, attention, language, visuospatial and executive function. However, a significant interaction (*APOE* x LLPA) provided strong evidence for benefits of LLPA on delayed memory trajectories among carriers. Likewise, although significant PA/cognitive associations were limited to the non-carrier strata in

majority of analyses, non-significant positive trends remained visible among carriers. Possibly with a longer follow-up period and larger sample size, positive trends observed among carriers may too become statistically significant, further endorsing PA as a viable preventative approach among both *APOE*ε4 non-carrier and carrier individuals. Without the measurement of AD pathology, these findings do not indicate PA as a means for AD risk reduction nor can we postulate that these findings indicate dementia risk reduction. However, on the back of previously discussed research, these results add to the literature advocating that higher levels of aerobic activity (in this case; ≥2 occasions of moderate/vigorous activity weekly) in either midlife and/or late-life promotes better cognitive performance in older age. Findings also support that these associations be dependent upon *APOE*ε4 carrier status, age, sex and possibly even as a function of plasma Aβ levels. Inclusion of AD biomarker data in future research will clarify the role of PA in either reducing AD pathology or promote resilience to existing pathology – again possibly dependent upon PA timing and dose alongside the modifying role of non-modifiable risk factors.

Incorporating the use of AD biomarkers within large-scale studies and/or among disadvantaged populations would benefit from low-cost and minimally invasive biomarker acquisition, such as blood or saliva, which are now beginning to emerge [329, 550-552]. This study has been able to implicate $A\beta_{1-42}$ plasma levels as a potentially meaningful indicator (even when coupled with possession of the *APOE*ε4 risk allele) of cognitive health and its possible moderation by PA. Nonetheless, further research in terms of identifying the temporal role of peripheral $A\beta_{1-42}$ in the context of dementia and as a possible proxy of brain and/or CSF Aβ is required. Findings in this thesis portray an encouraging outlook, connecting this biomarker with cognitive function in older age and possibly an early indicator of AD risk. However, BDNF appeared to play little to no role in mediating or moderating PA/cognitive associations in this project. Given previous research, these findings are surprising and may highlight methodological limitation in the current study, such as a reduced sample size or measurement error.

Novel, theoretically integrative and uniquely informative data have been reported in this thesis. Findings reported and discussed throughout this thesis may contribute toward devising experimental studies aimed at producing congruent and translatable outcomes. Such specificity of effects, if replicated within an experimental setting, can lead to precision intervention 'prescriptions' among cognitively healthy younger and older adults. For example, younger *APOE*ε4 non-carrier individuals may be advised to adopt aerobic activities at least twice weekly, during midlife and maintain such into late-life to substantially improve their cognitive vitality in older age. Those at increased risk, such as possessing the ε4 allele and being older in age (i.e. 70+), may be

prescribed a more intensive program including combinations of higher aerobic activity alongside strengthening exercises.

Existing evidence lacks the detail required to enable such exercise prescriptions for maximising cognitive benefits among individuals of differing phenotypes, nor are the underlying molecular and cellular mechanisms fully understood. Indeed, this study failed to identify the role of BDNF in PA/cognitive associations. Nevertheless, current evidence strongly suggests that PA promotes maintained cognitive health in older age, even among risk individuals – and critically, the magnitude of reported benefit exceeds that of pharmaceutical approaches [512]. Physical activity exerts a major public health importance in its ability to slow cognitive decline and reduce dementia risk, especially until measures directly assisting AD are discovered. Parenthetically, if the emergence of a disease modifying treatment were to be identified, new implications may be realised. For example, the emergence of a treatment could also lead to individuals living during the symptomatic stage of the disease for longer, placing considerable strain on medical cost and access [553]. Additionally, whether the medical care infrastructure could accommodate the surge in demand for screening and treatment brings about important policy implications regarding funding priorities. Therefore, from a public health standpoint, prevention of the syndrome (dementia) rather than the disease (AD), through focussing on modifiable lifestyle factors and promoting neural reserve capacity would be more cost effective and yield preferable long-term benefits [10].

Communicating the benefits of PA to health care providers and the general public is a vital next step. However, recognising that a large proportion of the older population is sedentary [554], how to successfully engage older adults and change behaviour toward a more active lifestyle will be challenging. These challenges need to be tackled via a worldwide multi-disciplinary collaborative approach between key stakeholders; researchers, healthcare workers, industry, policy-makers and non-government organisations, who should strive toward a consistent and fluid standard of recommendation. PA recommendation should aim to achieve individually catered prescriptions according to health and demographic profiling. This strategy may optimise engagement by those at increased AD risk and promote sustained health behaviour change.

A united goal toward initiating cost-effective, feasible and sustainable prevention strategies is pertinent. Such strategies should be aimed toward maintaining cognitive vitality and reducing risk of dementia plus other co-morbid diseases in older age. Given the ability of PA to consistently mitigate multiple health concerns, it should be considered a leading treatment strategy to delay or prevent cognitive decline in older age. Results presented in this thesis contribute toward the pool of growing knowledge surrounding PA in mitigating pre-clinical cognitive decline in older age, specifically, how this mitigation plays out as a function of non-modifiable risk profiling. Further

research to supplement that which has been reported here will aid in developing a precision lifestyle intervention for maintaining cognitive health in older age.

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Appendices

Contributions relating to the design, data collection and analysis of the Chariot: Pro Main study

The Chariot: Pro Main Study was funded by Janssen Research and Development, LLC (Janssen R&D, LLC is a pharmaceutical company of Johnson & Johnson) (ClinicalTrials.gov Identifier NCT02114372).

Candidate contribution

- Supported with preparation of study documents for submission and ethical approval
- Supported with preparation of online ethics submission internally (JRCO) and externally (NRES)
- Data management process set-up and oversight
- Supported with participant recruitment (during first three months of the study)
- Participant information consent and data collection: neurocognitive assessments, medical history, anthropometrics, self-reported questionnaires
- Data management – auditing source documentation, addressing data queries and/or delegating queries raised via study sponsor (worked alongside two-three other team members at any one time)

Collaborations

- Psychology team – Assistant psychologists and psychologists partook in neurocognitive assessment and neurocognitive data cleaning
- Data entry team – involved in entry of all study data from source to an online clinical research management system
- Medical team – nurses, doctors and phlebotomists were involved in bio-sample collection (blood, urine and saliva) and medical history
- Coordination of everyday study activities – study site coordinator managed the logistics of study visit flow and maintenance of the clinical environment (including stock management)
- Administrative and reception – greeting participants and supporting with study paperwork and filing
- Recruitment team – dedicated to recruiting participants onto the CHARIOT: PRO study
- Lab technician – processing of all biological samples for shipment to biobank and *APOE* genotyping lab

Peripheral biomarker analyses

Candidate contribution

- Funding acquisition (ICL seed funding in collaboration with co-applicant)
- Sample retrieval and shipment
- Assisted in sample assaying (via SIMOA)

Collaboration

- Sample assaying (via SIMOA) conducted within a collaborator lab primarily by a senior lab technician: Sylvia De Jong

Appendix A. Chapter 2: Systematic Review

A.1 Study selection criteria based on the PICOS framework [339]

Population

Inclusion

- At least one outcome measure at aged ≥60 years old
- Cognitively healthy (subjective memory complaints acceptable)
- *APOEε4* genotype status must be known and included in the study analysis as a moderator or stratified by carrier / non-carrier status
- Age related illnesses known not to impair cognition may be included

Exclusion

- Those who's final outcome measure is at an age <60 years old
- *APOEε*4 status is unknown
- Those with conditions that would preclude the performance of the physical activity intervention, e.g. those with functional limitations.
- Dementia and / or MCI patient populations (unless included in a case-control study) or those with a history or condition linked with a high chance of cognitive dysfunction (e.g. stroke, traumatic brain injury, etc).

Intervention / exposure

Inclusion

- RCT intervention to be aerobic, resistance or other form of exercise known to improve fitness (i.e. Tai Chi)
- Multi-domain intervention studies may be included only in cases where sub-strata are independent (i.e. an independent PA intervention sub-group).
- Baseline cognitive assessment must be completed prior to and following intervention or likewise for observational studies
- Observational cohort studies in which PA has been assessed either via subjective reporting or an objective measure including:
    - Self-report questionnaires, or questions relating to physical activity of any variety
    - Objective measurement such as via accelerometer or pedometer.
    - A measure of cardiorespiratory fitness such as maximal oxygen consumption may be included for sub-analysis.

Exclusion

- Multi-domain intervention studies whereby effect of intervention sub-domains (i.e. PA, cognitive activity, diet) may not be observed independently.
- Baseline cognitive assessment is completed during only one of prior or after intervention and likewise for observational studies.
- RCT's utilising a PA intervention not linked to improving fitness
- Observational cohort studies not adequately reporting a viable assessment of physical activity

Control

Inclusion

- Control group can be either: no-treatment control; a stretch or balance control; a control involving health education/social activity/mental activity

Exclusion

- Absence of control group in an experimental study design

Outcome

The *primary outcomes* concern:

- Incidence of dementia / AD or mortality as a result of dementia / AD
- Global cognitive functioning
- Domain specific cognition including, but not limited to:
  - Executive function
  - Attention
  - Language
  - Visuospatial functioning
  - Immediate memory
  - Delayed memory

The *secondary outcomes* concern:

- Peripheral, brain or CSF AD-related biomarkers will be extracted if concurrently reported with cognitive outcome measures.
- Incidence of MCI / CIND or other dementia's (e.g. VaD)
- Whether sex or age modifies the association between exposure and outcome

Study design

Inclusion criteria

- Randomized controlled trial (either at individual or group/cluster level)
- Prospective or retrospective observational study, including
  - Nested case-control studies (within cohort studies only)
  - Case-cohort studies
- Reported outcomes from at least 1 time point after randomisation for RCT's or baseline activity measurement for observational studies.
- Must assess *APOE*-ε4 carrier status with regard to moderation and/or effect of intervention on cognition / risk of dementia among carriers versus non-carriers

Exclusion criteria

- Any studies that do not report on the association between the exposure and outcomes investigated will be excluded
- Any other type of study design, including cross-sectional and case-control studies not nested in a cohort study
- Does not include assessment of physical activity on outcome as a factor of *APOEε4* carrier status
- Does not reported outcomes from at least 1 time point after randomisation for RCT's or baseline activity measurement for observational studies.

A.2 Search Strategy

MEDLINE search strategy – 337 hits (03/04/2018)

1. Motor activity/ or exp exercise/ or exp muscle strength/ or exp physical endurance/ or physical exertion/ or exp physical fitness
2. Physical activit*.mp.
3. Exerci?*.mp.
4. Endurance training.mp.
5. Resistance training.mp.
6. Zumba .mp.
7. Motor active*.mp.
8. Run*.mp.
9. Walk*.mp.
10. Fitness.mp.
11. Aerobic.mp.
12. Leisure activit*.mp.
13. Cycl*.mp.
14. Tai chi.mp.
15. Danc*.mp.
16. Swim*.mp.
17. Gym*.mp.
18. Yoga .mp.
19. stair* and climb*.mp.
20. physical inactivit*.mp.
21. sport*.mp.
22. bicycle*.mp.
23. jog*.mp.
24. anaerobic*.mp.
25. “high intensity interval training”.mp.
26. Metabolic equivalent.mp.
27. Energy expenditure.mp.
28. Sedentary lifestyle/
29. Sedentary .mp.
30. Sitting .mp
31. Sit .mp.
32. Exp cognition/or exp executive function/ or exp learning/ or exp thinking
33. Memory/ or memory, episodic/ or memory, long-term/ or memory, short-term/ or mental recall/ or “recognition (psychology)”/ or repetition priming/ or “retention (psychology)”/or spatial memory
34. Cognit*.mp.
35. Executive function*.mp.
36. Memory*.mp.
37. Dementia.mp.
38. Alzheimer*.mp.
39. Mild cognitive impairment.mp.
40. Exp animals/ not humans.sh
41. Apolipoproteins E/ or apolipoprotein E4
42. APOE*.mp.

43. Apolipoprotein*.mp.
44. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31
45. 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39
46. 41 or 42 or 43
47. (44 and 45 and 46) not 40

EMBASE search strategy – 1002 hits (03/04/2018)

1. Exp physical activity
2. Exp exercise
3. Physical activit* .mp.
4. Exerci?* .mp.
5. Resistance training/ or muscle strength/ or endurance .mp.
6. Exp exercise/ or aerobic exercise/ or anaerobic exercise/ or aquatic exercise/ or circuit training/ or closed kinetic chain exercise/ or dynamic exercise/ or endurance training/ or exercise intensity/ or high intensity interval training/ or leg exercise/ or muscle exercise/ or pilates/ or resistance training/
7. Motor active* .mp.
8. Run* .mp.
9. Walk* .mp.
10. Fitness .mp.
11. Aerobic .mp.
12. Leisure activit* .mp.
13. Cycl* .mp.
14. Tai Chi/
15. Danc* .mp.
16. Swim* .mp.
17. Gym* .mp.
18. Zumba
19. Stair* and climb* .mp.
20. Physical inactivit* .mp.
21. Sport* .mp.
22. Bicycle* .mp.
23. Jog* .mp.
24. Anaerobic* .mp.
25. “high intensity interval training” .mp.
25. Yoga .mp.
26. “high intensity interval training” .mp.
27. Metabolic equivalent .mp
28. Energy expenditure .mp.
29. Sitting/
30. Sedentary lifestyle/
31. Sedentary .mp.
32. Sitting .mp.
33. Sit .mp.
34. Exp cognition.

35. Exp thinking
36. Exp learning
37. Cognition/ or attention/ or cognitive reserve/ or learning/ or memory/ or orientation/ or perception/
38. Cognit* .mp.
39. Executive function .mp.
40. Memory* .mp.
41. Dementia* .mp.
42. Alzheimer* .mp.
43. Mild cognitive impairment .mp.
44. Apolipoprotein E/ or apolipoprotein E4
45. APOE* .mp.
46. Apolipoprotein* .mp.
47. Exp animal/not humans .sh.
48. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33
49. 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43
50. 44 or 44 or 45 or 46
51. (48 and 49 and 50) not 47

PsychInfo search strategy – 172 hits (03/04/2018)

1. Exp exercise/ or physical activity/ or aerobic exercise/ or weightlifting/ or yoga/ or health behaviour/ or movement therapy/ or physical fitness/ or weight control/
2. Physical activit* .mp.
3. Exerci?* .mp.
4. Endurance training .mp.
5. Resistance training .mp.
6. Motor active* .mp.
7. Run*.mp.
8. Walk* .mp.
9. Fitness .mp.
10. Aerobic .mp.
11. Strength .mp.
12. Leisure activit* .mp.
13. Cycl* .mp.
14. Tai chi.mp.
15. Danc* .mp.
16. Swim* .mp.
17. Gym* .mp.
18. Stair* and climb* .mp.
19. Physical inactivit* .mp.
20. Sport* .mp.
21. Bicycle* .mp.
22. Jog* .mp.
23. Anaerobic* .mp.
24. “high intensity interval training” .mp.
25. Metabolic equivalent .mp.
26. Energy expenditure .mp.
27. Zumba .mp.
28. *sedentary behaviour/

29. Sedentary .mp.
30. Sitting .mp.
31. Cognitive assessment/
32. Exp cognitive ability/
33. memory/ or autobiographical memory/ or early memories/ or episodic memory/ or explicit memory/ or implicit memory/ or long term memory/ or memory consolidation/ or memory decay/ or retrospective memory/ or short term memory/ or spatial memory/ or "spontaneous recovery (learning)"/ or verbal memory/ or visual memory/ or cued recall/ or free recall/ or learning/ or retention/
34. Cognit* .mp.
35. Executive function* .mp.
36. Memory* .mp.
37. Dementia* .mp.
38. Alzheimer* .mp.
39. Mild cognitive impairment .mp.
40. Exp animal/not humans .sh.
41. Apolipoprotein E/ or apolipoprotein E4
42. APOE* .mp.
43. Apolipoprotein*.mp.
44. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30
45. 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39
46. 41 or 42 or 43
47. (44 and 45 and 46) not 40

International Clinical Trials Registry Platform (ICTRP) search strategy - 625 hits, 32 results available (04/03/2018)

Title Exercis* OR physical activity OR fitness OR endurance training OR resistance training OR motor activit* OR run* OR walk* OR jog* OR aerobic OR cycl* OR danc* OR gym OR swim* OR anaerobic OR sedentary OR sitting AND cognit* OR memory OR executive function OR dementia OR Alzheimer* OR mild cognitive impairment AND APOE OR apolipoprotein* NOT animal

Condition cognit* OR memory OR executive function OR dementia OR Alzheimer* OR mild cognitive impairment

Intervention Exercis* OR physical activity OR fitness OR endurance training OR resistance training OR motor activit* OR run* OR walk* OR jog* OR aerobic OR cycl* OR danc* OR gym OR swim* OR anaerobic OR sedentary OR sitting

CENTRAL search strategy – 93 hits, 39 reviews and 54 trials (04/03/2018)

1. MeSh exp Exercise
2. Physical activity
3. Exercise
4. Endurance training
5. Resistance training
6. Zumba
7. Motor activity
8. Run*

9. Walk*
10. Fitness*
11. Aerobic
12. Leisure activity
13. Cycle*
14. Tai chi
15. Dance*
16. Swim*
17. Gym*
18. Yoga*
19. Physical inactivity
20. Sport*
21. Bicycle*
22. Jog*
23. Anaerobic
24. Sedentary lifestyle/
25. Sedentary
26. Sitting
27. MeSH descriptor exp cognitive reserve/ or exp cognition/ or exp memory/ or exp executive function
28. Cognit*
29. Executive function*
30. Memory* .mp.
31. Dementia* .mp.
32. Alzheimer* .mp.
33. Mild cognitive impairment .mp.
34. MeSH exp Apolipoprotein
35. APOE* .mp.
36. Apolipoprotein* .mp.
37. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or17 or 18 or19 or 20 or 21 or 22 or 23 or 24 or 25 or 26
38. 27 or 28 or 29 or 30 or 31 or 32 or 33
39. 35 or 36
40. 37 and 38 and 39

ClinicalTrials.gov search strategy – 19 hits (04/03/2018)

(Exercise OR "physical activity" OR fitness OR run OR walk OR jog OR aerobic OR cycle OR anaerobic OR sedentary OR sitting) AND (cognition OR memory OR executive function OR dementia OR mild cognitive impairment) AND (apolipoprotein OR APOE) | Completed studies

A.3 Data extraction template

RCT extraction template

Study (date, country)	Design (cohort/ duration)	Total N (%ε4 +ve)	Participants (mean age (SD) yrs/CH/SMH/MCI/%F)	Exercise type (duration, bout, frequency)/assessment	PA data format	Duration/frequency of follow-up	Cognitive tests (domains assesse
Quantification of outcome	Biomarker measure	APOE-ε4 carrier outcome	APOE-ε4 non-carrier outcome	Biomarker outcome	Statistical technique/ covariates	Summary of findings	

Observational study extraction template

Study (date, country)	Design (cohort/ duration)	Total N (%ε4 +ve)	Participants (mean age (SD) yrs/CH/SMH/MCI/%F)	Exercise type (duration, bout, frequency)/assessment	PA data format	Duration/frequency of follow-up	Cognitive tests (domains assesse
Quantification of outcome	Biomarker measure	APOE-ε4 carrier outcome	APOE-ε4 non-carrier outcome	Biomarker outcome	Statistical technique/ covariates	Summary of findings	

A.4 Quality and bias assessment tools

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation ✸
   b) yes, eg record linkage or based on self reports
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases ✸
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls ✸
   b) hospital controls
   c) no description

4) Definition of Controls
   a) no history of disease (endpoint) ✸
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for _____ (Select the most important factor.) ✸
   b) study controls for any additional factor ✸ (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   a) secure record (eg surgical records) ✸
   b) structured interview where blind to case/control status ✸
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description

2) Same method of ascertainment for cases and controls
   a) yes ✸
   b) no

3) Non-Response rate
   a) same rate for both groups ✸
   b) non respondents described
   c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average _____ (describe) in the community ✸
   b) somewhat representative of the average _____ in the community ✸
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort

2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort ✸
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure
   a) secure record (eg surgical records) ✸
   b) structured interview ✸
   c) written self report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes ✸
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for _____ (select the most important factor) ✸
   b) study controls for any additional factor ✸ (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment ✸
   b) record linkage ✸
   c) self report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) ✸
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for ✸
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) ✸
   c) follow up rate < ____% (select an adequate %) and no description of those lost
   d) no statement

Cochrane Risk of Bias Tool for RCT's

Study detail (author / title)		
Domain	**Support for judgement**	**Author's judgment**
Selection bias		
Random sequence generation		
Allocation concealment		
Performance bias		
Blinding of participants		
Blinding of personnel		
Detection bias		
Blinding of outcome assessment		
Attrition bias		
Incomplete outcome data		
Reporting bias		
Selective reporting		
Other bias		
Other sources of bias		

RESULTS

Table A.5. Summary of excluded studies following full-text review (n=39)

Study	Reason for exclusion
Lautenschlager *et al*., (2008)	MCI cohort
Baker *et al*., (2013)	MCI cohort
Barha *et al*., (2016)	VCI (vascular cognitive impairment) cohort
Brown *et al.,* (2011)	Editors review the results from Rovio *et al.* (2005)
Kivipelto *et al.* (2008)	PA results already reported by Rovio *et al.* (2005)
Knorr *et al.,* (2009)	A review
Ng *et al.,* (2014)	Result already reported by Niti *et al.,* (2008)
Chen *et al.,* (2012)	No explicit assessment of PA
Etnier *et al.* (2018)	Intervention without a control group
Solomon *et al.* (2018)	Did not assess PA independently of other interventions
Head *et al.,* (2011)	Cognition / incidence of dementia not assessed
Stillman *et al. (2017)*	Cognition / incidence of dementia not assessed
Liang et al. (2010)	Cognition / incidence of dementia not assessed
Etnier *et al,* (2007)	Cross-sectional design
Etnier et al. (2007)	Cross-sectional design
Obesian *et al.* (2009)	Cross-sectional design
Rodriquez *et al.* (2018)	Cross-sectional design
Porto *et al.* (2016)	*APOE*ε4 genotype status not assessed
Larson et al., (2006)	*APOE*ε4 genotype status not assessed
Scarmeas *et al.* (2009)	*APOE*ε4 genotype status not assessed
Welsh-Bohmer *et al*.(2009)	*APOE*ε4 genotype status not assessed
Brown *et al.* (2013)	*APOE*ε4 genotype status not assessed
Wang *et al.* (2013)	*APOE*ε4 genotype status not assessed
Jessen *et al.* (2014)	*APOE*ε4 genotype status not assessed
Okonkwo *et al.* (2014)	*APOE*ε4 genotype status not assessed
Shah *et al.* (2014)	*APOE*ε4 genotype status not assessed
Simada *et al.* (2014)	*APOE*ε4 genotype status not assessed
Dougherty *et al.* (2017)	*APOE*ε4 genotype status not assessed
Lin *et al.* (2017)	*APOE*ε4 genotype status not assessed
Reijs et al., (2017)	*APOE*ε4 genotype status not assessed
Tsai *et al.* (2017)	*APOE*ε4 genotype status not assessed
Buchman *et al.* (2012)	*APOE*ε4 genotype status not assessed
Horie et al., (2016)	*APOE*ε4 genotype status not assessed
De Bruijen *et al* (2012)	*APOE*ε4 genotype status not assessed
Shah et al., (2012)	*APOE*ε4 genotype status not assessed
Shah *et al*. (2012)	*APOE*ε4 genotype status not assessed
Meng et al., (2013)	*APOE*ε4 genotype status not assessed
Borenstein *et al.* (2012)	*APOE*ε4 genotype status not assessed
Boyle *et al.* (2009)	*APOE*ε4 genotype status not assessed

A.6 Study Quality Assessment of included RCT's

Risk of Bias assessment for RCT's

Stonnington *et al* (2013) [342]		
Domain	**Support for judgement**	**Author's judgment**
Selection bias		
Random sequence generation	No information provided.	Unclear risk
Allocation concealment	No information provided.	Unclear risk
Performance bias		
Blinding of participants	Was not feasible to blind participants to their group allocation, but outcome unlikely to be influenced	Low risk
Blinding of personnel	Trainers and research staff (other than outcome assessors) not blinded	High risk
Detection bias		
Blinding of outcome assessment	No information provided	Unclear risk
Attrition bias		
Incomplete outcome data	No information provided	Unclear risk
Reporting bias		
Selective reporting	No information provided.	Unclear risk
Other bias		
Other sources of bias	Insufficient information provided	Unclear risk

Legault *et al*. (2011) [343]		
Domain	**Support for judgement**	**Author's judgment**
Selection bias		
Random sequence generation	Stated participants were randomized into four groups via the equal probability method.	Low risk
Allocation concealment	No information provided.	Unclear risk
Performance bias		
Blinding of participants	Was not feasible to blind participants to their group allocation, but outcome unlikely to be influenced	Low risk

Blinding of personnel	Trainers and research staff (other than outcome assessors) not blinded	High risk
Detection bias		
Blinding of outcome assessment	Trial stated it was "single-blinded", so as participants were not able to be blinded, the outcome assessors must have been.	Low risk
Attrition bias		
Incomplete outcome data	Analysis conducted according to the ITT principle. 2/18 participants in the PA group were excluded as they did not return for their 4-month visit. 1/18 participants in the PA group and 1/19 participants in the combined intervention group were excluded for not attending any of the center-based training sessions.	Low risk
Reporting bias		
Selective reporting	Insufficient information provided.	Unclear risk
Other bias		
Other sources of bias	Unlikely	Low risk

Pereira *et al.* (2014) [341]		
Domain	**Support for judgement**	**Author's judgment**
Selection bias		
Random sequence generation	No information provided.	Unclear risk
Allocation concealment	No information provided.	Unclear risk
Performance bias		
Blinding of participants	Was not feasible to blind participants to their group allocation, but outcome unlikely to be influenced	Low risk
Blinding of personnel	Trainers and research staff (other than outcome assessors) not blinded	High risk
Detection bias		
Blinding of outcome assessment	No information provided	Unclear risk
Attrition bias		
Incomplete outcome data	No information provided	Unclear risk
Reporting bias		
Selective reporting	No information provided.	Unclear risk
Other bias		
Other sources of bias	Insufficient information provided	Unclear risk

A.7 Quality assessment for prospective observational studies – The Newcastle-Ottawa Quality Assessment Scale for cohort and case-control studies

Schuit *et al.,* (2001) [114]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Representative of a male cohort only (no women included). Non-respondent survey showed non-respondents had a lower SES and a worse health status in terms of stroke, disabilities and poor self-rated health (unclear whether this was significant)	-
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-administered questionnaire, validated and designed for retired men	-
Demonstration that outcome of interest was not present at start of study	Yes. MMSE administered at baseline. Study was looking at a *decline* in MMSE from baseline to follow-up, so outcome would not have been present at baseline.	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, education level, smoking status, alcohol, baseline cognition, CVD, Type 2 Diabetes, ADL and self-reported health	**
Outcome		
Assessment of outcome	MMSE administered at home by trained staff	*
Was follow-up long enough for outcomes to occur	Follow-up was 3 years.	*
Adequacy of follow up cohorts	390/560 men re-examined (7 had died, but no description of others lost) Follow up rate=70%. Complete information on PA at baseline and cognitive functioning at follow-up was available for 347 subjects.	-
	Total (out of 9 *'s)	5

