# Examining sex differences in cognitive ageing and lifetime risk factors for dementia

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I, Louisa Patricia Needham, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

## Abstract

**Background:** Mechanisms of increased female dementia risk are currently unclear given a paucity of research explicitly examining sex differences in cognitive and brain ageing.

**Methods:** Using data from a mostly cognitively unimpaired cohort, the 1946 British Birth Cohort and its neuroscience sub-study, Insight 46, sex differences in life course cognitive performance and neuroimaging indicators of ageing-, vascular-, and Alzheimer's Disease pathology-related brain health at age ~70 are examined (Chapter 3). A cumulative measure of lifetime modifiable dementia risk factor exposures is derived and tested for associations with later-life cognition and brain health in males and females (Chapter 4). The extent to which the female-specific menopause transition associates with later-life cognitive and brain health outcomes is examined (Chapter 5). Multivariable regression analyses test the extent to which life course socioeconomic, health, lifestyle, and genetic *(APOE*-ε4) variables contribute to associations in both sexes.

**Results:** Females showed cognitive performance advantages across a range of assessments from childhood to later-life; socioeconomic and educational disparities suppressed female advantages. At age 70, males had smaller relative brain volumes and females had greater levels of cerebral small vessel disease (cSVD) pathology (Chapter 3). Modifiable dementia risk factor exposures, greater in males, negatively associated with later-life cognitive performance in both sexes and with smaller brain volumes in males, independently of early cognitive, socioeconomic, and *APOE*-ε4 predictors of risk exposures (Chapter 4). Later menopause age associated with better later-life cognitive and brain health outcomes in women (e.g. larger brain volumes), with associations partly explained by childhood cognition and health-related variables, respectively (Chapter 5).

**Conclusions:** Sex differences in cognitive resilience to brain ageing and cSVD, and in the life course sociocultural and biological mechanisms underlying cognitive resilience, were shown. Findings advocate for the importance of sex- and gender-based analyses to understand life course pathways to dementia in males and females.

## Impact statement

Evidence from this thesis that demonstrated sex differences in cognitive performance and brain health reinforces initiatives which advocate the value of sex- and gender-based analyses (SGBA) in health research. For example, the Medical Science Sex and Gender Equity (MESSAGE) initiative aims to publish a sex and gender policy framework for the UK health research sector.[1] The findings in this thesis also provide evidence to support improved research on women's health issues, given that menopause timing was associated with later-life cognitive performance and brain health. Evidence from these analyses and the wider scientific literature indicates that female-specific reproductive factors, including but not limited to menopause, hormone therapy use, and pregnancy, could contribute to sex differences in later-life outcomes. The work presented in this thesis supports recommendations for improved data collection regarding women's health issues in cohort studies, which is often insufficient to facilitate meaningful analyses.

This thesis also advocates for policies to provide better support for women, which could potentially reduce the burden of women's health issues on later-life outcomes. An example of such policy is the Women's Health Strategy for England, which aims to improve the ways in which the health and care system listens to women, providing improved access to relevant information and healthcare support. Demonstration that gendered socioeconomic and educational inequalities typically favouring males could limit female opportunities to develop and maintain protective structural and functional brain resources also highlights how policies to reduce such inequalities could benefit female outcomes. Such policies might include improving educational access, supporting women to return to work after having children, and encouraging men to take on greater child rearing responsibilities.

The need for public health policies aimed at reducing dementia risk factor exposures is also reiterated through evidence that greater lifetime exposures to modifiable risks associated with adverse later-life cognitive and brain health outcomes. Sex-stratified analyses demonstrated that greater risk exposures could accelerate brain ageing particularly in males, while brain ageing in females was more strongly associated with female-specific reproductive ageing. The development of effective public health policies to prevent or delay dementia onset in both sexes will require careful consideration for the specific variables which increase

dementia risk in each sex and when in the life course interventions might be most beneficial, which could differ between males and females.

Findings from this thesis have been disseminated through national and international conference poster presentations (Alzheimer's Association International Conference/AAIC Neuroscience Next 2020, Wellcome Longitudinal Studies 2021, AAIC 2021, Organisation for the Study of Sex Differences 2023, AAIC 2023) and various internal seminar talks, including participation in the 2023 UCL Institute of Cardiovascular Science 3-minute thesis competition. Analyses from Chapter 5, specifically the examination of menopause age associations with later-life cognitive performance measures, have been published in a peer-reviewed academic journal (*Maturitas*, 2023). By invitation, findings from Chapter 4 (examining cumulative dementia risk factor exposures) will be presented in international talks for the Alzheimer's Association Sex and Gender Professional Interest Area and the UCL Dementia Research Centre in April and June 2024, respectively.

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Louisa P Needham conceived and designed the study, performed the analyses, wrote the first draft of the manuscript, contributed to the interpretation of the results and critically revised the manuscript.

Kirsty Lu contributed to the interpretation of the results and critically revised the manuscript.

Jennifer M Nicholas supervised the statistical analyses, contributed to the interpretation of the results and critically revised the manuscript.

Jonathan M Schott contributed to the interpretation of the results and critically revised the manuscript.

Marcus Richards conceived and designed the study, contributed to the interpretation of the results and critically revised the manuscript.

Sarah-Naomi James conceived and designed the study, contributed to the interpretation of the results and critically revised the manuscript.

4. **In which chapter(s) of your thesis can this material be found?**

   Chapter 5i

5. **e-Signatures confirming that the information above is accurate** (this form should be co-signed by the supervisor/ senior author unless this is not appropriate, e.g. if the paper was a single-author work)

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# Table of Contents

# List of Tables

## List of Figures

# Abbreviations

| | |
|---|---|
| **Aβ** | Beta-amyloid |
| **ACE-III** | Addenbrooke's Cognitive Examination – 3rd edition |
| **AD** | Alzheimer's disease |
| **APOE** | Apolipoprotein E |
| **CRS** | Cumulative risks score |
| **cSVD** | Cerebral small vessel disease |
| **CVD** | Cardiovascular disease |
| **DSST** | Digit-symbol substitution test |
| **FA** | Fractional anisotropy |
| **FNAME** | Face-name associative memory examination |
| **GHQ-28** | 28-item general health questionnaire |
| **GMV** | Grey matter volume |
| **HT** | Hormone therapy |
| **MCI** | Mild cognitive impairment |
| **MD** | Mean diffusivity |
| **MICE** | Multiple imputation by chained equations |
| **MMSE** | Mini-mental state examination |
| **MRC** | Medical research council |
| **MRI** | Magnetic resonance imaging |
| **NART** | National adult reading test |
| **NAWM** | Normal appearing white matter |
| **NSHD** | National survey of health and development |
| **PA** | Physical activity |
| **PACC** | Preclinical Alzheimer's cognitive composite |
| **PAF** | Population attributable fraction |
| **PET** | Positron emission tomography |
| **QC** | Quality control |
| **RCT** | Randomised controlled trial |
| **ROI** | Region of interest |
| **SEP** | Socioeconomic position |
| **SUVR** | Standardised uptake value ratio |
| **TBI** | Traumatic brain injury |
| **TBV** | Total brain volume |
| **TIV** | Total intracranial volume |
| **WMHV** | White matter hyperintensity volume |

# 1.0. Introduction

Dementia is more common in women than in men; two-thirds of people with Alzheimer's Disease (AD; the most common form of dementia) are female.[2] Sex differences in dementia features (e.g. symptoms, biomarkers, progression)[3] suggest more complex explanations for increased female risk than higher female life expectancy alone, but the mechanisms underlying greater female risk are not yet well understood.

Traditionally, much dementia research has included only males or under-represented females, limiting our knowledge of dementia in females. More recently, the introduction of policies to include both sexes in research has increased female inclusion, but most studies simply adjust for sex in their analyses and do not explicitly examine potential sex differences. With the advent of emerging dementia treatments, understanding how dementia differs between males and females will be useful for treatment effectiveness and for identifying individuals who might benefit most. Additionally, identifying groups with the greatest risk can inform targeted strategies for dementia prevention, which may prolong improved quality of life and reduce the need for costly treatments which can induce side-effects. There is increasing recognition that while dementia primarily occurs at older ages, underlying pathology develops over several decades before symptoms emerge,[4] hence a life course approach to understanding dementia processes and risks is necessary.

Given the long preclinical phase in dementia, when there are pathological brain changes but preserved cognitive and functional abilities, subtle changes in performance on cognitive assessments and across multi-modal neuroimaging indicators of brain health could help us to understand the pathways leading to diagnosable dementia. This thesis examines data from a generally cognitively unimpaired cohort – the 1946 British birth cohort (the MRC National Survey of Health and Development/NSHD) and its neuroscience sub-study, Insight 46 – up to age ~70, when dementia diagnoses remain rare. NSHD is an age homogenous cohort studied since birth which is geographically representative of the UK and is comprised of ~50% females. A vast array of sociocultural, biological, and cognitive measures is available across multiple timepoints, including multiple indicators of brain health obtained using combined PET-MRI neuroimaging at age ~70 (Insight 46 only).

In this thesis, a life course approach is used to examine whether different biological and lifestyle factors measured throughout life associate with later-life cognition and brain health in males and females. Sex differences in the longitudinal trajectories of cognitive change over time are not examined given that such analyses have previously been conducted in NSHD; females showed slower decline in processing speed performance between ages 43 and 69, while there were no sex differences in verbal memory performance trajectories.[5] Lifetime factors which both males and females can be exposed to are considered and examined for sex differences in their associations with later-life outcomes, alongside consideration of sex-specific reproductive factors, namely menopause, which might contribute to female cognitive and brain ageing.

## 1.1. The life course approach

With acknowledgement that dementia, while typically presenting at older ages, develops throughout life, a life course approach is important for elucidating how sex differences in dementia occur. Some key concepts in life course epidemiology are the sensitive period and accumulation models.[6]

The sensitive period model outlines that exposures within a specific time frame have stronger effects than outside that period. This is distinct from a critical period, where exposures have little to no influence outside of a limited time window.[6] Many, but not all, critical periods are developmental. For instance, maternal health behaviours (e.g. smoking, diet) during pregnancy influence foetal brain development,[7] with lasting impacts throughout life. Relevant to this thesis is the concept that midlife may be a sensitive period, particularly for females. As will be discussed in Section 1.11, the female menopause transition has been proposed as a neurological transition state which could represent a period of vulnerability for female brains to develop dementia pathology.[8]

The accumulative model contrasts from sensitive period perspectives in that exposures at any stage of life are proposed to influence health outcomes, and such exposures have additive effects on outcomes.[6] There can be a 'chain of risk' whereby one exposure leads to another and so on, such that the number of risk (or protective) exposures continually increases.[6] For example, poorer socioeconomic circumstances during childhood could lead to poorer diet and

reduced educational attainment, increasing the risk for adverse health behaviours such as smoking and physical inactivity, which then further increase the risk for cardiovascular disease. The effects of an exposure in midlife thereby represent the summed effects of all previous exposures up until that point.[9]

In reality, sensitive period and accumulation models are not mutually exclusive. For example, early-life as a sensitive period for brain development may set in motion a subsequent chain of events which accumulate throughout life; poor brain development *in utero* or during early infancy could limit later educational attainment, reducing occupational opportunities in adulthood. In midlife, menopause could increase female sensitivity to cardiovascular risks (e.g. female hypertension risk increases at the time of menopause),[10] which may trigger a cascade of further adversities (e.g. reduced physical activity, social isolation) beyond the menopause transition.

The sensitive period and accumulative models of life course epidemiology are inherently difficult to test. Support for a chain of risks would require causal evidence for each step in the chain. The sensitive period model can only be proven if the assumption holds that, apart from the timing of exposure, all else is equal including the level of exposure and consistent covariables. While not explicitly tested, the concepts of sensitive periods of vulnerability to dementia risks, accumulation of risks throughout the life course, and their interrelationships are drawn on throughout this thesis.

## 1.2. Defining sex and gender

The terms sex and gender are often used interchangeably, and there are ongoing debates around how sex and gender should be defined, particularly regarding whether biological sex is in fact binary[11] and how gender can be measured.[12] For the purposes of this thesis, where the focus is on sex differences, sex refers to biologically defined characteristics (e.g. anatomical features, sex chromosomes, hormones) which are traditionally considered to distinguish males from females. Gender is self-identified and refers to the social constructs of masculinity and femininity, which lie on a spectrum.[13,14] Many sociocultural exposures are gendered and may interact with biological sex. For example, traditional gender roles are such that women take on childrearing, caregiving, and domestic responsibilities, while men work

to earn money and provide for their families. Gendered sociocultural factors can vary across cultures and generations.

## 1.3. Defining cognition

Cognition refers to a complex set of functions giving rise to knowledge, skills, thoughts, and behaviours. The main cognitive domains are perception, motor control, memory, attention, executive function, and language.[15]

Cognitive ageing is the decline in cognition experienced in late adulthood, as part of normal ageing. Crystalised abilities (knowledge and skills retained from previous experiences)[16] typically remain intact with advancing age. Tests of such abilities include assessments of general knowledge, vocabulary and reading comprehension (e.g. National Adult Reading Task/NART). Fluid abilities (those facilitating perception, interpretation, and responses to environmental stimuli)[16] tend to decline from midlife onwards.[17,18] Fluid ability assessments typically involve problem solving or rapid responses (e.g. mental rotation and choice reaction tasks).

## 1.4. Defining dementia

Dementia is a syndrome, typically occurring approximately after age 65 years, whereby cognitive function declines faster than expected for normal ageing and causes functional impairment.[19] Dementia is caused by various conditions affecting the brain, including disease and injury, with dementia sub-types having different causes and underlying pathology. AD, responsible for 60-70% of world-wide dementia cases,[19] is characterised by the pathological hallmarks of extracellular beta-amyloid (Aβ) plaques and intracellular neurofibrillary tau tangles. These pathologies, which can disrupt neuronal functioning and contribute to brain atrophy,[20] typically first appear in the hippocampi and entorhinal cortex. Memory impairment is therefore often, although not always, an initial symptom of AD. Cognitive impairment can first present as mild cognitive impairment (MCI), when patients experience cognitive decline beyond expected with normal ageing but daily living is not significantly disrupted. Amnestic MCI primarily affects memory, while non-amnestic MCI affects other cognitive domains.[21] In

dementia, as damage spreads throughout the brain cognitive impairment becomes more severe and more cognitive functions are affected.[22]

The presentation of cognitive impairment may differ across dementia sub-types, including frontotemporal dementia (FTD), Parkinson's Disease Dementia (PDD), Lewy Body Dementia (LBD), and vascular dementia. FTD, characterised by progressive symptoms which vary according to variant (e.g. executive function impairments with behavioural variant, speech expression difficulties with progressive non-fluent aphasia), often has a younger age at onset than most other dementias and has greater levels of genetic heritability.[23] PDD is associated with a movement disorder caused by a loss of dopaminergic neurons in the substantia nigra, leading to symptoms including bradykinesia, rigidity, resting tremor, gait disturbance, and speech difficulties.[24] LBD, which can co-occur with Parkinson's Disease, is characterised by abnormal accumulation of α-synuclein (Lewy Body) protein and, in addition to cognitive decline, can include symptoms such as visual hallucinations, depression, and Parkinsonism motor symptoms.[25]

Vascular dementia is caused by reduced blood flow to the brain, with symptoms varying dependent on the location of brain damage.[26] Large vessel disease (e.g. atherosclerosis, occlusion/stroke) causes neuronal injury and grey matter infarcts (tissue death due to reduced blood supply).[27] Small vessel disease (e.g. microbleeds, pathologic protein deposition) leads to axonal injury and white matter infarcts.[27] White matter hyperintensities, regions of intense signal on MRI, are examined in this thesis as indicators of axonal loss or ischaemic damage related to cerebral small vessel disease (cSVD).[28,29] There is a growing understanding of the vascular contributions to cognitive impairment and dementia (VCID), with cerebrovascular comorbidities often found across dementia sub-types.[30] For example, vascular and AD pathology comorbidities include reduced cerebral blood flow associated with Aβ deposition, impaired Aβ clearance with cerebrovascular changes, and increased risk of AD with greater levels of white matter hyperintensities.[30,31] While the directionality of these associations is unclear, it is important to understand how cerebrovascular changes might contribute to the development of dementia pathology and clinical symptoms.

### 1.4.1. Dementia diagnosis

There are three stages of dementia: preclinical, prodromal, and diagnosable dementia.[31] Preclinical dementia is defined by the presence of dementia pathology (e.g. Aβ) but the absence of symptoms. For AD, there is a long preclinical phase whereby pathological proteins are detected several years or decades prior to symptom onset.[4] The prodromal phase is MCI, which increases dementia risk, but some individuals with MCI never progress to dementia and cognition can return to normal.[21] Diagnosable dementia, according to the DSM-V (Diagnostic and Statistical Manual of Mental Disorders, fifth edition), requires significant decline in one or more cognitive domain, impacting the individual's ability to independently complete everyday activities.[32]

Dementia is typically diagnosed by a GP or specialists at a memory clinic. A clinical history is obtained, including informant (e.g. family member) accounts, to understand the patient's symptoms and their impact on daily living.[32] Clinical tests are also administered, such as the General Practitioner Assessment of Cognition (GPCOG),[33,34] to examine the patient's cognitive abilities across cognitive domains. Brain scans are not typically used in dementia diagnosis, particularly if dementia is the likely diagnosis based on clinical tests, but MRI can help to identify dementia sub-type (e.g. atrophy in the frontal and temporal lobes can indicate FTD) or to rule out other causes (e.g. brain tumour).[35] Indeed, dementia is a diagnosis of exclusion, whereby it must be determined that other neurological conditions (e.g. depression) do not better explain the clinical symptoms.[32]

Existing methods to detect pathological proteins, such as PET neuroimaging or cerebrospinal fluid (CSF) measures of Aβ and tau, for example, are not widely used in clinical settings due to cost and practicality limitations.[32] Work is ongoing to develop effective blood-based biomarkers of dementia;[36] plasma phosphorylated tau (p-tau), for example, shows promising associations with AD.[37] The feasibility of using plasma p-tau in clinical settings is being investigated, with potential benefits for detecting early dementia and for identifying individuals who might benefit from new AD treatments.

## 1.5. Measuring brain health

Historically, brain health was measured only *post-mortem*, but the development of neuroimaging and other (e.g. CSF and blood-based biomarkers) techniques now facilitate *in vivo* assessments of brain health and the presence of dementia pathologies.

Structural MRI measures of total brain volume (TBV) and hippocampal volume are some of the most well-established neuroimaging indicators of brain health. Smaller volumes indicate greater levels of brain atrophy, which gradually occurs with age and is accelerated by neurodegenerative processes linked with dementia.[38]

Cortical thickness also reduces with age and cortical atrophy accelerates with dementia, with the pattern of thinning across brain regions varying depending on dementia type.[18] MRI signatures of AD have been derived, comprising cortical regions of interest across which thinning is characteristic of AD.[19,20]

White matter hyperintensities on MRI, which indicate cSVD and associate with increased dementia risk,[28,29] were previously manually counted but can now be measured using an automated pipeline to generate white matter hyperintensity volume (WMHV).[39] There is also increasing recognition that changes in the microstructural integrity of normal appearing white matter (NAWM) could be a precursor to the development of white matter hyperintensities,[40] with changes in NAWM also linked with ageing[41] and cognitive decline.[42] Measures of NAWM are relatively new and asses axonal integrity using MRI techniques such as diffusion tensor imaging (DTI) which measures the diffusion properties of water molecules within NAWM; greater diffusivity indicates reduced microstructural integrity.[42]

PET neuroimaging uses radioactive tracers to detect the presence of different molecules within the brain. Tracers which bind to Aβ or tau proteins have been relatively recently introduced, facilitating *in vivo* neuroimaging which can detect preclinical levels of AD pathology.[43]

With advances in neuroimaging techniques, multimodal methodologies such as combined MRI and PET imaging can now be used to examine the underlying disease processes of dementia, including how vascular and other pathologies develop across different dementia phases and sub-types.[44] These techniques will be useful for detecting the early, preclinical phases of dementia; with the advent of new treatments aimed at reducing levels of

pathological proteins in the brain, early dementia screening could identify individuals who are most likely to benefit from such treatments. Additionally, neuroimaging methodologies sensitive to subtle brain changes can be valuable endpoints in clinical trials aiming to slow or reverse dementia processes.

## 1.6. Resilience, reserve, and resistance

The overlapping concepts of resilience, reserve, and resistance, although an evolving field, can offer a framework through which individual differences, including sex differences, in cognitive abilities and susceptibility to brain ageing and dementia may be understood.[45] Resistance refers to the ability to avoid pathologies; some individuals may show low levels of pathology despite high levels of risk.[46] In contrast, resilience refers to the brains' ability to maintain cognitive function in spite of brain ageing and pathology, encompassing various mechanisms through which individuals may be better able to manage the impacts of pathology: cognitive reserve, brain reserve, and brain maintenance.[45] Cognitive reserve describes the adaptability of functional brain processes while brain reserve refers to structural brain characteristics (e.g. number of neurons and synapses) which can protect against the effects of pathology. Brain maintenance reflects the plasticity of the brain, whereby genetics and lifestyle have the potential to enhance brain structure and function. Therefore, across the life course there are opportunities for risk and protective factors to limit or enhance the ability of the brain to avoid pathology (resistance) and to cope with pathology (maintain cognitive function and brain structure; reserve) when it does emerge.

## 1.7. Sex differences in cognition

Whether males and females differ in their cognitive abilities has traditionally been a topic of scientific and popular interest. A wealth of studies have been conducted, although limitations, particularly in older studies, include the impact of researcher bias and failure to consider societal, gender-based influences such as stereotypical beliefs about men and women's abilities.[47,48]

Nonetheless, the most consistent sex differences reported are male advantages in spatial abilities and female advantages in verbal abilities. Males typically outperform females on mental rotation tasks with moderate effect sizes across the life course, but other spatial assessments (e.g. spatial visualisation, spatial perception) show only small male advantage.[49] The most consistently reported female advantage is in verbal fluency, although effect sizes are also typically small, and meta-analyses do not support statistically significant sex differences in crystallised verbal abilities such as vocabulary or reading comprehension.[47] The description of variations between male and female cognitive task performance as sex differences should be interpreted with caution, given that most 'differences' refer to mean performance levels while there is still a large amount of overlap in performance scores for males and females.[47] Additionally, the extent to which biological sex explains observed differences in cognitive performance between males and females is debateable, with evidence showing that when the gendered context of task instructions is altered, sex differences in task performance can be removed. For instance, describing a mental rotation task as assessing stereotypically female skills (e.g. handicraft abilities) removed the male advantage in mental rotation task performance.[50]

There is also a lack of consensus regarding whether males and females vary in their rates of cognitive decline with advancing age. Across five US cohorts[51] (median baseline age=58 years, median follow-up=7.9 years) no sex differences in the rate of memory decline were detected but overall cognitive state and executive function declined faster in women. Conversely, in the English Study of Longitudinal Ageing (ELSA; mean baseline age men=64.6 years, women=65.0 years), cognitive state, executive function and memory declined slower in women, over 8 years.[52] Data from NSHD also showed slower decline in women on repeated search speed measures between ages 43 and 69 years, although there were no sex differences in rates of verbal memory decline.[5] A systematic review of longitudinal research on cognition in people aged 60 years and over (follow-up range 5-17 years) concluded that men and women have similar rates of age-related cognitive decline, although it remains unclear whether sex differences in cognitive decline are present after age 80.[53] Inconsistent findings across studies could reflect variations in sample age ranges, since rates of age-related cognitive decline might not be linear. Additionally, there may be cultural differences in gendered exposures between samples; in the US women might be less likely to work than

women in the UK, which might contribute to varied sex differences in cognitive decline between the two countries.

Societal gender roles also vary over time, reflected by generational variations in socioeconomic opportunities, including educational access which has traditionally been limited for women but has increased over time.[46] Such generational changes have implications for sex differences in cognitive performance. For example, in the ELSA and Whitehall II UK-based cohorts, secular improvements in women's access to education associated with better female performance on verbal memory and verbal fluency tasks, and females in more recent generations (with greater access to education) outperformed males on verbal fluency while males outperformed females in earlier-born generations.[54] The impact of societal influences on cognition demonstrates that cognitive performance differences between males and females are not wholly biologically determined, and that observed sex differences may not be consistent across cultures or generations, given variations in gendered sociocultural exposures.

### 1.7.1. Summary

Overall, findings demonstrate that men and women have similar abilities in most cognitive domains, in agreement with the gender similarities hypothesis[55] which also states that observed differences can vary dependent on factors such as the context of performance measurement. When sex differences in mean performance are seen they tend to be subtle with external, gendered societal influences offering some explanation for the differences. It is also unclear whether males and females differ in their rate of age-related cognitive decline. Most longitudinal research addresses cognitive change from late adulthood onwards; it is unclear whether there are sex differences in cognitive change at midlife, a time when many risk factors for dementia are present.[56] Further, the cognitive tests typically assessed in epidemiological studies may not be sensitive to detect subtle, early dementia-related cognitive decline or potential sex differences in such changes.

## 1.8. Sex differences in the brain

After accounting for brain size, which is typically larger in males throughout life, sex explains only 1% of the variance in regional brain volumes.[57] Historical reports of greater hippocampal volume in males than females, when brain size was not accounted for,[47] do not therefore represent a robust sex difference in hippocampal volumes.

Some evidence does, however, indicate sex differences in levels of brain activation, even when cognitive task performance is equal. For instance, females are reported to have greater global cerebral blood flow, measured using SPECT (single photon emission computed tomography) and PET imaging techniques, during both rest and cognitive activities including when no significant sex differences in cognitive task performance are detected.[58,59] Females are also reported to have greater levels of cerebral glucose metabolism than males,[58] although evidence has shown variations in females' regional glucose metabolism across menstrual cycle phases,[60] indicating a potential role of sex hormones on brain activation. Despite such evidence though, most sex differences research does not report or consider how menstrual cycle phases in females of reproductive age might contribute to observed sex differences or similarities.

Although sex differences in Aβ are not consistently reported in people with and without cognitive impairment or dementia,[61] sex differences in other neuroimaging indicators of dementia-related pathology have been identified. For instance, females are found to have poorer NAWM microstructural integrity than males,[62-64] and females reportedly have greater WMHV,[65] particularly at older ages when women are post-menopausal.[66] Additionally, in the longitudinal Rotterdam study (n=5,286, mean baseline age=64.4 years, mean follow-up=5.2 years), males started to show increases in WMHV at younger ages than females,[67] further indicating that trajectories of cSVD differ between the sexes. Importantly, health and lifestyle behaviours which can show gendered differences in prevalence (e.g. physical inactivity is typically greater in women, alcohol consumption is higher in men) have been linked with indicators of cerebrovascular disease; sociocultural exposures may therefore contribute to observed sex differences in brain health.[68]

Males in the Rotterdam study have also shown earlier and faster decline in global and regional grey matter volumes than females, although significant sex-by-age interactions were not

detected.[67] The Baltimore Longitudinal Study of Ageing (n=617, mean baseline age=71.2 years, up to 20 years follow-up) did, however, find a significant sex difference in the annual rates of decline in TBV and grey matter volume (GMV) which were faster in males than females.[69] Similar to discrepancies in cognitive sex differences (Section 1.7.), these variations across studies might be explained by non-linear age-related trajectories of brain health, and by sociocultural differences between samples. For example, variations in educational access and occupations might contribute to differential levels of brain maintenance between males and females.

### 1.8.1. Summary

Although evidence for sex differences in regional brain volumes is weak, there is support for sex differences in some neuroimaging indicators of dementia-related pathology and in rates of brain ageing. Despite greater levels of brain activation than males, females show poorer cerebrovascular health (indicated by reduced NAWM microstructural integrity and greater WMHV). Males show earlier and faster rates of WMHV increases and brain atrophy, but it is possible that at older ages and post-menopause, female trajectories of brain ageing accelerate. Sociocultural exposures which differ between genders, including health and lifestyle behaviours, are important to consider when elucidating the potential mechanisms underlying brain health sex differences.

## 1.9. Sex differences in dementia

While overall rates of dementia are more frequent in females than males, largely driven by greater AD prevalence in females,[2] sex differences can vary by dementia type; LBD, PDD, and behavioural variant FTD are more frequent in males, and primary progressive aphasia FTD is more prevalent in females.[70] Variations in dementia symptoms and rate of progression between sexes are also recognised. Female PDD patients are more likely to have visuospatial difficulties, while males more commonly experience verbal fluency and emotion recognition problems.[24] In AD dementia, females typically experience greater cognitive impairment than males, with cognitively normal female advantages in verbal memory, for example, reversed in AD patients.[61] Female AD patients also show faster rates of cognitive decline than male AD

patients, although this could be partially explained by females typically receiving AD diagnoses at later disease stages than males.[61] Such sex differences demonstrate that there are likely variations and nuances in dementia development processes and mechanisms between males and females. Although these differences are not yet well understood, some theories have been proposed.

A common perspective is that females have greater dementia risk due to their greater longevity – dementia incidence and prevalence rates increase with age and females live longer than males,[71] even with AD diagnoses.[72] When accounting for age, studies in the US have not found sex differences in dementia rates,[73,74] giving some support to the theory of female longevity. However, several studies in Europe and Asia have found higher dementia rates in females even when accounting for age,[75] meaning that female longevity did not fully explain increased female dementia risk in these populations. Societal influences which vary across cultures are therefore important considerations, including gendered differences in education, occupations, caregiving responsibilities, and health and lifestyle behaviours (e.g. diet, smoking, physical activity). The role of survivor bias must also be considered and forms part of the debate as to whether female longevity explains dementia sex differences; cardiovascular disease increases dementia risk, but mortality rates from cardiovascular disease are higher in males than females, meaning that males surviving to older ages have better cardiovascular health and therefore lower cardiovascular risks for dementia.[61,71]

Interestingly, longevity has been linked with sex chromosomes across species, with organisms carrying two X chromosomes surviving for longer.[76] A study using AD mouse models demonstrated that genetic sex (XX[female], XY[male]) had a greater impact on survival than phenotypic sex (expression of testes or ovaries), and that this was driven by the presence of two X chromosomes rather than absence of the Y chromosome.[77] Females are potentially afforded some level of resilience from having two X chromosomes, possibly explaining greater female longevity, even with AD. However, females can experience gene dosage complications. Across different female cells, one X chromosome is randomly inactivated to regulate gene dosage, but some genes escape inactivation.[78,79] While this is an evolving field of research, it is intriguing that many genes found to consistently escape inactivation are immune related.[79]

The immune system has a role in dementia, with neuroinflammation contributing to dementia pathology. In AD, microglia (the brain's immune cells) are activated in response to Aβ but

become less efficient at Aβ clearance after prolonged periods of activation. In a downward spiral, microglia are chronically activated and release toxins which damage neurons, leading to neurodegeneration.[80] Widely reported sex differences in immune responses, with females having stronger immune responses and higher incidence of autoimmune conditions than males,[81] has led to the theory that variations in the immune system could underly dementia sex differences. Indeed, microglia activation is found to mediate the relationship of Aβ with tau deposition only in females,[82] demonstrating sex differences in immune-related mechanisms for the development of dementia pathologies.

Immune responses have also been linked with sex hormones, with stronger responses in females observed only after puberty, when both males and females experience a surge in sex hormone levels.[81] Given that variations in sex hormone levels are among the biological features distinguishing males from females (males have higher androgen levels, females have higher oestrogen and progestogen levels), much literature (discussed further in Section 1.11.) has focused on sex hormone contributions to dementia sex differences. Briefly, sex hormone receptors are found throughout the body, including the brain, and oestrogen in particular is known to have protective effects in the brain and the cardiovascular system.[83,84] Additionally, females have more marked hormonal changes than males throughout the life course including the menstrual cycle, pregnancy, and menopause, demonstrating female-specific processes which could contribute to female brain health and dementia risk. The menopause transition, as will be discussed in Section 1.11., has received much attention; falling levels of neuroprotective oestrogen during menopause are proposed to increase the vulnerability of the ageing female brain to the development of dementia pathologies.[8] Women going through the menopause transition can also experience a range of symptoms including 'brain fog', hot flushes, sleep disturbance, and low mood. These can have implications for women's work lives, social relationships and lifestyle behaviours including engagement with physical activity, which may further contribute to female dementia risk.[85]

Finally, while much sex differences literature focuses on why dementia risk is increased in females, another approach is to consider why males have reduced risk, drawing on the concept of resilience (Section 1.6.). Cognitive reserve can be built via various lifetime experiences including education, occupational complexity, and SEP, which are traditionally higher in men, meaning that men generally have greater opportunities to build cognitive

reserve than women.[86] It has also been shown that, for each additional unit of AD pathology (measured *post-mortem* in 141 members of the Religious Orders Study), the odds of clinical AD increased 3-fold in males compared with 20-fold in females, demonstrating greater male resilience to AD pathology.[87] Additionally, in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, increases in CSF AD biomarkers (Aβ-42 and total tau) associated with longitudinal hippocampal atrophy and decline in executive function performance (average 2.5 year follow-up) to a greater extent in females than males, and this was exacerbated among females with low education levels.[88] While some evidence indicates greater cognitive resilience to AD pathology in males, further sex stratified research is needed to understand how males develop greater resilience and whether interventions to improve female opportunities to build cognitive and brain reserve could reduce overall female dementia risk.[86]

### 1.9.1. Summary

Various theories for dementia sex differences have been proposed, which are unlikely to be mutually exclusive. For example, immune responses are linked with sex chromosomes and hormonal changes.[81] Theories highlight biological distinctions between males and females, indicating that some sex-specific processes (e.g. menopause in females) could contribute to dementia sex differences. However, there is also a role for sociocultural, health, and lifestyle factors which both sexes can be exposed to, with gendered variations in such exposures possibly contributing to sex differences in resilience mechanisms.

## 1.10. Dementia risk factors

Several factors have been identified which can increase individuals' risk of developing dementia. Some are non-modifiable, while others have the potential to be modified.

### 1.10.1. Individual characteristics

Genetics can influence dementia risk; *APOE* is strongly associated with dementia, particularly AD.[89-91] *APOE* codes for apolipoprotein E (ApoE), a lipid transporter protein involved in maintenance of the central nervous system (CNS) and Aβ clearance, and has three alleles –

ε2, ε3, and ε4.[92] One copy of the *APOE* ε4 allele (ε4 heterozygous) increases AD risk 2- to 3-fold, while two copies (ε4 homozygous) increases risk 12-fold, compared with ε3 homozygotes.[89-91] While genetic risk is traditionally non-modifiable, gene therapy techniques are a potential route through which the AD risk associated with *APOE*-ε4 could be modified. An ongoing clinical trial is investigating whether a viral vector carrying the ε2 allele, which is protective against AD pathology, can safely increase expression of the ApoE ε2 protein in ε4 homozygotes with Aβ pathology and MCI or moderate dementia diagnoses.[93]

Other individual characteristics linked with dementia risk include age and sex. Age is the greatest dementia risk factor (risk doubles every five years after age 69),[94] and female sex associates with increased risk, given greater dementia rates in females than males.[2] Further demonstrating exacerbated dementia risk in females, ε4 heterozygous females are also found to have a higher AD risk than heterozygous males between ages 65 and 75 years,[95] highlighting that there are interrelationships between different risk factors.

### 1.10.2. Health and lifestyle risks

The 2020 Lancet commission on dementia prevention, intervention and care[56] reviewed existing literature and identified twelve modifiable factors across the life course which were most convincingly associated with dementia risk. The authors estimated that if all twelve risks were removed, up to 40% of worldwide dementia cases could be delayed or prevented.

Low education was identified as an early-life (<45 years) risk. Midlife (age 45-65 years) risks are: hearing impairment, traumatic brain injury (TBI), hypertension, high alcohol consumption, and obesity. Later-life (>65 years) risks are: smoking, depression, social isolation, physical inactivity, diabetes, and exposure to high levels of air pollution.[56] Some risk factors, such as low education, hearing impairment, and social isolation, represent how reduced opportunities for cognitive stimulation could limit the development and maintenance of cognitive reserve which is protective against dementia.[45,96,97] Others reflect the impact of cardiovascular health on brain health. For instance, persistent high blood pressure in midlife associates with reduced brain volumes and greater WMHV,[98] and low physical activity associates with increased dementia risk particularly among individuals with existing cardiovascular comorbidities.[99] Some risks reflect trauma and insults to the brain

which could initiate neuroinflammatory processes. For example, TBI is associated with neuroinflammation and tau deposition, and smoking and air pollution could lead to toxic particles impacting the brain, also contributing to neuroinflammation.[56,100]

While the Lancet risks are based on an extensive review of available literature, it is worth noting that the twelve risks identified are not exhaustive. It is probable that other health and lifestyle factors contribute to dementia risk, but further research is required to provide convincing evidence for an association and clarity on how interventions could reduce risk. For example, sleep disturbance has been associated with various indicators of dementia-related pathology including neuroinflammation and tau and Aβ deposition.[56,101] Both short (<5 hours) and long (>10 hours) sleep duration associate with dementia risk, but the mechanisms for how sleep disturbance contributes to dementia processes are currently unclear and evidence for the effects of sleep disturbance medications (e.g. hypnotics, benzodiazepines) is mixed.[56,102]

There is evidence that reducing modifiable risk factors can have cognitive benefits. The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) is a randomised controlled trial (RCT) of a 2-year multidomain lifestyle intervention comparing regular health advice (control group) with a combination of dietary, exercise, cognitive training and vascular risk monitoring interventions (intervention group), among older-aged (60-77 years at baseline) individuals at increased risk of dementia.[103] Over two years from baseline, the intervention group showed a 25% greater improvement in neuropsychological test battery performance than the control group,[104,105] showing that multidomain lifestyle interventions can be beneficial in maintaining or improving cognitive performance in old age, which could protect against cognitive decline.

Further recognising interrelationships between dementia risk factors, dementia risk scores have been derived to quantify the impact of multiple risk factors on overall dementia risk and to predict individuals' level of dementia risk. Some scores, such as the lifestyle for brain health index (LIBRA),[106] include only modifiable risk factors. Others, such as the cardiovascular risk factors, ageing, and dementia (CAIDE) score,[107] include modifiable and non-modifiable risks. While increased dementia risk scores have been associated with poorer cognitive performance and with poorer neuroimaging measures of brain health, including greater WMHV and smaller brain volumes,[108] existing risk scores are not found to accurately predict

future dementia.[109] Several predictive dementia risk scores have been created, but to develop effective scores with useful research and clinical applications, there needs to be consideration for whether the risks included can be measured practically and, ideally, incorporate modifiable risks such that interventions might reduce dementia risk.[110]

### 1.10.3. Sex differences in dementia risk factors

A recent study quantified midlife exposures to eight of the twelve Lancet risks in an online survey sample, finding that males had more risk exposures but that the adverse effect of increasing risks on a composite measure of cognitive performance was greater in females than males.[111] Additional studies assessing whether the impact of multiple lifetime risk factor exposures differs between the sexes are, however, currently lacking.

There are established sex differences in the prevalence of some individual risk factors. For example, depression and obesity are more common in women,[112-116] while smoking, high alcohol consumption and hypertension are more prevalent in men.[117-121] However, such differences are not always consistent across the life course. For example, males have greater cardiovascular risks and develop cardiovascular disease (CVD) at younger ages than females, who show increases in CVD at midlife and post-menopause such that the incidence of CVD in later-life is similar between males and females.[122,123]

Aside from sex differences in prevalence, it is also useful to understand whether the dementia risk associated with each factor is equal in males and females. Some studies suggest that hypertension-associated dementia risk differs by sex and age at hypertension onset. In one study (n=5,646, 45.2% female; mean follow-up=15.3 years), mid-adulthood (mean age=44 years) hypertension associates with increased female all-cause dementia risk, while early adulthood (mean age=33 years) hypertension does not associate with risk in either sex.[124] However, others report that mid-adulthood (<65 years) hypertension associates with increased male AD risk, while late adulthood (≥65 years) hypertension does not associate with AD risk in either sex (n=848,505, 42.2% female; mean baseline age women=53.6 years, men=51.9 years; follow-up=14 years).[125] Variations in the type of dementia examined and sample ages might explain discrepancies between studies, but also highlight how sex

differences in risks are not necessarily consistent across the life course and highlight the value of considering nuances in risks associated with different dementia types.

Indeed, sex differences in diabetes associations with dementia-related outcomes differ across studies examining different dementia types and across cognitively impaired and cognitively intact cohorts. In a pooled analysis of over 2 million individuals, diabetes-associated vascular dementia risk was 19% higher in women than in men, but non-vascular dementia diabetes-related risk did not differ by sex.[126] Within the ADNI cohort (n=911, 46% female; average baseline age women=72.2 years, men=73.9 years; average follow-up=4.1 years), no sex differences in prediabetes-associated dementia risk (not specified by dementia sub-type) were found, while in MCI patients only women showed a prediabetes-associated decline in glucose metabolism, and executive function and language performance.[127] Conversely, in a cognitively intact cohort, no significant associations of diabetes status with global or domain-specific cognitive performance were found in either sex.[128] In Insight 46, a mostly cognitively unimpaired cohort, hyperglycaemia (a marker of diabetes) associated with smaller TBV only in females.[129] Together, studies suggest that diabetes-associated dementia and cognitive outcomes are worse for women than men, but the associated risks may vary according to type of cognitive impairment and whether baseline cognition is intact.

Some studies have examined sex differences in risk profiles. In the Italian Longitudinal Study of Ageing (n=2,501, 56.3% female; mean baseline age=71.3 years), sex-stratified analyses indicated that baseline older age, lower education, heart failure, Parkinsonism, mild depressive symptoms, and family history of dementia associated with increased male dementia incidence at follow-up (median 7.8 years). Female dementia incidence associated with older age, lower education, current smoking, lower BMI, and mild and severe depressive symptoms.[130] In a French population-based cohort (n=6,892; mean baseline age women=74.3 years, men=74.0 years), male risks for conversion from MCI to dementia over 4 years of follow-up were *APOE*-ε4, stroke, low education, age and loss of instrumental activities of daily living (IADL), although IADL loss is a requirement for dementia ascertainment. Female risks were similar, except stroke was not a risk while subclinical depression and anticholinergic drug use were.[131] In the UK Biobank (n=502,226, 54.4% female; mean baseline age women=56.3 years, men=56.7 years; median follow-up=11.8 years), being overweight or obese associated with higher dementia risk in women than in men, as did increased systolic

blood pressure.[132] These sex-stratified analyses indicate that dementia risk profiles may differ between sexes, although which factors are included in male and female risk profiles remains unclear. Additionally, given that these studies only assessed risk factors present in mid- or later-adulthood, the relevance of risk factor timing in the life course remains unclear and it is difficult to determine whether the factors identified indicate dementia risks or prodromal features of dementia.

### 1.10.4. Summary

Several dementia risks are identified, but there is currently insufficient evidence to fully understand sex-by-risk interactions on later-life cognitive, dementia and brain health outcomes. The 2020 Lancet commission outlined twelve modifiable risk factors spanning early-, mid-, and later-life, but most existing research only considers mid- or late-adulthood risks, omitting earlier risks, and it is unclear whether sex interacts with the age at which dementia risks are present. The prevalence of some individual risk factors differs by sex, indicating how sociocultural norms around health and lifestyle behaviours (e.g. smoking, alcohol consumption, physical activity) could contribute to dementia sex differences. Risks also interact with one another, and while several dementia risk scores have been created to quantify multiple risk exposures, there is debate over which risk factors should be included, with no consensus on how risk profiles might differ by sex, and existing scores do not perform well in predicting dementia.

## 1.11. The role of menopause in female cognitive and brain ageing

While variations in the prevalence and influences of lifetime risk factors which both sexes can be exposed to can provide some insight into dementia sex differences, the role of sex-specific exposures are also of interest. Marked hormonal events in females (e.g. menarche, pregnancy, menopause) represent female-specific experiences. Menopause, which typically occurs in midlife[133] and can involve neurological symptoms,[8] has received much attention regarding female dementia risk.

### 1.11.1. The menopause transition

Menopause marks the end of a woman's reproductive period and is reached after 12 consecutive months without menstrual bleeding.[134] It usually occurs between age 45-55 years,[133] but is considered premature before age 40 years, early before age 45, and late after age 55.[135-137]

The Stages of Reproductive Ageing Workshop (STRAW) criteria[138] outline five broad stages of reproductive ageing characterised by changes in menstrual cycle length and hormonal levels: late reproductive, early menopausal transition, late menopausal transition, early postmenopausal, and late postmenopausal. Perimenopause refers to the early and late menopausal transition; the time between the start of the transition through to menopause being reached which can last 2 to 8 years, with a mean duration of 5 years.[139] During perimenopause, oestrogen levels gradually decline and remain low post-menopause.[134] As the body adjusts to reduced hormone levels, women may experience symptoms including hot flushes, sleep disturbance, depressive affect, mood fluctuations and cognitive impairment.[8,140] Such symptoms can have adverse impacts on social relationships, performance at work, and the ability or motivation to engage in physical activities.[85] Hormone therapy (HT) can help to alleviate symptoms by replacing falling endogenous hormones with hormonal medications, although there is debate around the risks and benefits of HT, which will be discussed in Section 1.11.5.

While natural menopause involves a gradual decline in sex hormone levels over several years, menopause can be surgically induced resulting in a more acute fall in oestrogen levels. Reasons for surgery might include treatment or prevention of certain cancers, or to reduce symptoms of conditions such as endometriosis.[141] Surgeries which can induce menopause include hysterectomy (removal of the uterus) and oophorectomy (removal of one or both ovaries), which may be combined or performed independently. When both ovaries are conserved, oestrogen decline is more gradual than when one or both ovaries are removed.[142]

### 1.11.2. Menopause and cognition

#### *1.11.2.1. Cognitive symptoms during the menopause transition*

Around two-thirds of women experience subjective cognitive impairments ('brain fog') during menopause,[143] including concentration and memory problems.[144] Objective measures of cognitive performance correlate with subjective reports, although women typically still perform within normative ranges throughout the menopause transition.[143,145-147]

Additional impacts of the menopause transition on performance at work and social relationships, coupled with a general lack of awareness for how menopause can impact women, could induce additional stressors during the transition,[85] further affecting cognitive function. Cognitive difficulties might also be secondary to other neurological menopausal symptoms (e.g. sleep disturbance),[146,148] which typically subside post-menopause.[149] Suggesting that cognitive difficulties during menopause are transient, perimenopausal women are shown to experience more cognitive difficulties than pre- or post-menopausal women,[146] and perimenopausal women are shown to have poorer ability to learn from previous experiences (with fewer practise effects on repeated verbal memory and processing speed tasks over 4 years) than pre- and post-menopausal women.[145] Conversely, another study with 14 years of follow-up reported worsening verbal memory throughout successive menopause stages (independently of age, race, BMI, education and baseline cognition), contradicting the argument that cognition 'recovers' post-menopause.[147] Unlike studies which do not find poorer cognition post-menopause, this study did not include women taking hormonal medication - a difference which might explain discrepant findings given some evidence for cognitive differences between hormonal contraceptive or HT users and non-users.[145]

#### *1.11.2.2. Long-term cognition post-menopause*

Long-term cognitive outcomes post-menopause are generally found to be poorer in women who undergo surgical rather than natural menopause,[150,151] but indications for surgical menopause may confound such associations. For example, cardiovascular risk factors associated with later-life cognition[152] can also increase the likelihood of surgically induced menopause.[153]

Surgical menopause, by definition, occurs at earlier ages than natural menopause, hence it can be difficult to parse the effects of surgery from menopause timing, and many studies examining the effects of menopause timing on long-term outcomes post-menopause exclude women who had a surgical menopause. A review of 13 studies (4 of which included surgical menopause) found that, overall, earlier age at menopause associated with poorer cognitive performance in later-life, although no cognitive domains were consistently reported across studies.[154]

Most research relies on retrospective self-reports of menopause timing, which may induce error; women with poorer cognition or cognitive impairment might have difficulty recalling their age at menopause. Kuh et al. (2018)[155] tested associations between prospectively reported age at menopause and cognitive performance within the 1946 British birth cohort. Age at natural or surgical menopause did not associate with processing speed at any age between 43-69 years, but later age at natural menopause associated with better verbal memory performance across all timepoints. Associations remained after accounting for prospectively measured covariables including BMI, education, occupation, and childhood cognition, although there was some attenuation with childhood cognition which is shown to associate with menopause age[156,157] and with later life cognition.[158,159] Childhood cognition, which may reflect upstream developmental processes and represent a proxy indicator of lifetime oestrogen exposure,[156] is therefore an important confounder of menopause-cognition associations, but most studies are unable to account for this.

### 1.11.3. Menopause and brain health

Brinton et al. (2015)[8] propose that perimenopause, while primarily a reproductive transition, is also a neurological transition, given the neural nature of some menopausal symptoms (i.e. sleep circadian rhythm disruption, thermoregulation difficulties). Disruption in the brain oestrogen system during menopause is hypothesised to impair neural metabolism, leaving the brain vulnerable to further neural decline. However, determining whether hormonal changes lead to brain changes or vice versa is difficult given the synergistic roles of the brain and reproductive organs in hormone production via the hypothalamus-pituitary-gonadal (HPG) axis.[160] A small cross-sectional study (n=40)[161] found that some regional grey matter volumes were smaller in post- than pre-menopausal women, with volumes positively

correlated with oestradiol levels in both groups, demonstrating potential hormonal mechanisms for volumetric differences.[161] However, most studies do not include hormone measurements.

There is evidence of cerebral, structural, metabolic, and functional changes across menopause stages. Over 3 years of follow-up, peri- and post-menopausal women showed greater increases in Aβ levels, alongside faster rates of memory performance decline, than pre-menopausal women and age-matched males.[162] Levels of glucose metabolism and some grey matter volumes are also found to be reduced in peri- and post-menopausal women compared with pre-menopausal women and age-matched males, although over a 2 year follow-up levels of decline stabilised post-menopause and GMV in the precuneus increased.[163] UK Biobank research (excluding women with hysterectomy or bilateral oophorectomy, HT use or stroke) also showed that TBV and hippocampal volumes were greater in post- than in pre-menopausal women,[164] supporting 'recovery' of or improvements in brain volume post-menopause. However, older postmenopausal UK Biobank women also showed faster TBV decline over time than older premenopausal women,[164] suggesting that menopause contributes to accelerated brain ageing. This could have implications for female health in older age, with accelerated reductions in structural brain reserve potentially increasing female vulnerability to brain pathologies and functional impairments. The timing of health and lifestyle dementia risk factor exposures (Section 1.10.2) in relation to menopause are therefore important. For example, the female brain could be more sensitive to the adverse impacts of hypertension during menopause than during other life stages. Indeed, evidence associating cardiovascular risks with increased markers of cerebrovascular disease in females was strongest for midlife cardiovascular risks.[40]

UK Biobank data also shows that later menopause age associated with smaller TBV and hippocampal volumes,[164] seemingly contradicting evidence suggesting that later menopause is beneficial for postmenopausal cognition and that early menopause might increase dementia risk.[151,154] However, these neuroimaging measures were not analysed alongside cognitive outcomes, hence conclusions on whether the reported associations are linked with cognition in a beneficial or detrimental manner cannot be inferred.

Overall, neuroimaging research suggests that the menopause transition associates with changes in GMV and neurodegenerative markers such as Aβ load and glucose metabolism.

Few studies have linked brain changes with hormonal measures, making conclusions about the potential mechanisms driving brain changes difficult. Additionally, neuroimaging studies do not consistently assess cognitive performance; we cannot infer whether menopause-associated brain changes underlie menopause-associated cognitive changes. Further, most neuroimaging samples discussed have strict inclusion and exclusion criteria, generally limiting small samples to illness-free individuals (e.g. diabetes, CVD, neuropsychiatric conditions excluded), restricting the generalisability of results.

### 1.11.4. Menopause and dementia risk

A large meta-analysis[95] found increased female AD risk in the years following menopause (<75 years) compared with men of similar age. This difference in risk disappeared at older ages (to age 85), suggesting that late midlife to early old age is a time of vulnerability (or sensitive period) for AD risk in women. However, research assessing dementia risk associated with menopause age and type does not draw clear conclusions.

A review and meta-analysis did not find overall support for an association of menopause age with dementia risk, although several studies showed significant associations indicating increased risk with earlier age at menopause.[154] Another meta-analysis did not detect an overall association of surgical menopause and dementia risk, but there was evidence for increased dementia risk in women who underwent surgical menopause at an early age (≤45 years).[151] The association of early surgical menopause with dementia risk could be confounded by other health conditions, although most studies do not ascertain the reason for surgical menopause, and by social disadvantage which is linked with earlier menopause age.[165,166] Heterogeneity across methodologies, with variations in the covariates accounted for and in inclusion and exclusion criteria, might also disguise relationships between menopause and dementia risk present in some groups but not others.

An additional challenge is the time lag between the menopause transition and the age of dementia onset. Post-menopause, other health and lifestyle factors may further influence dementia risk. For example, women may retire from work, take up new hobbies or interests, or take on additional caring responsibilities (e.g. for elderly parents). Additionally, menopause has been consistently associated with cardiovascular risk factors such as obesity, diabetes,

and hypertension.[10,167,168] In females, CVD is rare before age 50 but cases rapidly increase in later years.[167] As a major transition occurring at midlife, menopause might contribute to female midlife cardiovascular risk, particularly since falling oestrogen levels might induce metabolic changes (e.g. reduced glucose metabolism) and changes in body fat distribution.[167] Social disadvantage could, however, confound menopause-cardiovascular associations, being linked with earlier age at menopause[165,166] and increased risk of heart disease.[169]

### 1.11.5. Effects of hormone therapy

There are discrepancies between observational studies and RCTs examining the effects of HT on cognitive performance and dementia risk. Observational studies tend to find benefits of HT use,[170] while RCTs typically report null or adverse effects.[171] Observational HT studies could have a healthy user bias, since HT users typically have better health than non-users.[172] RCTs can overcome this issue through random allocation of HT or placebo, but RCT participants are likely to be healthier than the general population due to strict exclusion criteria.

Perhaps the most well-known HT RCT is the Women's Health Initiative (WHI) trial of conjugated equine oestrogen (CEE) plus medroxyprogesterone acetate (MPA) in healthy postmenopausal women (mean baseline age 63.3 years), which was stopped early after 5.2 years when risks (primarily invasive breast cancer) were found to outweigh the benefits.[173] A second arm of the trial assessing unopposed CEE in healthy postmenopausal women without a uterus (mean baseline age=63.6 years) was also stopped early, due to increased stroke risk.[174] WHI follow-up sub-studies have found evidence for faster cognitive decline,[175,176] increased dementia risk,[177,178] and frontal lobe and hippocampal atrophy, particularly among women with poorer cognition at baseline,[179,180] in HT compared with placebo groups. However, the association of HT with dementia risk reported in the WHIMS (WHI Memory Study) sub-study was only detected for the CEE plus MPA arm; unopposed CEE was not significantly associated with dementia risk.[178]

The WHI trials assessed HT initiated in women 10 years, on average, post-menopause, meaning that results are not generalisable to women initiating HT closer to menopause. Indeed, a sub-group analysis of WHI participants aged 50-55 at trial medication initiation (mean time since final menstrual period/FMP 6.5 years) did not find effects of HT on cognitive

performance 7.2 years after discontinuation of trial medication.[181] Additionally, no differences in cognitive outcomes between HT and placebo groups were found in another trial (the Kronos Early Estrogen Prevention Cognitive and Affective Study; KEEPS) which examined the effects of HT use over 4 years in healthy, early post-menopausal women without hysterectomy (mean baseline age 52.6, average time since FMP 1.4 years).[182] Observationally, evidence from Cache County (a population-based dementia study of elderly residents) demonstrated a proximal effect whereby HT use associated with reduced AD risk if taken within 5 years of menopause.[171] The effects of HT on cognitive and dementia outcomes therefore seem to differ by when treatment is initiated in relation to menopause timing, supporting the sensitive period hypothesis which postulates that HT is beneficial if taken within a window of opportunity close to menopause.[183,184] Complimentary to this, the healthy cell bias theory proposes that exogenous oestrogen can have beneficial effects if cells are well-functioning and healthy, but may be harmful if cell functioning has already started to decline[185,186] (e.g. due to neuroprotective oestrogen decline during menopause).[187]

Many different forms of HT are available, with different formulations, dosages, and administration methods, and women may vary in how long they take HT for.[188] Such variations in HT use, which are rarely recorded with sufficient detail in observational studies and which vary across RCTs, mean that there is currently an overall lack of consensus regarding HT effects on cognition, dementia risk and brain health. Indeed, a recent examination of available data in the UK Biobank revealed a nuanced picture of HT associations with MRI outcomes; longer HT duration associated with older predicted brain ages (brains appeared older than expected for chronological age), greater WMHV, and smaller hippocampal volumes, and HT users with hysterectomy or oophorectomy history showed younger predicted brain ages than those without such surgeries.[189]

### 1.11.6. Summary

Associations between female hormones and later life cognitive and brain health outcomes are complex and poorly understood. Biological plausibility for such associations is mainly focused on the role of oestrogen in the brain. Little attention is given to the effects of non-oestrogen hormones (e.g. progesterone, luteinising hormone, follicle-stimulating hormone) which also fluctuate throughout life. There is also a lack of prospective research utilising

cognitive, neuroimaging and hormonal measures in combination, hence the mechanisms of associations with female hormonal events are unclear.

HT could modify associations between female hormonal events (i.e. menopause type and timing) and later-life outcomes in ways that we do not yet understand. Most studies do not record detailed information of HT use, meaning that analyses have generally not accounted for variations in dosage, formulation, duration, or timing of initiation. Those which have examined some of these variations demonstrate complex relationships of HT use with later-life outcomes.

Most research assessing the associations of menopause with later-life cognition and dementia risk is also unable to account for childhood cognition, which is shown to be an important confound, potentially representing a proxy measure of lifetime oestrogen exposure.[156] Beyond hormonal mechanisms though, the menopause transition and its associated symptoms can have health and lifestyle implications for women, which may contribute to the accumulation of dementia risk factor exposures (such as those outlined in Section 1.10.2.) during and post-menopause. Reducing symptoms, for example through HT medications, might limit subsequent accumulation of risk exposures. Given the time lag between menopause in midlife and later-life cognition or dementia, other health, lifestyle, and sociocultural factors during and after midlife could mediate or otherwise explain associations.

## 1.12. Summary and identification of gaps in the literature

Sex differences are reported in dementia prevalence, symptoms, and progression rates, with variations across dementia types.[3] Several theories of dementia sex differences have been proposed which consider the influence of biological factors specific to each sex and the role of sociocultural and lifestyle factors which can have gendered patterns and potentially differential impacts on cognitive ageing and dementia processes between males and females. However, the mechanisms underlying sex differences in cognitive ageing, brain ageing, and dementia pathology are not yet fully understood, due to an overall paucity of research explicitly testing sex differences or conducting sex-stratified analyses; only recently have policies been introduced to encourage the inclusion of sex as a biological variable in research.

There has been interest in studying sex differences in cognitive functions and brain structure, but historically studies have suffered from researcher bias, and have been used to justify or reinforce gender stereotypes. While understanding sex differences in cognition and the brain is useful for determining how males and females might differ regarding cognitive and brain ageing processes, such research must be treated with caution so as not to encourage potentially negative gender stereotypes. Additionally, there must be acknowledgement that the identification of sex differences does not necessarily represent a dichotomy between males and females; differences typically refer to statistically significant differences in mean values, while there may still be a degree of overlap in the distribution of values attributed to males and females.[47] Evidence also shows that cognitive sex differences can be modified by the nature of task instructions (e.g. whether tasks are presented within a stereotypically male or female context)[50] and by secular changes in access to education,[54] demonstrating that there are societal influences on cognitive sex differences and that such differences are not biologically pre-determined, highlighting that there are interrelationships between sex and gender. While sex differences in cognitive performance have been examined at various ages, there is no clear narrative for how patterns in cognitive performance sex differences across domains might change across the life course. Creating such a narrative will be useful to identify potential societal, lifestyle, or biological influences on cognitive sex differences, which could inform how brain ageing and dementia processes might vary between sexes.

Advances in technologies which can detect and measure *in vivo* biomarkers of dementia have led to the concept of preclinical dementia, whereby early dementia pathologies develop prior to the onset of noticeable cognitive and functional symptoms. Relatively new multimodal neuroimaging approaches, such as combining PET and MRI, facilitate a holistic approach to understanding the processes underlying dementia. Examining whether and how various *in vivo* indicators of brain health differ between the sexes will be valuable in elucidating the possible differential pathways to dementia in males and females. Indeed, neuroimaging studies have demonstrated poorer cerebrovascular health in females than in males, with females demonstrating poorer NAWM microstructural integrity and greater WMHV (indicative of greater cSVD) than males.[62-64] However, it is currently unclear how such sex differences in brain health relate to differences in cognitive functions, with few studies assessing sex differences in neuroimaging indicators of brain health alongside differences in cognitive

performance. Considering cognitive and brain health sex differences in parallel is necessary for understanding how males and females might differ in the functional changes associated with brain ageing or dementia-related pathology, particularly in relation to cognitive resilience.

Several dementia risk factors have been identified, some of which may limit opportunities for the development or maintenance of reserve (e.g. low education, social isolation), while others can impact cerebrovascular health (e.g. hypertension, obesity).[56] Dementia risk scores have been created which acknowledge that there are interrelationships between risks, but they do not accurately predict future dementia[109] and do not incorporate all possible modifiable and non-modifiable risks for dementia. Notably, existing risk scores typically focus on risks present at one point in time, primarily during midlife, and do not quantify cumulative risk exposures throughout the life course. Additionally, few studies have explicitly examined sex differences in the associations of dementia risk scores with later-life cognitive, brain health or dementia outcomes. Existing risk scores also fail to incorporate gendered sociocultural variations in risk factors (e.g. alcohol consumption is greater in men, physical inactivity is greater in women), or sex-specific risks (e.g. female reproductive factors).

As a female-specific transition, menopause has received much attention as a potential contributor of female cognitive and brain ageing. It is hypothesised that reductions in oestrogen, which has neuroprotective properties, during menopause leaves the female brain vulnerable to the effects of neural ageing and acts as a 'tipping point' for the development of dementia-related pathologies.[8] Evidence has shown that, overall, earlier ages at menopause associate with poorer cognitive performance in later-life,[154] possibly reflecting prolonged effects of reduced oestrogen levels. However, few studies have examined associations of menopause age with neuroimaging indicators of brain health and dementia-related pathology, so the neural mechanisms through which menopause timing associates with cognitive performance remain unclear. There is also heterogeneity across studies assessing menopause timing and later-life outcomes, with variations in the cognitive domains examined, such that no cognitive domains have been consistently associated with menopause age across studies. Many studies also exclude women who underwent surgical menopause or who took HT, possibly reflecting the current lack of understanding for how HT associates with later-life brain health outcomes given variations in HT dosages, formulations, and durations of

use, for example, which are often poorly recorded in observational studies. Additionally, most research examining associations of menopause timing rely on retrospective reports of menopause age, which could induce error; women who experience cognitive impairments could be more likely to incorrectly recall their age at menopause. Further, evidence has shown that early-life factors are important contributors to menopause timing and could confound associations with later-life outcomes; better childhood cognition associates with later menopause age[156,157] and improved later-life cognition,[158,159] and attenuates associations of menopause age with later-life cognition.[155] However, most studies assessing menopause timing are unable to account for such early-life covariables due to unavailability of life course data.

# 2.0. Data and analytical strategy

## 2.1. Aims and objectives

The overall aim of this PhD is to examine sex differences in lifetime risks for dementia, within the context of a mostly cognitively unimpaired cohort (the MRC NSHD and its neuroscience sub-study, Insight 46). The purpose is to develop an understanding for how different socioeconomic, lifestyle, and biological factors throughout the life course might contribute to the increased risk of developing dementia observed in females over males.

Sex differences in dementia risk factors which both males and females can be exposed to are examined, alongside consideration of how female-specific exposures (namely, timing of the menopause transition) associate with cognition and brain health in later-life.

Cognitive and brain health outcomes are examined at an age (69-71 years) when pathological brain health is expected to be accumulating while diagnosable, symptomatic dementia is rare. Assessing both cognition and neuroimaging measures is useful to help understand the possible pathological pathways underlying cognitive differences.

Although cognitive performance measures are available across the life course, this thesis focuses on cross-sectional rather than longitudinal analyses. This is because true repeated measures, that is cognitive assessments comparable across time points, are the assessments of processing speed and verbal memory administered between ages 43 and 69 (see Section 2.3.2.). As described in Section 1.7., sex differences in these cognitive performance trajectories have already been examined by others.[5]

This thesis consists of three empirical sections, broadly asking:

A. Are there sex differences in cognitive performance and brain health measures across the life course? (Chapter 3)
B. Do associations of lifetime dementia risk factors with later-life cognition and brain health differ between males and females? (Chapter 4)
C. To what extent does menopause timing contribute to later-life cognitive and brain health outcomes in females? (Chapter 5)

The objectives for each section are as follows, with greater detail and hypotheses outlined in the relevant chapters.

**Empirical section A (Chapter 3):** Generate a descriptive overview of cognitive and brain health sex differences and similarities across different life stages within the NSHD and Insight 46 cohorts. The purpose is to inform about which cognitive domains and brain health measures might show male or female advantages and to highlight when in the life course any sex differences are more evident. Sex differences are examined accounting for socioeconomic, educational, and lifestyle factors, to determine the extent to which sex differences can be explained by societal and environmental influences. Additionally, since the dementia risk associated with *APOE*-ε4 is greater in females,[95] whether sex differences are modified by *APOE*-ε4 status is examined to determine whether *APOE* genotype also associates with greater adverse cognitive performance and brain health measures in females throughout the life course.

**Empirical section B (Chapter 4):** A cumulative risks score (CRS) is derived from life course data, quantifying lifetime exposures to each of the twelve modifiable risk factors outlined in the Lancet commission (Section 1.10.2.). The objective is to examine the extent to which early-life socioeconomic and genetic factors predict lifetime exposures to modifiable dementia risks, and whether cumulative risk exposures associate with later-life cognitive performance and brain health differently in males and females. This is to inform whether the associations of exposures to non-sex-specific risk factors with later-life outcomes are greater in one sex over the other.

**Empirical section C (Chapter 5):** The objective is to examine how prospectively reported age at menopause, as a female-specific hormonal transition, associates with cognitive performance across a range of domains and with multi-modal neuroimaging markers of brain health at age ~70 years, and whether associations can be explained by relevant early cognitive, sociodemographic, reproductive, and health-related covariables. The aim is to build an understanding of menopause as a possible contributor to increased female dementia risk and variations in cognitive and brain ageing between women.

## 2.2. Introduction to the datasets

### 2.2.1. NSHD

The MRC National Survey of Health and Development (NSHD) began as a maternity survey to investigate the costs of childbirth and to assess maternity services. All 16,695 births in England, Wales, and Scotland during one week in March 1946 were included. A nationally representative cohort of 5,362 (2,547 females), stratified by occupational social class, was selected for follow-up from single births to married mothers; all babies whose fathers had agricultural or non-manual occupations were selected, and a random 1 in 4 sample of babies whose fathers had a manual occupation were included.[190] There have been over 25 data collections, using a combination of questionnaires and clinical assessments to generate a rich dataset including prospective socioeconomic, health, and cognitive variables spanning the life course. Questionnaires were completed by mothers, teachers, school nurses, and doctors prior to 1966, and completed by study members themselves thereafter. In childhood, follow-up data collections were completed approximately every 2 years until age 15. In adulthood, the main data collections took place at ages 26, 36, 43, 53, 60-64, and 69.

For this thesis, data up to age 70 was used. This included a postal questionnaire at age 68, followed by a home visit at age 69. A total of 2,638 (1,318 female) completed at least one of these, representing 94% of the target sample (n=2,816). Participation rates did not differ by sex, but higher participation rates were associated with having a non-manual occupation, higher educational attainment, and higher cognitive ability in childhood.[191] Of the 2,546 study members who were not contacted for this data collection, 18% were known to have died, 12% had previously withdrawn, 11% had emigrated, and 7% had been untraceable for over 5 years.[191]

#### *2.2.1.1. Women's Health in the Middle Years*

During midlife, 1,752 female NSHD study members took part in the Women's Health in the Middle Years survey.[192] Women completed 9 postal questionnaires between ages 43-54 years, providing detailed prospective data on women's reproductive health at midlife, relevant for Chapter 5 analyses. As will be detailed in Section 5.0., variables of interest included age at

menopause, whether menopause occurred naturally or if it was surgically induced, and whether women had used menopausal HT.

