Sex differences in the relationship between glycaemic traits and brain and cognitive health.

Nasrtullah Fatih

A thesis submitted to University College London for the degree of Doctor of Philosophy

Supervisors: Professor Alun Hughes, Dr Sarah-Naomi James, Professor Nish Chaturvedi, Dr Victoria Garfield

MRC Unit of Lifelong Health and Ageing, Institute of Cardiovascular Science
University College London

2024

Declaration Statement

‘I, Nasrtullah Fatih, 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.'

Nasrtullah Fatih

Date: 28/06/24

UCL Research Paper Declaration Form
referencing the doctoral candidate's own published work(s)

Please use this form to declare if parts of your thesis are already available in another format, e.g., if data, text, or figures:

- *have been uploaded to a preprint server*
- *are in submission to a peer-reviewed publication*
- *have been published in a peer-reviewed publication, e.g., journal, textbook.*

This form should be completed as many times as necessary. For instance, if you have seven thesis chapters, two of which containing material that has already been published, you would complete this form twice.

1. **For a research manuscript that has already been published** (if not yet published, please skip to section 2)

   a) **What is the title of the manuscript?**

   Sex-related differences in whole brain volumes at age 70 in association with hyperglycaemia during adult life

   b) **Please include a link to or doi for the work**

   https://doi.org/10.1016/j.neurobiolaging.2021.09.008

   c) **Where was the work published?**

   Neurobiology of Ageing

   d) **Who published the work?** (e.g., OUP)

   Elsevier

   e) **When was the work published?**

   April 2022

   f) **List the manuscript's authors in the order they appear on the publication**

   Nasrtullah Fatih, Nish Chaturvedi, Christopher A Lane, Thomas
   D. Parker, Kirsty Lu, David M. Cash, Ian
   B. Malone, Richard Silverwood, Andrew Wong, Josephine Barnes, Carole
   H. Sudre, Marcus Richards, Nick C. Fox, Jonathan
   M. Schott, Alun Hughes, Sarah-Naomi James

g) **Was the work peer reviewed?**

Yes

h) **Have you retained the copyright?**

Yes

i) **Was an earlier form of the manuscript uploaded to a preprint server?** (e.g., medRxiv). If 'Yes', please give a link or doi)

No

If 'No', please seek permission from the relevant publisher and check the box next to the below statement:

☐
*I acknowledge permission of the publisher named under **1d** to include in this thesis portions of the publication named as included in **1c**.*

2. **For a research manuscript prepared for publication but that has not yet been published** (if already published, please skip to section 3)

a) **What is the current title of the manuscript?**

Click or tap here to enter text.

b) **Has the manuscript been uploaded to a preprint server?** (e.g., medRxiv; if 'Yes', please give a link or doi)

Click or tap here to enter text.

c) **Where is the work intended to be published?** (e.g., journal names)

Click or tap here to enter text.

d) **List the manuscript's authors in the intended authorship order**

Click or tap here to enter text.

e) **Stage of publication** (e.g., in submission)

Click or tap here to enter text.

3. **For multi-authored work, please give a statement of contribution covering all authors** (if single-author, please skip to section 4)

Nasrtullah Fatih, Sarah-Naomi James and Alun Hughes were involved in the conceptualisation of the study. Thomas Parker, Chris Lane, and Kirsty Lune performed the data collection, Nasrtullah Fatih was involved in the formal analysis of the data. Sarah-Naomi James and Alun Hughes, Nish Chaturvedi and Nasrtullah Fatih were involved in the interpretation of the data. Nasrtullah Fatih drafted the manuscript and Alun Hughes, Sarah-Naomi James, Nish Chaturvedi, Chris Lane, Thomas Parker, Kirsty Lu, David Cash, Ian Malone, Richard Silverwood, Andrew Wong, Josephine Barnes, Carole Sudre, Marcus Richards, Nick Fox, and Jonathan Schott critically reviewed and amended the manuscript.

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

   Chapter 3

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)

   Candidate

   Nasrtullah Fatih

   Date:

   20/06/24

   Supervisor/ Senior Author (where appropriate)

   Alun Hughes

   Date

   20/06/24

Acknowledgements

I would like to thank the participants part of the MRC National Survey of Health And Development and Insight 46 participants for dedicating their lives to help scientific research. They have and continuously contribute to our understanding of human health through its various stages. This gratitude extends to the research who have been involved in the collection of data throughout all these years.

I would like to say a huge thank you to my supervisors Professor Alun Hughes, Dr Sarah-Naomi James, Dr Victoria Garfield, and Professor Nish Chaturvedi. They've been exceptional mentors during the most important phase of my research career. Alun and Sarah particularly have been there for me throughout the process and have played an unmeasurable contribution in my development as a scientist, so I am immensely grateful to them. I also would like to thank Dr Richard Silverwood and Dr George Ploubidis for their statistical expertise and the University College London Dementia Research Centre team for their processing of the neuroimaging data including Dr Carole Sudre and Jo Barnes. I would also like to thank Professor Marcus Richards for his feedback.

I am grateful for my father. He's sacrificed a lot throughout his life to create the circumstances that allowed me to study at a doctoral level. I also would like to dedicate my thesis to my grandma Halima who sadly did not get to see me graduate.

Funding

The work in this thesis was funded by the Medical Research Council (MRC).

Abstract

Evidence shows that diabetes is a risk factor for dementia.[1] But how diabetes-related mechanisms affect brain health in the general population is still poorly understood. Answering this entails examining whether glycaemia and its related markers are associated with specific brain changes (e.g., Alzheimer's disease-like pathology, damage to the brain's microvasculature) or cognitive impairments.

In line with the growing evidence highlighting important sex differences in metabolic and neurological health, these associations were examined separately in males and females. To combine different scientific approaches (e.g., using genetics and time-sensitive mediation analysis) when examining these relationships, two different samples with unique characteristics and phenotyping, National Survey of Health and Development and UK Biobank, were used.

In National Survey of Health and Development, poorer glycaemia and insulin resistance were associated with lower brain volume measures in females but not in males. This relationship was not mediated by systemic inflammation.

In the UK Biobank sample, glycaemia-brain volume associations appeared to be non-linear with both low and high glycaemia being associated with smaller brain volumes. There were some suggestions of increased susceptibility in these relationships for females although analysis was complicated by the non-linear associations observed.

A polygenic risk score for glycaemia was not convincingly associated with imaging markers of brain health, although the weakness of the genetic instrument limited the conclusions that could be drawn. There were no convincing associations between glycaemia and cognitive outcomes in either sample.

Impact statement

In 2020, The Lancet Commission on Dementia Prevention, Intervention and Care emphasised diabetes as one of the 12 modifiable risk factors that increases dementia risk.[1] Despite this, the precise nature of how diabetes-related pathophysiology may impact the brain across the general population is still poorly understood. Notably, this thesis takes a novel stance when examining these relationships as it aims to answer these questions by investigating males and females separately. This is particularly important in the context of growing evidence showing differences in metabolic and neurological health between males and females. Through taking different statistical and modelling approaches, this thesis aims to understand the relationship between glycaemia (and its related traits) and brain health, examining the nuances within these complex relationships in two flagship UK-based population-based studies.

A central finding of this thesis is that poorer glycaemic health throughout life was more robustly associated with poorer later-life brain health in NSHD. In UK Biobank, there were again some suggestions of increased susceptibility in these relationships in females, although results were complicated by the non-linear associations observed. Such findings have important implications as they reinforce the idea of sex differences in the relationship between metabolic and diabetes-related health and differing impacts on the brains of males and females.

This thesis offers thought-provoking findings about the nature of these relationships and can inspire future research. It highlights the need for all future research exploring similar associations to examine whether any effects differ by sex. Important mechanistic questions regarding what may be driving this increased vulnerability in females naturally follow. In this thesis, the potential mediating role of systemic inflammation was explored but other mechanisms related to cardiovascular health or oxidative stress may also be important in the glycaemia-brain relationship. Further neuroimaging questions are warranted to investigate whether these associations persist or aggravate as participants get older and are more likely to develop dementia.

Since diabetes is a modifiable risk factor, this opens an important route for intervention.

To ensure dissemination and impact from this body of work, four of the chapters will be sent for publication. Chapter 3 has already been published in Neurobiology of Ageing. Chapter 4 will be shortly sent to European Journal of Endocrinology. Chapter 5 will be sent to Lancet Diabetes & Endocrinology. Chapter 6 will be sent to PLOS one. Some of the work in this thesis has also been published in abstracts at Diabetes UK professional conference, presented at The Welcome conference for longitudinal studies, and locally at UCL. Some work from this thesis will be submitted to Alzheimer's Research UK 2025.

Table of Contents

Table of Tables

Table of Figures

Abbreviation List

3T: 3 Tesla (magnetic field strength)
AD: Alzheimer's disease
APOE: Apolipoprotein E
ASL: arterial spin labelling
Aβ: beta-amyloid
BaMoS: Bayesian Model Selection, an automated WMH segmentation tool
BMI: body mass index
CBF: cerebral blood flow
CFI: comparative fit index
CI: confidence interval
CMB: cerebral microbleed
CRP: C-reactive protein
CVD: cerebrovascular disease
DSST: digit symbol substitution test
ER: oestrogen receptors
FA: fractional anisotropy
FLAIR: fluid attenuated inversion recovery MRI
fMRI: functional MRI
FNAME-12A: 12-item Face-Name Associative Memory Exam
GIF: geodesic information flow, an in-house method of MRI segmentation
GLycA: **Glycoprotein acetyls**
GLM: generalised linear model
GWAS: genome wide association study
HOMA IR: Homeostatic Model Assessment for Insulin Resistance
HOMA B: Homeostatic Model Assessment for beta cell function
HV: hippocampal volume
IL-6: Interleukin 6
LMDR: logical memory delayed recall
LOD: Level of detection

LRT: Likelihood Ratio Test

MR: Mendelian randomisation

MRA: Missing at random

MCA: medial cerebral artery

MCI: mild cognitive impairment

MD: mean diffusivity

MMSE: mini-mental state examination

MRC: Medical Research Council

MRI: magnetic resonance imaging

NAWM: normal appearing white matter

NDI: neurite density index

NFTs: neurofibrillary tangles

NHS: National Health Survey

NMR: Nuclear Magnetic Resonance

NODDI: neurite orientation dispersion and density index

NSHD: National Survey of Health and Development

ODI: orientation dispersion index

OR: odds ratio

PDD: Parkinson's disease dementia

PET: positron emission tomography

QC: quality control

RMSEA: root mean square error of approximation

SBP: systolic blood pressure

SD: standard deviation

SEP: socioeconomic position

SVD: small vessel disease

SUVR: standardised uptake value ratio

T1: MRI spin–lattice or longitudinal relaxation time

T2: MRI spin-spin or transverse relaxation time

TE: MRI echo time

TIV: total intracranial volume

TOD: target organ damage

TNF-α: tumour necrosis factor-α

TR: MRI repetition time

UCL: University College London

VaD: vascular dementia

VCI: Vascular cognitive impairment (VCI)

WAIS-R: Wechsler Adult Intelligence Scale-Revised

WASI: Wechsler Abbreviated Scale of Intelligence

WBV: whole brain volume

WMH: white matter hyperintensity

WMHV: white matter hyperintensity volume

WMS-R: Wechsler Memory Scale-Revised

1. General Introduction

1.1 Ageing and the diabetes-brain connection

Advances in medical research have improved public health standards and increased worldwide life expectancy. A recent report by the United Nations predicts that the number of people above the age of 80 is expected to triple in the next 30 years.[2] An increasing ageing population, however, is giving rise to a number of age-related pathological diseases and complications. Ageing is a primary risk factor for many conditions such as dementia.[3] Dementia, now referred to as major neurocognitive disorder, describes a cognitive syndrome with progressive functional loss.[4,5] Estimates suggest that the global prevalence for the condition was 57.4 million people with this number projected to increase to 152.8 million by 2050.[6] In the absence of effective treatments and growing evidence indicating that the pathological onset of the disease may occur many years before it is clinically diagnosed,[7–9] there is a need to better understand the mechanisms that may underlie this. An important review of meta-analyses has identified numerous modifiable risk factors across life such as those relating to metabolic health including diabetes.[1] Diabetes-related pathology and mechanisms have been associated with an increased risk of developing all-type dementia, with around 3% of all cases being attributed to this metabolic disease.[10] As will be discussed in this chapter, both conditions are prevalent and share common risk factors, but the precise mechanisms through which the pathophysiology of diabetes (e.g., glycaemia) relates to brain health in the general population is still yet to be elucidated.