Niti *et al.,* (2008) [198]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants voluntarily invited to participate, limited to a geographically defined area in the South-East region of Singapore. All participants were Chinese, limiting generalizability.	-
Selection of non-exposed cohort	N/A	N/A

Ascertainment of exposure	Self-reported questionnaire	-
Demonstration that outcome of interest was not present at start of study	Yes: assessment of baseline MMSE. Average = 27.2 (3.2), those with cognitive impairment (MMSE <24) excluded at the start of the study	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, education, gender, APOE-ε4 status, functional status, no. comorbidities, presence of vascular risk factors/events, depressive symptoms, smoking and alcohol consumption and baseline MMSE score.	**
Outcome		
Assessment of outcome	Cognitive assessments performed by trained research nurses	*
Was follow-up long enough for outcomes to occur	Median follow up was 1.5 yrs	-
Adequacy of follow up cohorts	847/2611 lost to follow up (follow up rate 67%). Stated as a high lost to follow-up rate. Participants remaining had better cognitive function and were more active in LTPA.	-
	Total (out of 9 *'s)	4

Woodard *et al.,* (2012) [200]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants recruited via newspaper advertisements (volunteers), so will be limited to those who read newspapers (may differ in meaningful ways from those who do not e.g. yrs of education may differ). Half of participants were selected based on family history of AD, so the representation of *APOE*ε4 carriers was greater in this sample than the general population. All participants were Caucasian.	-
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-reported	-
Demonstration that outcome of interest was not present at start of study	Participants were required to be cognitively intact and non-demented upon study entry and were excluded if they had an MMSE score or <25, in addition to other measures of cognition. Average MMSE of the participant cohort was 29.4 (0.8) for those who were stable and 28.9 (1.2) for those who were declining.	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for *APOE*ε4 status, baseline PA, *APOE*ε4 status by activity interaction, baseline hippocampal function and structure.	*

Outcome		
Assessment of outcome	Assessed using validates cognitive tests; MMSE, DRS-2, RAVLT Trials 1-5, RAVLT DR and GDS	*
Was follow-up long enough for outcomes to occur	1.5 years	-
Adequacy of follow up cohorts	Complete follow-up, all subjects accounted for	*
Total (out of 9 *'s)		4

Pizzie *et al* (2014) [208]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Cohort appears to be representative of the average older adult in the USA.	*
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Nurses Healthy Study Exercise Questionnaire (validated self-report questionnaire)	-
Demonstration that outcome of interest was not present at start of study	To be eligible, participants had to have a CDR of 0 (indicating cognitive normality) at baseline, which all participants fulfilled.	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controlled for cognitive performance at baseline, age, sex, education level, GDS score at baseline, parental history of AD, APOE-ε4 status	**
Outcome		
Assessment of outcome	Cognitive assessments performed by trained research nurses	*
Was follow-up long enough for outcomes to occur	Follow up length was 6 yrs	*
Adequacy of follow up cohorts	91/173 baseline participants included in longitudinal analysis (no information on what happened to the other 82). Follow up rate = 53%.	-
Total (out of 9 *'s)		6

Tsai *et al* (2016) [199]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants recruited from the health check-up program at the National Taiwan University Hospital.	-

	No further information is given regarding derivation of the cohort.	
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-reported questionnaire	-
Demonstration that outcome of interest was not present at start of study	Montreal Cognitive Assessment, Wechsler Memory Scale-Third Edition, TMT and verbal fluency test administered at baseline.	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Multivariate analysis and stratification analysis by physical function, sex, age groups and *APOE*ε4 status	**
Outcome		
Assessment of outcome	Montreal Cognitive Assessment, Wechsler Memory Scale-Third Edition, TMT and verbal fluency test administered at follow-up	*
Was follow-up long enough for outcomes to occur	Follow-up was 2 years.	*
Adequacy of follow up cohorts	Follow-up rate: 79.8% (reasons for incomplete follow-up data not known)	*
Total (out of 9 *'s)		6

Thibeau *et al.* (2017) [248]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants selected to be relatively healthy and free of neurodegenerative disease (may possess risk-reducing factors), so may only be somewhat representative of older adults.	-
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-report questionnaire (VLS-ALQ)	-
Demonstration that outcome of interest was not present at start of study	Exclusionary criteria: baseline MMSE of <24 (cut off for impaired cognition). Baseline: average MMSE of 28.68	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for vascular health and BMI (preformed analysis with these covariates and found that none affected the main results, therefore final models did not include covariates)	-
Outcome		

Assessment of outcome	Four validated EF indicators used: Hayling sentence completion test, Stroop test, Brixton spatial anticipation test and Colour Trails Test part 2	*
Was follow-up long enough for outcomes to occur	Follow up length was 9-years	*
Adequacy of follow up cohorts	Retention rates by cohort range from 74%-89%	*
Total (out of 9 *'s)		4

de Souto Barreto *et al.* (2018) [207]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Representative of a male and female cohort. Enrolled from numerous memory clinics (13). Appears to be truly representative of the average older adult in the community.	*
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-reported questionnaire	-
Demonstration that outcome of interest was not present at start of study	COWAT, CNT, WAIS-DSST, TMT-A, TMT-B, CDR-SB, MMSE administered at baseline and cohort average scores were above the cut-off for cognitive impairment	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for time and demographic variables; age, sex and education level.	**
Outcome		
Assessment of outcome	Validated cognitive tests administered at several points after baseline using the same methods of assessment at baseline	*
Was follow-up long enough for outcomes to occur	Follow-up was 3 years.	*
Adequacy of follow up cohorts	Complete follow up, all subjects accounted for.	*
Total (out of 9 *'s)		7

Podewils *et al* (2005) [113]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Eligible persons drawn from the Health Care Financing Administration Medicare enrolments list. Drawn from 4 US communities. Both Caucasian and African American participants.	*
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Information collected by trained interviewers using a modified Minnesota Leisure Time Activity Questionnaire (validated).	*
Demonstration that outcome of interest was not present at start of study	MMSE 3MS administered at baseline. Average of 92.2 (6.3) for the whole cohort, which is above the cut-off for cognitive impairment.	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, gender, race, APOE-ε4, education level ADL, social factors, baseline MMSE and white matter grade	**
Outcome		
Assessment of outcome	Multistage process of neurological examination using validated tests	*
Was follow-up long enough for outcomes to occur	Average of 5.4 years follow up (range was 0.03-8.4 years)	*
Adequacy of follow up cohorts	Complete follow-up	*
Total (out of 9 *'s)		8

Rovio (2005) [347]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants randomly selected from the survivors of 4 separate, independent, population-based random samples examined within a previous study.	*
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-reported	-
Demonstration that outcome of interest was not present at start of study	Insufficient information	-
Comparability		

Comparability of cohorts on the basis of design or analysis	Controls for sociodemographic variables (e.g. age at re-examination, sex, education), follow-up time, locomotor disorders, vascular risk factors at midlife, history of vascular disorders e.g. MI, stroke, diabetes at re-examination, APOE status, smoking status and alcohol drinking	**
Outcome		
Assessment of outcome	Cognitive status screened with MMSE, and those scoring <24 were referred for further neurological and neuropsychological examination (diagnosis according to DSM-IV).	*
Was follow-up long enough for outcomes to occur	Average follow-up length was 21 years	*
Adequacy of follow up cohorts	Follow-up rate to re-examination: 72.5% (n=1449) (reasons for non-participants not given).	-
Total (out of 9 *'s)		5

Rovio *et al* (2007) [348]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants randomly selected from the survivors of 4 separate, independent, population-based random samples examined within a previous study.	*
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-reported	-
Demonstration that outcome of interest was not present at start of study	Insufficient information	-
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls age, sex, education, follow-up, time and locomotor symptoms, main occupation, during life, income at midlife, other subtypes of PA (LTPA, occupational and commuting PA), BMI, serum, total cholesterol, SBP, APOE-ε4 status, vascular disorders at re-examination, (MI, stroke and diabetes) and smoking status at baseline	**
Outcome		
Assessment of outcome	Cognitive status screened with MMSE, and those scoring <24 were referred for further neurological and neuropsychological examination (diagnosis according to DSM-IV).	*
Was follow-up long enough for outcomes to occur	Average follow-up length of 20.9 (SD 4.9) years	*

Adequacy of follow up cohorts	Follow-up rate to re-examination: 72.5% (n=1449) (reasons for non-participants not given). 20% had missing information in independent variables, leading to final analysis of 1158	-
Total (out of 9 *'s)		5

Taaffe *et al* (2008) [350]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants were Japanese American men only, living on a single island in Hawaii.	-
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-reported	-
Demonstration that outcome of interest was not present at start of study	Participants excluded if they had dementia at baseline or had a CASI score of <74. Dementia free participants had an average CASI score of 88.1 (6.7) at baseline.	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, baseline CASI score, education, APOE- ε4 status, BMI, smoking, midlife, systolic and diastolic BP, hypertension, cholesterol, diabetes, prevalent CHD and depression	**
Outcome		
Assessment of outcome	Outcome assessed using CASI, and diagnosis assigned by a consensus panel consisting of a neurologist and additional physicians, dementia diagnosed according to DSM-IIIR criteria and AD according to NINCDS-ADRDA, and VaD according to the California AD and Treatment Centres criteria	*
Was follow-up long enough for outcomes to occur	Mean follow-up length was 6.1 yrs	*
Adequacy of follow up cohorts	All participants account for	*
Total (out of 9 *'s)		6

Paillard-Borg (2009) [346]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants recruited from a single district of Stockholm, Sweden so may lack representativeness of wider older populations. No information given regarding other aspects of representativeness.	-

Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Personal interview by trained nurses	*
Demonstration that outcome of interest was not present at start of study	Participants living in an institution or with a MMSE score of <24 were excluded. Participants had an average MMSE score of 27.5 (1.45) at baseline (above the cut off for cognitive impairment)	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, sex, education level, comorbidity, cognitive and physical functioning, living arrangements and depressive symptoms	**
Outcome		
Assessment of outcome	Assessment involved personal and family history collected by nurses, a clinical examination by physicians and psychological tests administered by trained personnel.	*
Was follow-up long enough for outcomes to occur	Median follow up length was 7.8 yrs	*
Adequacy of follow up cohorts	All subjects accounted for	*
Total (out of 9 *'s)		7

Chen *el al* (2012)* [344]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Is the case definition adequate?	No information provided	-
Representativeness of the cases	AD patients recruited from three teaching hospitals in northern Taiwan, but no further information regarding the representativeness of cases is given.	-
Selection of controls	Recruited from health check-ups and volunteers of the hospital during the same period as the cases	-
Definition of controls	No description of source	-
Comparability		
Comparability of cases and controls on the basis of design or analysis	Controls for age, sex, APOE-ε4 status, education level, marital status and cigarette smoking	**
Exposure		
Ascertainment of exposure	Information obtained from a self-reported questionnaire	-

Same method of ascertainment for cases and controls	No information given	-
Non-response rate	No information given	-
Total (out of 9 *'s)		2

Luck *et al.* (2014) [345]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants were recruited from by GPs in six study centres across Germany and 90% of elderly German people have regular contact with a GP.	*
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Structured interview	*
Demonstration that outcome of interest was not present at start of study	Being dementia free at baseline was set as eligibility criteria.	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, gender, education level, alcohol consumption, smoking status, MMSE score, mental activity and comorbidity at follow-up (diabetes, hypertension, CVD, TIA, stroke, TBI, hyperlipidemia, hypercholesterolaemia, hypothyroidism, depression)	**
Outcome		
Assessment of outcome	Assessed via a structured interview and status of participants was agreed at consensus conferences including interviewer and experienced geriatric psychiatrist.	*
Was follow-up long enough for outcomes to occur	Follow-up length was 6 years	*
Adequacy of follow up cohorts	All subjects accounted for	*
Total (out of 9 *'s)		8

Strand *et al* (2015) [354]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Is the case definition adequate?	Dementia cases identified form death certificates in which a dementia diagnosis was specified in their death certificate, either as the underlying cause of as a contributory cause.	-

Representativeness of the cases	Sample deemed to be representative of the Norwegian population regarding APOE allele frequencies (similar to those reported in the Norwegian twin study). As cases were drawn from death certificates, they may not be representative of those living with dementia.	-
Selection of controls	Community controls, drawn from birth year strata	*
Definition of controls	No description	-
Comparability		
Comparability of cases and controls on the basis of design or analysis	Controls for gender, education, BMI, total and HDL, cholesterol, smoking, PA, diabetes, history of heart disease and general health status	**
Exposure		
Ascertainment of exposure	No description	_
Same method of ascertainment for cases and controls	Yes	*
Non-response rate	Same rate for both groups	*
Total (out of 9 *'s)		5

Tolpannen *et al* (2015) [352]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants randomly selected from the survivors of 4 separate, independent, population-based random samples examined within a previous study	*
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-reported	-
Demonstration that outcome of interest was not present at start of study	Insufficient information	-
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, sex, level of education, marital status, smoking, BMI, cardiorespiratory and musculoskeletal, disease and work-related PA in midlife	**
Outcome		
Assessment of outcome	Outcome assessed via cognitive screening using validated MMSE, further neurological examination and diagnosed according to DSM-IV criteria	*

Was follow-up long enough for outcomes to occur	Mean follow-up length was 29.3 yrs (range 11-36)	*
Adequacy of follow up cohorts	Follow-up rate to re-examination: 72.5% (n=1449) (reasons for non-participants not given). Follow-up rate to second re-examination: 63.7%. Final analysis of 1511 persons who had participated in at least 1 re-examination.	-
Total (out of 9 *'s)		5

Yang *et al* (2014) [353]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Is the case definition adequate?	AD and VaD cases were recruited form neurology clinics after being diagnosed by a neurologist using validated assessment.	*
Representativeness of the cases	Representativeness of sample not discussed and not obvious.	-
Selection of controls	Recruited from an elderly patient's health check-up program and volunteers form the hospital during the same period.	-
Definition of controls	No history of any sub-types of dementia (assessed by CDR).	*
Comparability		
Comparability of cases and controls on the basis of design or analysis	Controls for age, sex, APOE-ε4 status, education level, marital status and cigarette smoking	**
Exposure		
Ascertainment of exposure	Information obtained from a self-reported questionnaire	-
Same method of ascertainment for cases and controls	Yes, same questionnaire and duration of retrospect (1 year)	*
Non-response rate	0%	*
Total (out of 9 *'s)		6

Krell-Roesch *et al* (2016) [230]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants were volunteers. Majority of the population of Olmsted County from which the participants were drawn is of Caucasian decent.	-
Selection of non-exposed cohort	N/A	N/A

Ascertainment of exposure	Self-reported questionnaire	-
Demonstration that outcome of interest was not present at start of study	Participants had to be cognitively normal at baseline (based on published normative data developed on this community)	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, sex, education level, medical comorbidity and depression.	**
Outcome		
Assessment of outcome	Assessed from a neurological evaluation, a risk factor assessment interview and neuropsychological testing	*
Was follow-up long enough for outcomes to occur	Follow up length was 3.2 yrs (median)	*
Adequacy of follow up cohorts	222 withdrew prior to follow-up, 37 died before follow-up and 118 had no follow-up visit. Follow up rate= 83%	*
Total (out of 9 *'s)		6

Tan *et al* (2017) [351]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants (both in original and offspring cohorts) were volunteers. Taken from a single town (Framingham) in Massachusetts, so may lack representativeness of wider populations.	-
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-reported	-
Demonstration that outcome of interest was not present at start of study	Eligibility criteria included being dementia free at baseline	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, sex, APOE-ε4, high-school degree, plasma homocysteine, BP, antihypertensive medication, total cholesterol, current smoking status, prevalence of CVD, diabetes, stroke and atrial fibrillation.	**
Outcome		
Assessment of outcome	Assessed from neurologist examination, and various secure records	*
Was follow-up long enough for outcomes to occur	Follow up length was 10 yrs	*

Adequacy of follow up cohorts	Complete follow up	*
Total (out of 9 *'s)		6

Fenesi *et al* (2017) [337]		
Domain	**Support for judgement**	**Author's judgment**
Selection		
Representativeness of exposed cohort	Participants appear to be representative of the average older adult in the community drawn from both urban and rural areas in 10 Canadian provinces.	*
Selection of non-exposed cohort	N/A	N/A
Ascertainment of exposure	Self-report question	-
Demonstration that outcome of interest was not present at start of study	Participants removed at baseline if they had a positive dementia diagnosis or cognitive impairment with no dementia	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for APOE-ε4 status, age, sex, years of education, prior heart attack, prior stroke, diabetes, high BP, depression and smoking	**
Outcome		
Assessment of outcome	Clinical assessment by a nurse, neurological examination by a physician and an independent blind assessment by a psychometrist	*
Was follow-up long enough for outcomes to occur	Follow up length was 5 yrs	*
Adequacy of follow up cohorts	Complete follow up	*
Total (out of 9 *'s)		7

Shih *et al* (2018) [349]		
Domain	**Support for judgement**	**Author's judgement**
Selection		
Representativeness of exposed cohort	The cohort was truly representative of older Hispanics residing in the Sacramento valley (>89% of eligible households that were enumerated has at least one person who participated in the study). Participants were Latino only, so not fully representative of all older adults.	-
Selection of non-exposed cohort	N/A	N/A

Ascertainment of exposure	Self-reported	-
Demonstration that outcome of interest was not present at start of study	Sample participants were removed if they had dementia or CIND at baseline. Cognitive performance was assessed at bassline using 3MSE and SEVLT (both average scores for the cohort were above the cut-off for cognitive impairment)	*
Comparability		
Comparability of cohorts on the basis of design or analysis	Controls for age, gender, years of education, country of birth, primary language, smoking status, alcohol use, hypertension, CVD and type of occupation.	**
Outcome		
Assessment of outcome	Assessed by a team of neurologists and neuropsychologists.	*
Was follow-up long enough for outcomes to occur	Follow up length was 10 yrs	*
Adequacy of follow up cohorts	Complete follow up	*
Total (out of 9 *'s)		6

A.8 Details of PA questionnaires utilised within included observational studies

Questionnaire	Specifications	Utilised
The Zutphen Study Questionnaire [555]	Frequency and Duration of walking and cycling Previous year based on seasonal changes	Schuit *et al.* (2001)
Modified Minnesota Questionnaire [556]	Frequency / duration / intensity Previous 2-weeks	de Souto Barreto *et al.* (2018) Niti *et al.* (2008) Podewils *et al.* (2005) Krell-Roesch *et al.* (2016)
International Physical Activity Questionnaire (IPAQ) [557]	Frequency / duration / intensity Previous 7-days	Tsai *et al.* (2016)
Nurses' Health Study Exercise Questionnaire (NHSEQ) [558]	Frequency / duration During an average week over the previous year	Pizzie *et al* (2014)
Stanford Brief Activity Survey (SBAS) [559]	Frequency / duration / intensity During an average week over the previous year	Woodard *et al*. (2012)
1985 National Health Interview Study (Available from: https://www.psc.isr.umich.edu/dis/data/catalog/detail/526		Krell-Roesch *et al.* (2016)
VLS-Activity Lifestyle Questionnaire (VLS-ALQ) [560]	Frequency and intensity of Gardening; exercise activity such as jogging, swimming; outdoor activities such as fishing, sailing or backpacking; recreation sport such as tennis, bowling or golf Over a period of 2-years	Thibeau *et al.* (2017)

Appendix B. Chapter 3: Methods

Appendix B.1 Participant information sheet and informed consent form.

Participant Information Sheet and Informed Consent Form

The CHARIOT:PRO Study

Protocol Number: REGISTRYALZ001/Research Related Activity (RRA-11823)

Protocol Title: Cognitive Health in Ageing Register: Investigational, Observational, and Trial studies in dementia research (CHARIOT): Prospective Readiness cOhort Study (PRO)

Study Doctor: **Lefkos Middleton, MD**

School of Public Health, Imperial College London

Charing Cross Campus

St. Dustan's Rd.

London, W6 8RP, UK

A prospective observational study to better understand cognitive and functional changes over time, and factors influencing healthy brain ageing and progression to dementia.

A description of this clinical trial will be available at http://www.ClinicalTrials.gov as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the study results. You can search this Web site at any time.

You are being invited to take part in a research study, called the CHARIOT:PRO (Prospective Readiness Cohort) Study.

Before you decide whether or not you want to take part, it is important for you to understand what this study is, why it is being undertaken and what it will involve. Please take the time to read the information carefully. If you have any questions or do not understand something in this information sheet, please ask the study doctor or the study staff. You should feel free to discuss the study with anyone you choose, in order to better understand the study and your options. Please do not sign the consent form unless your questions have been answered and you decide to take part in the study.

If you have any questions:

Study Manager: Dr Chinedu Udeh-Momoh

Contact Details: CHARIOT: PRO Study Team - chariotpro.study@imperial.ac.uk / 020 3313 0620

1. Why have I been invited?

You are being asked to participate in CHARIOT:PRO because you are a member of the CHARIOT register (Cognitive Health in Ageing Register: Investigational, Observational and Trial Studies in dementia research), or were self-referred, and have expressed interest in being part of research studies for dementia prevention and have provided your consent to receive information about such studies.

For this study, we are inviting volunteers aged 60-85 years with *no* history or diagnosis of dementia. The study is planned to involve 700 people at Imperial College Clinical Research Centre at Charing Cross Hospital and the hope is that you will be a participant in this study for up to four years.

2. What is the purpose of the study?

Alzheimer's disease is, by far, the most frequent cause of dementia; as such, characterized by an relentless progressive memory and cognitive decline leading to loss of the ability of conducting daily routine activities. The course of the disease varies considerably for each person and the early disease stages are usually characterised by mild and sometimes barely noticeable changes in memory and thinking, which can easily be interpreted as normal age-related forgetfulness. Individuals who display a mild but noticeable decline in cognitive abilities such as memory and other thinking skills are at greater risk of developing dementia due to Alzheimer's disease. Research has shown that these subtle cognitive deficits, also known as Mild Cognitive Impairment, can be used to predict an individual's risk to progress to dementia.

Although we understand the important link between Mild Cognitive Impairment and Alzheimer's disease, we have yet to fully understand the factors that contribute to cognitive and functional decline and how these factors can be used to predict change over time on an individual basis. We therefore plan to measure memory and cognition, collect information through self-reported questionnaires on lifestyle and diet from individuals whose cognition and memory abilities are presently intact. This information will be collected over a period of several years in order to gain a better insight into the factors influencing and markers of cognitive decline to Alzheimer's disease. We also aim to explore how genetic and other biological information can be used to predict the onset and progression of the disease.

3. Do I have to take part?

It is entirely up to you whether you would like to participate in this study. We will describe the study and go through this information sheet with you. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time without giving a reason. Please note that being in a research study is not part of your routine medical care. This will not affect the standard of care you receive from your own GP.

4. What do I have to do if I take part?

If you would like to participate in this study, we would first like to have a conversation with members of our study team to make sure that this study is right for you. We may ask you questions about your current and past health, any medication you may be on and whether you are currently involved in any other studies.

If this study seems suitable, we will invite you to visit us at Charing Cross Hospital, London. During this first visit, the study doctor or the study staff will speak with you about the study and answer any questions you may have regarding the content of this information sheet. If you decide to participate in the study, you will be asked to sign an informed consent form.

We expect the total time for your first visit to be around 3½ hours. This is because we want to find out a lot about you for the study. You will be asked to complete questionnaires about your lifestyle and diet, medical history, family history, health and any concerns or potential risk factors for early Mild Cognitive Impairment – these might take up to 1½ hours. We appreciate that you may have questions whilst filling the forms and we will be there to clarify any questions. Then one of our researchers will take you through some assessments about your thinking, learning and memory, decision making and language. These will include questions and paper-based exercises.

You will additionally be asked to provide blood, urine and saliva samples. Further information about the reason for this and what it will be used for is below. You will be asked to refrain from eating or drinking coffee, for a specified period of time prior to your visit if samples are to be collected on that day. We will provide refreshments at your visit.

At this initial visit, we will also ask you to bring a form of photo ID, or other identification. We would like to keep a copy of this on your record and refer back to it at future visits. It is a requirement for us to ensure the integrity of our data, and will be kept separately to any study data collected. When you finish the study or are no longer taking part, we will destroy this copy of your ID.

We will also ask you to provide your National Health Service (NHS) number, in order to access and review your medical records. This will allow for a comprehensive overview of your past and present medical and medication history. Your detailed medical and medication history is important for us to maximally use the data derived from your study visits, such as the information collected through the lifestyle questionnaires, the findings of the cognitive and memory tests, as well as the imaging studies. It will also enable us to collect any subsequent data from electronic health records in primary or secondary care within NHS in the future, to determine if any study outcome measures or variables correlate with future development of conditions associated with memory problems. Your NHS number will be stored in a separate database following latest industry standards for encryption to ensure optimal data protection.

Please note that this first visit is also a "Screening visit". The study team will review the data gathered at this first meeting and check that you are still suitable for the study. We will inform you, as soon as possible, if for any reason it is not appropriate to continue in the study.

For those people continuing in the study, we will then invite you back for a follow-up visit within 1 month, to gather additional information from you. This will be a similar length to the first visit and may include questions, computer-based and paper-based exercises. After that, we plan to invite you back every 6 months for the next 4 years, for visits that may take up to 3 and 5 hours. We will be able to let you know what to expect for each upcoming visit.

If you have any questions or concerns about your treatment or health over the course of the study, you should discuss this with your GP as usual.

5. What happens to my blood samples and why are they needed?
Your blood samples will be used for DNA analysis. DNA is the substance in your cells which contains information you inherited from your parents. Your DNA contains "genes" or inheritable factors which are the building plan for your physical characteristics such as eye colour, hair colour and height. Having one or a combination of variations of DNA in genes may increase or decrease a person's risk of developing Alzheimer's disease.
We will collect one tube of blood (about 2 teaspoons or 10mL) from a vein in your arm at the first visit or the next possible visit. This will take about 5-10 minutes. We will send this blood sample to a research laboratory where it will be used to obtain your DNA. The DNA will be analyzed or "genotyped" by a research laboratory, possibly outside the UK to characterise the specific DNA variants of the genes that will be included in the analysis. Only Imperial College London and Janssen R&D, LLC will receive and store the results of this analysis. Your results will be coded and will not identify you by name.
6. Why are additional blood, urine and saliva samples needed?
At the first visit and on each subsequent yearly visit, we will also collect 5 tubes of blood (about 8 teaspoons or 40mL), urine and saliva that will be kept under laboratory conditions in special freezers for future research. These samples will be retained by Imperial College London indefinitely and enable us to use state-of-the-art technologies to conduct future research projects related to Alzheimer's disease, for example to identify or validate biological and genetic markers that would correlate with either cognitive and memory findings of our assessments or factors influencing risk of dementia that may help predict Alzheimer's disease in those without symptoms and identify interventions that may help prevent Alzheimer's disease. Imperial College London will ensure that your samples are kept securely and be de-identified. All such projects using your samples will be reviewed independently before being allowed to use your biological fluids. Furthermore, to protect your privacy, your samples will be de-identified and labelled only with your study number; the scientists potentially doing the later research will not know your identity.