### 2.2.2. Insight 46

Insight 46 is the NSHD neuroscience sub-study which aims to identify brain changes associated with healthy ageing and to detect brain changes which might predict who is at increased risk of dementia. The third wave of data collection is currently underway, but this thesis uses data from the first wave which assessed 502 (49% female) study members between ages 69 and 71. Wave I incorporated an enhanced cognitive test battery, with assessments sensitive to cognitive changes linked with dementia (Section 2.3.2.), and simultaneous MRI and PET neuroimaging which provided well established indicators of brain health including TBV, GMV, WMHV, and Aβ load through validated pipelines (Section 2.3.3.).[193]

To maximise the life course data available for analyses alongside Insight 46 data, NSHD study members were eligible to take part in Insight 46 if they had attended a clinic visit at age 60-64 and had available relevant data across childhood and adulthood. Relevant variables included assessments of cognitive function, mental health, educational attainment, cardiovascular function, and physical activity levels, amongst others, as further detailed in Lane et al. (2017).[193] Of the 1,322 eligible study members, 779 indicated a willingness to attend a clinic visit in London and were invited to take part. Once data collection was underway, eligibility criteria were relaxed to ensure that the target sample of 500 participants was reached; an additional 62 participants who did not have a previous measure of lung function, smoking, or physical exercise were invited. The final sample of 502 participants represented 60% of the 841 NSHD study members invited; 24% refused, 3% temporarily refused, 1% did not respond, 0.4% died, 3% cancelled a visit, and 8% were excluded due to PET-MRI contraindications including claustrophobia and metal implants.[194]

Higher educational attainment, non-manual socioeconomic position, higher cognitive abilities, not smoking, and higher self-rated health were associated with increased likelihood of Insight 46 participation, across all stages of recruitment (i.e. eligibility, willingness to attend a London clinic, attending clinic).[194] Insight 46 participants therefore have greater SEP,

educational attainment, cognitive function, and general health than the wider NSHD cohort. Insight 46 participation was not, however, predicted by sex or *APOE*-ε4 status.[194]

## 2.3. Measures

A range of health, lifestyle, genetic, and socioeconomic variables measured throughout the life course are included in the analyses of this thesis and are detailed in the relevant analytical chapters. Several variables, including cognitive and neuroimaging outcome measures, are common across the analyses and are outlined below.

### 2.3.1. Sex

As outlined in Section 1.2., sex refers to the biological characteristics which distinguish males from females. Participant sex within NSHD is defined as sex recorded at birth and is a binary measure: male or female. Throughout the analyses in this thesis which include both sexes, male is the reference category.

### 2.3.2. Cognitive performance

The intellectual and cognitive abilities of NSHD study members have been assessed at several timepoints across the life course. Tests of verbal and non-verbal abilities were administered at ages 8, 11, and 15. Functional cognitive abilities were assessed at ages 43, 53, 60-64, and 69. Table 1 summarises the tests administered at each timepoint, and further details can be found at https://closer.ac.uk/cross-study-data-guides/cognitive-measures-guide/nshd-cognition/

Insight 46 participants completed an additional comprehensive neuropsychological test battery during wave I of data collection (age ~70). A range of domains including memory, executive function, visuospatial function, and cognitive state were assessed in tests designed to detect subtle cognitive changes associated with dementia, outlined in Table 2.

In Chapter 3 sex differences in performance across all cognitive assessments are examined. In Chapters 4 and 5, later-life cognitive outcomes (those measured at age 69 and Insight 46 wave I) are used as cognitive outcome measures.

**Table 1**. Summary of the abilities tests and functional cognitive assessments administered during NSHD data collection waves. Prior to age 43 years only verbal and nonverbal abilities were assessed; functional cognitive abilities have been assessed from age 43 onwards. Further details for each test can be found at https://closer.ac.uk/cross-study-data-guides/cognitive-measures-guide/nshd-cognition/

| Age (year) | Test name | Test description | Domains assessed | Outcome measures |
|---|---|---|---|---|
| 8 (1954) | Picture intelligence | 60 items across 3 tasks:<br>a) Choose the image which is the odd-one out of a series<br>b) Select which image, from a choice of 5, completes a series<br>c) Select, from a choice of 5, an image which corresponds to a rule (e.g. foot is to shoe as head is to hat). | Non-verbal reasoning | One point per correct answer (0-60) |
| | Sentence completion | Choose, from a selection of 5 words, the correct word to complete each sentence (35 sentences). | Verbal ability, reading comprehension | One point per correct sentence (0-35) |
| | Word reading | Participants read a list of 50 words aloud. | Verbal ability, pronunciation | One point per word correctly pronounced (0-50) |
| | Vocabulary | Participants were asked the meaning of each of the 50 words presented in the word reading task. | Verbal ability, word comprehension | One point per correct response (0-50) |
| | Overall cognitive performance | Summary measure of performance across the four tests, devised by National Foundation for Educational Research (NFER),[195] administered at age 8. | Verbal and non-verbal abilities | Z-score average performance across all four tests |
| 11 (1957) | General ability test | Choose the correct word or shape/symbol to complete a series. | Verbal and non-verbal reasoning | One point per correct series (0-80) |
| | Arithmetic test | 20 mechanical sums and 30 problem questions assessing the ability to add, subtract, multiply, and divide. | Verbal (problem questions) and non-verbal (mechanical sums) abilities | One point for each correct solution (0-50) |
| | Word reading | As at age 8 | | |
| | Vocabulary | As at age 8 | | |
| | Overall cognitive performance | Summary measure of performance across the four | Verbal and non-verbal abilities | Z-score average performance |

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| | | tests, devised by NFER,[195] administered at age 11. | | across all four tests |
| 15 (1961) | The Alice Heim Group Ability Test (AH4)[196,197] | A 130-item test of verbal and non-verbal abilities including: series completion, mental arithmetic, vocabulary, and reasoning. | Verbal and non-verbal ability | One point per correct answer (0-130) |
| | Watts-Vernon Reading Test[196,198] | Choose, from a selection of 5 words, the correct word to complete each sentence (35 sentences). | Verbal ability, reading comprehension | One point per correct sentence (0-35) |
| | Mathematics test[196] | A 47-item test assessing arithmetic, geometry, trigonometry, and algebra. | Mathematical abilities | One point per correct item (0-47) |
| | Overall cognitive performance | Summary measure of performance across the three tests administered at age 15. | Verbal and non-verbal abilities | Z-score average performance across all three tests |
| 26 (1972) | Watts-Vernon Reading Test[198] | As at age 15, but with 10 additional items of increased difficulty. | Verbal ability, reading comprehension | One point per correct sentence (0-45) |
| 43 (1989) | Peg placement[199] | Time to move 10 pegs in a wooden peg board from one hole to an adjacent one. Five trials per hand. | Motor speed and praxis | Mean speed across 5 trials, per hand |
| | Picture recall[200] | Participants were given 30 seconds to look at 5 cards, each with a unique picture, and were asked ~20 minutes later to recall what was on the cards. | Visual, non-verbal memory | One point for each correctly recalled picture (0-5) |
| | Verbal learning/word list recall test[201] | 15 words presented (one word every 2 seconds). Participants write down as many words recalled as possible. A different word list was given to each half of the cohort. | Verbal memory | One point for every correctly recalled word, over three trials (0-45) |
| | Timed letter search/letter cancellation test[200] | Cancellation task with 2 different target letters embedded among non-target letters in three blocks. Participants were asked to cross out as many target letters as possible, as quickly as possible, within a block over 1-minute. There were three 1-minute trials. | Processing speed | Number of letters scanned in each 1-minute trial |
| 53 (1999) | Verbal learning/word list recall test[201] | As at age 43, with the addition of a delayed recall (90 seconds) condition. Participants were | Verbal memory | One point for every correct word recalled |

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| | | presented a different word list to the one seen at age 43. | | (0-45 immediate recall; 0-15 delayed recall) |
| | Timed letter search/letter cancellation test[200] | As at age 43 but only one trial, with letters covering a full page. | Processing speed | Number of letters scanned |
| | National Adult Reading Test (NART)[202] | Ability to read aloud and correctly pronounce 50 irregular words of increasing difficulty. | Verbal ability, reading | Number of errors, inverted such that higher scores indicate better performance (0-50) |
| | Verbal fluency animal naming test[203] | Category fluency: name as many different animals as possible in one minute. | Verbal fluency | Total number of animals named |
| | Prospective memory | Participants were informed that, later in the interview, they would be given an envelope and asked to write a name and address on it. On receipt of the envelope, they were to remember to turn it over, seal it, and write their initials on it. When handed the envelope, participants were asked to write "John Brown, 42 West Street, Bedford". | Prospective verbal memory | 3-point ordinal score:<br>3=both actions completed without prompting<br>2=One action completed without prompting<br>1=No actions completed without prompting |
| 60-64 (2006-2010) | Verbal learning/word list recall test[204] | As at age 53 | | |
| | Timed letter search/letter cancellation test[205] | As at age 53 | | |
| | Reaction time[206] | Simple RT: press a key (using one finger only) as quickly as possible when '0' or '8' appeared on the screen. 20 trials.<br>Choice RT: numbers '1', '2', '3', and '4' would appear on screen, participants were to press the corresponding keys as | Simple reaction time<br>Choice reaction time | Mean reaction time of correct trials for both tasks |

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| | | quickly as possible, using both hands. 40 trials. | | |
| 69 (2015) | Verbal learning/word list recall test[207] | As at age 53 | | |
| | Timed letter search/letter cancellation test[207] | As at age 53 | | |
| | Addenbrooke's Cognitive Examination-third edition (ACE-III)[208,209] | A validated screening test of overall cognitive state, comprising tests of: attention and orientation, language, memory, verbal fluency, visuospatial function. NSHD participants were administered the ACE-III by iPad (ACEMobile http://www.acemobile.org). A paper version was used when iPad testing was not possible. | Cognitive state (verbal and non-verbal ability)<br>Attention & orientation<br>Language<br>Memory<br>Verbal fluency<br>Visuospatial function | One point per correct item. Total score (max 100) is a sum of scores across all sub-domains: attention & orientation (0-18), language (0-26), memory (0-26), verbal fluency (0-14), visuospatial function (0-16) |
| | Finger tapping[210] | With palm down and fingers extended, participants were asked to tap a lever with their index finger as fast as possible for 10 seconds; both hands tested separately. | Psychomotor speed | Number of taps per hand |

***Table 2.*** *Summary of tests and outcomes derived from the Insight 46 wave I cognitive test battery, assessed at ages 69-71.*

| Task | Description | Domains assessed | Outcome measures |
|---|---|---|---|
| PACC: Total score is the mean z-score across four component tasks (MMSE, Logical Memory delayed recall, DSST, FNAME-12A) | | | |
| Mini-Mental State Examination (MMSE)[158,193,211] | Test of cognitive state including tests of attention, orientation in space and time, memory, language, executive function, and visuospatial function. | Overall cognitive state | Total score out of 30 |
| Logical Memory delayed recall[158,193,212] | Administrator reads a short story aloud; participants recall what they can after an approximate 20-minute delay. | Episodic memory | Total score out of 25 |
| Digit-Symbol Substitution Test (DSST)[158,193,213,214] | Participants fill in a worksheet as quickly and accurately as possible within 90 seconds, using a code table pairing numbers with different symbols. | Processing speed<br>Associative learning<br>Attention<br>Psychomotor speed<br>Executive function | Total number of symbols completed |
| Face-name associative memory examination (FNAME-12A)[158,193,215] | Participants learn and recall twelve face-name and face-occupation pairs. | Associative, episodic memory | Total names recalled plus total occupations recalled |
| Additional computerised cognitive tasks | | | |
| Matrix reasoning[158,193,216] | Participants are presented with grids of geometric shapes with a section missing. They then select the missing piece from a choice of five. | Non-verbal reasoning | Total number of correct trials |
| Choice reaction time[193,217] | Participants are presented with words or arrows indicating the direction they should respond (left/right) as quickly as possible, using a button box. | Choice Reaction Time; Executive function | Intra-individual variability in reaction times (IIVrt), correct trials only: (SD / mean RT) (Z-score inversed so that higher scores indicate better performance, i.e. less RT variability) |
| Response inhibition[193] | Participants are cued to respond, as quickly as | Executive function: | Proportional difference in RT between correct congruent |

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| | possible using a button box, to the direction indicated by words or arrows (left/right) presented simultaneously (congruently or incongruently). | response inhibition | and incongruent trials: ((mean RT incongruent trials – mean RT congruent trials) / mean RT congruent trials) (Z-score inversed so that higher scores indicate better performance, i.e. less slowing of RT for incongruent vs. congruent trials) |
| Visuomotor integration[40,193] | Participants use a stylus to trace a circle on a tablet screen as quickly and accurately as possible, under direct (hand and tablet visible) and indirect (hand and tablet hidden, circle viewed on vertical screen) visual feedback conditions. Administered as a dual-task, alongside serial subtraction. | Executive functions: visuomotor integration, error detection and correction<br>Psychomotor speed<br>Attention | Mean number of errors per rotation across dual-task trials (z-score inversed so higher scores indicate better performance, i.e. fewer errors)<br><br>Mean number of rotations across dual-task trials<br><br>Mean subtraction rate (numbers per second) across dual-task trials |
| "What was where?" visual working memory[193,218] | Either one or three fractal objects are presented in random locations on a touch screen. Participants choose which of two fractals they have previously seen and drag it to the location they think it was presented. | Visual memory<br>Visual memory binding<br>Attention<br>Visuospatial function | Identification rate: proportion of fractals correctly identified across all trials<br><br>Mean localisation error: distance from the correct target location, across all trials (z-score inversed so higher scores indicate better performance, i.e. smaller localisation error)<br><br>Swap error rate: percentage of correctly identified fractal trials in which a distractor fractal location is chosen (z-score inversed so higher scores indicate better performance, i.e. lower error rate) |

*PACC=Preclinical Alzheimer's Cognitive Composite; MMSE=mini-mental state examination; DSST=digit-symbol substitution test; FNAME=face-name associative memory examination; RT=reaction time; IIVrt=intra-individual variability in reaction times; SD=standard deviation*

### 2.3.3. Brain health

Brain health measures were derived from simultaneous PET-MRI neuroimaging completed by Insight 46 wave I participants at age 69-71. Throughout this thesis, the multimodal neuroimaging measures examined include those which represent AD- (Aβ, hippocampal volume, cortical thickness), non-specific ageing- (TBV), and vascular-related (WMHV, NAWM metrics) pathways to dementia.

These neuroimaging variables were derived, using validated pipelines, by neuroimaging experts at the UCL Dementia Research Centre, who provided guidance in using these variables within this thesis. The methodologies for deriving the relevant measures for this thesis are described below.

#### *2.3.3.1. Imaging protocol*

Of the 502 Insight 46 participants, 471 completed PET-MRI scanning.[191] The full imaging protocol has been previously described.[193] In brief, a single Biograph mMR 3T PET-MRI scanner (Siemens Healthcare, Erlangen) was used, with simultaneous acquisition of dynamic PET-MRI data, including volumetric (1·1 mm isotropic) T1-weighted and T2-weighted Fluid Attenuated Inversion Recovery (FLAIR) sequences. PET data were acquired continuously in list mode, during and following injection of 370 MBq $^{18}$F florbetapir (Avid Radiopharmaceuticals, Philadelphia, PA).

#### *2.3.3.2. Volumetric measures*

Volumetric T1-weighted and FLAIR images underwent visual quality control (QC) before processing using automated pipelines, previously summarised in Lane et al. (2017),[193] which generated, among other measures, total brain volume (TBV; $cm^3$), total intracranial volume (TIV; $cm^3$) and hippocampal volume ($cm^3$). For these analyses, hippocampal volume was taken as the mean volume of the left and right hippocampi.

#### *2.3.3.3. White matter hyperintensity volume (WMHV)*

The Bayesian Model Selection (BaMoS) algorithm,[39] followed by visual QC, was used to segment white matter hyperintensities from 3D T1 and FLAIR images to generate a global

WMHV which includes subcortical grey matter but excludes infratentorial regions.[98] For analyses assessing WMHV as an outcome, participants who failed BaMoS QC were excluded (n=3).

#### *2.3.3.4. Beta-amyloid*

Amyloid (Aβ) burden was assessed over a 10-minute period, around 50 minutes after injection of florbetapir PET tracer. Global standardised uptake value ratio (SUVR) was calculated from cortical regions of interest, normalised to eroded subcortical white matter, providing a continuous measure of cerebral Aβ levels. SUVR in Insight 46 is non-normally distributed (Appendix A Figure 1), with most individuals showing low levels of Aβ. A binary indicator of amyloid load was therefore also examined as an outcome variable, to compliment continuous SUVR analyses; positive or negative Aβ status was determined using a Gaussian mixture model applied to SUVRs, taking the 99th percentile of the lower Gaussian as the cut-off (0·6104).[98]

#### *2.3.3.5. Normal appearing white matter (NAWM)*

As described in James et al. (2023),[40] multi-shell diffusion MRI (dMRI) was used to derive diffusion maps indicating NAWM microstructural integrity. For the purposes of these analyses, mean diffusivity (MD) and fractional anisotropy (FA) maps were of interest. MD measures the level of diffusion in all directions, with greater MD representing more water dispersal and therefore reduced microstructural integrity.[26] FA represents the directionality of diffusion, with lower FA indicating more streamlined movement of water molecules along axons, showing better microstructural integrity.[26] All images underwent visual QC, with images failing QC excluded from analyses (n=59). Diffusion maps were converted to z-score maps, via comparison with a sub-set of participants with limited WMHV (<1ml), and a mean z-score over each NAWM mask was calculated for each diffusion metric (i.e. MD, FA).

#### *2.3.3.6. Cortical thickness*

Cortical thickness estimation was performed using a modified version of the standard automated pipeline in FreeSurfer version 6.0.[219] Regions of interest (ROI) were the surface area-weighted averages of the frontal, temporal, parietal, and occipital lobes, and the Harvard composite signature of AD cortical thinning (ADsig Harvard).[220] The ADsig Harvard comprises

areas of the frontal, temporal and parietal regions: entorhinal cortex, parahippocampus, inferior parietal lobe, pars opercularis, pars orbitalis, pars triangularis, inferior temporal lobe, temporal pole, precuneus, supramarginal gyrus, superior parietal lobe, and superior frontal lobe.[220]

### 2.3.4. Covariables

The specific covariables included in each analysis are detailed in the relevant chapters. Some covariables common across analyses are outlined below.

#### *2.3.4.1. APOE genotype*

Blood samples taken at age 53 were analysed to ascertain *APOE* genotype from the single nucleotide polymorphisms (SNPs) rs429358 and rs7412. Genotype was categorised according to *APOE*-ε4 allele carrier status: ε4 carriers (ε4 homozygous or heterozygous) or non-carriers.

#### *2.3.4.2. Educational attainment*

Educational attainment was recorded as the highest educational qualification achieved by age 26 and by age 43. Categories were derived from the Burnham Scale:[221,222] no qualifications, sub-GCE or sub-Burnham C (vocational only), GCE O level or Burnham C (GCSE) or equivalent, GCE A level or Burnham B or equivalent, and degree or higher. These categories were further collapsed for use in these analyses, as detailed in the relevant chapters.

#### *2.3.4.3. Socioeconomic position (SEP)*

SEP was categorised according to the UK Registrar General social classifications:[223] unskilled, partly skilled, skilled manual, skilled non-manual, intermediate, professional. Binary coding grouped the first three categories as manual (lower SEP) and the remaining categories as non-manual (higher SEP). Childhood SEP was determined according to the father's occupational social class at ages 4, 11, or 15, and thereafter SEP was derived from participants' own occupational social class from age 15 to 53. Overall adulthood SEP was derived by using SEP at age 53, or 43, and updating it with previous SEP if missing.

#### *2.3.4.4. Childhood cognitive ability*

Childhood cognitive ability, which is a predictor of later-life cognitive ability,[159] is a key covariable included in these analyses and represents a strength of the NSHD cohort, since other datasets typically lack prospective measures of cognitive ability in childhood. As shown in Table 1, childhood cognitive ability is derived for ages 8, 11, and 15 as an averaged, standardised z-score across all assessments completed at the respective age. In this thesis, cognitive ability at age 8, as the earliest indicator of cognitive ability, is taken to indicate childhood cognitive ability, with data from age 11 or 15 used if missing at age 8.

## 2.4. Analyses

Analyses were conducted in Stata (versions detailed in relevant chapters), and the details of each analytical sample and analytical method are outlined in each empirical section of this thesis (Chapters 3, 4, 5). Some methodology was shared between chapters, which is briefly outlined here.

### 2.4.1. Descriptive analyses

Variables in each analytical dataset were checked for normality and outliers by visually inspecting histograms. Where outcome variables were non-normally distributed, bootstrapping with 1000 replications was applied to the analyses for that outcome, to calculate non-parametric, bias-corrected confidence intervals.

Continuous variables were summarised using mean and standard deviation statistics. Categorical variables were described using frequencies and percentages. Descriptive statistics were compared between males and females using t-tests and chi-squared tests for continuous and categorical variables, respectively, with $p<0.05$ taken to indicate a statistical sex difference.

### 2.4.2. Regression modelling

Multivariable regression modelling is the main statistical method used throughout this thesis to test associations of life course variables with cognitive performance and brain health

outcomes. An inferential framework was adopted to identify relevant confounding, mediating, and competing exposure variables, with models adjusted for covariables accordingly. Linear regression was used for continuous outcome variables, and logistic regression for binary outcomes (e.g. Aβ status). The estimated associations were described using regression coefficients or odds ratios, and 95% confidence intervals, using bias-corrected confidence intervals for bootstrapped analyses. Non-linearity was assessed using quadratic terms, and effect modifications were examined by including relevant interaction terms, deemed significant at the $p<0.10$ level.

To maximise the use of available data, maintain statistical power, and reduce bias due to missing data, multiple imputation by chained equations (MICE) was used across analyses to account for missing covariable data, under the assumption that data were missing at random. Each MICE analysis included 50 imputations, except for analyses combining bootstrapping with MICE in Chapter 5. Imputation models included variables which might predict missingness (auxiliary variables), and all outcome, covariate, and predictor variables relevant to each analytical model. Continuous variables were imputed using Gaussian normal regression, and categorical variables using logistic or ordinal regression techniques.

# 3.0. Empirical section A: Sex differences in lifetime cognitive performance and later-life neuroimaging outcomes in NSHD and Insight 46

The purpose of this chapter is to provide a descriptive narrative of sex differences in cognitive performance across the life course and of sex differences in a range of neuroimaging indicators of brain health measured in later-life, within the NSHD and Insight 46 cohorts. Multivariable regression modelling was used to compare outcome measures in females with measures in males, while considering the potential role of socioeconomic, education, and lifestyle factors, and whether sex differences were modified by *APOE*-ε4 status. Females tended to perform better than males on most cognitive assessments throughout life and into older age, despite showing poorer cerebrovascular brain health (specifically, increased markers of cSVD) aged ~70, demonstrating female cognitive resilience to cerebrovascular pathology. There was evidence that sociocultural factors contributed to cognitive sex differences, with higher education level being especially beneficial for cognitive advantages in females. Primarily at later ages, there were also larger adverse associations of *APOE*-ε4 with cognitive performance in females. Overall, these analyses suggest that females could be more susceptible than males to some sociocultural and genetic factors linked with dementia risk and that, in females, cognitive function persists despite subtle brain changes.

## 3.1. Introduction

As discussed in Section 1.7., sex differences in cognition have been extensively examined and there are discrepancies in the findings, but the most consistent differences reported are male advantages in spatial abilities and female verbal memory advantages.[47] Evidence for sex differences in brain volumes is also mixed (Section 1.8.), but *in vivo* MRI demonstrates that males typically have larger brains throughout life, explaining the majority of variance in regional volumes.[57,58] There are reports of greater brain activation in females, indicated by greater cerebral blood flow,[58] who also reportedly show greater WMHV[65,66] and poorer NAWM[62,63] microstructural integrity than males, particularly at older ages. Conversely, sex differences in the AD hallmark, Aβ, are not found.[61]

Evidence demonstrates that cognitive sex differences are not robust against environmental influences, with task training or the gendered context of a task shown to remove sex differences in cognitive task performance,[50,224] indicating that differences are not necessarily biologically pre-determined. Indeed, lifestyle factors – including smoking and physical activity (PA), more common and frequent in males[225,226] – have been associated with cognitive and neuroimaging measures.[227–229] SEP and education, typically lower in females,[230,231] are also positively associated with cognitive performance and neuroimaging outcomes.[232,233] Sex differences in genetic risk for dementia might also contribute to cognitive and brain health sex differences. Despite an equal distribution of the *APOE*-ε4 genetic risk allele for AD between the sexes, the dementia risk associated with ε4 is greatest in females[90,95] and, compared with male ε4 carriers, female carriers have greater levels of brain pathology, poorer cognitive performance, and faster cognitive decline over time.[95,234] Understanding whether and how males and females differ in cognitive abilities and measures of brain health across the life course can help to identify how socioeconomic, lifestyle, and biological factors present at each life stage potentially underlie sex differences in later-life dementia risk.

Some, but not all, cognitive measures assessed throughout the life course in NSHD and Insight 46 (Section 2.3.2.) have been examined for sex differences. For instance, in 1968, Douglas et al.[235] reported a consistent female advantage in verbal skills from age 8 to 15. No sex differences in non-verbal abilities at ages 8 and 11 were found, but at 15 males showed non-verbal and mathematics advantages.[235] In another study looking at predictors of cognitive trajectories from age 43 to 69, females consistently outperformed males on repeated

measures of verbal memory and search speed across timepoints. While no sex differences in rates of verbal memory performance decline over time were detected, females showed slower decline in search speed task performance.[5] Within Insight 46 at age ~70, studies examining predictors of cognitive performance across a range of tasks found that females performed better than males on the PACC and each of its four sub-tests,[158] although no sex differences in matrix reasoning[158] or reaction time task performance[217] were detected. Another study primarily aiming to assess the effects of *APOE*-ε4 and Aβ pathology on visual working memory within Insight 46 did detect a dissociation whereby females showed better object identification and poorer recall of object locations than males.[218]

Insight 46 females have also shown younger predicted brain ages than males, based on volumetric neuroimaging measures.[236] When excluding individuals with neurological conditions, women had poorer NAWM integrity than men, but this was largely explained by differences in WMHV,[237] suggesting that alterations in NAWM and white matter hyperintensity may be part of an overlapping pathological process. However, in Insight 46 participants without neurological conditions, there was limited evidence for sex differences when looking at total WMHV itself.[158] In the same analyses, no sex differences in Aβ load were detected.[158]

While these NSHD and Insight46 studies have reported sex differences in papers with a wider scope, not specifically aiming to examine sex differences, sex differences across cognitive and imaging metrics have not yet been collated into one space to provide a comprehensive narrative of sex differences across the life course. Additionally, no direct assessments of socioeconomic and lifestyle covariable roles or potential *APOE*-ε4 effect modifications on sex differences have been completed.

### 3.1.1. Objectives and research questions

This work tests for sex differences in cognitive performance measures assessed throughout life in the NSHD and Insight 46 cohorts, and for sex differences in neuroimaging indicators of brain health at age ~70 within Insight 46. The aim is to inform which, if any, cognitive domains and brain health measures show male or female advantages, and when in the life course any cognitive sex differences are more evident. A descriptive overview of cognitive and brain

health sex differences across different life stages within the NSHD and Insight 46 cohorts is generated.

The following research questions are addressed:

1. Are there sex differences in cognitive performance measured across the life course and in brain health outcomes measured aged ~70?
2. Are sex differences explained by socioeconomic, education, and lifestyle covariables?
3. Are sex differences modified by *APOE*-ε4 status?

### 3.1.2. Hypotheses

Given previous reports outlined in Section 1.0., males and females were hypothesised to perform similarly across most cognitive domains, with subtle male advantages in spatial tasks[49] and female advantages in verbal ability assessments, namely verbal fluency.[238] Patterns of cognitive sex differences were expected to be similar across the life course, given that childhood cognition predicts later-life cognitive performance,[159] and previous NSHD evidence for consistent female processing speed and verbal memory advantages between ages 43 and 69.[5] Analyses were expected to replicate previous Insight 46 findings of no sex differences in Aβ or WMHV,[158] poorer NAWM integrity in females,[237] and larger brain volumes in females (given younger female predicted brain ages derived from volumetric MRI measures).[236]

Socioeconomic, education, and lifestyle factor adjustments were expected to influence estimated sex differences in cognitive performance and brain health measures, reflecting positive effects of education, SEP, and PA (all typically greater in males) and negative effects of smoking (greater in males) on outcomes.[54,228,229]

Sex-by-*APOE* interactions were expected to be evident in later-life, when subtle cognitive and brain health changes related to dementia could be beginning to emerge. Female *APOE*-ε4 carriers were expected to show poorer outcomes than male carriers, particularly for outcome measures associated with AD: memory domains, performance on a cognitive composite designed to detect subtle pre-clinical cognitive changes associated with AD, and Aβ pathology.

## 3.2. Analytic method

### 3.2.1. Analytic sample

At each timepoint, NSHD males and females were included in analyses if they had available data for at least one cognitive outcome at that timepoint. Insight 46 participants were included if they had available data for at least one cognitive assessment completed at wave I of data collection (age 69-71) and, for neuroimaging outcomes, if they underwent wave I PET-MRI neuroimaging and had available data for at least one neuroimaging outcome of interest. The maximal samples available at each timepoint are presented in Table 3.

***Table 3.*** *Maximal samples at each timepoint.*

| Age | Max N | % Female |
|---|---|---|
| **8** | 4,269 | 48.4 |
| **11** | 4,032 | 47.8 |
| **15** | 4,019 | 47.8 |
| **26** | 3,713 | 49.5 |
| **43** | 3,237 | 50.1 |
| **53** | 2,956 | 51.0 |
| **60-64** | 2,216 | 52.3 |
| **69** | 2,140 | 51.0 |
| **Insight 46 wave I cognition (~70)** | 502 | 49.0 |
| **Insight 46 wave I neuroimaging (~70)** | 470 | 48.7 |

### 3.2.2. Cognitive outcome measures

As outlined in Section 2.3.2., NSHD participants were administered tests of verbal and non-verbal abilities at ages 8, 11, and 15, while functional cognitive abilities were assessed at ages 43, 53, 60-64, and 69, summarised in Table 1. Insight 46 participants completed an additional comprehensive neuropsychological test battery aged 69-71 (Table 2), assessing a range of domains including memory, executive function, visuospatial function, and cognitive state.

All cognitive outcome measures were standardised to the analytical sample for each outcome, generating z-scores to facilitate comparison across different cognitive domains. Where necessary (i.e. for measures of error rates), z-scores were inverted such that higher z-scores represented better cognitive performance, across tasks.

### 3.2.3. Neuroimaging outcome measures

As outlined in Section 2.3.3., Insight 46 wave I participants underwent combined PET-MRI neuroimaging, generating a range of brain health and dementia-related pathology measures at age ~70. For these analyses, sex differences in TBV, hippocampal volume, WMHV, continuous amyloid SUVR, Aβ positivity status, NAWM microstructural integrity measures (FA and MD) and cortical thickness across ROI (frontal, occipital, parietal, temporal, the Harvard AD signature region)[220] are examined.

For analyses assessing WMHV as an outcome, participants who failed BaMoS QC were excluded (n=15; 53.3% female). Participants whose scans failed NAWM QC (n=59; 49.2% female) were excluded from NAWM analyses.

### 3.2.4. Covariables

Previous literature has demonstrated that socioeconomic, education, and lifestyle factors can differ between males and females. For example, men typically had greater levels of education,[230] higher occupational SEP,[231] greater levels of PA,[225,239,240] and higher levels of smoking[226,241] than women in this era. Evidence has also linked such factors with cognitive outcomes and with neuroimaging measures.[227–229,232,233] Therefore, in these analyses sociodemographic (education, SEP) and lifestyle (PA, smoking) covariables prospectively recorded throughout the life course were adjusted for, to examine the potential contribution of such factors in cognitive and neuroimaging sex differences.

Highest educational attainment by ages 26 and 43 (Section 2.3.4.2.) was categorised as: no qualifications, ordinary qualifications (O levels or equivalent), or advanced (A levels or higher).

SEP (Section 2.3.4.3.) was categorised as: unskilled, partly skilled, skilled manual, skilled non-manual, intermediate, professional. Overall childhood SEP reflects the most recent social class recorded at age 15, or at ages 11 or 4 if missing at 15. Overall adulthood SEP was derived at age 53 and 43, taking the most recent social class recorded and updating with previous SEP if missing at 53 or 43.

Participation in leisure time PA was recorded at ages 43, 53, 60, and 69 using the EPIC Physical Activity Questionnaire-2,[242] which assessed the frequency of participation in sports, vigorous leisure activities or exercise in the previous month (age 43) or the previous 4 weeks (ages 53,

60-64, 69). PA was not assessed during childhood or at age 26. As in previous work in this cohort,[227] responses at each age were categorised as: not active (no PA/month), moderately active (1-4 times/month), or most active (≥5 times/month).

Smoking pack years – a measure determined by multiplying the number of cigarette packs smoked per day by the number of years smoked – was based on self-reported frequencies at ages 26, 43, 53, and 60-64. The measure was not available during childhood or at age 69.

*APOE* genotype (Section 2.3.4.1.) indicated ε4 carrier or non-carrier status.

### 3.2.5. Statistical analyses

Analyses were conducted using Stata version 17.0.

Continuous raw cognitive performance scores, neuroimaging outcomes, and covariables were summarised by calculating the range, mean, and standard deviation (SD) for each variable, for males and females separately. For categorical variables, the percentage of participants in each category was calculated for males and females. Independent samples t-tests and chi-squared tests examined sex differences in continuous and categorical covariables, respectively.

For all cognitive outcome measures, binary sex (male/female) was included as the predictor variable in linear multivariable regression models, with male as the reference category. Initial models (M0) were completely unadjusted for NSHD cognitive assessments from age 8 through to 69, while models assessing Insight 46 (age 69-71) cognitive outcomes were adjusted for age at cognitive testing due to the two-year range required to complete Insight 46 data collection.

For neuroimaging outcomes, linear multivariable regression models with sex as the predictor examined sex differences in TBV, hippocampal volume, continuous SUVR, NAWM measures of microstructural integrity (MD, FA), and all cortical thickness ROI. Multivariable logistic regression was used to examine the odds ratio for being Aβ positive in females compared with males. Sex differences in WMHV, which has a skewed distribution, were examined using a generalised linear model with gamma distribution log link. All models were adjusted for age at scan and, for volumetric outcomes only (TBV, hippocampal volume, WMHV), for TIV.

Associations between sex and outcome measures were firstly examined in minimally adjusted models (M0). Next, models were individually adjusted for SEP (M0+SEP), education

(M0+education), smoking (M0+smoking), and PA (M0+PA) covariables available at each timepoint, as outlined in Table 4. Adjustments were recorded either concurrent to the outcome or at the most recent timepoint prior to the outcome measure, and SEP models for outcomes measured at age 43 onwards were also adjusted for an overall measure of childhood SEP.

Finally, for participants with available *APOE*-ε4 data, effect modification by *APOE*-ε4 status was examined by including a sex-by-*APOE* interaction term in minimally adjusted models. If the interaction was significant at the $p<0.1$ level, subsequent analyses were stratified by *APOE*-ε4 carrier status.

To account for missing SEP, education, smoking, and PA covariable data, multiple imputation was applied to the analytical sample at each timepoint (Section 2.4.2.) The imputation models included sex and the outcome measures relevant to the sample at each timepoint, in addition to other covariables included in the analytical models. Auxiliary variables included BMI and smoking status measured closest to the outcome timepoint, and the Townsend deprivation index measured in 1999.

Where cognitive outcome measures were not normally distributed, bootstrapping was applied (Section 2.4.1.) to the analyses of minimally adjusted models, including *APOE* interaction models. When adjusting for covariables, changes in effect sizes were of interest so to avoid the need to combine bootstrapping with multiple imputation, which is computationally intensive, bootstrapping was not applied to adjusted models.

***Table 4.*** *Summary of model adjustments at each timepoint.*

| Age (year) | M0 | M0+SEP | M0+Education | M0+PA | M0+Smoking |
|---|---|---|---|---|---|
| **8 (1954)** | Unadjusted | Paternal SEP, age 4 | - | - | - |
| **11 (1957)** | Unadjusted | Paternal SEP, age 11 | - | - | - |
| **15 (1961)** | Unadjusted | Paternal SEP, age 15 | - | - | - |
| **26 (1972)** | Unadjusted | Paternal SEP, up to age 15 | Education, age 26 | - | Smoking, age 26 |
| **43 (1989)** | Unadjusted | Paternal SEP, up to age 15 + SEP 15-43 | Education, age 43 | PA, age 43 | Smoking, age 43 |
| **53 (1999)** | Unadjusted | Paternal SEP, up to age 15 + SEP 15-53 | Education, age 43 | PA, age 53 | Smoking, age 53 |
| **60-64 (2006-10)** | Unadjusted | Paternal SEP, up to age 15 + SEP 15-53 | Education, age 43 | PA, age 60-64 | Smoking, age 60-64 |
| **69 (2015)** | Unadjusted | Paternal SEP, up to age 15 + SEP 15-53 | Education, age 43 | PA, age 69 | Smoking, age 60-64 |
| **69-71 (2015-18) Cognition** | Age at cognitive testing | Paternal SEP, up to age 15 + SEP 15-53 | Education, age 43 | PA, age 69 | Smoking, age 60-64 |
| **69-71 (2015-18) Neuroimaging** | Age at scan + TIV [TBV, hippocampal volume, WMHV only] | Paternal SEP, up to age 15 + SEP 15-53 | Education, age 43 | PA, age 69 | Smoking, age 60-64 |

*SEP=socioeconomic position; PA=physical activity; TIV=total intracranial volume; TBV=total brain volume; WMHV=white matter hyperintensity volume*

## 3.3. Results

### 3.3.1. Participant characteristics

Mean performance scores on each cognitive assessment across timepoints are summarised for males and females in Table 5 and mean neuroimaging measures from Insight 46 wave I (aged ~70 years) are summarised in Table 6.

As shown in Table 7, which presents summary data for the covariables measured at each timepoint, females had overall higher occupational classifications in adulthood. However, the highest classifications (professional and intermediate) had greater proportions of males than

females. Educational attainment was significantly greater in males than females, and significantly more males than females obtained further qualifications in adulthood between ages 26 and 43 (n=228 males vs. 163 females; $\chi^2$=21.43; p<0.01). Females had significantly greater levels of physical inactivity than males at age 43, but not at subsequent ages. At younger ages, males had greater levels of smoking than females, but this diminished over time and was no longer statistically significant at age 60-64, possibly explained by an overall decline in the number of smokers (41.54% of respondents were current smokers at age 26, compared with 12.13% at age 60-64). There was a trend (p<0.1) for a greater proportion of *APOE*-ε4 carriers in males than females. For Insight 46 participants, there was no difference in age at visit or age at scan between males and females, but males had significantly greater TIV than females.

***Table 5.*** *Mean, standard deviation, and range of raw performance scores for each cognitive assessment completed by NSHD and Insight 46 males and females at several ages throughout the life course.*

| | | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | **Cognitive assessment** | **N** | **Mean** | **SD** | **Min** | **Max** | **N** | **Mean** | **SD** | **Min** | **Max** |
| **8yrs** | Picture intelligence | 2,203 | 40.17 | 9.50 | 0 | 60 | 2061 | 40.21 | 9.47 | 0 | 59 |
| | Sentence completion | 2,196 | 13.73 | 8.02 | 0 | 34 | 2,062 | 14.71 | 7.48 | 0 | 34 |
| | Word reading | 2,196 | 16.37 | 10.56 | 0 | 49 | 2,062 | 17.71 | 9.91 | 0 | 45 |
| | Vocabulary | 2,196 | 16.52 | 6.04 | 0 | 36 | 2,062 | 16.01 | 5.93 | 0 | 40 |
| | Age 8 overall cognition (z-score) | 2,197 | -0.02 | 0.87 | -2.61 | 2.68 | 2059 | 0.02 | 0.85 | -2.61 | 2.52 |
| **11yrs** | Word reading | 2,101 | 35.96 | 11.08 | 0 | 50 | 1924 | 36.94 | 9.82 | 0 | 50 |
| | Vocabulary | 2,101 | 30.30 | 7.48 | 0 | 47 | 1924 | 29.64 | 7.41 | 0 | 49 |
| | Arithmetic | 2,102 | 26.15 | 12.05 | 0 | 50 | 1921 | 26.66 | 11.39 | 0 | 50 |
| | General ability | 2,106 | 43.76 | 15.94 | 0 | 80 | 1924 | 46.38 | 15.72 | 0 | 78 |
| | Age 11 overall cognition (z-score) | 2,101 | -0.03 | 0.90 | -3.01 | 1.74 | 1921 | 0.03 | 0.86 | -2.91 | 1.96 |
| **15yrs** | Watts-Vernon | 2,097 | 24.67 | 6.69 | 0 | 35 | 1915 | 24.25 | 6.57 | 0 | 35 |
| | General ability | 2,097 | 74.14 | 20.52 | 0 | 125 | 1918 | 73.97 | 19.62 | 5 | 125 |
| | Mathematics | 2,098 | 15.83 | 10.82 | 0 | 46 | 1915 | 12.55 | 9.20 | 0 | 42 |
| | Age 15 overall cognition (z-score) | 2,094 | 0.05 | 0.93 | -2.93 | 2.17 | 1912 | -0.08 | 0.85 | -2.78 | 2.07 |
| **26yrs** | Watts-Vernon | 1,874 | 33.11 | 8.21 | 0 | 45 | 1839 | 32.48 | 8.31 | 0 | 45 |
| **43yrs** | Word learning | 1,528 | 23.89 | 6.25 | 0 | 42 | 1531 | 25.55 | 6.43 | 6 | 41 |
| | Search speed | 1,577 | 332.90 | 77.16 | 127 | 450 | 1573 | 354.71 | 74.70 | 25 | 450 |
| | Peg speed | 1,575 | 104.12 | 16.80 | 77 | 516 | 1588 | 104.87 | 14.35 | 78 | 362 |
| | Picture memory | 1,608 | 4.34 | 0.82 | 0 | 5 | 1616 | 4.47 | 0.75 | 0 | 5 |
| **53yrs** | Word learning | 1,408 | 22.97 | 6.22 | 4 | 40 | 1478 | 24.84 | 6.25 | 3 | 41 |
| | Search speed | 1,438 | 272.67 | 75.42 | 91 | 591 | 1493 | 289.24 | 75.84 | 64 | 591 |
| | NART | 1,369 | 34.41 | 9.66 | 2 | 50 | 1455 | 34.21 | 9.42 | 1 | 50 |
| | Animal naming | 1,442 | 23.74 | 6.73 | 1 | 52 | 1506 | 23.40 | 7.09 | 1 | 62 |
| **60-64yrs** | Word learning | 1,023 | 23.04 | 5.92 | 6 | 41 | 1127 | 25.36 | 6.08 | 4 | 43 |
| | Search speed | 1,039 | 261.14 | 71.39 | 98 | 591 | 1143 | 271.78 | 71.72 | 112 | 591 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | MRT | 1,033 | 287.53 | 71.03 | 41 | 836 | 1134 | 284.84 | 66.07 | 185 | 849 |
| | IIVrt | 1,033 | 0.23 | 0.12 | 0 | 2 | 1134 | 0.22 | 0.11 | 0 | 1 |
| **69yrs** | Word learning | 1,011 | 21.14 | 5.98 | 0 | 39 | 1063 | 23.15 | 5.99 | 6 | 40 |
| | Search speed | 1,038 | 256.37 | 74.32 | 70 | 591 | 1073 | 268.02 | 73.56 | 60 | 591 |
| | Finger tapping | 1,005 | 49.92 | 10.91 | 15 | 87 | 1041 | 44.94 | 10.20 | 11 | 74 |
| | ACE-III total | 849 | 91.33 | 5.84 | 53 | 100 | 913 | 91.69 | 6.17 | 59 | 100 |
| | ACE-III verbal fluency | 1,028 | 10.86 | 2.17 | 1 | 14 | 1073 | 11.14 | 2.05 | 1 | 14 |
| | ACE-III language | 849 | 25.28 | 1.15 | 19 | 26 | 916 | 25.26 | 1.18 | 16 | 26 |
| | ACE-III attention | 865 | 16.86 | 1.71 | 6 | 18 | 921 | 16.59 | 1.97 | 5 | 18 |
| | ACE-III memory | 865 | 23.17 | 2.98 | 7 | 26 | 922 | 23.73 | 2.71 | 12 | 26 |
| | ACE-III visuospatial | 861 | 15.13 | 1.19 | 9 | 16 | 917 | 14.98 | 1.37 | 4 | 16 |
| **69-71yrs (Insight 46)** | PACC total z-score | 256 | -0.17 | 0.71 | -3.49 | 1.72 | 246 | 0.18 | 0.71 | -3.48 | 1.67 |
| | MMSE z-score | 256 | -0.07 | 1.03 | -7.17 | 0.74 | 246 | 0.08 | 0.97 | -6.18 | 0.74 |
| | DSST z-score | 255 | -0.15 | 1.00 | -2.76 | 3.03 | 246 | 0.16 | 0.98 | -2.28 | 3.31 |
| | Logical memory delayed z-score | 256 | -0.22 | 1.00 | -3.10 | 2.57 | 246 | 0.23 | 0.95 | -3.10 | 3.11 |
| | FNAME total z-score | 256 | -0.23 | 0.99 | -2.91 | 1.51 | 244 | 0.25 | 0.96 | -3.40 | 1.62 |
| | Matrix reasoning z-score | 256 | -0.02 | 1.04 | -4.07 | 1.42 | 246 | 0.02 | 0.96 | -3.87 | 1.62 |
| | IIVrt | 256 | 0.12 | 0.03 | 0.04 | 0.23 | 245 | 0.13 | 0.03 | 0.06 | 0.22 |
| | Response inhibition | 256 | 0.13 | 0.11 | -0.15 | 0.82 | 244 | 0.14 | 0.12 | -0.09 | 0.88 |
| | Visuomotor rotations | 249 | 0.99 | 0.80 | 0.00 | 6.16 | 234 | 1.00 | 1.02 | 0.00 | 12.04 |
| | Visuomotor errors | 249 | 5.45 | 3.60 | 0.63 | 29.16 | 234 | 5.18 | 4.11 | 0.60 | 35.84 |
| | Visuomotor subtraction rate | 249 | 0.55 | 0.17 | 0.19 | 1.01 | 234 | 0.45 | 0.15 | 0.12 | 1.08 |
| | Visual working memory ID rate | 246 | 0.82 | 0.09 | 0.46 | 1.00 | 240 | 0.83 | 0.09 | 0.58 | 1.00 |
| | Visual working memory localisation error | 246 | 6.55 | 1.95 | 3.18 | 12.94 | 240 | 7.03 | 2.00 | 2.75 | 13.83 |
| | Visual working memory swap error rate | 246 | 0.20 | 0.11 | 0.00 | 0.50 | 240 | 0.19 | 0.11 | 0.00 | 0.54 |

*SD=standard deviation; NART=National Adult Reading Test; MRT=mean reaction time; IIVrt=intra-individual variability in reaction times; ACE-III=Addenbrooke's Cognitive Examination 3rd edition; PACC=pre-clinical Alzheimer's Cognitive Composite; MMSE=mini-mental state examination; DSST=digit-symbol substitution test; FNAME=face-name associative memory examination; ID=identification*

***Table 6.*** *Mean, standard deviation, and range of neuroimaging measures in Insight 46 males and females measured at age ~70 years.*

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Neuroimaging measure** | **N** | **Mean** | **SD** | **Min** | **Max** | **N** | **Mean** | **SD** | **Min** | **Max** |
| TBV ($cm^3$) | 239 | 1151.35 | 86.69 | 945.90 | 1493.86 | 229 | 1046.75 | 82.49 | 818.60 | 1265.16 |
| Hippocampal Volume ($cm^3$) | 239 | 3.25 | 0.33 | 2.42 | 4.27 | 229 | 3.00 | 0.30 | 2.06 | 3.72 |
| WMHV ($cm^3$) | 234 | 4.77 | 5.09 | 0.27 | 33.67 | 221 | 5.47 | 5.78 | 0.35 | 32.78 |
| SUVR | 235 | 0.57 | 0.08 | 0.45 | 0.87 | 227 | 0.56 | 0.07 | 0.47 | 0.85 |
| Amyloid positive [N(%)] | | | 47 (20%) | | | | | 39 (17.18%) | | |
| Amyloid negative [N(%)] | | | 188 (80%) | | | | | 188 (82.82%) | | |
| NAWM FA (z-score) | 218 | 0.00 | 0.26 | -0.92 | 0.72 | 206 | -0.11 | 0.24 | -0.76 | 0.48 |
| NAWM MD (z-score) | 218 | 0.16 | 0.37 | -0.60 | 1.77 | 206 | 0.23 | 0.34 | -0.55 | 1.23 |
| CT Harvard ADsig (mm) | 239 | 2.68 | 0.08 | 2.39 | 2.87 | 229 | 2.68 | 0.08 | 2.42 | 2.88 |
| CT Frontal (mm) | 239 | 2.76 | 0.09 | 2.18 | 2.95 | 229 | 2.75 | 0.09 | 2.36 | 2.92 |
| CT Occipital (mm) | 239 | 2.20 | 0.10 | 1.90 | 2.46 | 229 | 2.18 | 0.09 | 1.94 | 2.38 |
| CT Parietal (mm) | 239 | 2.46 | 0.08 | 2.13 | 2.67 | 229 | 2.48 | 0.08 | 2.19 | 2.67 |
| CT Temporal (mm) | 239 | 2.87 | 0.08 | 2.55 | 3.15 | 229 | 2.85 | 0.09 | 2.57 | 3.09 |

*SD=standard deviation; TBV=total brain volume; WMHV=white matter hyperintensity volume; SUVR=standardised uptake value ratio; NAWM=normal appearing white matter; FA=factional anisotropy; MD=mean diffusivity; CT=cortical thickness; ADsig=Alzheimer's Disease signature region*

***Table 7.*** *Summary of male and female participant characteristics for covariables measured at different timepoints across the life course.*

| | Males | | Females | | Test of difference | |
|---|---|---|---|---|---|---|
| **Variable** | **N** | **%** | **N** | **%** | **t/$\chi^2$** | **p-value** |
| Father's social class age 4 | 2,354 | | 2,144 | | 1.32 | 0.93 |
| Professional | 136 | 5.78 | 125 | 5.83 | | |
| Intermediate | 387 | 16.44 | 361 | 16.84 | | |
| Skilled non-manual | 432 | 18.35 | 392 | 18.28 | | |
| Skilled manual | 732 | 31.1 | 689 | 32.14 | | |
| Partly skilled | 505 | 21.45 | 437 | 20.38 | | |
| Unskilled | 162 | 6.88 | 140 | 6.53 | | |
| *High (professional to non-manual)* | 955 | 40.57 | 878 | 40.95 | 0.07 | 0.79 |
| *Low (manual to unskilled)* | 1,399 | 59.43 | 1,266 | 59.05 | | |
| Father's social class age 11 | 2,039 | | 1,883 | | 4.86 | 0.43 |
| Professional | 125 | 6.13 | 112 | 5.95 | | |
| Intermediate | 409 | 20.06 | 354 | 18.80 | | |
| Skilled non-manual | 314 | 15.40 | 289 | 15.35 | | |
| Skilled manual | 683 | 33.50 | 650 | 34.52 | | |
| Partly skilled | 371 | 18.20 | 374 | 19.86 | | |
| Unskilled | 137 | 6.72 | 104 | 5.52 | | |
| *High (professional to non-manual)* | 848 | 41.58 | 755 | 40.10 | 0.90 | 0.34 |
| *Low (manual to unskilled)* | 1,191 | 58.41 | 1,128 | 59.90 | | |
| Father's social class age 15 | 1,945 | | 1,798 | | 3.20 | 0.67 |
| Professional | 135 | 6.94 | 114 | 6.34 | | |
| Intermediate | 458 | 23.55 | 391 | 21.75 | | |
| Skilled non-manual | 274 | 14.09 | 264 | 14.68 | | |
| Skilled manual | 618 | 31.77 | 583 | 32.42 | | |
| Partly skilled | 351 | 18.05 | 349 | 19.41 | | |
| Unskilled | 109 | 5.60 | 97 | 5.39 | | |
| *High (professional to non-manual)* | 867 | 44.58 | 769 | 42.77 | 1.24 | 0.27 |
| *Low (manual to unskilled)* | 1,078 | 55.42 | 1,029 | 57.23 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Childhood social class | 2,452 | | 2,221 | | 2.38 | 0.80 |
| Professional | 155 | 6.32 | 135 | 6.08 | | |
| Intermediate | 483 | 19.70 | 424 | 19.09 | | |
| Skilled non-manual | 385 | 15.70 | 344 | 15.49 | | |
| Skilled manual | 808 | 32.95 | 746 | 33.59 | | |
| Partly skilled | 449 | 18.31 | 433 | 19.50 | | |
| Unskilled | 172 | 7.01 | 139 | 6.26 | | |
| *High (professional to non-manual)* | 1,023 | 41.72 | 903 | 40.66 | 0.54 | 0.46 |
| *Low (manual to unskilled)* | 1,429 | 58.28 | 1,318 | 59.34 | | |
| Overall social class age 15 to 43 | 2,119 | | 2,039 | | **871.56** | **<0.01** |
| Professional | 228 | 10.76 | 28 | 1.37 | | |
| Intermediate | 795 | 37.52 | 618 | 30.31 | | |
| Skilled non-manual | 202 | 9.53 | 801 | 39.28 | | |
| Skilled manual | 640 | 30.20 | 159 | 7.80 | | |
| Partly skilled | 192 | 9.06 | 337 | 16.53 | | |
| Unskilled | 62 | 2.93 | 96 | 4.71 | | |
| *High (professional to non-manual)* | 1,225 | 57.81 | 1,447 | 70.97 | **78.31** | **<0.01** |
| *Low (manual to unskilled)* | 894 | 42.19 | 592 | 29.03 | | |
| Overall social class age 15 to 53 | 2,144 | | 2,056 | | **798.02** | **<0.01** |
| Professional | 246 | 11.47 | 36 | 1.75 | | |
| Intermediate | 769 | 35.87 | 638 | 31.03 | | |
| Skilled non-manual | 213 | 9.93 | 767 | 37.31 | | |
| Skilled manual | 644 | 30.04 | 170 | 8.27 | | |
| Partly skilled | 204 | 9.51 | 334 | 16.25 | | |
| Unskilled | 68 | 3.17 | 111 | 5.40 | | |
| *High (professional to non-manual)* | 1,228 | 57.28 | 1,441 | 70.09 | **74.37** | **<0.01** |
| *Low (manual to unskilled)* | 916 | 42.72 | 615 | 29.91 | | |
| Educational attainment by age 26 | 2,308 | | 2,124 | | **126.54** | **<0.01** |
| None attempted | 930 | 40.29 | 835 | 39.31 | | |
| Vocational or GCSE-level | 484 | 20.97 | 732 | 34.46 | | |
| A-level or higher | 894 | 38.73 | 557 | 26.22 | | |
| Educational attainment by age 43 | 2,401 | | 2,216 | | **91.95** | **<0.01** |

| | | | | | | |
|---|---|---|---|---|---|---|
| None attempted | 797 | 33.10 | 782 | 35.29 | | |
| Vocational or GCSE-level | 605 | 25.20 | 787 | 35.51 | | |
| A-level or higher | 999 | 41.61 | 647 | 29.20 | | |
| Physical activity age 43 | 1,634 | | 1,627 | | **19.45** | **<0.01** |
| None | 794 | 48.59 | 904 | 55.56 | | |
| 1-4 times | 386 | 23.62 | 367 | 22.56 | | |
| 5 or more times | 454 | 27.78 | 356 | 21.88 | | |
| Physical activity age 53 | 1,465 | | 1,520 | | 3.80 | 0.15 |
| None | 705 | 48.12 | 772 | 50.79 | | |
| 1-4 times | 273 | 18.63 | 245 | 16.12 | | |
| 5 or more times | 487 | 33.24 | 503 | 33.09 | | |
| Physical activity age 60-64 | 1,047 | | 1,141 | | 1.39 | 0.50 |
| None | 683 | 65.23 | 717 | 62.84 | | |
| 1-4 times | 137 | 13.09 | 162 | 14.20 | | |
| 5 or more times | 227 | 21.68 | 62 | 22.96 | | |
| Physical activity age 69 | 1,260 | | 1,341 | | 3.16 | 0.21 |
| None | 761 | 60.40 | 811 | 60.48 | | |
| 1-4 times | 142 | 11.27 | 178 | 13.27 | | |
| 5 or more times | 357 | 28.33 | 352 | 26.25 | | |
| *APOE*-ε4 status | 1,335 | | 1,351 | | 3.88 | 0.05 |
| Non-carrier | 909 | 68.09 | 967 | 71.58 | | |
| Carrier | 426 | 31.91 | 384 | 28.42 | | |
| Smoking pack years age 26 (Mean(SD)[range]) | 1,568 | 2.33(2.81) [0, 19.50] | 1,551 | 1.65(2.33) [0, 15.90] | **7.36** | **<0.01** |
| Smoking pack years age 43 (Mean(SD)[range]) | 1,579 | 2.38(3.68) [0, 21.00] | 1,590 | 1.79(2.93) [0, 24.50] | **5.04** | **<0.01** |
| Smoking pack years age 53 (Mean(SD)[range]) | 1,434 | 2.80(4.76) [0, 25.00] | 1,491 | 2.05(3.79) [0, 31.25] | **4.75** | **<0.01** |
| Smoking pack years age 60-64 (Mean(SD)[range]) | 1,410 | 1.33(3.21) [0, 22.50] | 1,402 | 1.11(2.67) [0, 20.00] | 1.97 | 0.05 |
| Insight 46 age at visit (Mean(SD)[range]) | 256 | 70.64(0.69) [70.56, 70.73] | 246 | 70.66(0.68) [70.57, 70.4] | -0.28 | 0.78 |
| Insight 46 age at scan (Mean(SD)[range]) | 241 | 70.66(0.68) [69.25, 71.78] | 230 | 70.67(0.67) [69.27, 71.86] | -0.18 | 0.86 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Insight 46 TIV ($cm^3$) (Mean(SD)[range]) | 239 | 1519.62(1274.26) [1274.26, 1938.77] | 229 | 1343.16(92.47) [1114.35, 1558.05] | **19.16** | **<0.01** |

*SD=standard deviation; GCSE=general certificate of secondary education; APOE=apolipoprotein E; TIV=total intracranial volume.*

*Tests of difference (t-tests for continuous variables, $\chi^2$ for categorical variables) are highlighted in bold when significant at the p<0.05 level.*

### 3.3.2. Cognitive performance in childhood and young adulthood

Unadjusted model estimates comparing male and female performance on cognitive ability tests completed by NSHD participants at ages 8, 11, 15 and 26 are presented in Figure 1, which demonstrates that females performed better than males on most assessments at ages 8 and 11, while males performed better on most assessments at age 15 and 26.

Some notable patterns of sex differences across childhood and young adulthood include:

i. *Changing advantage in overall cognition:* Significant sex differences in overall cognition z-scores at age 8 were not detected, while at 11 there was a trend for better overall cognitive performance in females than in males (β=0.05; 95% CI 0.00, 0.11), and at 15 males performed better than females overall (β=-0.13[-0.19, -0.08]). At 11, females performed better than males on the general ability test (β=0.17[0.10, 0.23]) but at 15 no sex differences were detected in general ability test performance.

ii. *Changing advantage in sentence completion task and arithmetic task:* At age 8, females performed better than males on the sentence completion task (β=0.13[0.07, 0.19]), but at 15 there was a trend for better male performance on a similar sentence completion assessment (the Watts-Vernon test: β=-0.06; bias-corrected 95% CI -0.13, 0.00), and at age 26, males performed significantly better than females on this task (β=-0.08; bias-corrected 95% CI -0.14, -0.01). As previously found by Douglas et al. (1968),[235] no sex difference was detected in arithmetic performance at age 11, but at 15 males showed better performance on the mathematics test than females (β=-0.32[-0.38, -0.26]).

iii. *Consistent female advantage on word reading task:* Females consistently performed better than males on the word reading task at ages 8 (β=0.13; bias-corrected 95% CI 0.07, 0.019) and 11 (β=0.09; bias-corrected 95% CI 0.03, 0.16).

iv. *Consistent male advantage on vocabulary task:* There was a consistent male advantage in vocabulary test performance at ages 8 (β=-0.09[-0.15, -0.03]) and 11 (β=-0.09[-0.15, -0.03]).

Adjustments for SEP (Table 8) did not substantially change model effect estimates for cognitive outcomes measured at ages 8, 15, and 26. However, SEP adjustment at age 11 revealed a trend for better female than male performance on the arithmetic test (β=0.06[0.00, 0.11]) and

the trend for better overall cognitive performance at age 11 in females became statistically significant (β=0.06[0.01, 0.11]), reflecting positive confounding. At 26, adjusting for education attenuated the male advantage in Watts-Vernon task performance, while accounting for smoking slightly strengthened the male advantage, demonstrating negative confounding from smoking.

No effect modification by *APOE*-ε4 status was detected for cognitive outcomes measured at ages 8, 11, or 26, but at 15 a sex-by-*APOE* interaction was detected for mathematics performance (Table 9) whereby a stronger male advantage was observed in *APOE*-ε4 carriers (β=-0.46; bias-corrected 95% CI -0.61, -0.31) than in non-carriers (β=-0.28; bias-corrected 95% CI -0.38, -0.18) (Table 10).

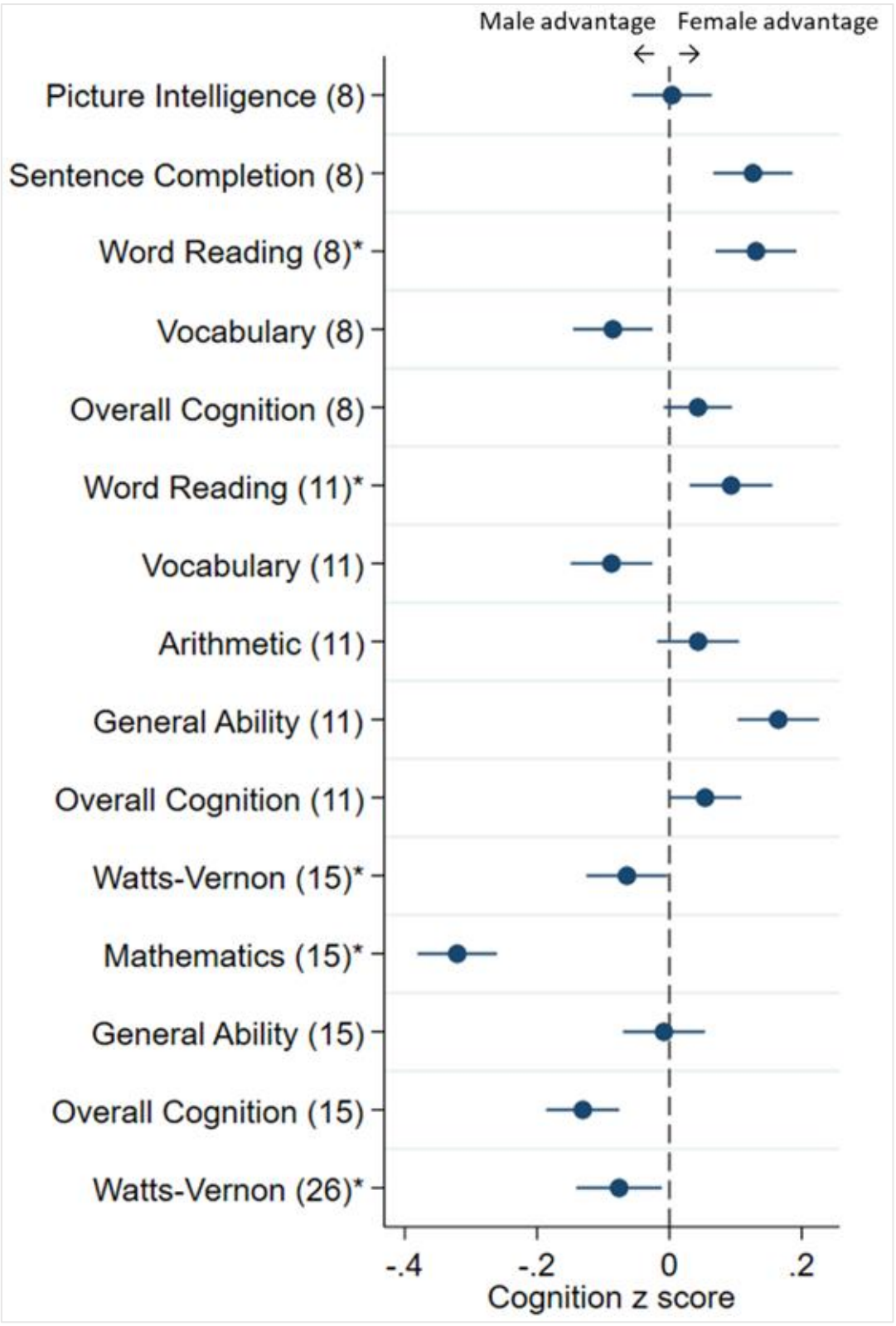


***Figure 1.*** *Unadjusted (M0) model estimates and 95% confidence intervals for cognitive assessments administered during childhood (ages 8, 11, and 15) and young adulthood (age 26).*

*Reference category: males.*

**Bootstrapping applied: confidence intervals are bias-corrected*

### 3.3.3. Cognitive performance ages 43 to 69

As shown in Figure 2, unadjusted model estimates indicate that, overall, females performed better than males on most cognitive assessments from age 43 to 69.

Some notable patterns in sex differences include:

i. *Persistent female advantages on word list recall and timed letter search tasks:* From age 43 through to age 69, females outperformed males on repeated measures of these tasks.

ii. *Changes in verbal fluency advantages:* No sex differences in verbal fluency performance were detected at age 53, but at age 69 females showed advantages on the ACE-III verbal fluency sub-domain.

iii. *No sex differences in cognitive state at age 69:* Males and females did not significantly differ in their ACE-III total performance.

iv. *Male task advantages observed only at age 69:* Males performed significantly better than females on the finger tapping task at age 69, and on the ACE-III attention and orientation and visuospatial sub-domains.

SEP adjustments (Table 8) strengthened female advantages in word list recall and timed letter search performance at ages 43, 60-64, and 69, prospective memory performance at age 54 and ACE-III sub-domains at age 69. SEP adjustments attenuated male advantages in ACE-III attention and orientation, and visuospatial domains at age 69.

Adjusting for education (Table 8) attenuated male advantages in ACE-III attention and orientation and visuospatial sub-domain performance at 69, while female performance advantages across all ages were strengthened. At 53, adjusting for education revealed a statistically significant female advantage on the NART ($\beta$=0.11[0.05, 0.18]).

Smoking adjustments (Table 8) had minimal impact on effect estimates for cognitive outcomes at ages 43, 60-64 and 69, but at age 53 smoking adjustments slightly attenuated female performance advantages across tasks. Across ages and cognitive measures, adjustments for PA did not substantially alter model estimates (Table 8).

*APOE*-ε4 effect modification was detected for word list recall (age 53 and 69), prospective memory (age 53), verbal fluency (age 53), ACE-III total (age 69), and ACE-III memory (age 69) (Table 9). Stratifying these models by *APOE*-ε4 status (Table 10) demonstrated that, of these memory tasks that initially showed female advantage (word list recall, prospective memory, ACE-III memory), the female advantage was stronger in ε4 non-carriers than in carriers. Conversely, the verbal fluency task at age 53 showed a male advantage only in ε4 carriers.