1.2 "Normal ageing": cognition and brain health

1.2.1 Brain Ageing

In order to better understand the effects of aberrant ageing on the brain, I first must describe "normal ageing". Postmitotic cells, such as those that make up brain tissue, are particularly sensitive to the effects of ageing. Mounting evidence indicates that

ageing results in a number of functional and anatomical changes to the brain. A review of 57 longitudinal imaging studies showed that there is a 0.2-0.5% annual decrease of total brain volume across the adult lifespan.[11] The volume and/or weight of the brain declines with age at a rate of around 5% per decade after age 60[11] with this reduction speeding from age 70.[12] By age 90 (or over), brains are found to weigh around ~10% (or 150g) less than at age 50.[13]

Both cross-sectional and longitudinal studies suggest that some brain areas are more susceptible to ageing than others with the frontal and parietal cortices showing the steepest rates of age-related decline.[14,15] Frontal lobe volume is estimated to decline 0.9-1.5% per year with volume loss affecting both grey matter (GM) and white matter (WM).[16] A recent study aggregating data from 100,000 brain imaging scans reported a decline of both GM and WM throughout a 100-year span with some evidence that the reduction in GM during late-life was steeper.[17]

Some studies suggest that there are some sex-specific differences in WM and GM during ageing.[18–20] For example, a study of 1,172 healthy older adults (aged 65-82) showed that females experienced a higher rate of GM atrophy (−4.7 cm^3/year, −0.91%/year) compared to males (−3.3 cm^3/year, −0.65%/year), suggesting greater anatomical vulnerability in females. But some regions such as the hippocampus showed an accelerated rate of GM atrophy with age in both sexes, highlighting its specific susceptibility to aging processes after 65 years of age.[20]

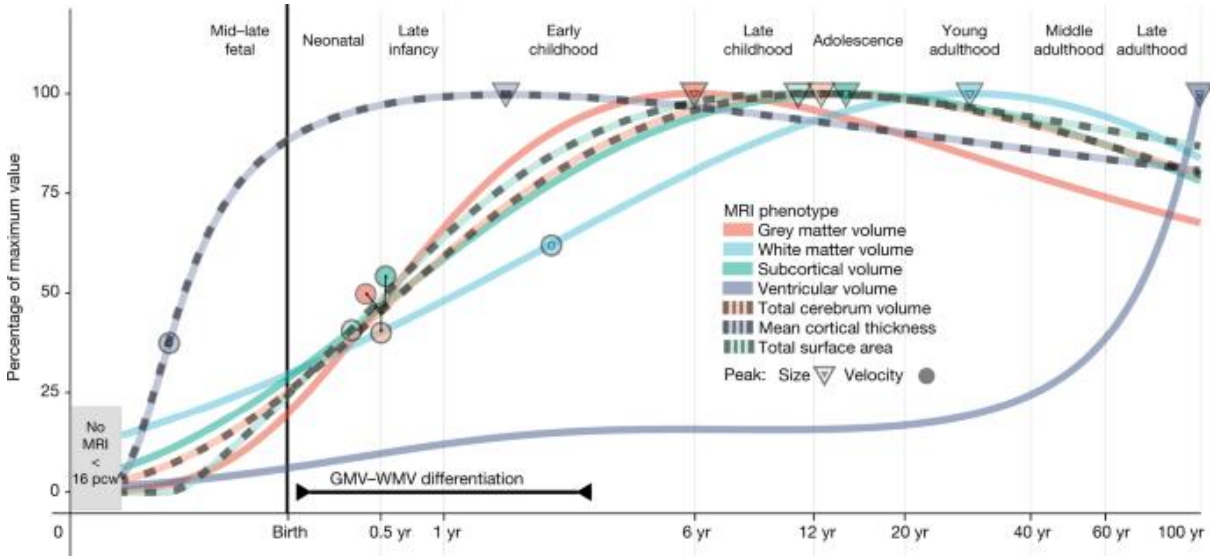


Figure 1.1: Representing volumetric brain changes throughout human life. Both grey matter and white matter volume decline as one ages but the decline in late life appears to be more severe for grey matter volume.[17]

In addition, ageing comes with cerebrovascular changes to the brain that affect small vessels and cerebral blood flow (CBF).[21,22] The ageing brain may thus begin to accrue microbleeds, loss of myelinated axons and lesions to WM tissue in the periventricular and deep subcortical areas. For example, white matter hyperintensities (WMH), a surrogate marker of small vessel disease (SVD), are uncommon under the age of 30 but can be detected in around 90% of the population by age 65.[22,23] These issues are discussed further below in the context of vascular disease (e.g., neurocognitive disorder of vascular origin or vascular cognitive impairment).

There is also evidence of age-related changes at the intracellular or local circuitry level. This includes the formation of Alzheimer's hyperphosphorylated tau neurofibrillary tangles (NFTs), amyloid plaques, Lewy bodies and α-synuclein.[13,24] Cerebrovasculature may also be affected with ageing, with evidence indicating a narrowing in vessel size, stiffening, and thickening of the vessel walls and a reduction in the number of capillaries. [25] These changes are associated with a great prevalence of WMH.[24] These are described in more details below.

1.2.2 Cognitive ageing

The neurobiological changes that underlie “normal ageing” are typically accompanied by some degree of cognitive deficit. This being said, it is important to note that there is considerable variety in the degree of functional loss observed across the population and that not all cognitive abilities are equally vulnerable to senescence.[26] Cognitive decline describes a worsening of complex human processes such as mentalising, planning and problem solving.

The scientific literature usually splits cognitive functions into two distinct categories: fluid and crystallised abilities. Fluid abilities support the active processing of new information and thus capture cognitive domains such as memory, reasoning, and processing speed. In contrast, crystallised abilities refer to abilities that use learned skills and knowledge such as language and visual perception.[27] Research has demonstrated that fluid abilities are most susceptible to ageing, with studies indicating that they peak in early adulthood and begin to decline before age 50.[28,29] In contrast, crystallised abilities can remain well-preserved, even in the face of mild dementia and only begin to decline much later in life.[30,31] Since it is those fluid cognitive abilities that typically decline earlier in life, research into cognitive decline has focused on assessing domains of memory, executive function, speed of processing and visuospatial function. Epidemiological studies have found that factors such as childhood cognition, individual and paternal educational attainment, occupational complexity and adult occupational class influence the trajectory of cognitive decline.[32]

It is important to note that there are limited studies that have been able to explore the effect of ageing on cognition and brain volume in the same sample from the general population. Similarly, very few studies allow investigating changes in brain and cognitive health across the entire life course. This implies that research into changes in cognitive and brain health in the context of normal ageing can be biased depending on the study design used. This can be due to bias in the recruitment or misclassification of participants. For example, individuals who are ill or have less social and financial support could be less likely to enrol in research studies. Evidence for this

can be seen in the UK Biobank where participants have been found to be healthier, smoke less and have a lower incidence of disease than the general UK population.[33] This is discussed in more detail in Chapter 2.5.1.

1.3 Cognitive impairment and dementia

Pathological aging includes cognitive, behavioural and functional impairments that exceed the expected “normal” age-related decline such as those characteristic of neurogenerative diseases such as dementia. Individuals considered in the “transitional stage” showing cognitive deficits but failing to meet the diagnostic criteria for dementia have mild forms of the respective neurocognitive disorder (e.g., mild cognitive impairment (MCI)). MCI is associated with a higher conversion risk into dementia, however there is considerable heterogeneity in the underlying biological mechanisms behind the condition and how it progresses into the full-blown disease across the population.[34–36]

The clinical assessment for dementia is comprehensive and multifaceted. It involves a two-part process that first aims to diagnose a person with the condition and then secondly identify the cause of it. These processes include a combination of an investigation into the patient’s history and assessments of mental and physical abilities.[37] The full history of a patient may be obtained directly from the individual and/or suitable informants (such as relatives) to gain an insight into any behavioural or cognitive change that the patient may have experienced. Mental state examinations will also be carried out, aiming to identify the presence of clinical symptoms such as those of depression, psychosis, physical illness as well as self-neglect and agitation. These may include a range of assessments of cognitive function, such as the Mini-Mental State Examination (MMSE), a standard test that captures temporal and spatial orientation, attention, registration, recall, language and working memory.[38] Clinicians then use thresholds to ascertain cognitive impairments (e.g., for the MMSE, a score below 23 from 30 is considered to reflect impaired cognition). A physical examination is also required to study other conditions that may affect the functioning of the central nervous system (CNS). For example, assessing blood pressure and focal neurological

signs may help identify a neurocognitive disorder of vascular origin. Clinicians assess hearing and vision to exclude impairments that may exaggerate cognitive dysfunction. Other screening typically involves a full blood count, erythrocyte sedimentation rate, serum B12 and folate, urea and electrolyte levels, and tests of liver and thyroid function. The definitive diagnosis of Alzheimer's disease (AD) requires post-mortem analysis of brain tissue for pathological markers. This contrasts with neurocognitive disorders of vascular origin (formerly known as vascular dementia), which despite several attempts do not have generally accepted neuropathological criteria.[39]

Several conditions may cause dementia or major neurocognitive disorders, with the AD aetiology at the heart of around 70% of cases.[40] Other common causes of dementia include vascular cognitive impairment (VCI), dementia of Lewy bodies (DLB), frontotemporal dementia (FTD), and Parkinson's disease dementia (PDD).

1.4 Risk factors for dementia

The last decades of research have uncovered a number of predictive risk factors for dementia and cognitive impairment. These include non-modifiable (e.g., age, apolipoprotein E (APOE) status, sex and ethnicity) and modifiable factors (e.g., hypertension, obesity, smoking, physical activity, and hearing loss). A more comprehensive list consisting of 12 modifiable risk factors for dementia has previously been described in detail in the report by the Lancet Commission on Dementia Prevention, Intervention and Care.[1]

1.4.1 Non-modifiable factors

Sex is an important non-modifiable factor for dementia. It is discussed in detail in section 1.5.

Increasing age, as mentioned above, is predictive of poorer brain health, with each decade of ageing being associated with a higher risk of being diagnosed with dementia. One meta-analysis observed an exponential increase in dementia rate that doubled every 5 years from age 65 to 90.[41] However, another meta-analysis of 12

studies suggested that incident rates of dementia slow down with increasing age tripling every 5 years before age 63, doubling every 5 years between ages 64 and 75 and increasing by 1.5 times by age 85.[42]

Genetic predisposition is an important factor in cognitive decline. The *APOE* e4 gene (*APOE* ε4) has been found to confer an elevated risk for AD and its related pathology.[43,44] Very briefly, the APOE protein is present in several classes of lipoprotein particles and is involved in lipid homeostasis.[45] In the CNS, APOE is mainly produced by astrocytes transporting cholesterol to neurons via *APOE* receptors (*APOER*).[46] The human *APOE* gene exists as three polymorphic alleles—ε2, ε3 and ε4—having a worldwide frequency of ~8%, ~78% and 14%, respectively.[47] Being an *APOE* ε4 carrier is the strongest genetic risk factor for sporadic AD and amnestic MCI.[44] Carriers of the ε4 allele have a higher risk for AD as well as typical earlier age of onset for the condition, compared to non-carriers.[48] Around 40% of all individuals with AD carry at least one copy of the allele.[44] One copy of the ε4 allele increases the risk of developing AD by about 3.7 times whereas two copies increase the risk up to 12 times.[49] Carrying the ε2 allele (*APOE* ε2), the least common one, is thought to have a protective effect as it reduces the risk of AD by about 40%.[50] Importantly, some studies suggest that the genetic driven-vulnerability caused by having two copies of *APOE* varies across populations, being weaker in African Americans (OR: 5.7) and Hispanics (OR: 2.2) but stronger in Japanese individuals (OR: 33.1). There is also evidence that *APOE* may confer sex-specific effects on the brain since females with the ε4 polymorphism have been found to have a higher risk for AD, a more rapid cognitive decline as well increased burden of NFTs compared to males.[47,51,52]

A recent systematic review and meta-analysis also identified ethnicity as an important risk factor with ethnic differences in AD incidence. Black individuals were found to have a higher risk of developing dementia compared to White individuals, with a pooled risk ratio of 1.33, while Asian individuals have a lower risk with a pooled risk ratio of 0.86. There was no significant difference in dementia incidence between Latino

and White individuals.[53] The complex relationship between ethnicity and AD risk is still not well understood.

1.4.2 Modifiable factors

The recent Lancet Commission for Dementia Prevention, Intervention and Care identified 12 risk factors that could prevent or delay up to 40% of dementia cases.[1] These included risk factors related to early life (e.g., low education), midlife (e.g., hypertension, obesity, hearing loss, brain injury, and alcohol abuse) and late life (e.g., smoking, depression, living a sedentary lifestyle, social isolation, diabetes and air pollution).