This research may lead to the development of eventual diagnostic tests for the early stages of Alzheimer's disease or effective treatments for this disease, which are not available to-date. It is possible that the study Sponsor may work with third parties including other companies and other research institutions to conduct the testing and research, and may share your samples and/or data with them. Some of this research may involve looking at the features (including genes) of your samples. However, as noted above, to protect your privacy, your samples will be de-identified and labeled only with your study number; the scientists potentially doing the later research will not know your identity. Your samples may also be sent to other members of the Johnson & Johnson group of companies, to contractors working for trem and to regulatory authorities.
Some or all of your samples may also be kept and used for future research. This will allow for the scientific research to be done in the future as new discoveries are made. The site will ensure that your samples are kept securely. You will not be informed when they are destroyed and you will not be paid for any use of your samples, results, or inventions made from research on them. You are providing your samples, for use by the Sponsor and Imperial College.
You can withdraw your consent for your samples to be used for this or future research by informing the study manager or a member of the study team. This can be done at the time you leave the main study or at any time thereafter, for any reason. Your study doctor will then arrange for destruction of all your samples.
If you withdraw your consent for samples to be kept and used at some time after the study is over, it is possible that your study doctor may have already discarded the records that link your unique participant ID to your de-identified samples. In this case, your samples would no longer be linked to you and it would not be possible to find your samples to destroy them.

7. Will I be made aware of any test results?
We have a duty of care to share any non-experimental test results with you if our findings indicate any clinically relevant information. If there are any clinically relevant findings the study team will contact you to arrange for you to have a discussion with one of the study doctors about what they may mean. At that time we would also ask your permission to pass this information onto your GP.
The results of the DNA genetic study are experimental; they will be used for research purposes only and, as they have no proven diagnostic value, will not be shared with you. As outlined above, this test will be conducted by a research laboratory. The test is not used in usual clinical care and has no diagnostic value for dementia, or for any other disease at the present time., However, they will be extremely valuable in the analysis of our research data allowing us to evaluate the various factors possibly influencing risk to Mild Cognitive Impairment and early Alzheimer's disease.
8. How long will my participation in the study last?
As we wish to observe cognitive changes over time, this study is intended to continue up to four years during which time you would be required to attend a visit once every six months.
You may have to leave the study early even if you want to continue. This could happen if:

- For any reason the study closes early, for example if funding becomes unavailable, we will let you know and explain the reason. We will use all data and samples collected to date.
- Due to safety issues, the study doctor or study staff believe it is best for you to stop being in the study
- You do not follow instructions about the study, such as completing the required questionnaires and measures, resulting in potential concerns for your safety
- You fail to return for protocol-required visits
- You refuse to complete the protocol-required assessments and questionnaires, as above
- For example, you develop a neurological degenerative or other disease that is exclusionary for participation in the stud7

9. What are the potential benefits of taking part in this study?

We will be assessing your cognition and everyday daily life functioning using standardised assessments and we will inform you and (with your approval) your GP if there are any clinically significant results. The frequency of evaluation provided in this study is above and beyond that you would expect to receive under normal stand of care in the UK. This is a research study and therefore any further diagnoses will need to be performed by your GP.

Furthermore, we hope that your contribution to the results obtained from this study may lead to effective risk prediction models, future research, and to the potential development of prevention strategies and new treatments for early memory problems and dementias, such as dementia due to Alzheimer's disease.

10. What are the potential disadvantages of taking part in this study?

It is important to remember that this is a purely observational study which does not prescribe or recommend a particular treatment. We would like to point out some potential disadvantages or risks in participating in this study:

- Whilst we will do everything possible to make your study visits comfortable, we recognize that we are asking a significant time commitment.
- The study will involve taking a blood sample during your first visit and subsequent yearly visits. This may cause slight discomfort, pain, and there is a small risk of fainting, bruising and/or infection at the place where the needle is inserted
- You may experience anxiety due to performance of study required memory or other tests and questionnaires

We will hold a lot of confidential data about you and we are taking several measures (listed in section 12 below) to ensure that your information remains confidential. Please do not hesitate to ask questions or raise any concerns at your study visit. In between visits, you can also call us on 020 3311 0620 or email us: chariotpro.study@imperial.ac.uk

11. What if I get hurt or sick while I am in this study?

If you have an illness or injury during the course of this study, you should seek medical attention via your GP or local A&E as you would normally. At your next study visit you will be asked to notify the study team about any injury, illness or other health concern since your last visit. If you think that anything might affect your participation in the study, you can contact us at any time to discuss.

As this study is purely observational, it is unlikely that you will get hurt or sick by participating. If you are harmed by taking part in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Study Manager (details on the first page). If you are still not satisfied with the response, you may contact the Imperial College Joint Research Office. The Sponsor, Janssen R&D, LLC, holds insurance policies for this research project and has agreed to provide compensation if they are at fault.

12. Will my taking part in this study be kept confidential?

Yes, we will keep all the information which is collected about you during the course of the research strictly confidential. At the first visit you will be assigned a unique participant ID to ensure confidentiality. This will be used instead of your name to identify any samples, information or data we have collected from you throughout the study sessions and on all paperwork. Your unique participant ID will not be identifiable to anyone other than the Imperial College researchers and principal investigator involved in this study at the site you have attended your visits. The ID will be stored separately from any identifiable information, such as name, contact details and NHS number.

All documents and electronic data will be stored securely in a locked cabinet and on secure, encrypted computer systems, accessible only to the study team and Sponsor's monitors, in accordance with the Data Protection Act 1998. All forms and questionnaires will be logged with your unique participant ID. Any subsequent reports, publications or analyses will remain anonymous.

Under the Data Protection Act you are entitled to see what is recorded about by applying in writing to the Study Manager – contact details on the first page.

13. What if relevant new information becomes available?

It is unlikely that new information will emerge during this study that affects your participation, however if we do receive new information the researcher will tell you and discuss whether you should continue in the study. If you decide not to carry on, the researcher will make arrangements for you to be withdrawn from the study.

14. What are the alternatives to participation?
The only alternative to participation in this study is not to participate or to withdraw from the study. This is your decision and will not affect any standard care you receive.

15. What will happen if I don't want to carry on with the study?
You can withdraw from this study at any time, by informing the Study Coordinator in writing, without giving a reason and without this affecting your continuing care.
If you leave the study early for any reason, we may use and distribute any non-identifiable data that we have already collected up to that time this may include any data that we have received from analysis of your samples.
We will ask for your consent to retain your samples for genetic analysis and future research purposes.
If you leave the study early but have not withdrawn your consent, you may be asked to return for a final exit visit, if this is agreeable to you. At this visit you will be asked for your reason for leaving the study, and to undertake all study procedures that have not been completed within the past 6 months. You are under no obligation to attend this visit.

16. What will happen to the results of the research study?
This study is expected to unravel important scientific findings that may have significant impact for future clinical studies, including trials of novel drugs for the prevention or cure of dementias, such as Alzheimer's disease. Therefore, we plan to make our findings available in the public domain through publication in highly regarded peer-reviewed scientific journals. Such publications of results may be made available to study participants upon request through contacting a member of the NEA research team using the number provided.
We will use these results to help us identify cognitive markers that are predictive of the risk of developing dementia or the presence of early stages of Mild Cognitive Impairment due to Alzheimer's disease, and consider preventative measures from them and new treatments. We may also use these results to identify other areas of research for the prevention of Alzheimer's disease.
We will also present these results at scientific conferences and may write about this study in our 'CHARIOT dementia prevention research register' newsletter. However, no-one involved in this study will be identified in any report or publication.
The study team may also use the data collected in this study to invite you to take part in other research studies that you would be eligible for.

17. Will it cost anything to be in this study?

No, and we will compensate reasonable travel expenses and provide refreshments, so that you are not out of pocket from taking part in this study. Please discuss any queries with the study team. There are no additional costs to you because of your participation in this study.

18. Who is organising and funding the research?
This research is being organised by Imperial College London and Janssen Research and Development, LLC *(Janssen R&D, LLC is a pharmaceutical company of Johnson & Johnson)*, the Sponsor that is funding this study.

19. Who has reviewed the study?
This study has been looked at by a group of people, called a Research Ethics Committee to protect participants' safety, rights, wellbeing and dignity, as well as the CHARIOT:PRO study team and by research teams of the Sponsor Organization. This study has also been approved by the National Research Ethics Service (NRES) committee.

20. Further information and contact details
If you would like further information or have any questions about this research study or about the CHARIOT register please give us a call on 0203 311 0620 or send an email to: chariotpro.study@imperial.ac.uk. We are happy to answer any questions you have.

Thank you for considering your involvement in this research study and for taking the time to read this information sheet. If you agree to participate please sign the informed consent form on the next page in the presence of someone from the study team.

Subject ID: _____

Participant Consent to Participant Information Sheet **Informed Consent Form**	**Participant Initials**
1. I confirm that I have read and understood this Informed Consent Form. I have been given enough time to consider the information, ask questions about the study and my questions have been answered to my satisfaction.	
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without having to give a reason and without my medical care or legal rights being affected.	

Participant Consent to Participant Information Sheet Informed Consent Form	Participant Initials
3. I agree to answer all the questions posed during the study to the best of my knowledge.	
4. I understand that data collected during the study may be looked at by individuals from Imperial College London for study management purposes, the Sponsor's designees for monitoring purposes; and regulatory authorities, where required by law. No identifiable data will be published, or accessed outside the study team and monitors. I give permission for these individuals to have access to my study records.	
5. I agree to my GP being informed of my participation in the study.	
6. I understand that my National Health Service (NHS) number will be collected and stored in a separate database following latest industry standards for encryption/data protection. I understand that this will enable subsequent data collection from my electronic health records in primary or secondary care within NHS in the future by researchers from Imperial College, for the purpose of high scientific quality, ethically approved research.	
7. I understand that responsible individuals from the Imperial research team and designated personnel from Janssen R&D may look through relevant sections of my medical records, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my medical records.	
8. I understand that my participation will be recorded in the CHARIOT register. Information collected in the study may be used to keep my contact and demographic details on CHARIOT register up to date.	
9. Researchers from Imperial College London and Janssen R&D LLC will have access to my data for research and analysis. My data will not directly identify me, and will be used for this study as detailed in the participant information sheet.	
10. I agree to a genetic test of my blood to be conducted by a research laboratory, and understand that the derived information will be used in the analysis of factors influencing risk for dementia such as Alzheimer's disease. I understand that I will not receive results from these tests.	
11. I agree to give additional blood, urine, and saliva samples to Imperial College London and the Sponsor for the purpose of future research as described in the Participant Information Sheet. I understand that these samples will be used for research such as the identification and validation of biological and genetic markers that would correlate with either cognitive changes or other factors influencing risk of dementia.	
12. By signing this consent I agree to the transfer of my de-personified data, (including health-related data collected during this clinical research study, and race or ethnic origin information)/ samples to countries outside of the UK and European Union (EU).	
13. If commercial products were developed as a result of this study, I will not profit financially from such a product.	

Participant Consent to Participant Information Sheet Informed Consent Form	Participant Initials
14. I agree to take part in this study.	
15. I understand that I will receive a copy of this Participant Information Sheet and signed Informed Consent Form	

Name of participant (your name): _____

Signature: _____ Date:_____

(DD/MMM/YYYY)

Name of Researcher obtaining consent: _____

Signature: _____ Date: _____

(DD/MMM/YYYY)

Table B.2 Eligibility Criteria

Inclusion

1. Between 60-85 years-old inclusive
2. Willing and able to give written informed consent
3. Meets the definition for one of the following groups:
    - *High risk:* Participants whose age- and education-adjusted baseline cognitive performance is 0.6-1.5 SD (inclusive) below normal on the RBANS Immediate and/or Delayed Memory Index and one other non-memory cognitive domain of 0.6-1.5 SD (inclusive) below normal.
    - *Medium risk (amnestic):* Participants whose age- and education-adjusted baseline performance is 0.6-1.5 SD (inclusive) below normal on the RBANS Immediate and / or Delayed Memory Index with all other RBANS nonmemory domains within normal limits operationally defined as better than 0.6 SD below normal.
    - *Medium risk (nonamnestic):* Participants whose age- and education-adjusted baseline cognitive performance is better than 0.6 SD below normal on the RBANS Immediate and Delayed Memory Index, and one or more other RBANS domains are 0.6 to 1.5 SD (inclusive) below normal
    - *Low risk cohort:* Participants whose age- and education-adjusted baseline cognitive performance on the RBANS is within normal limits, as defined for this study as all RBANS domains greater than 0.6 SD below normal.
    - Participants for whom an age- and education-adjusted baseline RBANS domain index score is more than 1.5 SD below normal will be adjudicated for inclusion.
4. Be willing and able to adhere to the prohibitions and restrictions specified in this protocol
5. Fluent in English

Exclusion

1. Concurrent participation in a clinical trial. Enrolment in the noninterventional trials is permitted only if the safety of the participant and integrity of the CHARIOT-PRO study data are not compromised by any of the following:
    - High participant burden through enrolment in multiple studies
    - Blood draw requirements beyond the permissible amount according to local recommended blood donation limits
    - Cumulative exposure to radiation that exceeds local recommended exposure limits
    - Exposure to neuropsychological measures in CHARIOT-PRO that may impact participant performance (e.g., learning effects)
2. Dementia diagnosis or MCI diagnosis
3. Past or current use of memantine or cholinesterase inhibitors
4. Diagnosis of other neurologic disease or conditions known to cause or be associated with dementia, such as Parkinson's disease
5. A history of traumatic brain injury with residual neurological deficit
6. History of stroke or transient ischemic attach (TIA)
7. History of seizure, excluding febrile seizures in childhood

8. Current diagnosis of significant psychiatric illness, as per the Diagnostic & Statistical Manual of Disorders, 4th Edition – Text Revision (DSM-IV-TR) (including but not limited to major depressive disorders and anxiety disorders) and is in an acute phase/episode; or the subject has a current diagnosis or history of schizophrenia or bipolar disorder

9. Reversible causes of dementia such as B12 deficiency, metabolic problems and/or nutritional deficiencies (e.g. folic acid)

10. History of hydrocephalus at any time

11. Uncontrolled hypo- or hyperthyroidism at enrolment

12. Chronic use of medications known to impair cognition such as sedatives, pain medications or anticonvulsants. Additionally, use within 48 hours or 4 times the half-life, whichever is longer, before the baseline cognitive assessments is prohibited.

13. At baseline, any clinically significant chronic illness, for example, hepatic impairment or renal insufficiency, or cardiovascular, pulmonary, gastrointestinal, endocrine, neurological, rheumatologic, immunologic, infectious skin and subcutaneous tissue disorders, or metabolic disturbance that is likely to result in rapid deterioration of the participant's condition or affect their safety during the study

14. Clinically significant infection within 30 days (e.g., persistent or acute infection such as a urinary tract infection or upper respiratory infection) of study entry

15. Self-reported HIV infection

16. History of alcohol or drug dependence or abuse as defined by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM IV) criteria within the last 3 years

17. Any physical disability that would prevent completion of study procedures or assessments (e.g., blindness or significant visual impairment, deafness or significant hearing impairment, non-AD-related speech impairment, or sensory or motor dysfunction)

18. Participants whose age- and education-adjusted baseline cognitive performance > 1.5 SD below normal on any RBANS index scores (note: when any screening scores are more than 1.5 below normal, the Sponsor's Medical Monitor and/or designee(s) will adjudicate and approval enrolment; and if participants are enrolled, they will be assigned to the appropriate risk group)

B.3 Screening visit data collection

- Demographics
    - Date of birth
    - Gender
    - Ethnicity
    - Marital status
    - Family history of dementia
    - Education level
    - Occupation
    - Occupations history
- Medical history such as:
    - Cerebrovascular accident
    - Seizures
    - Thrombotic conditions
    - Confirmed myocardial infarction and symptoms of angina
    - Congestive heart failure
    - Other dementias (e.g., vascular, Lewy body, frontotemporal)
    - Congestive heart failure
    - Other central nervous system conditions that may cause progressive deficits in memory and cognition
    - Systemic conditions known to cause dementia
    - Substance abuse
    - Major psychiatric illness
    - Hypertension
    - Diabetes
    - Medication-related side effects: drowsiness, confusion and anorexia
    - Falls and syncope
    - Concussion(s) or traumatic brain injuries
    - Incontinence
    - Infections
    - Pulmonary disease
    - Kidney disease
    - Osteoarthritic conditions
    - Vision loss
    - Hearing loss
- Concomitant medications (name, dose and frequency)
- AD-related information
    - Subjective cognitive complaints including duration
    - Results from diagnostic tests and procedures (if performed)
- Neuropsychological testing
- Self-reporting questionnaires
    - Depression and anxiety
    - Sleep
    - Nutrition
    - Physical activity
    - Health status
    - Work productivity
    - Midlife health behaviours (within the Imperial Lifestyle Questionnaire)
    - Functional abilities
    - Current and past smoking and alcohol consumption

Appendix B.4 Physical activity scale for the elderly (PASE)

Validity and Reliability

The validity and reliability of the PASE were first established in a random non-demented sample of 277 older adults aged 65-100 years old (mean age = 74, SD not reported) [389]. Item weights were derived and a component score was developed via a 3-day PA monitor, diary and global self-reporting; all of which were regressed on responses to the PASE and has since been re-validated across multiple studies [504, 561, 562]. PASE scores were also validated through comparing physiological and health status data in the home, whereby scores were significantly correlated with balance (r=0.33), grip strength (r=0.37), leg strength (r=0.25), resting heart rate (r=-0.34), age (r=-0.34), self-assessed health status (r=-.034), and Sickness Impact Profile scores (r=-0.42). Likewise, further correlation between PASE scores and sociodemographic and medical conditions hypothesised to effect PA levels were reported. They found significant correlations between PASE and age, sex, employment status, cancer, hypertension, and chronic respiratory disorder. The pattern of statistically significant correlations across a variety of health and physiological measures provides strong evidence for convergent validity of the PASE scoring algorithm. Test-retest reliability over three to seven weeks were strong, with a coefficient of 0.75 (95% CI = 0.69 – 0.80). The PASE scores also exhibited temperature-related seasonal variation, where the highest levels of PA reported during summer and lowest during winter.

A later study involving non-demented older adults (65 years plus) reported congruent results, whereby age was negatively correlated with PASE scores (r=-0.21), alongside systolic blood pressure (r=0.18), positively correlated with balance (r=0.20) and peak oxygen uptake (r=0.20) [561]. A further two studies found a significant correlation between PASE questionnaire outcome scores with a Physical Activity Ratio (PAR = Total Energy Expenditure/Resting Metabolic Rate) with a medium (r=0.36) to high effect size (r=0.68) [562, 563].

PASE scoring instructions and manual can be found here:

https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&cad=rja&uact=8&ved=2ahUKEwiUg9TetMjiAhUwVBUIHUudCKUQFjABegQIAxAC&url=https%3A%2F%2Fmeetinstrumentenzorg.blob.core.windows.net%2Ftest-documents%2FInstrument4142%2FPASE%2520handl.pdf&usg=AOvVaw1zjThqIo2R2akEbKePcKMq

Appendix B.5: Perl 5 script for SPSS output tabulation

Written by Sam Bessey in collaboration with the candidate (CR)

January 2019

```
use Data::Dumper qw(Dumper);
use File::Copy;
my $flag==0;
my $siglvl="";
my $depVar = "";
my $cleanBuffer="";
my $cleanQuery ="";
my $prefix ="";
my @searchTerms = ();
my %consolidated;
my $bothDone=0;
my $expectInputs=0;
my @legacyTerms=();
my@prefixArr=();
my @finalOutputOrderRows=();
my @finalOutputOrder =();
my $conffile="";
my $regParam="";
my $permitWrite = "Y";
my $tidy = "";
print "Please enter a config file: ";
$conffile = <>;
chomp ($conffile);
my %config = do 'config/'.$conffile;
my @listOfFiles = ($config{file1},$config{file2});
my @listOfTables = ($config{thistable1},$config{thistable2});
$modelId = $config{modelName};
@finalOutputOrder = split (',', $config{finalOutputOrder});
@finalOutputOrderRows = split (',', $config{finalOutputOrderRows});
my $finalOut = "final/".$modelId."-model.csv";
$expectInputs = $config{numberOfFiles};
while ($bothDone < $expectInputs)
{
  my $tempInput = @listOfFiles[$bothDone];
  $file = "input/".$tempInput;
  print "Is this file 'below the separator'? ";
  $gcurve = <>;
  chomp($gcurve);
  if ($gcurve eq "n")
  {
    #NEXT IS LLPA MAIN EFFECTS
  #  @searchTerms = ("[PASEtert2=2.00]","[PASEtert2=1.00]","Time","TimeSqr");
    @searchTerms  = split (',', $config{searchTerms1});
    #print @searchTerms[0].@searchTerms[1].@searchTerms[2].@searchTerms[3]."END";
    @legacyTerms = @searchTerms;
    #print "Enter a prefix (Like APoECarrier): ";
```

```
  $prefix =$config{prefix1};
  #chomp($prefix);
  push @prefixArr, $prefix;
  #print @prefixArr[0];
}
if ($gcurve eq "y")
{
  @searchTerms = split (',', $config{searchTerms2});
#  print "Enter a prefix (Like APoEnonCarrier): ";
  $prefix =$config{prefix2};
#  chomp($prefix);
  push @prefixArr, $prefix;
  #$prefix = '2-';
  if( @legacyTerms ~~ @searchTerms )
  {
    print "Identical terms for both runs found\n";
  }
}
my $output = "staging/".$prefix.$modelId.".tsv";
print "Set to ".$modelId;
open(IN, '<'.$file) or die $!;
while(<IN>)
{
    if ($_=~m/95\%\ Confidence\ Interval[\t\n]/)
    {
      #print "Found a match\n";
      $flag=1;
    }
    if ($flag == 1 && $_=~m/^\t*\n/)
    {
      $flag--;
      if ($flag==0)
      {
        print "End of table found: ".$depVar."\n";
        $cleanBuffer .= "\n".$depVar;
        $depVar =~ s/ /-/g;
        print "Writing to file ".$depVar."...\n";
        open(OUT, '>temps/'.$depVar.".tsv") or die $!;
        print OUT $cleanBuffer."\n";
       # print $cleanBuffer;
       # print "\n\n==========\n\n";
        $cleanBuffer ="";
        close (OUT);
      }
    }
    $_ =~ s/95\%\ Confidence\ Interval\n//g;
    $_ =~ s/\t\t\t\t\t\t//g;
    if($flag==1)
    {
    #  print "FLAG IS1-Here:".$_;
      if (@listOfTables[$bothDone] ne "" && $_!~m/95\%\ Confidence\ Interval[\t\n]/)
      {
        #print "\n\n\n\nMATCH FOUND!!!!!".@listOfTables[$bothDone]."?\n\n\n\n\n\n\n";
```

```
      my $tableID = @listOfTables[$bothDone];
      if ($_=~m/\Q$tableID/)
      {
        $permitWrite ="Y";
        $_=~ s/$tableID//g;
      }
      else {
      if ($_=~m/^[\w\-]/)
      {
        $permitWrite ="N";
        if ($_=~m/Dependent Variable/)
        {
          $permitWrite ="Y";
        }
      }
    }
    }
    #next unless $_ =~m/\-99/;
    #print "HEY\n";
    if ($_ =~m/\w\.* Dependent Variable\:\ (.*)?/)
    {
      $depVar = $1;
      $depVar =~ s/[^a-zA-Z0-9 ]//g;
    }
    $_=~ s/^\t([a-zA-Z0-9\[].*)/$1/g;
    if ($permitWrite eq "Y")
    {
      $cleanBuffer .= $prefix.$_;
    }
  }
}
close(IN);
close(OUT);
@files = <temps/*>;
foreach $filename (@files) {
  #my $filename = "Global-Cog.tsv";
  open(IN, '<'.$filename) or die $!;
  my %results = ();
  $filename =~ s/-/ /g;
  $filename =~ s/^.*?\///g;
  my $printDepVar = substr($filename, 0, -4);
  print "Writing ".$printDepVar." to staging file...\n";
  while(<IN>)
  {
    chomp;
    foreach my $k (@searchTerms)
    {
      $cleanQuery = "^".$prefix.$k;
      $cleanQuery =~ s/([\[\]\=\*])/\\$1/g;
      $cleanQuery.="\t";
      if($_=~m/$cleanQuery/)
      {
        push @{ $results{$k} }, split /\t/, $_;
```

```
          }
        }
      }
      open(OUT, '>>'.$output) or die $!;
      print "Writing from".$output."\n";
      keys %results;
      print OUT ($modelId."\t\t\n".$printDepVar."\t\tSig\n");
      foreach my $k (@searchTerms)
      {
        if ($results{$k}[5] < .08)
        {
          $siglvl = "x";
        }
        if ($results{$k}[5] < .05)
        {
          $siglvl = "*";
        }
        if ($results{$k}[5] < .01)
        {
          $siglvl = "**";
        }
        if ($results{$k}[5] < .001)
        {
          $siglvl = "***";
        }
        my   $middleCol   =   sprintf('%.2f',   $results{$k}[1])."   [".sprintf('%.2f',   $results{$k}[6]).",
".sprintf('%.2f', $results{$k}[7])."]".$siglvl;
        my $sigCol = sprintf('%.2f', $results{$k}[5]);
        print OUT ($k."\t".$middleCol."\t".$sigCol."\n");
        $siglvl = "";
        $consolidated.=$consolidated{$printDepVar}{ $prefix.$k } = $middleCol;
      }
      close (OUT);
    }
    $bothDone++;
    $permitWrite = "Y";
}
print Dumper \%consolidated;
print "\n\nRAW DATA OBJECT OUTPUT ABOVE - DON'T FORGET TO CHECK FOR ERRORS!\n\n";
open(FINAL, '>final/'.$modelId.'.tsv') or die $!;
print "Writing final model file to tsv for Excel...\n";
keys %consolidated;
my $thing="";
for my $pfx (@prefixArr) {
  print FINAL $pfx."\t";
  foreach my $k (@finalOutputOrder)
  {
    print FINAL $k."\t";
  }
  print FINAL "\n";
  for $hashMeasure(@finalOutputOrderRows)
  {
    print FINAL $hashMeasure."\t";
    for $hashDepVar( @finalOutputOrder )
```

```
    {
      print $hashDepVar.$thing.$hashMeasure.":".$consolidated{$hashDepVar}{$pfx.$hashMeasure}."\n";
      print FINAL "$consolidated{$hashDepVar}{$pfx.$hashMeasure}\t";
    }
    print FINAL "\n";
  }
}
close (FINAL);
print "Completed Successfully. Check if it is all OK, then we can tidy up.\n";
print "Do you want to tidy up? ";
$tidy = <>;
chomp ($tidy);
if ($tidy eq "y")
{
  print ("\nOK!\n");
  move ('config/'.$conffile, 'config/checkdone/'.$conffile) or die $!;
  my $xx;
    while ($xx < $expectInputs)
    {
      move ('input/'.@listOfFiles[$xx], 'input/checkdone/'.@listOfFiles[$xx]) or die $!;
    }
}
```

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Appendix B.7: Quadratic time and interactions with PA: example and interpretation

A quadratic term in linear regression may be necessary when the relationship between the outcome and the independent variable is not a straight line. That is, when the cloud of data exhibits a pattern that suggests non-linearity.