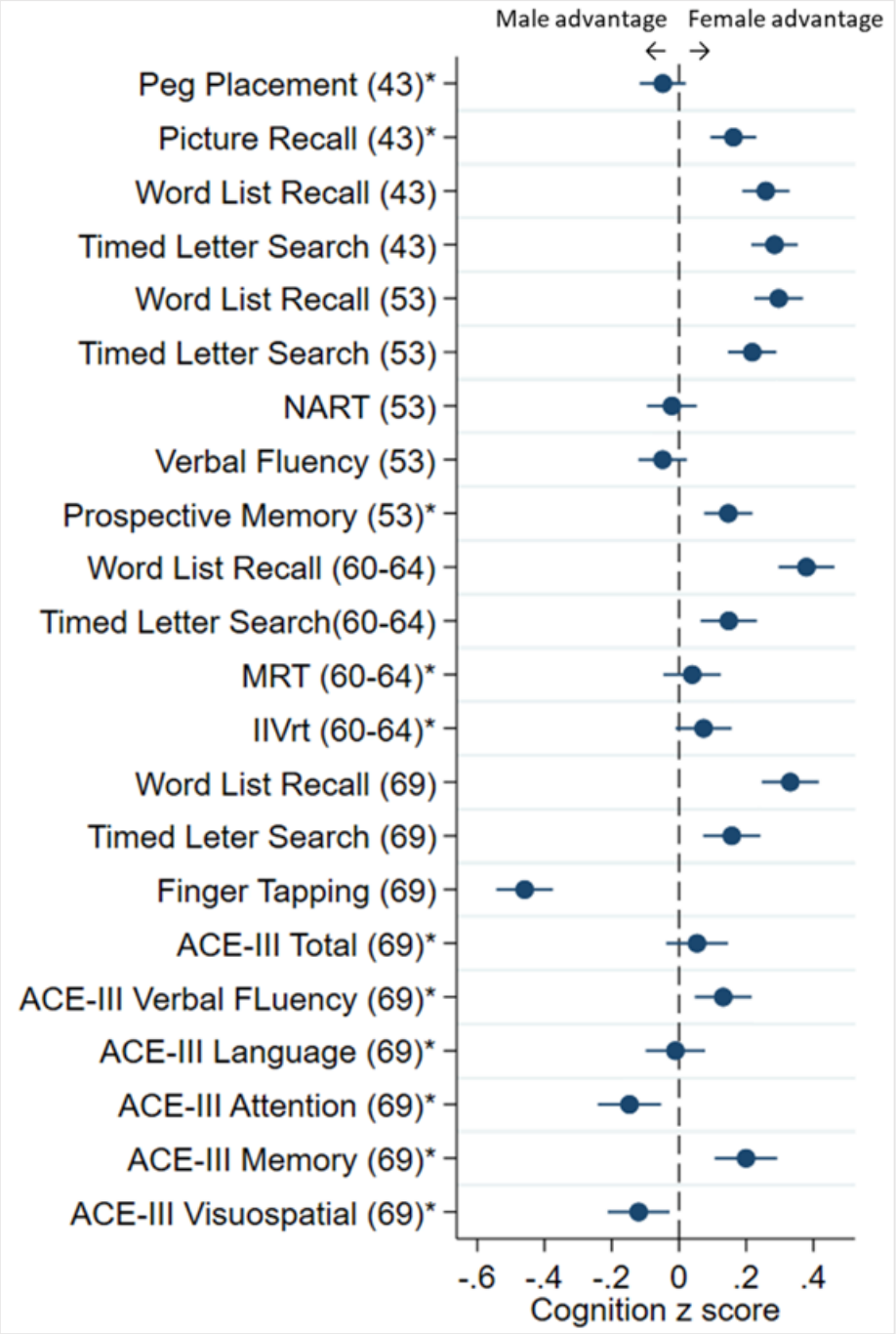


***Figure 2.*** *Unadjusted (M0) model estimates and 95% confidence intervals for cognitive assessments administered at ages 43, 53, 60-64, and 69.*

*Reference category: males.*
**Bootstrapping applied: confidence intervals are bias-corrected*
*NART=national adult reading test; MRT=mean reaction time; IIVrt=intra-individual variability in reaction times; ACE-III=Addenbrooke's cognitive examination 3rd edition*

### 3.3.4. Insight 46 wave I cognitive measures (age ~70)

Females performed better than males on the PACC sub-tests, while few sex differences were detected for additional computerised tasks (Figure 3). Males had significantly greater visuomotor integration task subtraction rates (β=-0.59[-0.76, -0.41]) and fewer visual working memory localisation errors (β=-0.24[-0.42, -0.06]) than females.

With adjustments for SEP (Table 8), female advantages on the PACC total and its sub-domains increased. The male advantage for visuomotor subtraction rate was slightly strengthened with SEP adjustment, while the effect for better male object localisation was attenuated. Adjusting for education strengthened most female advantages and slightly attenuated male advantages. Model estimates were not substantially altered with smoking or PA adjustments.

Effect modification by *APOE*-ε4 status was detected for PACC total, logical memory, visual working memory localisation error, and visual working memory swap error (Table 9). In stratified analyses of these models (Table 10), female advantages on the PACC total and logical memory tasks were detected only in ε4 non-carriers. Male non-carriers showed better object localisation than female non-carriers, and female ε4 carriers showed fewer visual working memory swap errors than male carriers.

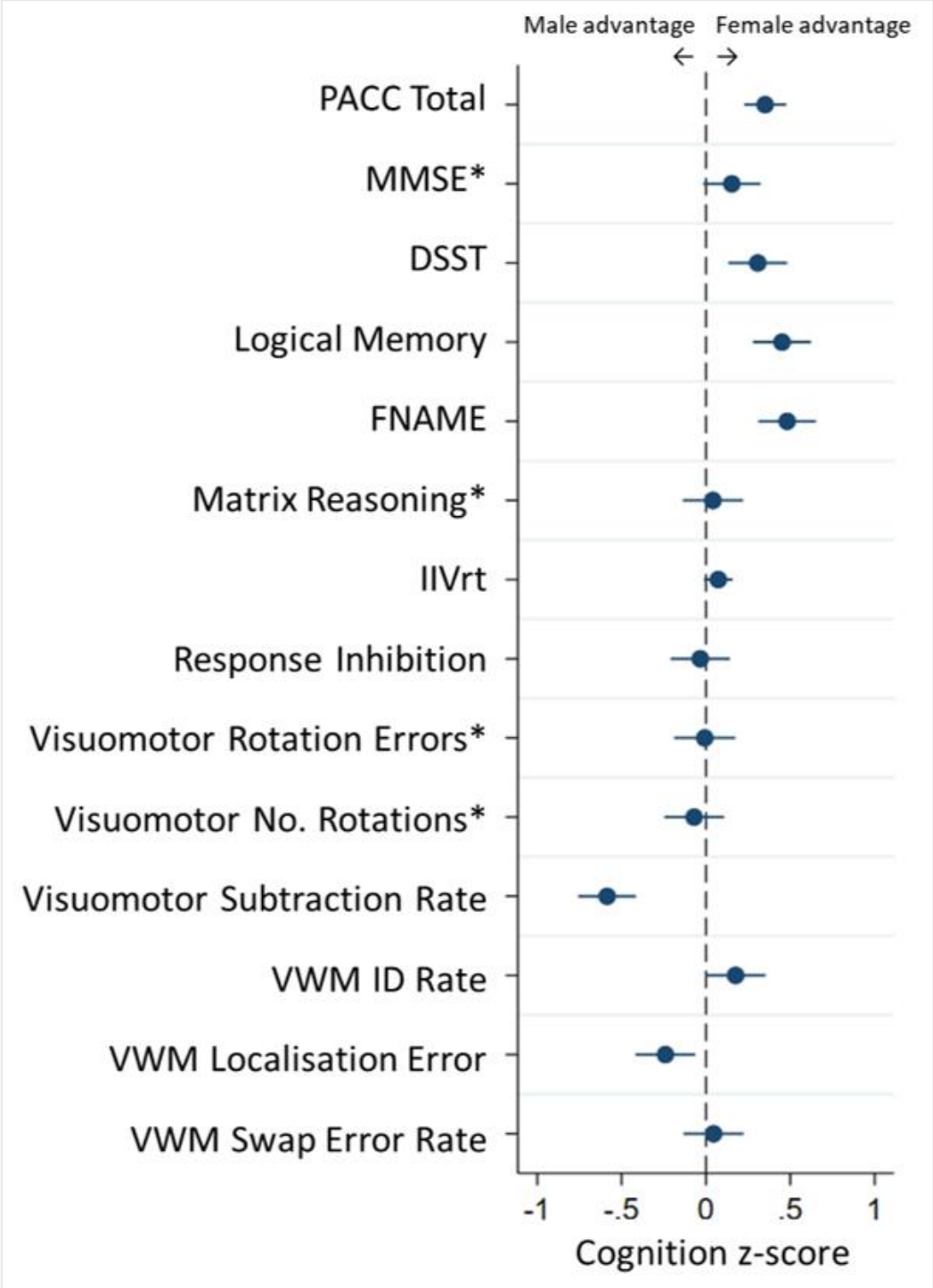


***Figure 3.*** *Unadjusted (M0) model estimates and 95% confidence intervals for cognitive assessments administered at Insight 46 wave I (age ~70).*

*Reference category: males.*

**Bootstrapping applied: confidence intervals are bias-corrected*

*PACC=pre-clinical Alzheimer's cognitive composite; MMSE=mini-mental state examination; DSST=digit-symbol substitution test; FNAME=face-name associative memory examination; IIVrt=intra-individual variability in reaction times; VWM=visual working memory; ID=identification*

### 3.3.5. Insight 46 wave I neuroimaging measures (age ~70)

#### *3.3.5.1. Volumetric measures*

With minimal adjustments, females had, on average, a 23.45cm$^3$ greater TBV, relative to TIV, than males (95% CI 12.65, 34.27). This effect was strengthened with adjustment for SEP, but adjustments for education, smoking, and PA did not substantially alter the model estimates (Table 8; Figure 4). No effect modification by *APOE*-ε4 status was detected (Table 9).

No sex difference in hippocampal volume was detected, nor did model adjustments alter the effect estimates (Table 8; Figure 4). No effect modification by *APOE*-ε4 status was detected (Table 9).

#### *3.3.5.2. White matter hyperintensity volume (WMHV)*

With minimal adjustments, females had, on average, 33% greater WMHV than males (relative log change=1.33[1.02, 1.73]). This effect was slightly increased with adjustments for SEP and PA while adjustments for education and smoking did not alter the effect estimate (Table 8; Figure 4). No effect modification by *APOE*-ε4 status was detected (Table 9).

#### *3.3.5.3. Amyloid*

No sex differences in continuous SUVR or in amyloid status were detected, nor were there substantial alterations in effect estimates with covariable adjustments (Table 8; Figure 5). No effect modifications by *APOE*-ε4 status were detected (Table 9).

#### *3.3.5.4. Normal appearing white matter (NAWM)*

With minimal adjustments, females had lower FA (β=-0.11[-0.16, -0.06]) and greater MD (β=0.07[0.00, 0.14]) than males, indicating poorer NAWM microstructural integrity in females. Adjusting for covariables did not substantially alter effect estimates (Table 8; Figure 6).

Effect modifications by *APOE*-ε4 status were detected for FA and MD (Table 9). Stratified analyses (Table 10) showed that, in ε4 carriers, there were no sex differences in FA or MD, while in non-carriers females had lower FA (β=-0.13[-0.19, -0.08]) and greater MD (β=0.11[0.03, 0.19]) than males.

#### *3.3.5.5. Cortical thickness*

As shown in Figure 7, no sex differences were detected in Harvard AD signature region or frontal cortical thickness, but there were trends for thinner occipital (β=-0.02[-0.03, 0.00]) and temporal (β=-0.02[-0.03, 0.00]) cortices and thicker parietal cortices (β=0.02[0.00, 0.03]) in females than males. Adjustment for SEP attenuated effects for occipital and temporal cortices while education, smoking, and PA adjustments did not alter effect estimates (Table 8; Figure 7). No effect modifications by *APOE*-ε4 status were detected (Table 9).

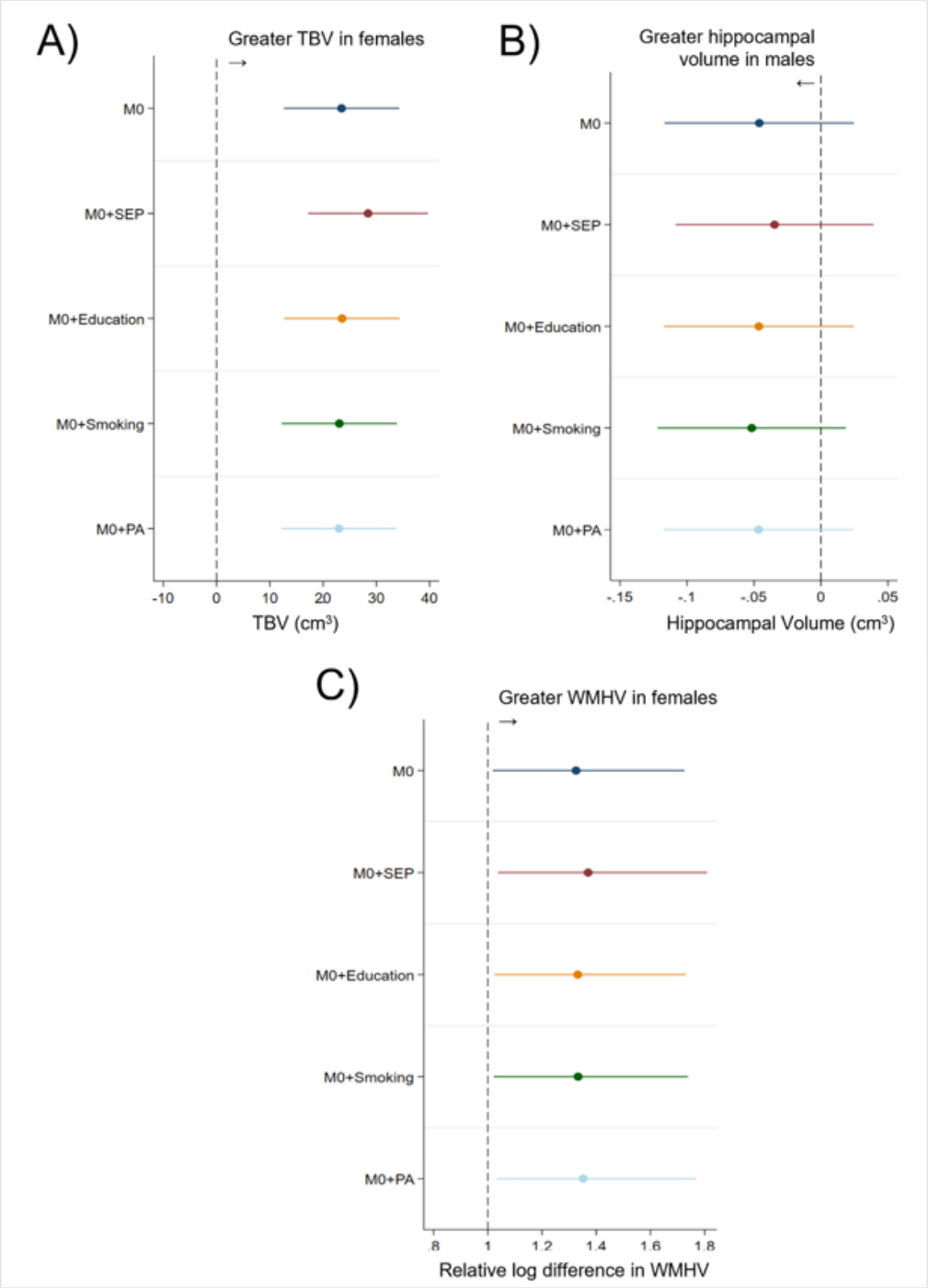


***Figure 4.*** *Model estimates and 95% confidence intervals showing the difference in: A) mean total brain volume (TBV; cm³); B) hippocampal volume (cm³); C) relative log difference in white matter hyperintensity volume (WMHV) between males and females, with minimal adjustments and with additional adjustments for socioeconomic and lifestyle covariables.*

*Reference category: males.*

*SEP=socioeconomic position; PA=physical activity*

*M0=age at scan + total intracranial volume (TIV); SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 60-64; PA=frequency of PA at age 69*

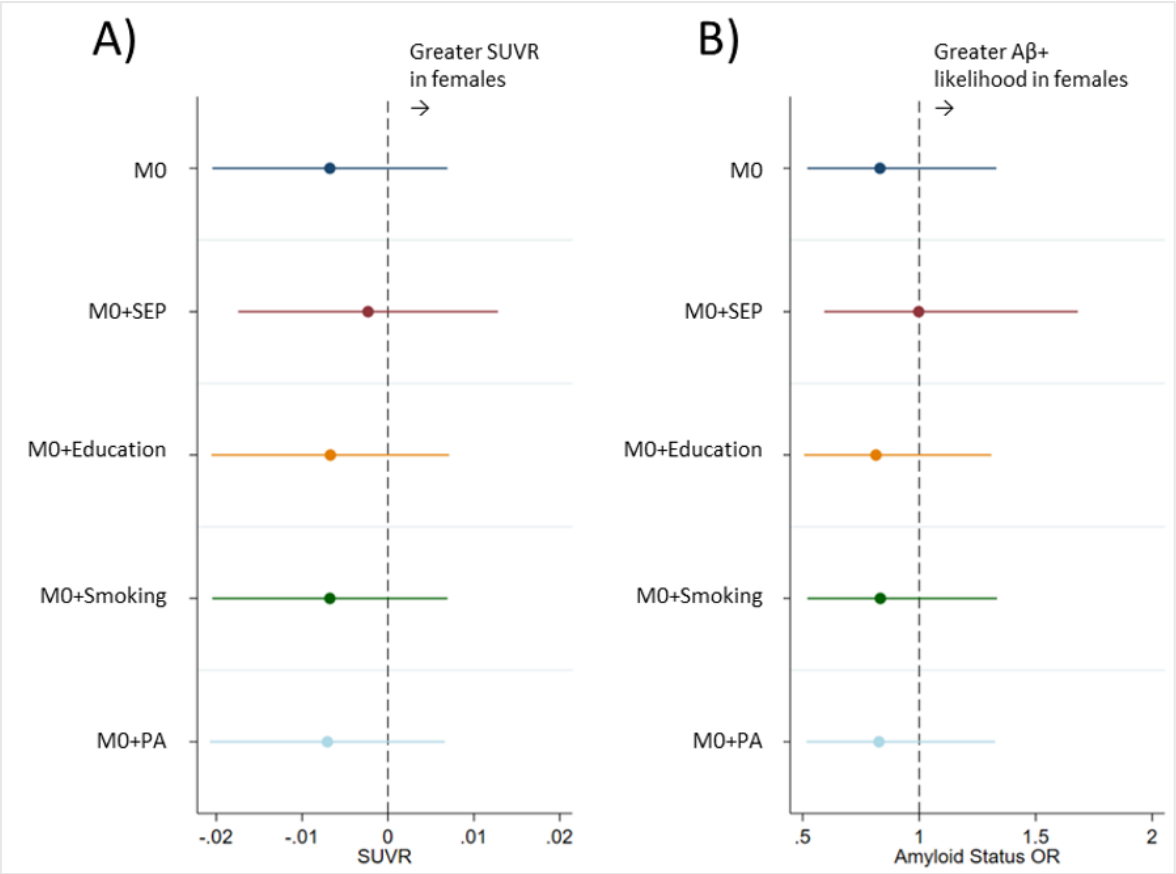


***Figure 5.*** *Model estimates and 95% confidence intervals showing the difference in A) amyloid standardised uptake value ratio (SUVR) and B) the difference in odds ratio for being amyloid positive, between males and females, with minimal adjustments and with additional adjustments for socioeconomic and lifestyle covariables.*

*Reference category: males.*

*SUVR=standardised uptake value ratio; Aβ+=beta-amyloid positive; OR=odds ratio; SEP=socioeconomic position; PA=physical activity*

*M0=age at scan; SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 60-64; PA=frequency of PA at age 69*

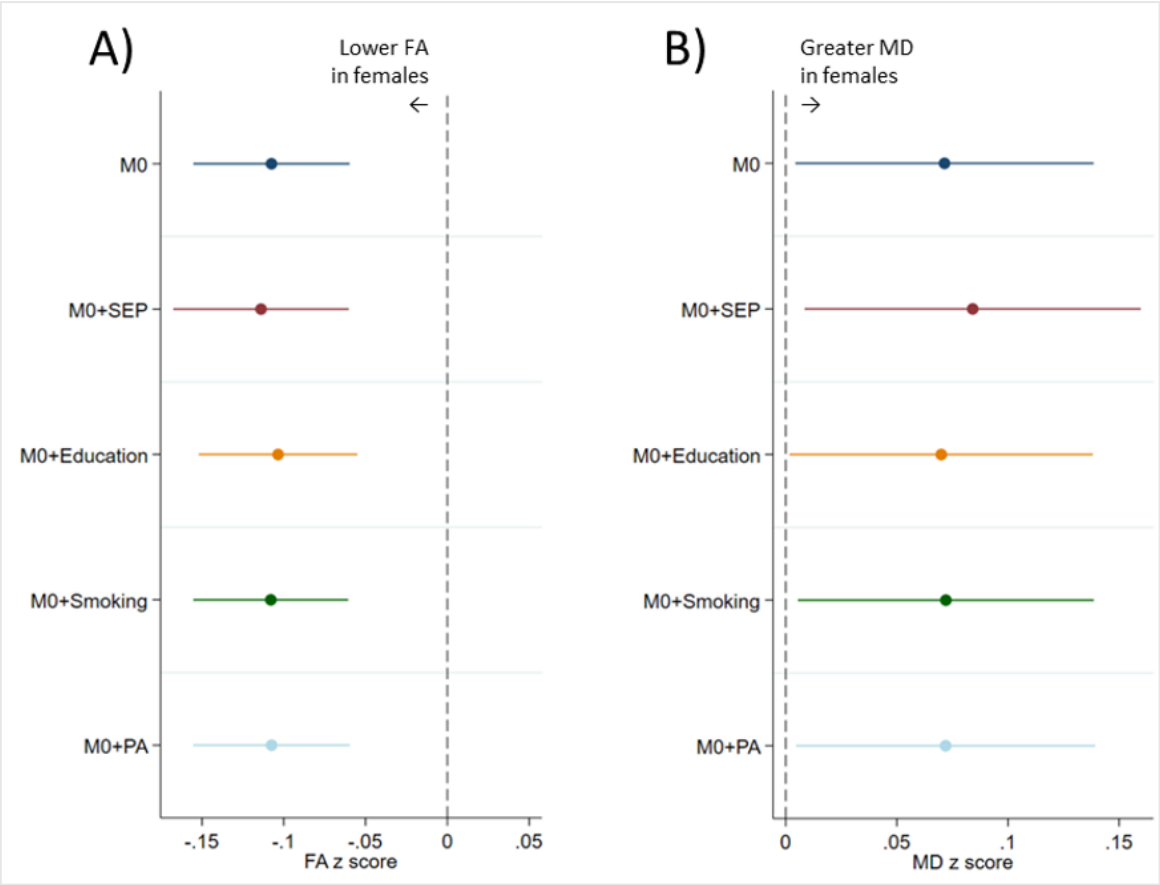


***Figure 6.*** *Model estimates and 95% confidence intervals showing the difference normal appearing white matter A) fractional anisotropy and B) mean diffusivity measures between males and females, with minimal adjustments and with additional adjustments for socioeconomic and lifestyle covariables.*

*Reference category: males.*

*NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; SEP=socioeconomic position; PA=physical activity*

*M0=age at scan; SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 60-64; PA=frequency of PA at age 69*

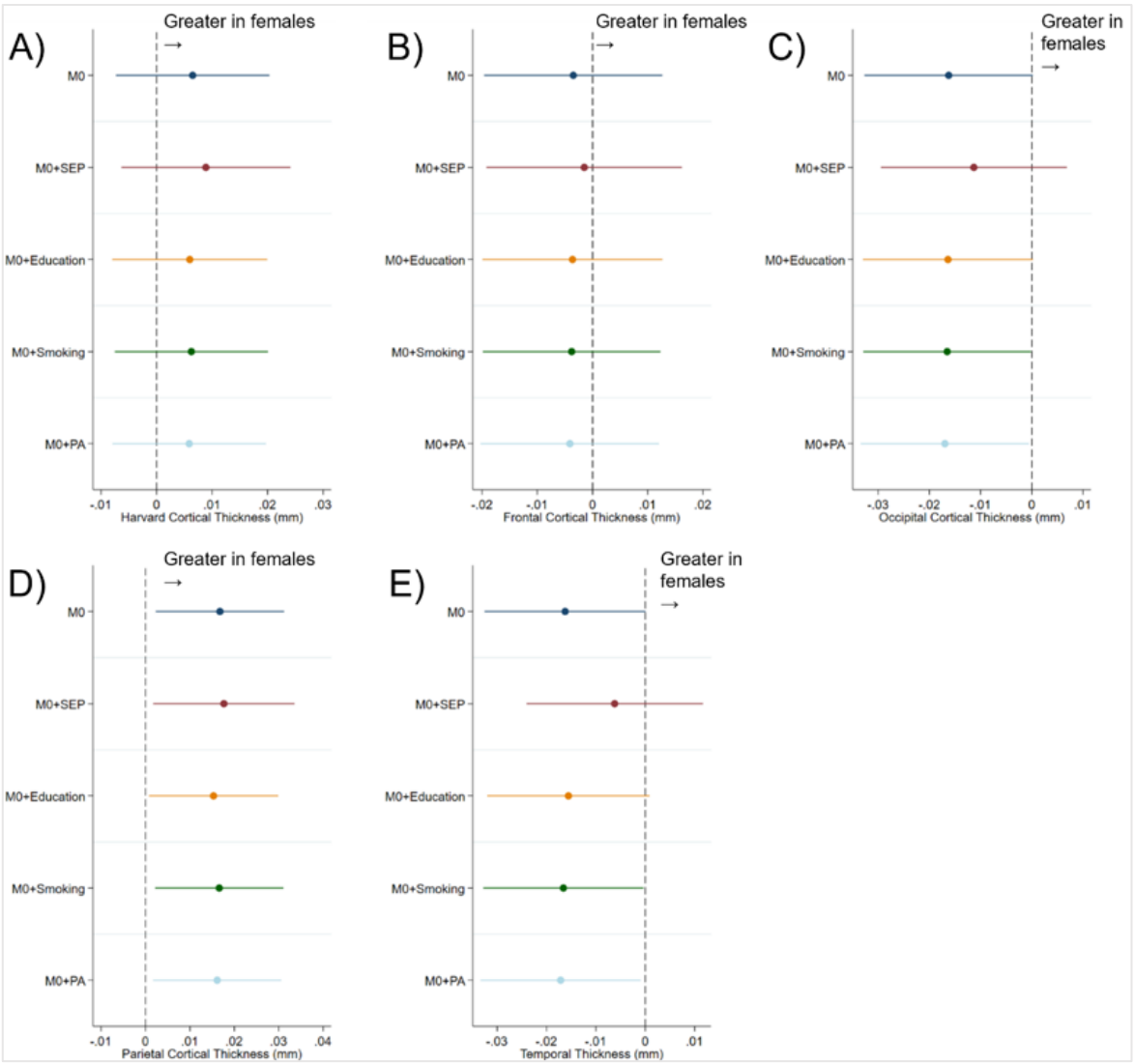


***Figure 7.*** *Model estimates and 95% confidence intervals showing the difference cortical thickness regions of interest measures between males and females, with minimal adjustments and with additional adjustments for socioeconomic and lifestyle covariables. A) Harvard Alzheimer's Disease signature; B) Frontal cortex; C) Occipital cortex; D) Parietal cortex; E) Temporal cortex.*

*Reference category: males.*

*SEP=socioeconomic position; PA=physical activity*

*M0=age at scan; SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 60-64; PA=frequency of PA at age 69*

***Table 8.*** *Model estimates for the effects of sex on cognitive performance or neuroimaging measures across the life course. Estimates from minimally adjusted models (M0) and models adjusted for socioeconomic and lifestyle covariables applicable at each timepoint are presented.*

| Age | Outcome variable | N | M0 (β) | M0+SEP (β) | M0+Education (β) | M0+Smoking (β) | M0+PA (β) |
|---|---|---|---|---|---|---|---|
| **8** | Picture intelligence | 4,264 | 0.00 | 0.00 | N/A | N/A | N/A |
| | Sentence completion | 4,258 | 0.13 | 0.13 | | | |
| | Word reading* | 4,258 | 0.13 | 0.13 | | | |
| | Vocabulary | 4,258 | -0.09 | -0.09 | | | |
| | Overall cognition age 8 | 4,255 | 0.04 | 0.04 | | | |
| **11** | Word reading* | 4,025 | 0.09 | 0.10 | N/A | N/A | N/A |
| | General ability | 4,030 | 0.17 | 0.18 | | | |
| | Vocabulary | 4,025 | -0.09 | -0.08 | | | |
| | Arithmetic | 4,023 | 0.04 | 0.06 | | | |
| | Overall cognition age 11 | 4,022 | 0.05 | 0.06 | | | |
| **15** | General ability | 4,015 | -0.01 | 0.00 | N/A | N/A | N/A |
| | Watts-Vernon* | 4,012 | -0.06 | -0.06 | | | |
| | Mathematics* | 4,013 | -0.32 | -0.31 | | | |
| | Overall cognition age 15 | 4,006 | -0.13 | -0.12 | | | |
| **26** | Watts-Vernon* | 3,713 | -0.08 | -0.07 | 0.00 | -0.11 | N/A |
| **43** | Word list recall | 3,059 | 0.26 | 0.30 | 0.36 | 0.24 | 0.29 |
| | Timed letter search | 3,150 | 0.28 | 0.32 | 0.32 | 0.27 | 0.30 |
| | Peg placement* | 3,163 | -0.05 | -0.03 | -0.03 | -0.06 | -0.04 |
| | Picture recall* | 3,224 | 0.16 | 0.16 | 0.19 | 0.16 | 0.18 |
| **53** | Word list recall | 2,886 | 0.30 | 0.30 | 0.40 | 0.27 | 0.31 |
| | Timed letter search | 2,931 | 0.22 | 0.22 | 0.26 | 0.20 | 0.22 |
| | NART | 2,824 | -0.02 | 0.02 | 0.11 | -0.05 | -0.01 |
| | Verbal fluency | 2,948 | -0.05 | -0.01 | 0.02 | -0.06 | -0.04 |
| | Prospective memory* | 2,924 | 0.15 | 0.18 | 0.17 | 0.14 | 0.15 |
| **60-64** | Word list recall | 2,150 | 0.38 | 0.45 | 0.47 | 0.37 | 0.37 |
| | Timed letter search | 2,182 | 0.15 | 0.17 | 0.19 | 0.14 | 0.15 |
| | MRT* | 2,167 | 0.04 | 0.08 | 0.07 | 0.04 | 0.03 |
| | IIVrt* | 2,167 | 0.07 | 0.06 | 0.08 | 0.07 | 0.07 |
| **69** | Word list recall | 2,074 | 0.33 | 0.36 | 0.41 | 0.32 | 0.34 |
| | Timed letter search | 2,111 | 0.16 | 0.17 | 0.19 | 0.15 | 0.16 |
| | Finger tapping | 2,046 | -0.46 | -0.47 | -0.45 | -0.47 | -0.46 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | ACE-III total* | 1,762 | 0.05 | 0.11 | 0.14 | 0.04 | 0.06 |
| | ACE-III verbal fluency* | 2,101 | 0.13 | 0.16 | 0.19 | 0.13 | 0.13 |
| | ACE-III language* | 1,765 | -0.01 | 0.02 | 0.05 | -0.02 | -0.01 |
| | ACE-III attention & orientation* | 1,785 | -0.15 | -0.07 | -0.12 | -0.15 | -0.15 |
| | ACE-III memory* | 1,786 | 0.20 | 0.21 | 0.26 | 0.19 | 0.20 |
| | ACE-III visuospatial function* | 1,778 | -0.12 | -0.10 | -0.06 | -0.13 | -0.12 |
| **Insight 46 cognition (age ~70)** | PACC total | 502 | 0.35 | 0.41 | 0.39 | 0.35 | 0.34 |
| | MMSE* | 502 | 0.15 | 0.21 | 0.19 | 0.15 | 0.14 |
| | DSST | 501 | 0.31 | 0.36 | 0.34 | 0.30 | 0.30 |
| | Logical memory | 502 | 0.45 | 0.50 | 0.48 | 0.44 | 0.45 |
| | FNAME | 500 | 0.48 | 0.56 | 0.53 | 0.48 | 0.47 |
| | Matrix reasoning* | 502 | 0.04 | 0.16 | 0.09 | 0.04 | 0.04 |
| | IIVrt | 501 | -0.13 | -0.13 | -0.12 | -0.13 | -0.13 |
| | Response inhibition | 500 | -0.03 | -0.06 | -0.02 | -0.04 | -0.04 |
| | Visuomotor integration rotation errors* | 483 | -0.01 | 0.07 | 0.01 | -0.01 | -0.01 |
| | Visuomotor integration number of rotations* | 483 | -0.07 | -0.12 | -0.07 | -0.07 | -0.07 |
| | Visuomotor integration subtraction rate | 483 | -0.59 | -0.61 | -0.57 | -0.60 | -0.59 |
| | Visual working memory ID rate | 486 | 0.18 | 0.19 | 0.18 | 0.17 | 0.18 |
| | Visual working memory localisation error | 486 | -0.24 | -0.19 | -0.23 | -0.24 | -0.25 |
| | Visual working memory swap errors | 486 | 0.05 | 0.12 | 0.05 | 0.05 | 0.04 |
| **Insight 46 neuroimaging (age ~70)** | TBV ($cm^3$) | 468 | 23.46 | 28.43 | 23.55 | 23.05 | 22.97 |
| | Hippocampal volume ($cm^3$) | 468 | -0.05 | -0.04 | -0.05 | -0.05 | -0.05 |
| | WMHV (relative log change) | 455 | 1.33 | 1.37 | 1.33 | 1.33 | 1.35 |
| | SUVR | 462 | -0.01 | 0.00 | -0.01 | -0.01 | -0.01 |
| | Amyloid status [OR; ref:Aβ negative] | 462 | 0.83 | 1.00 | 0.81 | 0.83 | 0.83 |
| | NAWM FA (z-score) | 411 | -0.11 | -0.11 | -0.10 | -0.11 | -0.11 |
| | NAWM MD (z-score) | 411 | 0.07 | 0.08 | 0.07 | 0.07 | 0.07 |
| | CT ADSig (mm) | 468 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | CT Frontal (mm) | 468 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | CT Occipital (mm) | 468 | -0.02 | -0.01 | -0.02 | -0.02 | -0.02 |
| | CT Parietal (mm) | 468 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| | CT Temporal (mm) | 468 | -0.02 | -0.01 | -0.02 | -0.02 | -0.02 |

*SEP=socioeconomic position; PA=physical activity; N/A=not applicable; NART=national adult reading test; MRT=mean reaction time; IIVrt=intra-individual variability in reaction times; ACE-III=Addenbrooke's cognitive examination 3rd edition; PACC=pre-clinical Alzheimer's cognitive composite; MMSE=mini-mental state examination; DSST=digit-symbol substitution test; FNAME=face-name associative memory examination; ID=identification; TBV=total brain volume; WMHV=white matter hyperintensity volume;*

*SUVR=standardised uptake value ratio; OR=odds ratio; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; ADSig=Alzheimer's Disease signature*

*Reference category: males.*

*Yellow cells indicate female advantages, significant at the p<0.05 level. Blue cells indicate male advantages, significant at the p<0.05 level.*

**Outcome variable is not normally distributed. The model estimates presented in this table are from analyses which did not have bootstrapping applied. While the model estimates are interpretable, parametric 95% confidence intervals and p-values for these outcomes cannot be interpreted. Cells are highlighted if bias-corrected 95% confidence intervals from M0 analyses (see Figures 1-3) did not cross zero.*

*Age 8: SEP=father's social class at participant age 4*

*Age 11: SEP=father's social class at participant age 11*

*Age 15: SEP=father's social class at participant age 15*

*Age 26: SEP=overall childhood social class; Education=highest educational attainment up to age 26; Smoking=smoking pack years to age 26*

*Age 43: SEP= overall childhood social class + overall own social class from age 15 to 43; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 43; PA=frequency of PA at age 43*

*Age 53: SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 53; PA=frequency of PA at age 53*

*Age 60-64: SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 60-64; PA=frequency of PA at age 64*

*Age 69: SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 60-64; PA=frequency of PA at age 69*

*Insight 46 cognition: M0=age at visit; SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 60-64; PA=frequency of PA at age 69*

*Insight 46 neuroimaging: M0=age at scan + TIV; SEP= overall childhood social class + overall own social class from age 15 to 53; Education=highest educational attainment up to age 43; Smoking=smoking pack years to age 60-64; PA=frequency of PA at age 69*

***Table 9.*** *Significance p-values and 95% confidence intervals for sex-by-APOE interaction terms, for each cognitive and neuroimaging outcome. Interaction terms were included in minimally adjusted models assessing the effects of sex on outcome measures (M0).*

| Age | Outcome variable | Lower CI | Upper CI | P |
|---|---|---|---|---|
| **8** | Picture intelligence | -0.04 | 0.30 | 0.13 |
| | Sentence completion | -0.26 | 0.08 | 0.29 |
| | Word reading* | -0.20 | 0.16 | - |
| | Vocabulary | -0.26 | 0.08 | 0.31 |
| | Overall cognition age 8 | -0.16 | 0.13 | 0.82 |
| **11** | Word reading* | -0.26 | 0.09 | - |
| | General ability | -0.21 | 0.13 | 0.66 |
| | Vocabulary | -0.28 | 0.07 | 0.24 |
| | Arithmetic | -0.27 | 0.08 | 0.27 |
| | Overall cognition age 11 | -0.23 | 0.07 | 0.30 |
| **15** | General ability | -0.20 | 0.14 | 0.74 |
| | Watts-Vernon* | -0.32 | 0.04 | - |
| | Mathematics* | **-0.34** | **0.01** | - |
| | Overall cognition age 15 | -0.27 | 0.04 | 0.14 |
| **26** | Watts-Vernon* | -0.23 | 0.12 | - |
| **43** | Word list recall | -0.27 | 0.08 | 0.27 |
| | Timed letter search | -0.24 | 0.10 | 0.43 |
| | Peg placement* | -0.13 | 0.18 | - |
| | Picture recall* | -0.23 | 0.09 | - |
| **53** | Word list recall | **-0.36** | **-0.03** | **0.02** |
| | Timed letter search | -0.26 | 0.07 | 0.27 |
| | NART | -0.27 | 0.07 | 0.26 |
| | Verbal fluency | **-0.32** | **0.01** | **0.06** |
| | Prospective memory* | **-0.31** | **0.02** | - |
| **60-64** | Word list recall | -0.34 | 0.04 | 0.12 |
| | Timed letter search | -0.25 | 0.15 | 0.56 |
| | MRT* | -0.22 | 0.14 | - |
| | IIVrt* | -0.13 | 0.23 | - |
| **69** | Word list recall | **-0.47** | **-0.07** | **0.01** |
| | Timed letter search | -0.26 | 0.13 | 0.53 |
| | Finger tapping | -0.12 | 0.27 | 0.47 |
| | ACE-III total* | **-0.43** | **0.00** | - |
| | ACE-III verbal fluency* | -0.27 | 0.11 | - |
| | ACE-III language* | -0.22 | 0.20 | - |
| | ACE-III attention & orientation* | -0.34 | 0.11 | - |
| | ACE-III memory* | **-0.53** | **-0.09** | **-** |
| | ACE-III visuospatial function* | -0.29 | 0.18 | - |
| **Insight 46 cognition (age ~70)** | PACC total | **-0.54** | **0.01** | **0.06** |
| | MMSE* | -0.72 | 0.11 | - |
| | DSST | -0.60 | 0.17 | 0.27 |
| | Logical memory | **-0.70** | **0.05** | **0.09** |
| | FNAME | -0.63 | 0.13 | 0.19 |
| | Matrix reasoning* | -0.17 | 0.57 | - |
| | IIVrt | -0.70 | 0.06 | 0.10 |
| | Response inhibition | -0.25 | 0.53 | 0.48 |
| | Visuomotor integration rotation errors* | -0.44 | 0.21 | - |
| | Visuomotor integration number of rotations* | -0.43 | 0.32 | - |
| | Visuomotor integration subtraction rate | -0.40 | 0.36 | 0.91 |

| | | | | |
|---|---|---|---|---|
| | Visual working memory ID rate | -0.29 | 0.49 | 0.61 |
| | Visual working memory localisation error | **0.04** | **0.81** | **0.03** |
| | Visual working memory swap errors | **0.06** | **0.84** | **0.02** |
| **Insight 46 neuroimaging (age ~70)** | TBV ($cm^3$) | -24.27 | 11.12 | 0.47 |
| | Hippocampal volume ($cm^3$) | -0.18 | 0.05 | 0.26 |
| | WMHV (relative log change) | 0.54 | 1.31 | 0.45 |
| | SUVR | -0.02 | 0.04 | 0.44 |
| | Amyloid status [OR; ref:Aβ negative] | 0.50 | 3.59 | 0.57 |
| | NAWM FA (z-score) | **-0.01** | **0.20** | **0.09** |
| | NAWM MD (z-score) | **-0.28** | **0.01** | **0.08** |
| | CT ADSig (mm) | -0.01 | 0.05 | 0.19 |
| | CT Frontal (mm) | -0.01 | 0.06 | 0.15 |
| | CT Occipital (mm) | -0.01 | 0.06 | 0.13 |
| | CT Parietal (mm) | -0.01 | 0.06 | 0.11 |
| | CT Temporal (mm) | -0.02 | 0.05 | 0.48 |

**Bootstrapping applied; confidence intervals are bias-corrected and p-values are non-interpretable*

*All cognitive measures are presented as standardised z-scores.*

*Reference category: males.*

*Bold text indicates significant sex-by-APOE interactions (p<0.1 or bias-corrected confidence intervals not crossing by greater than .02).*

*CI=confidence interval; NART=national adult reading test; MRT=mean reaction time; IIVrt=intra-individual variability in reaction times; ACE-III=Addenbrooke's cognitive examination 3rd edition; PACC=pre-clinical Alzheimer's cognitive composite; MMSE=mini-mental state examination; DSST=digit-symbol substitution test; FNAME=face-name associative memory examination; ID=identification; TBV=total brain volume; WMHV=white matter hyperintensity volume; SUVR=standardised uptake value ratio; OR=odds ratio; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; ADSig=Alzheimer's Disease signature*

***Table 10.*** *Model estimates and 95% confidence intervals for the association between sex and cognitive performance or neuroimaging measures, stratified by APOE-ε4 status, from minimally adjusted models (M0).*

| | | ***APOE*-ε4 carriers** | | | | | ***APOE*-ε4 non-carriers** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | **Outcome** | **N** | **β** | **Lower CI** | **Upper CI** | **P** | **N** | **β** | **Lower CI** | **Upper CI** | **P** |
| **15** | Mathematics* | 684 | -0.46 | -0.61 | -0.31 | - | 1,609 | -0.28 | -0.38 | -0.18 | - |
| **53** | Word list recall | 787 | 0.16 | 0.02 | 0.30 | 0.03 | 1,818 | 0.35 | 0.26 | 0.44 | <0.01 |
| | Verbal fluency | 797 | -0.15 | -0.29 | -0.02 | 0.03 | 1,856 | 0.01 | -0.09 | 0.10 | 0.92 |
| | Prospective memory* | 794 | 0.05 | -0.10 | 0.19 | - | 1,841 | 0.20 | 0.09 | 0.29 | - |
| **69** | Word list recall | 555 | 0.10 | -0.007 | 0.27 | 0.24 | 1,277 | 0.37 | 0.26 | 0.48 | <0.01 |
| | ACE-III total* | 470 | -0.17 | -0.34 | 0.02 | - | 1,076 | 0.07 | -0.05 | 0.19 | - |
| | ACE-III memory* | 475 | -0.06 | -0.24 | 0.15 | - | 1,092 | 0.26 | 0.15 | 0.37 | - |
| **Insight 46 cognition (age ~70)** | PACC total | 148 | 0.17 | -0.07 | 0.42 | 0.17 | 352 | 0.43 | 0.29 | 0.58 | <0.01 |
| | Logical memory | 148 | 0.25 | -0.06 | 0.55 | 0.11 | 352 | 0.56 | 0.35 | 0.77 | <0.01 |
| | VWM localisation error | 144 | 0.08 | -0.26 | 0.41 | 0.64 | 340 | -0.35 | -0.56 | -0.14 | <0.01 |
| | VWM swap errors | 144 | 0.38 | 0.06 | 0.70 | 0.02 | 340 | -0.07 | -0.29 | 0.14 | 0.51 |
| **Insight 46 neuroimaging (age ~70)** | NAWM FA (z-score) | 119 | -0.04 | -0.13 | 0.05 | 0.37 | 290 | -0.13 | -0.19 | -0.08 | <0.01 |
| | NAWM MD (z-score) | 119 | -0.03 | -0.16 | 0.11 | 0.72 | 290 | 0.11 | 0.03 | 0.19 | 0.01 |

**Bootstrapping applied; confidence intervals are bias-corrected and p-values are non-interpretable*

*All cognitive measures are presented as standardised z-scores.*

*Reference category: males.*

*APOE=apolipoprotein E; CI=confidence interval; ACE-III=Addenbrooke's cognitive examination 3rd edition; PACC=pre-clinical Alzheimer's cognitive composite; VWM=visual working memory; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity*

## 3.4. Discussion

### 3.4.1. Key findings

Differences in mean performance on a range of cognitive assessments were detected between males and females, at several timepoints throughout the life course. Overall, females had better performance on tasks assessing verbal and memory domains, while males performed better on tasks of non-verbal and visuospatial domains. Female cognitive performance advantages were generally strengthened with adjustment for socioeconomic and education covariables, which tended to attenuate male cognitive performance advantages. Lifestyle covariables (smoking, PA) did not substantially contribute to cognitive performance sex differences. Most sex differences in cognitive performance were not modified by *APOE*-ε4 status, but effect modifications were detected mainly at older ages and indicated that female memory performance advantages were stronger in ε4 non-carriers than in carriers.

Some sex differences in mean neuroimaging measures of brain health at age ~70 were detected; females had larger TBV relative to head size, but also greater WMHV and poorer NAWM microstructural integrity. Socioeconomic, education, and lifestyle covariables did not substantially contribute to sex differences in neuroimaging measures.

### 3.4.2. Interpretation of findings

Overall, these analyses align with previous reports of male advantages in non-verbal, visuospatial cognitive domains and female advantages in verbal and memory domains.[47] Here, and elsewhere, the reported effect sizes for such differences are small and there is substantial overlap in the distribution of male and female task performance.[47,49] Nonetheless, repeated replication of such differences in mean performance suggests that there are meaningful, albeit subtle, variations in non-verbal and verbal abilities between the sexes.

Interestingly, females showed an advantage in sentence completion performance at age 8 while males performed better on similar reading comprehension tasks at ages 15 and 26. While most research reports female reading comprehension advantages,[243] some evidence shows that boys can perform better when using phonological rather than analytical or context-driven strategies,[243]

highlighting how variations in cognitive strategies potentially underlie some observed cognitive sex differences. Indeed, in these analyses males did better on rule-based tasks such as mathematics, while females performed better on tasks of episodic memory which were more reliant on contextual processing. It is possible that some aspect of education, which could not be accounted for, contributed to the change in reading comprehension sex differences over time. For example, there could have been a bias in how males and females were taught during the 1950s, possibly with greater emphasis on preparing boys for employment or higher education while girls were taught more domestic skills, given prevalent traditional gender roles at that time.[244]

Indeed, education is repeatedly identified as an important explanatory factor in cognitive sex differences,[245,246] with secular improvements in women's access to education being associated with better female performance on cognitive tasks, namely verbal memory and fluency.[54] Being representative of post-war Britain, NSHD females unsurprisingly had lower education levels than males. Since accounting for education level strengthened female cognitive advantages while weakening male advantages, these analyses reinforce the notion that reduced female access to education can suppress female cognitive abilities. Adjusting for SEP had a similar effect; although more females than males had overall skilled non-manual occupations, females were outnumbered in the highest-level occupations. Greater engagement in cognitively stimulating activities, such as those linked with occupation and higher education levels, are thought to improve cognitive performance and reduce dementia risk by improving cognitive reserve[86] (Section 1.6.). It has been suggested that traditionally lower education and SEP in females could contribute to greater rates of AD in females,[247] emphasising the importance of understanding how and whether modifiable environmental and social factors contribute to cognitive variability between the sexes.

The current findings did not, however, support a role of smoking and PA lifestyle factors in cognitive sex differences. While such factors have previously been associated with cognitive performance,[228,229] associations appear to be driven by extremes (i.e. heavy smoking and physical inactivity)[227,248] and evidence for sex differences in how these factors link with dementia risk is mixed.[247] It should be noted that, within NSHD and Insight 46, sex differences in PA were only

detected at age 43 and that smoking sex differences diminished over time, with no significant difference at age 60-64, although this could be a result of limited power to detect sex differences given that few participants (n=291) were current smokers at age 60-64. The relative lack of sex differences in these lifestyle factors could offer some explanation for their lack of contribution to differences in cognitive performance.

The role of biological variability between males and females must also be considered. For instance, puberty typically occurs between age 8 and 14, with girls experiencing puberty at younger ages than boys.[249] Increasing sex hormone levels during this time could have contributed to the changing pattern of cognitive sex differences during childhood, particularly given that sex hormones are linked with pubertal changes in brain organisation.[250] The parietal and occipital lobes, subserving sensory and visuospatial processing,[251,252] tend to mature before the frontal and temporal lobes[253] which subserve higher level cognitive abilities such as attention, problem solving, and memory.[254,255] Reflecting the generally earlier age of puberty onset in girls than boys, females typically reach peak grey matter volumes at younger ages than males,[253] which could explain why females tended to perform better than males on cognitive tasks at younger ages during adolescence (8-11 years), but not at later ages (15-26 years). Additionally, with increasing age during adolescence, the hippocampus – rich in oestrogen receptors and involved in memory processing – is found to increase in volume only in females, while the amygdala – rich in androgen receptors and involved in attention and emotion processing – increases in volume only in males.[253,256] Potential advances in attentional abilities in males during adolescence, might also contribute to general improvements in male task performance at later stages of adolescence.

Hormonal changes at midlife could also have contributed to variations in verbal fluency sex differences over time. While literature consistently reports a female advantage in verbal fluency,[47] here a female advantage was only detected at age 69 and not at 53, although an effect modification by *APOE*-ε4 showed a male advantage at age 53 in ε4 carriers. During midlife, women experience declining oestrogen levels as they transition through menopause, typically aged 45-55.[133] The transition has been associated with cognitive changes, and 'brain fog' is a commonly reported symptom referring to cognitive difficulties, including word finding problems.[143] Word finding difficulties linked with menopause could therefore have suppressed

female verbal fluency performance during midlife. However, that female advantages in repeated measures of verbal memory were consistently detected from age 43 to 69, corroborating previous analyses in this cohort,[5] demonstrates that females still maintain some verbal cognitive advantages throughout midlife and into later-life.

While there is no clear explanation for better male ε4 carrier verbal fluency performance at 53, it is possible that any adverse effect of ε4 on verbal fluency performance was stronger in females, and that this effect could have been exacerbated during midlife. Most sex-by-*APOE*-ε4 interactions on cognitive performance were detected in later-life, in agreement with literature generally detecting negative associations of ε4 with cognition in later-life[257] but null associations at younger ages.[258] Some positive associations of ε4 with cognition have, however, been reported. Insight 46 ε4 carriers showed better object identification and localisation on the visual working memory task than non-carriers,[218] while other positive associations tend to be reported at younger ages.[259,260] It has been hypothesised that ε4 has some evolutionary advantages, possibly related to inflammatory responses protective against pathogens,[261] and only now that human lifespan has increased are the deleterious effects of ε4 in later-life observed.[262] Thus far, sex differences in the potential antagonistic pleiotropy of *APOE*-ε4 have not been explored, but if inflammatory processes are involved then greater immune responses in females[81] might contribute to sex differences. Overall, current results show that female cognitive advantages, particularly in memory domains, were stronger in ε4 non-carriers than in carriers, indicating that ε4 presence could suppress female cognitive abilities, particularly at older ages.

Conversely, only female ε4 non-carriers showed poorer microstructural integrity, although these *APOE*-ε4 interactions were attenuated when models were adjusted for WMHV (log-transformed) and TIV (MD p=0.14; FA p=0.20). The volume of cSVD present therefore explains some of this relationship; those with greater cSVD could have less NAWM, with poorer microstructural integrity than those with lower cSVD burden. Previous work in this cohort showed that poorer NAWM microstructural integrity in females was largely explained by differences in WMHV, suggesting that alterations in NAWM and white matter hyperintensities indicating cSVD may be part of an overlapping pathological process.[263] The links between NAWM and WMHV are corroborated in the current results, given that females also show greater WMHV than males.

However, previous Insight 46 analyses, which excluded individuals with neurological conditions, did not find sex differences in WMHV.[158] While, in the current analyses, excluding scans which failed BaMoS QC did exclude participants with multiple sclerosis (n=2) from WMHV analyses, these discrepant findings highlight that the presence of neurological conditions explain greater female WMHV in this cohort. Nonetheless, the detection of sex differences in NAWM and WMHV measures linked with cSVD[264] suggests a role for cerebrovascular factors underlying sex differences in white matter pathology.[291] Indeed, midlife cardiovascular risks only associated with NAWM measures in females in Insight 46.[237] In agreement with existing literature,[158,265] no sex differences in AD-related brain pathology (Aβ load, hippocampal volume) were detected within Insight 46. It is possible that cerebrovascular changes precede the development of AD pathology, as proposed by the vascular theory of AD which postulates that vascular dysfunction contributes to amyloid accumulation and neurodegeneration.[266]

While most neuroimaging sex differences detected seem to indicate poorer outcomes in females (i.e. females showed poorer NAWM integrity, greater WMHV, and thinner occipital and temporal cortices), females did have greater TBV which is generally thought to be beneficial, given that brain atrophy is a marker of dementia.[20] While literature reports mixed evidence for sex differences in TBV, meta-analysis does show overall support for greater TBV in males,[267] although most existing evidence is restricted to younger samples, aged 18-59.[267] In older, preclinical AD populations, there is suggestion that brain volume increases could precede brain atrophy.[268] If so, greater TBV at age ~70 could be interpretated as disadvantageous, which would align with the indication from other neuroimaging outcomes that Insight 46 females have overall poorer brain health than males aged ~70.

Despite finding support for the contribution of socioeconomic and education factors to cognitive sex differences, there did not appear to be a role for such factors in neuroimaging sex differences, nor did lifestyle covariables contribute. Education levels and SEP might influence the cognitive strategies individuals use to complete tasks, with different strategies potentially arising from the same underlying brain structures. Indeed, literature does not always correlate neuroimaging outcomes with cognitive performance measures.[269] For instance, James et al. did not find associations between Insight 46 PACC performance and NAWM measures.[237] Higher levels of

brain pathology could be required before cognitive functions are affected, highlighted by dementia pathology beginning up to 20 years prior to symptom onset.[270] In this mostly cognitively unimpaired cohort, the finding that females showed better cognitive performance but poorer cerebrovascular brain health than males at age ~70 seems counterintuitive, but potentially indicates that cognitive function persists in spite of subtle brain changes, at least in females. Better female performance on most cognitive assessments throughout the life course indicates greater cognitive reserve in females, and larger female TBV at age ~70 could reflect greater female brain reserve. Greater levels of cognitive and structural brain resources could, therefore, support female cognitive resilience to cSVD pathology.

### 3.4.3. Strengths and limitations

The main strength of these analyses is the use of an age-homogenous sample and the availability of prospective life course data collected over seventy years, facilitating an examination of cognitive sex differences at specific ages and an opportunity to observe patterns in sex differences over time. The potential contribution of age-specific socioeconomic and lifestyle covariables were also considered, a strength over many studies reliant on retrospectively reported data which may not capture any changes in such sociocultural factors throughout the life course. Additionally, cognitive sex differences were considered alongside sex differences in a range of dementia-related neuroimaging measures in later-life. This is useful for developing an understanding of the patterns of brain ageing which potentially underlie sex differences in dementia.

However, as is the case with most longitudinal research, the impact of sample attrition over time must be acknowledged; at each successive timepoint of interest, sample sizes for both males and females declined. Given that participation withdrawal or death is greater among individuals from more disadvantaged backgrounds and with poorer general health, sample attrition induced a survivor or retention bias. Further, the results of these analyses should be considered within the generational and cultural context of the cohort, an entirely white British cohort representative of mainland Britain in 1946 which may not be generalisable to other populations. Secular changes in education policies and societal views on traditional gender roles could mean that the

contribution of sociodemographic and lifestyle factors to sex differences is not equal across generations.

There is also a need to be mindful of potential collider bias in these analyses given that education, SEP, and lifestyle variables were individually adjusted as mediators. For instance, if an unmeasured confounder associates with sex as the exposure variable, with cognitive performance as the outcome variable, and with smoking as the mediating variable, then smoking would be a shared outcome (a collider) of the unmeasured confounder and sex. Adjusting for smoking could therefore open a back door path where the unmeasured confounder explains the associations between sex and cognitive performance.

### 3.4.4. Summary

This examination of life course cognitive and brain health sex differences demonstrates that, in NSHD, females generally perform better than males on some cognitive assessments throughout the life course, but in later-life show poorer cerebrovascular brain health, specific to cSVD. Despite greater cSVD pathology, female cognitive advantages were maintained at age ~70 years, demonstrating female cognitive resilience to pathology. Sex differences in cognitive performance were not necessarily biologically pre-determined, with evidence for a contribution of sociodemographic variables; most notably, higher education levels were especially beneficial for female cognitive advantages. Some evidence for modifying effects of *APOE*-ε4 were detected, particularly at later ages, indicating a potentially stronger adverse impact of being an ε4 carrier for females, notably for memory task performance. Overall, these analyses reinforce that, while females typically perform better than males on some cognitive tasks, they may be more susceptible to some sociocultural and genetic factors linked with dementia risk.

# 4.0. Empirical section B: Examining sex differences in cumulative lifetime dementia risks and their association with later-life cognitive function and brain health.

The primary purpose of this chapter is to examine whether life course modifiable dementia risk exposures differentially associate with cognition and brain health in later-life between males and females. A cumulative risks score (CRS) was derived from NSHD prospective life course data pertaining to each of the twelve modifiable dementia risk factors outlined in the 2020 Lancet commission on dementia prevention, intervention, and care;[56] higher CRS indicated a greater number of risk factor exposures across the life course, up to age 69. Unadjusted regression modelling assessed whether early-life and genetic variables (childhood cognition, childhood SEP, puberty timing, *APOE* genotype) associated with CRS; higher childhood cognition and SEP, and *APOE*-ε4 carrier (vs. non-carrier) status associated with lower CRS. Multivariable regression models accounting for these early-life predictors of CRS showed adverse associations of higher CRS with cognitive performance at age 69 in both sexes; associations with cognitive state were equal in both sexes, females showed slightly stronger associations with verbal memory, and an association with processing speed was only detected in males. Greater CRS associated with smaller total brain- and hippocampal volumes only in males, while there was no evidence for CRS relationships with AD- (Aβ) or cSVD-related (WMHV) pathology in either sex. There was no evidence for effect modifications by *APOE* genotype in either sex. Key findings were replicated when CRS was calculated up to midlife (age 53). Overall, these analyses showed that, beyond early-life and genetic (*APOE*) factors, greater life course exposures to modifiable dementia risks associated with later-life outcomes indicative of more advanced cognitive and brain ageing, particularly in males.

## 4.1. Introduction

As outlined in Section 1.0., there is increasing recognition that a life course approach is required to understand dementia risks, and that risks are interrelated.[111] The renowned 2020 Lancet commission on dementia prevention, intervention and care convened experts to identify the most convincing modifiable risk factors spanning early- (<45 years), mid- (45-65 years) and later-life (>65 years).[56] The twelve risks identified are: low education, hearing impairment, traumatic brain injury (TBI), hypertension, high alcohol consumption, obesity, smoking, depression, social isolation, physical inactivity, diabetes, and exposure to high levels of air pollution.[56] Although the causal mechanisms underlying the association of each factor with dementia risk are not fully established, the Lancet commission provides the most comprehensive overview of life course risk factors linked with dementia, using the best available evidence to date. A range of dementia risk scores have been developed to try to quantify the effects of multiple risk factors and to predict future risk of developing dementia.[106,107] While such scores have poor predictive accuracy,[109] they have been associated with later-life cognitive and brain health outcomes; higher scores, indicating more risk exposures, associated with poorer outcomes.[108,271] However, sex differences in the cumulative effects of multiple dementia risks are largely understudied.

Some studies have examined sex differences in the associations of individual cardiometabolic risk factors with later-life outcomes, generally demonstrating greater adverse effects in females than males. For example, midlife hypertension had been associated with poorer cognitive performance on the MMSE and greater dementia risk in women but not in men.[124,272] In the UK Biobank, being overweight or obese associated with increased dementia risk, compared with being a healthy weight, only in women.[132] Similarly, in NSHD at age 60-64, only in women did greater BMI associate with poorer performance on the letter search task assessing processing speed.[206]

Only one study to date, which derived a dementia risk score based on the Lancet commission identified risks, examined sex differences in the effects of multiple risk exposures. The online survey of 22,117 18-89 year-olds asked about eight of the twelve risks (low education, hearing loss, TBI, alcohol or substance abuse, hypertension, smoking, diabetes, depression). A dose-response effect was observed in males and females whereby each additional risk associated with poorer performance on a cognitive composite comprising tests of spatial working memory,

associative memory, processing speed, and executive function including inhibitory control and set shifting.[111,273] Females had fewer risk exposures than males across all ages, but the negative cumulative effect of multiple risk factors on associative memory performance was greater in females than in males.[111] Similar results were found when risks were weighted by the population attributable fractions (PAFs) outlined in the Lancet commission.[111,273]

While dementia risks have been identified across the life course, only one childhood risk is included in the Lancet risks (low education). Given the time lag between early-life exposures and dementia risk in later-life, the role of early-life factors in dementia risk can be controversial. However, early-life experiences are important for developmental processes including brain development and for building cognitive and brain reserve.[274] Additionally, as proposed by the accumulative model of life course epidemiology[6] (Section 1.1.), life course risks can be downstream results of early-life and developmental factors. For example, poorer SEP in childhood is linked with greater adversities including poorer housing quality, diet, and reduced access to education and healthcare, which are associated with poorer health including increased cardiovascular risks (e.g. obesity, hypertension) and riskier lifestyle behaviours (e.g. physical inactivity, smoking, high alcohol consumption).[275,276] Studies examining the effects of multiple dementia risks have not incorporated such early-life predictors of dementia risks in their analyses, owing to a lack of available prospective early-life data. It is also unclear whether *APOE* genotype modifies the extent to which each modifiable risk factor contributes to dementia risk, which is important to consider given greater ε4 associated dementia risk in females than males.[95]

Existing studies imply that exposure to increasing numbers of modifiable dementia risk factors associates with poorer cognitive and brain health outcomes, prior to dementia onset, and there is preliminary evidence that such adverse effects of multiple risk exposures could be exacerbated in females. However, existing studies have only measured risks present at a single timepoint, not considering the potential cumulative impacts of risks experienced throughout the whole life course. Additionally, the extent to which early-life factors predict subsequent exposures to dementia risk factors and explain the associations of such risk factors with later-life outcomes remains unclear. Sex differences in how multiple risk factor exposures associate with later-life

cognitive and brain health outcomes remain largely understudied, and the mechanisms underlying risk factor associations with outcomes in males and females are unclear.

### 4.1.1. Objectives and research questions

This work capitalises on the broad range of prospective life course data available within NSHD, which provides a unique opportunity to examine early determinants of lifetime risk exposures. A cumulative measure of lifetime modifiable dementia risk factor exposures (a cumulative risks score/CRS) spanning from birth to age 69 is derived in an age-homogenous population-based sample. CRS is based on the twelve risks outlined in the 2020 Lancet commission and weighted according to the Lancet risk-specific population PAFs.[56] The overall aims are to test whether cumulative risk exposures and early-life determinants of CRS differ between males and females, and whether there are sex differences in how cumulative risks associate with later-life cognitive and brain health measures, reflecting AD (Aβ, hippocampal volume), vascular (WMHV), and non-specific ageing (TBV) pathways to dementia. Whether early-life (childhood cognition, childhood SEP, puberty timing) and genetic (*APOE*-ε4 status) factors predict CRS is examined, and the extent to which these predictors contribute to associations of CRS with later-life outcomes in males and females is assessed, including whether associations are modified by *APOE*-ε4 status. Additionally, whether use of risk-modifying treatments (anti-hypertensive medication, hearing aids, diabetes medication, anti-depressant medication) mitigate associations is considered. Secondary analyses examine whether associations of CRS with later-life outcomes are detected when risks are measured up to midlife (age 53) rather than up to age 69, and whether there are associations of a non-weighted lifetime CRS with later-life cognitive and brain health outcomes.

The following research questions are addressed:

**Part A – CRS distribution and predictors:**

1) What is the distribution of lifetime CRS in a population-based cohort, including the contribution of individual risk factors, in males and females?

2) How are early-life factors (childhood cognition, childhood SEP, puberty timing, *APOE*-ε4 genotype) related with lifetime CRS in males and females?

**Part B – Associations of CRS with later-life cognitive performance and brain health**

1) Does lifetime CRS associate with later-life cognitive performance and brain health, and do associations differ between males and females?
2) Are associations of CRS with later-life outcomes modified by *APOE*-ε4 carrier status?
3) Do associations remain after accounting for early-life predictors of lifetime CRS?
4) Does the use of risk-modifying treatments (hearing aids, anti-hypertensive medications, anti-depressant medications, diabetes medications) mitigate relationships of lifetime CRS with later-life cognition and brain health?

**Part C – Secondary analyses**

1) Do key associations of CRS with cognitive and brain health outcomes remain when using a risk score capturing risk exposures up to midlife (age 53)?
2) Does the pattern of findings remain when using a non-weighted CRS?

### 4.1.2. Hypotheses

Females were expected to have lower CRS than males, given previous cross-sectional quantification of some of the Lancet risks.[111] Associations of CRS with later-life cognitive performance and brain health measures were expected to indicate adverse effects of increased risk factor exposures, to a greater extent in females. Adverse early-life factors (e.g. poorer childhood SEP, lower childhood cognition) were expected to associate with lower CRS, since downstream effects of early-life factors, for example childhood SEP, on lifetime health and lifestyle behaviours have previously been demonstrated.[275,276] Additionally, given associations of early-life factors with later-life outcomes (e.g. childhood cognition predicts later-life cognitive performance),[159] adjustments for early-life predictors of CRS were hypothesised to attenuate associations of CRS with later-life outcomes. *APOE*-ε4 carriers were anticipated to show greater

adverse associations of increased CRS with outcomes, given that ε4 is the risk allele for dementia (Section 1.10.1.).

## 4.2. Analytic method

### 4.2.1. Analytic sample

Males and females from NSHD were included in analyses if they had available data for cognitive measures assessed at age 69 (ACE-III total score, word list recall, timed letter search; maximum n=2140). Insight 46 participants were included if they had completed the PACC at data collection wave I (aged ~70 years) or if they had available data for the neuroimaging outcomes of interest (TBV, hippocampal volume, WMHV, amyloid SUVR; maximum n=502).