Individuals with lower education were 1.6 times more likely to develop dementia, based on a Weighted Population Attributable Fraction (Weighted PAF). it was estimated that 7.1% of dementia cases in the population can be attributed to inadequate education.[1] Higher levels of education can alter the shape of our cognitive trajectory delaying the onset of cognitive symptoms and protecting us against neurodegenerative diseases.[54]

Metabolic-related risk factors such as hypertension, obesity, physical inactivity and smoking together were predicted to account for 10.4% of dementia cases.[1] This is supported by findings demonstrating that the treatment and management of cardiometabolic health is an effective protective approach reducing dementia risk.[55] For example, one study found that the risk of dementia and AD can be lowered by 28% and 45% respectively with physical activity.[56] Exercise is neuroprotective and has been associated with preserved brain volumes, particularly in the hippocampus which is crucially involved in memory and learning and is characteristically vulnerable in AD.[57] It has been found that current smokers have a 30% increased risk of developing all-cause dementia, 40% higher risk for Alzheimer's disease and 38% higher risk for VaD. Furthermore, the risk of all-cause dementia increased with every 20 cigarettes smoked.[58] Diabetes is also an important metabolic modifiable risk factor for dementia.[1]

Its associations with brain health and conditions such as dementia is discussed further below.

1.5 Sex as a risk factor for dementia

Sex differences have been reported in the incidence of dementia. Studies of European cohorts have shown that the prevalence of AD is 7.02 per 1000 person-years in males vs 13.25 per 1000 person-years in females.[59] Individual participant data from the COSMIC Consortium, revealed that the risk of all-cause dementia was higher in females.[60] There were however significant country-level variations with age-adjusted rates of dementia found to be the highest among the lowest-to-middle income countries. Another meta-analysis has suggested that although there were no sex-specific differences in the prevalence of age-specific dementia, AD prevalence was higher for females.[61] This higher risk in females was observed at various ages including between 60-64, when it was triple that of males as evidenced by the incidence rate was 0.6 per 1000 person-years for males (Confidence Intervals (CI): 0.4-1.0) and 1.8 per 1000 person-years in females (CI: 1.2 to 2.7). This is consistent with recent data from the Alzheimer's Association which found that 3.3 million of the 5.2 million people with AD in the United States (US) are female.[62] Some of this excess has been attributed to the different survival rates between males and females. Newer evidence suggests that these differences may be due, at least in part, to biological and socio-cultural mechanisms. The impact of these on brain health are still poorly understood. In regard to brain pathology, some studies suggest a larger brain size in males than in females both in post-mortem and vivo imaging studies,[63,64] although the extent to which this is independent from differences in body size is unclear.

1.6 The cognitive and neurological markers of neurocognitive disorders

1.6.1 Introduction

The latest Diagnostic and Statistical Manual (DSM) Fifth Edition (DSM-V) has replaced the term dementia with neurocognitive disorders. This term broadly captures a spectrum of cognitive and functional conditions (e.g., AD and vascular disease) that form the basis of the clinical diagnosis made.[5] In this section, the different causes of these conditions and their manifestation will be discussed with a specific focus on the AD and vascular disease subtypes. The other prevalent subtypes such as neurocognitive disorders with Lewy bodies and frontotemporal lobar degeneration are comparatively infrequent. These will not further be covered in this thesis.

1.6.2 Alzheimer's disease

AD is the single biggest cause of dementia/neurocognitive disorder accounting for over 50-70% of clinically diagnosed cases. It is typically diagnosed in the eighth or ninth decades of life, but early onset forms of the disease may be diagnosed as early as the fifth decade.[65] Average duration of survival is about ~5-7 years after the onset of dementia,[66] but this varies widely depending on the age of onset, the severity of the cognitive impairment experienced and the presence of comorbid diseases, as well as other factors.[67]

The two main pathological hallmarks of AD are extracellular deposition of Aβ and intraneuronal NFTs in the brain. Downstream of these neuropathological markers is synaptic and neuronal degeneration, which manifests as macroscopic atrophy that can be picked up by magnetic resonance imaging (MRI). Clinically, AD is associated with impairments in episodic memory affecting the ability to learn and remember new information.[68] These cognitive symptoms are associated with early NFT in medial lobe structures, such as the hippocampus and entorhinal cortex (discussed further below).[69] Other impairments include deficits in visuospatial abilities[70] and language.[71]

AD brains also show considerable atrophy across a range of structures. These include regions of the frontal and temporal cortices which show both enlarged sulci and atrophy to the gyri.[72] Atrophy of the temporal cortices can reflect damage to regions of the medial temporal lobe such as the amygdala and the hippocampus, with the latter being crucially important for memory formation and learning. Several MRI studies have reported that total hippocampal volume (HV) are 25% lower in clinical AD patients than healthy controls.[73] Hippocampal atrophy rates have also been reported to be more than 3% higher in AD patients than in controls.[74] HV loss correlates with the severity of cognitive disorders and episodic memory deficits in MCI and AD.[75,76] Despite being one of its cardinal features, hippocampal atrophy is known to lack specificity for AD, and it may lack both sensitivity and specificity at the MCI stage since it can be present in non-AD forms of dementia, like VaD,[77,78] semantic dementia,[79] PDD[78] and frontotemporal lobar degeneration.[80]

Tissue loss has also been reported in posterior cortical regions in areas such as the precuneus, posterior cingulate gyrus and temporo-parietal cortex.[72,81–83] Despite such loss, other regions such as primary motor and somatosensory cortices have been found to be preserved.[72] Early cerebral atrophy in AD is thought to be due primarily to neuronal loss[84] but it is worth noting that some of these macroscopic changes such as the cortical atrophy observed in the frontal lobe are not specific to AD and may overlap with some other conditions as well as, to some extent, with natural ageing processes.

1.6.3 Neuropathological markers of AD

Aβ deposits consist of aggregated Aβ primarily found in GM tissue. Aβ plaques consist of toxic polypeptide aggregates composed of 39 to 43 amino acids (Aβ40 and Aβ42). These are generated through proteolytic cleavage of a larger amyloidal precursor protein (APP).[85] These plaques are spherical lesions and can usually found in the basal neocortex, spread into the neighbouring neocortical areas and the hippocampal formation, and finally appear in primary neocortex.[86] The evolution of Aβ plaques is not thought to correspond to disease stage nor is it specific for AD since amyloidal plaques can be observed in the brain during “normal ageing”.[87,88]

NFTs are fibrillary intracytoplasmic structures of abnormally hyperphosphorylated tau proteins.[89] Tau is a protein crucial for the stability, assembly and maintenance of axonal microtubules.[90] Tau can be measured using techniques such as positron emission tomography (PET) and cerebrospinal fluid (CSF) analysis. The former is discussed in more detail later.

The progression of NFTs in the human brain in the context of AD can be described by the Braak and Braak stages. This staging system is based on the distribution and severity of NFTs within different regions of the brain.[69] NFTs first typically appear in the medial region of the perirhinal cortex before progressively spreading to the limbic area, followed by areas of the neocortex. Tau pathology in AD appears to correlate with disease stages and is required for a neuropathological diagnosis of AD.[91] NFTs in AD are closely associated with cognitive decline and brain atrophy.[92,93] The amount and distribution of NFTs has been found to correlate with the severity and duration of dementia.[94]

The understanding of the manifestation of these AD biomarkers is constantly evolving. There is now evidence of significant pathophysiological changes occurring in the prodromal stage, long before clinical symptoms manifest.[23,72] A sigmoid-shaped trajectory of biomarker changes has been proposed with amyloid-related pathology being followed by tau and neurodegenerative markers.[95] Revised models of this prioritise change across these trajectories (rather than clinical symptoms), consider inter-subject variability and modify the temporal ordering of biomarkers by recognising the possibility that Aβ and tau proteinopathies may occur independently in late-onset AD.[95] This is consistent with the idea that AD progresses as a continuum, starting with brain pathology in asymptomatic individuals, advancing through stages of increasing pathology and eventually resulting in clinical symptoms.

More recently, the “A/T/N” system for categorising AD biomarkers into three binary groups has been proposed: "A" stands for amyloid beta (Aβ) deposition (amyloid PET or CSF Aβ42), "T" for tau pathology (CSF phosphorylated tau or tau PET), and "N" for neurodegeneration (CSF total tau, FDG-PET, or structural MRI). Each category is

scored as positive or negative to create various combinations such as A+/T+/N−, A+/T−/N−, etc. Each combination represents a different biomarker profile, making this system a comprehensive framework for describing and studying AD or other related pathologies. The “A/T/N” system effectively addresses several key issues in AD research. It neatly integrates newly developed tau PET tracers, which measure NFTs that correlate strongly with clinical impairments, and accommodate for the uncertainty in the causal relationship between amyloid and tau pathologies by not assuming a specific temporal order of events. By providing a flexible and unbiased classification that is independent of consensus diagnostic criteria, it comprehensively represents all clinical states.[96]

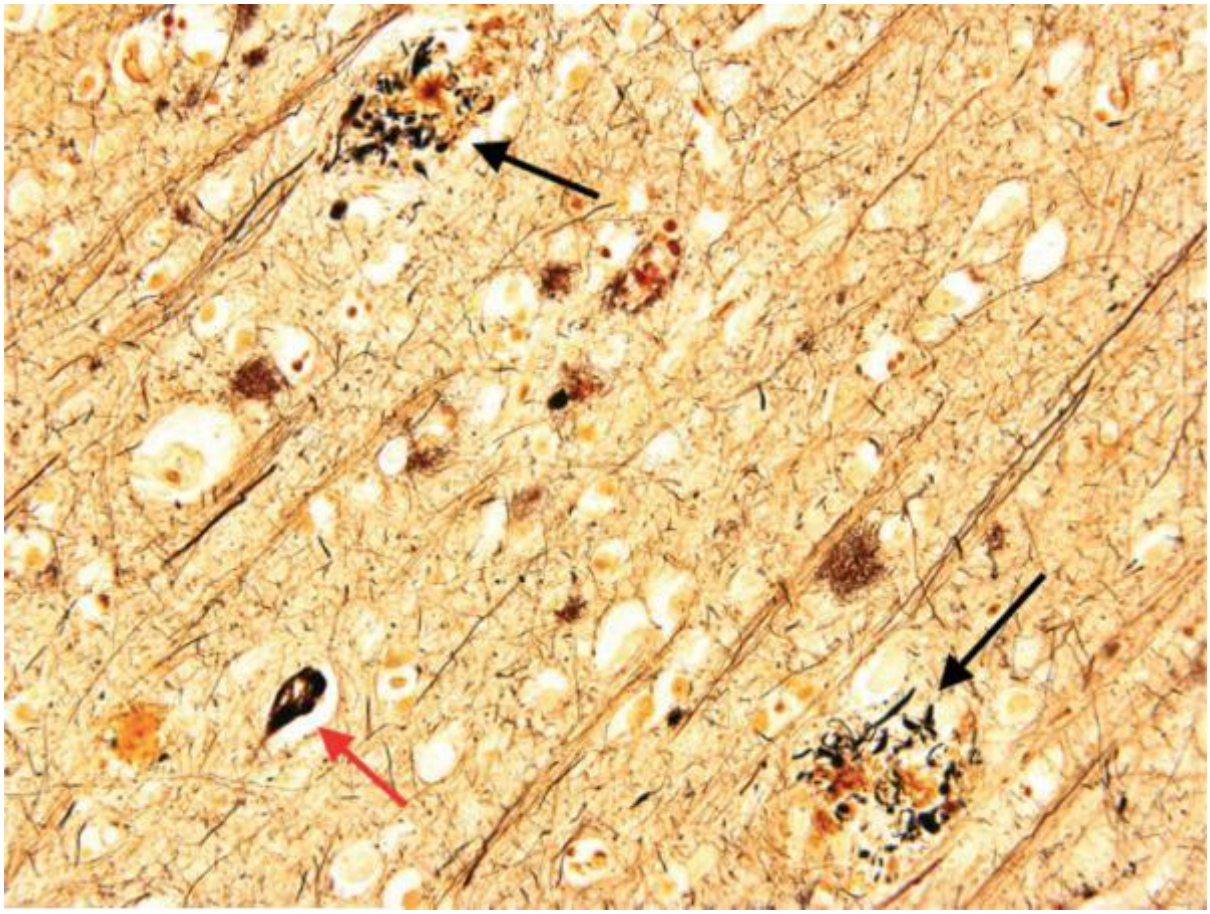


Figure 1.2: Photomicrograph of tissue from the temporal cortex of a patient with Alzheimer’s disease. A neuritic plaque is shown by a black arrow and a neurofibrillary tangle by a red arrow (Source: published work by Perl).[72]