Below are the results of fitting a polynomial mixed-models regression model exploring the association between physical activity and cognitive trajectories and whether time modifies these associations (i.e. do cognitive trajectories differ as a function of physical activity levels). The predictors in the model are time, time^2, LLPA (late-life PA), plus the two interactions.

The quadratic time variable determines which way the curve is bending. Negative indicates concave and positive indicate convex. In this case, the time^2 variable is negative (-0.46), which indicates a concave shape. This is reflected in the graph below. The coefficient indicates the change in the rate of change (i.e. the curvature) with a higher number indicating a sharper curve. The linear time variable indicates the linear rate of change when controlling for this curvature. A positive coefficient indicates a positive increase of global cognition over time (e.g. global cognitive scores are increasing by 2.70 [95% CI 2.00, 3.40] points per six-months).

The next step is to determine whether cognitive trajectories differ as a function of physical activity levels (LLPA). To do this, an interaction with time, time^2 and LLPA is added as independent variables (i.e. LLPA x time and LLPA x time^2) – all within the same model. This allows for both the linear and quadratic component of the curvature to be modelled together.

Table B.7. Mixed-models regression on the association between late-life leisure PA and global cognition

Parameter	Coefficients [95% CI]
Time	2.70 [2.00, 3.40]*
Time^2	-0.46 [-0.61, -0.30]*
LLPA	1.64 [-0.47, 3.75]
LLPA x time	-1.51 [-2.86, -0.16]*
LLPA x time^2	0.40 [0.08, 0.70]*

Based on the tabulated data above, the 'effect' of time can be evaluated by working out the change of the outcome (score) on the different time 'moments'. If the interaction is significant, the differences in trajectories among high LLPA versus low LLPA are significant.

In this example, we have an interaction between a dummy (0/1) and time which is 'continuous'.

The model looks like

$$y = \alpha + \beta_1 time + \beta_2 time^2 + \beta_3 X_2 + \beta_4 time X_2 + \beta_5 time^2 X_2$$

With $time \wedge X_2 = LLPA$

Suppose LLPA (0/1)

For X_2=0 the regression is :

$$y = \alpha + \beta_1 time + \beta_2 time^2$$

For this group (LLPA=0) intercept is α, slope β_1 and quadratic term β_2

For X_2=1 the regression is:

$$\begin{aligned} y &= \alpha + \beta_1 time + \beta_2 time^2 + \beta_3 + \beta_4 time + \beta_5 time^2 \\ &= (\alpha + \beta_3) + (\beta_1 + \beta_4) time + (\beta_2 + \beta_5) time^2 \end{aligned}$$

For this model where LLPA = 1, then intercept is $(\alpha + \beta_3)$, slope, $(\beta_1 + \beta_4)$ and quadratic term $(\beta_2 + \beta_5)$.

The expected difference between LLPA=0 and LLAP=1 (the "effect of LLPA=1") is exactly:

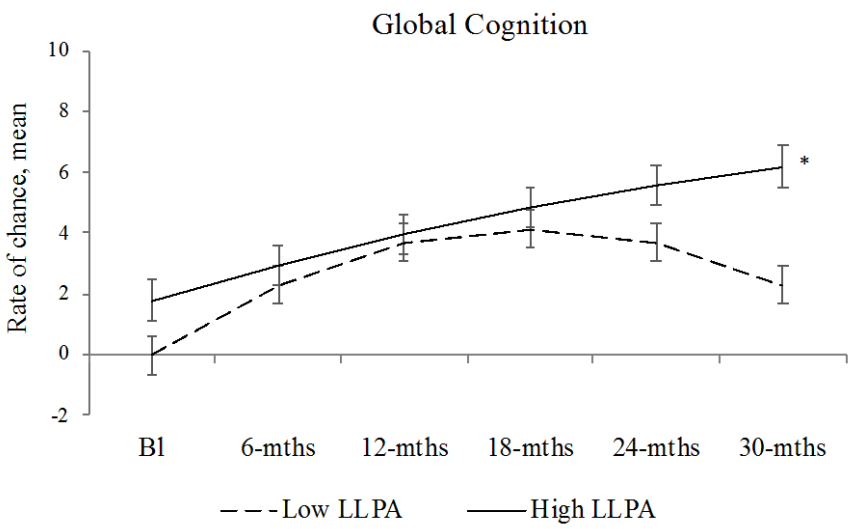
$$\beta_3 + \beta_4 time + \beta_5 time^2$$

If $\beta_5 < 0$ then we can say that effect of X1 is small for low X2 and largest for intermediate values of X2

It is important to note that time is composed by two parts, time and time square; hence, the regression is re-written as:

$$y = \alpha + (\beta_1 + \beta_4 X_2) time + (\beta_2 + \beta_5 X_2) time^2 + \beta_3 X_2$$

It is apparent that X2 and time (and X2 and time square) both have an effect on each other. The single interpretation of the quadratic component interaction, *B*=0.40 [95% CI 0.08, 0.70] is equivalent to looking at a partial result, and is not informative, but should be analysed within the framework defined in the formula.



Therefore, the most informative method of interpretation is to graph the results and explain the interaction visually. The above graph indicates that by inclusion of LLPA x time and LLPA x time^2 interactions in the mixed-effects models regression, a significant difference between groups in linear, *B*=-1.51 [-2.86, -0.16] and quadratic, *B*=0.40 [95% CI 0.08, 0.70] global cognitive trajectories over 30-months is identified. Inspection of the slopes (above) indicate those reporting high LLPA exhibit continued improvements in global cognitive scores over 30-months, whereas those reporting low LLPA initially show cognitive improvement until 18-months, after which they begin declining.

Appendix C. Chapter 4: Late-life Physical Activity, *APOEε4* and multi-domain Cognition

Time, *APOE*, Age and Sex main effects on cognition

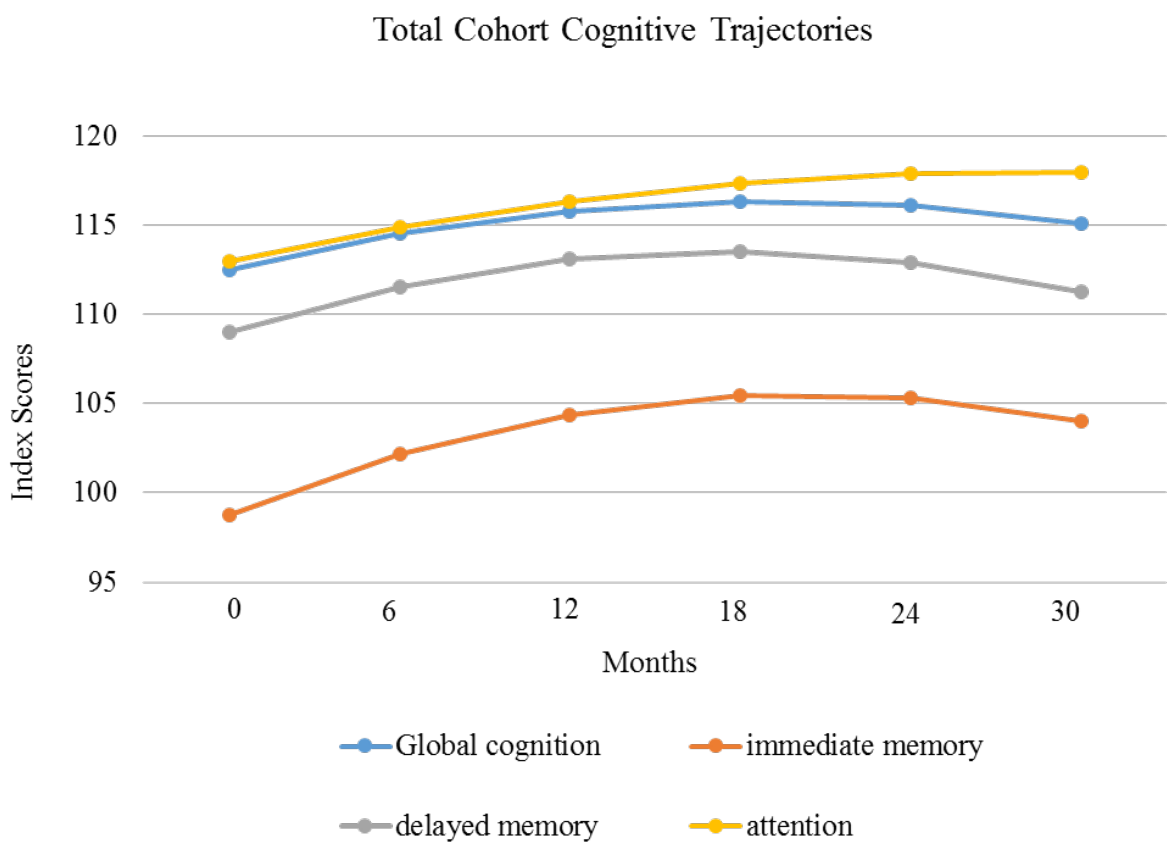


Figure C.1 Multivariate adjusted cognitive trajectories over 30-months in the overall cohort. A statistically significant linear and quadratic time effect on cognitive trajectories were identified for global cognition, immediate memory, delayed memory and attention index scores over 30-months. Improvements in cognitive performance gradually wane by 18-months, after which there appears to be reduced cognitive performance which accelerates between 18- to 30-months, with the exception of attention index scores which continue to improve. Adjusted for age, sex, education, BMI, hypertension, hypercholesterolemia, T2 diabetes, smoking, alcohol, time, time^2.

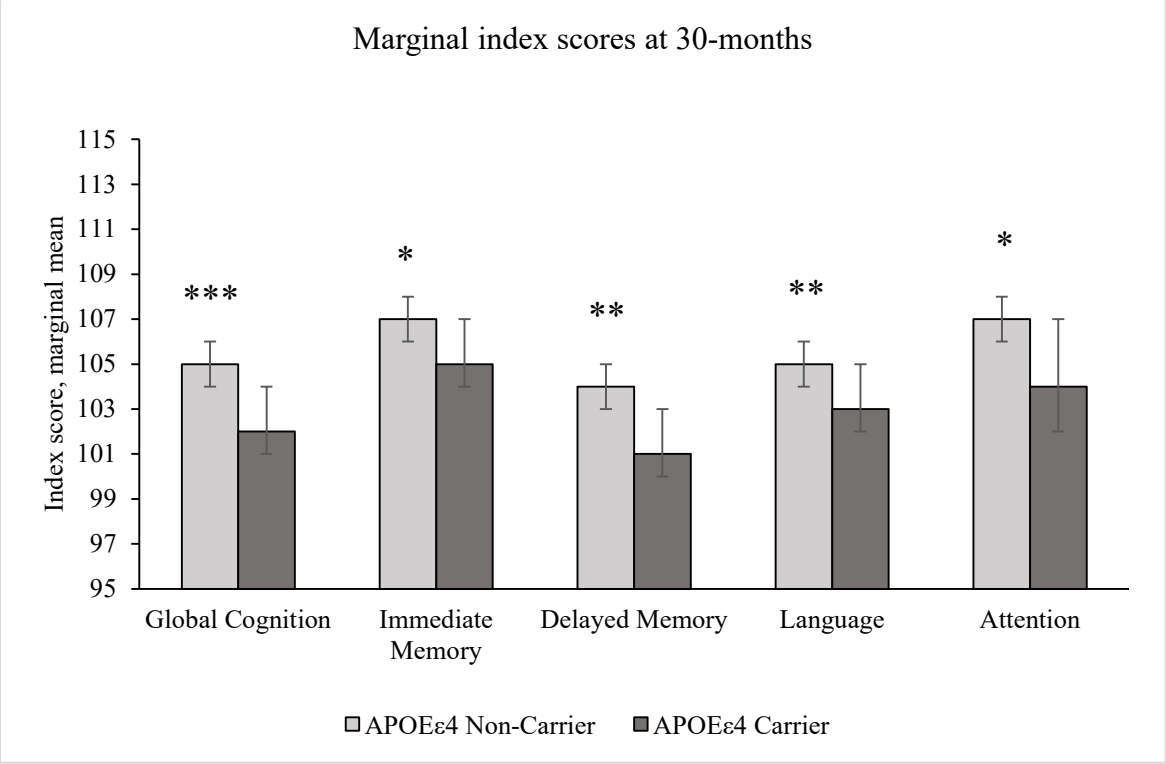


Figure C.2 Marginal mean cognitive outcome scores at 30-months within carriers versus non-carriers. Overall mean cognitive index scores for global cognition, immediate memory, delayed memory, language and attention were significantly higher in carriers compared to non-carriers. *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$, indicating significant differences between carriers and non-carriers within each cognitive domain. Error bars represent 95% CI's. Adjusted for time, time^2, age, sex, education, hypertension, hypercholesterolemia, BMI, type II diabetes, smoking and alcohol intake

Table C. Sample size (N) across time

	Baseline				6-months				12-months			
	Total	**M/F**	**NC/Car**	**<70/>70**	**Total**	**M/F**	**NC/Car**	**<70/>70**	**Total**	**M/F**	**NC/Car**	**<70/>70**
Total	901	406/495	579/322	579/322	607	264/343	450/141	392/215	514	222/292	376/125	331/183
EPA Tertiles												
T1	304	146/158	210/63	190/114	198	89/109	155/39	128/70	164	75/89	124/36	111/53
T2	296	123/173	204/73	178/118	211	86/125	153/51	129/82	179	73/106	132/42	107/72
T3	301	137/164	204/70	211/90	198	89/109	142/51	135/63	171	74/97	120/48	113/58
MLPA												
Low	568	242/326	387/129	365/203	380	154/226	227/76	248/132	313	125/188	227/77	204/109
High	310	154/156	216/69	199/111	211	102/109	159/49	133/78	284	188/96	140/43	117/69
ML/LL PA												
L-ML/L-LL PA	483	207/287	329/108	299/184	324	134/201	240/72	206/118	260	106/165	188/63	164/96
L-ML/H-LL PA	93	45/49	65/23	70/23	63	30/34	48/15	46/17	59	28/32	44/15	44/15
H-ML/L-LL PA	174	87/95	119/41	109/65	113	54/67	82/30	66/47	102	51/59	77/24	60/42
H-ML/H-LL PA	128	70/64	90/27	86/42	91	51/46	71/18	63/28	78	43/41	58/18	53/25

Abbreviations: M, Male; F, Female; NC, *APOE*ε4 Non-carrier; C, *APOE*ε4 carrier; <70, less than 70-years old; >70-years old; EPA, everyday physical activity; MLPA, midlife physical activity; L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity

Table C (continued). Sample size (N) across time

	18-months				24-months				30-months			
	Total	**M/F**	**NC/Car**	**<70/>70**	**Total**	**M/F**	**NC/Car**	**<70/>70**	**Total**	**M/F**	**NC/Car**	**<70/>70**
Total	901	406/495	579/322	579/322	607	264/343	450/141	392/215	514	222/292	376/125	331/183
EPA Tertiles												
T1	304	146/158	210/63	190/114	198	89/109	155/39	128/70	164	75/89	124 / 36	111 / 53
T2	296	123/173	204/73	178/118	211	86/125	153/51	129/82	179	73/106	132 / 42	107 / 72
T3	301	137/164	204/70	211/90	198	89/109	142/51	135/63	171	74/97	120 / 48	113 / 58
MLPA												
Low	568	242/326	387/129	365/203	380	154/226	227/76	248/132	313	125/188	227 / 77	204 / 109
High	310	154/156	216/69	199/111	211	102/109	159/49	133/78	284	188/96	140 / 43	117 / 69
ML/LL PA												
L-ML/L-LL PA	483	207/287	329 / 108	299 / 184	324	134/201	240 / 72	206 / 118	260	106/165	188 / 63	164 / 96
L-ML/H-LL PA	93	45/49	65 / 23	70 / 23	63	30/34	48 / 15	46 / 17	59	28/32	44 / 15	44 / 15
H-ML/L-LL PA	174	87/95	119 / 41	109 / 65	113	54/67	82 / 30	66 / 47	102	51/59	77 / 24	60 / 42
H-ML/H-LL PA	128	70/64	90 / 27	86 / 42	91	51/46	71 / 18	63 / 28	78	43/41	58 / 18	53 / 25

Abbreviations: M, Male; F, Female; NC, *APOE*ε4 Non-carrier; C, *APOE*ε4 carrier; <70, less than 70-years old; >70-years old; EPA, everyday physical activity; MLPA, midlife physical activity; L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity

Table C.1 Time, *APOE,* sex and age main effects in the overall cohort and stratified by *APOEε4* carrier status

	Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Main effects							
*APOE*ε4	3.02 [1.13, 5.04]***	2.12 [0.15, 4.01]*	2.41 [0.66, 4.12]**	1.20 [-0.88, 3.32]	2.01 [0.24, 3.83]*	2.72 [0.33, 5.12]*	1.22 [-1.63, 3.91]
Sex	4.43 [2.75, 6.11]***	7.22 [5.52, 8.93]***	4.54 [3.06, 6.01]***	-3.64 [-5.46, -1.82]***	5.51 [3.98, 7.04]***	0.93 [-1.12, 2.99]	-0.14 [-2.57, 2.29]
Age	0.06 [-1.61, 1.74]	-0.76 [-2.46, 0.94]	0.38 [-1.09, 1.85]	1.07 [-0.74, 2.89]	0.42 [-1.11, 1.95]	-0.74 [-2.80, 1.31]	3.40 [1.01, 5.79]***
Time	2.32 [1.74, 2.93]***	3.93 [3.11, 4.72]***	3.33 [2.72, 3.90]***	-0.08 [-1.01, 0.87]	0.50 [-0.30, 1.33]	1.90 [1.24, 2.62]***	.170 [-1.03, 1.41]
Time^2	-0.36 [-0.49, -0.22]***	-0.58 [-0.79, -0.40]***	-0.52 [-0.66, -0.38]**	-0.17 [-0.38, 0.05]	-0.06 [-0.24, 0.13]	-0.19 [-0.35, 0.03]**	0.08 [-0.23, 0.39]
Time interactions							
APOE x time	0.19 [-1.22, 1.64]	0.01 [-1.82, 1.83]	-0.89 [-2.33, 0.51]	-	0.52 [-1.30, 2.33]	-0.51 [-2.13, 1.14]	-
APOE x time^2	-0.03 [-0.33, 0.28]	0.11 [-0.29, 0.50]	0.20 [-0.11, 0.51]	-	-0.09 [-0.50, 0.32]	0.07 [-0.29, 0.43]	-
Sex x time	0.45 [0.03, 0.88]*	0.56 [0.00, 1.10]*	0.43 [-0.01, 0.87]†	0.83 [0.16, 1.51]**	-0.99 [-1.52, -0.43]**	0.26 [-0.24, 0.76]	-0.79 [-1.62, 0.00]*
Sex x time^2	0.21 [-0.06, 0.48]	0.03 [-0.33, 0.38]	0.22 [-0.06, 0.50]	-0.06 [-0.48, 0.39]	0.46 [0.10, 0.82]**	0.19 [-0.13, 0.51]	-0.11 [-0.74, 0.51]
***APOE*ε4 Carrier**							
Time	2.12 [0.89, 3.20]***	3.81 [2.32, 5.33]***	3.70 [2.42, 4.91]***	-0.06 [-2.02, 1.83]	-0.14 [-1.70, 1.42]	2.32 [0.87, 3.74]***	-1.10 [-3.43, 1.11]
Time^2	-0.32 [-0.57, 0.06]*	-0.62 [-0.97, -0.29]***	-0.62 [-0.90, -0.35]***	-0.12 [-0.54, 0.30]	0.06 [-0.28, 0.39]	-0.24 [-0.55, 0.07]	0.50 [-0.09, 1.10]
***APOE*ε4 Non-Carrier**							
Time	2.41 [1.71, 3.12]***	3.92 [3.02, 4.84]***	3.12 [2.44, -0.64]	-0.09 [-1.23, 1.02]	0.67 [-0.27, 1.62]	1.83 [0.96, 2.63]***	0.69 [-0.72, 2.12]
Time^2	-0.37 [-0.53, -0.21]***	-0.55 [-0.76, -0.34]***	-0.48 [-0.64, -0.32]	-0.18 [-0.43, 0.07]	-0.08 [-0.30, 0.13]	-0.18 [-0.37, 0.01]†	-0.09 [-0.46, 0.29]

*p<0.05, **p≤.01, ***p≤.001. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, T2 diabetes, smoking and alcohol. Abbreviations: *APOEε4*, Apolipoprotein epsilon 4; CI, Confidence Interval. *APOEε4* carrier = reference; Male = reference; Time treated as a continuous variable.

Table C.2 Time and *APOE* main effects stratified by males versus females and those ≥ 70 versus those < 70 years of age

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
<70 years old							
APOE	2.52 [0.12, 4.92]*	1.13 [-1.21, 3.47]	1.76 [-0.23, 3.74]	0.61 [-2.00, 3.22]	2.11 [-0.08, 4.3]*	2.87 [-0.09, 5.83]*	1.53 [-1.73, 4.75]
Time	2.84 [2.40, 3.51]***	4.32 [3.31, 5.34]***	3.4 [2.62, 4.11]***	0.2 [-1.01, 1.43]	0.40 [-0.52, 1.44]	2.83 [1.92, 3.65]***	0.83 [-0.61, 2.23]
Time^2	-0.4 [-0.6, -0.2]***	-0.60 [-0.82, -0.35]***	-0.52 [-0.71, -0.36]***	-0.2 [-0.52, 0.00]	0.00 [-0.22, 0.21]	-0.30 [-0.52, -0.11]***	0.00 [-0.33, 0.41]
≥70 years old							
APOE	3.63 [0.27, 6.99]*	3.51 [-0.01, 7.01]*	3.15 [0.00, 6.30]*	2.05 [-1.64, 5.74]	1.71 [-1.26, 4.67]	2.18 [-1.87, 6.23]	-0.19 [-5.27, 4.89]
Time	1.52 [0.57, 2.48]**	3.20 [2.00, 4.54]***	3.22 [2.11, 4.24]***	-0.71 [-2.34, 0.82]	0.31 [-1.00, 1.64]	0.88 [-0.37, 1.92]	-1.41 [-3.6,3 0.81]
Time^2	-0.31 [-0.52, -0.10]**	-0.63 [-0.91, -0.34]***	-0.62 [-0.82, -0.34]***	0.00 [-0.41, 0.35]	-0.16 [-0.43, 0.25]	-0.09 [-0.35, 0.16]	0.31 [-0.32, 0.93]
Female							
APOE	4.03 [1.33, 6.74]*	2.21 [-0.34, 4.73]	3.46 [1.31, 5.61]*	2.24 [-0.34, 4.73]	3.16 [0.7, 5.62]*	2.41 [-0.96, 5.79]	2.74 [-1.01, 6.49]
Time	2.20 [1.4, 3.1]***	4.23 [3.2,0 5.21]***	3.22 [2.41, 3.90]***	0.36 [-0.94, 1.70]	-0.83 [-1.91, 0.27]	1.94 [0.91, 2.81]***	-0.11 [-1.72, 1.44]
Time^2	-0.28 [-.47, -.10]**	-0.59 [-0.81, -0.36]***	-0.44 [-0.62, -0.27]***	-0.18 [-0.48, 0.11]	0.16 [-0.10, 4.01]	-0.14 [-0.36, 0.07]	0.07 [-0.35, 0.48]
Male							
APOE	2.18 [-0.69, 5.06]	2.30 [-0.78, 5.38]	1.29 [-1.45, 4.03]	2.32 [-0.78, 5.38]	0.92 [-1.54, 3.39]	3.18 [-0.16, 6.51]	-0.27 [-4.32, 3.79]
Time	2.50 [1.70, 3.40]***	3.62 [2.42, 4.85]***	3.54 [2.61, 4.57]***	-0.69 [-2.00, 0.65]	2.01 [0.87, 3.11]***	2.43 [1.43, 3.41]**	0.21 [-1.63, 2.01]
Time^2	-0.47 [-0.67, -0.28]***	-0.59 [-0.86, -.031]***	-0.65 [-.87, -.43]***	-0.13 [-0.44, 0.18]	-0.29 [-0.55, -0.04]*	-0.34 [-.057, -0.10]***	0.19 [-3.21, 5.21]

*$p\leq0.05$, **$p\leq.01$, ***$p\leq.001$. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, T2 diabetes, smoking and alcohol. Abbreviations: *APOEε4*, Apolipoprotein epsilon 4; CI, Confidence Interval. *APOEε4* carrier = reference; Male = reference; Time treated as a continuous variable.