### 4.2.2. Lifetime cumulative risks score (CRS)

A cumulative score of risk factors experienced across the life course was derived based on available data within NSHD for the twelve modifiable risk factors identified in the 2020 Lancet commission:[56] low education, hearing loss, TBI, hypertension, high alcohol consumption, obesity, smoking, depression, social isolation, physical inactivity, air pollution, diabetes. To ensure equal variance in the number of times each risk could be counted, the data were collapsed into three timepoints; earlier-life (up to and including age 36 years), midlife (age 43 to 53 years), and later-life (age 60 to 69 years). For each risk, except for low education and TBI, the number of timepoints in which the risk was present was counted, giving a maximum 'score' of three per risk. Low educational attainment up to age 43 was assigned a score of three, with the assumption that education level (measured according to the Burnham scale, see Section 2.3.4.2.) remained low in later-life. Low education was indicated by having 'no qualifications attempted' by age 43, or by age 26 if data were unavailable at 43 (n=2). Ever having had a TBI up to age 60 was also assigned a score of three, since it was not possible to distinguish the age at which a TBI had occurred or the number of TBIs experienced.

To calculate a weighted lifetime CRS per individual, each risk factor score was multiplied by the risk-specific PAF outlined in the 2020 Lancet commission[56] (Table 11) and then summed across all twelve risk factors. This value was divided by the maximum possible PAF (1.2), to generate a final CRS between the values of 0 and 1, whereby 1 indicates that all risks were present at all timepoints (early-, mid-, and later-life). CRS was calculated only for participants who had available data at early-, mid-, and later-life timepoints for each risk (whole-NSHD n=1509, 51% female; Insight 46 n=389, 48% female). Details for how each risk was measured are presented in Table 11.

***Table 11.*** *Description of how each risk factor included in the cumulative risks score (CRS) was measured, defined, and scored.*

| Timepoint | Age at measurement | Measure | Description | Scoring |
|---|---|---|---|---|
| **Low education** | | | | **Weighting (PAF): 0.07** |
| Life course | Up to age 43 | No qualifications | Based on self-reported highest qualification level achieved by age 43, or by age 26 if data were unavailable at 43, classified according to the Burnham Scale.[221] | Score 3 if:<br>- Low education up to age 43 |
| **Hearing impairment** | | | | **Weighting (PAF): 0.08** |
| Earlier-life (≤ age 36) | Childhood (6-15 years) | Doctor assessed hearing as 'poor' | Doctor's assessed children's hearing at ages 6, 7, 11, and 15, giving a rating of either poor, good, or excellent. | Score 1 if:<br>- Hearing rated as 'poor' at age 6, 7, 11, OR 15 |
| Midlife (age 43-53) | 43 | Difficulty following a conversation | Self-reported "Do you have great difficulty following a conversation if there is background noise, for example, a TV, radio or child playing (wearing your hearing aid)?" | Score 1 if:<br>- Difficulty following a conversation at age 43, OR<br>- Difficulty following a conversation OR hearing during testing at age 53 |
| | 53 | Difficulty following a conversation | Self-reported "Do you find it very difficult to follow a conversation if there is background noise (without a hearing aid)?" | |
| | | Difficulty hearing during testing | Study nurse recorded whether the study member had hearing difficulty during testing. | |
| Later-life (age 60-69) | 60-64 | Difficulty following a conversation | Self-reported "In the last 12 months have you had a problem with hearing conversation in a noisy room?" | Score 1 if:<br>- Difficulty following a conversation OR hearing over the phone OR hearing during testing at age 60-64, OR<br>- Difficulty following a conversation OR hearing over the phone OR hearing during testing at age 69 |
| | | Difficulty hearing over the phone | Self-reported "In the last 12 months have you had a problem with hearing over the phone?" | |
| | | Difficulty hearing during testing | Study nurse recorded whether the study member had hearing difficulty during testing. | |
| | 69 | Difficulty following a conversation | Self-reported "In the last 12 months have you had difficulty hearing conversation in a noisy room?" | |
| | | Difficulty hearing over the phone | Self-reported "In the last 12 months have you had difficulty hearing over the phone?" | |
| | | Difficulty hearing during testing | Study nurse recorded whether the study member had difficulty hearing during testing. | |
| **Traumatic Brain Injury (TBI)** | | | | **Weighting (PAF): 0.03** |
| Life course | Up to age 53 | Ever had a TBI | Self-reported "Have you ever been knocked unconscious by a blow to the head?" | Score 3 if:<br>- Ever reported having lost consciousness after a head injury, up to age 60-64 |
| | Up to age 60-64 | Had a TBI since age 53 | Self-reported "Since 1999 have you been knocked unconscious?"<br>Responses to this question were used to update information obtained at age 53, to indicate whether participants had ever had a TBI up to age 60-64. | |

| **Hypertension** | | | | **Weighting (PAF): 0.02** |
|---|---|---|---|---|
| Earlier-life (≤ age 36) | 36 | BP >140/90mmHg | Blood pressure was measured twice using a Hawksley random zero sphygmomanometer with regular (12×23 cm) upper arm cuff. A correction was made for arm circumference. | Score 1 if:<br>- BP >140/90mmHg at age 36 |
| Midlife (age 43-53) | 43 | BP >140/90mmHg | Blood pressure was measured as at age 36. | Score 1 if:<br>- BP >140/90mmHg OR self-reported hypertension at age 43, OR<br>- BP >140/90mmHg OR self-reported hypertension at age 53 |
| | | Self-reported high BP | "Have you had high blood pressure?" | |
| | 53 | BP >140/90mmHg | Blood pressure was measured twice, while seated and after 5 minutes of rest, using the validated automated Omron HEM-705 (Omron Corp., Tokyo, Japan) digital oscillometric sphygmomanometer. | |
| | | Self-reported high BP | Indicates "hypertension" in response to "Have you had any kind of blood pressure problems in the last 10 years?" | |
| Later-life (age 60-69) | 60-64 | BP >140/90mmHg | Blood pressure was measured as at age 53. | Score 1 if:<br>- BP >140/90mmHg OR self-reported hypertension at age 60-64, OR<br>- BP >140/90mmHg OR self-reported hypertension at age 69 |
| | | Self-reported Doctor diagnosed high BP | "Has a doctor told you that you have high blood pressure?" | |
| | 69 | BP >140/90mmHg | Blood pressure was measured 3 times, while seated and after 5 minutes of rest, using the validated automated Omron 907-HEM digital oscillometric sphygmomanometer. | |
| | | Self-reported Doctor diagnosed high BP | Indicated "hypertension" in response to "Since 2006 have you been told by a doctor that you have blood pressure problems?" | |
| **High alcohol consumption** | | | | **Weighting (PAF): 0.01** |
| Earlier-life (≤ age 36) | 36 | Problematic drinking | A question included in the present state examination (PSE)[277] asked "Is alcohol in any way a problem for you?" | Score 1 if:<br>- Responded 'yes' to problematic drinking OR >21 units/week at age 36 |
| | | Alcohol units >21/week[56] | Average daily alcohol units were recorded in diet diaries. This was converted into weekly alcohol units. | |
| Midlife (age 43-53) | 43 | CAGE[278] score ≥2 | 4-item screening tool for alcohol use disorder scored from 0-4, where scores of 2 or more indicate possible problem drinking. | Score 1 if:<br>- CAGE ≥2 OR >21 units/week at age 43, OR<br>- CAGE ≥2 OR >21 units/week at age 53 |
| | | Alcohol units >21/week | Participants self-reported how many measures of spirits, wine, and beer they had consumed in the past 7 days. This was later converted to an overall measure of alcohol units consumed within the 7 days. | |
| | 53 | CAGE score ≥2 | As at age 43. | |
| | | Alcohol units >21/week | As at age 43. | |
| Later-life (age 60-69) | 60-64 | CAGE score ≥2 | As at age 43 and 53. | Score 1 if: |
| | | Alcohol units >21/week | As at age 43 and 53. | |

| | | | | |
|---|---|---|---|---|
| | 69 | Alcohol Use Disorders Identification Test[279] (AUDIT) score ≥20 | 20-item questionnaire scored from 0-40, where scores of 20 or more indicate possible alcohol dependence. | - CAGE ≥2 OR >21 units/week at age 60-64, OR<br>- AUDIT ≥20 OR >21 units/week at age 6 |
| | | Alcohol units >21/week | As at age 43, 53, and 60-64. | |
| **Obesity** | | | | **Weighting (PAF): 0.01** |
| Earlier-life (≤ age 36) | 26 | BMI ≥ 30.0 | BMI calculated from self-reported weight (stones, later converted to kg) divided by height (feet, later converted to m) squared: kg/m$^2$ | Score 1 if:<br>- BMI ≥ 30.0 at age 26 OR 36 |
| | 36 | BMI ≥ 30.0 | BMI calculated from weight (kg) and height (m) measured at study visit: kg/m$^2$ | |
| Midlife (age 43-53) | 43 | BMI ≥ 30.0 | As at age 36. | Score 1 if:<br>- BMI ≥ 30.0 at age 43 OR 53 |
| | 53 | BMI ≥ 30.0 | As at age 36 and 43. | |
| Later-life (age 60-69) | 60-64 | BMI ≥ 30.0 | As at age 36, 43, and 53. | Score 1 if:<br>- BMI ≥ 30.0 at age 60-64 OR 69 |
| | 69 | BMI ≥ 30.0 | As at age 36, 43, 53, and 60-64. | |
| **Smoking** | | | | **Weighting (PAF): 0.05** |
| Earlier-life (≤ age 36) | 20 | Current smoker | Self-reported smoking status as current, ever, or never. | Score 1 if:<br>- Current smoker at age 20, 25, 31, OR 36 |
| | 25 | Current smoker | As at age 20. | |
| | 31 | Current smoker | As at age 20 and 25. | |
| | 36 | Current smoker | As at age 20, 25, and 31. | |
| Midlife (age 43-53) | 43 | Current smoker | As at age 20, 25, 31, and 36. | Score 1 if:<br>- Current smoker at age 43 OR 53 |
| | 53 | Current smoker | As at age 20, 25, 31, 36, and 43. | |
| Later-life (age 60-69) | 60-64 | Current smoker | As at age 20, 25, 31, 36, 43, and 53. | Score 1 if:<br>- Current smoker at age 60-64 OR 68 |
| | 68 | Current smoker | As at ages 20, 25, 31, 36, 43, and 53. | |
| **Depression** | | | | **Weighting (PAF): 0.04** |
| Earlier-life (≤ age 36) | Childhood (age 13 and 15)[a] | Internalising rating ≥2.085[280] | Sum of teacher ratings for emotional problems at 13 and 15. | Score 1 if:<br>- Internalising rating ≥2.085 in childhood, OR<br>- PSE ≥5 at age 36 |
| | 36 | PSE ≥5 | Present State Examination,[277] scored 0 to 28. | |
| Midlife (age 43-53) | 43 | PSF ≥23 | Psychiatric Symptom Frequency scale[281] (excluding suicide question), scored 0-90 | Score 1 if:<br>- PSF ≥23 at age 43, OR<br>- GHQ ≥5 at age 53 |
| | 53 | GHQ ≥5 | GHQ-28,[282] scored 0-28 | |
| Later-life (age 60-69) | 60-64 | GHQ ≥5 | GHQ-28, scored 0-28 | Score 1 if:<br>- GHQ ≥5 at age 60-64,<br>- GHQ ≥5 OR self-reported depression at age 69 |
| | 69 | GHQ ≥5 | GHQ-28, scored 0-28 | |
| | | Self-reported depression | Since 2006, have you had depression? | |
| **Social isolation** | | | | **Weighting (PAF): 0.04** |
| Earlier-life (≤ age 36) | 26 | Unmarried and living alone | Participants self-reported their marital status and how many people, including themselves, lived in their household. | Score 1 if:<br>- Unmarried and living alone at age 26, OR<br>- Unmarried and living alone at age 36 |

| | | | | |
|---|---|---|---|---|
| | 36 | Unmarried and living alone | As at age 26. | |
| Midlife (age 43-53) | 43 | Unmarried and living alone | As at age 26 and 36. | Score 1 if:<br>- Unmarried and living alone OR small network at age 43, OR<br>- Unmarried and living alone OR small network at age 53 |
| | | Small social network size (None) | Self-reported "How many friends or relatives do you meet and talk to socially on a regular basis?" | |
| | 53 | Unmarried and living alone | As at age 26, 36, and 43. | |
| | | Small social network size (None) | Self-reported "How many friends or relatives do you see once a month or more?" | |
| Later-life (age 60-69) | 60-64 | Unmarried and living alone | As at age 26, 36, 43, and 53. | Score 1 if:<br>- Unmarried and living alone OR small network OR low contact frequency at age 60-64, OR<br>- Unmarried and living alone OR small network OR low contact frequency at age 69 |
| | | Small social network size (None | Self-reported "How many relatives or friends do you see once a month or more?" | |
| | | Low social contact frequency (Never/almost never) | Self-reported "Thinking of all your relatives or friends, how often do you regularly visit or are visited by these people?" | |
| | 69 | Unmarried and living alone | As at age 26, 36, 43, 53, and 60-64. | |
| | | Small social network size (None) | Self-reported "How many relatives and/or friends do you see once a month or more?" | |
| | | Low social contact frequency (Never/almost never) | Self-reported "Thinking of all you friends/relatives, how often do you regularly visit or are visited by any of these people?" | |
| **Physical inactivity** | | | | **Weighting (PAF): 0.02** |
| Earlier-life (≤ age 36) | 36 | No participation in physical activity/month | Self-completed modified validated Minnesota leisure time physical activity questionnaire (frequency of participation in a range of physical activities per month).[227] | Score 1 if:<br>- No physical activity/month at age 36 |
| Midlife (age 43-53) | 43 | No participation in physical activity/month | EPIC Physical Activity Questionnaire-2 (frequency of participation in sport, vigorous leisure activities or exercise in the previous month).[227] | Score 1 if:<br>- No physical activity/month at age 36 OR 53 |
| | 53 | No participation in physical activity/month | EPIC Physical Activity Questionnaire-2 (frequency of participation in sport, vigorous leisure activities or exercise in the previous 4 weeks).[227] | |
| Later-life (age 60-69) | 60-64 | No participation in physical activity/month | As at age 53. | Score 1 if:<br>- No physical activity/month at age 60-64 OR 69 |
| | 69 | No participation in physical activity/month | As at age 53 and 60-64. | |
| **Exposure to high air pollution[b]** | | | | **Weighting (PAF): 0.02** |
| Earlier-life (≤ age 36) | Childhood (birth – age 11) | Heavy pollution | Pollution index based on domestic local coal consumption, categorised as heavy or low-moderate.[283,284] | Score 1 if:<br>- High pollution index in childhood |
| Midlife (age 43-53) | 43 | Geocoded residential $NO_2$ estimate > 41.5 μg/$m^3$, as | 1991 $NO_2$ air pollution maps, based on a land use regression (LUR) model from the Automatic Urban and Rural Network/AURN, Defra. | Score 1 if:<br>- $NO_2$ estimate > 41.5 μg/$m^3$ at age 43 OR 53 |

| | | | | |
|---|---|---|---|---|
| | | indicated in the Lancet Commission.[56] | | |
| | 53 | Geocoded residential $NO_2$ estimate > 41.5 µg/$m^3$ | 2001 $NO_2$ air pollution maps, based on a LUR model from the RGI project. | |
| Later-life (age 60-69) | 64 | Geocoded residential $NO_2$ estimate > 41.5 µg/$m^3$ | 2010 $NO_2$ air pollution maps, based on a LUR model from the European Study of Cohorts for Air Pollution Effects (ESCAPE). | Score 1 if:<br>- $NO_2$ estimate > 41.5 µg/$m^3$ at age 64 |
| **Diabetes[c]** | | | | **Weighting (PAF): 0.01** |
| Earlier-life (≤ age 36) | Up to age 36 | Self-reported diabetes up to age 36 | "Have you had diabetes?" | Score 1 if:<br>- Report diabetes up to age 36 |
| Midlife (age 43-53) | Up to age 43 | Self-reported diabetes up to age 43 | "Have you had diabetes?" | Score 1 if:<br>- Report diabetes up to age 36 OR up to age 43 OR up to age 53 |
| | Up to age 53 | Self-reported diabetes up to age 53 | "Diabetes at any point up to and including 1999" | |
| Later-life (age 60-69) | Up to age 60-64 | Self-reported diabetes diagnosed by GP at age 60-64, or self-reported at up to 53 | "Diabetes diagnosed by GP" | Score 1 if:<br>- Report diabetes up to age 36 OR up to age 43 OR up to age 53 OR up to age 60-64 OR up to age 69 |
| | Up to age 69 | Self-reported Doctor diagnosed diabetes at age 69, or self-reported up to 60-64 | "Ever self-reported doctor diagnosed diabetes" | |

*PAF=population attributable fraction; TBI=traumatic brain injury; BP=blood pressure; PSE=present state examination; AUDIT=alcohol use disorders identification test; BMI=body mass index; PSF=psychiatric symptom frequency scale; GHQ=general health questionnaire; LUR=land use regression; AURN=automatic urban and rural network; RGI=Ruimte voor Geoinformatie*

*a: At ages 13 and 15, teachers were asked to complete questionnaires (a forerunner of the Rutter A scale[285]) giving ratings for the study members' internalising problems (anxiety, timidity, fearfulness, diffidence, avoidance of attention).[280] Ratings from age 13 and 15 were summed, and childhood internalising caseness was indicated by an overall rating of 2.085 or more, based on previous NSHD studies.[286]*

*b: In adulthood, estimated nitrogen dioxide ($NO_2$) levels – identified as a key pollution measure in the Lancet commission[56] – were derived from land use regression (LUR) models. For $NO_2$ (µg/$m^3$) at age 43 (1989), 1991 Great Britain-wide $NO_2$ air pollution maps (200m x 200m; based on a LUR model using data from the Automatic Urban and Rural Network/AURN, Defra) were overlaid on maps of participants residential addresses in 1989. For $NO_2$ at age 53 (1999), 2001 air pollution maps (100m x 100m) based on a LUR models from the RGI project (grant RGI-137, Dutch program Ruimte voor Geoinformatie) were overlaid on maps of participant's residential addresses in 1999. $NO_2$ concentrations for participants geocoded addresses in 2010 were estimated from a LUR model derived as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE).[287]*

*c: Since diabetes is non-curable, presence of diabetes at an earlier age also indicated persistent presence of the risk at subsequent timepoints.*

### 4.2.3. Cognitive outcomes

At age 69, the cognitive outcomes used for these analyses were the ACE-III total score, word list learning (verbal memory), and timed letter search (search speed) measures. For the Insight 46 sample at age ~70, cognitive performance was assessed using the PACC total score. Raw scores for each assessment were standardised to the analytical sample, generating z-scores. Further details for each cognitive assessment have previously been outlined in Section 2.3.2. (Tables 1 and 2).

### 4.2.4. Neuroimaging outcomes

As outlined in Section 2.3.3., Insight 46 participants underwent PET-MRI neuroimaging at age ~70. For these analyses, the neuroimaging outcomes of interest were TBV, hippocampal volume, WMHV, continuous Aβ SUVR, and Aβ positivity status. For WMHV, participants who failed BaMoS QC were excluded (n=16; 56.3% female).

### 4.2.5. Early-life and genetic covariables

Variables hypothesised to associate with CRS and later-life outcomes are childhood cognition, childhood SEP, *APOE*-ε4 status (each described in Section 2.3.4.), and puberty timing. Childhood cognition and SEP, previously associated with later-life cognitive performance,[159,205] have been shown to be predictive of general health and health behaviours in adulthood.[275,276] Given the role of sex hormones in brain organisation, neural development and the cardiovascular system, puberty timing – when sex hormone levels surge – is hypothesised to contribute to cognitive and brain reserve processes, and to variations in cardiovascular health.[81,83,84] The *APOE*-ε4 allele is strongly associated with AD dementia,[288] more so in females,[95] and has been linked with increased risk of cardiovascular disease.[289]

Puberty timing was determined from age at menarche for females, grouped as very early (age ≤11 years), early (age 12), average (age 13), or late (age ≥13), as outlined in previous NSHD analyses.[290] A binary variable indicated later puberty for menarche at age 13 or older, and earlier puberty for menarche younger than age 13. Male puberty timing was ascertained via school doctor assessment at age 14-15, rating development as pre-pubertal, early puberty,

advanced puberty, or fully mature, whereby those assessed as fully mature had the earliest puberty timing.

Childhood cognition at age 8 years was standardised to the sample at the time of cognitive testing. Childhood SEP up to age 15, was categorised as manual or non-manual (Section 2.3.4.3.). *APOE*-ε4 carrier status indicated ε4 carriers and non-carriers (Section 2.3.4.1.).

Risk-modifying treatments including hearing aid use and anti-hypertensive, anti-depressant, and diabetes medications were considered as potentially mediating factors. Summary variables indicating ever or never use of each of these risk-modifying covariables were derived from life course data. At ages 11 and 15, hearing aid use was reported during doctor examinations. At ages 43, 53 and 69, participants self-reported if they wore a hearing aid. Anti-hypertensive medications, prescribed for high blood pressure or heart failure, were self-reported by participants at home visits or via postal questionnaire at ages 36, 43, 53, 60-64, and 69. Anti-depressant medications, including tricyclics, monoamine-oxidase inhibitors, and selective serotonin reuptake inhibitors were self-reported via home visits or postal questionnaires at ages 31, 36, 43, 53, 60-64, and 69. Diabetic medications, including insulin and oral diabetic agents, treatments for low blood sugar, and diabetic testing kits, were self-reported at home visits or on postal questionnaires at ages 31, 36, 43, 53, 60-64, and 69. For all medication types, use was categorised as using or not using treatment for each assessment.

### 4.2.6. Statistical analyses

Analyses were conducted using Stata version 18.0.

As presented in Tables 12 and 13, the mean, standard deviation, and range of continuous variables were calculated for males and females. T-tests and chi-squared tests examined sex differences in continuous and categorical variables, respectively, including examination of sex differences in total CRS and in mean scores for each risk factor. All analyses were sex-stratified, to determine how associations of CRS, later-life outcomes, and early-life covariables might differ between males and females.

The association of each early-life covariable with CRS was examined using unadjusted linear regression models, in the whole-cohort and Insight 46 samples.

Associations of CRS with cognitive outcome measures, TBV, hippocampal volume, and continuous SUVR were assessed using linear multivariable regression models. Multivariable logistic regression models examined associations of CRS with Aβ status, and WMHV was assessed using generalised linear models with gamma distribution log link, due to the skewed distribution of WMHV.

Associations of CRS with later-life cognitive performance and brain health outcomes were first examined in minimally adjusted models (M0). Models assessing cognitive outcomes at age 69 were completely unadjusted, while those assessing Insight 46 outcomes were adjusted for age at cognitive testing (PACC) or age at scan (neuroimaging outcomes), given the two-year range required to complete Insight 46 data collection. Additionally, models examining volumetric neuroimaging outcomes (TBV, hippocampal volume, WMHV) were adjusted for TIV.

Potential modifying effects of *APOE*-ε4 genotype on associations of CRS with later-life outcomes were examined by including a CRS-by-*APOE*-ε4 status interaction term in minimally adjusted models, interpreting significant interactions at the $p<0.10$ level. To determine whether associations of CRS and later-life outcomes were independent of early-life predictors of CRS, subsequent models cumulatively adjusted for early-life covariables found to associate with CRS. Where associations of CRS with outcomes were detected, post-estimation tests of difference assessed whether model coefficients significantly differed between males and females.

For outcomes found to associate with CRS, fully adjusted models were further adjusted for the use of risk-modifying treatments individually (fully adjusted model + hearing aids; fully adjusted model + anti-hypertensive medications; fully adjusted model + anti-depressant medications; fully adjusted model + diabetes medications) and all together (fully adjusted model + all risk-modifying treatments), to identify whether such treatments impact the associations of risk factor exposures with later-life outcomes.

To address missing data in CRS and covariables, multiple imputation was used (Section 2.4.), with imputation models stratified by sex. For models assessing the ACE-III outcome, which has a skewed distribution, bootstrapping was applied (Section 2.4.); multiple imputation was not applied for ACE-III models.

Non-linear associations of CRS with key outcomes (ACE-III total in the whole sample; TBV in Insight 46) were assessed by including a quadratic term for CRS in fully adjusted models. No evidence for non-linear associations was found (p>0.1).

### 4.2.7. Secondary analyses

To address the issue of reverse directionality from some risk factor variables which can be a part of the dementia prodrome (e.g. low BMI, depression)[291,292] and given an interest in midlife as a period of vulnerability, especially for females,[8] cumulative risk exposures up to midlife (age 53) were also calculated. Midlife CRS was ascertained using the same criteria as lifetime CRS, but each risk factor was scored from 0 to 2, based on data recorded in early-life (up to age 36) and midlife (ages 43 and 53) only. The PAF-weighted risk-specific scores were again summed across all twelve risk factors and divided by the new maximum possible PAF (0.8), generating a midlife CRS between the values of 0 and 1. Midlife CRS was calculated only for participants who had available data at early- and mid-life timepoints for each risk (whole-NSHD n=1,604, 52% female; Insight 46 n=420, 49% female). Fully adjusted sex-stratified multivariable models examined the associations of midlife CRS with outcome variables that were significantly associated with lifetime CRS.

While the PAF weighting applied to each risk factor allows for variances in the effects of each risk, the PAFs calculated in the Lancet commission may not be accurate and could also vary by sex. A non-weighted lifetime CRS was therefore also calculated, summing the risks present across each timepoint (early-, mid-, and later-life). This sum of risks was divided by 36 (the maximum possible risk count) to generate a value between 0 and 1. Fully adjusted sex-stratified multivariable models examined associations of non-weighted lifetime CRS with cognitive performance at age 69 (ACE-III, verbal memory, search speed), and neuroimaging outcome measures aged ~70 (TBV, hippocampal volume, WMHV, SUVR).

## 4.3. Results

### 4.3.1. Participant characteristics

Participant characteristics for the whole-cohort sample, with available data for at least one cognitive outcome at age 69 (n=2140; 51.1% female), are presented in Table 12. T-tests

demonstrated that females performed better than males on the word list learning and timed letter search tasks, but there were no sex differences in total ACE-III performance, in agreement with findings from Chapter 3 (Section 3.0.). Chi-squared tests showed that males had higher levels of anti-hypertensive medication use, while females were more likely to have taken anti-depressant medications. Puberty timing was also more likely to have been classed as later in females (first period at 12.83 years or older) than in males (pre-pubertal or in early puberty at age 14-15), although differences in how puberty is measured between males and females mean that this is not a direct comparison.

Table 13 presents participant characteristics for the 502 (48.3% female) participants in the Insight 46 sample. Unadjusted t-tests showed greater PACC performance in females than males, greater TBV, hippocampal volume and TIV in males, and no sex differences in WMHV or amyloid measures. As in the whole-cohort, chi-squared tests demonstrated greater anti-hypertensive medication use in males, greater anti-depressant use in females, and later puberty timing in females.

### 4.3.2. Lifetime cumulative risks score (CRS) distribution and characteristics

For the whole-cohort sample, lifetime CRS was calculated for 1,509 (51.0% female) participants who had available data at all timepoints, for all risk factors. Mean CRS was 0.25 (SD 0.13), indicating that participants on average were exposed to 25% of the maximum possible lifetime modifiable dementia risk. The CRS distributions for males and females are shown in Figure 8; female mean CRS was 0.25 (SD 0.13), which did not significantly differ from that of males (mean 0.26 (SD 0.13); p=0.188; Table 12). Figure 9 shows the distribution of scores for each risk factor, where higher scores indicate a greater number of timepoints in which the risk was present. T-tests examining sex differences in mean scores per risk showed that hearing impairment, TBI, hypertension, and high alcohol consumption were more often recorded in males than females, while females reported depression and physical inactivity more than males.

For the Insight 46 sample, CRS was calculated for 389 (48.3% female) participants, with a mean score of 0.21 (SD 0.10), indicating that participants on average were exposed to 21% of the maximum possible lifetime modifiable dementia risk, which was significantly lower than in the

whole-cohort sample (one-sample t-test $p<0.001$). The distributions of CRS by sex are presented in Figure 10; males had significantly higher scores (mean=0.23 (SD 0.10)) than females (mean=0.20 (SD 0.11); $p=0.013$; Table 13). As in the whole-cohort sample, hearing impairment, TBI, hypertension, and high alcohol consumption were more common in males than females while depression was more common in females, although within Insight 46 there was no sex difference in physical inactivity (Figure 11).

***Table 12.*** *Participant characteristics for NSHD participants with available data for at least one cognitive outcome measure at age 69.*

| | Male 1,047 (48.9%) | | Female 1,093 (51.1%) | | |
|---|---|---|---|---|---|
| **Variable** | **N** | **Mean(SD); range/%** | **N** | **Mean(SD); range/%** | **Test p-value[a]** |
| Lifetime cumulative risks score | 740 | 0.26(0.13); 0.00, 0.76 | 769 | 0.25(0.13); 0.02, 0.68 | 0.188 |
| ACE-III total score | 849 | 91.33(5.84); 53, 100 | 913 | 91.69(6.17); 59, 100 | 0.205 |
| Word list learning (number of recalled words) | 1,011 | 21.14(5.98); 3, 39 | 1,063 | 23.15(5.99); 6, 40 | **<0.001** |
| Timed letter search (number of letters scanned) | 1,038 | 256.38(74.32); 70, 591 | 1,073 | 268.02(73.56); 60, 591 | **<0.001** |
| Hearing aid use | | | | | |
| Never | 918 | 87.7% | 985 | 90.1% | 0.072 |
| Ever | 129 | 12.3% | 108 | 9.9% | |
| Anti-hypertensive medication | | | | | |
| Never | 677 | 64.7% | 808 | 73.9% | **<0.001** |
| Ever | 370 | 35.3% | 285 | 26.51% | |
| Anti-depressant medication | | | | | |
| Never | 945 | 90.3% | 890 | 81.4% | **<0.001** |
| Ever | 102 | 9.7% | 203 | 18.6% | |
| Diabetes medication | | | | | |
| Never | 951 | 90.8% | 1,012 | 92.6% | 0.140 |
| Ever | 96 | 9.2% | 81 | 7.4% | |
| Puberty staging | | | | | |
| Earlier | 492 | 56.5% | 351 | 39.1% | **<0.001** |
| Later | 379 | 43.5% | 547 | 60.9% | |
| Childhood cognition (8yrs)[b] | 970 | 0.089(0.83); -1.90, 2.50 | 1,010 | 0.16(0.80); -2.12, 2.47 | 0.079 |
| Childhood SEP | | | | | |
| Manual | 526 | 52.7% | 542 | 52.8% | 0.956 |
| Non-manual | 473 | 47.3% | 485 | 47.2% | |
| *APOE*-ε4 status | | | | | |
| Non-carrier | 624 | 68.1% | 692 | 71.3% | 0.137 |
| Carrier | 292 | 31.9% | 279 | 28.7% | |

*[a]Continuous variables: p-value from linear regression t-test. Categorical variables: p-value from Pearson chi-squared test. Bold text indicates a significant (p<0.05) sex difference.*

*[b]Standardised (z-score) to sample at the time of cognitive testing.*

*SD=standard deviation; ACE-III=Addenbrooke's cognitive examination 3rd edition; SEP=socioeconomic position; APOE=apolipoprotein-e.*

***Table 13.*** *Participant characteristics for the Insight 46 sub-sample.*

| | Male 201 (51.7%) | | Female 188 (48.3%) | | |
|---|---|---|---|---|---|
| **Variables** | **N** | **Mean(SD); range/%** | **N** | **Mean(SD); range/%** | **Test p-value[a]** |
| Lifetime cumulative risk score | 201 | 0.23(0.10); 0.04, 0.50 | 188 | 0.20(0.11); 0.02, 0.50 | **0.013** |
| ACE-III total score | 214 | 93.30(4.08); 79, 100 | 207 | 93.46(5.10); 70, 100 | 0.722 |
| Word list learning (number of recalled words) | 253 | 22.95(5.67); 5, 38 | 237 | 24.48(5.76); 9, 40 | **0.003** |
| Timed letter search (number of letters scanned) | 252 | 262.87(66.05); 138, 487 | 237 | 271.52(72.45); 138, 591 | 0.168 |
| PACC[b] | 256 | -0.17(0.71); -3.49, 1.72 | 246 | 0.18(0.71); -3.48, 1.67 | **<0.001** |
| TBV ($cm^3$) | 239 | 1,151.35(86.69); 945.90, 1,493.86 | 229 | 1,046.75(82.49); 818.60, 1,265.16 | **<0.001** |
| Hippocampal volume ($cm^3$) | 239 | 3.26(0.33); 2.42, 4.27 | 229 | 3.00(0.30); 2.06, 3.72 | **<0.001** |
| WMHV ($cm^3$) | 234 | 4.77(5.09); 0.27, 33.67 | 221 | 5.47(5.78); 0.35, 32.79 | 0.174 |
| SUVR | 235 | 0.57(0.08); 0.45, 0.87 | 227 | 0.56(0.07); 0.47, 0.85 | 0.329 |
| Amyloid status | | | | | |
| Negative | 188 | 80.0% | 188 | 82.8% | 0.436 |
| Positive | 47 | 20.0% | 39 | 17.2% | |
| Hearing aid use | | | | | |
| Never | 222 | 86.7% | 221 | 89.8% | 0.278 |
| Ever | 34 | 13.3% | 25 | 10.2% | |
| Anti-hypertensive medications | | | | | |
| Never | 170 | 66.4% | 193 | 78.5% | **0.003** |
| Ever | 86 | 33.6% | 53 | 21.5% | |
| Anti-depressant medications | | | | | |
| Never | 242 | 94.5% | 208 | 84.6% | **<0.001** |
| Ever | 14 | 5.5% | 38 | 15.4% | |
| Diabetes medication | | | | | |
| Never | 238 | 93.0% | 230 | 93.5% | 0.814 |
| Ever | 18 | 7.0% | 16 | 6.5% | |
| Puberty staging | | | | | |
| Earlier | 125 | 55.1% | 95 | 43.6% | **0.015** |
| Later | 102 | 44.9% | 123 | 56.4% | |
| Childhood cognition (8yrs)[b] | 256 | 0.36(0.75); -1.60, 2.50 | 246 | 0.42(0.73); -1.59, 2.47 | 0.353 |
| Childhood SEP | | | | | |
| Manual | 106 | 41.4% | 110 | 45.6% | 0.341 |
| Non-manual | 150 | 58.6% | 131 | 54.4% | |
| *APOE*-ε4 status | | | | | |
| Non-carrier | 171 | 67.3% | 181 | 73.6% | 0.126 |
| Carrier | 83 | 32.7% | 65 | 26.4% | |

| | | | | | |
|---|---|---|---|---|---|
| Age at scan (years) | 241 | 70.66(0.68); 69.25, 71.78 | 230 | 70.70(0.67); 69.27, 71.86 | 0.860 |
| Age at cognitive testing (years) | 256 | 70.64(0.69); 69.23, 71.73 | 246 | 70.66(0.68); 69.27, 71.84 | 0.782 |
| TIV ($cm^3$) | 239 | 1,519.62(105.93); 1,274.26, 1,938.77 | 229 | 1,343.16(92.47); 1,114.35, 1,558.05 | **<0.001** |

*[a]Continuous variables: p-value from linear regression t-test. Categorical variables: p-value from Pearson chi-squared test. Bold text indicates a significant (p<0.05) sex difference.*

*[b]Standardised (z-score) to sample at the time of cognitive testing.*

*SD=standard deviation; PACC=pre-clinical Alzheimer's cognitive composite; TBV=total brain volume; WMHV=white matter hyperintensity volume; SUVR=standardised uptake value ratio; SEP=socioeconomic position; APOE=apolipoprotein-e; TIV=total intracranial volume.*

### 4.3.3. Predictors of risk score

Unadjusted associations of early-life factors with lifetime CRS are presented in Table 14.

In the whole-cohort sample, males and females showed associations of higher childhood cognition and higher childhood SEP with lower CRS. In males, *APOE*-ε4 carriers had lower CRS than non-carriers. While not statistically significant, females showed the same association of *APOE*-ε4 with CRS. Puberty timing was not associated with CRS in males or females.

In the Insight 46 sample, no associations of early-life factors with CRS were found in males or females.

Childhood cognition, childhood SEP, and *APOE*-ε4 are included as confounding variables in subsequent adjusted models.

***Table 14.*** *Model estimates and 95% confidence intervals showing the estimated effects of early-life covariables on cumulative risks score, from unadjusted linear regression models.*

| Cohort | Sex | Value | Childhood cognition (8yrs)[a] | Childhood SEP | Puberty timing | *APOE*-ε4 status |
|---|---|---|---|---|---|---|
| **Whole-cohort** | **Males (n=1047)** | **β** | -0.040 | -0.060 | 0.016 | -0.021 |
| | | **95% CI** | -0.050, -0.029 | -0.077, -0.043 | -0.003, 0.034 | -0.040, -0.002 |
| | | **p-value** | <0.001** | <0.001** | 0.10 | 0.03* |
| | **Females (n=1093)** | **β** | -0.046 | -0.050 | 0.009 | -0.019 |
| | | **95% CI** | -0.057, -0.034 | -0.068, -0.033 | -0.011, 0.028 | -0.038, 0.001 |
| | | **p-value** | <0.001** | <0.001** | 0.37 | 0.06 |
| **Insight 46** | **Males (n=256)** | **β** | 0.006 | -0.004 | -0.008 | 0.007 |
| | | **95% CI** | -0.012, 0.024 | -0.032, 0.023 | -0.037, 0.020 | -0.022, 0.035 |
| | | **p-value** | 0.49 | 0.75 | 0.56 | 0.65 |
| | **Females (n=246)** | **β** | -0.008 | -0.015 | 0.002 | -0.014 |
| | | **95% CI** | -0.029, 0.013 | -0.047, 0.017 | -0.031, 0.035 | -0.049, 0.020 |
| | | **p-value** | 0.44 | 0.35 | 0.89 | 0.42 |

* p<0.05 ** p<.01

[a]*Childhood cognition z-score is standardised to the sample at the time of cognitive testing.*

*Reference categories: childhood SEP = manual, puberty timing = earlier, APOE-ε4 status = non-carrier*

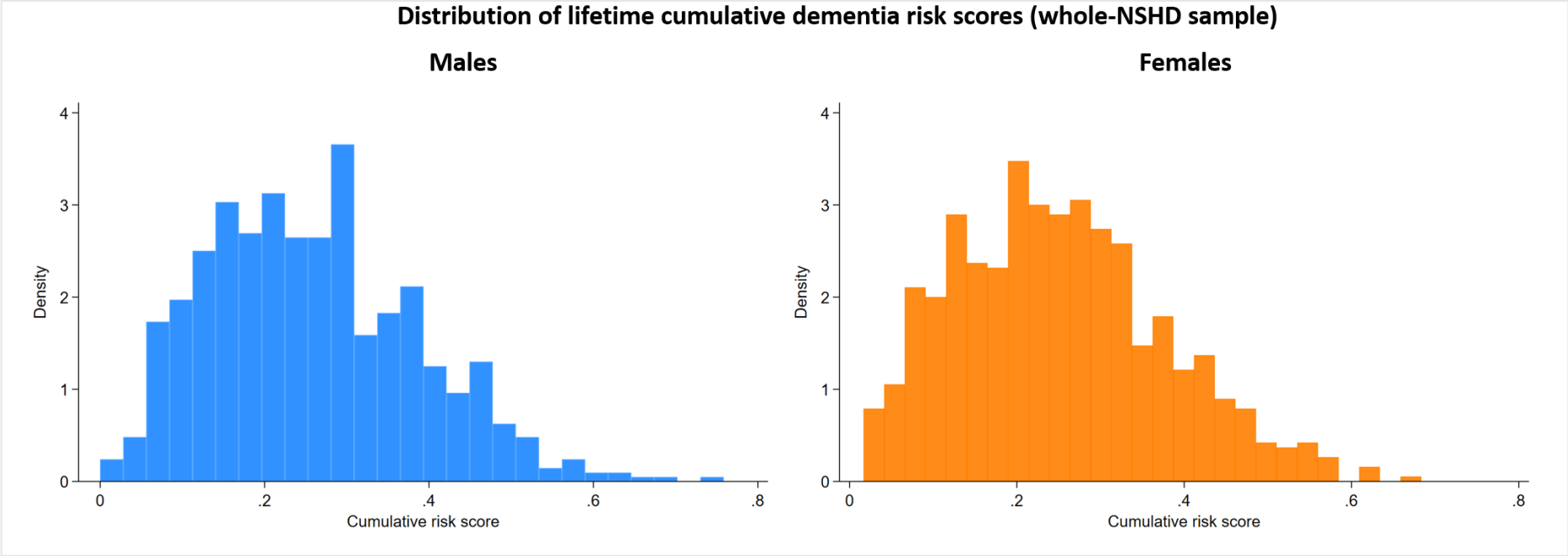


***Figure 8.*** *Distribution of lifetime cumulative dementia risk scores in males (N=740) and females (N=769), in the whole-NSHD analytical sample.*

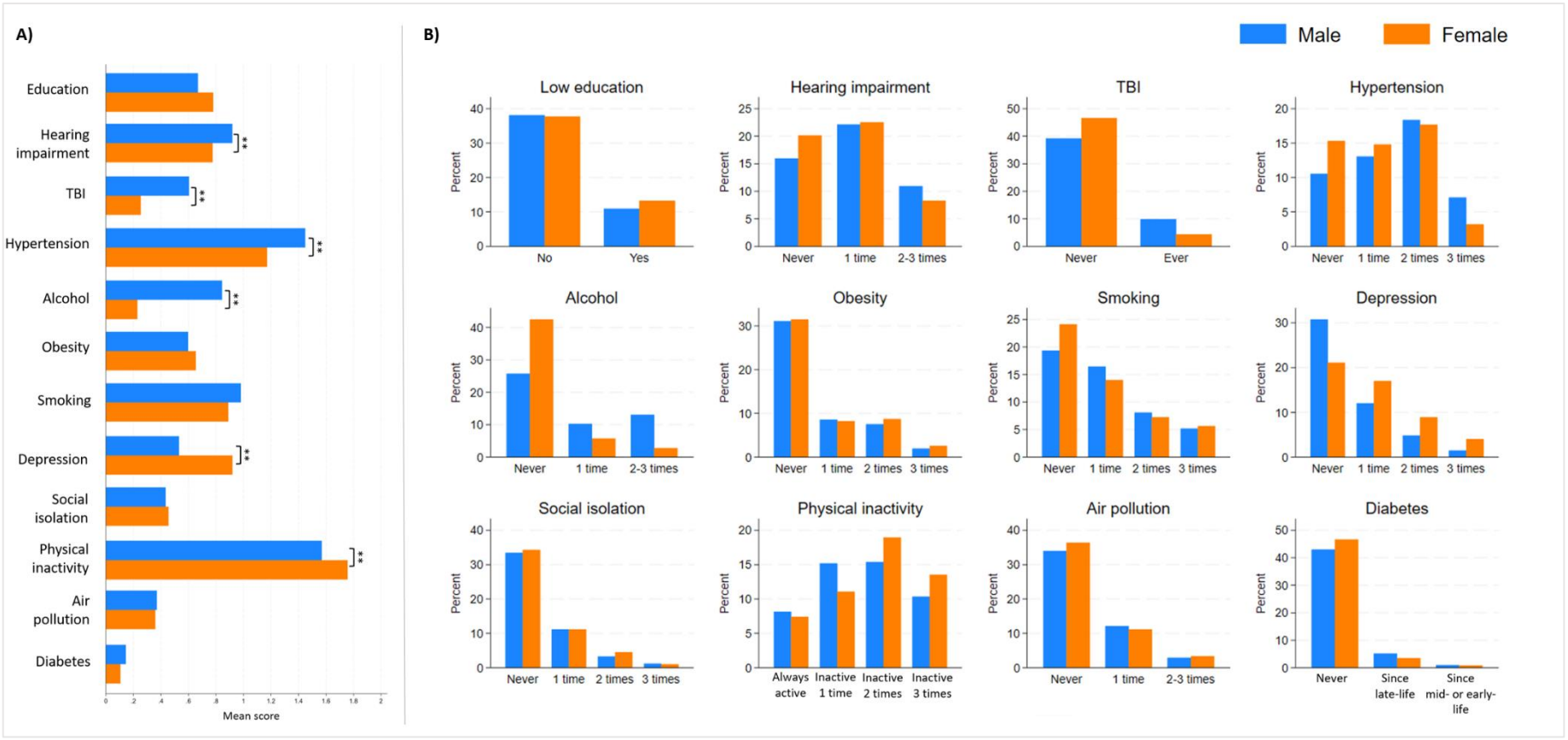


***Figure 9.*** *Distribution of raw scores per risk factor, in males (N=740) and females (N=769) in the whole-NSHD analytical sample. The maximum score per risk is 3. Low education 'No'=0, 'Yes'=3; TBI 'Never'=0, 'Ever'=3; diabetes 'Never'=0, 'Since late-life'=1, 'Since mid-life'=2, 'Since early-life'=3; all other risks are scored 0 to 3 where 0 means that the risk is never present and 3 means that the risk is present at three timepoints. For data protection reasons, some categories have been collapsed.* ***A)*** *Mean scores for males and females.* ***B)*** *Percentage of males and females with different levels of risk factor exposures. TBI=traumatic brain injury ** t-test p<0.01*

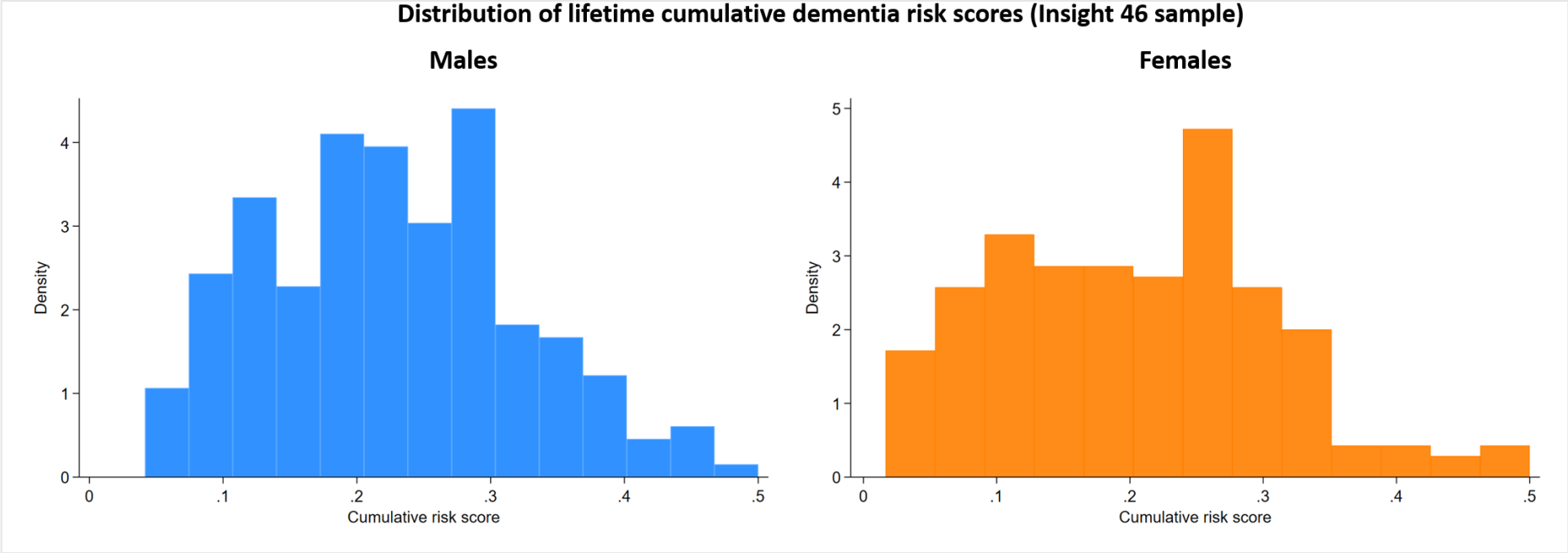


***Figure 10.*** *Distribution of lifetime cumulative dementia risk scores in males (N=201) and females (N=188), in the Insight 46 analytical sample.*

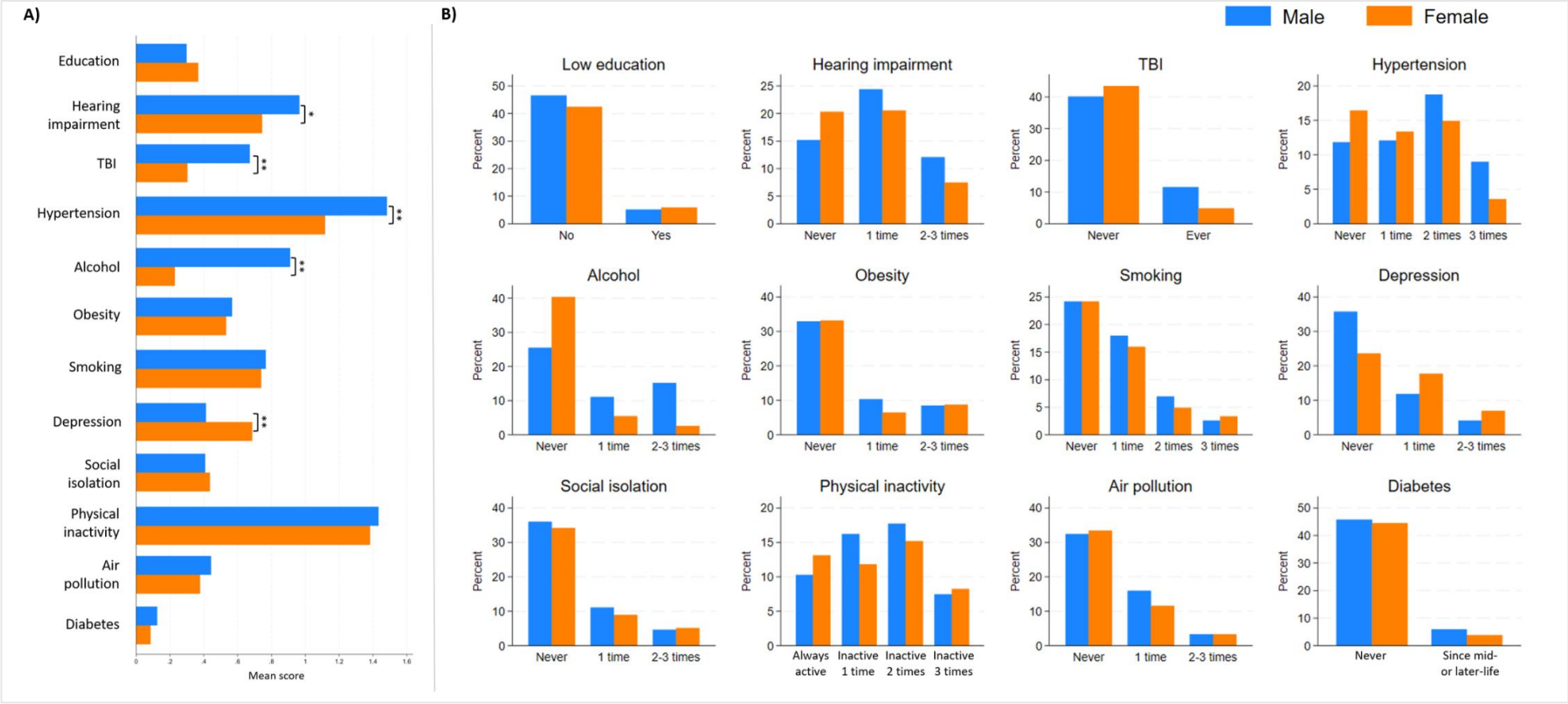


***Figure 11.*** *Distribution of scores per risk factor, in males (N=201) and females (N=188) in the Insight 46 analytical sample. The maximum score per risk is 3. Low education 'No'=0, 'Yes'=3; TBI 'Never'=0, 'Ever'=3; diabetes 'Never'=0, 'Since late-life'=1, 'Since mid-life'=2, 'Since early-life'=3; all other risks are scored 0 to 3 where 0 means that the risk is never present and 3 means that the risk is present at three timepoints. For data protection reasons, some categories have been collapsed. For diabetes, no participants had diabetes in early-life.* ***A)*** *Mean scores for males and females.* ***B)*** *Percentage of males and females with different levels of risk factor exposures. TBI=traumatic brain injury * t-test p<0.05; ** t-test p<0.01*

### 4.3.4. Associations of lifetime CRS with later-life cognitive performance and brain health measures

#### *4.3.4.1. Cognitive performance measures age 69*

Table 15 shows the estimated associations of lifetime CRS with each outcome measure in males and females. There was no evidence for effect modifications by *APOE*-ε4 status (Table 16).

With minimal adjustments, each unit increase in CRS associated with a 1.92 SD reduction in male verbal memory performance (95% CI -2.44, -1.40) and a 2.54 SD reduction in female verbal memory performance (95% CI -3.01, -2.07). Associations remained with adjustments for early-life predictors of CRS, although there was some attenuation, mainly driven by childhood cognition; adjustment for childhood cognition (M1) attenuated the estimated effect of CRS on verbal memory performance by 39% in males and 33% in females. Fully adjusted model effects are demonstrated in Figure 12; there was a trend-level difference in male and female effect estimates (F=3.25, p=0.07).

In males, increased CRS significantly associated with poorer search speed performance in males (M0 β=-1.79[95% CI -22.34, -1.25]) but not in females (M0 β=-0.50[-1.02, 0.04]). The association in males remained with adjustment for early-life predictors, although there was a 9% attenuation when accounting for childhood cognition. Fully adjusted effect estimates significantly differed between males and females (F=10.32, p<0.001), shown in Figure 12.

Increased CRS associated with poorer ACE-III performance in males (M0 β=-2.24[bias corrected 95% CI -2.84, -1.64]) and females (M0 β=-2.42[bias corrected 95% CI -3.16, -1.9]). There was some effect attenuation across adjusted models, with effects attenuating from M0 to M1 (childhood cognition) by 31% in males and 48% in females, but associations remained independently of early-life predictors of CRS. Fully adjusted model predictions, which did not substantially differ between males and females[a], are shown in Figure 12.

[a] Post-estimation test was not possible with bootstrapping applied. Running the post-estimation on multiple imputation estimates, without bootstrapping, showed no significant difference in estimates between males and females (F=0.08, p=0.78).

#### *4.3.4.2. Insight 46 cognitive performance and neuroimaging outcomes aged ~70*

Model estimations for the effect of lifetime CRS on outcomes are presented in Table 15. There was no evidence for effect modifications by *APOE*-ε4 status for any cognitive or neuroimaging outcomes (Table 16).

No association of CRS with PACC performance was found in males or females.

In males, each unit increase in CRS significantly associated with a 75.82cm$^3$ reduction in TBV (M0, 95% CI -138.44, -13.19), while females showed a trend-level association of increased CRS with reduced TBV (M0 β=-59.74[-118.96, -0.51]). The association in males remained independently of early-life predictors of CRS. Fully adjusted model predictions, which did not significantly differ between males and females (F=0.20, p=0.66) are shown in Figure 13.

Males also showed a significant association of increased CRS with reduced hippocampal volume (M0 β=0.69cm$^3$[-1.13, -0.24]), while no association was found in females. The male association remained significant with early-life covariable adjustments. Fully adjusted model predictions, which did not significantly differ between males and females (F=1.68, p=0.20), are demonstrated in Figure 13. To determine whether brain volume reductions associated with CRS were generalised across the brain, models of hippocampal volume were adjusted for TBV as a sensitivity analysis. This slightly attenuated the estimated effects of CRS on hippocampal volume (male M3+TBV β=-0.520[-0.959, -0.098], p=0.02; female M3+TBV β=-0.175[-0.536, 0.185], p=0.34).

No associations of CRS with WMHV or amyloid SUVR or Aβ status were detected in males or females.

***Table 15.*** *Model estimates and 95% confidence intervals for the association of cumulative risks score with standardised cognitive performance outcomes and neuroimaging outcomes, cumulatively adjusting for confounding variables.*

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | | **M0** | **M1** | **M2** | **M3** | **M0** | **M1** | **M2** | **M3** |
| **Verbal memory z-score** | **N** | 1011 | | | | 1063 | | | |
| | **β** | -1.921 | -1.167 | -1.052 | -1.051 | -2.539 | -1.703 | -1.617 | -1.674 |
| | **95% CI** | -2.439, -1.403 | -1.664, -0.671 | -1.553, -0.551 | -1.553, -0.549 | -3.013, -2.065 | -2.153, -1.253 | -2.069, -1.166 | -2.121, -1.226 |
| | **p** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** |
| **Search speed z-score** | **N** | 1038 | | | | 1073 | | | |
| | **β** | -1.794 | -1.634 | -1.584 | -1.571 | -0.495 | -0.303 | -0.256 | -0.248 |
| | **95% CI** | -2.338, -1.251 | -2.205, -1.064 | -2.165, -1.003 | -2.154, -0.988 | -1.024, 0.035 | -0.854, 0.248 | -0.813, 0.301 | -0.807, 0.310 |
| | **p** | <0.001** | <0.001** | <0.001** | <0.001** | 0.07 | 0.28 | 0.37 | 0.38 |
| **ACE-III total z-score**[a] | **N** | 595 | 585 | 578 | 525 | 644 | 641 | 630 | 575 |
| | **β** | -2.237 | -1.539 | -1.407 | -1.634 | -2.420 | -1.258 | -1.231 | -1.349 |
| | **95% CI** | -2.835, -1.647 | -2.11, -0.979 | -1.992, -0.827 | -2.299, -1.084 | -3.161, -1.786 | -1.786, -0.746 | -1.836, -0.718 | -1.925, -0.763 |
| | **p** | * | * | * | * | * | * | * | * |
| **PACC z-score** | **N** | 256 | | | | 246 | | | |
| | **β** | -0.067 | -0.188 | -0.129 | -0.145 | -0.404 | -0.247 | -0.197 | -0.222 |
| | **95% CI** | -1.075, 0.941 | -1.128, 0.753 | -1.053, 0.794 | -1.065, 0.775 | -1.324, 0.516 | -1.084, 0.591 | -1.039, 0.645 | -1.063, 0.619 |
| | **p** | 0.90 | 0.69 | 0.78 | 0.76 | 0.39 | 0.56 | 0.65 | 0.60 |
| **TBV** | **N** | 239 | | | | 229 | | | |
| | **β** | -75.815 | -75.352 | -75.612 | -76.941 | -59.737 | -59.869 | -58.843 | -58.362 |
| | **95% CI** | -138.436, -13.194 | -138.156, -12.548 | -138.658, -12.565 | -140.010, -13.872 | -118.964, -0.511 | -119.376, -0.362 | -118.324, 0.638 | -118.120, 1.397 |
| | **p** | 0.02* | 0.02* | 0.02* | 0.02* | 0.05 | 0.05 | 0.05 | 0.06 |
| **Hippocampal volume** | **N** | 239 | | | | 229 | | | |
| | **β** | -0.686 | -0.699 | -0.701 | -0.704 | -0.292 | -0.287 | -0.282 | -0.292 |
| | **95% CI** | -1.132, -0.241 | -1.147, -0.251 | -1.150, -0.251 | -1.154, -0.255 | -0.656, 0.072 | -0.652, 0.078 | -0.646, 0.083 | -0.658, 0.074 |
| | **p** | <0.001** | <0.001** | <0.001** | <0.001** | 0.12 | 0.12 | 0.13 | 0.12 |
| **WMHV** | **N** | 234 | | | | 221 | | | |
| | **β** | 1.226 | 1.121 | 1.121 | 1.138 | 1.929 | 1.929 | 1.890 | 1.891 |
| | **95% CI** | 0.261, 5.750 | 0.239. 5.255 | 0.238, 5.275 | 0.245, 5.287 | 0.471, 7.910 | 0.468, 7.954 | 0.451, 7.915 | 0.450, 7.951 |
| | **p** | 0.80 | 0.89 | 0.89 | 0.87 | 0.36 | 0.36 | 0.38 | 0.38 |
| **SUVR** | **N** | 235 | | | | 227 | | | |
| | **β** | -0.083 | -0.086 | -0.089 | -0.091 | 0.001 | -0.001 | -0.001 | 0.014 |
| | **95% CI** | -0.194, 0.028 | -0.197, 0.025 | -0.201, 0.023 | -0.200, 0.018 | -0.094, 0.096 | -0.095, 0.094 | -0.096, 0.094 | -0.075, 0.103 |
| | **p** | 0.14 | 0.13 | 0.12 | 0.10 | 0.98 | 0.99 | 0.99 | 0.75 |
| **Aβ status**[b] | **N** | 235 | | | | 227 | | | |
| | **OR** | 0.261 | 0.246 | 0.241 | 0.180 | 3.072 | 3.091 | 3.011 | 6.547 |
| | **95% CI** | 0.007, 9.780 | 0.006, 9.318 | 0.006, 9.307 | 0.003, 9.485 | 0.101, 93.451 | 0.100, 95.122 | 0.097, 93.235 | 0.151, 284.699 |
| | **p** | 0.47 | 0.45 | 0.44 | 0.40 | 0.52 | 0.52 | 0.53 | 0.33 |

[a]*Bootstrapping applied: confidence intervals are bias-corrected, p values are not applicable, models are not imputed.*

[b]*Logistic regression, coefficient presented is an OR for being amyloid positive compared with amyloid negative.*

**Evidence of association (bias-corrected confidence intervals do not cross zero); ** p<.01*

*M0: unadjusted (PACC adjusted for age at cognitive testing; neuroimaging outcomes adjusted for age at scan and TIV[TBV, hippocampal volume, WMHV only]); M1: M0+childhood cognition; M2: M1+childhood SEP; M3: M2+APOE-ε4 status*

***Table 16.*** *Interactive effects of cumulative risks score with APOE-ε4 status on cognitive performance and neuroimaging outcome measures, in males and females, in minimally adjusted models.*

| Outcome | | Males | Females |
|---|---|---|---|
| **Verbal memory z-score** | **N** | 654 | 686 |
| | **β** | 0.314 | 1.031 |
| | **95% CI** | -1.012, 1.640 | -0.257, 2.319 |
| | **p** | 0.64 | 0.12 |
| **Search speed z-score** | **N** | 669 | 693 |
| | **β** | 0.257 | -0.036 |
| | **95% CI** | -1.006, 1.521 | -1.356, 1.284 |
| | **p** | 0.69 | 0.96 |
| **ACE-III total**[a] | **N** | 540 | 587 |
| | **β** | 0.465 | 1.102 |
| | **95% CI** | -1.319, 1.865 | -0.458, 2.674 |
| | **p** | - | - |
| **PACC z-score** | **N** | 199 | 188 |
| | **β** | 0.064 | 0.025 |
| | **95% CI** | -2.167, 2.296 | -2.411, 2.461 |
| | **p** | 0.95 | 0.98 |
| **TBV** | **N** | 184 | 174 |
| | **β** | 41.969 | 85.751 |
| | **95% CI** | -111.015, 194.952 | -69.659, 241.162 |
| | **p** | 0.59 | 0.28 |
| **Hippocampal volume** | **N** | 184 | 174 |
| | **β** | -0.225 | -0.210 |
| | **95% CI** | -1.219, 0.769 | -1.170, 0.749 |
| | **p** | 0.66 | 0.67 |
| **WMHV** | **N** | 181 | 169 |
| | **β** | 0.470 | 2.889 |
| | **95% CI** | 0.012, 17.776 | 0.062, 134.618 |
| | **p** | 0.68 | 0.59 |
| **SUVR** | **N** | 181 | 172 |
| | **β** | -0.128 | 0.106 |
| | **95% CI** | -0.386, 0.130 | -0.143, 0.354 |
| | **p** | 0.33 | 0.40 |
| **Aβ status**[b] | **N** | 181 | 172 |
| | **OR** | 0.076 | 0.023 |
| | **95% CI** | 0.000, 423.196 | 0.000, 96.940 |
| | **p** | 0.56 | 0.38 |

*M0, minimally adjusted: PACC adjusted for age at cognitive testing; neuroimaging outcomes adjusted for age at scan and TIV (TBV, hippocampal volume, WMHV only); models assessing APOE interactions for other outcomes are unadjusted.*

[a]*Bootstrapping applied: confidence intervals are bias-corrected, p values are not applicable, models are not imputed.*

[b]*Logistic regression, coefficient presented is an OR for being amyloid positive compared with amyloid negative.*

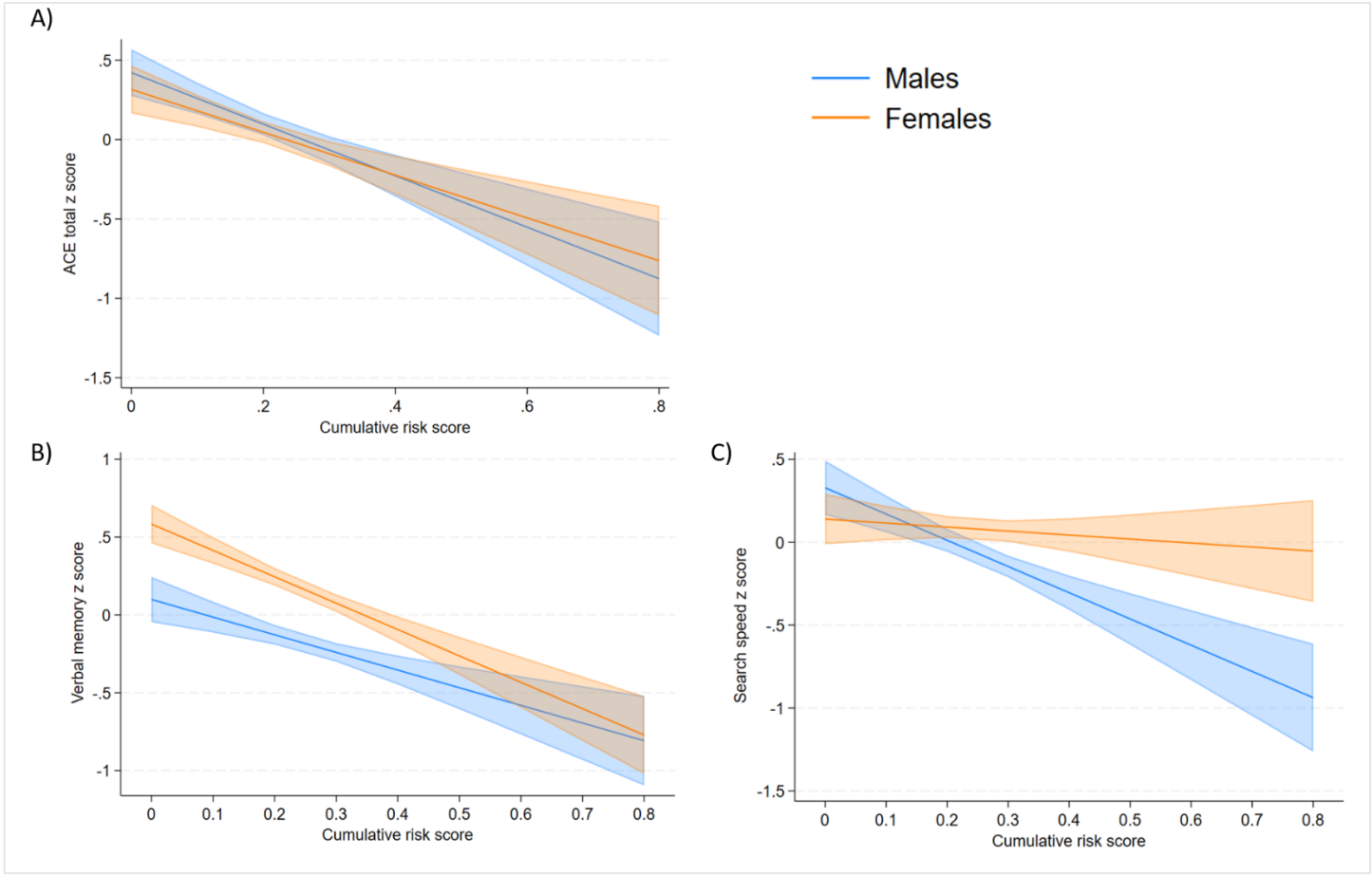


***Figure 12.*** *Predicted associations of cumulative risks score with standardised cognitive test performance at age 69 in males and females, in models fully adjusting for confounders (childhood cognition, childhood SEP, APOE-ε4 status). A) ACE-III: male n=525, female n=575. Bootstrapping is applied, confidence intervals are bias-corrected, and the model is not imputed. B) Word list recall: male n=1011, female n=1063. C) Timed letter search: male n=1038, female n=1073.*

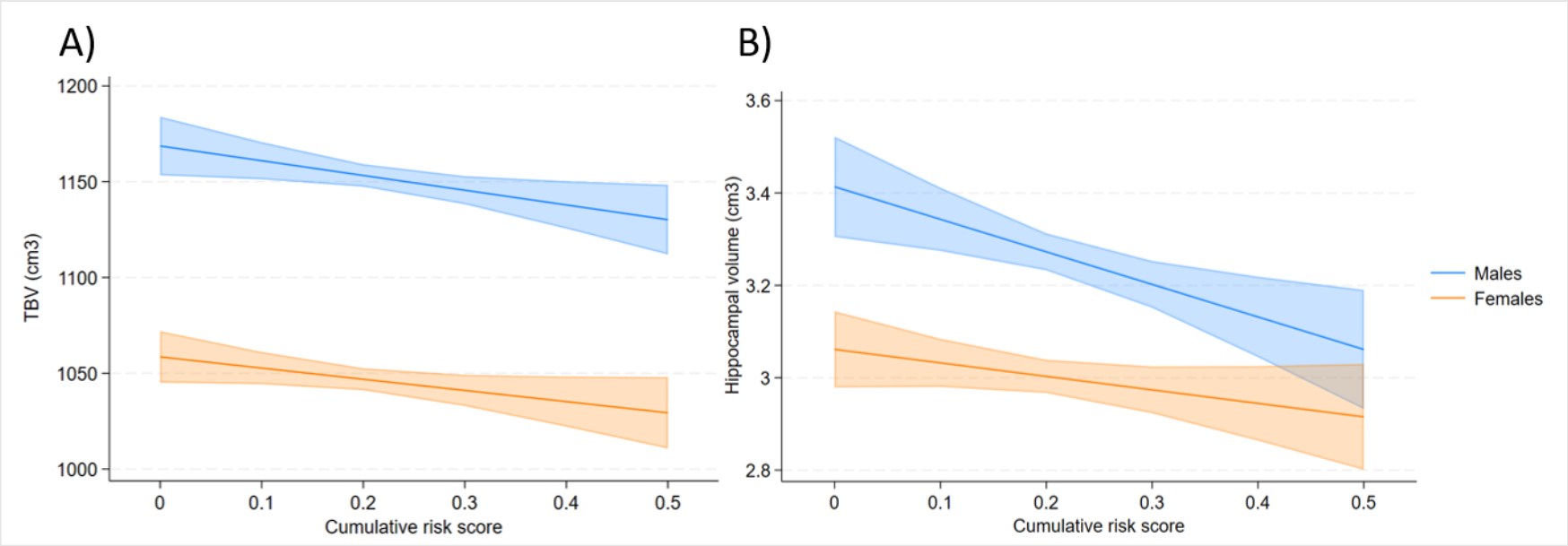


***Figure 13.*** *Predicted associations of cumulative risks score with A) total brain volume (TBV) and B) hippocampal volume at age ~70, in Insight 46 males (n=239) and females (n=229), adjusted for age at scan, total intracranial volume (TIV), childhood cognition, childhood socioeconomic position (SEP), and APOE-ε4 status.*

### 4.3.5. Use of risk-modifying treatments

When additionally accounting for ever use of risk-modifying treatments (hearing aid use, anti-hypertensive medication, anti-depressant medication, diabetes medications), all associations previously detected with adjustment for early-life predictors of lifetime CRS were maintained (Table 17).