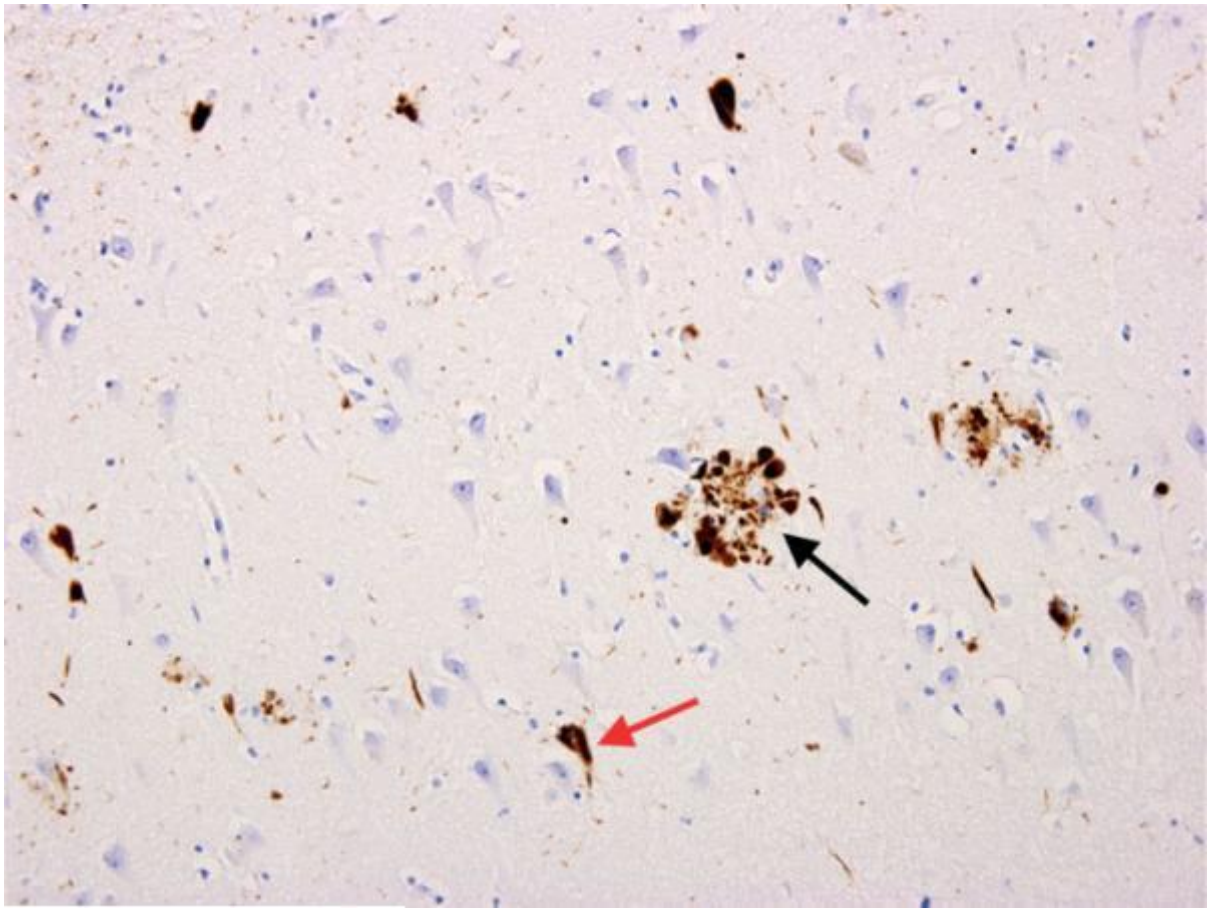


Figure 1.3: Photomicrograph of tissue from the temporal cortex of a patient with Alzheimer's disease.
A neurofibrillary tangle is shown by the red arrow whereas a dystrophic neurite that forms the outer rim of the senile neuritic plaques is singled out using a black arrow (Source: published work from Perl).[72]

1.6.4 Vascular cognitive disorders and vascular dementia

The DSM-V uses the global diagnostic criteria of vascular neurocognitive disorder to describe mild and major cognitive syndrome of vascular origin. The major syndrome, synonymous with vascular dementia (VaD), refers to a syndrome of vascular origin that accounts for around 15-17% of all dementia cases. This makes it the second leading cause of dementia after AD. The incidence for the condition has been found to increase with age, with some suggesting a doubling every 5–10 years after the age of 65 years.[97] However, the heterogenous nature of VaD has resulted in various criteria being developed. And more recently, the term VCI has been

proposed for its inclusivity of various cognitive conditions of vascular aetiology. The recently published guidelines from the Vascular Impairment of Cognition Classification Concensus Study (VICCCS) broadly defines VCI as clinically meaningful impairments that severely impact one's quality of life. The second criteria for mild or major VCI is imaging evidence of cerebrovascular disease. The criteria and new trends about VCI are discussed by Barbay and colleagues.[98]

In regard to its underlying mechanisms, any of the numerous aetiology causes of stroke (e.g., SVD, large artery atherosclerosis, or cardiac embolism) can result in VCI.[99] The most common cause of VCI is thought to be damage to small vessels (diameters of ~50 to 400μm), termed cerebral SVD. Damage to these smaller vessels (i.e., arterioles, capillaries and venules) has been found to affect the integrity of the blood–brain barrier (BBB), resulting in cerebral hypoperfusion and WM degeneration. SVD can manifest as WM lesions, lacunes and microbleeds. WMH of presumed vascular aetiology can present as multiple punctuate or periventricular lesions and subcortical lesions, which appear hypodense (on CT) or hyperintense (on MRI T2/ Fluid attenuated inversion recovery (FLAIR) imaging). Due to their appearance and location in deep GM and WM, WMHs are described as subcortical vascular disease. But the manifestation of SVD may affect the cortex resulting in both microscopic vascular lesions and cortical atrophy.

The clinical evolution of VCI can be often variable as it can represent a gradual deterioration triggered by a novel vascular insult (e.g., a stroke) or as a chronically deteriorating syndrome. The profile and temporal evolution of the cognitive deficits tied to VCI, and neurocognitive disorders of vascular origin are also variable. Cognitive decline may develop gradually, sequentially, or through a combination of both. Current diagnostic criteria no longer require the presence of memory impairment, which is typically characteristic of AD.[39] Brain lesions of vascular origin have been previously associated with impairments in executive function, processing speed and language abilities.[99,100] Another common finding in patients with VCI is a deficit in delayed recall of word lists and visual content.[100] It is generally accepted that VCI may occur more

commonly with prevalence rates of 30% in males compared to 25% in females.[101] This being said, VaD pathology may be decreasing over time due to improvements in vascular health.[102] As previously mentioned, WMHs are also reported in the general healthy population although their rates are much lower.[22]

1.6.5 Overlap of the AD and vascular subtype

Clinic-based studies show that many with dementia show mixed pathology.[103] This may be reflective of the high frequency of both vascular and AD-related pathology in the elderly as well as the shared risk factors between these conditions. Evidence from the Religious Orders Study and the Rush Memory and Aging Project revealed that mixed vascular and AD-type pathology was predominant in patients diagnosed with dementia.[103] Both individuals with AD have been found to show SVD (e.g., WMHs and lacunar infarcts) and those with VaD may exhibit amyloidosis and tau tangles.[104] These pathologies may interact as the combination of WMHs and Aβ pathology have been shown to negatively impact HV in elderly participants and increase dementia risk.[103,105] The volumetric changes typical of AD such as lower HV and global atrophy have been shown to also correlate with VaD.[106,107] It has been previously suggested that there is a multiplicative effect between vascular and AD-type pathology on cognition,[108] although more recent studies suggest that their effects are additive.[109–111] It is however likely that the association between the two are more complex. Vascular brain lesions may also lower the threshold of AD pathology required to induce dementia.[108,112,113] Novel autopsy data show that both large- and small-artery disease are associated with AD dementia, independently of infarcts.[114] In addition, vascular risk factors have been shown to be associated with damage to areas commonly damaged in AD/MCI, perhaps due to being watershed regions with strong vascular supply.[115] Overall, mixed neuropathological evidence highlights the complex mechanistic interplay between vascular issues and neurodegenerative diseases.

1.7 The brain – how to study it?

1.7.1 Introduction

The development of neuroimaging techniques has helped to bridge the gap in our understanding of how the brain changes during healthy ageing and disease. These safe and non-invasive methods have given us several indices of brain structure and function. In disease, some imaging criteria can help exclude certain non-degenerative pathologies or distinguish neurocognitive subtypes for diagnosis. These techniques are complimentary to cognitive and clinical assessments. Imaging techniques include, but are not limited to, MRI, computed tomography (CT), positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and functional near infrared spectroscopy (fNIRS). In addition, there are other promising blood biomarkers that are now being investigated in AD research, but these will not be discussed further in this thesis.

1.7.2 Overview of Magnetic Resonance Imaging:

MRI is a fundamental research and clinical tool used to investigate the anatomical structure of various parts of the body. In neuroscience, MRI is used to generate detailed images of the brain which can help identify cerebral markers associated with cognitive decline and neurological disease. It is considered safe and non-invasive since it does not contain ionising radiation (unlike CT and X-ray scans).

The description that follows is a simplification of the use of MRI since a comprehensive explanation would involve delving into quantum and “classical theory”, but these go beyond the scope of this thesis. Some of the concepts discussed below originate from the following journal.[116]

MRI is based on the magnetisation properties of atomic nuclei. In the presence of a strong magnetic field (typically of 1.5 to 7 Tesla), nuclei (protons) of hydrogen atoms found in fat and water within the human body act like tiny dipoles and align their spins either in parallel or antiparallel to the field. This creates a net magnetic field (known as

B_0). Once aligned, the spins of the hydrogen nuclei precess around the magnetic field line with the frequency of this rotation known as the Larmor frequency. Anti-parallel and parallel protons cancel each other out in terms of the magnetisation vector, but the surplus of parallel protons aligned with the magnetic field produces a “net magnetisation vector” M_Z.

The next step in MRI involves sending a short burst of radiofrequency (RF) energy which creates a rotation magnetic field at the same frequency as the angular frequency of the precessing protons. This disrupts the alignment of protons from their equilibrium resulting in some of them being tipped into a higher energy state (from parallel to anti-parallel), resulting in more anti-parallel protons and transversal magnetisation. When the RF pulse is switched off, the net magnetisation returns to equilibrium, protons relax back to their original, lower-energy state and electromagnetic energy is re-emitted and the nuclear magnetic resonance (NMR) signal is picked up by receiver coils of the MRI scanner placed around the participant. The emitted signals are crucial as they carry information about the local magnetic environment and properties of the tissues being imaged.

The timing and characteristics of the RF pulse is crucial in determining the contrast of the images produced and the specific information obtained in the MRI scan. By using a pulse sequence (multiple RF pulses) and modifying the time intervals between these different signals, researchers can influence the signal intensity and contrast of the final images produced. The time between the repetition of RF pulses is known as Time to Repeat (TR) whereas the time between the delivery of successive RF pulses and the receipt of the echo signal that follows is known as Time to Echo (TE). A short TR and TE generate T1-weighted images whereas a long TR and TE produce T2-weighted images.

T1-weighted images can be used to evaluate global and regional brain structure, tissue atrophy (with repetitive scans), as well as the assessment of tissue specific changes such as those characteristics of GM (affecting neurons, synapses, and the

damage and expansion of CSF spaces)[117] or those of WM (affecting white matter rarefaction, predominantly glial cells).

FLAIR is a variation of a T2-weighted imaging where the CSF signal is suppressed to allow better detection of lesions near fluid-filled spaces making it useful for detecting WM lesions, presumed to originate from demyelination and leukoaraiosis.[117] In addition, PET imaging can be used (and can be combined with MRI) to detect evidence of abnormal Aβ plaques and tauopathy. For example in Insight 46, a neuroscience and clinical sub-study of NSHD, a Biograph mMR 3 T PET/MRI scanner (Siemens Healthcare, Erlangen) was used to perform simultaneous acquisition of dynamic amyloid PET and MR data.[118] This can be particularly useful in reducing scanning time and participant exposure to radiation.