Table C.3 Mixed-effects models evaluating the association between late-life physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 1							
Physical activity tertiles							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-0.81 [-2.70, 1.09]	-0.38 [-2.27, 1.50]	-0.86 [-2.48, 0.76]	-0.53 [-2.52, 1.46]	0.19 [-1.47, 1.85]	-0.95 [-3.23, 1.33]	0.72 [-1.88, 3.33]
High PA	-0.42 [-2.33, 1.49]	-0.23 [-2.12, 1.67]	-0.97 [-2.60, 0.67]	-2.02 [-4.03, -0.02]*	0.99 [-0.69, 2.67]	1.19 [-1.11, 3.49]	-1.66 [-4.32, 1.00]
Time	2.53 [1.98, 3.08]***	4.00 [3.28, 4.72]***	3.41 [2.85, 3.97]***	0.18 [-0.68, 1.04]	0.48 [-0.25, 1.21]	2.22 [1.56, 2.87]***	0.36 [-0.73, 1.46]
Time^2	-0.39 [-0.52, -0.27]***	-0.61 [-0.78, -0.45]***	-0.55 [-0.68, -0.42]***	-0.20 [-0.40, -0.00]*	-0.04 [-0.20, 0.13]	-0.24 [-0.39, -0.09]**	0.09 [-0.20, 0.38]
Mod PA x time	0.47 [-0.88, 1.81]	0.79 [-0.96, 2.54]	0.93 [-0.44, 2.29]	-0.13 [-2.24, 1.97]	0.45 [-1.33, 2.22]	0.09 [-1.50, 1.69]	0.25 [-2.45, 2.94]
Mod PA x time^2	-0.11 [-0.41, 0.19]	-0.13 [-0.53, 0.27]	-0.27 [-0.58, 0.04]	0.06 [-0.42, 0.54]	-0.07 [-0.47, 0.33]	-0.03 [-0.39, 0.33]	-0.08 [-0.79, 0.63]
High PA x time	0.73 [-0.64, 2.09]	0.30 [-1.47, 2.08]	0.49 [-0.89, 1.88]	0.25 [-1.87, 2.38]	0.74 [-1.06, 2.54]	0.29 [-1.33, 1.91]	0.38 [-2.35, 3.11]
High PA x time^2	-0.16 [-0.47, 0.14]	-0.09 [-0.49, 0.31]	-0.17 [-0.48, 0.14]	0.03 [-0.45, 0.52]	-0.08 [-0.49, 0.33]	-0.11 [-0.48, 0.25]	-0.16 [-0.88, 0.55]
Leisure physical activity							
LLPA [a]	1.37 [-0.41, 3.16]	0.44 [-1.33, 2.21]	0.42 [-1.10, 1.95]	-0.15 [-2.03, 1.72]	1.73 [0.17, 3.29]*	2.31 [0.16, 4.45]*	1.29 [-1.13, 3.71]
LLPA x time	-1.50 [-2.76, -0.24]*	-2.83 [-4.47, -1.20]**	-1.50 [-2.78, -0.22]*	-1.05 [-3.02, 0.92]	0.21 [-1.45, 1.88]	0.29 [-1.20, 1.79]	-0.59 [-3.05, 1.88]
LLPA x time^2	0.36 [0.07, 0.65]*	0.68 [0.31, 1.06]***	0.38 [0.09, 0.68]*	0.16 [-0.30, 0.61]	-0.03 [-0.42, 0.36]	-0.02 [-0.37, 0.32]	0.16 [-0.49, 0.81]

†$p \leq .08$, *$p \leq 0.05$, **$p \leq .01$, ***$p \leq .001$. Model 1: Adjusted for time, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes; Model 3: adjusted for model 2 plus smoking and alcohol. [a] Low LLPA=reference. Abbreviations: PA, Physical activity; Mod, Moderate PA; LLPA; late-life leisure PA; ref, Reference variable; CI, Confidence Interval; LLPA, late-life leisure PA

Table C.3 (continued) Mixed-effects models evaluating the association between late-life physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 2							
Physical activity tertiles							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-1.18 [-3.06, 0.70]	-0.66 [-2.57, 1.24]	-0.96 [-2.61, 0.69]	-0.69 [-2.68, 1.30]	-0.01 [-1.69, 1.67]	-1.36 [-3.63, 0.92]	0.71 [-1.92, 3.33]
High PA	-0.59 [-2.49, 1.30]	-0.34 [-2.26, 1.58]	-1.02 [-2.68, 0.64]	-2.21 [-4.22, -0.21]*	0.88 [-0.81, 2.56]	1.23 [-1.06, 3.52]	-1.29 [-3.95, 1.38]
Time	2.54 [1.98, 3.11]***	3.96 [3.23, 4.69]***	3.36 [2.79, 3.93]***	0.10 [-0.78, 0.99]	0.46 [-0.28, 1.20]	2.30 [1.64, 2.96]***	0.16 [-0.95, 1.27]
Time^2	-0.40 [-0.53, -0.27]***	-0.60 [-0.77, -0.44]***	-0.55 [-0.68, -0.42]***	-0.19 [-0.40, 0.01]	-0.03 [-0.20, 0.14]	-0.26 [-0.41, -0.11]**	0.11 [-0.18, 0.41]
Mod PA x time	0.17 [-1.21, 1.55]	0.44 [-1.35, 2.22]	0.91 [-0.49, 2.31]	-0.20 [-2.35, 1.96]	0.07 [-1.74, 1.87]	-0.06 [-1.67, 1.56]	-0.05 [-2.79, 2.69]
Mod PA x time^2	-0.05 [-0.36, 0.27]	-0.07 [-0.47, 0.34]	-0.27 [-0.59, 0.05]	0.11 [-0.38, 0.60]	-0.00 [-0.42, 0.41]	-0.01 [-0.37, 0.36]	-0.04 [-0.76, 0.69]
High PA x time	0.54 [-0.85, 1.92]	0.10 [-1.70, 1.90]	0.48 [-0.94, 1.89]	0.31 [-1.86, 2.48]	0.54 [-1.27, 2.36]	-0.02 [-1.65, 1.61]	0.63 [-2.13, 3.38]
High PA x time^2	-0.12 [-0.43, 0.20]	-0.07 [-0.48, 0.34]	-0.15 [-0.47, 0.17]	0.04 [-0.46, 0.53]	-0.04 [-0.46, 0.37]	-0.04 [-0.41, 0.33]	-0.20 [-0.93, 0.52]
Leisure physical activity							
LLPA [b]	1.09 [-0.69, 2.87]	0.23 [-1.57, 2.02]	0.41 [-1.14, 1.97]	-0.51 [-2.39, 1.36]	1.79 [0.22, 3.36]*	1.87 [-0.29, 4.02]	1.38 [-1.04, 3.80]
LLPA x time	-1.59 [-2.87, -0.32]*	-2.80 [-4.46, -1.15]**	-1.48 [-2.78, -0.18]*	-1.11 [-3.12, 0.89]	0.08 [-1.60, 1.76]	-0.01 [-1.51, 1.49]	-0.24 [-2.73, 2.24]
LLPA x time^2	0.39 [0.09, 0.68]*	0.67 [0.29, 1.06]**	0.40 [0.10, 0.70]*	0.17 [-0.30, 0.63]	-0.01 [-0.40, 0.38]	0.05 [-0.29, 0.40]	0.11 [-0.55, 0.76]

†$p \leq .08$, *$p \leq 0.05$, **$p \leq .01$, ***$p \leq .001$. Model 1: Adjusted for time, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes; Model 3: adjusted for model 2 plus smoking and alcohol. Abbreviations: PA, Physical activity; Mod, Moderate PA; ref, Reference variable; CI, Confidence Interval; LLPA, late-life leisure PA

Table C.3 (continued) Mixed-effects models evaluating the association between late-life physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial** [a]	**Language** [a]	**Attention**	**Executive** [a]
Model 3							
Physical activity tertiles							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-1.42 [-4.61, 1.70]	-0.62 [-2.61, 1.42]	-1.12 [-2.84, 0.64]	-0.68 [-2.82, 1.52]	-0.13 [-1.94, 1.72]	-1.92 [-4.34, 0.51]	1.12 [-1.84, 3.92]
High PA	-0.22 [-2.21, 1.83]	-0.06 [-2.14, 2.01]	-0.90 [-2.63, 0.84]	-1.90 [-4.13, 0.26]	0.98 [-0.83, 2.82]	1.82 [-0.59, 4.33]	-0.66 [-3.53, 2.23]
Time	2.43 [1.82, 3.01]***	4.02 [3.22, 4.63]***	3.32 [2.74, 3.92]***	-0.10 [-1.02, 1.53]	0.44 [-0.34, 1.23]	2.14 [1.43, 2.84]***	0.03 [-1.23, 1.24]
Time^2	-0.37 [-0.50, -0.23]**	-0.59 [-0.77, -0.42]***	-0.53 [-0.67, -0.39]***	-0.16 [-0.37, 0.05]	-0.04 [-0.22, 0.14]	-0.23 [-0.39, -0.07]**	0.12 [-0.19, 0.43]
Mod PA x time	-0.02 [-1.54. 1.43]	0.17 [-1.73, 2.14]	0.93 [-0.56, 2.43]	-	-	0.01 [-2.43, 2.54]	-
Mod PA x time^2	0.02 [-0.31, 0.35]	0.04 [-0.39, 0.47]	-0.27 [-0.61, 0.07]	-	-	-0.00 [-0.39, 0.38]	-
High PA x time	0.31 [-1.83, 2.44]	-0.17 [-2.21, 1.73]	0.77 [-0.74, 2.32]	-	-	-0.36 [-2.84, 2.13]	-
High PA x time^2	-0.04 [-0.37, 0.29]	-0.00 [-0.43, 0.43]	-0.22 [-0.56, 0.12]	-	-	0.03 [-0.36, 0.42]	-
Leisure physical activity							
LLPA [b]	1.27 [-0.60, 3.13]	0.48 [-1.41, 2.37]	0.06 [-1.58, 1.69]	-0.80 [-2.81, 1.22]	2.33 [0.64, 4.01]**	2.28 [-0.00, 4.57] †	1.86 [-0.75, 4.48]
LLPA x time	-1.53 [-2.87, -0.19]*	-2.84 [-4.58, -1.10]**	-1.32 [-2.69, 0.06] †	-1.11 [-3.24, 1.01]	0.22 [-1.56, 2.00]	-0.15 [-1.74, 1.43]	-0.51 [-3.12, 2.10]
LLPA x time^2	0.39 [0.08, 0.70]*	0.69 [0.28, 1.09]**	0.36 [0.04, 0.68]*	0.21 [-0.28, 0.70]	-0.04 [-0.45, 0.37]	0.13 [-0.24, 0.49]	0.17 [-0.52, 0.86]

†p<.08;*p<.05, **p≤.01, ***p≤.001. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, T2 diabetes, smoking and alcohol. Abbreviations: PA, late-life Physical activity; *APOEε4*, Apolipoprotein epsilon 4; Mod; Moderate; CI, Confidence Interval. [a] IGC modelling not conducted due to NSS time and LLPA effect on cognition; [b] Low leisure PA=reference

Table C.4. Mixed-effects models evaluating the association between *APOEε4* carrier status, late-life physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 1							
***APOEε4* Car.**							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	0.34 [-3.79, 4.47]	1.26 [-2.70, 5.22]	0.68 [-3.44, 4.79]	2.26 [-2.26, 6.79]	-0.30 [-4.01, 3.40]	-1.54 [-6.39, 3.31]	3.07 [-2.41, 8.56]
High PA	1.91 [-2.36, 6.19]	1.86 [-2.21, 5.94]	0.11 [-4.14, 4.36]	-1.06 [-5.72, 3.59]	2.95 [-0.86, 6.76]	3.43 [-1.58, 8.44]	0.08 [-5.46, 5.62]
LLPA	0.82 [-3.03, 4.66]	0.23 [-3.45, 3.90]	0.55 [-3.28, 4.37]	1.03 [-3.20, 5.25]	1.41 [-2.04, 4.87]	-0.57 [-5.12, 3.98]	0.02 [-4.91, 4.95]
***APOEε4* Non-car.**							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-1.25 [-3.52, 1.02]	-0.99 [-3.20, 1.22]	-1.38 [-3.18, 0.42]	-1.40 [-3.73, 0.94]	0.61 [-1.32, 2.55]	-1.19 [-3.93, 1.55]	0.13 [-2.93, 3.20]
High PA	-1.07 [-3.37, 1.23]	-1.31 [-3.55, 0.93]	-0.88 [-2.70, 0.94]	-2.18 [-4.54, 0.19]	0.57 [-1.39, 2.53]	0.39 [-2.38, 3.16]	-1.58 [-4.73, 1.56]
LLPA	1.17 [-0.96, 3.30]	0.14 [-1.94, 2.21]	0.08 [-1.62, 1.77]	-0.38 [-2.57, 1.81]	1.20 [-0.61, 3.01]	2.94 [0.38, 5.51]*	1.66 [-1.18, 4.50]
Model 2							
***APOEε4* Car.**							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-0.63 [-4.70, 3.44]	0.23 [-3.73, 4.19]	0.22 [-3.90, 4.34]	1.72 [-2.81, 6.24]	-0.49 [-4.13, 3.16]	-2.58 [-7.41, 2.25]	2.63 [-2.95, 8.22]
High PA	1.58 [-2.60, 5.77]	1.52 [-2.54, 5.58]	-0.11 [-4.34, 4.13]	-0.95 [-5.57, 3.68]	2.59 [-1.15, 6.32]	3.55 [-1.42, 8.52]	0.12 [-5.48, 5.71]
LLPA	0.47 [-3.29, 4.23]	0.05 [-3.60, 3.70]	0.68 [-3.11, 4.48]	0.24 [-3.94, 4.42]	1.72 [-1.65, 5.09]	-1.28 [-5.81, 3.24]	-0.27 [-5.28, 4.74]
***APOEε4* Non-car.**							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-1.78 [-4.03, 0.47]	-1.34 [-3.58, 0.90]	-1.62 [-3.45, 0.20]	-1.69 [-4.02, 0.64]	0.26 [-1.70, 2.22]	-1.60 [-4.33, 1.13]	0.02 [-3.04, 3.09]
High PA	-1.27 [-3.54, 1.01]	-1.43 [-3.70, 0.84]	-0.95 [-2.80, 0.89]	-2.49 [-4.85, 0.13]	0.43 [-1.56, 2.41]	0.50 [-2.26, 3.26]	-1.23 [-4.37, 1.91]
LLPA	1.08 [-1.04, 3.20]	0.12 [-1.99, 2.23]	0.17 [-1.56, 1.89]	-0.56 [-2.76, 1.64]	1.31 [-0.53, 3.15]	2.71 [0.13, 5.28]*	1.87 [-0.97, 4.71]

†$p \leq .08$, *$p \leq 0.05$, **$p \leq .01$, ***$p \leq .001$.. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes; Model 3: adjusted for model 2 plus smoking and alcohol. Abbreviations: PA, Physical activity; *APOEε4*, Apolipoprotein epsilon 4; CI, Confidence Interval, LLPA, late-life leisure PA.

Table C.4. (Continued) Mixed-effects models evaluating the association between *APOEε4* carrier status, late-life physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 3							
***APOE*ε4 Carriers**							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-1.14 [-5.42, 3.11]	-0.23 [-4.41, 3.90]	-0.20 [-4.42, 3.93]	1.92 [-2.93, 6.70]	0.17 [-4.32, 4.64]	-3.62 [-8.62, 1.44]	4.10 [-1.93, 10.12]
High PA	1.81 [-2.73, 6.24]	1.74 [-2.62, 5.93]	0.17 [-4.13, 4.52]	-1.43 [-6.44, 3.62]	4.40 [-0.31, 9.01]†	4.13 [-1.10, 9.32]	0.87 [-5.13, 6.90]
LLPA	-0.51 [-4.64, 3.62]	-1.20 [-5.18, 2.79]	-0.92 [-4.90, 3.06]	-0.64 [-5.31, 4.04]	1.51 [-2.31, 5.34]	-1.05 [-6.01, 3.90]	0.66 [-4.95, 6.27]
***APOEε4* Non-car.**							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-1.90 [-4.21, 0.53]	-0.79 [-3.23, 1.62]	-1.62 [-3.51, 0.41]	-2.01 [-4.52, 0.50]	0.30 [-1.80, 2.41]	-2.13 [-5.01, 0.84]	-0.08 [-3.43, 3.30]
High PA	-0.80 [-3.24, 1.62]	-0.90 [-3.32, 1.50]	-0.87 [-2.91, 1.12]	-2.20 [-4.73, 0.33]	0.55 [-1.65, 2.73]	1.12 [-1.84, 4.01]	-0.72 [-4.13, 2.71]
LLPA	1.71 [-0.51, 3.94]	0.83 [-1.40, 3.07]	0.32 [-1.52, 2.16]	-0.32 [-2.65, 2.00]	2.00 [0.04, 3.96]*	3.06 [0.35, 5.78]*	2.25 [-0.80, 5.30]
Main effect interactions [a]							
APOE x Mod PA	0.96 [-3.84, 5.72]	1.01 [-3.70, 5.72]	1.12 [-3.14, 5.22]	4.36 [-0.75, 9.47]	-1.53 [-5.8, 2.8]	-0.52 [-6.32, 5.23]	3.11 [-3.70, 9.92]
APOE x High PA	2.89 [-2.02, 7.82]	3.23 [-1.64, 8.01]	0.86 [-3.43, 5.14]	0.89 [-4.32, 6.10]	2.01 [-2.44, 6.33]	3.81 [-2.02, 9.73]	0.53 [-6.34, 7.39]
APOE x LLPA	-0.79 [-5.26, 3.67]	-0.48 [-4.92, 3.95]	0.17 [-3.69, 4.04]	0.39 [-4.40, 5.18]	0.46 [-3.52, 4.44]	-3.22 [-8.63, 2.18]	-0.05 [-6.22, 6.13]

†$p \leq .08$, *$p < 0.05$, **$p \leq .01$, ***$p \leq .001$. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, T2 diabetes, smoking and alcohol. Abbreviations: PA, Physical activity; LLPA, late-life PA; *APOEε4*, Apolipoprotein epsilon 4; Mod; Moderate; ref, reference variable; CI, Confidence Interval. [a] Low PA ε4 carrier = reference group

Table C.5 Mixed-effects growth models evaluating the association between late-life physical activity and multi-domain cognitive trajectories among *APOEε4* carriers and non-carriers

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial [b]**	**Language [c]**	**Attention**	**Executive [b]**
Time interaction models [a]							
APOE x Mod PA x time	1.1 [-2.2, 4.5]	-2.7 [-7.0, 1.7]	2.4 [-1.0, 5.8]	-	-2.5 [-7.0, 2.0]	3.3 [-.69, 7.2]	-
APOE x Mod PA x time^2	-.23 [.97, .51]	.40 [-.56, 1.4]	-.43 [-1.2, .32]	-	.62 [-.37, 1.6]	-.74 [-1.6, .13]	-
APOE x High PA x time	-.31 [-3.7, 3.1]	-1.5 [-5.9, 2.9]	1.6 [-1.9, 5.1]	-	-5.4 [-9.9, -.86]	.58 [-3.4, 4.6]	-
APOE x High PA x time^2	-.04 [-.80, .71]	.13 [-.86, 1.1]	-.30 [-1.1, .48]	-	1.1 [.09, 2.1]	-.37 [-1.3, .52]	-
APOE x LLPA x time	1.63 [-1.58, 4.85]	-1.16 [-5.34, 3.01]	-3.83 [-7.12, -0.54]*	4.98 [0.44, 9.53]*	1.32 [-2.98, 5.62]	1.79 [-2.02, 5.59]	2.01 [-8.09, 4.07]
APOE x LLPA x time^2	-0.17 [-0.92, 0.58]	0.30 [-0.67, 1.28]	1.00 [0.23, 1.76]*	-0.88 [-2.01, 0.04]	-0.34 [-1.35, 0.67]	-0.35 [-1.24, 0.54]	0.71 [-0.87, 2.29]
***APOEε4* carrier**							
Mod PA x time	1.02 [-1.83, 3.87]	-1.51 [-5.25, 2.23]	2.8 [-.23, 5.8]†	-	-	2.36 [-1.03, 5.75]	-
Mod PA x time^2	-0.2 [-0.82, 0.42]	0.28 [-0.54, 1.1]	-.61 [-1.3, .04]†	-	-	-0.52 [-1.26, 0.21]	-
High PA x time	0.23 [-2.67, 3.13]	-0.89 [-4.7, 2.92]	2.1 [-1.0, 5.1]	-	-	0.33 [-3.12, 3.78]	-
High PA x time^2	-0.11 [-0.76, 0.53]	0.01 [-0.84, 0.86]	-.48 [-1.2, .20]	-	-	-0.25 [-1.02, 0.51]	-
LLPA x time	-0.21 [-3.00, 2.59]	-3.61 [-7.28, 0.06]†	-3.89 [-6.82, -0.95]*	4.02 [-0.51, 8.54]	1.22 [-2.42, 4.85]	1.21 [-2.14, 4.56]	-2.21 [-7.25, 2.82]
LLPA x time^2	0.25 [-0.40, 0.90]	0.90 [0.03, 1.76]*	1.05 [0.37, 1.74]**	-0.68 [-1.75, 0.38]	-0.29 [-1.15, 0.56]	-0.14 [-0.92, 0.65]	0.73 [-0.57, 2.02]
***APOEε4* Non-car.**							
Mod PA x time	-0.18 [-1.9, 1.55]	0.98 [-1.25, 3.22]	0.39 [-1.35, 2.13]	-	-	-0.94 [-2.97, 1.09]	-
Mod PA x time^2	0.04 [-0.35, 0.44]	-0.1 [-0.61, 0.42]	-0.18 [-0.58, 0.22]	-	-	0.22 [-0.24, 0.69]	-
High PA x time	0.33 [-1.41, 2.06]	0.26 [-1.98, 2.5]	0.28 [-1.47, 2.03]	-	-	-0.36 [-2.4, 1.68]	-
High PA x time^2	-0.04 [-0.43, 0.36]	-0.07 [-0.58, 0.44]	-0.15 [-0.55, 0.25]	-	-	0.13 [-0.33, 0.6]	-
LLPA x time	-1.95 [-3.50, -0.40]*	-2.58 [-4.59, -0.57]*	-0.24 [-1.81, 1.33]	-2.70 [-5.14, -0.26]*	-0.31 [-2.41, 1.80]	-0.63 [-2.46, 1.20]	-0.16 [-3.27, 2.94]
LLPA x time^2	0.44 [0.09, 0.80]*	0.62 [0.15, 1.08]**	0.09 [-0.27, 0.45]	0.49 [-0.07, 1.05]	0.09 [-0.40, 0.57]	0.22 [-0.20, 0.64]	0.03 [-0.79, 0.86]

†$p \leq .08$, *$p \leq 0.05$, **$p \leq .01$, ***$p \leq .001$. Adjusted for time, time^2 age, education, sex, BMI, hypertension, hypercholesterolemia, T2 diabetes, smoking and alcohol.

Abbreviations: PA, Physical activity; *APOEε4*, Apolipoprotein epsilon 4; Mod; Moderate; ref, reference variable; CI, Confidence Interval, LLPA, late-life leisure PA.

[a] Low PA ε4 carrier = reference group. [b] GCM not conducted due to NSS main effects of time in both carriers and non-carriers. [c] GCM not conducted due to NSS effect of time within non-carrier sub-group.

Table C.6. Mixed-effects models evaluating the association between sex, late-life physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Main effect interactions [a]							
Sex x Mod PA	-2.5 [-6.4, 1.5]	-3.8 [-8.8, .23]	.21 [-3.3, 3.7]	-1.3 [-5.6, 3.0]	.21 [-3.4, 3.8]	-3.0 [-7.8, 1.8]	-1.9 [-7.6, 3.8]
Sex x High PA	-1.8 [-5.8, 2.1]	-2.4 [-6.4, 1.7]†	-.11 [-3.6, 3.4]	-1.5 [-5.8, 2.8]	1.4 [-2.2, 5.0]	-2.9 [-7.7, 1.9]	-3.6 [-9.4, 2.1]
Sex x LLPA	-0.90 [-4.55, 2.74]	-1.31 [-5.01, 2.38]	-0.17 [-3.36, 3.01]	-1.52 [-5.46, 2.41]	0.76 [-2.53, 4.06]	-0.73 [-5.20, 3.73]	2.07 [-3.07, 7.21]
Time interactions [b]							
Sex x Mod PA x time	-1.2 [-.2.2, 1.1]	-.35 [-1.7, 1.0]	-.18 [-1.2, .90]	-2.5 [-4.2, 0.3]	-.48 [-1.9, .89]	-.63 [-1.9, .61]	-.18 [-2.2, 1.8]
Sex x mod PA x time^2	. -.42 [-1.1, .24]	-.55 [-1.4, .31]	.08 [-.60, .76]	.62 [-.43, 1.7]	-.63 [-1.5, .25]	-.58 [-1.4, .20]	-1.0 [-2.6, .53]
Sex x High PA x time	-.86 [-1.9, .18]	-.67 [-2.0, .67]	-.21 [-1.3, .885]	-1.9 [-3.6, 0.8]	.01 [-1.4, 1.4]	-.60 [-1.8, .64]	.24 [-1.7, 2.2]
Sex x High PA x time^2	-.31 [-.98, .35]	-.53 [-1.4, .34]	-.24 [-.93, .45]	.59 [-.48, 1.6]	-.29 [-1.2, .60]	-.57 [-1.4, .22]	.17 [-1.4, 1.7]
Sex x LLPA x time	-0.59 [-3.27, 2.09]	-1.70 [-5.18, 1.78]	-1.48 [-4.24, 1.27]	-0.25 [-4.51, 4.00]	0.70 [-2.86, 4.26]	2.05 [-1.13, 5.23]	-1.57 [-6.81, 3.67]
Sex x LLPA x time^2	0.17 [-0.45, 0.79]	-.18 [-0.62, -0.99]	0.26 [-0.38, 0.90]	0.18 [-0.81, 1.17]	-0.01 [-0.84, 0.82]	-0.41 [-1.15, 0.32]	0.22 [-1.16, 1.60]

†$p \leq .08$; *$p \leq 0.05$, **$p \leq .01$, ***$p \leq .001$. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol consumption. Abbreviations: CI, Confidence Interval; PA, late-life physical activity, LLPA, late-life leisure PA. [a] Low PA group as reference; [b] Linear time interactions are reported without time^2 interactions in model

Table C.7a. Mixed-effects growth models evaluating the association between late-life physical activity, *APOE4* carrier status and multi-domain cognitive trajectories among females

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Females							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-2.51 [-5.22, 0.23]	-2.5 [-5.0, .12]	-.97 [-3.2, 1.2]	-1.1 [-4.1, 1.9]	.18 [-2.4, 2.7]	-3.3 [-6.7, .12]	.48 [-3.4, 4.3]
High PA	-1.2 [-4.0, 1.54]	-1.4 [-4.0, 1.2]	-.96 [-3.2, 1.3]	-2.5 [-5.6, .53	1.5 [-1.1, 4.0]	.22 [-3.2, 3.7]	-2.0 [-6.0, 1.9]
LLPA	0.73 [-1.93, 3.40]	-0.27 [-2.77, 2.23]	-0.05 [-2.18, 2.08]	-1.57 [-4.50, 1.37]	2.36 [-0.08, 4.80]†	2.16 [-1.17, 5.48]	2.96 [-0.71, 6.64]
Growth curve models [a, b]							
Mod PA x time	.59 [-.14, 1.3]	.51 [.26, -.38]	-.12 [-.81, .57]	1.4 [.20, 2.5]*	.42 [-.56, 1.4]	.29 [-.56, 1.1]	.25 [-1.1, 1.6]
Mod PA x time^2	.19 [.41, -.25]	.28 [-.27, .83]	-.31 [-.75, .12]	-.20 [-.93, .53]	.35 [-.27, .97]	.25 [-.28, .78]	.40 [-.64, 1.4]
High PA x time	.50 [-.24, 1.2]	.11 [.81, -.77]	-.08 [-.77, .62]	1.5 [.32, 2.6]*	.43 [-.55, 1.4]	.12 [-.74, .97]	.20 [-1.1, 1.5]
High PA x time^2	.07 [-.38, .53]	.22 [-.34, .77]	-.15 [-.58, .28]	-.14 [-.87, .58]	.13 [-.49, .75]	.26 [-.26, .79]	-.47 [-1.5, .56]
LLPA x time	-1.86 [-3.80, 0.07] †	-3.63 [-5.96, -1.30]**	-2.12 [-3.94, -0.29]*	-1.13 [-4.20, 1.94]	0.31 [-2.29, 2.91]	0.75 [-1.49, 3.00]	-1.28 [-4.93, 2.37]
LLPA x time^2	0.50 [0.05, 0.94]*	0.78 [0.24, 1.32]**	0.51 [0.09, 0.94]*	0.29 [-0.42, 1.01]	-0.01 [-0.61, 0.60]	-0.05 [-0.56, 0.47]	0.26 [-0.72, 1.23]
***APOE* interaction [c]**							
APOE x Mod PA	0.37 [-6.67, 7.42]	-	2.2 [-3.39, 7.79]	-	-1.69 [-8.09, 4.71]	-	-
APOE x High PA	2.9 [-4.17, 9.97]	-	1.98 [-3.62, 7.58]	-	3.5 [-2.91, 9.9]	-	-
APOE x LLPA	1.88 [-4.50, 8.26]	2.65 [-3.33, 8.62]	1.54 [-3.51, 6.59]	1.27 [-5.69, 8.22]	-0.56 [-6.34, 5.23]	1.81 [-6.14, 9.76]	-0.43 [-9.06, 8.21]

†$p \leq .08$;*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol consumption. Abbreviations: CI, Confidence Interval; PA, Physical activity (late-life), LLPA, late-life leisure PA. [a] Low PA group as reference; [b] Linear time interactions are reported without time^2 interactions in model; [c] *APOEε*4 carrier group as reference.