When accounting for all risk-modifying treatments, the association of CRS with verbal memory was attenuated by 4% in males and 10% in females, compared with models fully adjusted for early-life factors. This was primarily driven by an attenuating effect of anti-depressant medication use.

In males, the significant association of CRS with search speed performance was attenuated by 2% when accounting for all risk-modifying treatments, but adjustment for hearing aid use increased effect estimates by 6%.

The association of CRS with ACE-III performance was attenuated by 3% in males and 10% in females when accounting for all risk-modifying treatments, primarily driven by an attenuating effect of anti-depressant medication use.

Accounting for all risk-modifying treatments strengthened the significant associations of CRS with TBV and hippocampal volume in males by 6% and 27%, respectively. For TBV this was primarily driven by a strengthening effect of anti-depressant medication, while hearing aid use primarily explained the strengthening effect for hippocampal volume.

***Table 17.*** *Model estimates and 95% confidence intervals showing the estimated association of cumulative risks score with standardised cognitive performance measures at age 69 and volumetric neuroimaging measures aged ~70 in males and females, with full adjustments for confounding variables and additional adjustments for the use of risk-modifying treatments.*

| | | Males | | | | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | | **M3** | **1** | **2** | **3** | **4** | **5** | **M3** | **1** | **2** | **3** | **4** | **5** |
| **Verbal memory z-score** | **N** | 1011 | | | | | | 1063 | | | | | |
| | **β** | -1.051 | -1.065 | -1.055 | -1.000 | -1.035 | -1.008 | -1.674 | -1.706 | -1.621 | -1.562 | -1.607 | -1.514 |
| | **95% CI** | -1.553, -0.549 | -1.576, -0.555 | -1.561, -0.549 | -1.527, -0.474 | -1.537, -0.532 | -1.545, -0.471 | -2.121, -1.226 | -2.159, -1.253 | -2.070, -1.172 | -2.019, -1.105 | -2.054, -1.160 | -1.978, -1.051 |
| | **p** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** |
| **Search speed z-score** | **N** | 1038 | | | | | | 1073 | | | | | |
| | **β** | -1.571 | -1.673 | -1.539 | -1.492 | -1.542 | -1.542 | -0.248 | -0.205 | -0.200 | -0.225 | -0.217 | -0.126 |
| | **95% CI** | -2.154, -0.988 | -2.261, -1.084 | -2.125, -0.953 | -2.102, -0.881 | -2.125, -0.959 | -2.159, -0.926 | -0.807, 0.310 | -0.771, 0.362 | -0.760, 0.361 | -0.797, 0.347 | -0.780, 0.346 | -0.711, 0.460 |
| | **p** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | 0.38 | 0.48 | 0.48 | 0.44 | 0.45 | 0.67 |
| **ACE-III total z-score[a]** | **N** | 525 | | | | | | 575 | | | | | |
| | **β** | -1.634 | -1.658 | -1.644 | -1.553 | -1.631 | -1.588 | -1.349 | -1.345 | -1.317 | -1.287 | -1.296 | -1.208 |
| | **95% CI** | -2.299, -1.084 | -2.378, -1.087 | -2.311, -1.074 | -2.255, -0.997 | -2.302, -1.093 | -2.313, -0.988 | -1.925, -0.763 | -1.895, -0.746 | -1.874, -0.730 | -1.854, -0.694 | -1.874, -0.698 | -1.762, -0.604 |
| | **p** | * | * | * | * | * | * | * | * | * | * | * | * |
| **TBV** | **N** | 239 | | | | | | 229 | | | | | |
| | **β** | -76.941 | -78.214 | -74.951 | -79.910 | -77.496 | -81.182 | -58.362 | -58.541 | -56.243 | -60.392 | -46.831 | -48.922 |
| | **95% CI** | - 140.010, -13.872 | - 143.881, -12.546 | - 139.476, -10.425 | - 144.436, -15.384 | - 140.290, -14.702 | - 149.861, -12.502 | -118.120, 1.397 | -118.902, 1.821 | -116.434, 3.948 | -120.982, 0.197 | -104.005, 10.342 | -107.772, 9.927 |
| | **p** | 0.02* | 0.02* | 0.02* | 0.02* | 0.02* | 0.02* | 0.06 | 0.06 | 0.07 | 0.05 | 0.11 | 0.10 |
| **Hippocampal volume** | **N** | 239 | | | | | | 229 | | | | | |
| | **β** | -0.704 | -0.815 | -0.742 | -0.741 | -0.703 | -0.894 | -0.292 | -0.318 | -0.284 | -0.300 | -0.265 | -0.301 |
| | **95% CI** | -1.154, -0.255 | -1.282, -0.348 | -1.202, -0.282 | -1.199, -0.284 | -1.154, -0.252 | -1.384, -0.405 | -0.658. 0.074 | -0.691, 0.055 | -0.654, 0.085 | -0.671, 0.071 | -0.635, 0.105 | -0.685, 0.084 |
| | **p** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | 0.12 | 0.09 | 0.13 | 0.11 | 0.16 | 0.12 |

*M3: (Neuroimaging outcomes adjusted for age at scan and TIV), childhood cognition, childhood SEP, APOE-ε4 status; 1=M3+hearing aid use; 2=M3+anti-hypertensive medication use; 3=M3+anti-depressant medication use; 4=M3+diabetes medication use; 5=M3+all risk-modifying treatments.*

*[a]Bootstrapping applied: confidence intervals are bias-corrected, p values are not applicable, models are not imputed.*

**Evidence of association (bias-corrected confidence intervals do not cross zero); ** p<.01*

### 4.3.6. Secondary analyses

#### *4.3.6.1. Cumulative risk exposures to midlife*

Midlife CRS was calculated for 1,604 (52% female) participants who had available data at both the early- (up to 36 years) and mid-life (ages 43 and 53) timepoints, for all risk factors. In the whole cohort, males (n=774) had a mean midlife CRS of 0.23 (SD 0.13, range 0-0.71), which did not significantly differ from that of females (n=830, mean 0.22, SD 0.13, range 0-0.69; p=0.105; Appendix B Figure 1). T-tests showed that mean scores per risk factor in the whole-cohort (Appendix B Figure 2) were higher in males than females for hearing impairment, TBI, hypertension, high alcohol consumption and smoking, while females had higher scores than males for obesity, depression, and physical inactivity.

Summary statistics showing mean total midlife CRS, mean scores per risk factor, and t-tests of sex differences in the Insight 46 sample are shown in Appendix B Table 1. Midlife CRS was calculated for 420 (49% female) Insight 46 participants. Males (n=215) had a mean midlife CRS of 0.19 (SD 0.10, range 0-0.48) which did not significantly differ from that of females (n=205, mean 0.17, SD 0.11, range 0-0.54; p=0.07). T-tests demonstrated that mean scores per risk were higher in males than females for TBI, hypertension and high alcohol consumption, while scores were higher in females for depression.

Estimated associations of midlife CRS with cognitive performance outcomes at age 69 and Insight 46 volumetric neuroimaging outcomes are presented in Table 18. As in the main analyses, independently of early-life predictors of lifetime CRS, increased midlife CRS associated with poorer verbal memory and ACE-III performance in males and females, poorer search speed performance in males only, and reduced hippocampal volume in males but not females. The negative association of lifetime CRS with TBV in males was replicated with midlife CRS, and a significant negative association of midlife CRS with TBV was also detected in females.

***Table 18.*** *Effect of midlife cumulative risks score on cognitive performance outcomes at age 69, and volumetric neuroimaging outcomes at age ~70 in males and females, with full model adjustments.*

| Outcome | | Males | Females |
|---|---|---|---|
| **Verbal memory z-score** | **N** | 1011 | 1063 |
| | **β** | -0.927 | -1.763 |
| | **95% CI** | -1.412, -0.441 | -2.227, -1.299 |
| | **p** | <0.001** | <0.001** |
| **Search speed z-score** | **N** | 1038 | 1073 |
| | **β** | -1.145 | -0.358 |
| | **95% CI** | -1.715, -0.576 | -0.920, 0.205 |
| | **p** | <0.001** | 0.21 |
| **ACE-III total[a]** | **N** | 572 | 625 |
| | **β** | -1.456 | -1.259 |
| | **95% CI** | -1.996, -0.881 | -1.762, -0.621 |
| | **p** | * | * |
| **TBV** | **N** | 239 | 229 |
| | **β** | -71.163 | -65.050 |
| | **95% CI** | -130.417, -11.909 | -122.135, -7.966 |
| | **p** | 0.02* | 0.03* |
| **Hippocampal volume** | **N** | 239 | 229 |
| | **β** | -0.571 | -0.110 |
| | **95% CI** | -0.991, -0.152 | -0.471, 0.252 |
| | **p** | 0.01* | 0.55 |

*<0.05 ** p<.01

[a]*Bootstrapping applied: confidence intervals are bias-corrected, p values are not applicable, models are not imputed.*

*Models are fully adjusted for: childhood cognition, childhood SEP, APOE-ε4 status [TBV and hippocampal volume also adjusted for age at scan and TIV]*

#### *4.3.6.2. Non-weighted lifetime cumulative risks score*

The sex-stratified distributions of non-weighted risk scores in the whole-cohort and Insight 46 samples are shown in Appendix B Figures 3 and 4. In the whole cohort, males (n=740) had a mean count of 9.11 (SD 3.72, range 0-23) risk factors across early-, mid- and later-life while females (n=769) had a mean count of 8.35 (SD 3.59, range 1-20). This translated to non-weighted risk scores of 0.25 (SD 0.10, range 0-0.64) in males and 0.23 (SD 0.10, range 0.03-0.56) in females, where the sex difference was significant (t-test p=0.001). A significant sex difference was also found in Insight 46 (p<0.001) where males had higher mean scores (0.24, SD 0.09, range 0.06-0.56, n=201) than females (0.19, SD 0.09, range 0.03-0.44, n=188).

The fully adjusted estimated effects of non-weighted lifetime CRS on each cognitive and neuroimaging outcome are presented in Table 19. As in the main analyses, increased risk exposures associated with poorer verbal memory and ACE-III performance in males and females, and with poorer search speed performance in males only, while there were no

associations of CRS with PACC performance in either sex. Similarly to the main analyses, a negative association of CRS with hippocampal volume was found in males only. The negative association of weighted CRS with TBV in males was replicated, and a significant negative association of non-weighted CRS on TBV in females was also detected. No associations of non-weighted CRS with WMHV were found, but in contrast to the main analyses a significant association of non-weighted CRS with amyloid SUVR was detected in males; increased risk exposures associated with reduced Aβ levels, although not sufficiently for an effect to be seen on the likelihood of having a positive Aβ load.

***Table 19.*** *Model estimates and 95% confidence intervals for the association of non-weighted lifetime cumulative risks score with standardised cognitive performance outcomes and neuroimaging outcomes, fully adjusted for early-life confounding variables.*

| Outcome | Values | Males | Females |
|---|---|---|---|
| **Verbal memory z-score** | **N** | 1011 | 1063 |
| | **β** | -0.827 | -1.923 |
| | **95% CI** | -1.432, -0.221 | -2.481, -1.366 |
| | **p** | 0.01* | <0.001** |
| **Search speed z-score** | **N** | 1038 | 1073 |
| | **β** | -2.085 | -0.585 |
| | **95% CI** | -2.772, -1.398 | -1.272, 0.102 |
| | **p** | <0.001** | 0.10 |
| **ACE-III total z-score**[a] | **N** | 525 | 575 |
| | **β** | -1.276 | -1.700 |
| | **95% CI** | -2.034, -0.569 | -2.404, -0.991 |
| | **p** | * | * |
| **PACC total z-score** | **N** | 256 | 246 |
| | **β** | 0.090 | -0.388 |
| | **95% CI** | -0.899, 1.080 | -1.344, 0.568 |
| | **p** | 0.86 | 0.42 |
| **TBV** | **N** | 239 | 229 |
| | **β** | -100.408 | -90.950 |
| | **95% CI** | -168.156, -32.659 | -158.224, -23.675 |
| | **p** | <0.001** | 0.01* |
| **Hippocampal volume** | **N** | 239 | 229 |
| | **β** | -0.585 | -0.273 |
| | **95% CI** | -1.068, -0.102 | -0.694, 0.147 |
| | **p** | 0.02* | 0.20 |
| **WMHV** | **N** | 234 | 221 |
| | **β** | 2.934 | 2.306 |
| | **95% CI** | 0.512, 16.804 | 0.440. 12.095 |
| | **p** | 0.23 | 0.32 |
| **SUVR** | **N** | 235 | 227 |
| | **β** | -0.137 | 2.845 |
| | **95% CI** | -0.253, -0.022 | 0.040, 202.861 |
| | **p** | 0.02* | 0.64 |
| **Aβ status**[b] | **N** | 235 | 227 |
| | **β** | 0.096 | 2.845 |
| | **OR** | 0.001, 7.047 | 0.040, 202.861 |
| | **p** | 0.28 | 0.63 |

[a]*Bootstrapping applied: confidence intervals are bias-corrected, p values are not applicable, models are not imputed.*

[b]*Logistic regression, coefficient presented is an OR for being amyloid positive compared with amyloid negative.*

**p<0.05 or bias-corrected confidence intervals do not cross zero; ** p<.01*

*M0: unadjusted (PACC adjusted for age at cognitive testing; neuroimaging outcomes adjusted for age at scan and TIV[TBV, hippocampal volume, WMHV only]); M1: M0+childhood cognition; M2: M1+childhood SEP; M3: M2+APOE-ε4 status*

## 4.4. Discussion

### 4.4.1. Key findings

A lifetime cumulative dementia risks score (CRS) was derived from life course data, quantifying exposures to modifiable dementia risk factors up to age 69. Sex differences in the frequency of some individual risk factors were found and, although males in the neuroimaging sub-study had greater CRS than females, there were no sex differences in CRS in the whole-cohort sample.

Early-life (childhood cognitive performance, childhood SEP) and genetic (*APOE*-ε4 status) factors were predictive of CRS in the whole-cohort sample. Independently of these predictors, males and females showed adverse associations of increased risk exposures with poorer cognitive performance at age 69, with equal effects on cognitive state (ACE-III) but stronger associations with verbal memory in females and processing speed in males. Greater cumulative risk exposures associated with reduced brain volumes at age ~70, particularly in males, but there were no associations with AD (Aβ) or cSVD (WMHV) pathology measures in either sex. There was no evidence that associations between CRS and outcomes were modified by *APOE*-ε4 status. Use of risk-modifying treatments did not have a substantial impact on the estimated effects of cumulative risk exposures on later-life outcomes.

Associations of lifetime cumulative risk exposures with later-life cognitive performance and brain volumes were replicated when risk exposures were measured only up until midlife, and when lifetime CRS was not weighted by PAFs.

### 4.4.2. Interpretation of findings

While males had a significantly higher count of lifetime risk exposures than females, the lack of sex difference in weighted lifetime CRS in the whole cohort sample reflects sex differences across individual risk factors assigned different weightings. That non-weighted lifetime CRS was significantly greater in males than females indicates that some of the risk-factor weightings could be sex-biased. Demonstrating sex differential patterns of risk, greater frequency of hearing impairment, TBI, hypertension and high alcohol consumption among males than females, and greater depression and physical inactivity in females, aligned with previous reports of sex differences in these risk factors.[120,293-297] However, significantly greater

weighted CRS in Insight 46 males than females seemed to be driven by greater levels of physical activity among Insight 46 females than in the whole cohort, in agreement with previous analyses demonstrating that greater physical activity predicted agreement to take part in the Insight 46 sub-study.[194]

Indeed, better general health and socioeconomic circumstances within Insight 46[194] likely explains reduced risk exposures in Insight 46 than in the whole cohort. Minimal variation in childhood cognitive performance and SEP, which are higher in Insight 46,[194] might also explain the lack of associations between early-life factors and lifetime CRS among Insight 46 participants. In the whole cohort, higher childhood cognition and SEP predicted lower CRS, reflecting how such early-life advantages can contribute to maintained health and social advantages throughout life,[205,298] in agreement with the accumulative model of life course epidemiology (Section 1.1.). While greater cognitive performance in childhood could have downstream effects on socioeconomic circumstances throughout life (e.g. improved education and career opportunities), greater early cognitive performance could also be indicative of developmental advantages prenatally or during infancy (e.g. diet in infancy), which may have lasting impacts on susceptibility to some health-related dementia risk factors such as obesity or hypertension, for example. However, puberty timing was not found to be predictive of CRS, implying that early-life sex hormone exposure did not influence the occurrence of modifiable risk factors throughout life; social advantage may be more predictive of lifetime dementia risk factor exposures than biological development.

Interestingly though, higher cumulative risk exposures were found in whole-cohort *APOE*-ε4 non-carriers than carriers, emphasising the strength of the *APOE*-ε4 association with dementia; while ε4 carriers might have fewer lifetime exposures to modifiable dementia risks, dementia rates are higher among carriers.[288] Nonetheless, associations between CRS and outcome measures were not modified by *APOE*-ε4 status, indicating that adverse associations of greater risk exposures with cognitive performance and brain health at age ~70 were not exacerbated in ε4 carriers. Conversely, the CAIDE population-based study found that a composite of midlife (mean age 50.6(6.0), range 39-64) lifestyle risks (physical inactivity, alcohol consumption, smoking, high dietary fat) associated with increased dementia risk ~21 years later (mean age 71.6(4.1), range 65-79) to a greater extent in ε4 carriers than non-carriers.[299] The extent to which ε4 status modifies the associations of lifestyle risks with later-

life dementia-related outcomes could depend on the risks examined, when these risks occur, and the age at which outcomes are assessed. In the current analyses, participants were younger than in the CAIDE study; it is possible that ε4 modifying effects are not detected in a preclinical cohort. Reasons for why NSHD ε4 carriers had fewer risk exposures than non-carriers are unclear. Pleiotropic effects of ε4 whereby some ε4 benefits are recognised at younger ages (e.g. better cognitive performance in young carriers than non-carriers)[262] could provide some explanation. There may also be a survivor bias in which only the healthiest ε4 carriers are retained in the cohort at age ~70, meaning that ε4 carriers with high risk factor exposures are not observed in the data collected at older ages. However, *APOE* genotype was not associated with cumulative risk exposures in the Insight 46 sub-sample, and lifestyle risks in the CAIDE study did not significantly differ between ε4 carriers and non-carriers. Additionally, in the population-based Rotterdam study, no *APOE* group differences (low-[ ε2/ε3, ε2/ε2], mid-[ ε3/ε3], high-dementia-risk[ε4/ε4, ε4/ε2, ε4/ε3]) were found in a count of protective health and lifestyle factors (smoking abstinence, no depression, no diabetes, regular physical activity, avoiding social isolation, adhering to a healthy diet including limited alcohol consumption), although BMI was lower and cholesterol levels were higher among ε4 carriers.[300] To understand the association of *APOE* genotype with lifetime cumulative risk exposures, further work is required to determine whether the association is robust across varied cohorts.

Although early-life predictors of CRS were found, adverse associations of greater risk exposures with later-life cognitive performance and brain volumes remained independently of these early-life predictors, reinforcing the value of interventions in adulthood to limit the adverse effects of risk exposures on later-life outcomes. Accounting for the use of risk-modifying treatments did not, however, substantially alter the significant associations of CRS with later-life outcomes, implying that treatments to control existing risk factors do not effectively mitigate against the adverse effects of multiple risk exposures. Nonetheless, anti-depressant use slightly attenuated the effects of CRS on memory performance and cognitive state, in agreement with previous findings of cognitive performance improvements with anti-depressant use.[301] Conversely, hearing aid use slightly strengthened the negative effects of CRS on processing speed performance and hippocampal volume. Previous evidence has found benefits of hearing aid use, including increased social interaction,[302,303] but adherence to

hearing aid use is generally poor [304] which potentially contributed to the negative confounding observed. While further work is needed to elucidate potential mediating effects of risk factor treatments, it is possible that interventions to prevent the occurrence of risk factors could be beneficial for later-life outcomes. Indeed, multidomain lifestyle intervention trials have shown promise in reducing risk factor incidence and improving cognitive performance. For example, the 2-year FINGER trial of combined dietary, exercise, cognitive training and vascular risk monitoring interventions showed improved cognitive performance on a neuropsychological test battery assessing executive function, processing speed and memory.[104,105]

Associations of increased cumulative modifiable risk exposures with reduced cognitive state at age 69 could reflect diminished cognitive resilience (Section 1.6.) to ageing, resulting from reduced opportunities for the development and maintenance of cognitive reserve throughout the life course. However, equal effects of CRS on cognitive state between sexes while there were sex variations in CRS effects on individual cognitive domains demonstrates that, although composite outcomes may not differ by sex, pathways to these outcomes may be different; the adverse effect of CRS on cognitive state could be driven by effects on memory in females and processing speed in males. As shown in Chapter 3, both these domains show female advantages throughout adulthood, with a larger female advantage in verbal memory performance. Although there was a slightly greater adverse effect of CRS on verbal memory performance in females than males, possibly representing greater CRS-associated reductions in female verbal memory cognitive resources given a higher starting point, this sex difference was not statistically significant. Conversely, the adverse effect of CRS on processing speed performance was only detected in males, demonstrating a male-specific adverse effect of greater lifetime risk exposures on a cognitive domain shown to be particularly vulnerable to the effects of ageing.[17] Previous NSHD analyses have, however, found adverse effects of increased BMI (at age 60-64, and between ages 36 and 43) on processing speed performance at age 60-64 only in females;[206] an individual risk factor association could be masked when measuring cumulative risk exposures, but there may also be differences in how risk factors associate with outcomes measured at different ages. Interestingly, longitudinal analyses of verbal memory and processing speed performance within NSHD showed faster age-related decline in processing speed performance in males than females, while there were no sex differences in rates of verbal memory decline between ages 43 and 69.[5] Taken together,

evidence suggests that increased exposure to modifiable dementia risk factors could contribute to accelerated cognitive ageing observed in males, although further work is required to determine if CRS associates with longitudinal cognitive trajectories.

Within the Insight 46 sub-sample, CRS was not found to associate with performance on a cognitive composite designed to detect subtle pre-clinical cognitive changes indicative of AD (PACC), possibly reflecting a lack of association for cumulative risk exposures with AD-specific cognitive changes. However, only PACC total was examined in these analyses; it remains possible that CRS associates with subtle cognitive changes assessed in the PACC sub-tests (Table 2), particularly given sex-specific findings for verbal memory and processing speed in the whole-cohort. Additionally, given better general health, socioeconomic circumstances, and cognitive performance within the Insight 46 cohort,[194] it is possible that Insight 46 participants had greater cognitive resilience than the whole cohort, buffering against the adverse effects of greater lifetime risk exposures. Indeed, a sensitivity analysis to examine the associations of CRS with ACE-III performance in the Insight 46 sub-sample (male n=163, female n=155; Appendix B Table 2) did not detect an association in males, and the significant effect in females was weaker than that found in the whole cohort.

However, it is also notable that CRS was not associated with any of the neuroimaging measures of specific dementia-related pathology (Aβ, WMHV) examined, further indicating that the estimated effects of lifetime risk exposures are not specific to indicators of possible dementia at age ~70. The lack of association between CRS, which includes several cardiovascular risks, and WMHV (a marker of cSVD) was surprising; the effects of cardiovascular risks on WMHV were possibly diluted by the inclusion of other non-cardiovascular risks which are not independently associated with WHMV. There was evidence for CRS associations with reduced brain volumes (stronger in males) which, alongside the male-specific association with processing speed, suggests that greater lifetime risk factor exposures exacerbate non-dementia-specific cognitive and brain ageing, particularly in males. Interestingly, males, but not females, showed an association of CRS with reduced hippocampal volume which remained independently of total brain volume, indicating that CRS-associated volume reductions are not evenly distributed throughout the male brain. Given a slightly stronger effect of CRS on verbal memory performance in females, it is surprising that CRS was not associated with female hippocampal volume, as a brain region important for memory

function.[305] However, a sensitivity analysis did not detect associations of CRS with verbal memory for either sex in the Insight 46 sub-sample (Appendix B Table 3), possibly reflecting improved cognitive resilience to hippocampal atrophy within the Insight 46 cohort. As the cohort ages, continued follow-up assessments[306] will be valuable in identifying whether and how cognitive resilience changes over time.

The potential effects of CRS on brain ageing to a greater extent in males than females is consistent with previous Insight 46 work which found older predicted brain age differences (PAD; the difference between chronological age and predicted brain age derived from T1-weighted MRI measures of grey matter, white matter, CSF and intracranial volume) in males.[236] In analyses adjusted for sex, greater PAD associated with increased WMHV and cardiovascular risk (measured using the Framingham risk score) at ages 36 and 69,[236] indicating a link between cerebrovascular disease and accelerated brain ageing in the Insight 46 cohort; future research examining CRS-PAD associations could inform whether lifetime risks, including but not exclusive to cardiovascular risk, differentially associate with brain ageing in males and females.

While the primary focus of the current analyses was on cumulative modifiable risk exposures throughout the life course, midlife is a proposed period of vulnerability,[8,307] and many of the Lancet risks (e.g. TBI, hypertension, obesity) are attributed to midlife.[56] Indeed, associations of lifetime CRS with later-life cognitive and volumetric neuroimaging outcomes were replicated when only modifiable risk exposures accumulated up to midlife were quantified, demonstrating that the adverse effects of risk factor exposures on later-life outcomes were already present by midlife. While this does not prove that midlife is a sensitive period, the finding does add to research demonstrating that risks up to and including midlife more strongly associate with brain health measures than risks in later-life. Cardiovascular risk measured in early adulthood (age 36) has been particularly associated with later-life neuroimaging outcomes (older PAD, reduced TBV, increased WMHV) in Insight 46, while cardiovascular risk at ages 53 and 69 showed progressively weaker associations with such outcomes.[236,308] Further work is needed to investigate whether risk exposures within each of the three timepoints included in the CRS (early-, mid-, later-life) differentially associate with later-life outcomes. This is also important given that the directionality of some health and lifestyle factors associated with dementia risk can reverse in later-life; for example,

hypotension and weight loss (lower BMI) at older ages could be part of the dementia prodrome.[309,310]

Associations of lifetime CRS with later-life cognitive and volumetric neuroimaging outcomes were also replicated when weightings per risk factor were removed, in agreement with previous studies which did not find differences in weighted and non-weighted Lancet risk exposure associations with cognitive performance.[111,273] An additional association detected between non-weighted CRS and cerebral amyloid levels in males, that was not found for weighted CRS, was in the unexpected direction, whereby greater risk exposure associated with lower levels of cerebral amyloid. Reasons for this are unclear, requiring independent validation.

### 4.4.3. Strengths and limitations

The strength of these analyses lies in the wealth of prospective life course data facilitating the quantification of lifetime exposures to each of the twelve modifiable dementia risks identified in the Lancet commission, where other studies have been cross-sectional and have not had available data for all twelve risks.[111,311] Importantly, the availability of prospective early-life data also enabled analyses accounting for early-life predictors of cumulative risk exposures, demonstrating that although early-life factors are important, there are opportunities throughout life to mitigate the adverse effects of cumulative risks on later-life outcomes.

While the inclusion of variables relating to lifetime use of risk-modifying treatments was informative, these variables were not independent from cumulative risk score. For example, diabetes medication use is indicative of having diabetes, and even if diabetes is controlled with medication, the diabetes diagnosis remains. Additionally, treatment use could reflect greater risk factor severity; anti-depressant medications are more likely to be prescribed for more severe depression,[312] for example. Adjustments for risk-modifying treatments were intended to inform whether the use of such treatments mitigated CRS-outcome associations, but such adjustments can be problematic. As described in Section 3.4.3., adjusting for a mediator can induce collider bias, particularly if there is unmeasured confounding. Further, Tobin et al. (2005)[313] demonstrated that, when examining determinants of blood pressure, adjusting for anti-hypertensive use as a covariate induced estimation bias whereby effect sizes

were reduced; individuals with higher underlying blood pressure (an unobserved measure) are more likely to be those taking anti-hypertensive medications which reduce observed blood pressure. In individuals who receive risk-modifying treatment, there are two components to the observed risk factor: i) the extent to which treatment reduces the risk factor, negatively associated with the risk (e.g. anti-hypertensive use reduces blood pressure), and ii) the underlying presence of the risk as a prerequisite for treatment, positively associated with the risk (e.g. high blood pressure increases likelihood of taking anti-hypertensive medication). Adjusting for treatment can therefore have minimal impact on effect estimates, or there may even be counterintuitive results. For example, if underlying blood pressure (ii) is higher than the blood pressure-lowering effect of anti-hypertensive medication (i), the overall association of treatment with blood pressure would be positive, implying that anti-hypertensive use increases blood pressure. One solution suggested by Tobin et al. is to apply a sensible constant to the observed risk factor measurement in treated individuals, based on prior knowledge of the treatment effect size (e.g. anti-hypertensives reduce blood pressure by 15mmHg). An avenue for future research addressing treatment effects could be to incorporate treatment effect weightings into the CRS, instead of adjusting for treatments as covariates.

Further work is also warranted to include more detailed investigation of medication types, adherence, severity of the risk requiring treatment, and the potential impact of treatment on other risk factors. The current analyses of risk-modifying treatments take a broad approach whereby multiple risk factors are combined in the CRS and adjustments are made for treatments which modify only some of these risk factors. This inadvertently leads to some illogical research questions, asking 'does the effect of depression on cognition go through anti-hypertensive use?', for example. In further work, risk-modifying treatment effects should be examined for individual risk factors.

While the cumulative risks score was derived to maximise the use of available life course data pertaining to each individual risk factor, some limitations in the categorisation of individual risks must be acknowledged. For instance, with no consistent measure of social isolation established,[56] proxies for social isolation were derived from structural social health measures[314] (marital and cohabitation status, social network size, social contact frequency); isolation indicated by any one of these measures was taken to represent social isolation during the relevant time-period. It is possible that, if someone is unmarried and living alone, they

might have a large social network outside of the home and are therefore not socially isolated, although evidence has shown that married people tend to have more social contact than single people, particularly among older generations.[56] Additionally, smoking was counted as a risk if participants indicated current smoking during each timepoint, but this categorisation does not indicate how heavily someone smoked, which has previously been negatively associated with cognitive performance.[248] There is, however, still value in quantifying the number of timepoints throughout life that individuals were smokers, and a level of smoking heaviness associated with dementia risk is not specified in the Lancet commission.[56]

It could be argued that collapsing dementia risk data into a single measure of cumulative risk exposures is disadvantageous given that the fine-grained detail of individual risks and their associations with later-life outcomes are diluted. However, there is value in quantifying multiple risk exposures throughout life given that, in reality, risk factors are interrelated. For instance, physical inactivity increases the risk of obesity, which is also associated with hypertension and diabetes.[315]

The Lancet commission acknowledges the interrelationships between risk factors and recognises that the contribution of each factor may not be equal.[56] While the risk-specific PAFs calculated in the Lancet commission were used to produce the weighted lifetime CRS in these analyses, these PAFs have not been validated and a particular limitation for these analyses is that PAFs were not sex-specific. Nonetheless, results from the secondary analyses of non-weighted CRS did not substantially differ from weighted CRS findings in males or females.

Another limitation is that, while the Lancet risks were identified as those with the most convincing evidence during an extensive review of dementia risks literature, this body of evidence is likely to be male-centric given a general lack of research explicitly testing for sex differences and that females have been traditionally underrepresented in research. Additionally, these twelve risks are unlikely to be exhaustive (see Section 1.10.2.). In fact, with the emergence of new evidence between the 2017[316] and 2020 Lancet reviews, three risks were added (heavy alcohol consumption, high air pollution exposure, TBI).[56] Even so, comparison of dementia risk scores within the Dunedin, New Zealand, population cohort did not find substantially improved predictions of midlife brain health (predicted brain age, WMHV, hippocampal volume) or objective or subjective cognitive decline when an extensive

list of 48 risk factors were measured,[271] indicating that measures of fewer, more selective dementia risk factors can still be informative.

Finally, there may be reverse directionality for some risk factors whereby there is uncertainty whether a factor contributes to dementia risk or if it forms part of the dementia prodrome. For example, depression in earlier-life is considered a risk for later dementia while depression in later-life could be a non-cognitive dementia feature.[292] However, the results from secondary analyses, which reduced the potential effects of bidirectionality by measuring risk factors only up until midlife, did not substantially differ from the main analyses.

### 4.4.4. Summary

Independently of early-life predictors of lifetime dementia risk factor exposures, there were adverse associations of accumulated risks with later-life cognitive performance and brain volumes in both sexes, which were already established by midlife. There were nuances in which cognitive domains more strongly associated with cumulative risk exposures between the sexes, and associations with reduced brain volumes were stronger in males. These findings demonstrate evidence for greater risk factor exposures with more advanced cognitive and brain ageing not specific to dementia-related pathology, particularly in males, reflecting reduced male resilience to life course health and lifestyle risks.

## 5.0. Empirical section C: Menopause and female later-life cognition and brain health

This section aims to examine how variations in menopause, as a female-specific hormonal transition, associate with cognition and brain health in later-life, to build an understanding of menopause as a possible contributor to increased female dementia risk, and to determine how risk for adverse cognitive and brain ageing might differ between women.

The primary research question addressed in this section is:

1. Does age at menopause associate with measures of cognitive performance and brain health in later-life?

The section is split into two empirical chapters:

5i. Menopause and later-life cognition (published in a peer reviewed journal)[317]

5ii. Menopause and later-life brain health

## 5i. Menopause and later-life cognition

In this section, associations between age at menopause and cognitive performance at age ~70 were examined to determine whether relationships were stronger for certain cognitive domains. NSHD and Insight 46 women with available data for menopause age and cognitive state (NSHD, ACE-III age 69) or PACC performance (Insight 46, age 69-71) were included in multivariable linear regression analyses assessing the relationships between menopause age and cognitive performance, and the extent to which associations could be explained by relevant early cognitive, sociodemographic, reproductive, and health-related covariables. Older age at menopause associated with better performance across all cognitive outcomes, most strongly for assessments of visual processing, and associative learning and memory. These associations were not modified by menopause type (natural or surgical) or *APOE*-ε4 status, but effect estimates were attenuated by life course covariables, most notably by childhood cognition. Additionally adjusting for HT use did not have a meaningful impact on effect estimates, implying that HT use did not mediate the relationship of menopause timing with post-menopausal cognitive performance.

### 5i.1. Introduction

As described in Section 1.11., endogenous oestrogen levels gradually decline during natural menopause, while surgeries to remove the uterus or one or both of the ovaries can cause an earlier and sometimes more dramatic decline in oestrogen levels.[142] Oestrogen has pleiotropic effects influencing both the reproductive axis and higher mental function,[305] and menopause is often accompanied by neurological symptoms including cognitive difficulties, particularly with memory and attention.[144] However, an understanding of the longer-term association between menopause and cognitive function in later-life is not yet established. There is conflicting evidence around how age at menopause, or taking menopausal HT, is associated with dementia risk, cognitive impairment or later-life cognitive function.[144,154] It is important to understand the association between menopause and well characterised cognitive function in later-life, prior to overt dementia symptoms, to help develop a better understanding of female cognitive ageing.

Evidence shows increased dementia risk among female carriers of the *APOE*-ε4 risk allele for AD, compared with male ε4 carriers, especially at later, post-menopausal ages.[95,318] However, evidence linking menopause age with *APOE* genotype is mixed.[319,320] Research examining interactions of *APOE* and menopause age on dementia risk and later-life cognitive performance is also lacking.

While meta-analysis does not support an overall link between menopause age and risk for developing dementia, most studies investigating the association between menopause and later-life outcomes are unable to account for childhood cognition, a key confound given higher childhood cognition predicts both later menopause age[156,157] and better cognitive performance in later-life.[158,159] Studies also typically lack prospectively recorded data for pre-menopausal covariables such as BMI and smoking. In addition, most studies have wide age ranges at cognitive testing and short follow-up periods. The NSHD and Insight 46 cohorts provide a unique opportunity to overcome some of these issues, with prospectively recorded data obtained over 70 years of follow-up in an age-homogenous cohort.

Previous NSHD work has investigated associations between menopause age and cognitive performance. Among women who were post-menopause by age 57 years, positive associations of menopause age with NART and verbal memory, but not processing speed, performance at age 54 have been detected. Associations attenuated with adjustments for

childhood cognition, previous task performance, and additional socioeconomic factors.[321] Small positive associations between age at natural menopause and better verbal memory performance, but not processing speed, from ages 43 to 69 were also found after accounting for lifetime factors, although the effect estimates attenuated with adjustment for childhood cognition.[155] A broader examination of which cognitive domains most strongly associate with menopause age has not yet been conducted, nor have clinically relevant cognitive assessments been examined in relation to menopause age.

### *5i.1.1. Objectives and research questions*

This work expands on previous NSHD analyses, by addressing the relationship between age at menopause and performance on clinically relevant cognitive assessments completed in later-life; a test of cognitive state at age 69 (ACE-III) and a composite measure, mainly used in clinical trials (PACC),[322] completed by Insight 46 participants. Overall task performance and sub-domain performance is assessed, to examine which cognitive domains associate with menopause age.

The following research questions are addressed:

1. Does age at menopause associate with cognitive performance on the ACE-III and PACC in later-life, and which cognitive domains show the strongest associations?
2. Are associations independent of a range of relevant confounders including early cognitive and sociodemographic factors, reproductive, and health-related factors?
3. Are associations modified by menopause type (natural or surgical)?
4. Are associations modified by *APOE*-ε4 status?
5. Does HT use contribute to associations of menopause age with later-life cognitive performance?

### *5i.1.2. Hypotheses*

Given previous evidence associating later menopause age with better cognitive performance, later menopause age was expected to positively associate with cognitive performance at age ~70, most notably for assessments of verbal abilities and memory. Early cognitive, sociodemographic, reproductive, and health-related factors were expected to attenuate

associations between menopause age and later-life cognition, in agreement with previous studies testing menopause age-cognition relationships within NSHD.[155] Given previous evidence for poorer cognitive and dementia-related outcomes in women who had surgically-induced menopause at earlier compared with later ages,[151] an effect modification by menopause type was anticipated whereby women who had surgical menopause at earlier ages would show poorer cognitive performance than women who had natural menopause at earlier ages. Although associations of *APOE* genotype with menopause age are unclear, an effect modification by *APOE*-ε4 status was hypothesised, particularly for domains linked with AD (i.e. memory). Since the ε4 dementia risk is greater in females than males, menopause age-cognition associations were expected to be stronger in carriers than non-carriers. The relationship between HT use and later-life cognitive performance is not yet well understood, hence a hypothesis for whether, and in what direction, adjustments for HT use might alter effect estimates cannot be drawn.

## 5i.2. Analytic method

### *5i.2.1. Analytic sample*

Between ages 43 and 54, 1,572 female NSHD study members completed annual postal questionnaires for the Women's Health in the Middle Years survey[192] (Section 2.2.1.1.), providing information on their age at menopause and whether they underwent a surgical or natural menopause. As outlined in Section 2.3.2., NSHD participants completed the ACE-III at age 69 (Table 1), and at wave I of Insight 46 data collection (age 69-71), participants completed a modified version of the PACC (Table 2). Women were included in these analyses if they had known age at menopause and available ACE-III data at age 69 (whole-cohort) or available PACC data at age 69-71 (Insight 46 wave 1) (Figure 14).

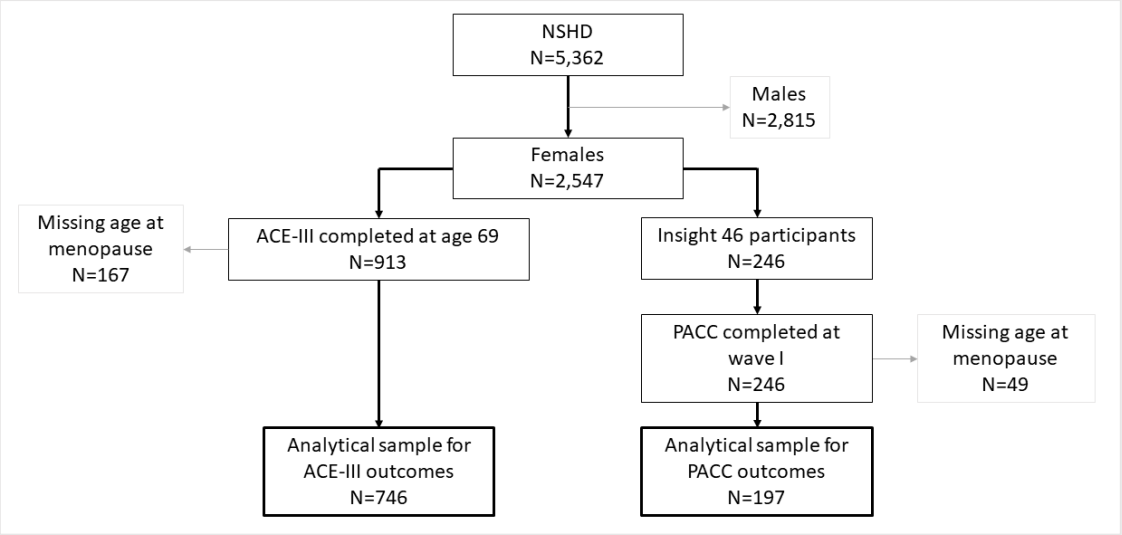


***Figure 14.*** *Flow chart demonstrating the sample selection for analyses.*

### *5i.2.2. Menopause age and type*

Age at menopause was ascertained for all menopause types as age at final menstrual period, indicated on self-reported questionnaires[192] as months since birth and later converted into years. Menopause type was recorded as natural if no hysterectomy or oophorectomy surgery was reported prior to the final menstrual period. Women who reported having a hysterectomy or unilateral or bilateral oophorectomy before reaching a natural menopause were categorised as having had a surgical menopause. Information on the type of surgeries women reported is presented in Table 20.

***Table 20.*** *Characteristics for women with available data on age at menopause and cognitive home assessment at age 69 (ACE-III), and Insight 46 sub-study neuropsychology assessment (PACC). The descriptive data included in this table have not been imputed.*

| | ACE-III completed (NSHD) | | PACC completed (Insight 46) | |
|---|---|---|---|---|
| **Variable** | **N** | **Mean(SD);range/%** | **N** | **Mean(SD);range/%** |
| Age of period cessation (years since birth) (mean(SD); range) | 746 | 49.81(5.95);28.75-62.50 | 197 | 49.89(5.71);30.25-60.50 |
| Natural menopause (mean(SD); range) | 541 | 51.95(4.06);34.50-61.92 | 134 | 52.47(3.22);40.50-59.50 |
| Surgical menopause (mean(SD); range) | 205 | 44.18(6.44);28.75-62.50 | 63 | 44.41(5.98);30.25-60.50 |
| ACE-III total raw score (mean(SD); range) | 746 | 91.79(6.01);62-100 | 167 | 93.28(5.27);70-100 |
| ACE-III attention & orientation raw score (mean(SD); range) | 746 | 16.61(1.95);5-18 | 167 | 16.74(1.90);8-18 |
| ACE-III language raw score (mean(SD); range) | 746 | 25.28(1.13);16-26 | 167 | 25.49(0.99);19-26 |
| ACE-III memory raw score (mean(SD); range) | 746 | 23.79(2.66);12-26 | 167 | 24.50(1.97);15-26 |
| ACE-III verbal fluency raw score (mean(SD); range) | 746 | 11.09(22.04);2-14 | 192 | 11.5(1.87);2-14 |
| ACE-III visuospatial function raw score (mean(SD); range) | 746 | 15.01(1.30);8-16 | 167 | 15.13(1.34);8-16 |
| PACC total raw score[a] (mean(SD); range) | 167 | 39.62(6.59);13.50-52 | 197 | 39.8(6.62);13.50-52.75 |
| PACC DSST raw score (mean(SD); range) | 167 | 48.80(10.24);24-76 | 197 | 49.15(10.11);24-76 |
| PACC FNAME-12A raw score (mean(SD); range) | 165 | 68.78(18.36);3-95 | 195 | 69.18(18.16);3-95 |
| PACC logical memory delayed raw score (mean(SD); range) | 167 | 12.17(3.27);0-20 | 197 | 12.39(3.41);0-23 |
| PACC MMSE raw score (mean(SD); range) | 167 | 29.23(1.10);23-30 | 197 | 29.28(1.04);23-30 |
| Childhood cognition z-score age 8[b] (mean(SD); range) | 693 | 0.16(0.80);-2.11-2.39 | 197 | 0.40(0.77);-1.59-2.47 |
| Childhood social class | 702 | | 192 | |
| Manual (%) | 377 | 53.70 | 94 | 48.96 |
| Non-manual (%) | 325 | 46.30 | 98 | 51.04 |
| Education (to age 26) | 709 | | 192 | |
| Ordinary (GCSE-level or below) (%) | 462 | 65.16 | 95 | 49.48 |
| Advanced (A-level or higher) (%) | 247 | 34.84 | 97 | 50.52 |
| Age at menarche (years since birth) (mean(SD); range) | 598 | 13.02(1.19);9-18.50 | 172 | 12.88(1.20);9.92-17.50 |
| Number of natural-born children | 642 | | 177 | |
| 0-2 children (%) | 420 | 65.42 | 115 | 64.70 |
| 3 or more children (%) | 222 | 34.58 | 62 | 35.03 |
| Menopause type | 746 | | 197 | |
| Natural (%) | 541 | 72.50 | 134 | 68.02 |
| Surgical (%) | 205 | 27.50 | 63 | 31.98 |
| Type of surgery | 205 | | 63 | |
| Hysterectomy only (%) | 101 | 49.27 | 34 | 53.97 |
| Unilateral oophorectomy (with/without hysterectomy) (%) | 21 | 10.24 | 5 | 7.94 |
| Bilateral oophorectomy (with/without hysterectomy) (%) | 83 | 40.49 | 24 | 38.09 |
| BMI at age 36 years ($kg/m^2$) (mean(SD); range) | 690 | 23.18(3.31);16.23-40.39 | 183 | 23.10(3.27);17.16-39.16 |
| Smoking pack years at age 36 years (mean(SD); range) | 679 | 1.12(2.01);0-10 | 183 | 0.84(1.72);0-7.50 |

| | | | | | |
|---|---|---|---|---|---|
| *APOE*-ε4 status | 665 | | | 197 | |
| ε4 present (%) | 195 | 29.32 | | 54 | 27.41 |
| ε4 absent (%) | 470 | 70.68 | | 143 | 72.59 |
| Affective symptoms age 69 (GHQ caseness) | 744 | | | 192 | |
| Yes (%) | 140 | 18.82 | | 24 | 12.50 |
| No (%) | 604 | 81.18 | | 168 | 87.50 |
| Age at Insight 46 cognitive testing (mean(SD); range) | N/A | | | 184 | 70.68(0.68);69.27-71.86 |
| Ever use of HT | 667 | | | 191 | |
| No (%) | 282 | 42.28 | | 58 | 30.37 |
| Yes (%) | 385 | 57.72 | | 133 | 69.63 |
| For HT users, age at first use | 384 | | | 132 | |
| ≤45 years | 83 | 21.61% | | 23 | 17.42% |
| 46-51 years | 231 | 60.16% | | 89 | 67.43% |
| ≥52 years | 70 | 18.23% | | 220 | 15.15% |
| For HT users, length of HT use | 377 | | | 130 | |
| <5 years (%) | 183 | 48.54 | | 61 | 46.92 |
| 5-10 years (%) | 139 | 36.87 | | 48 | 36.92 |
| >10 years (%) | 55 | 14.59 | | 21 | 16.15 |

*ACE-III = Addenbrooke's Cognitive Examination; PACC = Preclinical Alzheimer's Cognitive Composite; NSHD=National Survey of Health and Development; SD=standard deviation; DSST=Digit-Symbol Substitution Test; FNAME=Face-Name Associative Memory Examination; MMSE=Mini-Mental State Examination; GHQ= General Health Questionnaire; HT= Hormone Therapy*

*a: PACC total raw score Is the mean of scores across the four PACC sub-tests (DSST, FNAME-12A, logical memory delayed recall, MMSE) calculated for each participant. Where FNAME-12A data was missing, PACC total was calculated as the mean of scores across DSST, logical memory delayed recall and MMSE.*

*b: Z-score standardised to the sample at the time. If data are missing for cognition at age 8, values from age 11 (n=11) or age 15 (n=10) years were used instead.*

### *5i.2.3. Cognitive outcome measures*

As outlined in Section 2.3.2. (Table 1), NSHD participants completed the ACE-III, a measure of cognitive state, during home visits at age 69. Total ACE-III score (maximum 100) is the sum of scores across five cognitive sub-domains: attention and orientation (scored 0-18), verbal fluency (0-14), memory (0-26), language (0-26), and visuospatial function (0-16). Raw ACE-III total and sub-domain scores were standardised to the analytical sample.

Also described in Section 2.3.2., Insight 46 participants completed a comprehensive neuropsychology test battery[158,193] when aged between 69 and 71 (Table 2). This included a modified version of the PACC,[158] comprising of four sub-tests: the digit-symbol substitution test (DSST; Wechsler Adult Intelligence Scale-Revised)[213] assessing processing speed, associative learning, attention, and executive function; the 12-item face-name associative memory examination (FNAME-12A)[215] assessing associative, episodic memory; logical memory IIa (Wechsler Memory Scale-Revised)[212] assessing episodic memory; and the mini-mental state examination (MMSE),[211] a 30-point test of overall cognitive state. Raw scores on each sub-test were standardised to the analytical sample and averaged to generate a total PACC score for each participant.

### *5i.2.4. Covariables*

Covariables were selected based on previous analyses and evidence linking variables with menopause age or cognition.[155,157,158] Childhood cognition at age 8 years was the sum of four tests of verbal and non-verbal ability (Section 2.3.4.4.), standardised to the sample at the time of testing. If data were missing for cognition at age 8, available data for cognition at age 11 (n=11), or at age 15 years (n=10) were used instead. Childhood SEP up to age 15 (Section 2.3.4.3.) was categorised as manual or non-manual. Highest educational attainment up to age 26 (Section 2.3.4.2.) was categorised as ordinary (GCSE-level or equivalent) or below, or advanced (A-level or equivalent, or above). Age at menarche was recorded as years since birth, reported by a school doctor at age 14-15 years or self-reported at age 48.[323] Parity was indicated by self-reported number of natural-born children, excluding still births and miscarriages. Due to small proportions of nulliparous or single parity women in the whole-NSHD sample, this variable was categorised as 0-2 children, or 3 or more children. Menopause type was categorised as natural or surgical, as defined above (Section 5i.2.2.). Given some

evidence for a relationship between BMI and menopause timing (higher BMI, later menopause) and that obesity associates with an increased likelihood of surgical menopause,[324] BMI at age 36, negatively associated with cognitive performance at age 60-64 in this cohort,[206] was included as a covariable, recorded as a continuous value (kg/m$^2$). Smoking pack years was self-reported at age 36 years. *APOE*-ε4, linked with an earlier menopause age,[319] was categorised as ε4-present or ε4-absent (Section 2.3.4.1.). Affective symptom caseness at age 69 was determined using a cut-off of 5 or more on the 28-item GHQ.[282] Age at cognitive testing for Insight 46 participants was derived from the recorded age, in years, at which participants underwent neuroimaging. Ever or never use of any type of prescribed menopausal HT by age 69 was self-reported by questionnaire.

#### *5i.2.5. Statistical analyses*

Analyses were conducted using Stata version 17.0.

Associations between menopause age and z-score standardised cognitive outcomes (ACE-III total and sub-domains, and PACC total and sub-tests) were assessed using multivariable linear regression analyses, cumulatively adjusting for covariables. Unadjusted models (model 0/M0) were followed by adjustments for early cognitive and sociodemographic factors (M1:childhood cognition, childhood SEP, education), reproductive factors (M2:M1 plus age at menarche, parity, menopause type), and health-related factors (M3:M2 plus BMI, smoking, affective symptoms, *APOE*-ε4 status, and age at cognitive testing [Insight 46 only]).

The potential moderating role of menopause type was examined by testing for menopause age-by-type interactions on standardised ACE-III total and PACC total scores in fully adjusted models (M3). Similarly, menopause age-by-*APOE*-ε4 interactions were added to fully adjusted models to examine whether associations were modified by *APOE*-ε4 status.

The potential role of HT use in associations of menopause age with cognitive outcomes was assessed by further adjusting for HT (M3 plus HT). Whether HT use was associated with ACE-III total and PACC total scores was also examined without adjustments, and after accounting for menopause age.

In sensitivity analyses, ran for ACE-III total and PACC total, participants who scored less than 82 on the ACE-III, a threshold indicative of possible cognitive impairment,[325] were excluded (whole-cohort n=50; Insight 46 n=5). Additionally, since the surgical removal of both ovaries results in the cessation of all ovarian oestrogen production, in contrast to surgeries in which at least one ovary is conserved,[142] analyses excluding women who had bilateral oophorectomy (whole-cohort n=83; Insight 46 n=24) were conducted.

Multiple imputation (Section 2.4.) with 50 imputations (unless otherwise specified) was used to account for missing data in the covariables and, where applicable, in outcomes (PACC FNAME-12A only; missing n=2). Separate imputation models were run on the maximal analytical samples for the ACE-III (whole-cohort; n=746) and PACC (Insight 46; n=197) outcomes. The imputation models included the outcome measures available for each sample. For the whole cohort, these were ACE-III total and all ACE-III sub-domains. For Insight 46, these were PACC DSST, PACC FNAME-12A, PACC logical memory delayed recall, PACC MMSE, and ACE-III total. PACC total was passively generated as an average of the four PACC sub-tests, because of missing data for FNAME-12A (n=2), which was imputed using Gaussian normal regression in the Insight 46 sample. For both samples, the imputation models additionally included menopause age and additional covariables included in the analytical models (childhood cognition, childhood SEP, education, age at menarche, parity, menopause type, BMI, smoking, *APOE*-ε4 status, affective symptoms, age at cognitive testing [Insight 46 only], and HT use). No auxiliary variables were included due to the need for simplified imputation models to allow multiple imputation to be combined with bootstrapping (see below). For analyses testing menopause age-by-type and menopause age-by-*APOE*-ε4 interactions, these interactions were added to the imputation.

Where outcome measures were skewed (ACE-III total, ACE-III sub-domains, and MMSE), bootstrapping was applied (Section 2.4.). Bootstrapping was combined with multiple imputation by running the multiple imputation and analysis model on the imputed data within each bootstrap sample. To reduce processing demands 8 imputations were used, as this was deemed sufficient according to Monte Carlo Error estimations (Appendix C Table 1).

## 5i.3. Results

### *5i.3.1. Participant characteristics*

Data for menopause age was available for 1,378 women. Of these, 746 were still in the cohort and completed the ACE-III at age 69, and 197 Insight 46 participants completed the PACC (Figure 14). Two participants did not complete the FNAME-12A assessment due to technical problems and lack of time; multiple imputation was applied to account for these missing data (described above).

Table 20 displays participant characteristics. Mean age at menopause was comparable between the whole-cohort (mean=49.81 years, range 28.75-62.50) and the Insight 46 samples (mean=49.89 years, range 30.25-60.50), and between *APOE*-ε4 carriers and non-carriers in the whole-cohort (carriers mean menopause age=49.99 (SD=6.10), non-carriers=49.66 (5.89); t-test p=0.52) and in Insight 46 (carriers mean=49.50 (5.71), non-carriers=50.04 (5.66); t-test p=0.55). Women who had a surgical rather than natural menopause experienced menopause at a younger age (7.70 and 8.06 years younger, on average, for the whole-cohort and Insight 46 samples, respectively). Surgical menopause and HT use were more common among Insight 46 participants than in the whole-cohort. Consistent with a previous report,[194] cognitive scores were also generally higher in Insight 46 participants, as was childhood SEP and education.

Most women included in these analyses had used HT (57.72% and 69.63% in the whole-cohort and Insight 46, respectively; Table 20). Of the women who had used HT, most started taking HT between age 46 and 51 years (whole-cohort=60.16%; Insight 46=67.42%; Table 20), and less than 5 years was the most common length of HT use (whole-cohort=48.54%; Insight 46=46.92%; Table 20).

### *5i.3.2. Associations of menopause age with later-life cognitive performance*

Among women from NSHD who completed the ACE-III at age 69, positive associations were detected for later menopause age with better task performance on the ACE-III total and across all ACE-III sub-domains (n=746; Figure 15; Appendix C Table 2). In the unadjusted model, each 1-year increase in age at menopause associated with a 0.024 SD increase in standardised ACE-III total score (95% CI 0.012, 0.036), equating to 0.02 additional points for the ACE-III total raw

score (maximum 100). Attention and orientation was the only ACE-III outcome measure for which the unadjusted effect estimate was not significant.

As shown in Figure 15, the effect estimates for all ACE-III outcomes were attenuated after adjustments for early cognition and sociodemographic factors in model 1. Adjustments for reproductive factors in model 2 increased the effect estimates for ACE-III total and the language, verbal fluency, and visuospatial sub-domains. The association of menopause age with ACE-III memory performance was further attenuated and no longer significant after model 2 adjustments. With further adjustment for health-related factors in model 3, no significant associations of menopause age with any ACE-III outcomes remained, but the largest effect estimates were for ACE-III total (β=0.010[-0.004, 0.024]) and the memory (β=0.009[-0.006, 0.023]) and visuospatial function (β=0.013[-0.004, 0.026]) sub-domains.

For women in the Insight 46 sub-sample, later menopause age associated with better task performance on the PACC total and across all PACC sub-tests (n=197; Figure 16; Appendix C Table 3). In the unadjusted model, each 1-year increase in menopause age associated with a 0.029 SD increase in PACC total z-score (95% CI 0.011, 0.048), equating to 0.01 additional points on the PACC total raw score. MMSE was the only PACC sub-test for which the unadjusted effect estimate was not significant.

As shown in Figure 16, adjusting for early cognition and sociodemographic factors in model 1 attenuated the associations with PACC outcomes; no associations remained significant. Additionally adjusting for reproductive factors in model 2 increased the effect estimates for PACC total, DSST, and FNAME-12A. With full adjustments (M3) the largest effects were for FNAME-12A performance, remaining significant (β=0.037[0.005, 0.069]), and for DSST, although non-significant (β=0.031[-0.001, 0.062]). No significant associations remained for PACC total, logical memory delayed, nor MMSE performance.

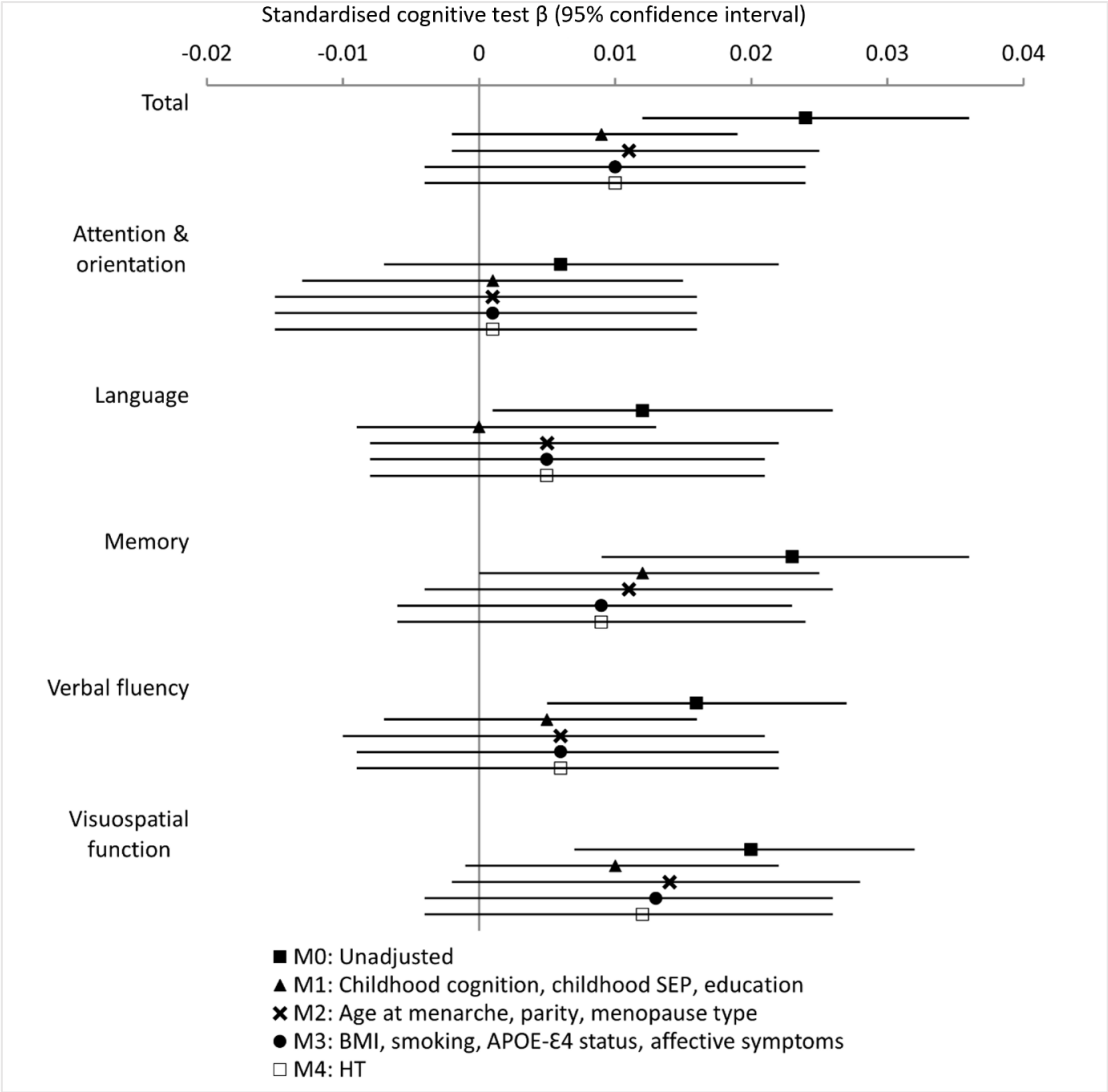


***Figure 15.*** *Model estimates and bootstrap bias-corrected 95% confidence intervals for the effect of 1-year increase in age at menopause on standardised z-scores for the Addenbrooke's Cognitive Examination (ACE-III; total score and sub-domains) at age 69 in the National Survey of Health and Development (NSHD) whole-cohort. N = 746.*

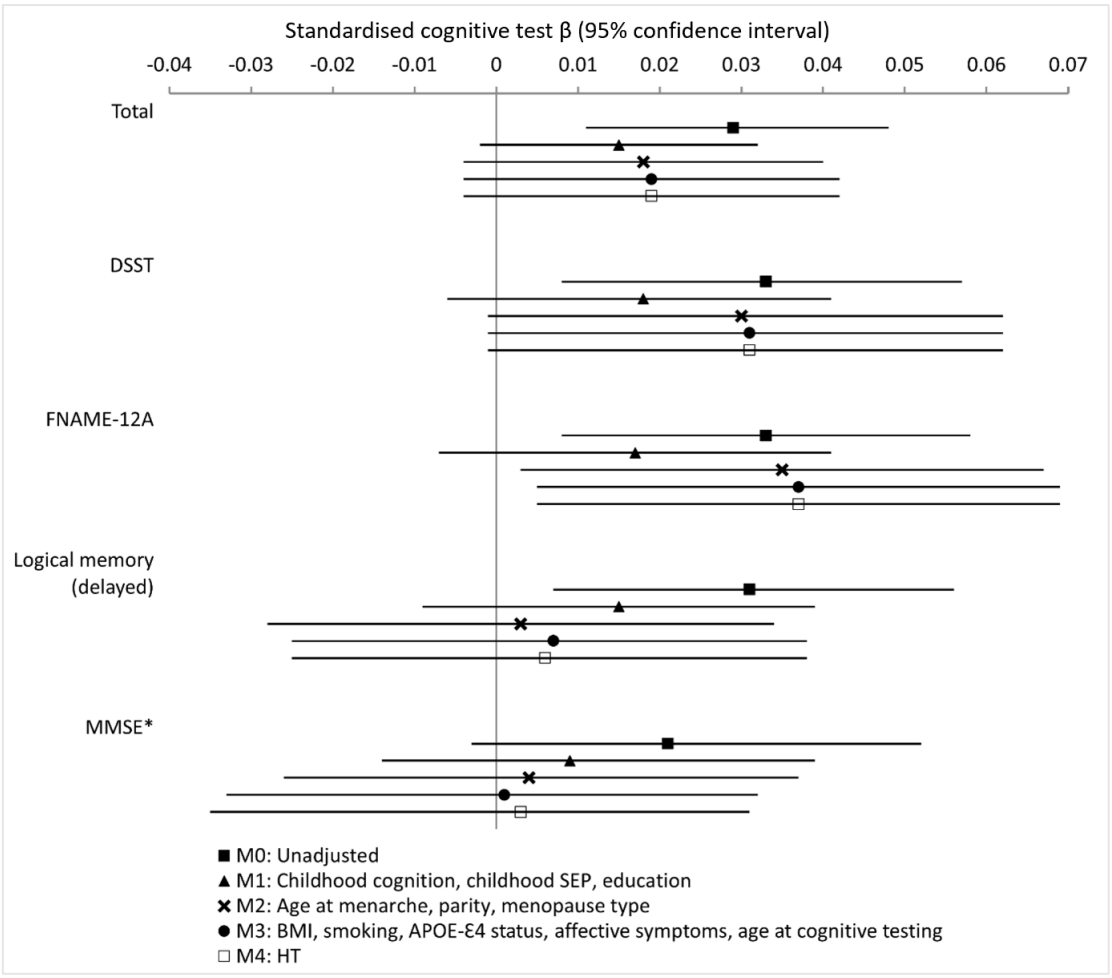


***Figure 16.*** *Model estimates and 95% confidence intervals for the effect of 1-year increase in age at menopause on standardised z-scores for the Preclinical Alzheimer's Cognitive Composite (PACC; total score and sub-tests) at age 69 to 71 in the Insight 46 sample. N = 197.*

*DSST = Digit-Symbol Substitution Test;*
*FNAME = Face-Name Associative Memory Examination;*
*MMSE = Mini-Mental State Examination*

**Bootstrap bias-corrected confidence interval.*

#### *5i.3.3. Effect modification by menopause type and APOE-ε4 status*

No interactive effects of menopause age-by-menopause type on standardised ACE-III total (95% CI -0.03, 0.02) nor PACC total (95% CI -0.073, 0.019; p=0.243) performance were detected (Appendix C Table 4). Additionally, no interactive effects of menopause age-by-*APOE*-ε4 status on ACE-III total (95% CI -0.009, 0.006) nor PACC total (95% CI -0.039, 0.037; p=0.949) were detected (Appendix C Table 5).