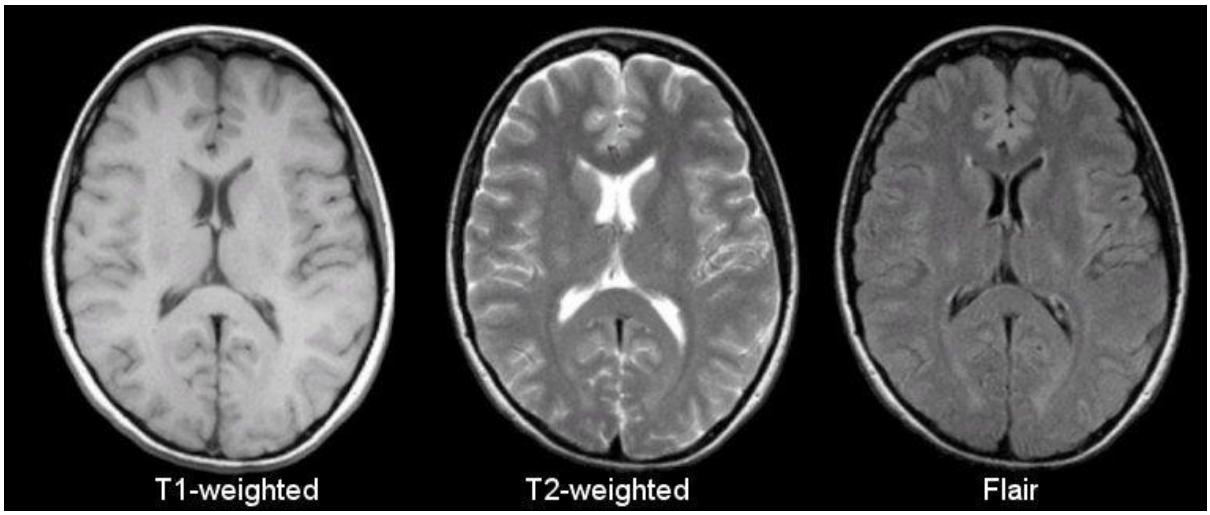


Figure 1.4: Example MRI scans using T_1, T_2 and FLAIR sequences.
T_1 weighted images can easily be distinguished by looking at the cerebrospinal fluid. For T_1 weighted-images, the cerebrospinal fluid appears dark whereas for T_2-weighted images it appears as bright (Source: case.edu website).[119]

1.7.3 Imaging of the structural brain in dementia: MRI indices of brain health

1.7.4 Global and regional brain volumes

Brain atrophy refers to brain volume loss not related to a specific macroscopic focal injury such as those that originate from trauma or infarction.[120] It can be generalised (representing widespread shrinkage throughout the brain), focal (e.g., localised shrinkage preferentially affecting the hippocampus), tissue specific (e.g., predominantly affecting GM) and can be further subdivided into subcortical atrophy (enlargement of the ventricles) and cortical atrophy (enlargement of the cortical sulci). It is best identified through T1-weighted images generated by an MRI machine. Neuropathological correlates of atrophy are heterogeneous and diverse including not only neuronal loss, but also cortical thinning, cerebrovascular disease, and white matter rarefaction (e.g., demyelination).[121]

As mentioned above, in addition to the distinction it makes between global and regional tissue, MRI can distinguish between GM and WM volumes. At the cellular level, both tissue types are composed of neurons; GM consists mainly of cell bodies, dendrites, axons, and synapses; while WM predominantly involves myelinated axons, glial cells, and vasculature. Theories have argued for both different mechanistic and complimentary roles for each tissue. For example, Twin Studies report that GM and WM share around 68% heritability whereas other evidence suggests a different transcriptomic profile of each tissue type, indicating cellular and functional heterogeneity between them and highlighting the importance of studying both tissue together as well as separately.[122,123]

Exploring WM changes in disease is important especially in the context of the noticeable shift from the neuron-centric view of the nervous system. Glial cells (that make up the majority of WM tissue) play an important role in a range of functions such as synaptic plasticity, cognition and brain health, with more recent evidence highlighting the role of glial cells in potentially exacerbating AD-related neuropathology.[124,125]

1.7.5 White matter hyperintensities

Since small vessels cannot be resolved by conventional MR imaging, parenchymal lesions are used as a proxy of SVD. WMHs are mostly bilateral and symmetrical hyperintense lesions detected on T2-weighted and FLAIR MR images.[120] They present as white matter degeneration characterised by neuronal loss, demyelination, and gliosis on neuropathologic examination. They are distributed in the periventricular and deep WM of the cerebral hemispheres, in the basal ganglia, and, less frequently in the posterior parts of the brain.[126]

Age is closely related to the onset and development of WMHs with their frequency in healthy subjects shown to be around 11–21% in those aged ~64 years, and 64–94% in those who are ~82 years.[127,128] This makes WMHs a common finding in the older general population.[22] WMHs have been strongly correlated with cerebrovascular disease and cardiovascular risk factors, although post-mortem studies have shown that their underlying histopathology is heterogeneous.[129] WMHs are also associated with cognitive, neurological, and functional symptoms such as walking difficulties and depression.[130,131] Some studies suggest that WMH burdens may differ by sex, with location of the lesions and cardiovascular history being key determining factors.[132] This being said, around 80% of the variation accounting for them remains unexplained.[133] WMHs can be assessed with visual rating scales, such as the Fazekas scale, Scheltens scale,[134] age-related white matter changes (ARWMCs) scale[135] and automated systems (e.g., BAMOS).[136] As previously discussed, WMHs are a cardinal biomarker of VaD although their role in contributing to or exacerbating AD is beginning to be recognised.[130,137] Since WMH are thought to mainly represent ischaemia-related demyelination and axonal loss due to damage to arteries and arterioles (i.e., SVD), the vivo observation of WMH lesions is crucial in guiding towards a VaD diagnosis. Neuroimaging and neuropathological studies however show that the aetiology of WMH may also include degenerative axonal loss caused by either cortical neuronal loss or Wallerian degeneration.[138] This highlights the complexity of the aetiologies that characterise WMHs.

1.7.6 Diffusor tensor imaging

Diffusor tensor imaging (DTI) is an imaging technique used to assess the microstructural properties of cerebral WM. DTI indexes water diffusion as an indirect measure of microstructural orientation and integrity of WM tracts in the brain.[139] The directionality and magnitude of these random water movements in brain tissue can be evaluated via multiple quantitative measures such as mean diffusivity (MD), transverse or radial diffusivity (RD), axial diffusivity, and the degree of anisotropic diffusion.[140] For example, fractional anisotropy (FA) is the summative direction of water diffusion within a voxel and is thought to be highly sensitive to microstructural change. MD captures the mean water diffusion rate with a higher value reflecting potential diseases such as oedema or necrosis.

Anisotropic diffusion and RD quantify water diffusion in a parallel and perpendicular direction to the principal direction of fibre tracts, respectively, thus thought to be reflective of axonal and myelin integrity.

Numerous DTI studies of AD and MCI participants have shown that greater cognitive impairment is associated with lower FA in a number of brain regions such as the corpus callosum, fornix, cingulum, superior longitudinal fasciculus (SLF), and inferior longitudinal fasciculus (ILF).[141–144] Diffusivity measures have also been shown to correlate with widely used clinical or cognitive assessments. Higher FA in the corpus callosum and cingulum and lower MD, AxD, and RD in temporal lobe regions were associated with MMSE scores, while FA, MD, AxD, and RD in the left cingulum correlated with scores from the Clinical Dementia Rating.[145] Interestingly, there was evidence that FA was the least sensitive measure for detecting differences between diagnostic groups and cognitive scores while RD and MD were more sensitive for detecting subtle differences in MCI groups.

Overall, DTI is an interesting novel research tool that can shed some light into the more subtle damage to the brain microstructure which may underlie cognitive decline as well as some of the pathologies that characterise dementia subtypes.

Recent years have seen an alternative to the DTI “signal” model designed to estimate microstructural complexity of neurons. One of the common microstructural models is Neurite Orientation Dispersion and density imaging (NODDI). The NODDI model estimates the signal in three tissue compartments for each voxel – intracellular, extracellular, and CSF compartments.[146] The intra-cellular compartment is composed of neurites (modelled as zero-radius sticks capturing restricted diffusion) with a distribution of directions that includes both an average direction and a spread of orientations around that direction. The output of the NODDI model produces values for: 1) neurite density index (NDI) capturing proportion of brain tissue occupied by neurites, 2) orientation dispersion index (ODI) representing the coherence of neurites by measuring variability in their orientation and 3) free water fraction (FWF) estimating CSF contamination.[147]

Advanced techniques such as NODDI can help provide detailed microstructural information that can reveal abnormalities in WM, known as normal-appearing white matter (NAWM) that may not be detectable with conventional MRI.

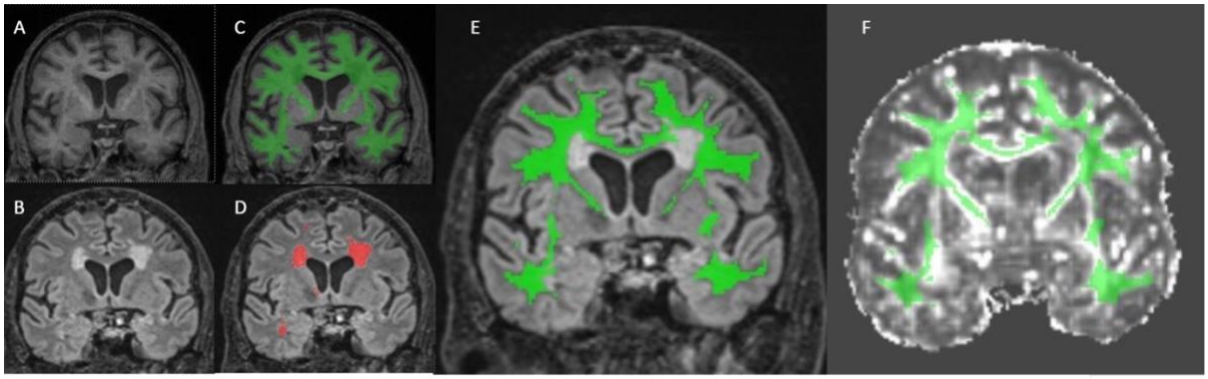


Figure 1.5: Tissue segmentation in Insight 46 **(**Source: published work by James and colleagues).[148]
The scans represent a (A) T_1-weighted (B) FLAIR (C) segmented images to create WM) (D) WMH mask (E) NAWM mask created by subtracting the WMH mask (F) The NAWM mask overlayed on the FA map in the T_1 space. The methods behind the scanning, preprocessing and postprocessing of these images are discussed in Chapter 2.

1.7.7 Overview of positron emission tomography (PET)

PET is an imaging technique that uses metabolically active compounds labelled with positron-emitting radioisotopes to assess physiological function. Very briefly, this technique requires the intravenous injection of a radioactive tracer (e.g., ^{18}F-amyloid PET ligand, ^{18}F-florbetapir) into a peripheral vein. Once injected, the tracer accumulates in the area of the body for which the molecule has specific affinity (e.g., the brain). The radioactive nuclei within the tracer decay through a process called positron emission, whereby the positron combines with an electron leading to an annihilation event.[149] This generates two gamma photons travelling in opposite directions at 180 degrees from each other which are recorded by an array of detectors arranged in a circular or ring-like structure around the patient. When these photons are recorded simultaneously, this creates “coincidence lines” which provide spatial information about the radiotracer's distribution in the body. The coincidence data, along with timing information in Time-of-Flight (TOF) PET are processed to generate detailed 3D images reflective of metabolic activity in tissue of the brain (e.g., brain), which can then be used to guide the diagnosis and management of medical conditions such as AD.

PET is invaluable in research as it provides comprehensive information on metabolic and physiological processes in the body. However, the spatial resolution of PET imaging is considerably lower than structural imaging techniques such as MRI/CT. This may be due to multiple reasons such as photons not always travelling precisely in opposite directions following an annihilation event or because the tracer mapping is dependent on where positrons and electrons combine not where the positrons are emitted. To make up for the imprecision of the anatomical data acquired, PET data is often registered to structural images by a different image modality. PET/CT scanners can be used to allow for the acquisition of a structural CT scan prior to the PET scan. The recent development of PET/MRI technology provides important benefits over PET/CT scanners.[150] This includes the simultaneous acquisition of MRI and PET scans saving scanning time and PET registration.[118]

1.7.8 βeta Amyloid

PET imaging biomarkers of metabolism have substantially enhanced our understanding of AD-specific neural changes in living human subjects.

The earliest imaging biomarker in this scheme is the presence of Aβ plaques in the precuneus/posterior cingulate and orbitofrontal cortex regions of the brain. ^{18}F-fluoro-deoxyglucose (FDG) PET was initially used to detect glucose uptake as a proxy marker of amyloid, but novel radiotracers such as ^{11}Pittsburgh compound-B (PIB)[151] and ^{18}F-florbetapir[152] have enabled the direct imaging of Aβ plaques. As discussed previously, age and APOE e4 are important risk factors for AD but these have also been associated with amyloid deposition.[153,154] Despite the high sensitivity of amyloid imaging in diagnosis of AD, there is approximately a 15 year time lag between Aβ deposition and the appearance of clinical symptoms in AD.[7] This means that by the time patients become symptomatic, the amyloid load may have reached its plateau phase thus limiting the utility of amyloid PET as a staging or prognostic biomarker of AD.