Table C.7b. Mixed-effects growth models evaluating the association between late-life physical activity, *APOE4* carrier status and multi-domain cognitive trajectories among males

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Males							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	0.01 [-0.29, 2.90]	1.65 [-1.63, 4.85]	-1.46 [-4.23, 1.45]	0.21 [-2.86, 3.34]	-0.13 [-2.7,4 2.42]	-0.21 [-3.62, 3.25]	2.25 [-2.14, 6.47]
High PA	0.66 [-2.26, 3.45]	1.33 [-1.91, 4.55]	-0.83 [-3.63, 1.96]	-1.46 [-4.34, 1.65]	0.30 [-2.27, 2.84]	3.24 [-0.20, 6.57]t	0.98 [-3.24, 5.26]
LLPA	1.96 [-0.66, 4.57]	1.38 [-1.52, 4.29]	0.16 [-2.37, 2.69]	0.07 [-2.69, 2.82]	2.29 [0.01, 4.58]*	2.72 [-0.37, 5.82]	0.98 [-2.74, 4.71]
Growth curve models [a, b]							
Mod PA x time	-0.62 [-1.32, 0.12]	0.18 [0.74, -0.86]	-0.34, [-1.27, 0.49]	-1.16 [-2.34, 0.00]	-0.14 [0.77, -1.16]	-0.35 [-1.25, 0.54]	0.13 [-1.36, 1.62]
Mod PA x time^2	-0.23 [-0.68, 0.24]	-0.27 [-0.93, 0.39]	-0.23 [-0.76, 0.29]	0.41 [-0.33, 1.17]	-0.27 [-0.88, 0.34]	-0.33 [0.25, -0.90]	-0.63 [0.29, -1.87]
High PA x time	-0.47 [-1.32, 0.26]	-0.54 [0.30, -1.63]	-0.33 [-1.23, 0.50]	-0.43 [-1.64, 0.70]	0.30 [0.52, -0.63]	-0.50 [-1.46, 0.38]	0.45 [-0.97, 1.95]
High PA x time^2	-0.25 [-0.72, 0.23]	-0.33 [-1.01, 0.36]	-0.39 [-0.94, 0.15]	0.44 [-0.32, 1.27]	-0.15 [-0.78, 0.47]	-0.32 [0.29, -0.90]	-0.32 [0.58, -1.56]
LLPA x time	-1.23 [-3.06, 0.60]	-1.91 [-4.52, 0.71]	-0.57 [-2.67, 1.52]	-0.87 [-3.78, 2.04]	-0.34 [-2.73, 2.06]	-1.26 [-3.49, 0.98]	0.23 [-3.54, 3.99]
LLPA x time^2	0.32 [-0.10, 0.75]	0.60 [-0.01, 1.20] ✣	0.25 [-0.24, 0.73]	0.11 [-0.56, 0.79]	-0.00 [-0.56, 0.55]	0.36 [-0.15, 0.88]	0.05 [-0.93, 1.04]
***APOE* interaction [c]**							
APOE x Mod PA	-	-	-	-	-	-	-
APOE x High PA	-	-	-	-	-	-	-
APOE x LLPA	-3.23 [-9.52, 3.07]	-3.19 [-9.95, 3.56]	-1.45 [-7.47, 4.58]	-0.61 [-7.27, 6.05]	1.66 [-3.75, 7.07]	-7.05 [-14.32, 0.22]	0.14 [-8.85, 9.12]

†p≤.08, *p≤0.05, **p≤0.01, ***p≤0.001. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol consumption. Abbreviations: CI, Confidence Interval; PA, Physical activity (late-life), LLPA, late-life leisure PA. [a] Low PA group as reference; [b] Linear time interactions are reported without time^2 interactions in model; [c] *APOEε*4 carrier group as reference.

Table C.8a Mixed-effects growth models evaluating the association between late-life physical activity, *APOE* carrier status and multi-domain cognitive trajectories among those <70 years of age

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
<70 years old							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-0.5 [-3.22, 2.01]	0.3 [-2.2, 2.8]	-0.5 [-2.6, 1.6]	0.6 [-2.1, 3.4]	-1.1 [-3.4, 1.3]	-0.4 [-3.5, 2.8]	0.8 [-2.7, 4.3]
High PA	-0.3 [-2.75, 2.23]	-0.6 [-3, 1.8]	-1.1 [-3.2, 0.9]	-1.4 [-4.1, 1.2]	0.6 [-1.6, 2.9]	2.3 [-0.7, 5.4]	0.2 [-3.2, 3.6]
LLPA	1.19 [-1.02, 3.40]	0.26 [-1.95, 2.46]	0.23 [-1.61, 2.08]	-0.81 [-3.25, 1.63]	2.36 [0.32, 4.40]*	1.95 [-0.82, 4.71]	2.23 [-0.80, 5.26]
Growth curve models [a]							
Mod PA x time	0.25 [-0.41, 0.91]	0.45 [-0.4, 1.29]	0.69 [-0.46, 1.84]	-	-	0.34 [-0.43, 1.11]	-
Mod PA x time^2	0.09 [-0.34, 0.51]	0.1 [-0.45, 0.66]	0.26 [-0.44, 0.95]	-	-	-0.03 [-0.53, 0.47]	-
High PA x time	0.19 [-0.44, 0.82]	-0.11 [-0.92, 0.71]	-0.01 [-1.2, 1.19]	-	-	0.21 [-0.53, 0.95]	-
High PA x time^2	-0.12 [-0.53, 0.28]	-0.26 [-0.79, 0.27]	0.6 [-0.15, 1.34]	-	-	-0.13 [-0.61, 0.35]	-
LLPA x time	-1.41 [-3.04, 0.23]	-2.43 [-4.54, -0.31]*	-1.45 [-3.07, 0.16]†	-0.09 [-2.65, 2.47]	-0.18 [-2.31, 1.96]	-0.49 [-2.40, 1.43]	-1.28 [-4.21, 1.65]
LLPA x time^2	0.35 [-0.03, 0.73] †	0.54 [0.05, 1.03]*	0.35 [-0.02, 0.72] †	-0.04 [-0.63, 0.56]	0.09 [-0.40, 0.59]	0.22 [-0.23, 0.66]	0.33 [-0.45, 1.10]
***APOE* moderation [b]**							
APOE x Mod EPA	-0.87 [-6.86, 5.12]	-	-	-	-2.88 [-8.3, 2.55]	-4.69 [-12.02, 2.64]	-
APOE x High EPA	0.52 [-5.43, 6.46]	-	-	-	1.93 [-3.46, 7.31]	-0.1 [-7.37, 7.17]	-
APOE x LLPA	0.28 [-5.01, 5.58]	-1.42 [-6.58, 3.74]	-0.55 [-4.93, 3.83]	2.60 [-3.15, 8.34]	0.07 [-4.74, 4.88]	0.16 [-6.36, 6.68]	-0.71 [-7.75, 6.33]

†$p \leq .08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol consumption. Abbreviations: CI, Confidence Interval; PA, Physical activity (late-life); LLPA, late-life leisure PA. [a] Low PA group as reference; [b] *APOEε*4 carrier group as reference.

Table C.8b Mixed-effects growth models evaluating the association between late-life physical activity, *APOE* carriers status and multi-domain cognitive trajectories among those 70 + years of age

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
≥70 years old							
Low PA	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)	.00 (ref)
Mod PA	-3.34 [-6.51, -0.17]*	-2.5 [-5.9, 0.9]	-2.4 [-5.4, 0.7]	-3.2 [-6.7, 0.2]	1.4 [-1.4, 4.3]	-4.71 [-8.50, -0.93]*	2.1 [-2.7, 7]
High PA	-0.40 [-3.79, 2.99]	1 [-2.6, 4.6]	-0.5 [-3.8, 2.7]	-3.1 [-6.8, 0.6]	1.3 [-1.8, 4.3]	0.67 [-3.38, 4.72]	-1.4 [-6.6, 3.8]
LLPA	1.23 [-2.15, 4.61]	1.15 [-2.43, 4.73]	-0.43 [-3.64, 2.78]	-1.18 [-4.83, 2.48]	1.78 [-1.21, 4.76]	3.11 [-0.94, 7.17]	0.65 [-4.33, 5.64]
Growth curve models [a]							
Mod PA x time	0.01 [-0.87, 0.89]	0.69 [-0.46, 1.84]	-0.10 [-0.72, 0.53]	-	-	-0.36 [-1.41, 0.69]	-
Mod PA x time^2	0.05 [-0.48, 0.58]	0.26 [-0.44, 0.95]	-0.35 [-0.94, 0.24]	-	-	0.37 [-0.30, 1.05]	-
High PA x time	-0.05 [-0.96, 0.87]	-0.01 [-1.2, 1.19]	-0.2 [-1.21, 0.82]	-	-	-0.79 [-1.88, 0.30]	-
High PA x time^2	0.16 [-0.4, 0.72]	0.6 [-0.15, 1.34]	-0.22 [-0.85, 0.40]	-	-	0.16 [-0.48, 0.79]	-
LLPA x time	-2.15 [-4.50, 0.20]†	-3.91 [-6.99, -0.84]*	-1.06 [-3.67, 1.56]	-3.90 [-7.73, -0.07]*	1.08 [-2.17, 4.34]	0.03 [-2.79, 2.85]	0.12 [-5.23, 5.46]
LLPA x time^2	0.50 [-0.04, 1.05]†	0.98 [0.26, 1.69]**	0.38 [-0.23, 0.98]	0.92 [0.02, 1.81]*	-0.39 [-1.15, 0.37]	-0.02 [-0.68, 0.63]	-0.01 [-1.44, 1.41]
***APOE* moderation** [b]							
APOE x Mod EPA	4.97 [-2.72, 12.65]	7.62 [-0.46, 15.69]	2.37 [-4.91, 9.65]	-	-	-	-
APOE x High EPA	6.61 [-1.69, 14.9]	9.98 [1.29, 18.66]*	4.25 [-3.59, 12.08]	-	-	-	-
APOE x LLPA	-2.49 [10.76, 5.79]	1.21 [-7.45, 9.86]	2.32 [-5.53, 10.00]	-4.84 [-13.92, 4.24]	1.75 [-5.56, 9.07]	-9.14 [19.03, 0.75]	1.62 [-10.73, 13.97]

†p≤.08, *p≤0.05, **p≤0.01, ***p≤0.001. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol consumption. Abbreviations: CI, Confidence Interval; PA, Physical activity (late-life), LLPA, late-life leisure PA. [a] Low PA group as reference; [b] *APOEε*4 carrier group as reference.

Appendix D. Chapter 5: Midlife Physical Activity and Cognition

Table D.1 Mixed-effects models evaluating the association between midlife physical activity and longitudinal cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 1							
MLPA[a]	2.83 [1.21, 4.45]**	1.70 [0.06, 3.33]*	1.65 [0.25, 3.05]*	2.23 [0.51, 3.94]*	1.77 [0.33, 3.21]*	2.47 [0.51, 4.44]*	2.83 [0.56, 5.09]*
MLPA x time	-0.90 [-2.08, 0.27]	-2.92 [-4.44, -1.40]***	-1.29 [-2.49, -0.10]*	-0.53 [-2.36, 1.29]	0.24 [-1.31, 1.80]	0.81 [-0.58, 2.21]	0.27 [-2.04, 2.57]
MLPA x time^2	0.25 [-0.02, 0.52] †	0.74 [0.39, 1.09]***	0.28 [0.01, 0.56]*	0.05 [-0.38, 0.47]	-0.02 [-0.38, 0.34]	-0.13 [-0.45, 0.19]	-0.13 [-0.74, 0.48]
Model 2							
MLPA	2.42 [0.80, 4.04]**	1.52 [-0.14, 3.17] †	1.67 [0.24, 3.09]*	1.65 [-0.07, 3.37] †	1.67 [0.22, 3.12]*	1.97 [-0.00, 3.94] †	2.63 [0.36, 4.90]*
MLPA x time	-0.88 [-2.07, 0.32]	-2.89 [-4.42, -1.35]***	-1.29 [-2.51, -0.07]*	-0.43 [-2.29, 1.43]	0.38 [-1.19, 1.94]	0.76 [-0.64, 2.17]	0.48 [-1.84, 2.80]
MLPA x time^2	0.25 [-0.03, 0.53] †	0.75 [0.39, 1.10]***	0.28 [-0.00, 0.56] †	0.04 [-0.39, 0.48]	-0.05 [-0.42, 0.31]	-0.13 [-0.45, 0.19]	-0.20 [-0.81, 0.41]
Model 3							
MLPA	2.67 [0.98, 4.36]**	1.65 [-0.09, 3.39] †	1.58 [0.09, 3.06]*	1.83 [-0.01, 3.66] †	1.82 [0.27, 3.37]*	2.52 [0.44, 4.60]*	3.00 [0.58, 5.41]*
MLPA x time	-0.68 [-1.93, 0.57]	-2.90 [-4.51, -1.28]***	-0.98 [-2.26, 0.31]	-0.24 [-2.21, 1.73]	0.70 [-0.96, 2.36]	0.79 [-0.69, 2.26]	0.40 [-2.04, 2.85]
MLPA x time^2	0.20 [-0.09, 0.49]	0.75 [0.38, 1.12]***	0.21 [-0.08, 0.51]	-0.01 [-0.46, 0.45]	-0.11 [-0.50, 0.27]	-0.15 [-0.49, 0.19]	-0.08 [-0.73, 0.56]

†$p \leq .08$, *$p \leq 0.05$, **$p \leq 01$, ***$p < .001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes; Model 3: adjusted for model 2 plus smoking and alcohol. Abbreviations: MLPA, Midlife physical activity; CI, Confidence Interval. [a] Low MLPA = comparator

Table D.2. Mixed-effects models evaluating the association between *APOEε4* carrier status, midlife physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 1							
APOEε4 Car.							
MLPA	3.55 [1.60, 5.49]***	1.98 [0.05, 3.90]*	1.77 [0.21, 3.33]*	2.90 [0.89, 4.91]**	2.17 [0.50, 3.83]*	3.00 [0.64, 5.36]*	4.14 [1.47, 6.81]**
MLPA x time	-1.35 [-3.78, 1.07]	-4.88 [-8.09, -1.67]**	-0.81 [-3.34, 1.72]	-0.03 [-4.01, 3.94]	-1.19 [-4.31, 1.93]	1.14 [-1.78, 4.05]	-1.65 [-6.08, 2.78]
MLPA x time^2	0.43 [-0.14, 0.99]	1.24 [0.49, 1.99]**	0.15 [-0.44, 0.74]	-0.05 [-0.98, 0.88]	0.35 [-0.38, 1.08]	-0.11 [-0.79, 0.56]	0.54 [-0.62, 1.69]
***APOEε4* Non-Car.**							
MLPA	2.23 [-1.36, 5.82]	1.35 [-2.12, 4.82]	2.11 [-1.48, 5.70]	2.94 [-1.02, 6.90]	0.77 [-2.52, 4.05]	1.33 [-2.94, 5.60]	0.39 [-4.36, 5.14]
MLPA x time	-0.89 [-2.26, 0.49]	-2.43 [-4.18, -0.68]**	-1.29 [-2.66, 0.08] †	-0.76 [-2.86, 1.33]	0.32 [-1.52, 2.16]	0.56 [-1.05, 2.18]	0.87 [-1.89, 3.63]
MLPA x time^2	0.23 [-0.09, 0.54]	0.60 [0.20, 1.01]**	0.30 [-0.02, 0.62] †	0.09 [-0.40, 0.57]	-0.07 [-0.49, 0.35]	-0.10 [-0.47, 0.27]	-0.34 [-1.07, 0.39]
Model 2							
APOEε4 Car.							
MLPA	3.15 [1.21, 5.08]**	2.06 [0.10, 4.01]*	1.94 [0.36, 3.53]*	2.41 [0.40, 4.43]*	1.81 [0.12, 3.50]*	2.40 [0.04, 4.77]*	4.10 [1.44, 6.76]**
MLPA x time	-1.06 [-3.53, 1.42]	-4.23 [-7.45, -1.00]*	-0.84 [-3.46, 1.79]	-0.42 [-4.51, 3.67]	-0.86 [-4.06, 2.34]	1.65 [-1.29, 4.58]	-1.71 [-6.25, 2.84]
MLPA x time^2	0.36 [-0.22, 0.94]	1.13 [0.37, 1.89]**	0.14 [-0.48, 0.75]	0.06 [-0.91, 1.03]	0.22 [-0.54, 0.97]	-0.25 [-0.93, 0.44]	0.46 [-0.73, 1.65]
***APOEε4* Non-Car.**							
MLPA	1.52 [-2.02, 5.06]	0.38 [-3.12, 3.87]	1.45 [-2.16, 5.07]	2.11 [-1.83, 6.05]	0.99 [-2.25, 4.23]	0.90 [-3.40, 5.20]	-0.09 [-4.95, 4.78]
MLPA x time	-0.93 [-2.33, 0.46]	-2.58 [-4.36, -0.79]**	-1.28 [-2.67, 0.12] †	-0.57 [-2.69, 1.56]	0.43 [-1.43, 2.29]	0.45 [-1.19, 2.09]	1.10 [-1.67, 3.86]
MLPA x time^2	0.24 [-0.08, 0.56]	0.64 [0.23, 1.06]**	0.29 [-0.04, 0.61]	0.06 [-0.43, 0.55]	-0.08 [-0.51, 0.35]	-0.09 [-0.47, 0.28]	-0.39 [-1.12, 0.35]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes; Low MLPA = reference. *APOEε*4 carrier = reference group.

Abbreviations: MLPA, midlife Physical activity; CI, Confidence Interval; *APOEε*4, apolipoprotein epsilon-4; Car, Carrier; Non-car., non-carrier

Table D.2. (Continued) Mixed-effects models evaluating the association between *APOEε4* carrier status, midlife physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 3							
APOEε4 Car.							
MLPA	1.86 [-1.82, 5.53]	0.69 [-2.93, 4.32]	1.52 [-2.08, 5.11]	2.99 [-1.20, 7.17]	1.16 [-2.31, 4.63]	0.85 [-3.59, 5.30]	0.09 [-4.96, 5.15]
MLPA x time	-0.75 [-3.38, 1.88]	-3.68 [-7.11, -0.24]*	-0.21 [-3.02, 2.59]	-0.28 [-4.59, 4.04]	-0.65 [-4.10, 2.80]	1.56 [-1.60, 4.72]	-2.29 [-7.07, 2.50]
MLPA x time^2	0.29 [-0.33, 0.91]	1.07 [0.25, 1.88]*	-0.01 [-0.68, 0.65]	-0.06 [-1.09, 0.97]	0.22 [-0.60, 1.04]	-0.23 [-0.98, 0.52]	0.72 [-0.53, 1.97]
***APOEε4* Non-Car.**							
MLPA	3.54 [1.51, 5.56]**	2.32 [0.25, 4.39]*	2.10 [0.41, 3.79]*	2.53 [0.40, 4.66]*	1.95 [0.14, 3.76]*	3.17 [0.68, 5.67]*	4.58 [1.75, 7.42]**
MLPA x time	-0.83 [-2.28, 0.62]	-2.92 [-4.78, -1.05]**	-1.06 [-2.53, 0.40]	-0.48 [-2.74, 1.78]	0.67 [-1.29, 2.63]	0.57 [-1.13, 2.28]	0.96 [-1.94, 3.86]
MLPA x time^2	0.22 [-0.11, 0.55]	0.70 [0.27, 1.13]**	0.23 [-0.10, 0.57]	0.06 [-0.46, 0.58]	-0.12 [-0.58, 0.33]	-0.13 [-0.52, 0.26]	-0.24 [-1.01, 0.53]
APOE Interactions [a]							
MLPA x *APOE*	-0.93 [-5.03, 3.18]	-0.68 [-4.82, 3.46]	-0.17 [-3.74, 3.41]	0.75 [-3.67, 5.17]	-0.45 [-4.16, 3.27]	-1.64 [-6.64, 3.36]	-4.49 [-10.28, 1.29]
MLPA x *APOE* x Time	-0.05 [-3.07, 2.98]	-0.90 [-4.82, 3.01]	0.74 [-2.38, 3.85]	0.01 [-4.77, 4.79]	-1.66 [-5.72, 2.40]	0.90 [-2.68, 4.48]	-3.40 [-9.14, 2.34]
MLPA x *APOE* x Time^2	0.10 [-0.61, 0.81]	0.40 [-0.52, 1.32]	-0.22 [-0.96, 0.51]	-0.07 [-1.20, 1.06]	0.39 [-0.57, 1.35]	-0.09 [-0.93, 0.75]	1.00 [-0.51, 2.51]

*p<0.05, **p≤.01, ***p≤.001. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol. Abbreviations: MLPA, midlife physical activity; *APOEε4*, Apolipoprotein epsilon 4; CI, Confidence Interval. ε4 carrier = reference group; Low MLPA = reference group. [a] Results from Mode 1 and 2 = Not statistically significant.

Table D.3. Mixed-effects models evaluating the association between sex, *APOE*ε4 status, midlife physical activity and longitudinal multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Main effect interactions [a]							
Sex x MLPA	1.46 [-1.90, 4.82]	0.92 [-2.54, 4.38]	0.72 [-2.23, 3.68]	1.36 [-2.29, 5.00]	1.31 [-1.77, 4.38]	0.78 [-3.35, 4.91]	-0.54 [-5.38, 4.29]
Sex x MLPA x time	-0.28 [-2.79, 2.22]	0.87 [-2.37, 4.11]	-0.92 [-3.51, 1.66]	0.45 [-3.50, 4.41]	-0.81 [-4.15, 2.52]	-0.01 [-2.98, 2.96]	-1.34 [-6.27, 3.59]
Sex x MLPA x time^2	-0.00 [-0.58, 0.57]	0.04 [-0.71, 0.78]	0.26 [-0.34, 0.85]	-0.35 [-1.27, 0.57]	0.06 [-0.72, 0.83]	-0.14 [-0.83, 0.54]	0.52 [-0.78, 1.82]
Females							
MLPA	2.25 [-0.11, 4.62] †	1.49 [-0.77, 3.75]	1.52 [-0.37, 3.42]	1.29 [-1.34, 3.91]	1.37 [-0.83, 3.57]	2.36 [-0.61, 5.33]	3.56 [0.26, 6.85]*
MLPA x time	-0.62 [-2.39, 1.15]	-3.31 [-5.44, -1.19]**	-0.63 [-2.31, 1.05]	-0.36 [-3.15, 2.44]	0.85 [-1.53, 3.23]	0.73 [-1.33, 2.79]	1.12 [-2.19, 4.43]
MLPA x time^2	0.24 [-0.17, 0.65]	0.76 [0.26, 1.25]**	0.13 [-0.26, 0.52]	0.17 [-0.49, 0.82]	-0.10 [-0.66, 0.45]	-0.06 [-0.53, 0.42]	-0.37 [-1.25, 0.51]
***APOE* Interactions**							
APOE x MLPA	-0.38 [-5.93, 5.17]	-2.11 [-7.43, 3.21]	-0.77 [-5.19, 3.65]	0.52 [-5.61, 6.65]	1.80 [-3.33, 6.93]	0.93 [-6.06, 7.93]	-5.26 [-12.97, 2.44]
Males							
MLPA	3.78 [1.34, 6.23]**	2.51 [-0.24, 5.26] †	2.10 [-0.28, 4.48]	2.61 [0.03, 5.20]*	2.75 [0.59, 4.90]*	3.29 [0.39, 6.20]*	2.79 [-0.77, 6.36]
MLPA x time	-0.89 [-2.64, 0.86]	-2.41 [-4.88, 0.05] †	-1.53 [-3.53, 0.46]	0.07 [-2.69, 2.83]	0.02 [-2.27, 2.30]	0.68 [-1.44, 2.81]	-0.33 [-4.00, 3.33]
MLPA x time^2	0.24 [-0.16, 0.64]	0.79 [0.22, 1.36]**	0.38 [-0.08, 0.84]	-0.18 [-0.82, 0.45]	-0.05 [-0.58, 0.48]	-0.19 [-0.68, 0.30]	0.17 [-0.79, 1.13]
***APOE* Interactions**							
APOE x MLPA	-1.41 [-7.50, 4.67]	1.11 [-5.48, 7.71]	0.85 [-4.99, 6.69]	1.57 [-4.84, 7.98]	-3.28 [-8.52, 1.96]	-5.09 [-12.17, 1.99]	-3.93 [-12.66, 4.80]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol consumption. Abbreviations: CI, Confidence Interval; MLPA, midlife physical activity; *APOE*ε4, apolipoprotein epsilon-4. Low MLPA group = reference;

Table D.4. Mixed-effects models evaluating the association between midlife physical activity, *APOE*ε4 carrier status and longitudinal cognition among those < 70 years versus ≥70 years of age

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
<70 years old							
MLPA	2.54 [0.48, 4.61]*	1.49 [-0.62, 3.59]	2.14 [0.40, 3.87]*	1.95 [-0.35, 4.24]	2.28 [0.34, 4.23]*	1.26 [-1.35, 3.87]	3.12 [0.21, 6.03]*
MLPA x time	-0.83 [-2.40, 0.73]	-2.71 [-4.72, -0.69]**	-1.51 [-3.06, 0.04]†	0.35 [-2.11, 2.82]	0.56 [-1.49, 2.62]	0.55 [-1.29, 2.39]	0.40 [-2.43, 3.23]
MLPA x time^2	0.31 [-0.05, 0.67]	0.76 [0.29, 1.23]**	0.34 [-0.02, 0.70]†	-0.07 [-0.65, 0.50]	0.04 [-0.44, 0.52]	-0.09 [-0.51, 0.34]	0.01 [-0.73, 0.75]
***APOE* interaction**							
APOE x MLPA	-0.62 [-5.59, 4.35]	0.05 [-4.91, 5.00]	1.32 [-2.81, 5.46]	1.25 [-4.18, 6.68]	-1.54 [-6.12, 3.05]	-2.56 [-8.74, 3.63]	-6.61 [-13.38, 0.16]
≥70 years old							
MLPA	2.23 [-0.64, 5.10]	1.43 [-1.62, 4.49]	0.26 [-2.47, 3.00]	1.35 [-1.76, 4.46]	0.63 [-1.91, 3.17]	3.90 [0.48, 7.32]*	2.84 [-1.39, 7.07]
MLPA x time	-0.35 [-2.38, 1.68]	-3.07 [-5.73, -0.41]*	0.04 [-2.23, 2.32]	-1.37 [-4.64, 1.90]	0.94 [-1.86, 3.74]	1.24 [-1.19, 3.66]	0.83 [-3.79, 5.45]
MLPA x time^2	-0.04 [-0.50, 0.43]	0.67 [0.06, 1.28]*	-0.03 [-0.56, 0.49]	0.13 [-0.62, 0.88]	-0.42 [-1.07, 0.23]	-0.29 [-0.84, 0.27]	-0.48 [-1.73, 0.77]
***APOE* interaction**							
APOE x MLPA	0.18 [-7.00, 7.36]	-1.34 [-8.87, 6.20]	-2.30 [-9.04, 4.44]	0.95 [-6.94, 8.84]	2.45 [-3.90, 8.81]	2.02 [-6.58, 10.62]	-0.31 [-10.97, 10.36]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Adjusted for time, time^2, age, education, sex, BMI, hypertension, hypercholesterolemia, type II diabetes, smoking and alcohol consumption.
Abbreviations: CI, Confidence Interval; MLPA, midlife physical activity; *APOE*ε4, apolipoprotein epsilon-4. Low MLPA group = reference;