#### *5i.3.4. The role of menopausal hormone therapy*

Compared with fully adjusted models (M3), further adjusting for HT use (M4) had little impact on the effect estimates for any outcomes (Figures 15 and 16; Appendix C Tables 2 and 3). There was no evidence of associations between HT use and ACE-III total nor PACC total performance (Appendix C Table 6).

#### *5i.3.5. Supplementary and sensitivity analyses*

Individually adjusting for each model 1 covariable (childhood cognition, childhood SEP, education) revealed that the attenuation of effect estimates was driven by childhood cognition (Appendix C Table 7).

Where negative confounding in model 2 was observed (ACE-III: total, language, verbal fluency, visuospatial function; PACC: total, DSST, FNAME-12A), individually adjusting for reproductive covariables (age at menarche, parity, menopause type) showed that the negative confounding was driven by menopause type (Appendix C Table 8). No menopause age-by-menopause type interactions were detected on these outcomes (Appendix C Table 9). Unadjusted regression models of menopause type on cognitive outcomes showed poorer cognitive performance in women with surgical compared with natural menopause, and these associations were negatively confounded when adjusting for menopause age (Appendix C Table 10).

Excluding women with possible cognitive impairment (total ACE-III score <82) did not substantially change the effect estimates for the association of menopause age with ACE-III total, although the estimates for PACC total were slightly attenuated (Appendix C Tables 11-14). Similarly, excluding women with bilateral oophorectomy did not substantially change the

effect estimates for the ACE-III total outcome, while the estimates for PACC total were slightly attenuated (Appendix C Tables 15-17).

## 5i.4. Discussion

### *5i.4.1. Key findings*

Later menopause age associated with better cognitive performance in later-life across all outcomes. Associations were strongest for the ACE-III memory and visuospatial function sub-domains, and the PACC DSST and FNAME sub-tests, demonstrating relationships of menopause age with visual processing, and associative learning and memory domains. Adjusting for early-life factors attenuated all effect estimates, driven by childhood cognition, and accounting for menopause type revealed negative confounding for some outcomes. No significant interactions with menopause type or *APOE*-ε4 status were detected, and further adjustment for HT use did not meaningfully alter the estimated effects.

### *5i.4.2. Interpretation of findings*

These findings support previous evidence for positive associations between age at menopause and cognitive outcomes,[154,157,321] with prolonged exposure to the neuroprotective benefits of endogenous oestrogen a hypothesised mechanism.[305] Additionally, these findings are consistent with previous evidence of small positive associations between menopause age and verbal memory performance in NSHD.[157] During the menopause transition, women often report memory problems,[144] and oestrogen receptors are found in high concentrations within the hippocampus, a brain region important for learning and memory.[305] However, the associations of menopause age with memory performance were not consistent across different memory assessments; the association with delayed episodic memory (logical memory delayed recall) in the Insight 46 sample was not particularly strong compared with other outcome measures. There are several memory types (e.g. episodic, associative, semantic) each differently assessed in the sub-tests included in these analyses. Associations between menopause age and memory performance could differ according to the type of memory assessed.

The association with a measure of processing speed in the Insight 46 cohort contrasts with previous evidence from NSHD where associations with processing speed were not detected.[157,321] The relationships could differ in the Insight 46 sub-sample compared with the whole-cohort, particularly given demographic differences.[194] However, the processing speed measure completed by Insight 46 participants (DSST) differs from the letter cancellation task completed between age 43 and 69 in the whole-cohort; [157,321] the DSST includes an associative learning component. The relationship with a measure of associative memory (FNAME-12A) in the Insight 46 cohort might also suggest that menopause age links with associative learning in later-life. Additionally, both the DSST and FNAME-12A are reliant on visual processing, in agreement with the whole-cohort association with visuospatial function.

As previously shown,[157,321] most associations were not independent of life course covariables and childhood cognition was a particularly important factor. Adjustment for childhood cognition, which predicts both menopause age[156,157] and later-life cognitive performance,[158,159] most strongly attenuated the associations compared with other covariables such as SEP and education. Upstream, developmental factors (e.g. genetic factors, pre-natal exposures, early-life experiences)[157,326] giving rise to childhood cognition could link the timing of menopause with later-life cognitive outcomes, and childhood cognition could be a proxy indicator of lifetime oestrogen exposure.[156]

The negative confounding by menopause type likely reflects negative associations of surgical, compared with natural, menopause and cognitive outcomes,[151] which contrasts the positive associations between menopause age and cognitive performance. Interestingly, the association between menopause age and memory performance at age 69 was explained by other reproductive factors, including menopause type; menopause type rather than timing could be important for memory in later-life, although surgical menopause is synonymous with earlier menopause. Differences in menopause age-cognition associations by menopause type were not detected, but there are differences between natural and surgical menopause beyond menopause timing. Surgeries inducing menopause lead to more rapid declines in oestrogen levels than during natural menopause; removal of both ovaries results in the most acute cessation of ovarian oestrogen production.[142] Nonetheless, excluding women who had a bilateral oophorectomy did not substantially change the results. Women with surgical menopause are also more likely to use HT, and to have poorer overall health and lower SEP

than women who have a natural menopause.[327,328] Whether and how the associations of menopause age with later-life cognitive outcomes might differ by menopause type is a complex topic which requires further investigation utilising different and larger cohorts. Understanding the potential impacts of surgical menopause on later-life outcomes, including cognitive abilities, will be important for informing clinical guidance encompassing the risks and benefits of such surgical interventions.

Additionally, the relationships between menopause age and later-life cognitive performance were not modified by *APOE*-ε4 status. Although there are some reports of earlier[319] and later[320] menopause timing in carriers than non-carriers, menopause age did not differ by *APOE* genotype in NSHD and Insight 46 women. Since adverse effects of *APOE*-ε4 tend to be more evident at older ages, continued follow-up of the NSHD and Insight 46 cohorts will be valuable to further examine the potential for interactions between menopause timing and *APOE* genotype. However, Chapter 3 analyses did reveal some sex-by-*APOE*-ε4 interactions on cognitive performance at older ages (53 onwards). It is possible that menopause timing is not an underlying mechanism exacerbating ε4-associated dementia risk in females.

#### *5i.4.3. Strengths and limitations*

The main strength of this work is the use of longitudinal, prospective life course data which provides a unique opportunity to account for early-life confounds such as childhood cognition. The age homogenous cohort is particularly beneficial given that the exposure variable (menopause age) was age dependent. However, the generalisability of these results to other generations could be limited given secular changes in women's access to education and in HT use, for example. While the potential contribution of HT use in relation to associations between menopause age and later-life cognition has been considered, with little evidence for potential mediating effects, differentiating between the different types of HT used was not possible. There is a need for more in-depth analyses of HT and cognition beyond the scope of these analysis, which might benefit from the inclusion of additional data sets, given potential variations according to dosage, formulation, duration of use, and age when HT is initiated.[329] While the effects reported here are small, these are consistent with other research, and some residual associations were still detected after adjustments. In future research, follow-up cognitive assessments in this cohort will facilitate further examination of associations

between menopause and cognitive decline and, as explored in Section 5ii, the availability of neuroimaging data within Insight 46 provides an opportunity to examine the potential neural mechanisms underlying these associations.

#### *5i.4.4. Summary*

These analyses provide further evidence that later age at menopause associates with better cognitive performance in later-life and identify that the associations are most notable for visual processing, and associative learning and memory domains. However, life course covariables, particularly childhood cognition, contribute to associations. Such factors are therefore important to consider when examining the potential mechanisms underlying relationships between menopause and female cognitive ageing.

## 5ii. Menopause and later-life brain health

The purpose of this section was to test relationships between age at natural menopause and brain health at age ~70, across a range of multi-modal neuroimaging measures. Women from Insight 46 who had a natural menopause, had available data for their age at menopause, and who completed PET-MRI imaging at Insight 46 wave I were included. Multivariable regression analyses tested non-linear associations, effect modifications by *APOE*-ε4 status, and accounted for relevant early cognitive, sociodemographic, reproductive, and health-related covariables. There was evidence for an inverted-U non-linear relationship of menopause age with AD pathology (Aβ), and later menopause age positively (and linearly) associated with larger TBV (indicating reduced non-specific brain ageing), but menopause age was not associated with hippocampal volume or measures of NAWM microstructural integrity. Some associations were modified by *APOE* genotype; in ε4 carriers but not in non-carriers, later menopause age associated with increased cortical thickness and reduced markers of cSVD (WMHV). Associations were mostly independent of life course covariables, including HT use, but blood pressure and smoking at age 36 partially explained associations with TBV and WMHV, respectively. Overall, these findings indicate that later age at natural menopause could be beneficial for later-life brain health, particularly in *APOE*-ε4 carriers. An alternative, but complementary, interpretation is that earlier age at natural menopause is detrimental for later-life brain health.

### 5ii.1. Introduction

Given that menopause age is associated with later-life cognitive performance measures (Section 5i.), it is plausible that brain structures and functions which subserve cognitive abilities will also be associated with menopause age. Indeed, menopause can be considered a neurological transition, given the neurological nature of some menopausal symptoms (e.g. hot flushes, sleep disturbance, brain fog).[8] Oestrogen also supports neural functioning and has neuroprotective properties (e.g. neuronal maintenance, Aβ production inhibition),[84] hence declining oestrogen levels during menopause are hypothesised to increase the vulnerability of the female brain to neuropathology and dementia processes. The female-specific process of menopause could therefore offer some explanation for greater dementia risk in females. However, the relationship between menopause and dementia-related brain health in later-life is not yet fully understood.

Some studies have demonstrated variations in brain health across menopause phases. Peri-menopausal women have showed smaller grey and white matter volumes, and lower energy metabolism than pre- and post-menopausal women, while post-menopausal women showed similar or greater brain volumes and energy metabolism levels than pre-menopausal women.[163] Longitudinally though, evidence shows faster brain ageing in post-menopausal women, with faster declines in TBV and glucose metabolism, and faster increases in WMHV and Aβ deposition than age-matched men and pre-menopausal women.[162,330] This supports the hypothesis that menopause accelerates neural ageing processes.

Although meta-analysis does not support an overall association of menopause age with dementia risk, several individual studies associated later menopause age with lower dementia risk,[154] and consistent positive associations with cognitive performance are reported,[154,156,157,317,331] including in this cohort (Section 5i.). Only a few studies have examined menopause age and brain health, reporting mixed results. In the UK Biobank, later age at natural menopause associated with smaller TBV and hippocampal volumes,[164] and with younger predicted brain ages.[332] In a small study (n=35), TBV was not linked with menopause age but poorer cerebrovascular function and greater WMHV were detected in women with late (≥53 years) compared with early (≤49 years) natural menopause.[333] For some, but not all, women, menopause could be a 'tipping point' for accelerated brain ageing and the development of pathology.[318] Indeed, peri- and post-menopausal *APOE*-ε4 carriers are shown

to, independently of age, have higher Aβ levels than pre-menopausal and male ε4 carriers.[163] While evidence linking *APOE*-ε4 status and menopause age is mixed,[319,320] ApoE is shown to have a role in ovarian sex hormone production and in the neural effects of oestrone.[334] Interactions of menopausal hormone changes and *APOE*-ε4 status on brain health are yet to be examined and, although Section 5i analyses did not find evidence for menopause age-by-*APOE*-ε4 interactions on later-life cognitive performance, such investigations are warranted.

Whether menopausal HT confers benefits or risks to later-life dementia-related outcomes is also unclear[170] and neuroimaging outcomes from RCTs are inconsistent[179,335] (Section 1.11.5.). Discrepant HT findings can be attributed to heterogeneity in dosages, formulations, duration of use, and the timing of HT initiation in relation to menopause. Given uncertainty over how HT might influence brain health outcomes, most research assessing menopause and brain health excludes women who have used HT, meaning that the potential role of HT in menopause-brain health associations is unclear.

#### *5ii.1.1. Objectives and research questions*

This work aims to examine the associations of menopause age with a range of multimodal neuroimaging markers of brain health reflecting dementia- (Aβ, hippocampal volume), non-specific ageing- (total brain volumes, cortical thickness), and vascular-related (NAWM microstructural integrity, WMHV) pathways in Insight 46 participants at age ~70 who underwent a natural menopause.

Learning from the previous analyses in Section 5i, there are some variations in the methodological approach taken here, namely that women who underwent surgical menopause are now excluded. This is in recognition of the distinctions between natural and surgical menopause; women with surgical menopause have underlying etiologies indicating a need for surgery, are more likely to use HT, and by definition have earlier ages at menopause than women who go through natural menopause.[336] Additionally, the possibility of non-linear associations is recognised given mixed findings in the relatively little research which has examined associations of menopause age with neuroimaging outcomes;[332,333] non-linear associations of menopause age with brain-health outcomes are now examined prior to subsequent analyses.

The following research questions are addressed:

1. Are there linear or non-linear associations of age at natural menopause with neuroimaging markers of brain health at age ~70?
2. Are associations moderated by *APOE*-ε4 status?
3. Are associations independent of relevant early-life, reproductive, and health-related covariables?
4. Does HT use contribute to associations of menopause age with neuroimaging markers of brain health at age ~70?

#### *5ii.1.2. Hypotheses*

Previous examinations of menopause age and brain health are scarce, but given positive associations with cognitive performance, it follows that later menopause age will also positively associate with brain health, particularly given evidence for faster brain ageing in post-menopausal women. As observed in Section 5i, life course covariables were expected to attenuate effect estimates, with little mediating effect of HT use. While effect modifications by *APOE*-ε4 status were not detected in Section 5i, some evidence linking menopause status and *APOE* genotype with Aβ levels leads to the hypothesis that relationships between menopause age and brain health, particularly AD-related measures, may be stronger in ε4 carriers than in non-carriers.

### 5ii.2. Analytic method

#### *5ii.2.1. Analytic sample*

Insight 46 females who also completed Women's Health in the Middle Years Survey questionnaires (Section 2.2.1.1.) were included in these analyses if they reported a natural menopause, had available data on their age at menopause, and if they underwent neuroimaging at wave I of Insight 46 data collection, generating a maximal analytical sample of 126 females (Figure 17).

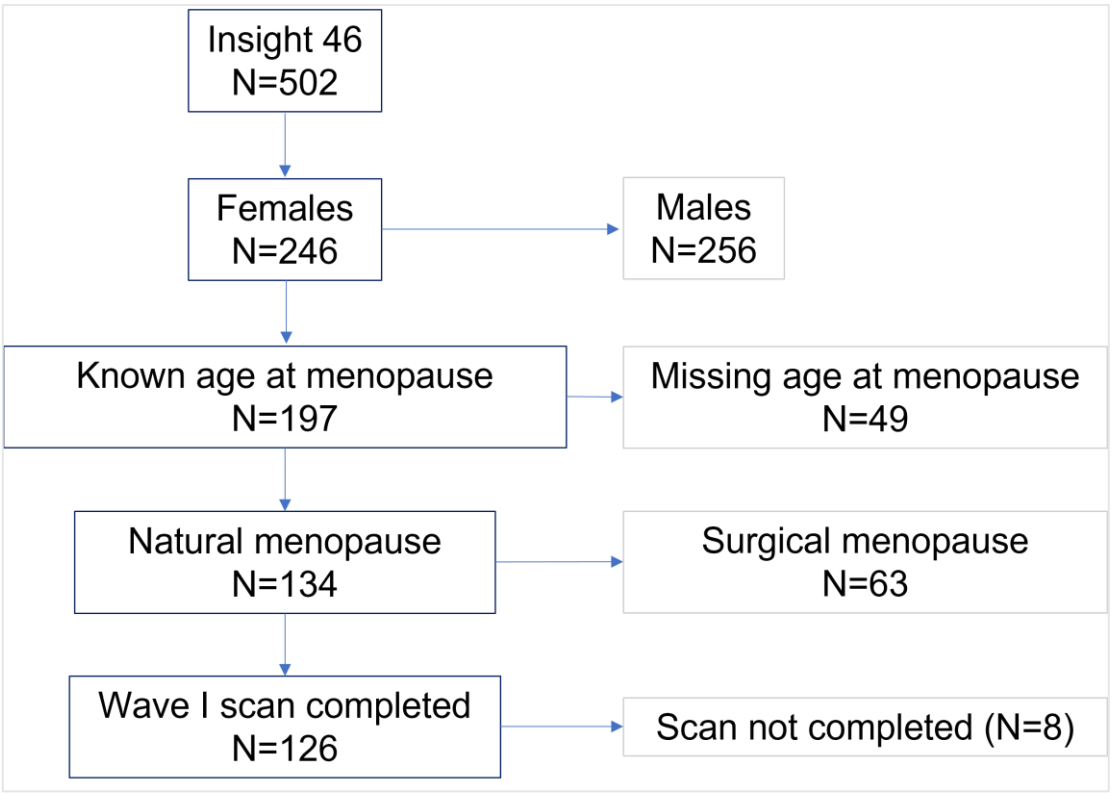


***Figure 17.*** *Flow chart demonstrating sample selection for analyses. Scan refers to combined amyloid PET-MRI.*

#### *5ii.2.2. Menopause age*

As in Section 5i., age at menopause was prospectively self-reported as age (years) since birth that menstrual periods stopped. Women indicated, via questionnaire, whether their periods had stopped naturally or if there was another reason for their cessation (e.g. surgery to remove the ovaries, Section 5i.2.2.).

#### *5ii.2.3. Neuroimaging outcome measures*

As outlined in Section 2.3.3., Insight 46 participants underwent combined PET-MRI neuroimaging aged ~70. For these analyses, the following outcomes were of interest: TBV, hippocampal volume, WMHV, continuous Aβ SUVR, Aβ status, NAWM microstructural integrity measures (FA and MD), and cortical thickness across ROI (frontal, occipital, parietal, temporal lobes, and the Harvard AD signature region).[220]

For analyses assessing WMHV as an outcome, participants who failed BaMoS QC were excluded (n=3). Participants whose scans failed NAWM QC (n=16) were excluded from NAWM analyses.

### *5ii.2.4. Covariables*

Lifetime covariables were selected based on previous analyses linking menopause age with later-life outcomes in this cohort[317] and on associations of covariables with menopause age and brain health measures.[158,326] Childhood cognition at age 8 years (Section 2.3.4.4.) was standardised to the sample at the time of testing. If data were missing for cognition at age 8, available data for cognition at age 11 (n=7), or at age 15 years (n=1) were instead used. Educational attainment up to age 26 (Section 2.3.4.2.) was categorised as ordinary (GCSE-level or equivalent) or below, or advanced (A-level or equivalent, or above). Adult SEP up to age 53 (Section 2.3.4.3.) was dichotomised to manual and non-manual. In contrast to Section 5i analyses which included a measure of childhood SEP, adulthood SEP is instead used here given evidence linking mid- but not early-life SEP with volumetric measures of brain health.[337] Age at menarche was recorded as years since birth, according to school doctor- or self-report (Section 5i.2.4.). Parity was indicated by self-reported number of biological children, excluding still births and miscarriages, coded as an ordinal variable. As in 5i. (Section 5i.2.4.), BMI and smoking pack years at age 36 were included as covariables, *APOE*-ε4 status was categorised as ε4 present or absent, and menopausal HT use by age 69 was categorised as ever or never used. Blood pressure at age 36 was included as an additional covariable in these analyses, given reported relationships between vascular health and brain health (specifically cSVD) which are particularly notable in females.[40] Seated blood pressure at age 36 was measured in the upper arm twice after 5 minutes of rest, using a Hawksley Random Zero sphygmomanometer.

### *5ii.2.5. Statistical analyses*

Analyses were conducted using Stata version 17.0.

Multivariable regression analyses were used to assess the associations of menopause age with brain health measures (TBV, hippocampal volume, continuous SUVR, NAWM MD and FA measures, and all cortical thickness ROI). Multivariable logistic regression was used to

examine the odds of being amyloid positive given each 1-year increase in menopause age. The association of menopause age with WMHV, which has a skewed distribution, was examined using a generalised linear model with gamma distribution log link. All minimally adjusted models (M0) were adjusted for age at scan. Models were also adjusted for TIV for volumetric measures (TBV, hippocampal volume, and WMHV).

Firstly, non-linear associations were examined by including a quadratic term for menopause age in fully adjusted models (M0+childhood cognition, education, SEP, puberty, parity, BMI, smoking and blood pressure at age 36, and *APOE*-ε4 status). Where non-linear associations were detected ($p<0.1$), subsequent analyses included a quadratic term for menopause age, otherwise subsequent analyses used linear models.

The potential moderating role of *APOE*-ε4 status was examined by testing for menopause age-by-*APOE*-ε4 interactions in fully adjusted models. Where interactions were significant ($p<0.1$), *post-hoc* analyses were conducted, stratifying by *APOE*-ε4 status.

The contribution of life course covariables was examined by cumulatively adjusting for early-life (M1:M0+childhood cognition, education, SEP), reproductive (M2:M1+puberty, parity), and health-related (M3:M2+smoking, BMI, and blood pressure at age 36, *APOE*-ε4 status) factors.

To examine whether HT contributed to associations of menopause age with brain health, HT use was further adjusted for (M3+HT). The relationship of HT use with neuroimaging outcomes was also examined in minimally adjusted models, and in models adjusting for menopause age, including HT use as the predictor variable where never use was the reference category.

Given evidence for sex differences in WMHV where women typically have greater WMHV levels than men (Chapter 3), and evidence that WMHV predicts cortical thickness in this cohort,[219] sensitivity analyses were ran whereby all models for non-WMHV outcomes were adjusted for WMHV. Further sensitivity analyses examined whether the main findings could be replicated when excluding the twelve women with clinically diagnosed neurological conditions (Table 21), since neurological changes associated with such conditions (e.g. greater WMHV in multiple sclerosis patients) could influence the estimated effects of menopause age on neuroimaging outcomes. Additionally, since only two women in the sample had an early menopause (aged <45 years), analyses were re-ran excluding these participants.

Multiple imputation with 50 imputations was used to impute missing data in covariables (Section 2.4.2.). Separate imputation models were run on the maximal analytic sample for the main analyses (n=126) and for the sensitivity analyses excluding 12 women with clinically diagnosed neurological conditions (n=114). The imputation models included all variables used in the analytic models (menopause age, all outcome variables, all covariables), quadratic terms for menopause age, interaction terms for menopause age and *APOE*-ε4 status, and auxiliary variables selected to improve predictions of imputed values (diastolic and systolic blood pressure at age 63, diastolic and systolic blood pressure at Insight 46 wave I age ~70, BMI at Insight 46 wave I, childhood social class, and smoking pack years at age 63). For each sample, missing values were imputed using Gaussian normal regression for TIV, age at scan, childhood cognition, puberty age, smoking pack years at age 36, BMI at age 36, and diastolic and systolic blood pressure at age 36. Missing values were imputed using logistic regression for adult social class, education, *APOE*-ε4 status, and HT use. Ordinal logistic regression was used to impute missing values for number of natural-born children.

## 5ii.3. Results

### *5ii.3.1. Participant characteristics*

The maximal sample, summarised in Figure 17, was 126 women with known age at natural menopause and who had undergone neuroimaging at Insight 46 wave I. Participant characteristics for the analytical sample are presented in Table 21. Mean age at menopause was not statistically different between *APOE*-ε4 carriers (mean 51.93 years, SD=2.84, n=36) and non-carriers (mean 52.67 years, SD=3.41, n=90) (t=1.14, p=0.256).

Most women in the sample had ever used HT (60.3%; Table 21). Of those who had used HT, most started HT between age 46 and 51 (69.5%; Table 21) and most took HT for less than 5 years (60.6%; Table 21).

***Table 21.*** *Participant characteristics for women with available age at natural menopause and neuroimaging outcome data, based on complete case data.*

| Variable | N | Mean(SD)/% | Range |
|---|---|---|---|
| Menopause age (years) | 126 | 52.5(3.3) | 40.5, 59.5 |
| TBV ($cm^3$) | 126 | 1043.6(82.2) | 818.6, 1265.2 |
| Hippocampal volume ($cm^3$) | 126 | 3.0(0.3) | 2.3, 3.7 |
| WMHV ($cm^3$) | 123 | 5.1(5.4) | 0.4, 32.8 |
| SUVR | 126 | 0.6(0.1) | 0.5, 0.9 |
| Aβ positive | 20 | 15.9% | - |
| Aβ negative | 106 | 84.1% | - |
| NAWM MD (z-score) | 115 | 0.2(0.3) | -0.6, 1.2 |
| NAWM FA (z-score) | 115 | -0.1(0.2) | -0.8, 0.5 |
| CT: ADsig Harvard(mm) | 126 | 2.7(0.1) | 2.4, 2.9 |
| CT: frontal(mm) | 126 | 2.8(0.1) | 2.4, 2.9 |
| CT: occipital(mm) | 126 | 2.2(0.1) | 1.9, 2.4 |
| CT: parietal(mm) | 126 | 2.5(0.1) | 2.3, 2.7 |
| CT: temporal(mm) | 126 | 2.9(0.1) | 2.6, 3.1 |
| TIV ($cm^3$) | 126 | 1340.0(92.9) | 1129.8, 1558.1 |
| Age at scan (years) | 126 | 70.7(0.7) | 69.5, 71.8 |
| Childhood cognition (z-score) | 126 | 0.5(0.8) | -1.6, 2.5 |
| SEP age 53 | | | |
| Manual | 11 | 8.7% | - |
| Non-manual | 115 | 91.3% | - |
| Educational attainment to age 26 | | | |
| Ordinary (GCSE-level/equivalent or below) | 61 | 48.8% | - |
| Advanced (A-level/equivalent or above) | 64 | 51.2% | - |
| Age at menarche (years) | 107 | 13.0(1.3) | 9.9, 17.5 |
| Number of natural-born children | | | |
| 0 | 15 | 12.0% | - |
| 1 | 11 | 8.8% | - |
| 2 | 64 | 51.2% | - |
| 3 | 28 | 22.4% | - |
| 4 | 7 | 5.6% | - |
| Smoking pack years age 36 | 117 | 0.9(1.7) | 0, 6.9 |
| BMI age 36 ($kg/m^2$) | 117 | 22.8(2.9) | 17.2, 36.1 |
| DBP age 36 (mmHg) | 116 | 74.1(11.6) | 43.6, 113.6 |
| SBP age 36 (mmHg) | 116 | 116.7(12.8) | 87.6, 150.3 |
| *APOE*-ε4 status | | | |
| ε4 non-carrier | 90 | 71.4% | - |
| ε4 carrier | 36 | 28.6% | - |

| | | | |
|---|---|---|---|
| HT use | | | |
| Never | 48 | 39.7% | - |
| Ever | 73 | 60.3% | - |
| For HT users, age at first use | | | |
| ≤45 | 6 | 8.3% | - |
| 46-51 | 50 | 69.5% | - |
| ≥52 | 16 | 22.2% | - |
| For HT users, HT duration | | | |
| <5 years | 43 | 60.6% | - |
| 5+ years | 28 | 39.4% | - |
| Clinical neurological condition | | | |
| None | 114 | 90.5% | - |
| Depression | 3 | 2.4% | - |
| Epilepsy | 2 | 1.6% | - |
| MS | 2 | 1.6% | - |
| PD | 2 | 1.6% | - |
| Stroke | 2 | 1.6% | - |
| MCI | 1 | 0.8% | - |

*Descriptive statistics for continuous variables are presented as mean, standard deviation, and range. For categorical variables, the percentage of participants in each category are presented.*

*TBV=total brain volume; WMHV=white matter hyperintensity volume; SUVR=standarised uptake value ratio;* Aβ*=beta-amyloid; NAWM=normal appearing white matter; MD=mean diffusivity; FA=fractional anisotropy; CT=cortical thickness; ADsig=Alzheimer's Disease signature; TIV=total intracranial volume; SEP=socioeconomic position; BMI=body mass index; DBP=diastolic blood pressure; SBP=systolic blood pressure; APOE=apolipoprotein e; HT=hormone therapy; MS=multiple sclerosis; PD=Parkinson's Disease; MCI=mild cognitive impairment*

#### *5ii.3.2. Amyloid*

There was evidence for a non-linear association of menopause age with SUVR (β=-0.001[95% CI -0.002,0.000], p=0.02; Appendix C Table 18) and with amyloid status (OR=0.91[0.83,1.00], p=0.06; Appendix C Table 18), but only marginal evidence for *APOE* interactions (SUVR p=0.10; amyloid status p=0.78; Appendix C Table 19). Women who had menopause earlier or later had lower SUVR (Figure 18) and less likelihood of being amyloid positive than women who had menopause in the mid-range. Cumulatively adjusting for covariables and HT use did not alter the estimated associations (Appendix C Table 21), and there were no associations of HT use with SUVR or Aβ status (Appendix C Table 24).

### *5ii.3.3. WMHV*

There was no evidence for a non-linear association of menopause age on WMHV (p=0.79; Appendix C Table 18), but there was a significant modifying effect of *APOE*-ε4 status (p=0.03; Figure 19; Appendix C Table 20). *APOE*-ε4 carriers (n=35) showed a 12% decrease in WMHV per 1-year increase in menopause age (M0 relative WMHV change=0.88[0.79,0.98], p=0.03) while non-carriers (n=88) showed a non-significant 4% WMHV increase (M0 relative WMHV change=1.04[0.98 1.10], p=0.21). Further adjusting for covariables (Figure 19; Appendix C Table 22) did not substantially alter the effect estimates for ε4 non-carriers, but in carriers there was an effect attenuation when adjusting for health-related factors (M3). Individually adjusting for M3 covariables demonstrated that this effect attenuation was mostly driven by adjustment for smoking (Appendix C Table 25). Additional adjustment for HT use did not alter the model estimates for ε4 carriers or non-carriers, and there was no association of HT use with WMHV (Appendix C Table 24).

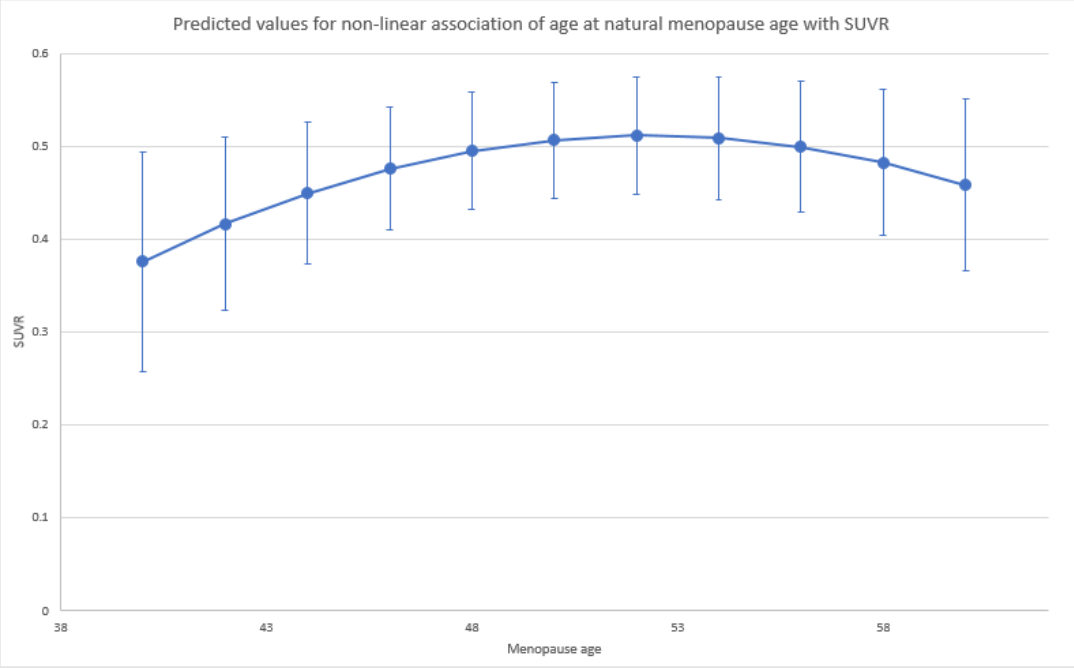


***Figure 18.*** *Predicted values and 95% confidence intervals for the non-linear association of age at natural menopause with amyloid SUVR, based on the fully adjusted model assuming mean values for continuous variables and reference categories for categorical variables (n=126).*

*Model adjusted for: age at scan, childhood cognition, education, SEP, puberty, parity, smoking, BMI, blood pressure, APOE-ε4 status*

*SUVR=standardised uptake value ratio; SEP=socioeconomic position; BMI=body mass index; APOE=apolipoprotein-e*

#### *5ii.3.4. TBV*

There was no evidence for a non-linear association of menopause age with TBV (p=0.57; Appendix C Table 18) or for effect modification by *APOE*-ε4 status (p=0.19; Appendix C Table 20). There was an overall positive association for increasing menopause age with greater TBV (Figure 20; M0:β=3.17[0.72,5.62], p=0.01), attenuated by adjustment for health-related covariables (Appendix C Table 23). Individually adjusting for M3 covariables revealed that this attenuation was mostly driven by blood pressure adjustments (Appendix C Table 26). Further adjustment for HT use did not alter the estimated association of menopause age with TBV (Appendix C Table 23), and there was no association of HT use with TBV (Appendix C Table 24).

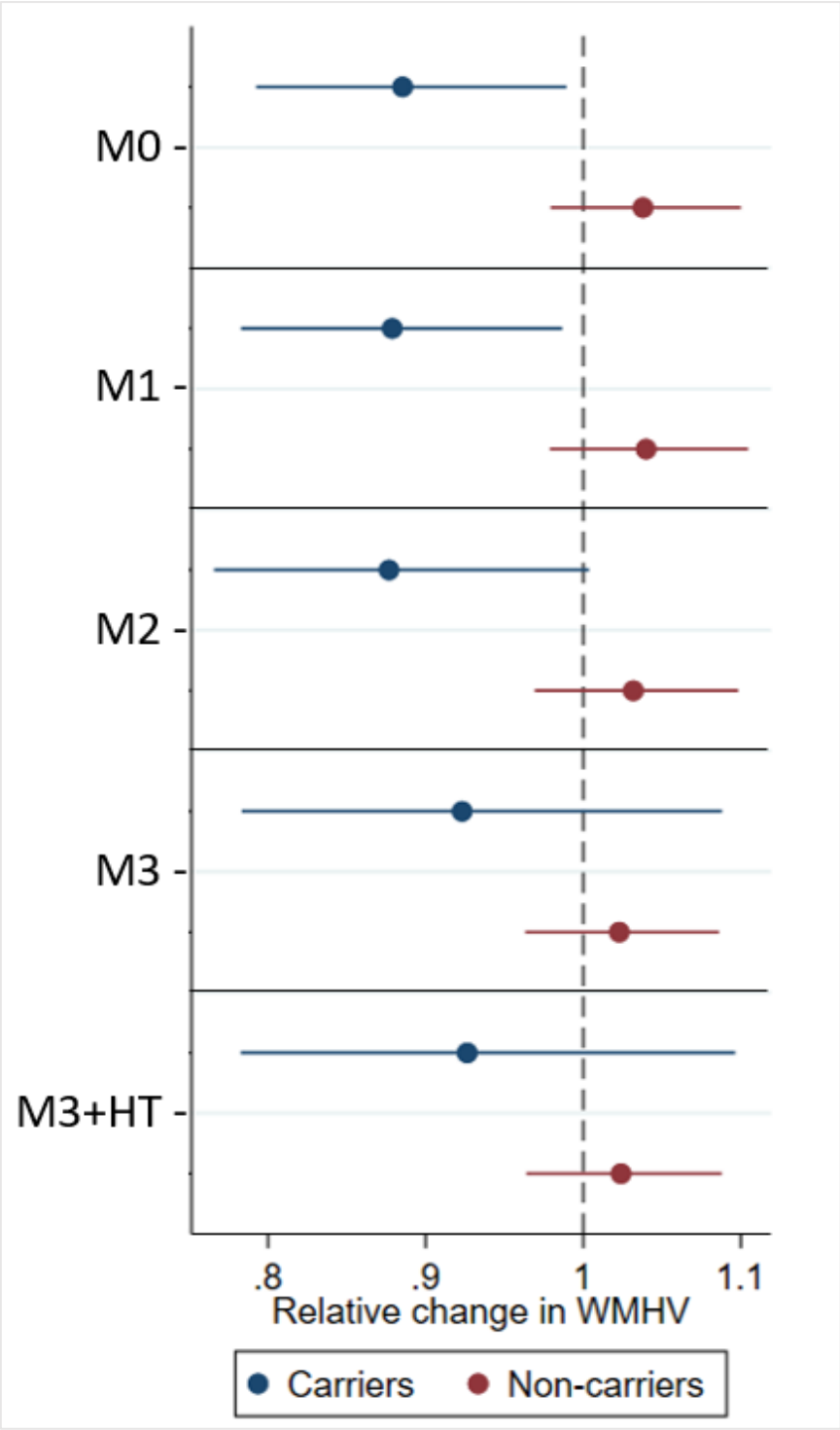


***Figure 19.*** *Model estimates and 95% confidence intervals demonstrating the predicted relative change in white matter hyperintensity volume (WMHV) per 1-year increase in menopause age, for APOE-ε4 carriers (n=35) and non-carriers (n=88), across models cumulatively adjusted for life course covariables.*

*M0:age at scan, TIV; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure*

*WMHV=white matter hyperintensity volume; HT=hormone therapy; TIV=total intracranial volume; SEP=socioeconomic position*

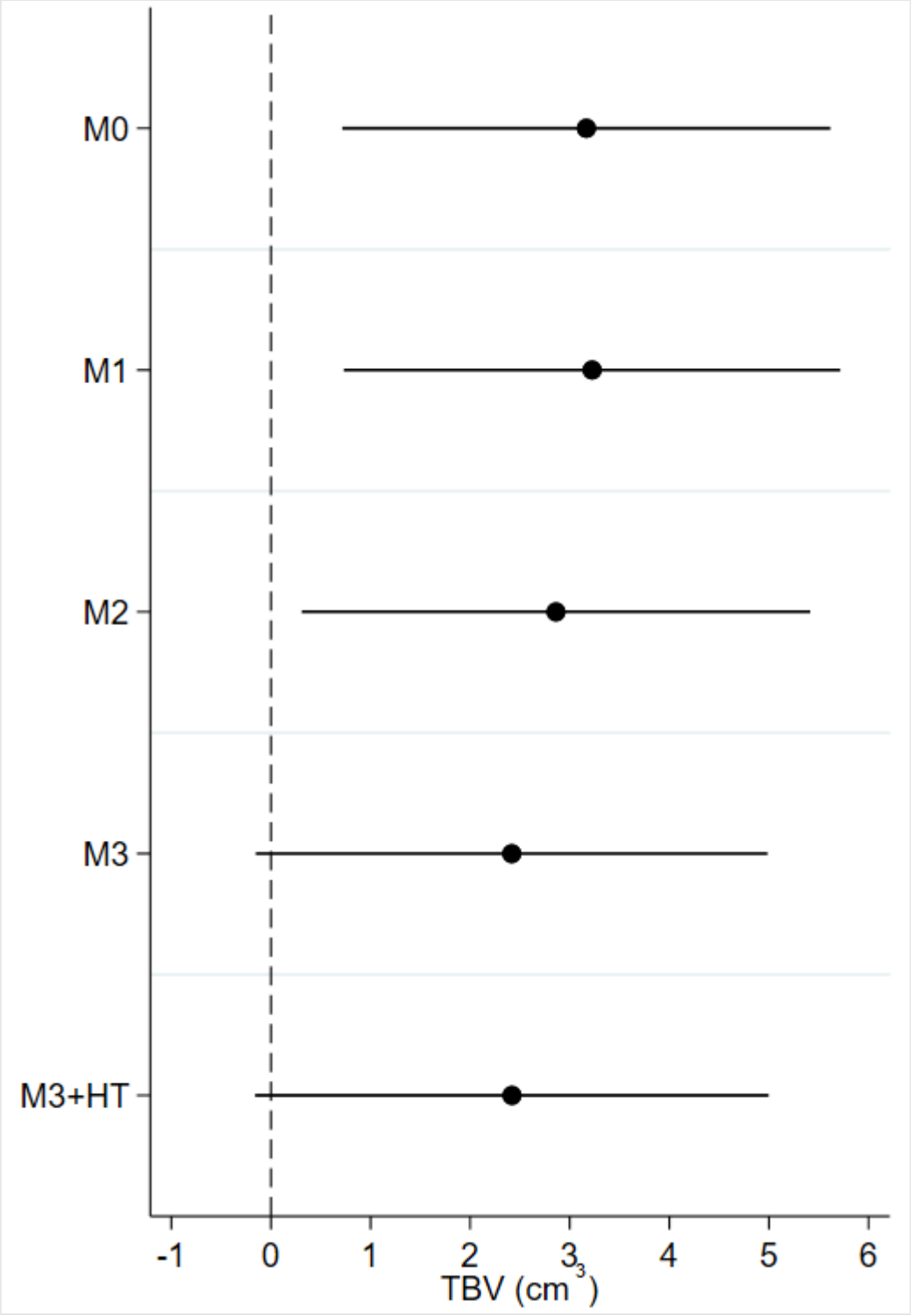


***Figure 20.*** *Model estimates and 95% confidence intervals demonstrating the predicted change in total brain volume (TBV) per 1-year increase in menopause age, for the pooled analytical sample (n=126) across models cumulatively adjusted for life course covariables.*

*M0:age at scan, TIV; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status*

*TBV=total brain volume; HT=hormone therapy; TIV=total intracranial volume; SEP=socioeconomic position*

#### *5ii.3.5. Hippocampal volume*

There was no evidence for non-linear associations (Appendix C Table 18), *APOE*-ε4 interactions (Appendix C Table 20), or for any meaningful association of menopause age with hippocampal volume (Appendix C Table 23). HT use was not associated with hippocampal volume (Appendix C Table 24).

#### *5ii.3.6. NAWM*

There was no evidence for non-linear associations (Appendix C Table 18), *APOE*-ε4 interactions (Appendix C Table 20), or associations of menopause age with NAWM FA or MD (Appendix C Table 23). HT use was not associated with NAWM measures (Appendix C Table 24).

#### *5ii.3.7. Cortical thickness*

There was no evidence for non-linear associations of menopause age with cortical thickness in any ROI (all $p>0.1$; Appendix C Table 18), but linear associations with all ROI were modified by *APOE*-ε4 status ($p<0.1$; Appendix C Table 20). Across all ROI, *APOE*-ε4 carriers (n=36) showed greater cortical thickness with increasing age at menopause (Table 22; Figure 21) while in non-carriers (n=90) cortical thickness was reduced with increasing menopause age (Table 22; Figure 21). For the frontal, occipital and temporal ROI, model estimates did not substantially change with cumulative adjustments for life course covariables (Figure 21, Appendix C Table 22). However, in ε4 carriers the positive association of menopause age with cortical thickness in the Harvard ADsig ROI was attenuated with adjustment for reproductive factors, driven by adjustment for parity, and the positive association with parietal cortical thickness was attenuated with adjustment for health-related factors (Figure 21, Appendix C Tables 27 and 28). Additionally adjusting for HT use did not substantially alter the effect estimates for ε4 carriers or non-carriers across all ROI (Figure 21), and HT use did not associate with cortical thickness in any ROI (Appendix C Table 24).

***Table 22.*** *Model estimates and 95% confidence intervals demonstrating the predicted change in cortical thickness regions of interest (ROI), based on minimally adjusted models (M0), for APOE-ε4 carriers (n=36) and non-carriers (n=90).*

| | ROI (mm) | β | Lower 95% CI | Upper 95% CI | P-value |
|---|---|---|---|---|---|
| ε4 carriers | Harvard ADsig | 0.009 | 0.002 | 0.017 | 0.02 |
| | Frontal | 0.007 | -0.003 | 0.016 | 0.13 |
| | Occipital | 0.006 | -0.004 | 0.017 | 0.24 |
| | Parietal | 0.011 | 0.004 | 0.019 | <0.01 |
| | Temporal | 0.010 | -0.001 | 0.020 | 0.06 |
| ε4 non-carriers | Harvard ADsig | -0.004 | -0.009 | 0.001 | 0.09 |
| | Frontal | -0.003 | -0.009 | 0.002 | 0.27 |
| | Occipital | -0.003 | -0.009 | 0.002 | 0.20 |
| | Parietal | -0.003 | -0.008 | 0.001 | 0.16 |
| | Temporal | -0.004 | -0.010 | 0.002 | 0.17 |

*M0:age at scan, TIV*

*ROI=regions of interest; CI=confidence interval; ADsig=Alzheimer's Disease signature region*

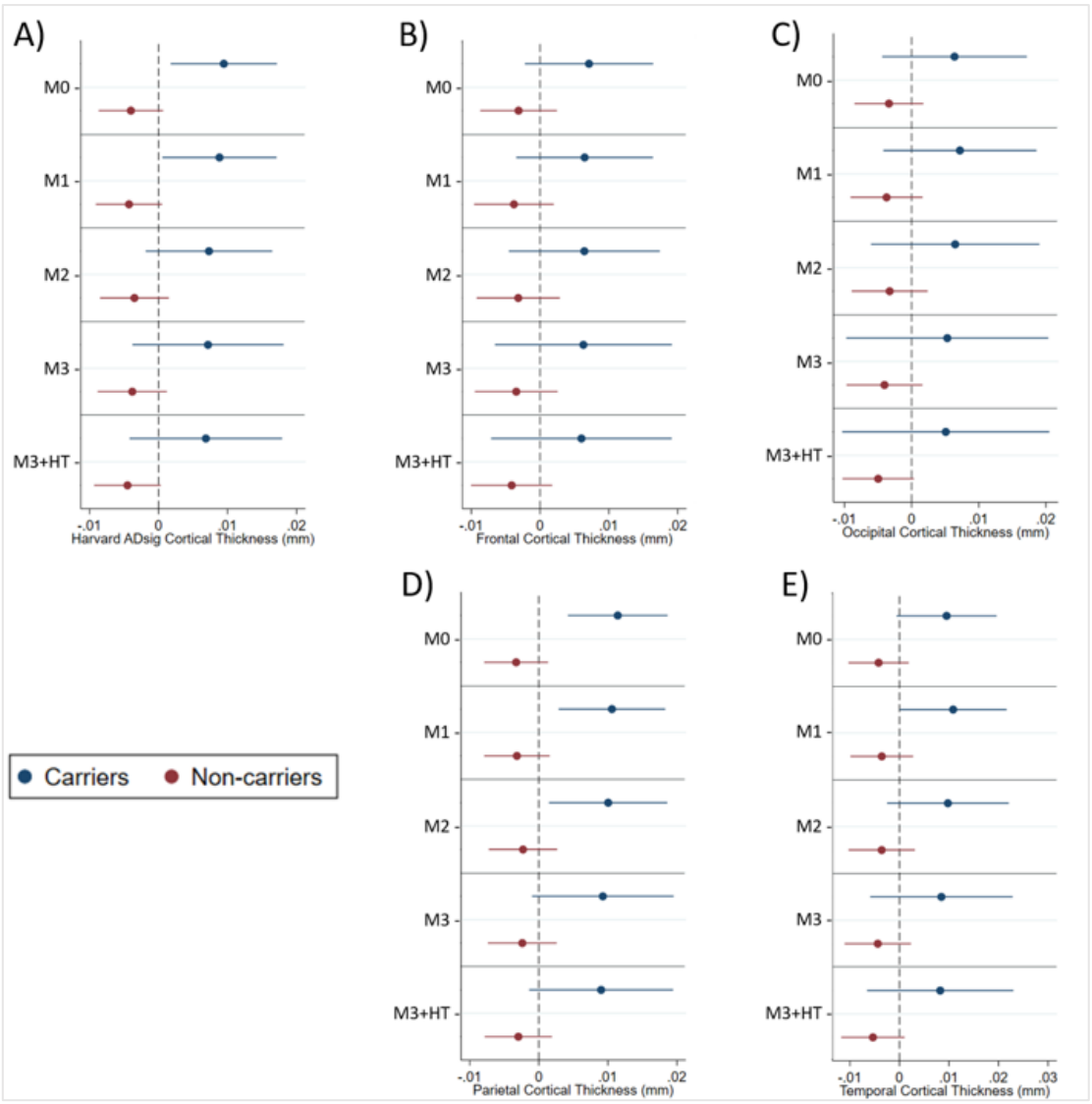


***Figure 21.*** *Model estimates and 95% confidence intervals demonstrating the predicted change in cortical thickness regions of interest (ROI; A=Harvard ADsig, B=Frontal, C=Occipital, D=Parietal, E=Temporal) per 1-year increase in menopause age, for APOE-ε4 carriers (n=36) and non-carriers (n=90), across models cumulatively adjusted for life course covariables.*

*M0:age at scan; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status[pooled analyses only]*

*ADsig=Alzheimer's Disease signature region; HT=hormone therapy; SEP=socioeconomic position*

#### *5ii.3.8. Sensitivity analyses*

When adjusting non-WMHV outcome models for WMHV, the results did not differ from the main analyses (Appendix C Tables 29-31).

In analyses excluding the twelve women with neurological conditions (Appendix C Tables 32-37), results for amyloid SUVR, WMHV, hippocampal volume, and most cortical thickness ROI did not substantially differ from the main analyses. For amyloid status there was no longer evidence for a non-linear association with menopause age (n=114; p=0.23). For TBV, a modifying effect of *APOE*-ε4 status was detected (n=114; p=0.08) whereby the positive association of later menopause age with greater TBV was stronger in ε4 carriers (n=35) than in non-carriers (n=79). There was also evidence of an *APOE*-ε4 interaction for NAWM measures (n=102; FA p=0.08, MD p=0.02). In ε4 carriers (n=30), no associations of menopause age with FA or MD were detected, but in non-carriers (n=72) later menopause age associated with reduced FA and increased MD. Therefore, in ε4 non-carriers increasing menopause age associated with poorer NAWM microstructural integrity. There was no longer a significant *APOE*-ε4 interaction for frontal cortical thickness (n=114; p=0.10).

When excluding the two women who had early menopause (aged <45 years; Appendix C Tables 38-43), non-linear associations of menopause age with SUVR and amyloid status were replicated, as were menopause age-by-*APOE*-ε4 interactive effects on WMHV and cortical thickness measures, except for frontal cortical thickness (n=124; p=0.111). As in the main analyses, no associations of menopause age with hippocampal volume or NAWM FA were detected. An interactive effect of menopause age and *APOE*-ε4 on TBV was detected (n=124; p=0.080) whereby ε4 carriers (n=36) showed a stronger positive relationship between later menopause age and greater TBV than non-carriers (n=88). An effect modification by *APOE*-ε4 was also detected for NAWM MD (n=109; p=0.081); in non-carriers (n=78) but not in carriers (n=31), later menopause age associated with greater MD.

### 5ii.4. Discussion

#### *5ii.4.1. Key findings*

As expected, based on previously reported associations of later menopause with better cognitive performance (Section 5i.),[154-156,317] overall positive associations of natural

menopause age with better brain health at age ~70 were found, such as greater total brain volumes with increasing menopause age. Some associations were modified by *APOE*-ε4 genotype. Cortical thickness, for example, was positively associated with menopause age in ε4 carriers while non-carriers showed non-significant negative associations. Overall, associations were independent of life course covariables, including HT use. However, midlife blood pressure and smoking partially explained some associations.

#### *5ii.4.2. Interpretation of findings*

These findings generally support the hypothesis that prolonged exposure to endogenous oestrogen with increasing menopause age is beneficial for later-life brain health. However, HT use - which indicates exogenous oestrogen exposure - was not found to contribute to menopause age associations with brain health, nor was HT use associated with the neuroimaging outcomes assessed. Variations in HT dosages, administration routes, formulations, durations, and timing of initiation in relation to menopause are sources of discrepancy within HT literature[170,329] and require further examination before conclusions can be drawn on the effects of HT on later-life brain health.

However, it is surprising that no association with hippocampal volume - a key region implicated in AD[338] - was detected despite previous evidence demonstrating the hippocampi as oestrogen-sensitive regions[339,340] and that, in this cohort, a cognitive domain most strongly linked with menopause age is memory (Section 5i.),[317] which is subserved by the hippocampi.[338] Although replication and further investigation is needed, this finding could indicate that menopause age is not associated with AD-specific regional pathology, rather with more global measures of brain health.

Later menopause age was linked with greater TBV, generally a positive indicator of brain health since TBV reduces with advancing age and in dementia,[341] contrasting previous reports of null or negative associations.[164,333] However, previous studies differed from the current analyses as they: excluded HT users, did not include women with menopause age younger than 45, and had larger age ranges at neuroimaging. The association detected with TBV here was slightly attenuated by adjustments for health-related factors, primarily driven by blood pressure at age 36. While evidence shows increases in blood pressure during and post-menopause,[342]

examination of pre-menopause blood pressure in relation to menopause age is scarce. Findings elsewhere do, however, demonstrate that higher blood pressure typically associates with smaller TBV,[98,343,344] which could explain the effect attenuation observed here.

In the existing literature, menopause age has not been examined in relation to Aβ, but these analyses demonstrate evidence for non-linear associations whereby continuous Aβ levels and the likelihood of being Aβ positive were lowest in women with the earliest and latest ages at menopause. Reasons for this finding are unclear and could be driven by methodological factors such as using a white matter reference region; these results require replication and further examination.

Although no moderating effects of *APOE*-ε4 status for amyloid measures were detected, ApoE - a lipid transporter protein closely linked with cholesterol[92] - is known to have a role in Aβ deposition and metabolism,[92,345] and *APOE*-ε4 genotype is linked with higher Aβ levels.[346] The evidence linking *APOE*-ε4 genotype with menopause age is mixed[319,320] and, in this sample, no significant differences in menopause age between ε4 carriers and non-carriers were detected. Nonetheless, some findings report links between ApoE, female reproductive factors, and brain health, indicating potential mechanisms through which *APOE* genotype might interact with menopause-brain associations. For instance, cholesterol levels are shown to increase across the menopause transition and post-menopause[347] and lower cholesterol levels pre-menopause have been linked with later age at menopause.[348] ApoE is also shown to facilitate neuroprotective effects of oestrogen while oestrogen can also stimulate ApoE production in the brain.[349]

For cortical thickness and WMHV, stronger positive associations of menopause age with brain health were detected in ε4 carriers than in non-carriers. Given that ε4 carriers are more prone to brain changes leading to dementia-related pathology,[350,351] having prolonged exposure to neuroprotective oestrogen (through having menopause at a later age) could be particularly beneficial to ε4 carriers in limiting the build-up of brain pathologies. Alternatively, an earlier age at menopause could be particularly detrimental for ε4 carriers.

Although research linking *APOE* genotype with menopause and cortical thickness is lacking, some research does indicate sex-specific associations of *APOE* genotype and cortical thickness; healthy female ε4 carriers showed greater cortical thinning than female non-

carriers, while healthy male carriers and non-carriers showed no differences in cortical thinning.[352] For white matter hyperintensities, indicative of cerebrovascular disease (specifically cSVD),[353] higher cholesterol levels associated with greater WMHV[354] provides some plausibility for cholesterol serving some mechanistic role in links between menopause, *APOE* genotype, and WMHV. However, the association of menopause age and WMHV in ε4 carriers was partially explained by smoking at age 36. Previous research has associated smoking with earlier age at menopause, possibly due to anti-oestrogen effects of tobacco,[355] and with increased WMHV which reflects adverse effects of smoking on vascular health.[356,357] Such associations could be reflective of smoking as a marker for poorer general health and social disadvantage, although adjustments for health-related factors such as hypertension did not attenuate the association of smoking with WMHV progression over a 10 year follow-up.[356]

When excluding women with neurological conditions, some *APOE* interactions were unmasked. *APOE*-ε4 carriers showed stronger positive associations of menopause age with TBV, and in non-carriers increasing menopause age associated with poorer NAWM microstructural integrity. It is worth noting that excluding women with neurological conditions did exclude one woman in the main analytic sample who had an early menopause (<45 years); changes in the results could reflect potential effects of early menopause, rather than the effects of neurological conditions. Indeed, a sensitivity analysis excluding two women who had menopause younger than age 45 demonstrated similar effect modifications by *APOE*-ε4 status. Nonetheless, previous analyses of Insight 46 data showed sex differences in NAWM; females had poorer FA and, specifically in females, higher systolic blood pressure and cardiovascular risk scores associated with poorer FA.[237] Further research is required to determine the mechanisms underlying menopause associations with NAWM microstructural integrity and how menopause, or other female reproductive variables, might contribute to poorer cerebrovascular health observed in females compared with males.

#### *5ii.4.3. Strengths and limitations*

A major strength of this research is the range of multimodal neuroimaging measures examined, facilitating an examination of which indicators of brain health do and do not associate with menopause age. This study also uses life course data prospectively recorded over 70 years, providing a valuable opportunity to examine prospectively recorded age at

menopause in relation to later-life brain health, where other studies are reliant on retrospectively recorded menopause age which does not offer the same level of accuracy and could also be prone to error.

However, as a sub-study of the NSHD cohort, Insight 46 has a limited sample size which is further reduced when confining analyses to females. This may limit the potential power to detect statistically significant relationships, particularly when interrogating potential differences in estimates stratified by *APOE*-ε4 genotype. Nonetheless, that some effect modifications by *APOE*-ε4 were detected is encouraging and provides justification for further research, including using larger cohorts. Additionally, as will be discussed in Section 6.3., there is a selection bias within Insight 46 whereby participants tend to have better general health and higher socioeconomic backgrounds than the whole-NSHD sample.[194] There is a need for the current findings to be replicated in more diverse cohorts, particularly given that Insight 46 (and the whole-NSHD cohort) is representative only of white British individuals born in 1946. Finally, while these analyses included women who took HT, with adjustments for HT use and examination of associations between HT use and neuroimaging outcomes, there is recognition that further research is needed to examine how variations in HT, such as dosages and timing of HT initiation in relation to menopause, might associate with later-life brain health.

#### *5ii.4.4. Summary*

Later age at natural menopause generally associated with better brain health at age ~70 (particularly with reduced non-specific brain ageing and fewer markers of cSVD) and some associations were stronger in *APOE*-ε4 carriers than in non-carriers. Most associations were independent from life course covariables, including HT use, although midlife smoking and blood pressure partially explained some relationships. These findings support the notion that later menopause age is beneficial for brain health given prolonged exposure to endogenous oestrogen. These findings can also be interpreted such that earlier menopause age, indicating reduced oestrogen exposure, increases risk for adverse brain health outcomes. Further research is needed to replicate these findings in other cohorts and to determine the underlying mechanisms.

## 5.1. Summary

Together, the analyses conducted in this empirical section demonstrate that, overall, later age at menopause is beneficial for later-life cognitive performance and brain health, aligning with previous research indicating that later age at menopause is protective. Alternatively, the same results may indicate that an earlier age at menopause is detrimental for later-life cognition and brain health. Not all associations were independent of life course covariables; notably, childhood cognition mostly explained associations of menopause age with later-life cognition, indicating some upstream, developmental factor might explain the associations, or perhaps higher childhood cognition is a proxy indicator for increased lifetime oestrogen exposure. While evidence was found for an effect modification by *APOE*-ε4 status in associations between menopause age and brain health measures, *APOE* genotype did not modify associations of menopause age with cognitive performance. A possible explanation for this discrepancy could be that dementia-related brain pathology develops and can be detected prior to the onset of cognitive symptoms. Since the NSHD and Insight 46 cohorts are mainly healthy and dementia free at the time of cognitive testing and neuroimaging, any differences in cognitive performance between ε4 carriers and non-carriers in relation to menopause age could be subtle and not detected. Further examination of associations between menopause age, *APOE* genotype, and cognitive performance assessed in subsequent data collection waves is warranted.

# 6.0. Discussion

## 6.1. Summary of key findings

This thesis examined sex differences in cognitive performance across the life course and in measures of brain health in later-life. There was consideration of how various developmental, sociodemographic, lifestyle, genetic, and sex-specific reproductive factors might contribute to later-life cognition and brain health, within a mostly cognitively unimpaired birth cohort. The findings of each analysis are discussed in the relevant chapters, with the key findings also summarised below.

A) A descriptive narrative of sex differences and similarities in cognitive performance across domains, throughout life, and in neuroimaging indicators of brain health in later-life was produced (Chapter 3). The role of socioeconomic, educational, and lifestyle (smoking, physical activity/PA) covariables in sex differences were examined, alongside the potential for *APOE*-ε4 genotype to modify sex differences.

   i. **Cognitive performance:** Differences in mean performance between males and females were detected across a range of cognitive assessments, at several timepoints throughout the life course. Females showed better performance on more cognitive tests than males, but most notably on tasks assessing verbal and memory domains. Males, on the other hand, tended to show non-verbal and visuospatial task advantages. When accounting for socioeconomic and educational covariables, which were higher in males, female cognitive performance advantages were strengthened while male advantages were weakened, reflecting negative and positive confounding, respectively. Lifestyle covariables (smoking, PA) did not substantially contribute to cognitive performance sex differences. Generally, sex differences in cognitive performance in younger adulthood were not modified by *APOE*-ε4 status, but some *APOE* effect modifications were detected at older ages, demonstrating stronger female memory performance advantages in ε4 non-carriers than in carriers.

   ii. **Brain health:** At age ~70 years, females showed larger total brain volumes (relative to head size) than males. Females also showed poorer cerebrovascular health (specifically, greater indicators of cerebral small vessel

disease/cSVD) than males, indicated by greater white matter hyperintensity volumes and poorer microstructural integrity of normal-appearing white matter (NAWM), but no sex differences were detected in measures associated with AD (hippocampal volume and Aβ levels). Socioeconomic, educational, and lifestyle covariables did not substantially contribute to sex differences in neuroimaging measures and effect modification by *APOE*-ε4 status was detected only for NAWM measures, whereby only female non-carriers showed poorer NAWM integrity.

B) To quantify lifetime exposures to twelve identified modifiable dementia risk factors, a cumulative risks score (CRS) was derived from life course data (Chapter 4). The extent to which early cognitive, reproductive, socioeconomic, and genetic factors associated with cumulative risk exposures, and whether CRS associated with later-life cognitive performance and brain health were compared in males and females. This was to determine whether the potential associations with non-sex-specific dementia risk factor exposures were stronger in one sex than the other. Interactive effects of *APOE*-ε4 status and CRS on later-life outcomes were also examined in both sexes.

   i. **Cumulative risk exposures:** In the whole cohort, males had more cumulative exposure to modifiable dementia risk factors across the life course than females. In both sexes, higher childhood cognitive performance, childhood SEP, and *APOE*-ε4 non-carrier status associated with greater CRS. These associations were not replicated in the neuroimaging sub-study sample.

   ii. **Cognitive performance:** Independently of genetic and early-life cognitive and socioeconomic predictors of CRS, greater cumulative risk exposures associated with poorer cognitive performance in males and females at age 69. Associations of CRS with cognitive state were equal between males and females, while the association with verbal memory performance was slightly (non-significantly) stronger in females, and the association with a measure of processing speed was only detected in males. No effect modifications by *APOE* genotype were found.

   iii. **Brain health:** In males and females, greater risk exposures associated with smaller TBV although this was stronger in males. Only males showed an

association of greater CRS with smaller hippocampal volume, and neither sex showed associations of CRS with measures of cSVD (WMHV) or AD pathology (Aβ). Associations were not modified by *APOE*-ε4 status.

C) Prospectively reported age at menopause, as a female-specific hormonal transition, was examined for its associations with cognitive performance across a range of domains and with neuroimaging measures of brain health (Chapter 5). The extent to which these associations could be explained by relevant early cognitive, sociodemographic, reproductive, genetic, and health-related covariables was also examined, alongside tests for whether *APOE*-ε4 genotype modified associations of menopause age with later-life outcomes.

   i. **Cognitive performance:** Later age at menopause associated with better performance across a range of cognitive domains, but the strongest associations were found for the domains of visual processing, associative learning, and memory. These associations were largely explained by covariables, most notably by cognitive performance in childhood, which is positively associated with menopause age and later-life cognitive performance. There was no evidence for interactive effects of *APOE*-ε4 genotype and menopause age.

   ii. **Brain health:** Later menopause age generally associated with better indicators of brain health (greater TBV, for example), although these were partly explained by covariables, particularly by health (blood pressure) and lifestyle (smoking) factors. Some evidence for interactive effects of *APOE* genotype was found whereby ε4 carriers showed beneficial associations of later menopause age with reduced WMHV and greater cortical thickness.

Figure 22, which will be referenced in the following sections of this discussion, summarises the interrelationships between each of the three empirical chapters and provides an illustrative schema of causal directions through which the findings can be interpreted.