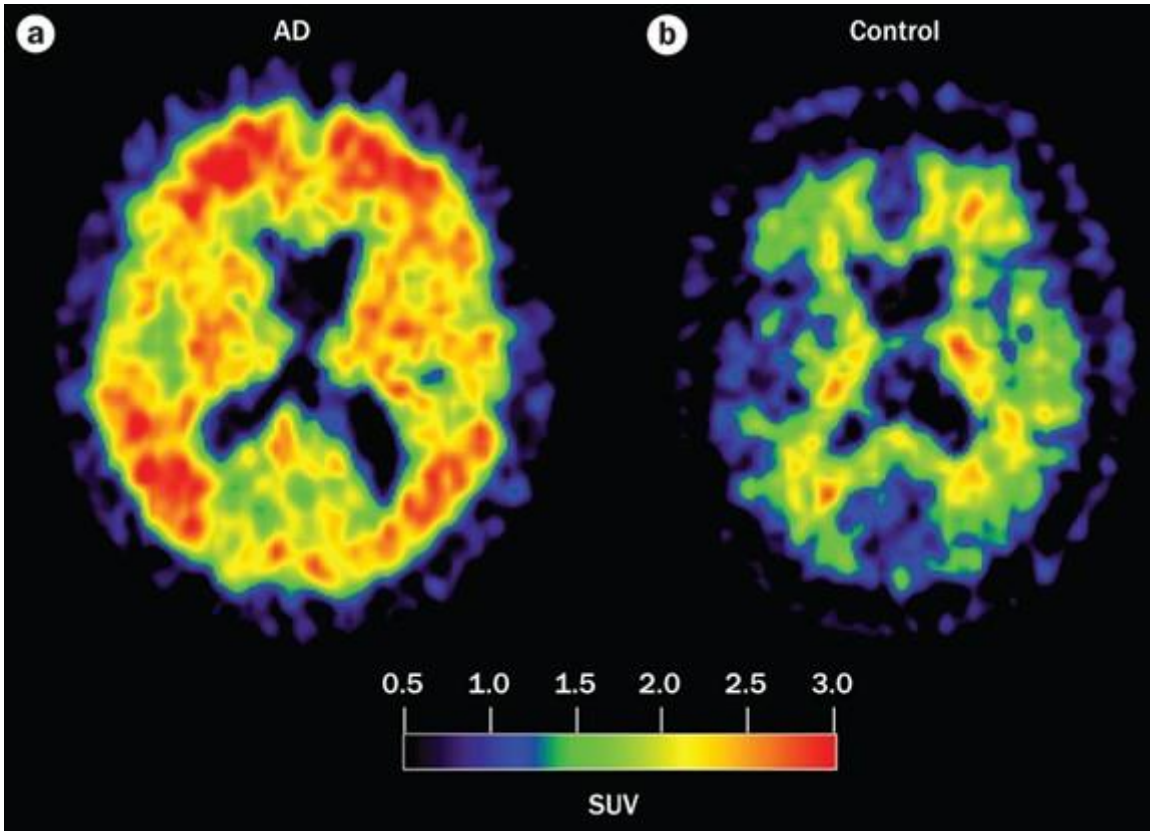


Figure 1.6: Images from positron emission tomography scans representing the difference in amyloid burden (as measured by ^{11}C-PIB) between Alzheimer's disease patients and healthy individuals.
In panel (a), scans from Alzheimer's disease patients show evidence of high ^{11}C-PIB retention, indicated by the prominent red areas signifying high retention. In contrast, panel (b) shows scans from age-matched, healthy control participants who show low ^{11}C-PIB retention, depicted in cooler colours on the scale. This is an example of how amyloid burden is quantified using positron emission tomography imaging (Source: published work by Nordberg and colleagues).[155]

1.7.9 Tau imaging

As previously mentioned, NFTs are a key neuropathological marker of AD. Their prevalence have been associated with a higher degree of cognitive decline[156] and the severity of dementia symptoms.[94] Thus tau-specific PET imaging is considered an important biomarker of cognitive decline and disease progression. Several first- and second-generation tau PET ligands have been developed and are currently in clinical research (see published work from Leuzy and colleagues for more information).[157] PET tau can have important diagnostic power when used in conjunction with other biomarkers of AD. For example, a patient who presents as amyloid positive (A+) but shows no indication of neurodegeneration on PET or structural MRI (N–), the detection of tau pathology may reflect an AD diagnosis or an advanced stage of the disease. On the other hand, if a patient is A+and N+without any evidence of tau, clinicians may consider neurodegeneration to be occurring as a consequence of non-AD pathology thus learning towards a mixed dementia syndrome.[158]

1.7.10 Brain indices of brain health

To summarise, brain imaging tools have given several indices of brain health. These include global, regional and tissue type specific brain volume measures, SVD-related markers such as WMHs and their microvascular counterparts originating from DTI and NODDI (e.g., FA, MD, ODI, NDI) and measures of neuropathology (e.g., PET amyloid and PET tau).

1.7.11 Other approaches

Other imaging tools include fMRI to measure task-related and resting state activity[159,160] as well as functional near infrared spectroscopic (fNIRS) imaging to study haemodynamic and inferred activation patterns in the context of brain pathology. Alternatively, other non-imaging biomarkers are also being investigated, e.g., plasma biomarkers for beta amyloid (Aβ42), total tau and phosphorylated tau.[161] These biomarkers have shown utility in helping differentiate the aetiology of dementia, assess

disease progression and enhance clinical trials. All of these approaches go beyond the scope of this thesis and will not be discussed further.

1.8 Diabetes mellitus

Diabetes mellitus (DM) describes a group of metabolic conditions characterised by chronic hyperglycaemia, a physiological state of elevated blood glucose levels. These conditions originate from defects in the production of and/or secretion to insulin.[162] Traditionally, DM has been split into two categories. Type I diabetes (T1D) describes a state of metabolic pathology driven by the autoimmune destruction of pancreatic β cells resulting in insulin deficiency. Accounting for 10-15% of DM cases, T1D is primarily diagnosed in children and adolescents.[163] The specific causes driving this autoimmune destructive response of β cells are still poorly understood, but genetic and environmental effects have been proposed.[164]

T2D is characterised by hyperglycaemia that arises primarily from a combination of insulin resistance (IR) in insulin-sensitive tissue as well a deficiency in insulin production by β cells.[165,166] It is thought to be driven by a complex interplay between genetic and environmental factors.[167] Some of these mechanisms are discussed in more detail below. A recent study estimated that in 2021, 529 million people worldwide were affected by DM with 96% of cases being those of the T2D subtype.[168] The same study projects that the number of cases is expected to more than double by 2050 which will amount to around 1.31 billion people. DM prevalence varies across geographical locations with high frequency reported in North Africa and the Middle East.[168] Studies within the United Kingdom (UK) and US also revealed excess T2D incidence in South Asian, African Caribbeans and African Americans.[169,170]

The complications of T2D are distinguished between acute and chronic. More acute complications include diabetic ketoacidosis (DKA), hypoglycaemia, acute infections and hyperglycaemia.[171] More chronic complications can be macrovascular (e.g., heart disease, stroke etc.) and microvascular affecting small blood vessels (e.g., retinopathy, nephropathy and neuropathy).[172]

1.8.1 Risk factors for T2D

Risk factors for T2D are categorised as modifiable or non-modifiable. Non-modifiable risk factors include age, sex, ethnicity, and family history of T2D. Age is an independent risk factor for T2D separate of important other well-known factors such as obesity. There is a considerable increase in T2D prevalence after the age of 40 with an important inflection point between the fourth and fifth decade of life. Individuals aged 70 with a body mass index (BMI) in the normal range however had similar prevalence for T2D to those aged 30 of normal BMI range.[173]

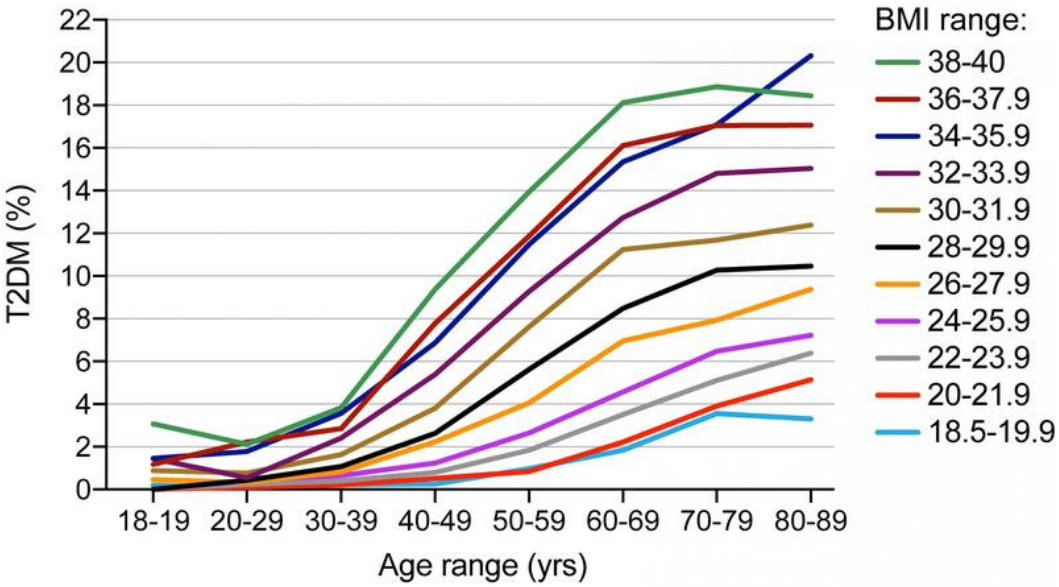


Figure 1.7: Visual demonstration that type 2 diabetes prevalence (%) increases with age across all individuals of all body mass index ranges (Source: published work by Fazeli and colleagues).[173]

Family history of T2D is recognized as an important risk factor for the disease: the odds of having diabetes were found to be considerably higher in those with a family history for the condition. The crude odds ratio (OR) for individuals with a family history of diabetes was 5.06 (CI: 4.37-5.85), suggesting that they were over five times more likely to have diabetes compared to individuals without such a history.[174]

Social factors such as lower childhood social class and lower education levels have been associated with higher rates of T2D.[175] Obesity and low physical activity are

among the most important modifiable risk factors for T2D since evidence shows that a healthy diet and active lifestyle reduce the risk of developing the condition.[176] More specifically, lifestyle change benefits showed a sustained 37% reduction in T2D risk (RR = 0.63, CI: 0.54, 0.74) in those who maintained these changes over a decade.

1.8.2 Sex differences in diabetes

There is growing evidence of sex differences in the pathogenesis of various metabolic diseases including diabetes. Globally, diabetes in early and midlife has been shown to be more prevalent in males than in females.[177] Peak age of onset also varies by sex with diabetes commonly being diagnosed earlier in males (65–69 years) compared to females (70–79 years).[178] This being said, postprandial hyperglycaemia increases to a larger extent in females as they age, contributing to a higher prevalence of undiagnosed diabetes in females after the age of 60, and of total diabetes after 70.[179] Sex-dimorphic differences in diabetes outcomes are thought to be complex and reflect differences in the roles that genetic and hormonal contributors play on pathophysiology, clinical manifestation and therapeutic response.[180,181]

Sex hormones may play an important role in metabolic health, especially during a period of change that is the menopause. Before this stage of prominent hormonal shift, oestrogen is protective of metabolic health by increasing insulin sensitivity, stimulate insulin secretion and protect against β cell apoptosis; 17β-oestradiol, a form of oestrogen, has been found to act on two oestrogen receptors (ERs), ERα and ERβ, with ERα playing a crucial role in β-cell survival. Premenopausal females are found to have higher skeletal muscle, hepatic insulin sensitivity and higher stimulated insulin. This is thought to, at least partially, account for females' lower fasting glucose and HbA_{1c} values.[180,182] Premature menopause on the other hand, is associated with an increased risk of T2D with hormonal replacement therapy (HRT) shown to delay the condition.[181,183] However, the hormonal changes during the menopause, may result in a parallel increase in HbA_{1c} values with changes in body composition suggesting that females are at increased risk of impaired glucose tolerance.[180] Beyond this,

psychosocial factors may also have a strong impact on sex differences in T2D development and complication. For example, prolonged night work was shown to be associated with a 46% higher risk of T2D in females compared to males.[184] Other factors such as low levels of education, low socioeconomic and occupational status, and low income are all also significant risk factors for the development of T2D, especially in females.[185,186]

There is growing evidence that there are sex-specific differences in target organ damage in the context of T2D.[187,188] Females showed greater target organ damage (TOD) compared to males over a 3.5-year follow-up across several vascular and renal measures, as shown by their annual deterioration in carotid intima-media thickness, carotid plaques and pulse wave velocity as well as changes in glomerular filtration rates. For example, carotid intima-media thickness increase in mm/per year was 0.018 in females and 0.0007 in males. There is also evidence of sex differences in microvascular disease in the context of T2D. Compared to people with normoglycaemia, males with T2D have been found to have a higher risk of sensory neuropathy and retinal microvascular damage than females.[188] Other studies suggest a greater risk of renal failure, renal insufficiency, greater neuropathic pain and nerve injury in females with T2D.[189,190] Currently, there is little evidence regarding sex differences in the impact of diabetes-related pathology on brain and cerebrovascular outcomes.

To summarise, some epidemiological evidence indicates that T2D may affect females and males differently and at different stages of the life course. Potential biological mechanisms include the changing protective role of sex hormones in metabolic wellbeing over the menopause, as well as differences in body composition that are linked to the processing of glucose and lipids differently in males and females.

1.8.3 Treatment of T2D

Treatment for T2D has undergone tremendous change in past decades. Before the discovery of insulin, dietary interventions such as diets rich in fat and protein and low

in carbohydrates were recommended.[191] During the 20th century, the use of pharmacotherapies, such as oral antihyperglycaemic drugs became prominent.