Appendix E. Chapter 6: Midlife to late-life Physical Activity and Cognition

Table E.1 Mixed effect models evaluating the association between midlife to late-life physical activity groups on late-life longitudinal cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 1							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-0.17 [-2.77, 2.44]	-1.18 [-3.79, 1.43]	0.12 [-2.13, 2.36]	-0.84 [-3.58, 1.90]	-0.02 [-2.31, 2.27]	0.93 [-2.22, 4.07]	-1.03 [-4.55, 2.49]
H-ML/L-LL PA	1.95 [-0.08, 3.99] †	0.89 [-1.16, 2.93]	1.65 [-0.11, 3.40]x	2.27 [0.12, 4.42]*	0.21 [-1.59, 2.01]	1.50 [-0.96, 3.95]	1.95 [-0.92, 4.81]
H-ML/H-LL PA	3.13 [0.84, 5.42]**	1.72 [-0.58, 4.02]	1.26 [-0.71, 3.23]	1.16 [-1.26, 3.57]	3.25 [1.23, 5.27]**	3.72 [0.95, 6.49]**	3.37 [0.20, 6.54]*
L-ML/H-LL PA x Time	-0.92 [-2.75, 0.91]	-2.24 [-4.59, 0.11] †	-0.69 [-2.55, 1.17]	0.26 [-2.58, 3.10]	-0.44 [-2.86, 1.98]	0.35 [-1.82, 2.52]	0.09 [-3.46, 3.65]
L-ML/H-LL PA x Time^2	0.25 [-0.16, 0.67]	0.65 [0.11, 1.18]*	0.26 [-0.16, 0.68]	-0.24 [-0.89, 0.40]	0.14 [-0.41, 0.69]	-0.05 [-0.54, 0.44]	-0.16 [-1.09, 0.77]
H-ML/L-LL PA x Time	-0.66 [-2.13, 0.80]	-2.36 [-4.25, -0.47]*	-0.93 [-2.42, 0.56]	0.23 [-2.05, 2.51]	-0.75 [-2.69, 1.19]	0.92 [-0.83, 2.67]	1.34 [-1.52, 4.21]
H-ML/L-LL PA x Time^2	0.22 [-0.11, 0.56]	0.72 [0.28, 1.15]**	0.21 [-0.13, 0.55]	-0.21 [-0.74, 0.31]	0.21 [-0.24, 0.66]	-0.13 [-0.53, 0.26]	-0.49 [-1.25, 0.26]
H-ML/H-LL PA x Time	-2.13 [-3.79, -0.47]*	-4.23 [-6.37, -2.08]***	-2.48 [-4.17, -0.79]**	-1.97 [-4.56, 0.62]	0.58 [-1.62, 2.79]	0.52 [-1.46, 2.49]	-0.12 [-3.40, 3.16]
H-ML/H-LL PA x Time^2	0.51 [0.13, 0.90]**	1.00 [0.50, 1.50]***	0.57 [0.17, 0.97]**	0.42 [-0.19, 1.03]	-0.14 [-0.66, 0.37]	-0.04 [-0.50, 0.42]	0.06 [-0.81, 0.93]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; *APOEε*4, apolipoprotein epsilon-4; CI, Confidence Interval. L-ML/L-LL PA= comparator

Table E.1 (Continued) Mixed effect models evaluating the association between midlife to late-life physical activity groups on late-life longitudinal cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 2							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-0.23 [-2.83, 2.37]	-1.26 [-3.91, 1.39]	0.07 [-2.22, 2.36]	-0.94 [-3.68, 1.81]	0.14 [-2.17, 2.45]	0.85 [-2.31, 4.01]	-0.43 [-3.95, 3.09]
H-ML/L-LL PA	1.75 [-0.29, 3.78]	0.79 [-1.29, 2.87]	1.61 [-0.18, 3.40] †	1.87 [-0.29, 4.02]	0.23 [-1.59, 2.04]	1.41 [-1.06, 3.87]	2.03 [-0.83, 4.90]
H-ML/H-LL PA	2.44 [0.16, 4.72]*	1.36 [-0.97, 3.69]	1.32 [-0.69, 3.32]	0.34 [-2.07, 2.75]	3.06 [1.03, 5.09]**	2.70 [-0.06, 5.47] †	3.11 [-0.06, 6.27] †
L-ML/H-LL PA x Time	-1.03 [-2.89, 0.84]	-2.10 [-4.50, 0.30]	-0.63 [-2.53, 1.27]	0.19 [-2.72, 3.09]	-0.67 [-3.12, 1.78]	-0.14 [-2.34, 2.06]	0.81 [-2.79, 4.41]
L-ML/H-LL PA x Time^2	0.29 [-0.13, 0.71]	0.62 [0.08, 1.17]*	0.28 [-0.16, 0.71]	-0.24 [-0.91, 0.42]	0.18 [-0.39, 0.74]	0.07 [-0.43, 0.57]	-0.31 [-1.25, 0.63]
H-ML/L-LL PA x Time	-0.62 [-2.13, 0.88]	-2.27 [-4.20, -0.34]*	-0.91 [-2.44, 0.62]	0.36 [-1.97, 2.70]	-0.63 [-2.60, 1.34]	0.82 [-0.95, 2.60]	1.74 [-1.17, 4.64]
H-ML/L-LL PA x Time^2	0.22 [-0.13, 0.56]	0.72 [0.27, 1.16]**	0.20 [-0.15, 0.55]	-0.22 [-0.76, 0.32]	0.17 [-0.29, 0.63]	-0.13 [-0.53, 0.28]	-0.63 [-1.40, 0.14]
H-ML/H-LL PA x Time	-2.13 [-3.80, -0.47]*	-4.15 [-6.30, -2.01]***	-2.44 [-4.14, -0.74]**	-1.85 [-4.45, 0.75]	0.57 [-1.63, 2.77]	0.35 [-1.61, 2.32]	0.21 [-3.06, 3.48]
H-ML/H-LL PA x Time^2	0.52 [0.13, 0.91]**	0.99 [0.49, 1.49]***	0.57 [0.18, 0.97]**	0.41 [-0.20, 1.02]	-0.14 [-0.66, 0.37]	-0.01 [-0.47, 0.45]	-0.01 [-0.87, 0.86]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; *APOE*ε4, apolipoprotein epsilon-4; CI, Confidence Interval. L-ML/L-LL PA= comparator

Table E.1 (Continued) Mixed effect models evaluating the association between midlife to late-life physical activity groups on late-life longitudinal cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 3							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-0.17 [-2.77, 2.44]	-1.18 [-3.79, 1.43]	0.12 [-2.13, 2.36]	-0.84 [-3.58, 1.90]	-0.02 [-2.31, 2.27]	0.93 [-2.22, 4.07]	-1.03 [-4.55, 2.49]
H-ML/L-LL PA	1.95 [-0.08, 3.99] †	0.89 [-1.16, 2.93]	1.65 [-0.11, 3.40] †	2.27 [0.12, 4.42]*	0.21 [-1.59, 2.01]	1.50 [-0.96, 3.95]	1.95 [-0.92, 4.81]
H-ML/H-LL PA	3.13 [0.84, 5.42]**	1.72 [-0.58, 4.02]	1.26 [-0.71, 3.23]	1.16 [-1.26, 3.57]	3.25 [1.23, 5.27]**	3.72 [0.95, 6.49]**	3.37 [0.20, 6.54]*
L-ML/H-LL PA x Time	-1.12 [-3.09, 0.86]	-1.94 [-4.50, 0.61]	-0.32 [-2.36, 1.72]	-0.36 [-3.48, 2.77]	-0.26 [-2.89, 2.38]	-0.82 [-3.16, 1.52]	0.18 [-3.65, 4.02]
L-ML/H-LL PA x Time^2	0.35 [-0.10, 0.80]	0.65 [0.06, 1.23]*	0.23 [-0.23, 0.70]	-0.08 [-0.79, 0.64]	0.09 [-0.52, 0.69]	0.27 [-0.26, 0.80]	-0.16 [-1.17, 0.85]
H-ML/L-LL PA x Time	-0.58 [-2.15, 0.99]	-2.05 [-4.08, -0.02]*	-0.54 [-2.16, 1.08]	0.07 [-2.41, 2.55]	-0.18 [-2.27, 1.91]	0.55 [-1.31, 2.41]	1.59 [-1.49, 4.66]
H-ML/L-LL PA x Time^2	0.20 [-0.15, 0.56]	0.70 [0.23, 1.16]**	0.13 [-0.24, 0.51]	-0.16 [-0.73, 0.41]	0.07 [-0.41, 0.55]	-0.10 [-0.52, 0.33]	-0.49 [-1.30, 0.33]
H-ML/H-LL PA x Time	-1.81 [-3.54, -0.09]*	-4.26 [-6.49, -2.03]***	-2.07 [-3.85, -0.29]*	-1.33 [-4.07, 1.40]	0.75 [-1.56, 3.05]	0.42 [-1.63, 2.46]	0.12 [-3.28, 3.51]
H-ML/H-LL PA x Time^2	0.46 [0.06, 0.86]*	1.01 [0.49, 1.53]***	0.48 [0.07, 0.90]*	0.29 [-0.35, 0.93]	-0.17 [-0.71, 0.37]	-0.00 [-0.48, 0.47]	0.09 [-0.80, 0.98]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; *APOE*ε4, apolipoprotein epsilon-4; CI, Confidence Interval. L-ML/L-LL PA= comparator

Table E.2 Mixed-effects models evaluating the association between mid- to late-life physical activity groups and longitudinal cognition within *APOE*ε4 carriers and non-carriers across three covariate adjusted models

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 1 *APOE4* carrier							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-0.67 [-6.10, 4.76]	-1.24 [-6.48, 3.99]	0.91 [-4.52, 6.34]	-2.97 [-8.90, 2.96]	1.86 [-3.10, 6.81]	-1.29 [-7.75, 5.16]	-2.94 [-9.75, 3.86]
H-ML/L-LL PA	2.43 [-1.93, 6.79]	1.22 [-2.99, 5.43]	2.93 [-1.43, 7.28]	1.54 [-3.24, 6.32]	0.92 [-3.06, 4.91]	2.31 [-2.87, 7.49]	-0.59 [-6.28, 5.10]
H-ML/H-LL PA	2.03 [-3.15, 7.21]	1.49 [-3.52, 6.50]	0.94 [-4.24, 6.12]	4.17 [-1.51, 9.85]	1.12 [-3.62, 5.86]	0.38 [-5.78, 6.54]	1.16 [-5.88, 8.20]
L-ML/H-LL PA x Time	1.77 [-1.68, 5.21]	-2.11 [-6.66, 2.45]	-3.32 [-6.89, 0.25]	7.04 [1.42, 12.66]*	1.36 [-3.07, 5.80]	1.43 [-2.72, 5.58]	-0.67 [-7.18, 5.85]
L-ML/H-LL PA x Time^2	-0.19 [-0.93, 0.56]	0.60 [-0.39, 1.60]	0.93 [0.16, 1.71]*	-1.41 [-2.64, -0.18]*	-0.29 [-1.26, 0.67]	-0.31 [-1.21, 0.58]	0.11 [-1.55, 1.76]
H-ML/L-LL PA x Time	-1.07 [-4.04, 1.89]	-4.48 [-8.41, -0.56]*	-0.22 [-3.29, 2.85]	1.34 [-3.51, 6.18]	-2.00 [-5.82, 1.83]	0.54 [-3.03, 4.11]	-0.84 [-6.34, 4.67]
H-ML/L-LL PA x Time^2	0.41 [-0.27, 1.09]	1.36 [0.45, 2.27]**	0.04 [-0.67, 0.75]	-0.52 [-1.64, 0.60]	0.53 [-0.35, 1.42]	0.07 [-0.75, 0.89]	0.26 [-1.19, 1.71]
H-ML/H-LL PA x Time	-1.35 [-4.98, 2.28]	-6.26 [-11.06, -1.46]*	-3.84 [-7.60, -0.08]*	0.42 [-5.51, 6.34]	1.07 [-3.61, 5.74]	2.38 [-1.99, 6.74]	-3.60 [-9.89, 2.69]
H-ML/H-LL PA x Time^2	0.41 [-0.27, 1.09]	1.36 [0.45, 2.27]**	0.04 [-0.67, 0.75]	-0.52 [-1.64, 0.60]	0.53 [-0.35, 1.42]	0.07 [-0.75, 0.89]	0.26 [-1.19, 1.71]
Model 1 *APOE4* non-carrier							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-0.14 [-3.25, 2.96]	-1.33 [-4.39, 1.74]	-0.38 [-2.86, 2.10]	0.28 [-2.92, 3.48]	-0.99 [-3.64, 1.65]	1.53 [-2.22, 5.29]	-0.06 [-4.23, 4.11]
H-ML/L-LL PA	2.72 [0.27, 5.18]*	1.25 [-1.17, 3.67]	1.73 [-0.23, 3.69]	3.70 [1.17, 6.23]**	0.47 [-1.62, 2.57]	1.59 [-1.37, 4.55]	3.74 [0.35, 7.14]*
H-ML/H-LL PA	3.37 [0.65, 6.10]*	1.51 [-1.18, 4.20]	1.22 [-0.96, 3.40]	0.86 [-1.95, 3.66]	3.26 [0.94, 5.59]**	4.59 [1.30, 7.88]**	4.25 [0.58, 7.92]*
L-ML/H-LL PA x Time	0.41 [-0.27, 1.09]	1.36 [0.45, 2.27]**	0.04 [-0.67, 0.75]	-0.52 [-1.64, 0.60]	0.53 [-0.35, 1.42]	0.07 [-0.75, 0.89]	0.26 [-1.19, 1.71]
L-ML/H-LL PA x Time^2	0.42 [-0.08, 0.92]	0.68 [0.04, 1.32]*	-0.06 [-0.56, 0.44]	0.22 [-0.55, 0.99]	0.33 [-0.34, 1.01]	0.06 [-0.53, 0.65]	-0.20 [-1.34, 0.93]
H-ML/L-LL PA x Time	-0.72 [-2.44, 0.99]	-1.82 [-4.01, 0.36]	-1.10 [-2.82, 0.62]	-0.35 [-2.97, 2.27]	-0.83 [-3.12, 1.47]	0.95 [-1.07, 2.98]	1.94 [-1.48, 5.35]
H-ML/L-LL PA x Time^2	0.21 [-0.19, 0.60]	0.54 [0.04, 1.04]*	0.25 [-0.14, 0.65]	-0.08 [-0.68, 0.52]	0.18 [-0.35, 0.71]	-0.17 [-0.63, 0.29]	-0.71 [-1.61, 0.18]
H-ML/H-LL PA x Time	-2.45 [-4.37, -0.53]*	-3.66 [-6.11, -1.21]**	-1.73 [-3.66, 0.19]	-2.78 [-5.72, 0.16]	0.18 [-2.40, 2.76]	-0.26 [-2.52, 2.00]	1.09 [-2.85, 5.04]
H-ML/H-LL PA x Time^2	0.55 [0.11, 0.99]*	0.89 [0.32, 1.45]**	0.40 [-0.04, 0.85]	0.53 [-0.15, 1.21]	-0.05 [-0.65, 0.54]	0.08 [-0.44, 0.60]	-0.33 [-1.38, 0.71]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; *APOE*ε4, apolipoprotein epsilon-4; CI, Confidence Interval. L-ML/L-LL PA= comparator

Table E.2 (Continued) Mixed-effects models evaluating the association between mid- to late-life physical activity groups and longitudinal cognition within *APOE*ε4 carriers and non-carriers

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 2 *APOE4* carrier							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-0.97 [-6.23, 4.28]	-1.40 [-6.57, 3.76]	0.76 [-4.61, 6.12]	-3.70 [-9.48, 2.08]	2.18 [-2.61, 6.97]	-1.65 [-8.02, 4.72]	-3.03 [-9.86, 3.79]
H-ML/L-LL PA	1.87 [-2.46, 6.20]	0.19 [-4.09, 4.46]	1.99 [-2.44, 6.42]	0.94 [-3.84, 5.73]	1.23 [-2.74, 5.19]	2.43 [-2.82, 7.68]	-0.89 [-6.77, 4.99]
H-ML/H-LL PA	1.11 [-3.95, 6.17]	0.62 [-4.36, 5.61]	0.69 [-4.48, 5.86]	2.76 [-2.83, 8.34]	1.41 [-3.22, 6.03]	-0.78 [-6.91, 5.36]	0.40 [-6.73, 7.53]
L-ML/H-LL PA x Time	2.08 [-1.34, 5.50]	-1.70 [-6.14, 2.75]	-3.28 [-6.88, 0.32]	7.14 [1.51, 12.78]*	1.57 [-2.85, 6.00]	1.62 [-2.45, 5.69]	-0.52 [-7.07, 6.03]
L-ML/H-LL PA x Time^2	-0.25 [-0.99, 0.49]	0.52 [-0.45, 1.48]	0.92 [0.15, 1.70]*	-1.42 [-2.65, -0.19]*	-0.34 [-1.30, 0.62]	-0.35 [-1.23, 0.53]	0.07 [-1.59, 1.73]
H-ML/L-LL PA x Time	-0.76 [-3.84, 2.32]	-3.66 [-7.67, 0.35] †	-0.20 [-3.44, 3.04]	0.68 [-4.40, 5.77]	-1.78 [-5.77, 2.21]	1.14 [-2.53, 4.80]	-0.72 [-6.47, 5.03]
H-ML/L-LL PA x Time^2	0.33 [-0.39, 1.06]	1.27 [0.33, 2.22]**	-0.01 [-0.77, 0.75]	-0.37 [-1.57, 0.83]	0.38 [-0.56, 1.32]	-0.08 [-0.94, 0.78]	0.04 [-1.50, 1.58]
H-ML/H-LL PA x Time	-0.91 [-4.51, 2.69]	-5.69 [-10.37, -1.01]*	-3.73 [-7.52, 0.06]	0.82 [-5.12, 6.76]	1.39 [-3.27, 6.06]	2.66 [-1.63, 6.95]	-3.48 [-9.79, 2.84]
H-ML/H-LL PA x Time^2	0.39 [-0.47, 1.26]	1.24 [0.11, 2.38]*	0.93 [0.02, 1.85]*	0.04 [-1.41, 1.48]	-0.34 [-1.47, 0.79]	-0.39 [-1.42, 0.65]	1.15 [-0.49, 2.79]
Model 2 *APOE4* non-carrier							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	0.08 [-3.04, 3.19]	-1.07 [-4.21, 2.06]	-0.31 [-2.85, 2.23]	0.53 [-2.70, 3.75]	-0.88 [-3.59, 1.82]	1.76 [-2.03, 5.56]	0.85 [-3.32, 5.03]
H-ML/L-LL PA	2.51 [0.07, 4.95]*	1.51 [-0.95, 3.97]	1.87 [-0.12, 3.86]	3.40 [0.87, 5.93]**	0.10 [-2.02, 2.22]	1.36 [-1.60, 4.33]	4.01 [0.64, 7.39]*
H-ML/H-LL PA	2.83 [0.13, 5.53]*	1.43 [-1.29, 4.15]	1.45 [-0.75, 3.65]	0.27 [-2.53, 3.06]	2.96 [0.62, 5.31]*	3.65 [0.36, 6.94]*	4.25 [0.61, 7.89]*
L-ML/H-LL PA x Time	-2.19 [-4.43, 0.04]	-2.27 [-5.15, 0.60]	0.74 [-1.51, 2.98]	-2.73 [-6.15, 0.69]	-1.77 [-4.76, 1.22]	-0.74 [-3.37, 1.89]	0.95 [-3.40, 5.30]
L-ML/H-LL PA x Time^2	0.50 [-0.02, 1.02]	0.69 [0.03, 1.36]*	-0.07 [-0.59, 0.45]	0.26 [-0.54, 1.06]	0.44 [-0.26, 1.14]	0.25 [-0.36, 0.87]	-0.38 [-1.55, 0.78]
H-ML/L-LL PA x Time	-0.79 [-2.53, 0.96]	-1.98 [-4.22, 0.25]	-1.06 [-2.81, 0.69]	-0.13 [-2.79, 2.53]	-0.70 [-3.02, 1.63]	0.72 [-1.33, 2.78]	2.37 [-1.06, 5.80]
H-ML/L-LL PA x Time^2	0.22 [-0.17, 0.62]	0.59 [0.07, 1.10]*	0.23 [-0.17, 0.63]	-0.12 [-0.73, 0.49]	0.18 [-0.36, 0.71]	-0.14 [-0.60, 0.33]	-0.81 [-1.71, 0.09]
H-ML/H-LL PA x Time	-2.56 [-4.49, -0.63]**	-3.70 [-6.17, -1.22]**	-1.70 [-3.63, 0.23]	-2.72 [-5.67, 0.23]	0.04 [-2.54, 2.62]	-0.48 [-2.75, 1.78]	1.44 [-2.48, 5.36]
H-ML/H-LL PA x Time^2	0.58 [0.14, 1.02]*	0.90 [0.33, 1.47]**	0.40 [-0.05, 0.84]	0.53 [-0.15, 1.21]	-0.02 [-0.61, 0.58]	0.12 [-0.41, 0.64]	-0.39 [-1.43, 0.65]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; CI, Confidence Interval. L-ML/L-LL PA= comparator

Table E.2 (continued) Mixed-effects models evaluating the association between mid- to late-life physical activity groups and longitudinal cognition within *APOE*ε4 carriers and non-carriers

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 3 *APOE4* carrier							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-3.79 [-9.51, 1.93]	-3.50 [-9.16, 2.17]	-1.69 [-7.30, 3.92]	-7.27 [-13.71, 0.84]	1.25 [-4.20, 6.71]	-2.97 [-9.92, 3.98]	-3.67 [-11.38, 4.05]
H-ML/L-LL PA	1.66 [-2.85, 6.16]	0.56 [-3.89, 5.02]	2.19 [-2.23, 6.61]	0.90 [-4.15, 5.96]	1.04 [-3.25, 5.33]	1.53 [-3.95, 7.00]	-1.55 [-7.69, 4.58]
H-ML/H-LL PA	1.01 [-4.31, 6.32]	-0.08 [-5.32, 5.16]	-0.64 [-5.85, 4.57]	3.59 [-2.36, 9.54]	1.48 [-3.57, 6.53]	-0.09 [-6.54, 6.37]	1.74 [-5.79, 9.26]
L-ML/H-LL PA x Time	0.70 [-3.13, 4.53]	-2.87 [-7.84, 2.10]	-4.13 [-8.19, -0.07]*	6.27 [0.00, 12.53]	1.98 [-3.06, 7.02]	-0.10 [-4.72, 4.53]	-1.30 [-8.53, 5.94]
L-ML/H-LL PA x Time^2	0.16 [-0.69, 1.01]	0.94 [-0.17, 2.04]	1.20 [0.30, 2.10]**	-1.13 [-2.52, 0.27]	-0.45 [-1.57, 0.68]	0.09 [-0.93, 1.11]	0.33 [-1.50, 2.17]
H-ML/L-LL PA x Time	-1.11 [-4.33, 2.10]	-3.28 [-7.46, 0.90]	0.10 [-3.30, 3.51]	-0.65 [-5.92, 4.63]	-1.22 [-5.46, 3.02]	0.63 [-3.25, 4.51]	-1.98 [-7.99, 4.03]
H-ML/L-LL PA x Time^2	0.42 [-0.32, 1.17]	1.25 [0.28, 2.22]*	-0.05 [-0.84, 0.74]	-0.14 [-1.37, 1.09]	0.27 [-0.72, 1.26]	0.04 [-0.86, 0.94]	0.53 [-1.08, 2.15]
H-ML/H-LL PA x Time	-0.22 [-4.21, 3.77]	-4.61 [-9.80, 0.57]	-2.79 [-7.02, 1.44]	2.04 [-4.50, 8.58]	1.18 [-4.08, 6.44]	2.35 [-2.47, 7.16]	-3.75 [-10.43, 2.93]
H-ML/H-LL PA x Time^2	0.24 [-0.76, 1.24]	1.08 [-0.23, 2.39]	0.69 [-0.37, 1.75]	-0.33 [-1.98, 1.33]	-0.23 [-1.56, 1.09]	-0.30 [-1.50, 0.91]	1.32 [-0.41, 3.06]
Model 3 *APOE4* non-carrier							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	0.28 [-2.96, 3.52]	-0.80 [-4.10, 2.50]	-0.52 [-3.22, 2.18]	0.41 [-2.98, 3.80]	-0.46 [-3.33, 2.41]	2.11 [-1.86, 6.09]	0.93 [-3.53, 5.39]
H-ML/L-LL PA	2.50 [-0.05, 5.05] †	1.26 [-1.34, 3.86]	1.79 [-0.34, 3.91]	3.25 [0.57, 5.93]*	-0.19 [-2.45, 2.08]	2.22 [-0.91, 5.35]	4.33 [0.72, 7.94]*
H-ML/H-LL PA	3.80 [0.99, 6.61]**	2.45 [-0.41, 5.31]	1.80 [-0.54, 4.14]	0.70 [-2.24, 3.63]	3.74 [1.25, 6.22]**	4.43 [0.98, 7.88]*	4.97 [1.10, 8.83]*
L-ML/H-LL PA x Time	-1.76 [-4.09, 0.56]	-1.63 [-4.62, 1.37]	1.28 [-1.08, 3.63]	-2.88 [-6.50, 0.74]	-1.38 [-4.53, 1.76]	-0.94 [-3.67, 1.80]	0.28 [-4.29, 4.85]
L-ML/H-LL PA x Time^2	0.42 [-0.12, 0.95]	0.54 [-0.16, 1.23]	-0.18 [-0.73, 0.36]	0.34 [-0.50, 1.18]	0.35 [-0.38, 1.08]	0.32 [-0.31, 0.95]	-0.27 [-1.50, 0.95]
H-ML/L-LL PA x Time	-0.72 [-2.54, 1.11]	-1.97 [-4.32, 0.39]	-0.71 [-2.56, 1.13]	-0.35 [-3.19, 2.49]	-0.41 [-2.88, 2.05]	0.64 [-1.51, 2.80]	2.28 [-1.35, 5.90]
H-ML/L-LL PA x Time^2	0.20 [-0.22, 0.61]	0.58 [0.04, 1.12]*	0.15 [-0.27, 0.58]	-0.06 [-0.71, 0.59]	0.11 [-0.45, 0.68]	-0.15 [-0.64, 0.34]	-0.68 [-1.64, 0.27]
H-ML/H-LL PA x Time	-2.35 [-4.33, -0.37]*	-4.18 [-6.74, -1.62]**	-1.45 [-3.47, 0.56]	-2.36 [-5.47, 0.74]	0.17 [-2.52, 2.86]	-0.25 [-2.59, 2.08]	1.18 [-2.86, 5.22]
H-ML/H-LL PA x Time^2	0.54 [0.09, 0.99]*	0.97 [0.38, 1.56]**	0.33 [-0.13, 0.79]	0.49 [-0.23, 1.20]	-0.04 [-0.66, 0.57]	0.10 [-0.44, 0.63]	-0.25 [-1.31, 0.82]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; CI, Confidence Interval. L-ML/L-LL PA= comparator