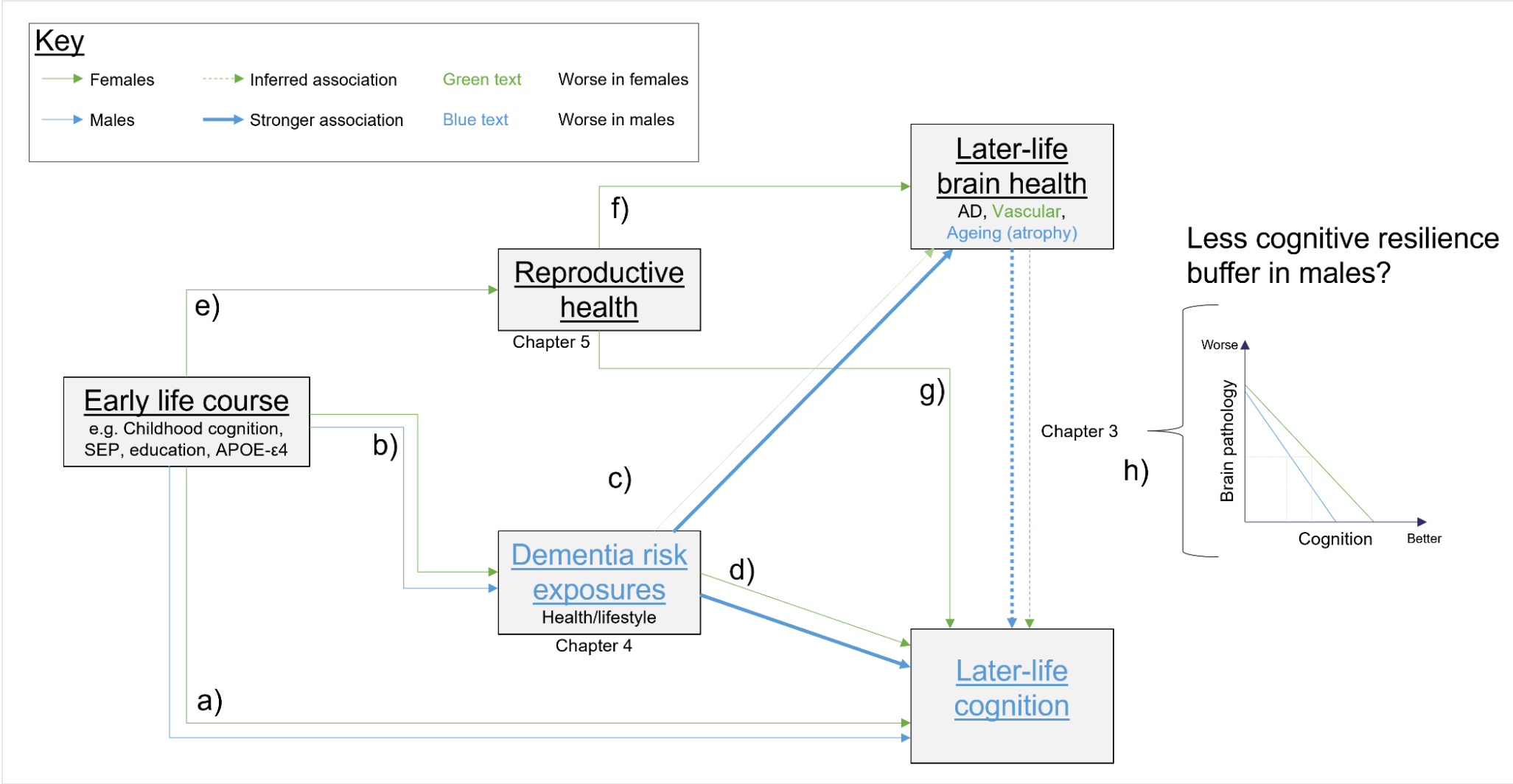


***Figure 22.****Illustrative schema of causal directions between life course variables, later-life brain health, and later-life cognitive performance in males and females, as described and assessed in each of the empirical chapters in this thesis.*

a) *Known early-life predictors of later-life cognitive performance; higher childhood cognition, higher educational attainment, and APOE-ε4 non-carrier (vs. carrier) status associated with better later-life cognitive performance on the Addenbrooke's Cognitive Examination-3rd edition (ACE-III).*[159]

b) *Greater childhood cognition and socioeconomic position (SEP), and APOE-ε4 carrier (vs. non-carrier) status associated with fewer lifetime dementia risk factor exposures (Chapter 4). Note that education was included in the cumulative risks score (CRS), so education was not further adjusted for in Chapter 4 analyses.*

c) *Greater dementia risk exposures (increased CRS) exacerbated brain ageing in males; males showed negative associations of increased CRS with smaller brain volumes (total brain volume and hippocampal volume), while females showed a trend-level association with total brain volume but no associations with hippocampal volume (Chapter 4).*

d) *Greater dementia risk exposures adversely associated with later-life cognitive performance, with more associations detected for males than females. Both sexes showed an equal negative association of CRS with cognitive state, females showed a slightly stronger association with verbal memory, while only males showed an association with processing speed performance (Chapter 4).*

e) *Higher childhood cognition associated with later menopause age*[156,157] *(Chapter 5).*

f) *Later menopause age associated with reduced brain ageing (greater brain volume) and with better cerebrovascular health (Chapter 5); in APOE-ε4 carriers, later menopause age associated with reduced white matter hyperintensity volume (WMHV).*

g) *Later menopause age associated with better later-life cognitive performance, although this is largely explained by childhood cognition*[155] *(Chapter 5).*

h) *Some existing evidence from Insight 46 has demonstrated associations of poorer brain health measures (amyloid positivity, greater WMHV, lower brain volumes) with poorer later-life cognitive performance, although sex differences in these associations are not explicitly examined.*[40,158,217,218,236]

*Females showed poorer cerebrovascular health, but better cognitive performance than males (Chapter 3), indicating a female cognitive resilience buffer against cerebrovascular pathology. As depicted in the illustrative line graph, reduced cognitive resilience buffer in males suggests a steeper brain health-cognition association in males such that, for a given level of brain pathology, males will show poorer cognitive function. Note that this graph is illustrative; brain-cognition associations could be non-linear and may differ once pathology reaches a certain threshold (see Section 6.2.6.).*

## 6.2. Interpretation of key findings

### 6.2.1. Sex differences in reserve and resilience

As outlined in Section 1.6., resilience is the ability to maintain cognitive function despite brain ageing and pathology. This is achieved through mechanisms including cognitive and brain reserve, which refer to cognitive adaptability and structural brain characteristics, respectively.[45] Depicted in Figure 22, the findings from this thesis indicate differences in how well males and females can maintain cognitive function with brain ageing and pathology, and maintain cognitive function and brain health with exposures to life course health and lifestyle risks.

In Chapter 3, females were found to maintain cognitive performance advantages into later-life, despite showing poorer cerebrovascular health (increased cSVD markers) than males. Insight 46 females therefore demonstrated cognitive resilience - cognitive abilities were maintained despite cSVD pathology. Given that females outperformed males on most cognitive assessments throughout the life course, it is plausible that females have increased cognitive reserve, with greater cognitive resources and functional networks which can be drawn on and adapted to minimise the functional impacts of brain pathologies. Although the direct associations of brain health and cognitive performance have not been assessed in this thesis, colleagues have assessed these associations in the Insight 46 sample, generally indicating poorer cognitive performance with more advanced brain ageing and pathologies. Excluding participants with cognitive impairment, poorer performance on the PACC at age ~70 has been associated with amyloid positivity, increased WMHV,[158] and older predicted brain age,[236] but not with NAWM[40] or total brain volume.[158] While sex differences in these associations have not been explicitly tested in this cohort, the relationship between brain health and cognitive performance is hypothesised to be stronger and steeper in males, as outlined in Figure 22h. In a non-dementia sample, at a given level of brain pathology, males are expected to show poorer cognitive ability than females, given less cognitive reserve to protect against the adverse effects of pathology on cognitive function.

Similarly, findings from Chapter 4 suggest that males are less able to resist the adverse effects of accumulated modifiable dementia risk factor exposures than females on cognitive performance (Figure 22c) and brain volumes (Figure 22d). Given that these associations are stronger in men,

and men were more likely to have greater exposure to modifiable dementia risks, cognitive and brain volume sex differences favouring women observed in Chapter 3 could reflect reduced cognitive and brain reserve in males, diminished by modifiable lifestyle risk exposures. Indeed, more male cognitive advantages were observed during childhood and young adulthood, when fewer risk exposures have been accumulated, than in later-life (Chapter 3). It also follows that fewer cumulative risk exposures in females, and reduced effects of cumulative risks on brain ageing (brain volumes) in females, contributes to the preservation of female structural brain resources (brain reserve), thereby facilitating female cognitive resilience to cerebrovascular pathology.

### 6.2.2. Brain ageing vs. cerebrovascular health

Findings from Chapters 3 and 4 demonstrate that more advanced brain ageing in males (smaller relative brain volumes) is linked with accumulated dementia risk exposures, while poorer brain health in females, specific to cSVD risk, is not. Although a measure of cumulative risk exposures was not associated with cSVD markers (WMHV), other Insight 46 analyses associated individual risk factors with later-life brain health. These relationships are most evident in females and particularly demonstrate links of midlife cardiovascular risks with later-life cerebrovascular health; glycemia (an indicator of diabetes) measured from age 53 to 69 associated with reduced TBV only in females,[129] and only in females did greater cardiovascular risk and blood pressure in midlife associate with poorer NAWM integrity.[40] Taken together, evidence indicates potential variations in how accumulated or individual risk factor exposures associate with age- and vascular-related brain health between the sexes; males show greater brain ageing vulnerability to accumulated risks, while females are more vulnerable to cerebrovascular impacts of individual risks, particularly cardiovascular risks present in midlife.

Nonetheless, both sexes did show adverse associations of accumulated risk exposures with later-life cognitive performance, albeit across more domains in males than females (Chapter 4). As shown in Figure 22(c,d,h), CRS-cognition associations could be mediated through CRS-associated brain ageing in males, but less so in females, in whom the potential pathways between

accumulated risks and cognitive performance are less clear. Aside from brain health, societal factors linked with cumulative health and lifestyle risks also warrant consideration. For instance, the traditional caregiving role of women could mean that men with greater risk factor exposures receive more support than women, who might manage their own health to a greater extent than men do. Women with greater cumulative risk exposures could take on additional burdens, such as managing health appointments and medications, which could provide stimulation to help maintain cognitive abilities, but could also divert cognitive resources away from the functional systems required to complete cognitive assessments. Additionally, female underrepresentation in clinical research means that the symptoms for some clinical conditions might not accurately reflect female clinical presentations. Females can therefore experience delayed diagnoses and subsequent treatments or support when they present with 'atypical' symptoms; the health worries associated with such delays could adversely impact cognitive functioning.

It is also possible that subtle effects of individual risks on brain health are diluted when these risks are included in a cumulative measure; the absence of significant CRS-brain health associations does not necessarily mean that mediating effects of brain health are not present in females. If there are such mediating effects, fewer CRS-cognition associations in females are assumed to reflect female cognitive resilience to cSVD. Further, female-specific exposures not included in the CRS could provide pathways through which accumulated risks may associate with brain health in females. For example, the menopause transition has been linked with several risks included in the CRS (e.g. hypertension, diabetes, weight gain, and obesity)[167] and Chapter 5 demonstrates associations of menopause age with TBV and cSVD. It is possible that menopause-related variables (e.g. timing, symptoms) interact with accumulated risks, providing a mechanism through which accumulated risks might influence female brain health.

### 6.2.3. Midlife and menopause as a sensitive period for females

As mentioned above, menopause, which typically occurs during midlife, has been associated with several cardiovascular risks, with peri- and post-menopausal women showing increased blood pressure and BMI, for example, than pre-menopausal women,[167] even when groups are age-

matched.[358] Additionally, cardiovascular risk prevalence is greater in males up until midlife, when female prevalence increases to match that of males.[167] Given that oestrogen has both vascular and neuroprotective properties,[83,84,359] declining oestrogen levels during menopause could contribute to increased cardiovascular risks and vulnerability of the brain to those risks. Menopause could therefore act as a trigger for cardiovascular risks and further risk factor accumulation by inducing a chain of risks. Female risk factor accumulation may be more concentrated at midlife and beyond, potentially with more rapid risk accumulation, while male risk exposures are more evenly dispersed across the life course, possibly exerting more prolonged influence on brain ageing (Chapter 4, Figure 22c). Indeed, Chapter 4 sensitivity analyses did demonstrate that risks accumulated up to and including midlife already associated with later-life brain volumes.

There were also parallels in the examination of the interrelationships between cumulative risk exposures and menopause timing. It was previously reported that later menopause age associated with better verbal memory performance but not with performance on an assessment of processing speed.[155] Similarly, cumulative risk exposures in females associated with poorer verbal memory performance but not with processing speed. While not tested in this thesis, it is possible that earlier menopause timing increases cumulative risk exposures by shifting the female sensitive period to cardiovascular risks and subsequent risk accumulation earlier. Interactive effects of cumulative risk exposures and menopause timing are therefore expected whereby women with earlier menopause are more likely to show stronger CRS associations with cognition (particularly verbal memory) and brain ageing (given positive associations of menopause age with TBV; Figure 22f) than women with later menopause timing.

### 6.2.4. The role of *APOE* genotype

Chapter 3 analyses showing reduced later-life memory performance in female *APOE*-ε4 carriers than non-carriers align with reports of greater *APOE*-ε4-associated dementia risk in females than males.[95] However, the mechanisms underlying this sex difference in ε4 dementia risk are not yet well understood.

Interestingly, Chapter 5 demonstrated menopause associations with cerebrovascular health only in ε4 carriers (later menopause age, reduced cSVD markers), indicating a greater sensitivity to menopause timing among carriers than non-carriers. Given the role of ApoE protein as a lipid transporter, including in the delivery of the cholesterol precursor needed for oestrogen synthesis,[92,360] there are biological mechanisms through which *APOE* genotype and menopause can plausibly interact. For example, adipose tissue (which increases at menopause) becomes the primary source of endogenous oestrogen post-menopause,[361] when ovarian oestrogen production declines. It is possible that earlier menopause timing results in an earlier switch to reliance on adipose-derived oestrogen, which could be disadvantageous for *APOE*-ε4 carriers, given that ε4 disrupts ApoE lipid transporter function.[92] In partial support of this hypothesis, there is some evidence for interactive effects of BMI and *APOE* genotype on female, but not male, predicted brain age.[362] However, higher BMI associated with younger predicted brain age in ε4 carriers, in the opposite direction to expected if ε4 disrupts adipose tissue oestrogen synthesis.

Alternatively, ε4 carriers metabolise glucose less efficiently than non-carriers and are more reliant on ketone bodies (produced from fatty acids)[363] as an energy source.[318] Glucose metabolism, supported by oestrogen, also declines during menopause,[163] hence greater reliance on ketone metabolism post-menopause could be exacerbated in ε4 carriers. Some literature suggests that over-reliance on ketone metabolism could leave brain white matter, as a ketone source, vulnerable to degeneration.[318,364] Therefore, ε4 carriers who undergo menopause at earlier ages could have prolonged over-reliance on ketone metabolism, adversely impacting white matter integrity.

While interactive effects of *APOE* with menopause age were found, there was no evidence for *APOE* interactions with cumulative risk exposures in either sex (Chapter 4). Taken together, these findings could indicate that ε4 contributes to female dementia risk via female-specific reproductive processes, such as menopause, rather than by increasing the vulnerability of the female brain to accumulated health and lifestyle risks which are not sex-specific. However, more work is clearly needed to understand the mechanisms through which *APOE*-ε4 genotype increases dementia risk more greatly in females.

### 6.2.5. Early-life and sociocultural influences

A particular strength of the NSHD cohort is the wealth of prospective data from across the life course, including early-life which many other studies of ageing are lacking. With previous NSHD analyses demonstrating associations of early-life variables, including childhood cognition and parental SEP, with later-life cognitive performance,[159] such early-life covariables were considered throughout the analyses in this thesis.

There were some variations in the extent to which early-life covariables explained associations of predictor variables with later-life cognition and brain health outcomes. Menopause-cognition associations were largely explained by early-life factors (primarily childhood cognition; Chapter 5) while cumulative risk exposure associations with cognitive and brain health measures were not (Chapter 4). As described in Chapter 5, childhood cognition could be a proxy measure of some upstream developmental process during infancy or *in utero,* possibly reflecting the role of sex hormones on brain development.[156] This could represent a sensitive period during which the pathways linking female reproductive and cognitive ageing are established. However, this thesis has not explicitly tested the sensitive period model, which would be methodologically challenging (see Section 1.1.). For example, repeated measures of endogenous and exogenous sex hormone exposures across the life course would be needed, and the effects of these exposures at different timepoints on later-life cognition compared.[6,365] This would establish whether early-life sex hormone exposures have stronger effects than the same exposures at other timepoints, but only under the assumption that the level of sex hormone exposure is equal at all timepoints, which given the known variations in sex hormone levels across the life course,[366] is not realistic.

Conversely, although early-life factors associated with cumulative risk exposures, there was an additional effect of lifetime risk exposures on later-life outcomes. Less advantageous early-life exposures (e.g. lower SEP) could induce a chain of risks (e.g. fewer educational and occupational opportunities, poorer diet), which may explain the associations of early-life factors with cumulative risk scores. The accumulative model proposes that it is the built-up effect of these chained risks which influence later-life outcomes rather than the initial (early-life) exposures alone, a notion which the Chapter 4 findings support.

It is important to note that many sociocultural variables included in these analyses are traditionally gendered in this cohort (e.g. lower educational and occupational attainment in women) and there could be variations between sexes in how early-life factors cascade to other sociocultural exposures throughout life. For example, although females had greater cognitive performance in childhood than males, educational attainment and occupational SEP were greater in males (Chapter 3). There is a controversial argument that educational attainment reflects baseline cognitive ability,[9] which may be at least partially true given positive associations of childhood cognition and educational attainment,[159] but higher educational attainment cannot be achieved if access to education is limited, which has traditionally been the case for women and for those with lower socioeconomic backgrounds.

Given that societal gender roles vary over time, the generational context of the NSHD cohort must be considered. People born in 1946 would have been entering the labour market in the 1960s, when the culture in Britain tended toward women taking on more household and childcare duties than men and, although female representation in the work force was increasing, men were the main breadwinners. When women did work, fewer hours were worked, and the job roles available tended to be lower skilled and lower paid than those available to men, hence there was less incentive for women to pursue higher education.[367,368]

Evidence from Chapter 3 demonstrates how gendered inequalities in education and SEP could suppress female cognitive advantages. Indeed, secular improvements in educational access across generations (as traditional gender roles become less prevalent within society)[369] have been associated with increased female memory and verbal memory advantages.[54] Traditional gendered socioeconomic inequalities, as observed within NSHD, could therefore limit opportunities to develop and maintain cognitive and brain reserve resources. With fewer opportunities for activities which support brain maintenance (e.g. through occupational complexity), females could show more rapid reductions than males in their existing reserve resources as they advance into older age, as will be discussed in the following Section (6.2.6.).

### 6.2.6. Pathways to greater female dementia risk

While this thesis has not explicitly examined sex differences in dementia diagnoses, variations in male and female pathways to cognitive and brain health measures up until an age at which dementia diagnoses remain relatively rare (~70 years) were tested in a mostly dementia-free, cognitively unimpaired cohort. At this age, there was evidence that females showed better cognitive and brain reserve than males, providing structural and functional brain resources through which females can cope with (exert cognitive resilience to) increased levels of cerebrovascular pathology. Although this does appear paradoxical to evidence showing greater dementia risk in females, sex differences in resilience pathways were demonstrated and it remains possible that changes in resilience with continued ageing, brain pathology progression and expression, and cognitive and functional impairment diagnoses, may also differ by sex.

Indeed, among MCI participants in the ADNI cohort (mean age at baseline 74 years), females showed greater decline than males on the AD Assessment Scale-Cognition score (ADAS-Cog) over 10 years.[370] Wider evidence has also shown that male AD patients outperform female AD patients across several cognitive domains including visuospatial abilities, language, and episodic memory.[371] Interestingly, a quantification of cognitive reserve among Aβ positive ADNI participants (baseline age 73.9 years) demonstrated differences in cognitive reserve-related cognitive decline across AD dementia stages (mean follow-up 2 years). Greater cognitive reserve (reflecting the extent to which ADAS-Cog performance was higher or lower than expected given indicators of brain health, e.g. GMV) associated with slower decline in memory and executive functioning in participants with unimpaired cognition and MCI, but with faster decline in AD participants.[372] Although sex differences were not examined, such findings do support the theory that greater baseline cognitive resilience slows cognitive decline in the preclinical phases of AD dementia, but once dementia becomes clinically manifest, cognitive resilience advantages are lost. Together, findings imply that, in older age when cognitive impairment and brain pathology becomes more prevalent, females lose their cognitive resilience advantage, leaving them vulnerable to more rapid functional decline. The relationship between brain health and cognitive function (e.g. Figure 22h) may, therefore, not be linear. If females show cognitive resilience advantages until a threshold of pathology is reached, then the female slope is expected to be

gentler than the male slope at lower levels of pathology, but to become steeper than the male slope at high pathology levels.

Alternatively, lifetime cognitive advantages in females, particularly in memory domains which are first affected in AD dementia, could result in delayed detection of dementia-related cognitive decline. Indeed, some evidence indicates that females receive dementia diagnoses at more advanced disease stages than males, with females showing lower (poorer) MMSE scores than males when initially diagnosed with AD.[373] This difference might also reflect a greater privilege for male clinical assessments; for example, female caregivers might be more likely to raise concerns about functional impairments, particularly since males are less likely to be widowed than females.[374] Delayed diagnoses in females could at least partially explain females' more rapid decline once dementia is diagnosed. Cognitive assessments widely used in clinic and research often have ceiling effects (e.g. MMSE) and lack the sensitivity to detect early signs of dementia, but the range of cognitive outcomes examined throughout this thesis include more sensitive measures with greater performance variability (e.g. PACC), with demonstrable sex differences in task performance. Further utilisation of such assessments could be valuable for detecting dementia at earlier stages, which would be beneficial for both sexes.

## 6.3. Methodological limitations

### 6.3.1. Multiple testing

Several cognitive and neuroimaging outcome measures have been investigated across multiple statistical models throughout this thesis, increasing the likelihood of detecting associations where there are none (type I error). Some methods commonly used to address this issue of multiple testing include adopting family-wise error or false discovery rates which control for the type I error rate across all comparisons or for a fraction of type I errors among the significant results, respectively.

However, such adjustments can be too conservative and increase the risk of type II errors, when null associations are incorrectly accepted. Additionally, corrections for multiple testing are not required when the variables examined are correlated because the overall number of hypotheses

tested is reduced. In this thesis, the main hypotheses focused on the themes of cognitive performance and neuroimaging indicators of brain health; the cognitive variables are correlated (e.g. better ACE-III performance associates with better performance on other cognitive assessments), as are the neuroimaging variables (e.g. larger TBV associates with greater WMHV). For these reasons, it was deemed unnecessary to correct for multiple testing in this thesis.

### 6.3.2. Non-dementia outcomes

Although the overall purpose of this thesis was to develop an understanding of how various life course factors might contribute to increased dementia risk in females, use of a mostly cognitively unimpaired cohort in their 70's precluded the opportunity for dementia diagnosis as an outcome measure. Nonetheless, Insight 46 neuroimaging measures of AD-related pathology (principally PET amyloid imaging) provide a valuable indication for the likelihood of later AD diagnosis; Aβ positive individuals are thought to be in the preclinical phases of AD dementia.[193,375] Throughout this thesis, there was a general lack of significant findings for Aβ outcomes, aligning with the norm across other Insight 46 analyses to date, whereby *APOE* genotype is the variable most consistently associated with Aβ: 60.8% of Aβ positive individuals are ε4 carriers, compared with 22.7% of Aβ negative individuals.[158] Such null findings could reflect the preclinical nature of the Insight 46 cohort at age ~70; Aβ levels may not have yet reached a threshold to noticeably impact functional abilities or neurodegenerative processes. Alternatively, risk factors (such as those identified in the Lancet commission)[56] could be linked with dementia via non-amyloid-related pathways including ageing- (e.g. brain volumes) or vascular-related (e.g. WMHV, NAWM microstructural integrity) pathways.

Further follow-up of the cohort is ongoing, including repeated cognitive testing, amyloid PET imaging and additional tau PET imaging.[306] Subsequent analyses of additional pathology data, including examination of cognitive and brain health trajectories alongside dementia diagnoses, which are expected to increase as the cohort advances into older age,[94] will be important to develop a full picture of life course sex differences in resilience pathways and to elucidate what this could mean for dementia risk in males and females.

It is also important to note that, alongside neuroimaging outcomes, there has been a focus on cognitive performance assessments to infer levels of functional abilities related to atypical ageing or dementia. While cognitive assessments are useful tools, with some validated measures (e.g. Memory Impairment Screen, 10-point Cognitive Screener) used in the clinical diagnosis of dementia,[376] performance on such assessments are not necessarily true to real life, everyday functioning.[377] For instance, some tasks are unusual and would not typically be encountered in daily life, and test anxiety could also influence performance. Further research could benefit from incorporating additional functional measures intrinsic to the clinical definition of dementia, such as ability to undertake activities of daily living which can be assessed by questionnaire administered to individuals and close informants such as family members.[378]

### 6.3.3. Data availability and biases

The vast amount of prospective data available spanning the life course is a strong advantage of NSHD and Insight 46, including the rarely available measures of cognition in childhood; but there are some limitations of birth cohort and secondary data analyses which should be acknowledged. First, the variables available at each data collection wave reflect the topics and research questions of interest at that time, which within NSHD have substantially evolved since the survey's conception as a maternity study. Therefore, variables of interest are not always available across all timepoints (e.g. physical activity was not measured before age 36). Some variables can also lack sufficient detail to address present day research questions; for example, age at HT initiation is only available in age group categories which hinders analyses assessing a sensitive period for HT use in relation to age at menopause.

Second, given that NSHD data spans over seven decades, where variables have been repeatedly measured, the measurement technique can vary over time and may not reflect current day best practices. For example, several different questionnaires were used across data collections to assess depressive affect, transitioning from the Present State Examination (PSE) to the Psychiatric Symptom Frequency (PSF) scale, and then to the 28-item General Health Questionnaire (GHQ-28) which is more widely used in clinical and research settings today.[379]

Third, NSHD study members were invited to participate in Insight 46 if they had a clinic visit at age 60-64, indicated they were willing to attend a clinic visit in London, and if they had available relevant data available across childhood and adulthood, as previously outlined elsewhere.[193] This was to maximise the life course data available for analyses alongside the additional Insight 46 data collected, but the approach has induced a sample selection bias. Compared with the wider NSHD cohort, Insight 46 participants are found to be generally healthier, to have better cognitive function, higher educational attainment, and to be from higher socioeconomic backgrounds.[194] Individuals who may be at the greatest risk for adverse outcomes, including dementia, are therefore underrepresented within Insight 46, limiting the generalisability of findings to the wider NSHD cohort and the general population, whereby associations of education and SEP, for example, with health-related outcomes may be underestimated. Indeed, that early-life variables associated with cumulative risk exposures in the whole-NSHD but not Insight 46 sample (Chapter 4) could be explained by less socioeconomic variability within Insight 46, as well as lower statistical power.

Fourth, attrition bias can induce similar problems. As is the case with most longitudinal studies, there is inevitable sample attrition over time due to various factors including loss of contact, emigration, and no longer wanting or being able to participate. Recent NSHD retention rates are, however, high; at age 69, 94% of the 2816 remaining study members took part in data collection.[191] High retention rates may limit the level of attrition bias, but there is still evidence for greater likelihood of participation among study members with higher SEP, educational attainment, childhood cognition, and better health.[191] The study members retained at older data collections could, therefore, be under representative of individuals who may be at increased risk of adverse outcomes.

Finally, and related to attrition bias, is the issue of survivor bias, whereby participants remaining in the study, particularly at older ages, are those with the best health since those with poorer health may withdraw due to illness or die. This is of particular concern for sex difference analyses given that females have a greater life expectancy than males. Indeed, within NSHD 20% of males were not contacted for the age 69 data collection because they had died, compared with 15% of women.[191] Other evidence also shows that males have greater cardiovascular disease mortality

rates than females at younger ages.[380,381] Therefore, the males remining in the study in later-life are likely to be those with the best cardiovascular health, while females with poorer cardiovascular health can still participate; surviving males could be healthier than surviving females, which could influence sex differences in later-life outcomes such as cognitive ageing, brain health, and dementia.

### 6.3.4. Generalisability

Some generalisability limitations linked with sample selection and attrition biases have been outlined in the previous Section (6.3.3.). This section will discuss some of the generalisability issues intrinsic to a birth cohort representative of the post-war population in Britain.

A key strength of NSHD is its very narrow age range, with all participants born in the same week of 1946, which minimises the influence of age variability on the reported associations with later-life outcomes. This also means that wider societal, political, environmental, and economic events (e.g. the 1970s financial crash, introduction of the internet) were experienced at the same age across study members. However, it follows that people of other generations experience different societal exposures, hence the relationships of life course variables and later-life outcomes could vary between generations that are not represented in NSHD data. As mentioned in Section 6.2.5., changing societal gender roles over time and secular improvements in educational access are particularly relevant when considering how sex differences in cognitive ageing and dementia risk might change across generations.[54] With continued reductions in gendered educational and occupational disparities,[382] it is hypothesised that females' heightened dementia risk could diminish when more recent generations reach older age, given greater opportunities for the development and maintenance of cognitive and brain reserve resources than females born in 1946 or earlier.

Additionally, NSHD is a White British cohort, hence it is not representative of non-British populations or other ethnic groups. This is a particularly relevant limitation for the generalisability of dementia sex differences research, given reported geographical variations in dementia incidence sex differences; evidence indicates that AD dementia incidence does not differ by sex

in the US, but in European countries females have higher incidence than males.[383] Cultural differences in health, lifestyle, and socioeconomic circumstances, for example, could contribute to such variations. Indeed, evidence has shown female cognitive disadvantages (including on attention and orientation, memory, and fluency tasks) in lower income counties, contrasting with findings of female cognitive advantages in high income countries.[384] Such sex differences are largely explained by gendered educational inequalities whereby women have reduced access to education, which are greatest in low-income countries.[384]

Further, ethnic diversity in Britain has vastly increased since 1946, but ethnic minorities are frequently underrepresented in research. Relatively little is therefore known about cognitive ageing and dementia risk in these groups, and much less about sex differences across ethnicities. When adjusting for sex, analyses of UK Biobank data have indicated increased dementia risk in Black compared with White participants, and equal dementia risk in South Asian and White participants.[385] Similarly, English medical record and mortality data shows increased dementia incidence in Black compared with White people, accounting for sex, and South Asian and Black people with dementia are shown to die at younger ages than White people with dementia.[386] In non-British samples, there is some evidence for cognitive and dementia sex differences which vary between ethnic groups. A US study of non-Hispanic White, Black, and Hispanic males and females found female memory advantages over males within the Black and Hispanic groups, while non-Hispanic White females outperformed all sex and ethnic groups.[387] Over an average 15 years of follow-up, decline in memory and visuospatial performance was also found to be steeper in Black females compared with non-Hispanic White females.[387] Elsewhere, AD risk is reported to be greater among Black American than non-Hispanic White adults,[388] and Black American females are reported to have the highest prevalence of cardiovascular risks linked with dementia (including hypertension and type 2 diabetes) than other ethnic or sex groups.[389] The intersectionality of sex and ethnicity is therefore an important issue which needs to be examined to understand cognitive and brain ageing, and dementia mechanisms within all groups of society, but this cannot be addressed in an ethnically homogenous cohort such as NSHD.

### 6.3.5. Sex and gender interrelationships

This thesis has focused on sex differences, referring to the biological characteristics distinguishing males from females.[13] While gender (self-identified and based on societal constructs of masculinity and femininity)[13] need not match biological sex, interrelationships between sex and gender are likely. Given the gendered nature of some variables (e.g. education, occupation, physical activity levels, smoking habits) linked with dementia and cognitive resilience, it will be important to consider how gender identity might modify sex differences in life course cognitive and brain ageing pathways.

In 2016, the Sex and Gender Equity in Research (SAGER) guidelines[14] were published to streamline and encourage the reporting of sex and gender in scientific research and outputs. While the guidelines recommend that sex should distinguish males and females based on biological characteristics, no specific recommendations are made for gender reporting. It is acknowledged that, although often misconceptualised as binary (masculine/feminine), gender identity lies on a spectrum.[14] The term transgender describes someone whose gender identity (internal sense of gender) or gender expression (public presentation of gender) does not conform to their sex assigned at birth; some people who identify as transgender might have a binary gender identity, while others might not. Individuals who identify as non-binary or gender fluid might identify as both, neither, or somewhere in-between the traditional masculine and feminine dichotomy.[390]

Although there is no current consensus for how best to measure gender in scientific research, this is an active topic of discussion. In 2007, the Canadian Women's Health Research Network outlined a gender framework suggesting four domains of gender-related variables through which researchers might be better able to conceptualise gender: gender identification (inner sense of self), gender role (behavioural norms), gender relations (interpersonal interactions), and institutionalised gender (distribution of power within society, including discrimination).[391,392] More recently, the GOING-FWD (Gender Outcomes International Group – to Further Well-being Development) consortium incorporated this framework into a five-step process developed to facilitate retrospective analyses of gender in relation to non-communicable disease outcomes.[12] With the development of gender-based frameworks and increased awareness of sex and gender

distinctions, researchers will be better equipped to address questions of sex and gender interactions in the coming years.

Regarding the findings presented in this thesis, gender identify could modify some of the sex differences observed. For example, according to the minority stress model, people who identify as gender diverse could experience stigmatisation and discrimination, leading to an accumulation of psychological stress which can induce negative impacts on mental and physical well-being.[390,393] Individuals who identify as gender diverse could, therefore, experience increased life course exposures to modifiable dementia risk factors and could lack the necessary support to manage multiple health and lifestyle adversities, given a lack of acceptance or understanding of gender diversity within society, including in clinical settings.

A recent examination of survey data from the United States has reported sex and gender differences in dementia risk scores, across age-matched groups.[394] Individuals assigned female at birth (female sex) showed lower midlife dementia risk (measured using CAIDE [ages 40-65] and LIBRA [age ≥50] scores) than those assigned male at birth (male sex), but females showed greater later-life AD risk (indicated by greater Australian National University-Alzheimer's Disease Risk Index/ANU-ADRI scores [age ≥ 55] scores) than males. While midlife dementia risk scores did not differ between gender identity groups, gender diverse cohorts (transgender women, transgender men, and non-binary adults) did show increased later-life AD risk (ANU-ADRI scores) compared with cisgender men and women. The authors postulate that social stigma and discrimination experienced by individuals expressing gender identities deviating from the social norms of binary masculinity and femininity matching biological sex, could be an important contributor to increased AD risk. Indeed, levels of depression were greatest among non-binary adults and transgender males, providing some support for the minority stress model.

Additionally, generational variations in the impacts of gender identity on health and lifestyle throughout the life course are anticipated. Only in more recent years has gender fluidity been increasingly recognised and accepted within society.[390] In an older cohort such as the NSHD, there could have been increased societal pressure for binary expression of gender identify, with gender

fluid individuals experiencing discrimination, or else suppressing their true gender identity, with implications for mental well-being and general health.

## 6.4. Policy and research implications

### 6.4.1. Sex- and gender-based analysis (SGBA) research policies

The findings from this thesis highlight that there are complicated sex differences in the life course pathways and resilience mechanisms to later-life cognitive function and brain health, reinforcing the importance of sex-aware research and therefore providing support for the implementation of sex- and gender-based analysis (SGBA) policies. Such policies encourage careful consideration of sex and gender throughout the research process including design (e.g. inclusion of both sexes), analyses (e.g. sex-disaggregated data), and reporting (e.g. clarity on how sex and gender were considered). The SAGER guidelines[14] are an example of an international framework to encourage better sex and gender reporting in research publications, but individual countries have also introduced policies to embed SGBA in their health-related research.

In 1993, the US National Institutes of Health (NIH) implemented the Women and Minorities as Subjects in Clinical Research policy[395] to ensure that women and minority groups were included in all clinical research, with success in that at least half of participants in recent NIH-funded clinical research are women.[396] The Canadian Institutes for Health Research followed in 2009, providing clear definitions for sex and gender, requiring grant applicants to report whether they had integrated sex or gender into their proposals, and later asking applicants to justify any omissions to do so.[397,398] The US NIH additionally introduced a policy in 2016 to embed sex as a biological variable (SABV) throughout the design, analysis, and reporting phases of all vertebrate animal and human studies, with researchers needing to provide strong justification for single-sex studies.[396,399] The UK has lagged behind, with the first medical research funder (the MRC) introducing a sex and gender policy only in July 2023.[400] However, there is increasing awareness across the UK research sector, with several organisations advocating for improved SGBA in health research. A collaboration of various stakeholders (researchers, regulators, funders, participants, publishers, clinicians, the Department for Health and Social Care) are currently designing a sex

and gender policy framework, due to be launched in 2024, as part of the Medical Science Sex and Gender Equity (MESSAGE) initiative.[1] Work is clearly ongoing to develop effective SGBA frameworks which can be readily adopted by the medical research community. Research specifically aiming to examine and understand sex differences in health outcomes, such as in this thesis, can contribute to the success of SGBA policies, including by helping to encourage a culture of change in the way that researchers approach sex and gender.

### 6.4.2. Women's health

This thesis reinforces the notion that female-specific reproductive processes can relate to female health-related outcomes in later-life, thereby advocating for an emphasis on women's health, which has traditionally been overlooked in research and health care. Although menopause was the primary female-specific reproductive factor examined in this thesis, there are several other female-specific health issues which were not examined, including pre-menstrual syndrome, endometriosis, and complications of pregnancy to name a few. Throughout the duration of this doctoral work, there has been an increase in public awareness and discussion of women's health issues, including the implementation of public health policies pertaining to women's health and wellbeing. For instance, the first Women's Health Strategy for England was launched in July 2022,[401] setting out plans for improvements in how the health and care system listens to women, aiming to boost health outcomes for women and girls. Priority areas include, amongst others, menstrual health and gynaecological conditions, fertility and pregnancy, menopause, mental health and wellbeing, and healthy ageing. New and planned initiatives under the strategy include the creation of women's health hubs, dedicated women's health areas on the NHS website, and increased accessibility to menopausal HT.[401,402] Media attention and advocacy from public figures, including TV presenters Davina McCall and Naga Munchetty, has also helped to reduce taboo surrounding women's health, widening the discussion around topics such as menstruation and menopause, including among men. Societal shifts in the understanding of women's health could improve support, for example within family networks and in the workplace, and thereby reduce the individual and societal burden of women's health issues.

The apparent wave of public and governmental support for women's health improvements should be taken as an opportunity to re-evaluate how best to record women's health data, improving the resources available to examine the determinants of women's health outcomes and treatment effectiveness. For instance, as highlighted in Chapter 5, our current understanding of HT effects on later-life cognition and brain health is poor, often hindered by inadequate data regarding HT timing, dosages, and administration methods. There should also be continued research examining women's health in relation to later-life health outcomes, to determine how recent policy implementations and societal attitude changes might alter associations as women across different generations age.

### 6.4.3. Reducing dementia risk exposures

The demonstration of adverse associations between increased lifetime exposures to the Lancet commission's twelve modifiable dementia risk factors and later-life cognitive and brain health outcomes adds to existing literature reporting dose-response effects of health and lifestyle risk factors.[108,111,271,273] Together, this associative evidence implies that reducing lifetime risk exposures could have benefits for later-life outcomes, and multi-domain lifestyle intervention trials have shown promise in this respect.[104,105] However, the development of public health policies to reduce accumulated risk exposures across the life course will need to consider how modifiable the identified risk factors truly are for individuals and governments. For instance, reducing air pollution exposure at an individual level would be difficult, with air pollution improvements requiring substantial worldwide efforts which could take several years to be realised.[403] Additionally, individuals who have multiple risk factor exposures are assumed to be those at the highest risk of dementia but also the least likely to engage with prevention strategies, given greater health and social barriers.[403]

There also needs to be consideration for when in the life course interventions might be most effective, including how this might differ between sexes and other societal groups. For example, if midlife, or more specifically the menopause transition, is indeed a sensitive period for females, then public health policies aimed at improving women's health outcomes might target women of

this age. Conversely, if males are vulnerable to more prolonged accumulation of risks across the life course, then policies targeting males earlier in life could be beneficial. In fact, the associations of early-life variables with accumulated risks (Chapter 4) do suggest that early-life could be a key target for policies to reduce lifetime accumulation of risks, particularly in males, thereby promoting healthier lifestyles throughout the entire life course.

This thesis also highlights how gendered socioeconomic and educational inequalities may limit female opportunities to develop and maintain the structural and functional brain resources needed to protect against abnormal brain ageing- and dementia pathology-related functional impairments. While this thesis demonstrates greater female cognitive resilience than males in a cohort at age ~70, how cognitive resilience might change in older age and as pathology levels increase remains to be determined. Nonetheless, policies aimed at reducing gendered disparities, including improving educational access especially in lower-income countries, could enhance female cognitive abilities and presumably also the resources supporting cognitive reserve. Other avenues for reducing gendered socioeconomic disparities might include supporting and encouraging women to return to work after having children in tandem with strategies to facilitate and encourage men to take on greater responsibilities for child rearing, and supporting women in work during the menopause transition. In addition to health benefits, such strategies could also have wider economic advantages associated with a stronger workforce.

## 6.5. Future directions

### 6.5.1. Cognitive resilience and risk factor exposure interrelationships

Further analyses should examine the extent to which potential sex differences in cognitive resilience might contribute to the observed sex differences in how cumulative dementia risk exposures associate with later-life cognitive and brain health outcomes. Three additional research questions to be addressed are: 1) Do males show reduced cognitive resilience than females at age ~70? 2) Are the associations of cumulative dementia risk factor exposures with cognitive performance mediated by brain health, and are mediating effects strongest in males? 3) Is

cognitive resilience modified by cumulative dementia risk factor exposures, and are such interactive effects strongest in males?

Question 1 can be addressed by testing for sex differences in the associations of neuroimaging measures of brain health and cognitive performance in later-life. The strongest brain-cognition associations are expected in males (Figure 22h) since greater cognitive resilience in females suggests a disconnect between brain health and cognitive function which would be less apparent in males.

Question 2 can be tested using sex-stratified mediation analyses. The mediating effects of brain health on CRS-cognition associations are expected to be strongest in males, given greater female resistance to brain ageing and pathology when exposed to dementia risk factors (Figure 22c) and presumed greater female cognitive resilience that buffers against reduced brain health (Figure 22h).

Question 3 would be assessed by testing for interactive effects of CRS and later-life brain health on cognitive performance. Those with greater risk factor exposures would be expected to show reduced resilience (i.e. stronger brain-cognition associations), with greater modifying effects in males than females since CRS more strongly associated with later-life outcomes in males (Figure 22c, d).

### 6.5.2. Addressing risk-modifying treatment use

As described in Section 4.4.3., there are limitations in adjusting for risk-modifying treatments, such as anti-hypertensive medications, when examining the associations of CRS with later-life outcomes. It is still important to consider how such treatments might contribute to these associations. One way to address this in further work could be to apply a weighting to each risk factor included in the CRS for which risk-modifying treatments exist. Weightings should be determined using existing literature and knowledge of the treatment effect size; for example, the extent to which blood pressure is reduced by anti-hypertensive medication.[313]

### 6.5.3. Integrating female reproductive health

Further analyses should examine how menopause timing and additional reproductive health variables might contribute to female cognitive resilience and associations of dementia risk factor exposures with later-life outcomes.

Findings from this thesis, and wider literature, imply that later menopause (reflecting prolonged endogenous oestrogen exposure) extends brain structure and function maintenance (promoting greater cognitive resilience), and possibly delays the accumulation of dementia risk factor exposures (Section 6.2.3.). The following questions can address these assumptions: 1) Does menopause timing modify cognitive resilience to brain ageing and pathology in later-life? 2) Is there an association between menopause age and cumulative risk exposures, and are there interactive effects of menopause age and risk factor exposures on later-life cognitive and brain health outcomes?

To test question 1, interactive effects of menopause age and brain health measures on cognitive performance should be examined. It is hypothesised that women with later menopause would show greater cognitive resilience, with weaker brain-cognition associations than those who experienced menopause at younger ages.

Addressing question 2, later menopause age is expected to associate with fewer dementia risk exposures. Menopause age-by-CRS interactions are hypothesised to reveal stronger associations of CRS with later-life cognition and brain health in women with earlier menopause ages.

More broadly, future research should also examine how additional female-specific reproductive exposures might influence female resilience mechanisms, as potential explanatory factors in observed sex differences. Indeed, there is evidence of GMV reductions associated with pregnancy, which are maintained for at least 2-years post-partum.[404] Hypertensive disorders of pregnancy are also of great interest, with long-lasting cerebrovascular changes (increased white matter hyperintensities and reduced NAWM microstructural integrity) and reduced cognitive functioning observed in women who had pre-eclampsia compared with normotensive pregnancies.[405-407] Additionally, while menopause age is informative, there will also be value in examining how menopausal symptoms relate to dementia risk exposures and later-life cognition

and brain health. For example, fewer or less severe symptoms could reflect a greater ability to maintain functional abilities with hormonal changes. Social relationships, work-related performance, and physical activities, for example, may therefore be better maintained, potentially minimising additional accumulation of health and lifestyle dementia risks.

Given the suggestion that menopause and perhaps other female reproductive transitions (e.g. menarche, pregnancy) could be sensitive periods for female brain health, future work could also examine whether women who have greater dementia risk factor exposures during these periods have poorer cognitive and brain health outcomes in later-life than those with fewer risk exposures during sensitive periods. For example, risk exposures in the five years either side of menopause could be quantified and examined for associations with later-life outcomes. The expectation would be that women with greater risk exposures would have poorer outcomes. Such analyses could be further extended by additionally quantifying risk exposures in males at ages matched to female menopause for comparison; adverse associations of increased risk exposures would be hypothesised to be most evident in females.

### 6.5.4. Longitudinal follow-up

This thesis has examined cross-sectional sex differences in cognitive performance and brain health measures, but a great strength of NSHD is that there are longitudinal measures (Table 1) which facilitate the examination of cognitive trajectories, necessary to understand variations in cognitive ageing and the underlying mechanisms. Given the inference that midlife could be a sensitive period for females (Section 6.2.3.), a further question to address is whether dementia risk factor exposures accumulated up to and including midlife (up to age 53) differentially associate with rates of verbal memory and processing speed decline in males and females from age 53 to 69, with careful consideration for how accumulated risks to midlife might interact with additional risk exposures during the follow-up period. Accumulated risks to midlife would be expected to associate with steeper cognitive performance decline in females, reflecting how midlife risk exposures might increase female vulnerability to accelerated cognitive ageing in the years preceding any dementia diagnosis.

Now aged 78, study members are continuing to participate in further waves of data collection for Insight 46.[306] As the cohort continues to advance into older age, when brain pathology levels and rates of dementia diagnoses are anticipated to increase, there is a valuable opportunity to further examine the life course pathways to dementia and how these differ by sex. To test the hypothesis that females show a rapid decline in their level of cognitive resilience once a threshold of brain pathology is reached, thereby contributing to greater dementia rates in females, follow-up analyses should re-examine the associations observed at age ~70 (Figure 22) at subsequent ages, in addition to testing for sex differences in cognitive and brain ageing trajectories from age 69 onwards, and how life course health, lifestyle, and reproductive variables might contribute to such trajectories in males and females. A challenge for such analyses is that the statistical power to detect dementia incidence could be low due to small sex-stratified sample sizes in future Insight 46 data collections. The Insight 46 selection bias, whereby Insight 46 participants have better general health than the wider NSHD cohort, might also mean that the proportion of dementia cases are lower than in the whole cohort or general population.

### 6.5.5. Examining survivor bias

Described in Section 6.3.3., survivor bias is a limitation of these analyses given attrition of the NSHD cohort over time. This is a particular concern for sex difference analyses since more female than male study members are retained at older ages, given higher male mortality rates at younger ages. In further research, the analyses included in this thesis could be re-run using inverse probability weighting. This method aims to recreate a representative sample of the initial cohort by assigning weights to individuals according to their likelihood of remaining in the cohort, based on their characteristics. For example, participants with higher SEP are more likely to have available ACE-III data at age 69 than those with lower SEP; participants with higher SEP would be given a lower weighting in the analysis, thereby increasing the proportional representation of participants with lower SEP who were more likely to be lost to follow-up. Comparing such weighted analyses to the current findings in this thesis can inform the extent to which survivor bias may have driven the findings, which is important to understand given the hypothesis that increased female life expectancy could contribute to females' increased dementia risk.

### 6.5.6. Varied cohorts

As discussed in Section 6.3.4., there are limitations in the generalisability of the findings presented in this thesis, primarily to non-British and non-White populations, and to other generations. Further research should therefore utilise more diverse cohorts to assess whether the associations observed in NSHD and Insight 46 (Figure 22) are replicated in different groups. Importantly, the intersectionality of sex and ethnicity should be tested, acknowledging that the presence or absence of sex differences (e.g. in level of cognitive resilience, or in the associations of cumulative risk exposures with later-life brain health) might not be equal across all ethnic groups.

Additionally, the sex differences in relationships between life course variables as presented in Figure 22 should be examined and compared between cohorts spanning generations (e.g. subsequent British birth cohorts; National Child Development Study/NCDS (1958), 1970 British Cohort Study/BCS70, Millennium Cohort Study/MCS (2000)). This will allow for trends to be observed over time, to determine whether societal changes in gender roles and gendered socioeconomic disparities translate into reduced sex differences in cognitive resilience, cognitive and brain ageing, and ultimately dementia diagnoses, although dementia outcomes will only be possible several decades in the future (particularly for MCS).

## 6.6. Conclusions

The findings of this thesis highlight that males and females differ in their abilities to maintain functional abilities with poorer brain health related to ageing and cerebrovascular disease at age ~70. Females showed cognitive resilience to increased levels of cerebral small vessel disease pathology, while males demonstrated reduced cognitive resilience to greater brain ageing (smaller MRI-measured brain volumes). The use of a life course approach incorporating early-life exposures, health and lifestyle variables identified elsewhere as dementia risk factors, and female-specific reproductive processes (menopause timing) revealed that exposures at different life stages differentially associate with later-life cognitive and brain health outcomes between males and females, thereby showing sex differences in the life course mechanisms underlying

cognitive resilience. There was also evidence to support previous reports that gendered socioeconomic and educational disparities may suppress female cognitive abilities. This thesis therefore advocates for the importance of sex- and gender-based analyses, which will be integral to building a deeper understanding of the sociocultural and biological processes related to the life course development and risk of dementia in males and females, ultimately helping to inform public health prevention strategies to minimise the future individual and societal burden of dementia in an ageing population.

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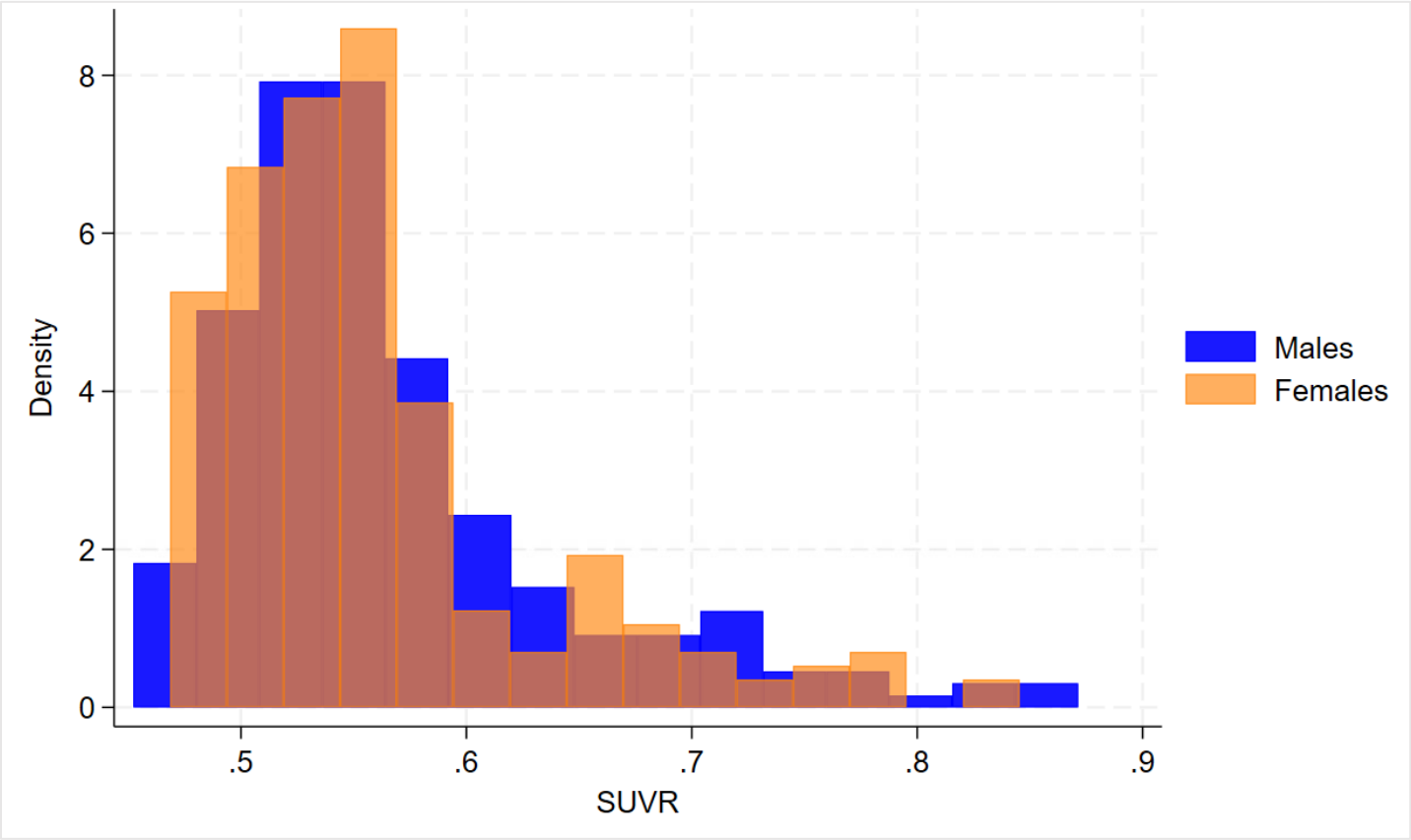
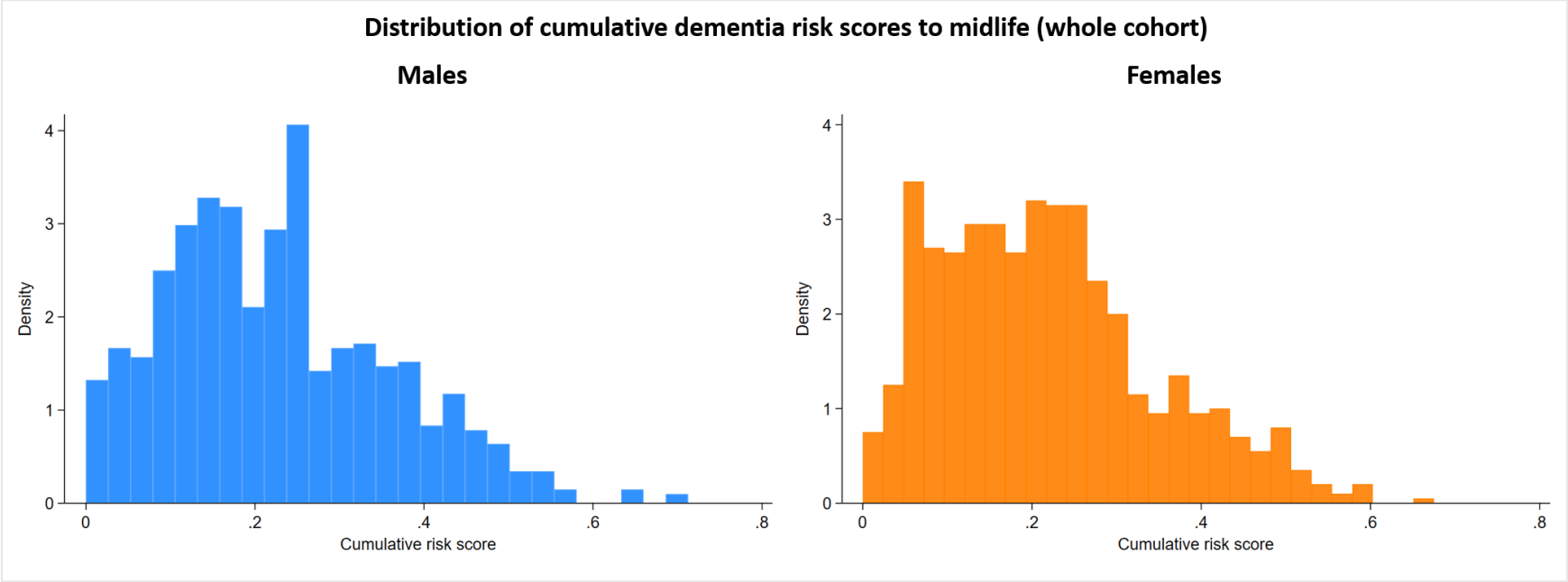
# Appendix A

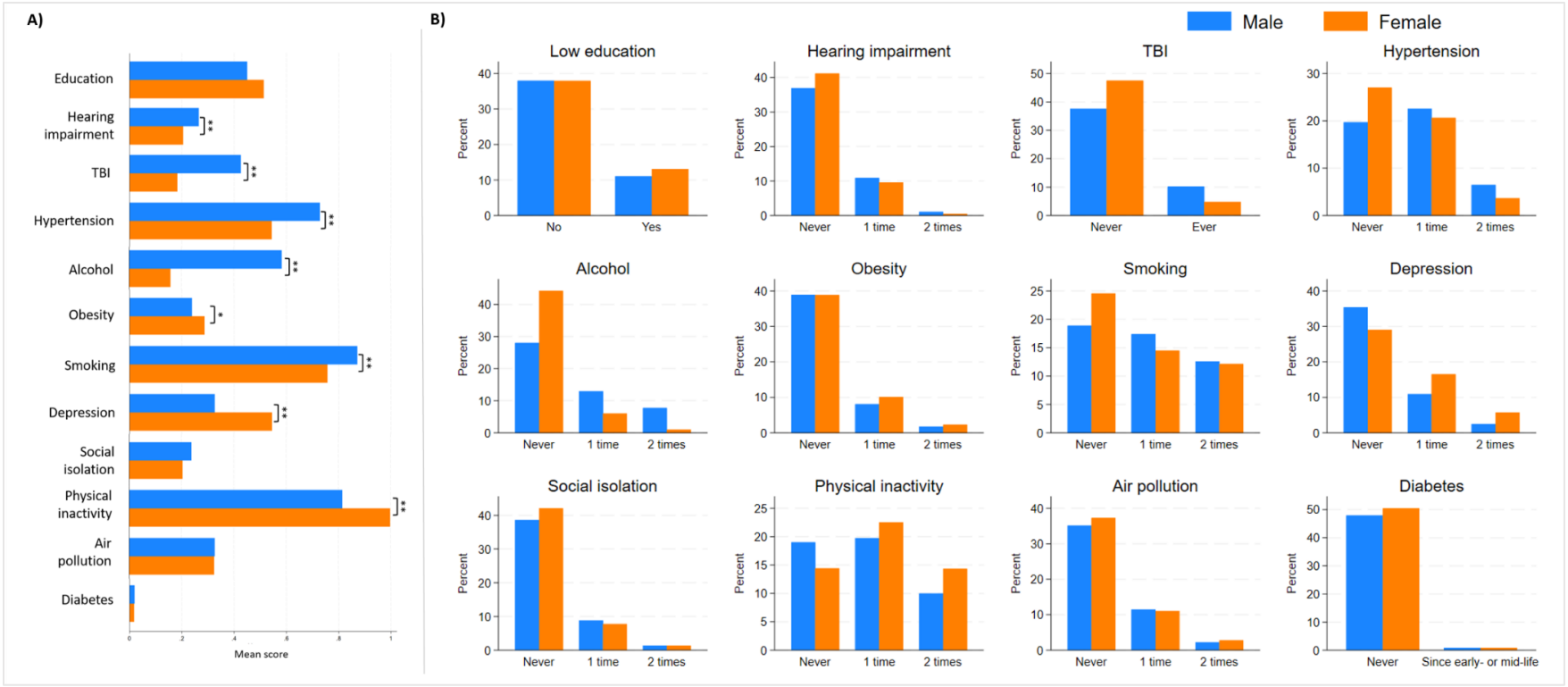



***Supplementary Figure 1.*** *Histogram displaying the distribution of continuous beta-amyloid standardised uptake value ratio (SUVR) in Insight 46 males (n=235) and females (n=227), measured using amyloid PET-MRI at age 69-71 during wave I of Insight 46.*

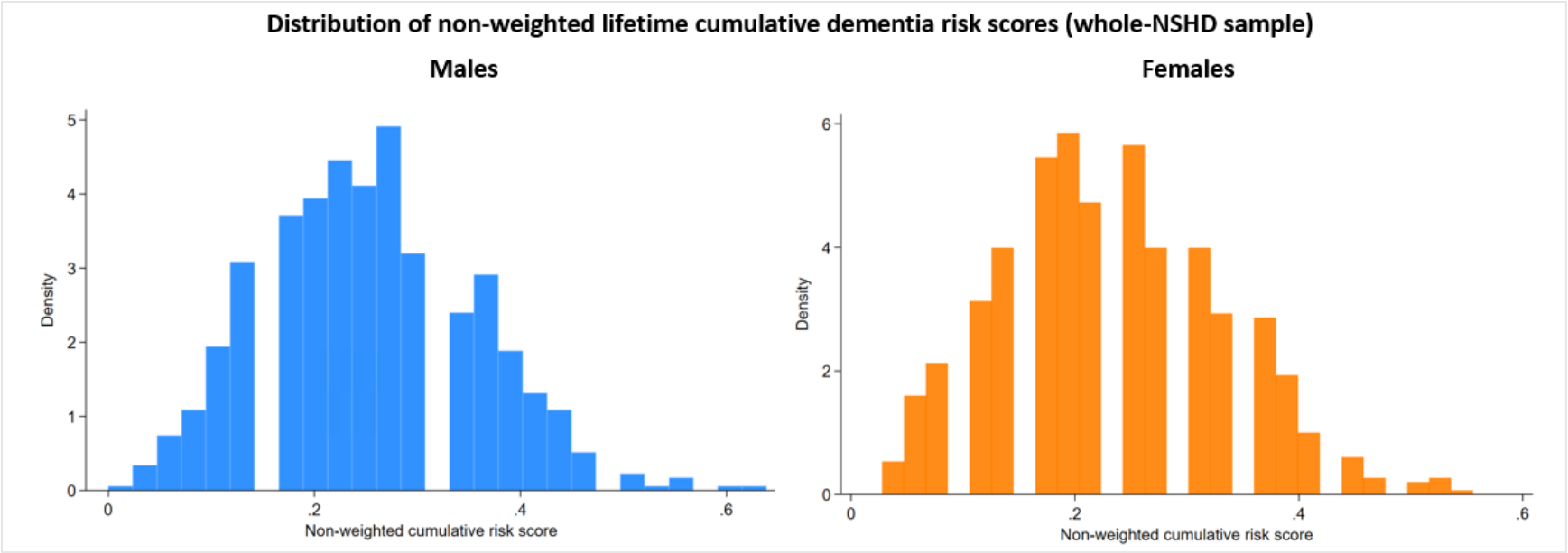
# Appendix B (Chapter 4 supplementary)



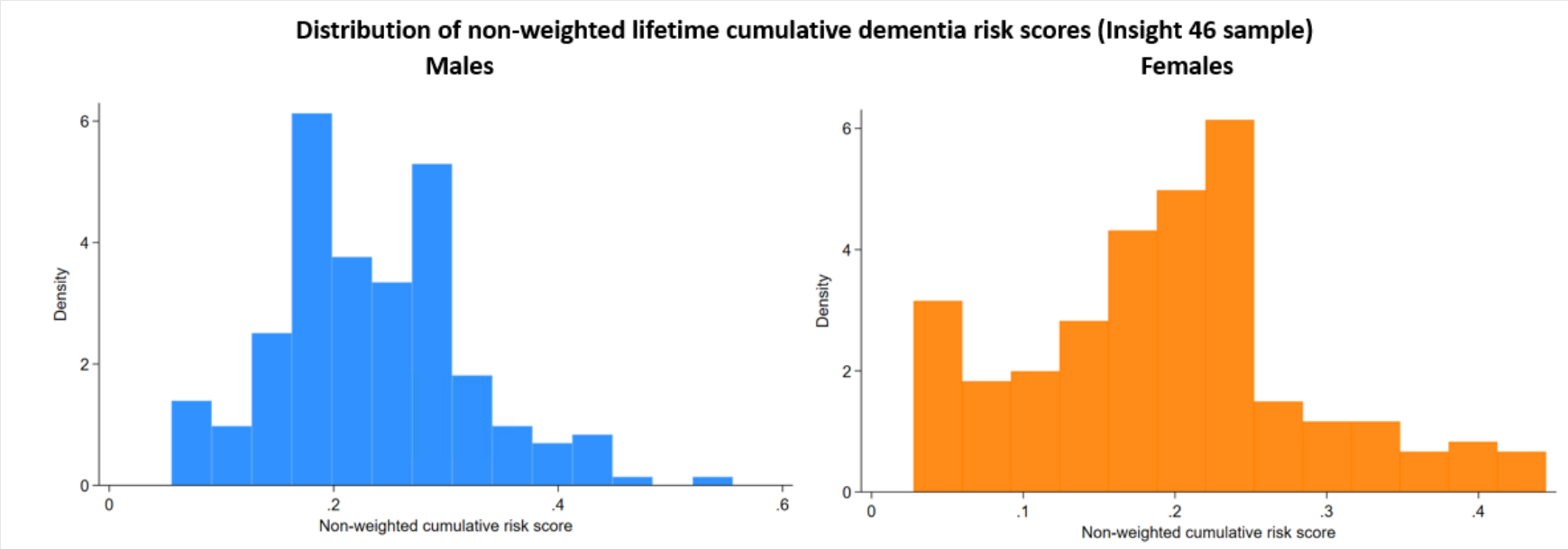

***Supplementary Figure 1.*** *Distribution of cumulative dementia risk scores up to midlife in males (N=774) and females (N=830), in the whole-cohort sample.*




***Supplementary Figure 2.*** *Distribution of scores to midlife per risk factor, in males (N=774) and females (N=830) in the whole cohort analytical sample. The maximum score per risk is 2. Low education 'No'=0, 'Yes'=2; TBI 'Never'=0, 'Ever'=2; diabetes 'Never'=0, 'Since mid-life'=1, 'Since early-life'=2 (for data protection reasons, diabetes categories have been collapsed); all other risks are scored 0 to 2 where 0 means that the risk is never present and 2 means that the risk is present at two timepoints.* ***A)*** *Mean scores for males and females.* ***B)*** *Percentage of males and females with different levels of risk factor exposures. TBI=traumatic brain injury. * t-test p<0.05; ** t-test p<0.01*




***Supplementary Figure 3.*** *Distribution of non-weighted lifetime cumulative dementia risk scores in males (N=740) and females (N=769), in the whole-NSHD analytical sample.*




***Supplementary Figure 4.*** *Distribution of non-weighted lifetime cumulative dementia risk scores in males (N=201) and females (N=188), in the Insight 46 analytical sample.*

***Supplementary Table 1.*** *Distributions of total midlife cumulative risks score (CRS) and individual risk factor scores in Insight 46 males and females.*

| | Male 215 (51.2%) | | Female 205 (48.8%) | | |
|---|---|---|---|---|---|
| **Variable** | **N** | **Mean(SD)** | **N** | **Mean(SD)** | **Linear regression t-test p-value[a]** |
| Midlife cumulative risks score; range | | 0.19(0.10); 0.00, 0.48 | | 0.17(0.11); 0.00, 0.54 | 0.073 |
| Low education | | 0.23(0.64) | | 0.26(0.67) | 0.566 |
| Hearing impairment | | 0.24(0.47) | | 0.21(0.43) | 0.386 |
| TBI | | 0.44(0.83) | | 0.21(0.61) | **<0.001** |
| Hypertension | | 0.74(0.72) | | 0.50(0.62) | **<0.001** |
| High alcohol consumption | | 0.65(0.76) | | 0.15(0.43) | **<0.001** |
| Obesity | | 0.22(0.50) | | 0.25(0.50) | 0.573 |
| Smoking | | 0.73(0.76) | | 0.65(0.75) | 0.235 |
| Depression | | 0.25(0.49) | | 0.43(0.59) | **<0.001** |
| Social isolation | | 0.26(0.49) | | 0.19(0.48) | 0.103 |
| Physical inactivity | | 0.72(0.72) | | 0.81(0.75) | 0.206 |
| Air pollution | | 0.37(0.56) | | 0.34(0.57) | 0.607 |
| Diabetes | | 0.02(0.15) | | 0.01(0.09) | 0.502 |