Metformin is currently considered the first line treatment for T2D. It is a biguanide derivative that is thought to work by supressing hepatic glucose production, increasing insulin sensitivity in muscles and facilitating glucose uptake in peripheral tissues.[192] Metformin does not affect β cell function.[193] Its mechanism of action is debated but a common view is that it inhibits the transport of glucose across the intestinal wall, reducing the amount of glucose entering the bloodstream, supressing glycogen synthesis in the liver and enhanceing glucose uptake in tissue.[194,195]

Metformin is often used in combination with lifestyle interventions and has been shown to promote weight loss, manage blood lipid concentrations and reduce mortality.[196] Other classes of antidiabetic drugs include sulfonylureas, alpha-glucosidase inhibitors, glinides, Dipeptidyl Peptidase-4 inhibitors (DPP-4), meglitinides, thiazolidinediones, incretin mimetics, sodium-glucose transporter 2 inhibitors and insulin.[197] The mechanisms of action vary from drug-to-drug. Sulfonylureas and meglitinides, for example, both increase the secretion of insulin from pancreatic β cells producing hyperinsulinemia to reduce glycaemic levels (albeit through slightly different mechanisms).

In many patients a single antihyperglycaemic drug may suffice initially, but people with T2D frequently require another drug with a different mechanism of action to achieve adequate control of their hyperglycaemia. Drugs that increase insulin levels independently of glucose concentration carry a risk of hypoglycaemia, whereas metformin does not.[198]

More recently, Glucagon-like peptide-1 (GLP-1) receptor agonists have been approved as a treatment for T2D and obesity. GLP-1 belongs to the family of gut-derived incretins that also include glucose-dependent insulinotropic polypeptides (GIP). These are hormones responsible for a range of glucoregulatory effects including glucose-dependent secretion of insulin and suppression of glucagon release.

GLP-1 inhibits glucagon secretion from a-cells which can lower glucose levels while GIP does not significantly inhibit glucagon levels and may even increase its secretion in certain contexts. Since GLP-1 has a more prominent effect on β cell proliferation and survival, compared to GIP, GLP-1 based treatments are favourable for the treatment of T2D.

In a healthy individual, the presence of food in the gastrointestinal tract triggers GLP-1 to be secreted by L-cells in the small intestine.[199,200] In people with T2D, the pathophysiological mechanisms of the condition cause the effects of GLP-1 to become dysfunctional, thereby impairing these glucoregulatory effects and of the postprandial insulin response.[201] GLP-1 receptor agonists can directly or indirectly target multiple defects at the core of T2D (e.g., decreased insulin secretion and decreased glucose uptake).[202,203] Since the effects of GLP-1 receptor agonists on insulin secretion are glucose-dependent, there is a low risk of hypoglycaemia.[204] The benefits of GLP-1 receptor agonists extend beyond their well-established effects on glycaemic control, showing their effectiveness for weight loss and blood pressure.[205,206]

1.8.4 Diagnosis of T2D

Based on the criteria set by the World Health Organization (WHO), T2D can be diagnosed from a fasting blood glucose concentration ≥7·0 mmol/L (126 mg/dL), a random blood glucose concentration ≥11·1 mmol/L (200 mg/dL), or a two hour plasma glucose concentration ≥ 11.1 mmol/l two hours after 75g anhydrous glucose in an oral glucose tolerance (OGT) test.[207] In the absence of symptoms, abnormal glycaemia must be present on two different occasions. A diagnosis of diabetes can also be made on the basis of a glycated haemoglobin A1c (HbA_{1c}) concentration above 48 mmol/mol (6.5%),[208] but a value of less than 48mmol/mol does not exclude diabetes diagnosed using glucose.

HbA_{1c} is a measure of long-term glycaemia (since the lifespan for red blood cells is around 120 days). However, haemolysis can impact the lifespan of red blood cells and thus affect the accuracy of HbA_{1c} measures. Other factors that may also invalidate this

variable include older age, alcohol consumption, smoking, iron deficiency, kidney disease, and high dietary choices.[209–213] This is particularly important as it suggests that the sensitivity of HbA_{1c} as a marker of glycaemia can vary by sex because sex-related differences in erythrocyte properties are commonly observed.[214] Differences in erythrocyte properties may affect HbA_{1c} values and result in the underestimation of values in males.[215] Iron and haemoglobin are negatively associated with HbA_{1c} but are not associated with fasting glucose. Iron deficiency, on the other hand, increases HbA_{1c} values.[216]

1.8.5 The limitation of current diabetes classifications

It is important to note that the diagnostic thresholds for T2D are based on the results from epidemiological studies which observed that, as glycaemic health gets worse, higher rates of vascular complications, particularly those of microvasculature nature increase. For example, there is evidence that higher HbA_{1c} levels, are associated with an increased risk of retinopathy.[217,218] This is consistent with other research indicating that interventions which reduce HbA_{1c} levels, lower the risk for retinopathy.[219] Nonetheless, some studies have shown that in the general population, the relationship between fasting glucose and risk is continuous, with no clear threshold.[220] Similar findings have been reported for HbA_{1c}.[221] While initiation of treatment from a clinician's perspective is inevitably a binary decision that must reflect the balance of benefit and risk, these findings suggest that there is value, particularly in a research context, in looking across the entire spectrum of glycaemia in relation to outcomes. This may be even more important when looking at brain outcomes since thresholds based on vascular outcomes might not predict neurological disease in the same way. Interestingly, there is minimal evidence that interventions that reduce HbA_{1c} have a beneficial effect on cognitive outcomes.[222]

1.8.6 Prediabetes

Prediabetes is defined as a state in which individuals have elevated blood glucose levels, which are not high enough to be classified as diabetes.[223] In the UK, it is defined

as a fasting plasma glucose ranging between 5.5 mmol/L to 6.9 mmol/L and HbA_{1c}: 42 to 47 mmol/mol (6.0 to 6.4%) although there is no internationally agreed prediabetes ranges.

It is characterised by impaired fasting glucose, impaired glucose tolerance or elevated HbA_{1c}. Prediabetes is associated with a number of co-morbidities and complications such as risk for developing stroke and heart disease.[224] Annual conversion from prediabetes to T2D is between 3 to 11%.[225] Through lifestyle and nutritional changes, individuals with pre-diabetes can return their blood glucose levels to normal and prevent or delay the development of T2D. Research has shown that lifestyle interventions are more cost-effective than medication in preventing and delaying the progression of prediabetes to diabetes.[226] A recent national diabetes statistic report from the CDC, showed that a higher percentage of males (41%) than females (32%) could be considered to have prediabetes based on their HbA_{1c} or glucose values (as per the CDC National Diabetes Statistics report 2023 (based on data from Bryan and colleagues)).[227]

1.9 Insulin and insulin signalling pathways

Insulin is an anabolic peptide hormone secreted by pancreatic β-cells in response to a glucose load (e.g., ingestion of food). Insulin binds to receptors located in the membrane of target cells. These target cells include hepatocytes (where insulin promotes glucose utilisation and suppression of glucose production), muscle and adipose tissue (for glucose uptake and synthesis) as well as brain cells.[228]

The insulin signalling pathway is a network of interconnected components and feedback loops that integrate signals from various sources to produce coordinated sets of cellular responses. It is described very briefly here based on description by Satiel and Kahn and Batista and colleagues.[229,230] The binding of insulin to a tyrosine kinase receptor, a transmembrane protein composed of two extracellular α and two β-

subunits, activates the intrinsic properties of the β-subunit, initiating conformational changes to its receptor inducing autophosphorylation of the β-subunits as well as other intracellular molecule which further increases kinase activity. This sets in motion a cascade of early intracellular signalling events including the phosphorylation of a family of proteins known as the insulin receptor substrate (IRS) on specific tyrosine residues creating binding sites which recruit intracellular signalling proteins such as phosphatidylinositol 3-kinase (PI3K) via the Src Homology (SH2) domain of the p85 regulatory subunit. The recruitment of PI3K to the membrane results in phosphatidylinositol 3,4,5-trisphosphate (PIP3) in the plasma membrane. PIP3 then serves as a docking site for phosphoinositide-dependent kinase-1 (PDK1) and Akt (protein kinase B), which both have pleckstrin homology (PH) domains. Akt has a series of downstream effects regulating a series of cellular processes (e.g., glycogen and protein synthesis) and promotes glucose transporter 4 (GLUT-4) to the cell surface of muscle and adipose tissue. The pathway influences liver gluconeogenesis and is connected to processes like cell growth and protein synthesis through the mTOR pathway. Several other signalling pathways are also activated.

The overall activation of these pathways is responsible of glucose uptake, metabolism, and cell survival. In muscle and adipose tissue, the insulin signalling pathway handles glucose uptake and storage, whereas in the liver it inhibits glucose production and enhances the synthesis of glycogen. Abnormalities to this complex signalling network such as dysfunction to subunit interactions and phosphorylation events are considered to be at the heart of IR and T2D.

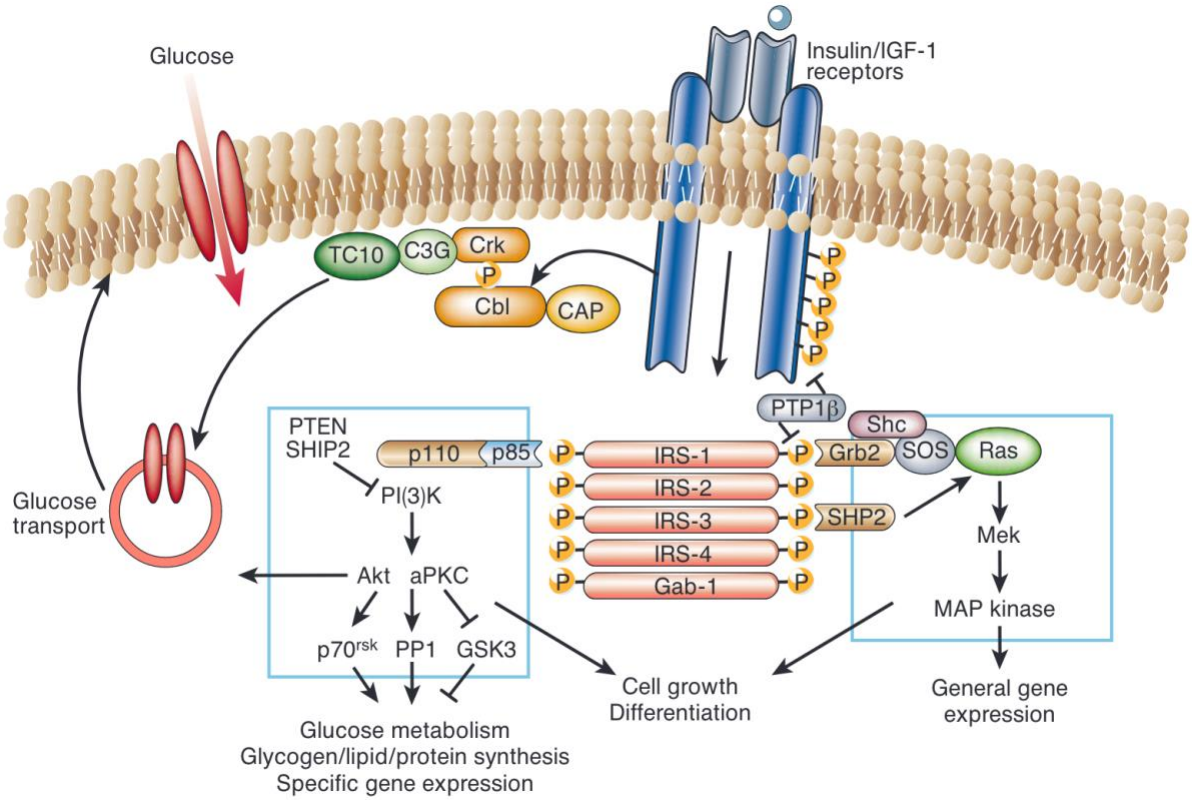


Figure 1.8: A visual representation of the insulin signalling pathway (source: published work by Satiel and Kahn).[229]

Insulin receptors are also found in high volumes in the brain with evidence of considerable variation in the locations where these are expressed. Animal studies uncovered a high insulin receptor density in regions such as the hypothalamus, hippocampus, cortices, striatum and cerebellum.[231–234] Such widespread receptor distribution across different brain regions suggests that insulin signalling is likely to play an important and diverse role in the brain. Moreover, insulin also influences gene expression, protein synthesis and is implicated in apoptosis (programme cell death) and autophagy (degradation of cellular components).