Table E.3 Mixed-effects models evaluating the effect modification of *APOE*ε4 carrier status and sex on the association between mid- to late-life physical activity groups and longitudinal cognition

	Beta-Coefficients [95% CI]						
Model 3	**Global Cognition**	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
***APOE* Interactions**							
L-ML/H-LL PA x *APOE*	-2.06 [-8.50, 4.38]	-1.01 [-7.51, 5.48]	0.61 [-5.00, 6.21]	-6.39 [-13.28, 0.51]	3.42 [-2.38, 9.22]	-4.05 [-11.89, 3.79]	-1.87 [-10.63, 6.90]
H-ML/L-LL PA x *APOE*	-0.17 [-5.29, 4.95]	-0.14 [-5.31, 5.03]	0.52 [-3.94, 4.98]	-2.05 [-7.54, 3.44]	1.63 [-2.99, 6.25]	0.08 [-6.15, 6.31]	-5.96 [-13.11, 1.19]
H-ML/H-LL PA x *APOE*	-0.97 [-6.76, 4.82]	-0.41 [-6.24, 5.43]	-0.81 [-5.85, 4.22]	3.84 [-2.35, 10.03]	-1.32 [-6.52, 3.89]	-3.34 [-10.39, 3.70]	-1.95 [-10.24, 6.33]
L-ML/H-LL PA x *APOE x* Time	2.43 [-2.09, 6.96]	-1.33 [-7.16, 4.50]	-5.53 [-10.17, -0.89]*	9.09 [0.96, 16.21]	3.41 [-2.65, 9.48]	0.85 [-4.51, 6.21]	-1.45 [-10.25, 7.34]
L-ML/H-LL PA x *APOE* x Time^2	-0.25 [-1.27, 0.76]	0.42 [-0.90, 1.73]	1.40 [0.36, 2.44]**	-1.46 [-3.07, 0.15]	-0.80 [-2.17, 0.57]	-0.24 [-1.44, 0.96]	0.62 [-1.65, 2.88]
H-ML/L-LL PA x *APOE* x Time	-0.46 [-4.20, 3.27]	-1.46 [-6.28, 3.36]	0.80 [-3.03, 4.64]	-0.44 [-6.33, 5.46]	-1.00 [-6.02, 4.02]	-0.05 [-4.48, 4.38]	-4.47 [-11.69, 2.75]
H-ML/L-LL PA x *APOE* x Time^2	0.24 [-0.63, 1.10]	0.70 [-0.42, 1.81]	-0.20 [-1.09, 0.69]	-0.04 [-1.42, 1.33]	0.18 [-0.98, 1.35]	0.19 [-0.83, 1.21]	1.28 [-0.66, 3.21]
H-ML/H-LL PA x *APOE* x Time	1.87 [-2.64, 6.37]	-0.69 [-6.51, 5.13]	-1.64 [-6.27, 2.98]	4.05 [-3.07, 11.16]	0.49 [-5.57, 6.55]	2.43 [-2.91, 7.76]	-4.95 [-12.99, 3.08]
H-ML/H-LL PA x *APOE* x Time^2	-0.25 [-1.36, 0.87]	0.16 [-1.28, 1.60]	0.43 [-0.72, 1.57]	-0.73 [-2.50, 1.04]	-0.09 [-1.60, 1.41]	-0.37 [-1.68, 0.95]	1.60 [-0.49, 3.70]
Sex Interactions							
L-ML/H-LL PA x Sex	-0.79 [-6.18, 4.61]	-1.65 [-7.19, 3.90]	-0.54 [-5.28, 4.20]	0.87 [-4.98, 6.71]	1.03 [-3.89, 5.95]	-2.44 [-9.06, 4.19]	3.39 [-4.23, 11.01]
H-ML/L-LL PA x Sex	-1.20 [-5.46, 3.07]	-1.01 [-5.40, 3.39]	-0.89 [-4.65, 2.86]	0.07 [-4.57, 4.71]	-0.97 [-4.87, 2.94]	-1.44 [-6.68, 3.80]	2.27 [-3.94, 8.47]
H-ML/H-LL PA x Sex	-1.73 [-6.38, 2.92]	-1.64 [-6.42, 3.13]	-0.45 [-4.54, 3.63]	-2.76 [-7.79, 2.27]	0.32 [-3.91, 4.55]	-1.13 [-6.85, 4.58]	2.21 [-4.42, 8.84]
L-ML/H-LL PA x Sex x Time	-3.41 [-7.37, 0.56]	-2.45 [-7.56, 2.67]	-3.44 [-7.52, 0.65]	-5.33 [-11.60, 0.94]	-1.97 [-7.26, 3.31]	3.47 [-1.23, 8.18]	-6.00 [-13.68, 1.68]
L-ML/H-LL PA x Sex x Time^2	0.67 [-0.24, 1.57]	0.30 [-0.87, 1.47]	0.82 [-0.11, 1.76]	0.94 [-0.51, 2.38]	0.33 [-0.88, 1.55]	-0.61 [-1.68, 0.47]	0.77 [-1.26, 2.80]
H-ML/L-LL PA x Sex x Time	-1.16 [-4.32, 2.00]	-0.98 [-5.06, 3.09]	0.22 [-3.03, 3.48]	-3.10 [-8.09, 1.89]	-0.91 [-5.12, 3.29]	0.65 [-3.11, 4.40]	-2.16 [-8.34, 4.02]
H-ML/L-LL PA x Sex x Time^2	0.26 [-0.47, 0.98]	-0.01 [-0.95, 0.92]	-0.02 [-0.76, 0.73]	0.73 [-0.42, 1.88]	0.13 [-0.84, 1.10]	0.16 [-0.70, 1.01]	0.03 [-1.61, 1.67]
H-ML/H-LL PA x Sex x Time	0.84 [-2.62, 4.31]	-1.16 [-5.63, 3.32]	0.69 [-2.88, 4.27]	0.66 [-4.83, 6.15]	2.17 [-2.46, 6.80]	0.94 [-3.17, 5.06]	0.77 [-6.03, 7.56]
H-ML/H-LL PA x Sex x Time^2	-0.08 [-0.89, 0.73]	-0.05 [-1.10, 0.99]	-0.30 [-1.13, 0.54]	0.26 [-1.02, 1.55]	-0.21 [-1.30, 0.87]	-0.13 [-1.09, 0.82]	-0.24 [-2.03, 1.55]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; CI, Confidence Interval. L-ML/L-LL PA= comparator

Table E.4 Mixed-effects models evaluating the association between mid- to late-life physical activity groups and longitudinal cognition within males and females

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Female							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-0.92 [-4.75, 2.90]	-2.15 [-5.80, 1.51]	-0.89 [-3.96, 2.17]	-1.30 [-5.56, 2.96]	0.68 [-2.87, 4.23]	0.23 [-4.57, 5.04]	1.50 [-3.77, 6.78]
H-ML/L-LL PA	1.69 [-1.29, 4.66]	0.68 [-2.16, 3.52]	1.51 [-0.88, 3.90]	2.11 [-1.20, 5.42]	0.01 [-2.75, 2.77]	1.57 [-2.17, 5.31]	3.72 [-0.46, 7.90]
H-ML/H-LL PA	2.01 [-1.37, 5.38]	1.00 [-2.21, 4.22]	0.95 [-1.76, 3.65]	-1.06 [-4.80, 2.68]	3.61 [0.50, 6.73]*	2.96 [-1.28, 7.21]	4.92 [0.20, 9.63]*
L-ML/H-LL PA x Time	-2.78 [-5.61, 0.04] †	-3.04 [-6.44, 0.35] †	-2.01 [-4.70, 0.67]	-2.79 [-7.26, 1.68]	-1.58 [-5.39, 2.22]	0.76 [-2.54, 4.06]	-2.65 [-8.00, 2.70]
L-ML/H-LL PA x Time^2	0.69 [0.05, 1.33]*	0.81 [0.04, 1.57]*	0.65 [0.05, 1.26]*	0.35 [-0.67, 1.36]	0.29 [-0.57, 1.16]	0.01 [-0.74, 0.75]	0.13 [-1.30, 1.57]
H-ML/L-LL PA x Time	-1.14 [-3.36, 1.07]	-2.45 [-5.12, 0.21] †	-0.46 [-2.56, 1.64]	-1.22 [-4.73, 2.29]	-0.86 [-3.84, 2.13]	0.74 [-1.84, 3.33]	0.91 [-3.21, 5.03]
H-ML/L-LL PA x Time^2	0.36 [-0.15, 0.87]	0.72 [0.10, 1.33]*	0.16 [-0.32, 0.65]	0.18 [-0.64, 0.99]	0.16 [-0.53, 0.85]	0.02 [-0.57, 0.62]	-0.58 [-1.69, 0.53]
H-ML/H-LL PA x Time	-1.41 [-3.93, 1.11]	-4.76 [-7.79, -1.73]**	-1.79 [-4.18, 0.61]	-0.71 [-4.70, 3.28]	1.47 [-1.92, 4.87]	0.72 [-2.22, 3.65]	0.73 [-3.93, 5.39]
H-ML/H-LL PA x Time^2	0.45 [-0.14, 1.04]	1.00 [0.29, 1.72]**	0.36 [-0.20, 0.93]	0.39 [-0.55, 1.33]	-0.21 [-1.02, 0.59]	-0.03 [-0.72, 0.66]	-0.09 [-1.31, 1.13]
Male							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	0.37 [-3.51, 4.24]	-0.05 [-4.39, 4.30]	-0.18 [-3.95, 3.58]	-1.99 [-6.07, 2.09]	0.31 [-3.10, 3.72]	2.89 [-1.70, 7.48]	-2.25 [-7.79, 3.29]
H-ML/L-LL PA	2.69 [-0.41, 5.79]	1.60 [-1.88, 5.08]	2.03 [-0.98, 5.04]	1.81 [-1.47, 5.08]	0.71 [-2.02, 3.45]	3.29 [-0.38, 6.96] †	0.55 [-4.02, 5.13]
H-ML/H-LL PA	4.30 [0.95, 7.65]*	3.18 [-0.57, 6.93]	1.48 [-1.78, 4.73]	1.94 [-1.58, 5.46]	3.94 [1.00, 6.89]**	4.42 [0.45, 8.38]*	2.73 [-2.09, 7.54]
L-ML/H-LL PA x Time	0.68 [-2.06, 3.42]	-0.57 [-4.43, 3.29]	1.48 [-1.64, 4.61]	2.60 [-1.73, 6.93]	0.41 [-3.18, 4.01]	-2.61 [-5.95, 0.73]	3.38 [-2.15, 8.92]
L-ML/H-LL PA x Time^2	0.02 [-0.61, 0.65]	0.50 [-0.39, 1.40]	-0.18 [-0.90, 0.55]	-0.59 [-1.60, 0.42]	-0.04 [-0.87, 0.80]	0.60 [-0.17, 1.37]	-0.64 [-2.08, 0.80]
H-ML/L-LL PA x Time	0.01 [-2.20, 2.23]	-1.46 [-4.57, 1.66]	-0.69 [-3.22, 1.84]	1.89 [-1.60, 5.38]	-0.02 [-2.91, 2.88]	0.09 [-2.61, 2.78]	3.02 [-1.61, 7.65]
H-ML/L-LL PA x Time^2	0.10 [-0.40, 0.61]	0.73 [0.01, 1.44]*	0.18 [-0.40, 0.76]	-0.56 [-1.36, 0.24]	0.04 [-0.62, 0.71]	-0.13 [-0.75, 0.48]	-0.62 [-1.83, 0.59]
H-ML/H-LL PA x Time	-2.21 [-4.56, 0.15] †	-3.55 [-6.87, -0.23]*	-2.41 [-5.10, 0.28] †	-1.38 [-5.12, 2.35]	-0.63 [-3.72, 2.46]	-0.23 [-3.10, 2.63]	-0.15 [-5.12, 4.83]
H-ML/H-LL PA x Time2	0.52 [-0.02, 1.06] †	1.05 [0.28, 1.82]**	0.65 [0.03, 1.27]*	0.13 [-0.74, 1.00]	-0.01 [-0.73, 0.71]	0.11 [-0.56, 0.77]	0.18 [-1.14, 1.49]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; CI, Confidence Interval. L-ML/L-LL PA= comparator

Table E.5 Mixed-effects models evaluating the association between mid- to late-life physical activity groups and longitudinal cognition within those ≥70 years versus those <70 years of age

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
<70 years old							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	-1.75 [-4.83, 1.33]	-2.38 [-5.52, 0.76]	-1.43 [-4.03, 1.17]	-2.77 [-6.20, 0.66]	0.38 [-2.51, 3.28]	-0.18 [-4.07, 3.72]	-1.66 [-5.88, 2.56]
H-ML/L-LL PA	0.86 [-1.78, 3.50]	0.06 [-2.63, 2.75]	1.44 [-0.79, 3.66]	1.20 [-1.74, 4.14]	0.16 [-2.32, 2.65]	0.23 [-3.10, 3.57]	0.56 [-3.21, 4.34]
H-ML/H-LL PA	3.50 [0.67, 6.33]*	2.06 [-0.81, 4.93]	1.91 [-0.47, 4.29]	1.23 [-1.90, 4.36]	4.20 [1.55, 6.85]**	2.92 [-0.66, 6.50]	5.17 [1.24, 9.11]*
L-ML/H-LL PA x Time	-1.31 [-3.63, 1.00]	-1.90 [-4.87, 1.08]	-0.33 [-2.62, 1.95]	0.06 [-3.59, 3.71]	-0.52 [-3.56, 2.52]	-1.43 [-4.16, 1.29]	0.15 [-4.05, 4.36]
L-ML/H-LL PA x Time^2	0.40 [-0.13, 0.92]	0.54 [-0.14, 1.22]	0.22 [-0.30, 0.74]	-0.14 [-0.98, 0.70]	0.24 [-0.45, 0.94]	0.39 [-0.23, 1.01]	-0.17 [-1.29, 0.94]
H-ML/L-LL PA x Time	-1.08 [-3.09, 0.93]	-2.12 [-4.70, 0.46]	-1.23 [-3.22, 0.76]	0.20 [-2.97, 3.37]	-0.04 [-2.68, 2.60]	-0.15 [-2.52, 2.22]	2.23 [-1.39, 5.85]
H-ML/L-LL PA x Time^2	0.43 [-0.03, 0.89]	0.79 [0.20, 1.38]**	0.33 [-0.12, 0.79]	-0.09 [-0.82, 0.64]	0.21 [-0.40, 0.82]	0.05 [-0.49, 0.59]	-0.50 [-1.45, 0.45]
H-ML/H-LL PA x Time	-1.67 [-3.81, 0.48]	-3.67 [-6.43, -0.92]**	-2.68 [-4.79, -0.56]*	0.19 [-3.19, 3.58]	0.46 [-2.37, 3.28]	-0.07 [-2.59, 2.45]	-0.27 [-4.13, 3.59]
H-ML/H-LL PA x Time^2	0.47 [-0.03, 0.96]	0.90 [0.26, 1.55]**	0.57 [0.08, 1.07]*	-0.04 [-0.84, 0.75]	0.00 [-0.66, 0.66]	0.14 [-0.45, 0.72]	0.21 [-0.79, 1.22]
≥ 70 years old							
L-ML/L-LL PA	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]	0.00 [Ref]
L-ML/H-LL PA	2.84 [-2.67, 8.34]	2.35 [-3.50, 8.20]	1.95 [-3.27, 7.16]	0.39 [-5.52, 6.31]	-0.08 [-4.91, 4.75]	5.17 [-1.38, 11.72]	3.53 [-4.64, 11.70]
H-ML/L-LL PA	2.49 [-0.96, 5.94]	1.32 [-2.36, 5.00]	1.32 [-1.95, 4.60]	2.45 [-1.29, 6.19]	-0.47 [-3.52, 2.58]	3.87 [-0.24, 7.98]	5.02 [-0.05, 10.09]
H-ML/H-LL PA	1.27 [-2.87, 5.41]	1.00 [-3.41, 5.40]	-0.94 [-4.86, 2.99]	-1.42 [-5.88, 3.04]	2.25 [-1.39, 5.89]	3.78 [-1.14, 8.71]	1.18 [-4.85, 7.22]
L-ML/H-LL PA x Time	-1.13 [-4.99, 2.73]	-2.23 [-7.27, 2.80]	-0.65 [-4.98, 3.68]	-1.85 [-8.09, 4.40]	0.82 [-4.53, 6.18]	-0.06 [-4.69, 4.56]	-0.74 [-9.16, 7.69]
L-ML/H-LL PA x Time^2	0.31 [-0.58, 1.20]	0.90 [-0.26, 2.07]	0.31 [-0.69, 1.31]	0.26 [-1.19, 1.71]	-0.44 [-1.69, 0.80]	0.04 [-1.03, 1.11]	0.02 [-2.15, 2.18]
H-ML/L-LL PA x Time	0.38 [-2.09, 2.85]	-1.65 [-4.87, 1.58]	0.73 [-2.04, 3.50]	-0.10 [-4.09, 3.89]	-0.26 [-3.68, 3.16]	1.78 [-1.18, 4.75]	1.20 [-4.40, 6.81]
H-ML/L-LL PA x Time^2	-0.22 [-0.78, 0.34]	0.45 [-0.29, 1.18]	-0.24 [-0.87, 0.39]	-0.30 [-1.21, 0.62]	-0.22 [-1.00, 0.57]	-0.37 [-1.04, 0.30]	-0.74 [-2.25, 0.78]
H-ML/H-LL PA x Time	-2.43 [-5.30, 0.45]	-5.45 [-9.20, -1.70]**	-1.02 [-4.24, 2.21]	-4.66 [-9.30, -0.02]*	1.08 [-2.90, 5.06]	0.91 [-2.54, 4.36]	0.47 [-6.19, 7.14]
H-ML/H-LL PA x Time^2	0.49 [-0.18, 1.16]	1.18 [0.31, 2.06]**	0.34 [-0.41, 1.09]	1.07 [-0.02, 2.15] †	-0.45 [-1.39, 0.48]	-0.24 [-1.04, 0.57]	-0.20 [-2.01, 1.61]

†$p \leq 0.08$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Model 1: Adjusted for time, time^2, age, education and sex, Model 2: adjusted for model 1 plus BMI, hypertension, hypercholesterolemia, T2 diabetes. Abbreviations: L-ML, low midlife; H-ML, high midlife; L-LL, low late-life; H-LL, high late-life; PA, physical activity; CI, Confidence Interval. L-ML/L-LL PA= comparator

Appendix F. Chapter 8-9: Physical activity, BDNF, Aβ1-42 and cognition

Table F.1 Mixed-models regression exploring the association and interactions between BDNF, MLPA and LLPA on multi-domain cognition

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
Model 1							
Main Effects [a]							
BDNF	-0.16 [-0.28, -0.04]**	-0.09 [-0.20, 0.02]	-0.07 [-0.15, 0.01]	-0.06 [-0.19, 0.06]	-0.09 [-0.19, -0.00]*	-0.19 [-0.31, -0.06]**	0.16 [0.01, 0.30]*
MLPA	0.15 [-2.94, 3.78]	0.91 [-2.18, 4.00]	2.60 [0.38, 4.82]*	3.40 [-0.14, 6.93] †	-0.36 [-3.05, 2.34]	-0.61 [-4.45, 3.23]	3.32 [-1.04, 7.69]
LLPA	-0.60 [-4.17, 2.97]	0.39 [2.98, -3.76]	-0.66 [-3.09, 1.77]	-1.02 [-4.82, 2.77]	0.39 [-2.47, 3.25]	-3.96 [-7.97, 0.05]*	-1.12 [-6.01, 3.76]
Independent Main Effects [b]							
BDNF [c]	-0.10 [-0.23, 0.02]	-0.07 [-0.18, 0.05]	-0.06 [-0.14, 0.02]	0.00 [-0.13, 0.13]	-0.07 [-0.17, 0.03]	-0.21 [-0.35, -0.07]**	0.16 [0.00, 0.31]*
MLPA	0.55 [3.98, -2.87]	0.91 [4.04, -2.23]	2.68 [4.92, 0.43]*	3.40 [6.94, -0.14]	-0.27 [-2.45, 2.98]	-0.06 [-3.89, 4.01]	3.26 [7.61, -1.09]
BDNF	-0.16 [-0.28, -0.04]**	-0.09 [-0.20, 0.02]	-0.07 [-0.15, 0.01]	-0.06 [-0.18, 0.06]	-0.10 [-0.19, -0.01]*	-0.17 [-0.30, -0.03]*	0.16 [0.01, 0.31]*
LLPA	0.28 [-4.00, 3.43]	0.73 [-4.19, 2.74]	-0.33 [-2.15, 2.81]	-0.73 [-3.13, 4.59]	0.83 [-3.74, 2.09]	-2.68 [-1.50, 6.85]	-1.54 [-3.34, 6.42]
Interactions [d]							
BDNF x Time	0.02 [-0.06, 0.10]	0.05 [-0.06, 0.15]	-0.01 [-0.07, 0.06]	0.04 [-0.07, 0.15]	0.03 [-0.05, 0.11]	0.02 [-0.06, 0.09]	-0.02 [-0.31, 0.26]
BDNF x Time^2	-0.00 [-0.02, 0.01]	-0.01 [-0.03, 0.01]	0.00 [-0.01, 0.01]	-0.01 [-0.03, 0.01]	-0.00 [-0.02, 0.01]	-0.00 [-0.02, 0.01]	0.00 [-0.05, 0.06]
BDNF x *APOE*	-0.09 [-0.34, 0.16]	-0.19 [-0.41, 0.04]	0.07 [-0.10, 0.24]	0.04 [-0.23, 0.31]	-0.01 [-0.21, 0.19]	-0.03 [-0.34, 0.28]	-0.08 [-0.38, 0.23]
BDNF x MLPA	-0.27 [-0.55, 0.01]	-0.31 [-0.57, -0.06]*	-0.07 [-0.26, 0.11]	0.16 [-0.13, 0.45]	-0.01 [-0.24, 0.21]	-0.12 [-0.46, 0.21]	-0.19 [-0.54, 0.17]
BDNF x LLPA	-0.22 [-0.54, 0.09]	-0.22 [-0.50, 0.07]	-0.04 [-0.24, 0.17]	-0.20 [-0.51, 0.12]	-0.16 [-0.40, 0.08]	-0.21 [-0.57, 0.16]	-0.18 [-0.56, 0.19]

[a] BDNF and MLPA / LL-LPA in separate models; [b] Interactions in separate models. Controlled for age, sex, education, time, time^2. Abbreviations: BDNF, brain derived neurotrophic factor; *APOE*, apolipoprotein epsilon 4 carrier / non-carrier; MLPA, midlife leisure physical activity; LLPA, late-life leisure physical activity; CI, confidence interval. Comparators: Low MLPA, Low LLPA, *APOE*ε4 carrier. †$p \leq .08$; *$p \leq .05$;

Table F.2. Mixed-models regression exploring the association and interactions between Aβ1-42, MLPA and LLPA on multi-domain cognition among APOEε4 carrier and non-carrier groups

	Beta-Coefficients [95% CI]						
	Global Cognition	**Immediate Memory**	**Delayed Memory**	**Visuospatial**	**Language**	**Attention**	**Executive**
***APOE*ε4 Carrier** [a, b]							
$A\beta_{1-42}$	-0.14 [-1.16, 0.88]	-0.42 [-1.36, 0.52]	-0.16 [-0.83, 0.50]	-0.05 [-1.18, 1.08]	-0.40 [-1.30, 0.51]	0.68 [-0.60, 1.96]	-1.04 [-2.35, 0.27]
$A\beta_{1-42}$ x MLPA	-0.01 [-2.29, 2.27]	-1.74 [-3.73, 0.26]	-0.71 [-2.10, 0.67]	-0.74 [-3.22, 1.74]	1.18 [-0.83, 3.19]	2.34 [-0.49, 5.18]	1.35 [-1.53, 4.23]
$A\beta_{1-42}$ x LLPA	-1.29 [-3.39, 0.80]	-1.11 [-3.05, 0.83]	-0.84 [-2.21, 0.52]	-1.51 [-3.84, 0.82]	-0.17 [-2.06, 1.71]	-0.82 [-3.48, 1.85]	-0.96 [-3.79, 1.87]
***APOE*ε4 Non-Carrier** [a, b]							
$A\beta_{1-42}$	-0.51 [-1.08, 0.07]	-0.18 [-0.71, 0.34]	-0.37 [-0.79, 0.06]	-0.57 [-1.14, -0.00]	-0.07 [-0.52, 0.37]	-0.60 [-1.32, 0.12]	-0.36 [-1.11, 0.38]
$A\beta_{1-42}$ x MLPA	1.27 [0.17, 2.38]*	0.46 [-0.61, 1.52]	1.21 [0.37, 2.06]**	1.26 [0.14, 2.38]*	0.17 [-0.75, 1.08]	0.94 [-0.52, 2.40]	0.35 [-1.18, 1.88]
$A\beta_{1-42}$ x LLPA	0.67 [-0.97, 2.31]	0.87 [-0.64, 2.39]	0.16 [-1.07, 1.39]	-0.47 [-2.12, 1.17]	-0.49 [-1.78, 0.79]	1.82 [-0.22, 3.86]	-0.31 [-2.37, 1.76]

[a] $A\beta_{1-42}$ as a continuous variable in all analysis. [b] Interactions in separate models. Controlled for age, sex, education, hypertension, hypercholesterolemia, type II diabetes, BMI, time and time^2. Abbreviations: $A\beta_{1-42}$, plasma amyloid-beta$_{1-42}$; *APOE*, apolipoprotein epsilon 4 carrier/non-carrier; MLPA, midlife leisure physical activity; LLPA, late-life leisure physical activity; CI, confidence interval. Comparators: Low MLPA, Low LLPA, *APOE*ε4 carrier. *$p \leq .05$; **$p \leq .01$