*Bold text indicates a significant (p<0.05) sex difference.*

*SD=standard deviation; TBI=traumatic brain injury*

***Supplementary Table 2.*** *Model estimates and 95% bias-corrected confidence intervals demonstrating the estimated fully adjusted effects of cumulative risks score (CRS) on ACE-III performance at age 69, standardised to the Insight 46 sample, in Insight 46 males and females. Models are fully adjusted for childhood cognition, childhood SEP, and APOE-ε4 status.*

| | **Males** | **Females** |
|---|---|---|
| **N** | 163 | 155 |
| **β** | -0.875 | -1.494 |
| **95% CI** | -2.383, 0.641 | -3.120, -0.033 |
| **p** | - | * |

**Significant effect, confidence interval does not cross zero*

*Bootstrapping applied: confidence intervals are bias-corrected, p values are not applicable.*

***Supplementary Table 3.*** *Model estimates and 95% confidence intervals demonstrating the estimated fully adjusted effects of cumulative risks score (CRS) on verbal memory performance at age 69, standardised to the Insight 46 sample, in Insight 46 males and females. Models are fully adjusted for childhood cognition, childhood SEP, and APOE-ε4 status.*

| | **Males** | **Females** |
|---|---|---|
| **N** | 197 | 180 |
| **β** | -0.783 | -0.684 |
| **95% CI** | -2.136, 0.571 | -2.043, 0.675 |
| **p** | 0.26 | 0.32 |

# Appendix C (Chapter 5 supplementary)

## 5i. Supplementary material

***Supplementary Table 1.*** *Monte Carlo Error (MCE) estimations generated from fully adjusted multivariable regression models for menopause age on standardised outcome measures, with multiple imputation by chained equations (MICE) applied using 8 imputations. MCE of the coefficient should be <10% of its standard error.*

| Outcome | | Beta | Standard error |
|---|---|---|---|
| ACE-III total | Model estimation | 0.010 | 0.006 |
| | MCE | 0.0002 | |
| ACE-III attention and orientation | Model estimation | 0.001 | 0.008 |
| | MCE | 0.0002 | |
| ACE-III language | Model estimation | 0.005 | 0.007 |
| | MCE | 0.0003 | |
| ACE-III memory | Model estimation | 0.009 | 0.007 |
| | MCE | 0.0002 | |
| ACE-III verbal fluency | Model estimation | 0.006 | 0.007 |
| | MCE | 0.0002 | |
| ACE-III visuospatial function | Model estimation | 0..012 | 0.007 |
| | MCE | 0.0002 | |
| MMSE | Model estimation | 0.002 | 0.016 |
| | MCE | 0.0004 | |

***Supplementary Table 2.*** *Model estimates and bootstrap 95% confidence intervals, corresponding with Figure 2, for the effect of 1-year increase in age at menopause on standardised z-scores for the Addenbrooke's Cognitive Examination (ACE-III; total score and sub-domains) at age 69 in the National Survey of Health and Development (NSHD) whole-cohort. N=746.*

| | M0 | | | M1 | | | M2 | | | M3 | | | M4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **β** | **Lower CI** | **Upper CI** | **β** | **Lower CI** | **Upper CI** | **β** | **Lower CI** | **Upper CI** | **β** | **Lower CI** | **Upper CI** | **β** | **Lower CI** | **Upper CI** |
| ACE-III total | 0.024 | 0.012 | 0.036 | 0.009 | -0.002 | 0.019 | 0.011 | -0.002 | 0.025 | 0.010 | -0.004 | 0.024 | 0.010 | -0.004 | 0.024 |
| ACE-III attention and orientation | 0.006 | -0.007 | 0.022 | 0.001 | -0.013 | 0.015 | 0.001 | -0.015 | 0.016 | 0.001 | -0.015 | 0.016 | 0.001 | -0.015 | 0.016 |
| ACE-III language | 0.012 | 0.001 | 0.026 | 0.000 | -0.009 | 0.013 | 0.005 | -0.008 | 0.022 | 0.005 | -0.008 | 0.021 | 0.005 | -0.008 | 0.021 |
| ACE-III memory | 0.023 | 0.009 | 0.036 | 0.012 | 0.000 | 0.025 | 0.011 | -0.004 | 0.026 | 0.009 | -0.006 | 0.023 | 0.009 | -0.006 | 0.024 |
| ACE-III verbal fluency | 0.016 | 0.005 | 0.027 | 0.005 | -0.007 | 0.016 | 0.006 | -0.010 | 0.021 | 0.006 | -0.009 | 0.022 | 0.006 | -0.009 | 0.022 |
| ACE-III visuospatial function | 0.020 | 0.007 | 0.032 | 0.010 | -0.001 | 0.022 | 0.014 | -0.002 | 0.028 | 0.013 | -0.004 | 0.026 | 0.012 | -0.004 | 0.026 |

β*= z-score standardised coefficient beta; CI=confidence interval (bootstrap bias corrected). Note that, because bootstrapping is applied, p-values are not applicable.*

*M0: unadjusted; M1: childhood cognition, childhood socioeconomic position, education; M2: M1+ age at menarche, parity, menopause type; M3: M2+ BMI, smoking, APOE-ε4 status, affective symptoms; M4: M3+ hormone therapy (HT)*

***Supplementary Table 3.*** *Model estimates and 95% confidence intervals, corresponding with Figure 3, for the effect of 1-year increase in age at menopause on standardised z-scores for the Preclinical Alzheimer's Cognitive Composite (PACC; total score and sub-tests) at age 69 to 71 in the Insight 46 sample. N=197.*

| | M0 | | | M1 | | | M2 | | | M3 | | | M4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| PACC total | 0.029 | 0.011, 0.048 | 0.002 | 0.015 | -0.002, 0.032 | 0.089 | 0.018 | -0.004, 0.040 | 0.112 | 0.019 | -0.004, 0.042 | 0.099 | 0.019 | -0.004, 0.042 | 0.099 |
| DSST | 0.033 | 0.008, 0.057 | 0.008 | 0.018 | -0.006, 0.041 | 0.147 | 0.030 | -0.001, 0.0622 | 0.058 | 0.031 | -0.001, 0.062 | 0.058 | 0.031 | -0.001, 0.062 | 0.058 |
| FNAME-12A | 0.033 | 0.008, 0.058 | 0.010 | 0.017 | -0.007, 0.041 | 0.164 | 0.035 | 0.003, 0.067 | 0.030 | 0.037 | 0.005, 0.069 | 0.023 | 0.037 | 0.005, 0.069 | 0.023 |
| Logical memory delayed recall | 0.031 | 0.007, 0.056 | 0.012 | 0.015 | -0.009, 0.039 | 0.222 | 0.003 | -0.028, 0.034 | 0.852 | 0.007 | -0.025, 0.038 | 0.684 | 0.006 | -0.025, 0.038 | 0.684 |
| MMSE* | 0.021 | -0.003, 0.052 | - | 0.009 | -0.014, 0.039 | - | 0.004 | -0.026, 0.037 | - | 0.001 | -0.033, 0.032 | - | 0.003 | -0.035, 0.031 | - |

β*=z-score standardised coefficient beta; CI=confidence interval; DSST=Digit-Symbol Substitution Test; FNAME=Face-Name Associative Memory Examination; MMSE=Mini-Mental State Examination*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable. Some bootstrap replications failed (M0: no failures. M1: 12 replication failures. M2: 10 replication failures. M3: 8 replication failures. M4: 16 replication failures).*

*M0: unadjusted; M1: childhood cognition, childhood socioeconomic position, education; M2: M1+ age at menarche, parity, menopause type; M3: M2+ BMI, smoking, APOE-ε4 status, affective symptoms, age at cognitive testing; M4: M3+ hormone therapy (HT)*

***Supplementary Table 4.*** *Model estimations for menopause age-by-menopause type (natural or surgical) interaction terms in fully adjusted models (M3) regressing menopause age (in years) on standardised Addenbrooke's Cognitive Examination (ACE-III) total and Preclinical Alzheimer's Cognitive Composite (PACC) total performance, in the National Survey for Health and Development (NSHD) whole-cohort and Insight 46 samples, respectively.*

| Outcome | N | Menopause Age x Menopause Type β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| ACE-III total* | 746 | -0.005 | -0.033 | 0.021 | - |
| PACC total | 197 | -0.027 | -0.073 | 0.019 | 0.243 |

β= *z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: natural menopause*

*M3: adjusted for childhood cognition, childhood socioeconomic position, education, age at menarche, parity, BMI, smoking, APOE-ε4 status, affective symptoms, age at cognitive testing [PACC total only]*

***Supplementary Table 5.*** *Model estimations for menopause age-by-APOE-ε4 status (ε4 carrier or non-carrier) interaction terms in fully adjusted models (M3) regressing menopause age (in years) on standardised Addenbrooke's Cognitive Examination (ACE-III) total and Preclinical Alzheimer's Cognitive Composite (PACC) total performance, in the National Survey for Health and Development (NSHD) whole-cohort and Insight 46 samples, respectively.*

| Outcome | N | Menopause Age x *APOE* β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| ACE-III total* | 746 | -0.001 | -0.002 | 0.002 | - |
| PACC total | 197 | -0.001 | -0.039 | 0.037 | 0.949 |

β= *z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: APOE-ε4 non-carrier*

*M3: adjusted for childhood cognition, childhood socioeconomic position, education, age at menarche, parity, menopause type, BMI, smoking, affective symptoms, age at cognitive testing [PACC total only]*

***Supplementary Table 6.*** *Model estimations and 95% confidence intervals for the effect of ever using menopausal hormone therapy (HT), compared with never using HT, on standardised Addenbrooke's Cognitive Examination (ACE-III) total and Preclinical Alzheimer's Cognitive Composite (PACC) total performance, in the National Survey for Health and Development (NSHD) whole-cohort and Insight 46 samples, respectively.*

| | | Unadjusted | | | | Adjusted for menopause age | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **Lower CI** | **Upper CI** | **P** | **β** | **Lower CI** | **Upper CI** | **p-value** |
| ACE-III total* | 746 | -0.041 | -0.157 | 0.147 | - | 0.022 | -0.151 | 0.191 | - |
| PACC total | 197 | -0.024 | -0.265 | 0.216 | 0.841 | 0.059 | -0.183 | 0.301 | 0.630 |

β= *z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: HT never used*

*Unadjusted: only the exposure (HT use) and the outcome are included in the model.*

*Adjusted for menopause age: Age at menopause is added to the model as a covariable. There are no additional covariables in the model.*

***Supplementary Table 7.*** *Model estimates and 95% confidence intervals, individually adjusting for early cognitive and sociodemographic covariables, for the effect of 1-year increase in menopause age on standardised z-scores for the Addenbrooke's Cognitive Examination (ACE-III; total score and sub-domains; n=746) the Preclinical Alzheimer's Cognitive Composite (PACC; n=197). Multiple imputation by chained equations (MICE) was applied with 50 imputations.*

To assess whether effect attenuations from M0 to M1 in the main analyses were driven by a particular variable included in M1, childhood cognition, childhood socioeconomic position, and education covariables were individually adjusted for. The model p-values and confidence intervals are not presented here, the interest is in the change in effect estimates when adjusting for each covariable.

| | **M0 (unadjusted)** | **M0 + childhood cognition** | **M0 + childhood SEP** | **M0 + education** |
|---|---|---|---|---|
| **Outcome** | **β** | **β** | **β** | **β** |
| ACE-III total | 0.024 | 0.011 | 0.019 | 0.018 |
| ACE-III attention and orientation | 0.006 | 0.001 | 0.005 | 0.003 |
| ACE-III language | 0.012 | 0.001 | 0.008 | 0.008 |
| ACE-III memory | 0.023 | 0.013 | 0.019 | 0.019 |
| ACE-III verbal fluency | 0.016 | 0.006 | 0.012 | 0.012 |
| ACE-III visuospatial function | 0.020 | 0.011 | 0.017 | 0.016 |
| PACC total | 0.029 | 0.014 | 0.025 | 0.025 |
| PACC DSST | 0.033 | 0.017 | 0.029 | 0.028 |
| PACC FNAME-12A | 0.033 | 0.016 | 0.028 | 0.027 |
| PACC logical memory delayed recall | 0.031 | 0.014 | 0.028 | 0.027 |
| PACC MMSE | 0.021 | 0.008 | 0.016 | 0.015 |

β*= z-score standardised coefficient beta; CI=confidence interval; DSST=Digit-Symbol Substitution Test; FNAME=Face-Name Associative Memory Examination; MMSE=Mini-Mental State Examination; SEP=socioeconomic position*

***Supplementary Table 8.*** *Model estimates and 95% confidence intervals, individually adjusting for reproductive covariables, for the effect of 1-year increase in menopause age on standardised z-scores for the Addenbrooke's Cognitive Examination (ACE-III) total and ACE-III language, verbal fluency, and visuospatial function sub-domains (n=746), and on standardised z-scores for the Preclinical Alzheimer's Cognitive Composite (PACC) total and digit-symbol substitution test (DSST) and face-name associative memory examination (FNAME) sub-tests (n=197). Multiple imputation by chained equations (MICE) was applied with 50 imputations.*

To assess whether negative confounding observed for ACE-III total, ACE-III language, ACE-III verbal fluency, ACE-III visuospatial function, PACC total, PACC DSST, and PACC FNAME-12A between M1 and M2 in the main analyses was driven by a particular variable included in M2, age at menarche, parity, and menopause type were individually adjusted for. The model p-values and confidence intervals are not presented here, as the interest is in the change in effect estimates when adjusting for each covariable.

| | **M1** | **M1 + age at menarche** | **M1 + parity** | **M1 + menopause type** |
|---|---|---|---|---|
| **Outcome** | **β** | **β** | **β** | **β** |
| ACE-III total | 0.009 | 0.009 | 0.010 | 0.012 |
| ACE-III language | 0.000 | 0.001 | 0.000 | 0.005 |
| ACE-III verbal fluency | 0.005 | 0.004 | 0.005 | 0.007 |
| ACE-III visuospatial function | 0.010 | 0.009 | 0.010 | 0.014 |
| PACC total | 0.015 | 0.016 | 0.014 | 0.018 |
| PACC DSST | 0.018 | 0.017 | 0.018 | 0.031 |
| PACC FNAME-12A | 0.017 | 0.019 | 0.016 | 0.034 |

β*= z-score standardised coefficient beta; CI=confidence interval; DSST=Digit-Symbol Substitution Test; FNAME=Face-Name Associative Memory Examination; MMSE=Mini-Mental State Examination*

*M1: adjusted for childhood cognition, childhood socioeconomic position, and education*

***Supplementary Table 9.*** *Menopause age-by-menopause type interactions, for outcomes which show negative confounding from menopause type in the main analyses. Model estimations for menopause age-by-menopause type (natural or surgical) interaction terms in fully adjusted models (M3) regressing menopause age (in years) on standardised Addenbrooke's Cognitive Examination (ACE-III) language, verbal fluency, and visuospatial fluency (N=746) and on standardised Preclinical Alzheimer's Cognitive Composite (PACC) digit-symbol substitution test (DSST) and face-name associative memory examination (FNAME-12A) (N=197).*

| Outcome | Menopause Age x Menopause Type β | Lower CI | Upper CI | P |
|---|---|---|---|---|
| ACE-III language* | 0.005 | -0.029 | 0.031 | - |
| ACE-III verbal fluency* | -0.014 | -0.047 | 0.014 | - |
| ACE-III visuospatial function* | 0.023 | -0.005 | 0.051 | - |
| PACC DSST | -0.045 | -0.110 | 0.020 | 0.171 |
| PACC FNAME-12A | -0.028 | -0.094 | 0.038 | 0.399 |

β*=z-score standardised coefficient beta; CI=confidence interval. Reference category: natural menopause*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*M3: adjusted for childhood cognition, childhood socioeconomic position, education, age at menarche, parity, BMI, smoking, APOE-ε4 status, affective symptoms, age at cognitive testing (PACC measures only)*

***Supplementary Table 10.*** *Model estimations for type of menopause on standardised Addenbrooke's Cognitive Examination (ACE-III) total and sub-domain scores and on standardised Preclinical Alzheimer's Cognitive Composite (PACC) total and sub-test scores. Multiple imputation is not applied because there is no missing data for the menopause age covariable.*

| | | Unadjusted | | | | Adjusted for menopause age | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **Lower CI** | **Upper CI** | **P** | **β** | **Lower CI** | **Upper CI** | **P** |
| ACE-III total* | 746 | -0.131 | -0.300 | 0.044 | - | 0.084 | -0.149 | 0.296 | - |
| ACE-III attention and orientation* | 746 | -0.031 | -0.223 | 0.135 | - | 0.023 | -0.174 | 0.208 | - |
| ACE-III language* | 746 | -0.013 | -0.182 | 0.139 | - | 0.122 | -0.085 | 0.335 | - |
| ACE-III memory* | 746 | -0.164 | -0.349 | 0.008 | - | 0.019 | -0.201 | 0.240 | - |
| ACE-III verbal fluency* | 746 | -0.096 | -0.253 | 0.056 | - | 0.051 | -0.178 | 0.244 | - |
| ACE-III visuospatial function* | 746 | -0.088 | -0.249 | 0.084 | - | 0.103 | -0.098 | 0.296 | - |
| PACC total | 197 | -0.216 | -0.444 | 0.013 | 0.064 | 0.039 | -0.262 | 0.339 | 0.799 |
| PACC DSST | 197 | -0.133 | -0.434 | 0.169 | 0.386 | 0.234 | -0.161 | 0.629 | 0.245 |
| PACC FNAME-12A | 195 | -0.104 | -0.406 | 0.199 | 0.500 | 0.249 | -0.153 | 0.652 | 0.223 |
| PACC logical memory delayed recall | 197 | -0.413 | -0.710 | -0.117 | 0.007 | -0.286 | -0.681 | 0.109 | 0.154 |
| PACC MMSE* | 197 | -0.223 | -0.606 | 0.082 | - | -0.098 | -0.485 | 0.312 | - |

β= *z-score standardised coefficient beta; CI=confidence interval; DSST=digit-symbol substitution task FNAME-12A=face-name associative memory examination; MMSE=mini-mental state examination*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: natural menopause*

## Model outputs from section 5i sensitivity analyses excluding women with ACE-III total <82

***Supplementary Table 11.*** *Model estimates and bootstrap 95% confidence intervals for the effect of 1-year increase in age at menopause on standardised z-scores for the Addenbrooke's Cognitive Examination (ACE-III) total score at age 69 in the National Survey of Health and Development (NSHD) whole-cohort, and the Preclinical Alzheimer's Cognitive Composite (PACC) at age 69 to 71 in the Insight 46 sample.*

| | | M0 | | | M1 | | | M2 | | | M3 | | | M4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| ACE-III total* | 696 | 0.015 | 0.005, 0.024 | - | 0.006 | -0.003, 0.014 | - | 0.011 | 0.001, 0.021 | - | 0.010 | 0.000, 0.021 | - | 0.010 | -0.001, 0.020 | - |
| PACC total | 192 | 0.026 | 0.010, 0.043 | 0.002 | 0.014 | -0.001, 0.030 | 0.072 | 0.015 | -0.006, 0.036 | 0.150 | 0.016 | -0.005, 0.036 | 0.133 | 0.016 | -0.005, 0.036 | 0.133 |

β= *z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable.*

*M0: unadjusted; M1: childhood cognition, childhood socioeconomic position, education; M2: M1+ age at menarche, parity, menopause type; M3: M2+ BMI, smoking, APOE-ε4 status, affective symptoms (+age at cognitive testing for PACC outcome only); M4: M3+ hormone therapy (HT)*

***Supplementary Table 12.*** *Model estimations for menopause age-by-menopause type (natural or surgical) interaction terms in fully adjusted models (M3) regressing menopause age (in years) on standardised Addenbrooke's Cognitive Examination (ACE-III) total and Preclinical Alzheimer's Cognitive Composite (PACC) total performance, in the National Survey for Health and Development (NSHD) whole-cohort and Insight 46 samples, respectively.*

| **Outcome** | **N** | **Menopause Age x Menopause Type β** | **Lower CI** | **Upper CI** | **P** |
|---|---|---|---|---|---|
| ACE-III total* | 696 | 0.007 | -0.012 | 0.029 | - |
| PACC total | 192 | 0.001 | -0.042 | 0.044 | 0.997 |

β=*z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: natural menopause*

*M3: adjusted for childhood cognition, childhood socioeconomic position, education, age at menarche, parity, BMI, smoking, APOE-ε4 status, affective symptoms, age at cognitive testing [PACC total only]*

***Supplementary Table 13.*** *Model estimations for menopause age-by-APOE-ε4 status (ε4 carrier or non-carrier) interaction terms in fully adjusted models (M3) regressing menopause age (in years) on standardised Addenbrooke's Cognitive Examination (ACE-III) total and Preclinical Alzheimer's Cognitive Composite (PACC) total performance, in the National Survey for Health and Development (NSHD) whole-cohort and Insight 46 samples, respectively.*

| Outcome | N | Menopause Age x *APOE* β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| ACE-III total* | 696 | -0.001 | -0.002 | 0.003 | - |
| PACC total | 192 | 0.004 | -0.030 | 0.038 | 0.813 |

β= *z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: APOE-ε4 non-carrier*

*M3: adjusted for childhood cognition, childhood socioeconomic position, education, age at menarche, parity, menopause type, BMI, smoking, affective symptoms, age at cognitive testing [PACC total only]*

***Supplementary Table 14.*** *Model estimations and 95% confidence intervals for the effect of ever using menopausal hormone therapy (HT), compared with never using HT, on standardised Addenbrooke's Cognitive Examination (ACE-III) total and Preclinical Alzheimer's Cognitive Composite (PACC) total performance, in the National Survey for Health and Development (NSHD) whole-cohort and Insight 46 samples, respectively.*

| | | Unadjusted | | | | Adjusted for menopause age | | | |
|---|---|---|---|---|---|---|---|---|---|
| Outcome | N | β | Lower CI | Upper CI | P | β | Lower CI | Upper CI | P |
| ACE-III total* | 696 | -0.063 | -0.177 | 0.056 | - | -0.023 | -0.158 | 0.097 | - |
| PACC total | 192 | -0.042 | -0.259 | 0.174 | 0.700 | 0.029 | -0.188 | 0.246 | 0.793 |

β=*z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: HT never used*

*Unadjusted: only the exposure (HT use) and the outcome are included in the model.*

*Adjusted for menopause age: Age at menopause is added to the model as a covariable. There are no additional covariables in the model.*

## Model outputs from section 5i sensitivity analyses excluding women who had surgical menopause via bilateral oophorectomy

***Supplementary Table 15.*** *Model estimates and bootstrap 95% confidence intervals for the effect of 1-year increase in age at menopause on standardised z-scores for the Addenbrooke's Cognitive Examination (ACE-III) total score at age 69 in the National Survey of Health and Development (NSHD) whole-cohort, and the Preclinical Alzheimer's Cognitive Composite (PACC) at age 69 to 71 in the Insight 46 sample.*

| | | M0 | | | M1 | | | M2 | | | M3 | | | M3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **95% CI** | **P** | **β** | **Lower CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| ACE-III total* | 663 | 0.023 | 0.011, 0.037 | - | 0.012 | -0.001, 0.021 | - | 0.015 | -0.001, 0.031 | - | 0.015 | -0.003, 0.030 | - | 0.014 | -0.001, 0.030 | - |
| PACC total | 173 | 0.030 | 0.010, 0.050 | 0.003 | 0.014 | -0.004, 0.032 | 0.136 | 0.011 | -0.014, 0.037 | 0.382 | 0.013 | -0.014, 0.039 | 0.343 | 0.013 | -0.014, 0.039 | 0.343 |

β= *z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable.*

*M0: unadjusted; M1: childhood cognition, childhood socioeconomic position, education; M2: M1+ age at menarche, parity, menopause type; M3: M2+ BMI, smoking, APOE-ε4 status, affective symptoms (+age at cognitive testing for PACC outcome only); M4: M3+ hormone therapy (HT)*

***Supplementary Table 16.*** *Model estimations for menopause age-by-APOE-ε4 status (ε4 carrier or non-carrier) interaction terms in fully adjusted models (M3) regressing menopause age (in years) on standardised Addenbrooke's Cognitive Examination (ACE-III) total and Preclinical Alzheimer's Cognitive Composite (PACC) total performance, in the National Survey for Health and Development (NSHD) whole-cohort and Insight 46 samples, respectively.*

| **Outcome** | **N** | **Menopause Age x *APOE* β** | **Lower CI** | **Upper CI** | **P** |
|---|---|---|---|---|---|
| ACE-III total* | 663 | -0.001 | -0.003 | 0.003 | - |
| PACC total | 173 | 0.008 | -0.032 | 0.047 | 0.710 |

β= *z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: APOE-ε4 non-carrier*

*M3: adjusted for childhood cognition, childhood socioeconomic position, education, age at menarche, parity, menopause type, BMI, smoking, affective symptoms, age at cognitive testing [PACC total only]*

***Supplementary Table 17.*** *Model estimations and 95% confidence intervals for the effect of ever using menopausal hormone therapy (HT), compared with never using HT, on standardised Addenbrooke's Cognitive Examination (ACE-III) total and Preclinical Alzheimer's Cognitive Composite (PACC) total performance, in the National Survey for Health and Development (NSHD) whole-cohort and Insight 46 samples, respectively.*

| | | Unadjusted | | | | Adjusted for menopause age | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **Lower CI** | **Upper CI** | **P** | **β** | **Lower CI** | **Upper CI** | **P** |
| ACE-III total* | 663 | -0.068 | -0.230 | 0.108 | - | -0.009 | -0.222 | 0.140 | - |
| PACC total | 173 | -0.041 | -0.297 | 0.215 | 0.752 | 0.031 | -0.226 | 0.287 | 0.814 |

β=*z-score standardised coefficient beta; CI=confidence interval*

**Bootstrapping applied; bias-corrected confidence intervals; p-values not applicable*

*Reference category: HT never used*

*Unadjusted: only the exposure (HT use) and the outcome are included in the model.*

*Adjusted for menopause age: Age at menopause is added to the model as a covariable. There are no additional covariables in the model.*

## 5ii. Supplementary material

***Supplementary table 18.*** *Model estimates and 95% confidence intervals demonstrating the predicted non-linear change in neuroimaging outcomes with each 1-year increase in menopause age. Models are fully adjusted and include a quadratic term for menopause age.*

| Outcome variable | N | Menopause age$^2$ β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| SUVR | 126 | -0.001 | -0.002 | 0.000 | 0.015 |
| Amyloid status [OR; reference=negative] | 126 | 0.914 | 0.833 | 1.002 | 0.055 |
| WMHV* | 123 | 0.999 | 0.988 | 1.009 | 0.786 |
| TBV* | 126 | -0.141 | -0.628 | 0.345 | 0.566 |
| Hippocampal volume* | 126 | 0.001 | -0.002 | 0.004 | 0.690 |
| NAWM FA | 110 | -0.002 | -0.005 | 0.001 | 0.132 |
| NAWM MD | 110 | 0.002 | -0.003 | 0.006 | 0.456 |
| CT: Harvard ADsig | 126 | 0.000 | -0.001 | 0.001 | 0.573 |
| CT: Frontal | 126 | 0.000 | -0.001 | 0.001 | 0.557 |
| CT: Occipital | 126 | 0.000 | -0.001 | 0.001 | 0.664 |
| CT: Parietal | 126 | 0.000 | 0.000 | 0.001 | 0.462 |
| CT: Temporal | 126 | 0.001 | 0.000 | 0.002 | 0.300 |

*Models are adjusted for age at scan, childhood cognition, education, SEP, puberty, parity, smoking, BMI, blood pressure, APOE-ε4 status [*and TIV]*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; TIV=total intracranial volume; SUVR=standardised uptake value ratio; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*

***Supplementary table 19.*** *Model estimates and 95% confidence intervals demonstrating the predicted interaction of APOE-ε4 status (reference=ε4 non-carrier) with non-linear change in amyloid SUVR and likelihood of being amyloid positive with each 1-year increase in menopause age. Models are fully adjusted and include a quadratic term for menopause age and APOE-ε4-by-menopause age interaction terms. N=126*

| Outcome variable | *APOE*-by-menopause age$^2$ β/OR | Lower CI | Upper CI | P |
|---|---|---|---|---|
| SUVR | -0.002 | -0.004 | 0.000 | 0.103 |
| Amyloid status* | 0.966 | 0.755 | 1.235 | 0.782 |

**Reference category=amyloid negative; OR*

*Models are adjusted for age at scan, childhood cognition, education, SEP, puberty, parity, smoking, BMI, and blood pressure.*

*SUVR=standardised uptake value ratio; CI=confidence interval; OR=odds ratio; SEP=socioeconomic position*

***Supplementary table 20.*** *Model estimates and 95% confidence intervals demonstrating the predicted interaction of APOE-ε4 status (reference=ε4 non-carrier) with linear change in neuroimaging outcomes with each 1-year increase in menopause age. The models are fully adjusted and include an APOE-ε4-by-menopause age interaction term. N=123*

| Outcome variable | N | *APOE*-by-menopause age β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| WMHV* | 123 | 0.868 | 0.767 | 0.982 | 0.025 |
| TBV* | 126 | 4.105 | -2.110 | 10.319 | 0.193 |
| Hippocampal volume* | 126 | -0.019 | -0.057 | 0.019 | 0.332 |
| NAWM FA | 110 | 0.020 | -0.021 | 0.060 | 0.341 |
| NAWM MD | 110 | -0.043 | -0.101 | 0.014 | 0.140 |
| CT: Harvard ADsig | 126 | 0.012 | 0.002 | 0.022 | 0.016 |
| CT: Frontal | 126 | 0.010 | -0.002 | 0.023 | 0.091 |
| CT: Occipital | 126 | 0.010 | -0.002 | 0.022 | 0.093 |
| CT: Parietal | 126 | 0.013 | 0.003 | 0.022 | 0.012 |
| CT: Temporal | 126 | 0.014 | 0.001 | 0.027 | 0.037 |

*Model is adjusted for age at scan, childhood cognition, education, SEP, puberty, parity, smoking, BMI, blood pressure [*and TIV]*

*CI=confidence interval; SEP=socioeconomic position; TIV=total intracranial volume; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*

***Supplementary table 21.*** *Model estimates and 95% confidence intervals demonstrating the predicted non-linear change in amyloid SUVR and likelihood of being amyloid positive with each 1-year increase in menopause age, with cumulative adjustments for life course covariables. Models include a quadratic term for menopause age. N=126*

| | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome variable** | **β/OR** | **95% CI** | **P** | **β/OR** | **95% CI** | **P** | **β/OR** | **95% CI** | **P** | **β/OR** | **95% CI** | **P** | **β/OR** | **95% CI** | **P** |
| SUVR | -0.001 | -0.002, 0.000 | 0.015 | -0.001 | -0.002, 0.000 | 0.012 | -0.001 | -0.002, 0.000 | 0.008 | -0.001 | -0.002, 0.000 | 0.015 | -0.001 | -0.002, 0.000 | 0.015 |
| Amyloid status* | 0.930 | 0.864, 1.001 | 0.052 | 0.933 | 0.868, 1.003 | 0.060 | 0.924 | 0.855, 0.998 | 0.045 | 0.914 | 0.833, 1.002 | 0.055 | 0.913 | 0.833, 1.001 | 0.053 |

**Reference category=amyloid negative; OR*

*M0:age at scan; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; HT=hormone therapy; SUVR=standardised uptake value ratio*

***Supplementary table 22.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in neuroimaging outcomes with each 1-year increase in menopause age among APOE-ε4 carriers and non-carriers), with cumulative adjustments for life course covariables.*

| | | | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***APOE*-ε4** | **Outcome** | **N** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| Carriers | WMHV* | 35 | 0.885 | 0.792, 0.989 | 0.032 | 0.879 | 0.783, 0.987 | 0.029 | 0.877 | 0.766, 1.004 | 0.057 | 0.923 | 0.783, 1.088 | 0.341 | 0.926 | 0.783, 1.096 | 0.374 |
| | CT: Harvard ADsig | 36 | 0.009 | 0.002, 0.017 | 0.018 | 0.009 | 0.001, 0.017 | 0.037 | 0.007 | -0.002, 0.016 | 0.113 | 0.007 | -0.004, 0.018 | 0.187 | 0.007 | -0.004, 0.018 | 0.209 |
| | CT: Frontal | 36 | 0.007 | -0.002, 0.016 | 0.130 | 0.006 | -0.003, 0.016 | 0.193 | 0.006 | -0.005, 0.017 | 0.237 | 0.006 | -0.007, 0.019 | 0.318 | 0.006 | -0.007, 0.019 | 0.348 |
| | CT: Occipital | 36 | 0.006 | -0.004, 0.017 | 0.236 | 0.007 | -0.004, 0.019 | 0.207 | 0.006 | -0.006, 0.019 | 0.296 | 0.005 | -0.010, 0.020 | 0.469 | 0.005 | -0.010, 0.021 | 0.497 |
| | CT: Parietal | 36 | 0.011 | 0.004, 0.019 | 0.003 | 0.011 | 0.003, 0.018 | 0.009 | 0.010 | 0.001, 0.019 | 0.024 | 0.009 | -0.001, 0.020 | 0.074 | 0.009 | -0.001, 0.019 | 0.086 |
| | CT: Temporal | 36 | 0.010 | -0.001, 0.020 | 0.064 | 0.011 | 0.000, 0.022 | 0.049 | 0.010 | -0.003, 0.022 | 0.113 | 0.008 | -0.006, 0.023 | 0.232 | 0.008 | -0.007, 0.023 | 0.256 |
| Non-carriers | WMHV* | 88 | 1.038 | 0.979, 1.100 | 0.209 | 1.040 | 0.979, 1.105 | 0.204 | 1.032 | 0.969, 1.098 | 0.326 | 1.023 | 0.963, 1.086 | 0.459 | 1.024 | 0.964, 1.088 | 0.442 |
| | CT: Harvard ADsig | 90 | -0.004 | -0.009, 0.001 | 0.092 | -0.004 | -0.009, 0.001 | 0.080 | -0.003 | -0.008, 0.001 | 0.166 | -0.004 | -0.009, 0.001 | 0.134 | -0.005 | -0.009, 0.000 | 0.067 |
| | CT: Frontal | 90 | -0.003 | -0.009, 0.002 | 0.267 | -0.004 | -0.010, 0.002 | 0.195 | -0.003 | -0.009, 0.003 | 0.299 | -0.003 | -0.009, 0.003 | 0.255 | -0.004 | -0.010, 0.002 | 0.167 |
| | CT: Occipital | 90 | -0.003 | -0.009, 0.002 | 0.195 | -0.004 | -0.009, 0.002 | 0.170 | -0.003 | -0.009, 0.002 | 0.253 | -0.004 | -0.010, 0.002 | 0.157 | -0.005 | -0.010, 0.000 | 0.066 |
| | CT: Parietal | 90 | -0.003 | -0.008, 0.001 | 0.161 | -0.003 | -0.008, 0.002 | 0.186 | -0.002 | -0.007, 0.003 | 0.358 | -0.002 | -0.007, 0.003 | 0.340 | -0.003 | -0.008, 0.002 | 0.228 |
| | CT: Temporal | 90 | -0.004 | -0.010, 0.002 | 0.173 | -0.004 | -0.010, 0.003 | 0.268 | -0.004 | -0.010, 0.003 | 0.291 | -0.004 | -0.011, 0.002 | 0.198 | -0.005 | -0.012, 0.001 | 0.100 |

*M0:age at scan, [*TIV]; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure*

*CI=confidence interval; SEP=socioeconomic position; TIV=total intracranial volume; HT=hormone therapy; WMHV=white matter hyperintensity volume; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*

***Supplementary table 23.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in neuroimaging outcomes with each 1-year increase in menopause age, with cumulative adjustments for life course covariables, with APOE pooled.*

| | | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| TBV* | 126 | 3.167 | 0.717, 5.617 | 0.012 | 3.224 | 0.732, 5.716 | 0.012 | 2.861 | 0.309, 5.414 | 0.028 | 2.417 | -0.152, 4.986 | 0.065 | 2.419 | -0.159, 4.998 | 0.066 |
| Hippocampal volume* | 126 | 0.008 | -0.007, 0.023 | 0.275 | 0.009 | -0.006, 0.024 | 0.260 | 0.010 | -0.005, 0.026 | 0.196 | 0.009 | -0.007, 0.025 | 0.262 | 0.009 | -0.007, 0.024 | 0.258 |
| NAWM FA | 110 | -0.008 | -0.022, 0.007 | 0.292 | -0.007 | -0.022, 0.008 | 0.357 | -0.007 | -0.023, 0.008 | 0.352 | -0.006 | -0.022, 0.010 | 0.447 | -0.006 | -0.023, 0.010 | 0.439 |
| NAWM MD | 110 | 0.012 | -0.009, 0.032 | 0.261 | 0.009 | -0.012, 0.030 | 0.405 | 0.008 | -0.015, 0.030 | 0.493 | 0.007 | -0.017, 0.030 | 0.575 | 0..007 | -0.016, 0.030 | 0.559 |

*M0:age at scan, [*TIV]; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status*

*CI=confidence interval; SEP=socioeconomic position; TIV=total intracranial volume; HT=hormone therapy; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity*

***Supplementary table 24.*** *Model estimates and 95% confidence intervals demonstrating the predicted difference in neuroimaging outcomes measures in hormone therapy (HT) ever users compared with never users.*

| | | M0 | | | | M0 + menopause age | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **Lower CI** | **Upper CI** | **P** | **β** | **Lower CI** | **Upper CI** | **P** |
| SUVR | 121 | 0.002 | -0.025 | 0.028 | 0.894 | 0.001 | -0.025 | 0.028 | 0.926 |
| Aβ status (OR; reference=negative) | 121 | 0.359 | 0.080 | 1.613 | 0.182 | 0.354 | 0.078 | 1.599 | 0.177 |
| WMHV* | 118 | 0.097 | -0.282 | 0.475 | 0.617 | 0.111 | -0.268 | 0.489 | 0.567 |
| TBV* | 121 | -4.492 | -21.559 | 12.575 | 0.603 | -5.445 | -22.200 | 11.309 | 0.521 |
| Hippocampal volume* | 121 | -0.078 | -0.174 | 0.019 | 0.115 | -0.081 | -0,177 | 0.016 | 0.101 |
| NAWM FA | 105 | 0.029 | -0.067 | 0.126 | 0.546 | 0.033 | -0.064 | 0.130 | 0.498 |
| NAWM MD | 105 | -0.028 | -0.162 | 0.106 | -0.679 | -0.033 | -0.168 | 0.101 | 0.623 |
| CT: Harvard ADsig | 121 | -0.015 | -0.042 | 0.011 | 0.261 | -0.015 | -0.041 | 0.012 | 0.282 |
| CT: Frontal | 121 | -0.014 | -0.046 | 0.017 | 0.367 | -0.014 | -0.046 | 0.018 | 0.384 |
| CT: Occipital | 121 | -0.029 | -0.060 | 0.002 | 0.062 | -0.029 | -0.059 | 0.002 | 0.069 |
| CT: Parietal | 121 | -0.012 | -0.038 | 0.014 | 0.361 | -0.012 | -0.038 | 0.015 | 0.375 |
| CT: Temporal | 121 | -0.028 | -0.062 | 0.006 | 0.101 | -0.027 | -0.061 | 0.006 | 0.111 |

*Reference category: Never use M0=scan age, [*TIV]*

*CI=confidence interval; TIV=total intracranial volume; HT=hormone therapy; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*

***Supplementary table 25.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in WMHV with each 1-year increase in menopause age among APOE-ε4 carriers, cumulatively adjusting for health-related (M3) covariables. N=35*

| | M2+smoking | | | M2+BMI | | | M2+SBP+DBP | | | M2+SBP | | | M2+DBP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| WMHV | 0.934 | 0.807, 1.080 | 0.356 | 0.880 | 0.772, 1.003 | 0.056 | 0.877 | 0.755, 1.020 | 0.088 | 0.877 | 0.773, 0.995 | 0.041 | 0.892 | 0.780, 1.021 | 0.097 |

*M0:age at scan, TIV; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure*

*WMHV=white matter hyperintensity volume; CI=confidence interval; SEP=socioeconomic position; TIV=total intracranial volume; SBP=systolic blood pressure; DBP=diastolic blood pressure*

***Supplementary table 26.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in TBV($cm^3$) with each 1-year increase in menopause age, cumulatively adjusting for health-related (M3) covariables. N=126*

| | M2+smoking | | | M2+BMI | | | M2+SBP+DBP | | | M2+SBP | | | M2+DBP | | | M2+*APOE*-ε4 status | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| TBV | 2.785 | 0.229, 5.342 | 0.033 | 2.860 | 0.298, 5.422 | 0.029 | 2.544 | 0.004, 5.084 | 0.050 | 2.746 | 0.191, 5.302 | 0.035 | 2.853 | 0.292, 5.415 | 0.029 | 2.796 | 0.227, 5.365 | 0.033 |

*M0:age at scan, TIV; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status*

*TBV=total brain volume; CI=confidence interval; SEP=socioeconomic position; TIV=total intracranial volume; SBP=systolic blood pressure; DBP=diastolic blood pressure*

***Supplementary table 27.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in cortical thickness(mm) in the Harvard ADsig ROI with each 1-year increase in menopause age among APOE-ε4 carriers, cumulatively adjusting for reproductive (M2) covariables. N=36*

| | M1+puberty | | | | M1+parity | | | |
|---|---|---|---|---|---|---|---|---|
| **Outcome variable** | **β** | **Lower CI** | **Upper CI** | **P** | **β** | **Lower CI** | **Upper CI** | **P** |
| Harvard ADsig | 0.009 | 0.000 | 0.017 | 0.043 | 0.008 | -0.001 | 0.017 | 0.098 |

*M0:age at scan; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure*

*Harvard ADsig=Harvard Alzheimer's Disease signature regions; ROI=regions of interest; CI=confidence interval; SEP=socioeconomic position*

***Supplementary table 28.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in cortical thickness(mm) in the parietal ROI with each 1-year increase in menopause age among APOE-ε4 carriers, cumulatively adjusting for health-related (M3) covariables. N=36*

| | M2+smoking | | | M2+BMI | | | M2+SBP+DBP | | | M2+SBP | | | M2+DBP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome variable** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| Parietal | 0.010 | 0.000, 0.019 | 0.045 | 0.010 | 0.001, 0.019 | 0.034 | 0.010 | 0.001, 0.019 | 0.039 | 0.010 | 0.001, 0.019 | 0.027 | 0.010 | 0.001, 0.019 | 0.033 |

*M0:age at scan; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure*

*Harvard ADsig=Harvard Alzheimer's Disease signature regions; ROI=regions of interest; CI=confidence interval; SEP=socioeconomic position; SBP=systolic blood pressure; DBP=diastolic blood pressure*

## Model outputs from section 5ii sensitivity analyses adjusting for WMHV

***Supplementary table 29.*** *Model estimates and 95% confidence intervals demonstrating the predicted non-linear change in amyloid SUVR and in the likelihood of being amyloid positive with each 1-year increase in menopause age, in fully-adjusted models (M3). Models include a quadratic term for menopause age. N=126*

| Outcome variable | Menopause age$^2$ β/OR | Lower CI | Upper CI | P |
|---|---|---|---|---|
| SUVR | -0.001 | -0.002 | 0.000 | 0.015 |
| Amyloid status* | 0.915 | 0.835 | 1.002 | 0.056 |

**OR; Reference category=amyloid negative*

*Models are adjusted for: age at scan, WMHV, childhood cognition, education, SEP, puberty, parity, smoking, BMI, blood pressure, APOE-ε4 status*

*CI=confidence interval; OR=odds ratio; HT=hormone therapy; SEP=socioeconomic position; WMHV=white matter hyperintensity volume; SUVR=standardised uptake value ratio*

***Supplementary table 30.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in neuroimaging outcomes with each 1-year increase in menopause age, in fully-adjusted models (M3).*

| Outcome variable | N | β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| TBV* | 126 | 2.404 | -0.179 | 4.986 | 0.068 |
| Hippocampal volume* | 126 | 0.009 | -0.007 | 0.024 | 0.278 |
| NAWM FA | 110 | -0.005 | -0.021 | 0.010 | 0.493 |
| NAWM MD | 110 | 0.005 | -0.017 | 0.028 | 0.634 |

*Models are adjusted for: age at scan, [*TIV], WMHV, childhood cognition, education, SEP, puberty, parity, smoking, BMI, blood pressure, APOE-ε4 status*

*CI=confidence interval; SEP=socioeconomic position; TIV=total intracranial volume; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity*

***Supplementary table 31.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in cortical thickness(mm) ROI with each 1-year increase in menopause age among APOE-ε4 carriers (n=36) and non-carriers (n=90), in fully-adjusted models (M3).*

| ***APOE*-ε4 status** | **Outcome variable** | **β** | **Lower CI** | **Upper CI** | **P** |
|---|---|---|---|---|---|
| Carriers | Harvard ADsig | 0.009 | -0.003 | 0.022 | 0.129 |
| | Frontal | 0.006 | -0.009 | 0.021 | 0.415 |
| | Occipital | 0.005 | -0.012 | 0.022 | 0.535 |
| | Parietal | 0.011 | -0.001 | 0.022 | 0.068 |
| | Temporal | 0.012 | -0.004 | 0.028 | 0.137 |
| Non-carriers | Harvard ADsig | -0.003 | -0.008 | 0.002 | 0.178 |
| | Frontal | -0.003 | -0.009 | 0.003 | 0.309 |
| | Occipital | -0.003 | -0.009 | 0.002 | 0.236 |
| | Parietal | -0.002 | -0.007 | 0.003 | 0.405 |
| | Temporal | -0.004 | -0.011 | 0.003 | 0.259 |

*Models are adjusted for: age at scan, WMHV, childhood cognition, education, SEP, puberty, parity, smoking, BMI, blood pressure*

*Harvard ADsig=Harvard Alzheimer's Disease signature regions; ROI=regions of interest; CI=confidence interval; SEP=socioeconomic position; WMHV=white matter hyperintensity volume*

## Model outputs from section 5ii sensitivity analyses excluding women with clinically diagnosed neurological conditions

***Supplementary table 32.*** *Model estimates and 95% confidence intervals demonstrating the predicted non-linear change in neuroimaging outcomes with each 1-year increase in menopause age. Models are fully adjusted and include a quadratic term for menopause age.*

| Outcome variable | N | Menopause age² β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| SUVR | 114 | -0.001 | -0.002 | 0.000 | **0.006** |
| Amyloid status {OR; reference=negative] | 114 | 0.980 | 0.948 | 1.013 | 0.232 |

*Models are adjusted for age at scan, [*TIV], childhood cognition, education, SEP, puberty, parity, smoking, BMI, blood pressure, and APOE-ε4 status.*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; TIV-total intracranial volume; SUVR=standardised uptake value ratio; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*

***Supplementary table 33.*** *Model estimates and 95% confidence intervals demonstrating the predicted interaction of APOE-ε4 status (reference=ε4 non-carrier) with non-linear change in amyloid SUVR with each 1-year increase in menopause age. The model is fully adjusted and includes a quadratic term for menopause age and APOE-ε4-by-menopause age interaction terms. N=114*

| Outcome variable | APOE-by-menopause age² β | Lower CI | Upper CI | p |
|---|---|---|---|---|
| SUVR | -0.001 | -0.004 | 0.001 | 0.303 |

*Models are adjusted for age at scan, childhood cognition, education, SEP, puberty, parity, smoking, BMI, and blood pressure.*

*CI=confidence interval; SEP=socioeconomic position; SUVR=standardised uptake value ratio*

***Supplementary table 34.*** *Model estimates and 95% confidence intervals demonstrating the predicted interaction of APOE-ε4 status (reference=ε4 non-carrier) with linear change in neuroimaging outcomes with each 1-year increase in menopause age. The models are fully adjusted and include an APOE-ε4-by-menopause age interaction term.*

| Outcome variable | N | *APOE*-by-menopause age β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| Amyloid status [OR; reference=negative] | 114 | 1.126 | 0.813 | 1.559 | 0.476 |
| WMHV* | 113 | 0.858 | 0.752 | 0.979 | 0.023 |
| TBV* | 114 | 5.551 | -0.7122 | 11.813 | 0.082 |
| Hippocampal volume* | 114 | -0.026 | -0.065 | 0.013 | 0.187 |
| NAWM FA | 102 | 0.037 | -0.005 | 0.078 | 0.082 |
| NAWM MD | 102 | -0.069 | -0.126 | -0.012 | 0.017 |
| CT: Harvard ADsig | 114 | 0.014 | 0.004 | 0.024 | 0.007 |
| CT: Frontal | 114 | 0.011 | -0.002 | 0.023 | 0.104 |
| CT: Occipital | 114 | 0.011 | -0.002 | 0.023 | 0.091 |
| CT: Parietal | 114 | 0.015 | 0.005 | 0.025 | 0.003 |
| CT: Temporal | 114 | 0.014 | 0.000 | 0.027 | 0.050 |

*Reference category=amyloid negative*

*Models are adjusted for age at scan, [*TIV], childhood cognition, education, SEP, puberty, parity, smoking, BMI, and blood pressure.*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; TIV=total intracranial volume; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*

***Supplementary table 35.*** *Model estimates and 95% confidence intervals demonstrating the predicted non-linear change in amyloid SUVR with each 1-year increase in menopause age, with cumulative adjustments for life course covariables. The model includes a quadratic term for menopause age. N=114*

| | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| SUVR | -0.001 | -0.002, 0.000 | 0.013 | -0.001 | -0.002, 0.000 | 0.015 | -0.001 | -0.002, 0.000 | 0.009 | -0.001 | -0.002, 0.000 | 0.006 | -0.001 | -0.002, 0.000 | 0.007 |

*M0:age at scan; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status*

*SUVR=standardised uptake value ratio; CI=confidence interval; SEP=socioeconomic position; HT=hormone therapy*

***Supplementary table 36.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in neuroimaging outcomes with each 1-year increase in menopause age, with cumulative adjustments for life course covariables.*

| | | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| Amyloid status [OR; reference=negative] | 114 | 1.018 | 0.903, 1.148 | 0.769 | 1.032 | 0.912, 1.167 | 0.617 | 1.023 | 0.900, 1.164 | 0.727 | 1..047 | 0.911, 1.204 | 0.516 | 1.047 | 0.911, 1.204 | 0.515 |
| Hippocampal volume* | 114 | 0.008 | -0.008, 0.024 | 0.310 | 0.009 | -0.007, 0.026 | 0.259 | 0.009 | -0.008, 0.026 | 0.299 | 0.007 | -0.010, 0.024 | 0.431 | 0.008 | -0.010, 0.025 | 0.389 |
| CT: Frontal | 114 | - 0.001 | -0.006, 0.004 | 0.656 | - 0.001 | -0.006, 0.004 | 0.634 | - 0.001 | -0.006, 0.005 | 0.788 | - 0.001 | -0.007, 0.005 | 0.739 | - 0.001 | -0.007, 0.005 | 0.759 |

*M0:age at scan, [*TIV]; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; HT=hormone therapy; TIV=total intracranial volume; CT=cortical thickness*

***Supplementary table 37.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in neuroimaging outcomes with each 1-year increase in menopause age among APOE-ε4 carriers and non-carriers, with cumulative adjustments for life course covariables.*

| | | | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***APOE*-ε4** | **Outcome** | **N** | **β** | **95% CI** | **p** | **β** | **95% CI** | **p** | **β** | **95% CI** | **p** | **β** | **95% CI** | **p** | **β** | **95% CI** | **p** |
| Carriers | WMHV* | 34 | 0.881 | 0.787, 0.987 | 0.028 | 0.876 | 0.778, 0.986 | 0.029 | 0.860 | 0.753, 0.982 | 0.026 | 0.910 | 0.779, 1.062 | 0.232 | 0.915 | 0.778, 1.075 | 0.280 |
| | TBV* | 35 | 5.717 | -0.204, 11.637 | 0.058 | 5.881 | -0.515, 12.277 | 0.070 | 5.258 | -1.664, 12.181 | 0.129 | 3.711 | -4.301, 11.723 | 0.341 | 3.774 | -4.534, 12.082 | 0.349 |
| | NAWM FA | 30 | 0.010 | -0.020, 0.040 | 0.485 | 0.007 | -0.027, 0.041 | 0.689 | 0.004 | -0.032, 0.03 | 0.829 | 0.003 | -0.037, 0.044 | 0.857 | 0.004 | -0.038, 0.046 | 0.838 |
| | NAWM MD | 30 | -0.028 | -0.062, 0.007 | 0.112 | -0.029 | -0.069, 0.011 | 0.145 | -0.029 | -0.077, 0.019 | 0.216 | -0.032 | -0.086, 0.023 | 0.228 | -0.032 | -0.088, 0.023 | 0.226 |
| | CT: Harvard ADsig | 35 | 0.009 | 0.001, 0.017 | 0.025 | 0.008 | -0.001, 0.016 | 0.069 | 0.007 | -0.002, 0.016 | 0.136 | 0.007 | -0.003, 0.017 | 0.173 | 0.007 | -0.004, 0.018 | 0.204 |
| | CT: Occipital | 35 | 0.006 | -0.005, 0.017 | 0.305 | 0.006 | -0.006, 0.018 | 0.287 | 0.005 | -0.007, 0.018 | 0.393 | 0.005 | -0.010, 0.020 | 0.497 | 0.005 | -0.011, 0.021 | 0.531 |
| | CT: Parietal | 35 | 0.011 | 0.004, 0.019 | 0.004 | 0.010 | 0.002, 0.018 | 0.016 | 0.010 | 0.001, 0.018 | 0.029 | 0.009 | -0.001, 0.019 | 0.072 | 0.009 | -0.002, 0.020 | 0.092 |
| | CT: Temporal | 35 | 0.008 | -0.002, 0.019 | 0.100 | 0.009 | -0.002, 0.020 | 0.098 | 0.009 | -0.004, 0.021 | 0.155 | 0.009 | -0.005, 0.023 | 0.211 | 0.009 | -0.006, 0.023 | 0.223 |
| Non-carriers | WMHV* | 79 | 1.053 | 0.985, 1.125 | 0.131 | 1.045 | 0.975, 1.121 | 0.210 | 1.034 | 0.961, 1.113 | 0.371 | 1.016 | 0.947, 1.091 | 0.656 | 1.018 | 0.949, 1.091 | 0.619 |
| | TBV* | 79 | 0.861 | -2.135, 3.856 | 0.569 | 1.244 | -1.826, 4.314 | 0.422 | 0.411 | -2.861, 3.682 | 0.803 | 0.388 | -2.878, 3.653 | 0.813 | 0.347 | -2.941, 3.634 | 0.834 |
| | NAWM FA | 72 | -0.024 | -0.043, -0.004 | 0.017 | -0.025 | -0.046, -0.004 | 0.018 | -0.029 | -0.052, -0.006 | 0.014 | -0.028 | -0.050, -0.005 | 0.019 | -0.028 | -0.051, -0.005 | 0.020 |
| | NAWM MD | 72 | 0.035 | 0.008, 0.0622 | 0.011 | 0.037 | 0.008, 0.065 | 0.012 | 0.039 | 0.007, 0.070 | 0.017 | 0.038 | 0.005, 0.070 | 0.023 | 0.038 | 0.006, 0.070 | 0.022 |
| | CT: Harvard ADsig | 79 | -0.006 | -0.011, -0.001 | 0.011 | -0.007 | -0.012, -0.002 | 0.008 | -0.006 | -0.012, -0.001 | 0.027 | -0.006 | -0.012, 0.000 | 0.038 | -0.006 | -0.012, -0.001 | 0.025 |
| | CT: Occipital | 79 | -0.005 | -0.011, 0.001 | 0.079 | -0.006 | -0.012, 0.001 | 0..074 | -0.005 | -0.011, 0.002 | 0.155 | -0.004 | -0.011, 0.002 | 0.179 | -0.005 | -0.011, 0.001 | 0.112 |
| | CT: Parietal | 79 | -0.006 | -0.011, -0.001 | 0.018 | -0.006 | -0.011, -0.001 | 0.016 | -0.005 | -0.011, 0.000 | 0.064 | -0.005 | -0.010, 0.001 | 0.101 | -0.005 | -0.010, 0.001 | -0.080 |
| | CT: Temporal | 79 | -0.005 | -0.012, 0.002 | 0.148 | -0.004 | -0.011, 0.003 | 0.219 | -0.005 | -0.012, 0.003 | 0.238 | -0.005 | -0.012, 0.003 | 0.226 | -0.005 | -0.013, 0.002 | 0.169 |

*M0:age at scan, [*TIV]; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure*

*CI=confidence interval; SEP=socioeconomic position; HT=hormone therapy; TIV=total intracranial volume; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*

## Model outputs from section 5ii sensitivity analyses excluding women with early menopause (age <45 years)

***Supplementary table 38.*** *Model estimates and 95% confidence intervals demonstrating the predicted non-linear change in amyloid standardised uptake value ratio (SUVR) and odds ratio for being amyloid positive, with each 1-year increase in menopause age. Models are fully adjusted and include a quadratic term for menopause age.*

| Outcome variable | N | Menopause age² β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| SUVR | 124 | -0.002 | -0.003 | -0.000 | **0.007** |
| Amyloid status {OR; reference=negative] | 124 | 0.915 | 0.834 | 1.003 | **0.058** |

*Models are adjusted for age at scan, childhood cognition, education, SEP, puberty, parity, smoking, BMI, blood pressure, and APOE-ε4 status.*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; SUVR=standardised uptake value ratio*

***Supplementary table 39.*** *Model estimates and 95% confidence intervals demonstrating the predicted interaction of APOE-ε4 status (reference=ε4 non-carrier) with non-linear change in amyloid SUVR and Aβ odds ratio, with each 1-year increase in menopause age. Models are fully adjusted and include a quadratic term for menopause age and APOE-ε4-by-menopause age interaction terms.*

| Outcome variable | N | *APOE*-by-menopause age² β | Lower CI | Upper CI | p |
|---|---|---|---|---|---|
| SUVR | 124 | -0.002 | -0.004 | 0.001 | 0.249 |
| Amyloid status {OR; reference=negative] | 124 | 0.969 | 0.759 | 1.237 | 0.800 |

*Models are adjusted for age at scan, childhood cognition, education, SEP, puberty, parity, smoking, BMI, and blood pressure.*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; SUVR=standardised uptake value ratio*

***Supplementary table 40.*** *Model estimates and 95% confidence intervals demonstrating the predicted interaction of APOE-ε4 status (reference=ε4 non-carrier) with linear change in neuroimaging outcomes with each 1-year increase in menopause age. The models are fully adjusted and include an APOE-ε4-by-menopause age interaction term.*

| Outcome variable | N | *APOE*-by-menopause age β | Lower CI | Upper CI | P |
|---|---|---|---|---|---|
| WMHV* | 121 | -0.140 | -0.270 | -0.010 | **0.035** |
| TBV* | 124 | 5.739 | -0.691 | 12.168 | **0.080** |
| Hippocampal volume* | 124 | -0.014 | -0.054 | 0.258 | 0.486 |
| NAWM FA | 109 | 0.028 | -0.014 | 0.070 | 0.188 |
| NAWM MD | 109 | -0.053 | -0.112 | 0.007 | **0.081** |
| CT: Harvard ADsig | 124 | 0.012 | 0.002 | 0.022 | **0.023** |
| CT: Frontal | 124 | 0.010 | -0.002 | 0.023 | 0.111 |
| CT: Occipital | 124 | 0.011 | -0.001 | 0.024 | **0.076** |
| CT: Parietal | 124 | 0.012 | 0.002 | 0.022 | **0.019** |
| CT: Temporal | 124 | 0.012 | -0.002 | 0.026 | **0.089** |

*Reference category=amyloid negative*

*Models are adjusted for age at scan, [*TIV], childhood cognition, education, SEP, puberty, parity, smoking, BMI, and blood pressure.*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; TIV=total intracranial volume; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; FA=fractional anisotropy; MD=mean diffusivity; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*

***Supplementary table 41.*** *Model estimates and 95% confidence intervals demonstrating the predicted non-linear change in amyloid SUVR and odds ratio for being amyloid positive, with each 1-year increase in menopause age, with cumulative adjustments for life course covariables. The models include a quadratic term for menopause age. N=124.*

| | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| SUVR | -0.001 | -0.003, -0.000 | **0.012** | -0.001 | -0.003, -0.000 | **0.015** | -0.002 | -0.003, -0.000 | **0.014** | -0.002 | -0.003, -0.000 | **0.007** | -0.002 | -0.003, -0.000 | **0.007** |
| Amyloid status {OR; reference=negative] | 0.930 | 0.864, 1.001 | **0.052** | 0.933 | 0.868, 1.003 | **0.061** | 0.924 | 0.855, 0.998 | **0.045** | 0.915 | 0.834, 1.003 | **0.058** | 0.913 | 0.833, 1.002 | **0.056** |

*M0:age at scan; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status*

*SUVR=standardised uptake value ratio; CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; HT=hormone therapy*

***Supplementary table 42.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in neuroimaging outcomes with each 1-year increase in menopause age, with cumulative adjustments for life course covariables.*

| | | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **N** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** | **β** | **95% CI** | **P** |
| Hippocampal volume* | 124 | 0.004 | -0.013, 0.020 | 0.651 | 0.004 | -0.013, 0.021 | 0.618 | 0.006 | -0.012, 0.024 | 0.505 | 0.004 | -0.014, 0.022 | 0.688 | 0.004 | -0.014, 0.022 | 0.634 |
| NAWM FA | 109 | -0.012 | -0.028, 0.003 | 0.123 | -0.012 | -0.028, 0.005 | 0.161 | -0.013 | -0.030, 0.005 | 0.147 | -0.011 | -0.028, 0.007 | 0.230 | -0.011 | -0.029, 0.007 | 0.217 |
| CT: Frontal | 124 | -0.001 | -0.006, 0.004 | 0.714 | -0.001 | -0.006, 0.004 | 0.739 | 0.000 | -0.005, 0.006 | 0.917 | 0.001 | -0.005, 0.007 | 0.756 | 0.001 | -0.005, 0.007 | 0.703 |

*M0:age at scan, [*TIV]; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure, APOE-ε4 status*

*CI=confidence interval; OR=odds ratio; SEP=socioeconomic position; HT=hormone therapy; TIV=total intracranial volume; NAWM=normal appearing white matter; FA=fractional anisotropy; CT=cortical thickness*

***Supplementary table 43.*** *Model estimates and 95% confidence intervals demonstrating the predicted linear change in neuroimaging outcomes with each 1-year increase in menopause age among APOE-ε4 carriers and non-carriers, with cumulative adjustments for life course covariables.*

| | | | M0 | | | M1 | | | M2 | | | M3 | | | M3+HT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **APOE-ε4** | **Outcome** | **N** | **β** | **95% CI** | **p** | **β** | **95% CI** | **p** | **β** | **95% CI** | **p** | **β** | **95% CI** | **p** | **β** | **95% CI** | **p** |
| Carriers | WMHV* | 35 | -0.122 | -0.233, 0.011 | **0.032** | -0.129 | -0.245, 0.013 | **0.029** | -0.129 | -0.265, 0.007 | 0.062 | -0.080 | -0.243, 0.084 | 0.341 | -0.074 | -0.240, 0.092 | 0.382 |
| | TBV* | 36 | 5.715 | -0.070, 11.499 | 0.053 | 5.869 | -0.393, 12.131 | 0.065 | 5.418 | -1.379, 12.216 | 0.113 | 4.012 | -3.636, 11.660 | 0.284 | 4.087 | -3.798, 11.971 | 0.289 |
| | NAWM MD | 31 | -0.026 | -0.067, 0.015 | 0.202 | -0.022 | -0.067, 0.024 | 0.342 | -0.026 | -0.081, 0.030 | 0.347 | -0.031 | -0.092, 0.030 | 0.299 | -0.031 | -0.095, 0.033 | 0.309 |
| | CT: Harvard ADsig | 36 | 0.009 | 0.002, 0.017 | **0.018** | 0.009 | 0.001, 0.017 | **0.037** | 0.007 | -0.002, 0.016 | 0.112 | 0.007 | -0.004, 0.018 | 0.193 | 0.007 | -0.004, 0.018 | 0.219 |
| | CT: Occipital | 36 | 0.006 | -0.004, 0.017 | 0.236 | 0..007 | -0.004, 0.019 | 0.207 | 0.006 | -0.006, 0.019 | 0.300 | 0.005 | -0.010, 0.020 | 0.475 | 0.005 | -0.010, 0.020 | 0.511 |
| | CT: Parietal | 36 | 0.011 | 0.004, 0.019 | **0.003** | 0.011 | 0.003, 0.018 | **0.009** | 0.010 | 0.001, 0.019 | **0.024** | 0.009 | -0.001, 0.019 | 0.076 | 0.009 | -0.002, 0.019 | 0.089 |
| | CT: Temporal | 36 | 0.010 | -0.001, 0.020 | 0.064 | 0.011 | 0.000, 0.022 | **0.049** | 0.010 | -0.002, 0.022 | 0.113 | 0.008 | -0.006, 0.022 | 0.241 | 0.008 | -0.007, 0.023 | 0.265 |
| Non-carriers | WMHV* | 86 | 0.045 | -0.027, 0.117 | 0.217 | 0.049 | -0.027, 0.126 | 0.204 | 0.034 | -0.046, 0.114 | 0.408 | 0.014 | -0.061, 0.089 | 0.715 | 0.014 | -0.061, 0.090 | 0.711 |
| | TBV* | 88 | 1.550 | -1.677, 4.776 | 0.342 | 1.751 | -1.588, 5.091 | 0.300 | 0.482 | -3.120, 4.084 | 0.791 | -0.006 | -3.623, 3.611 | 0.997 | -0.019 | -3.663, 3.626 | 0.992 |
| | NAWM MD | 78 | 0.027 | 0.000, 0.054 | 0.050 | 0.027 | -0.002, 0.055 | 0.067 | 0.028 | -0.004, 0.060 | 0.083 | 0.029 | -0.004, 0.061 | 0.088 | 0.029 | -0.004, 0.062 | 0.086 |
| | CT: Harvard ADsig | 88 | -0.004 | -0.010, 0.001 | 0.104 | -0.005 | -0.010, 0.001 | 0.097 | -0.004 | -0.010, 0.002 | 0.205 | -0.003 | -0.009, 0.003 | 0.255 | -0.004 | -0.009, 0.002 | 0.191 |
| | CT: Occipital | 88 | -0.005 | -0.011, 0.001 | 0.112 | -0.005 | -0.012, 0.001 | 0.092 | -0.005 | -0.012, 0.002 | 0.157 | -0.005 | -0.012, 0.002 | 0.138 | -0.006 | -0.012, 0.001 | 0.088 |
| | CT: Parietal | 88 | -0.003 | -0.009, 0.002 | 0.189 | -0.003 | -0.009, 0.002 | 0.249 | -0.002 | -0.008, 0.004 | 0.514 | -0.002 | -0.008, 0.004 | 0.565 | -0.002 | -0.008, 0.004 | 0.492 |
| | CT: Temporal | 88 | -0.003 | -0.010, 0.004 | 0.423 | -0.002 | -0.009, 0.006 | 0.652 | -0.001 | -0.010, 0.007 | 0.725 | -0.001 | -0.009, 0.007 | 0.737 | -0.002 | -0.009, 0.006 | 0.633 |

*M0:age at scan, [*TIV]; M1:M0+childhood cognition, education, SEP; M2:M1+puberty, parity; M3:M2+smoking, BMI, blood pressure*

*lCI=lower confidence interval; uCI=upper confidence interval; SEP=socioeconomic position; HT=hormone therapy; TIV=total intracranial volume; WMHV=white matter hyperintensity volume; TBV=total brain volume; NAWM=normal appearing white matter; MD=mean diffusivity; CT=cortical thickness; Harvard ADsig=Harvard Alzheimer's Disease signature regions*