1.9.1 The pathophysiology of T2D (IR and hyperglycaemia)

T2D is a complex metabolic disorder characterised by hyperglycaemia, a physiological state of elevated blood glucose. Hyperglycaemia is thought to occur as a consequence of impairments in the secretion of insulin or the action of insulin (or both). IR is defined as the impaired metabolic response to insulin in sensitive tissues such as the liver, adipose tissue and skeletal muscle.[235] In the early stage of the disease, reduced insulin sensitivity triggers hyperfunction of the β-cells in pancreatic islets causing them to continuously secrete insulin to maintain normoglycaemia. When compared to healthy controls, individuals with IR may show 3- to 4-fold higher rate of insulin section.[236] This compensatory hypersecretion of insulin may reflect both the expansion of β-cell mass and altered expression of key enzymes of β-cell glucose metabolism.[237] Initially, this state of hyperinsulinemia prevents hyperglycaemia in the early stages. However since β-cells are undergoing constant dynamic change as evidenced by the continued regeneration of islets and concurrent apoptosis, a disruption to this delicate balance can have severe consequences.[238] Gradually, β-cell function begins to decline and can no longer compensate for the decrease in insulin sensitivity,[238] inevitably resulting in hyperglycaemia.[239] Thus, β-cell dysfunction is considered to play a major role in T2D development, across the spectrum of hyperglycaemia, from prediabetes to overt diabetes.[240]

Abnormalities in a multitude of other interrelated mechanisms have been linked to IR pathogenesis in human and animal studies. These include primary abnormalities in

the insulin signal transduction pathway, circulating factors (such as tumour necrosis factor-α (TNF-α) and free fatty acids), perturbations of intracellular signalling molecules including PKC, ceramides, chronic inflammation, and oxidative stress. More recently, sustained hyperglycaemia, also referred as glucose toxicity, has been considered to be implicated in IR affecting both the secretion and sensitivity.[241] A number of studies have shown that IR can be produced by hyperglycaemia in muscle in vitro.[242] This feedback loop between hyperglycaemia and IR highlights the complex interplay of the underlying pathophysiological mechanisms that this metabolic state. Factors such as age, increased adiposity, decreased muscle mass and a reduction in physical activity have been proposed to contribute to the development of IR.[243]

1.9.2 Markers of IR and hyperglycaemia

Hyperglycaemia describes a state of elevated levels of blood glucose. It is thought to occur as a consequence of a disruption in the body's ability to produce insulin or a lack of sensitivity of tissue to it (i.e., IR). Hyperglycaemia results in the production of glycated haemoglobin. Very briefly, glycation is a non-enzymatic process resulting from an irreversible attachment of glucose and haemoglobin in a two-step process firstly involving the attachment of glucose at the N-terminal valine of the beta chain of haemoglobin to form a Schiff base and subsequent Amadori rearrangement of this structure form more stable Amaradori products or ketoamines.[244] The Amadori products persist for the lifespan of the red blood cells which can result in the formation of glycated haemoglobin.

The glycation of haemoglobin, particularly of haemoglobin A can be measured in blood via HbA_{1c}, a useful marker correlating with the level of ambient glycaemia over a 2-to-3-month period representing the lifespan of red blood cells.[245] Tests for HbA_{1c} are inexpensive and easily administered. The concentration of HbA_{1c} strongly predicts the risk of incident eye disease, heart failure, vascular, kidney, and nerve disease both in people with T1D and T2D.[246–248] A meta-analysis of 10 cohort studies involving over 7000 individuals with T2D showed that a 1% increase in HbA_{1c} was associated with a

significant 18% increase in the risk of coronary heart disease or stroke and a 28% increase in the risk of peripheral vascular disease.[248]

The gold standard test for IR is the hyperinsulinaemic-euglycaemic glucose clamp technique.[249,250] It is however time-consuming and difficult to use in large samples of people. There are a number of clinically useful surrogate measures of IR (or insulin sensitivity), including Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), Homeostasis Model Assessment of β-cell Function) (HOMA-B), QUICKI, serum triglycerides, leptin-adiponectin ratio, and triglyceride/HDL ratio.[251–253] Recently, similar measures were generated using an updated HOMA2 model which can be generated using a calculator.[254]

Sex differences in glucose metabolism, β-cell function, and insulin sensitivity have previously been reported. For example, females have been found to exhibit higher post-OGT glucose and lower fasting glucose levels partly due to their shorter stature affecting their glucose load.[255] Higher postprandial insulin and c-peptide concentrations in females may also indicate insulin secretion. GLP-1 levels are higher in females following an OGT test, driven by oestrogen, which also promotes β-cell function and survival.[256,257] Despite greater insulin sensitivity in skeletal muscle, females exhibit these markers under specific physiological conditions, such as post-load glucose levels and insulin response to meals, reflecting intrinsic biological differences between genders in metabolic regulation and glucose homeostasis. This perhaps highlights the importance of considering several markers of glycaemia and related traits when exploring sex differences in the associations.

Considering multiple glycaemic traits such as HbA_{1c}, fasting glucose, HOMA-IR, and HOMA-B allows for a thorough investigation of these differences. HbA_{1c} provides a chronic overview of glycaemic heath, fasting glucose may offer more insight into basal glucose levels, HOMA-IR assesses tissue IR, and HOMA-B can give a measure of beta-cell function. While none of these markers are perfect, their collective consideration may give a nuanced understanding of metabolic regulation, essential for

advancing research into understanding how the pathophysiological mechanisms that underlie metabolic conditions are associated with different health outcomes.

1.9.3 Hypoglycaemia

Hypoglycaemia is a state of low plasma glucose set at <70mg (<3.9 mmol/L) by the American Diabetes Association. It has been associated with a number of adverse events such as myocardial infarctions, arrythmias and stroke.[258,259] Risk factors of hypoglycaemia include fasting, alcohol consumption, and drugs that increase insulin secretion (e.g., sulfonylurea, thiazolidinediones and biguanide). There is evidence that hypoglycaemia (<3.0 mmol/L) can impair cognitive function consistently across adults with and without diabetes, independent of various clinical factors.[260–262]

1.9.4 Hyperglycaemia, IR and inflammation

The development of IR has been proposed to occur, at least partially, in response to the increased production of pro-inflammatory cytokines by adipose tissue in obesity, which may consequently have an inhibitory effect on insulin signalling pathways in various tissues. For example, TNF-α, which is highly expressed in adipose tissue of obese mice, has been hypothesised to induce IR.[263] The administration of exogenous TNF-α in animal studies has also been shown to cause IR, whereas the suppression of TNF-α improves insulin sensitivity.[263] It is thought that inflammatory cytokines such as TNF-α, interleukin-6 (IL-6) and interleukin-1β) enhance the expression of several proteins that suppress insulin signalling pathways which can reduce insulin sensitivity and increase the risk of IR developing. In turn, IR further induces inflammation, as evidenced by abnormal levels of fibrinogen, c-reactive protein (CRP), IL-6, plasminogen activator inhibitor-1 (PAI-1), and elevated white cell count with T2D.[264–268]

Interestingly, insulin has been found to exhibit anti-inflammatory properties reducing pro-inflammatory cytokines, improving endothelial function, and reducing oxidative stress.[269]

1.9.5 Insulin resistance, diabetes and the cerebrovasculature

Diabetes-related mechanisms such as obesity, IR and hyperglycaemia have been found to cause systemic vascular pathology in animal models. For instance, IR animal models with mild hyperglycaemia show significant medial thickening and hypertrophic remodelling.[270] This also includes vascular remodelling such as extracellular matrix deposition and increased wall thickness of middle cerebral arteries (MCA) occurring via endothelin-1 (ET-1) processes.[271] Some of the adverse remodelling of MCAs may be reversed by glycaemic control and ET-1 antagonists suggesting an important role of hyperglycaemia and ET-1 processes driving these vascular changes.[272] ET-1 mediated vascular changes are also important as ET-1 is a potent vasoconstrictor with proliferative effects on smooth muscle. The role of ET-1 has been recently reviewed as it may play an important role in the vascular complications of diabetes.[273] Hyperglycaemia may also damage the cerebrovasculature through other processes independent of ET-1, which include but are not limited to oxidative stress, increased production of cytokines and activation of the polyol pathways.

In addition, IR and hyperglycaemia can affect the physiology of blood vessels compromising myogenic reactivity, neurovascular uncoupling, and endothelial dysfunction. This can result in the disruption of the BBB integrity, produce changes in CBF and increase the risk of ischemic events as well as cerebral microbleeds. There is consistent evidence that links acute or chronic hyperglycaemia to neurovascular uncoupling as supported by the reduced response of retinal veins to flicker stimulation.[274] Genetic models of T2D have demonstrated that cerebral arteries isolated from diabetic animals tend to develop more myogenic tone than control animals which further indicates damage to endothelial function.[275,276]

It is well established that the risk and severity of neurological diseases, vascular cognitive impairment and AD are increased by metabolic disease such as T2D. A meta-analysis of 28 studies found that T2D was associated with a 73% increased risk of all-types dementia, a 56% increase of AD and a 127% increase of VaD.[277] Since CBF and BBB integrity are essential for brain homeostasis, and that cerebral

circulation is an early target in these conditions, it is highly likely that changes in cerebrovascular structure and physiology play an important role in the onset and progressive pathological cognitive decline in T2D.

1.9.6 The role of genes in hyperglycaemia, glucose and T2D

There is an important genetic contribution to T2D as evidenced by the higher concordance rate for diabetes in monozygotic than dizygotic twins.[278,279] Being born to one or both parents with diabetes increases the individual's lifetime risk for the condition by 40% and 70% respectively.[280]

Despite this, identifying genetic risk variants for T2D has been challenging due to the important contributory roles of environmental and lifestyle factors in the pathogenesis of the disease. The early focus of genetic studies was on linkage and candidate gene association studies. The former successfully identified familial variants for monogenic diabetes forms, such as MODY as well as discovery of genes like calpain 10 (CAPN10) and TCF7L2, found to be associated with T2D across multiple populations.[281–283] Candidate-gene studies identified PPARG and KCNJ11 as susceptibility genes, both becoming targets of anti-diabetes medications.

The arrival of Genome-Wide Association Studies (GWAS) marked a significant breakthrough in understanding the genetic basis of a complex condition such as T2D. GWAS is a powerful, biology-agnostic method that screens the entire genome of individuals with and without a condition or trait for common single-nucleotide polymorphisms (SNPs). This genetic approach was enabled by the completion of the Human Genome Project and the International HapMap project, which catalogued millions of SNPs and established patterns of genetic variation across people. The 1000 Genomes Project built on this work by expanding on the population studied and using high-throughput next-generation sequencing technologies to increase SNP information, allowing current GWAS to examine over 2 million SNPs. A SNP's association with a disease is determined by its higher frequency in cases versus

controls, with a stringent p value of 5×10^{-8} required for genome-wide significance to reduce false positives.

SNPs can contribute to the development and progression of disease by influencing the altering gene function, regulating gene expression and increasing translocational efficiency.[284] The first GWAS for T2D, conducted in a French cohort, identified novel associations at loci such as SLC30A8 and HHEX.[285] Since then, risk alleles have been reported to be associated with genes that regulate pancreatic β-cell development and function, insulin gene expression, secretion, and action.[286–288] To date, GWAS have identified nearly 40 susceptibility loci for T2D both in European and Asian populations. However, disparities in variant allele frequencies among populations from different ethnic groups or geographic regions may contribute to varied disease susceptibilities.[289] This is particularly a problem since most studies are limited by their inclusion of mainly individuals of European descent.

By aggregating multiple SNPs, a polygenic risk score (PRS) for a condition such as T2D can be created. A PRS outputs a numerical score that captures an individual's genetic predisposition for the condition. SNPs included in the PRS are assigned a weight based on their effect size, which captures the strength of its association with T2D. Thus, SNPs with larger effect sizes have greater weights and contribute more to the overall score. A higher genetic estimate for T2D can be considered a greater predisposition for developing the disease. Genetic influences on T2D would be expected to be more stable throughout life than measured HbA_{1c} because genetic risk factors are established at birth, remaining constant, thus providing a fixed baseline risk for developing T2D. By combining information from multiple genetic variants, a PRS can provide a more comprehensive assessment of genetic risk compared to looking at individual SNPs. PRSs have been shown to be useful for stratifying individuals into different risk categories for T2D and have helped identify individuals at high risk who may benefit from early interventions such as lifestyle modifications or pharmacological treatments aimed at preventing or delaying the onset of diabetes.[290]

More recently, GWAS have been conducted for continuous glycaemic traits identifying loci influencing beta-cell function, IR and HbA_{1c}.The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) is a collaborative effort focused on understanding the genetic basis of glucose and insulin regulation by pooling data from multiple GWAS.[291] Key achievements of the consortium include the discovery of loci associated with fasting glucose, fasting insulin, HOMA-β (a measure of β-cell function), and HOMA-IR (a measure of IR).

1.9.7 Risk factors

1.9.8 Diabetes and dementia – shared risk factors

Many predictors of T2D are also considered potentially modifiable risk factors for cognitive impairment and neurocognitive disorders (i.e., dementia). Factors such as hypertension, obesity, smoking, low physical activity, unhealthy diet, chronic alcohol consumption and high lipid and glucose levels have all shown to be associated with an increased incidence of T2D (reviewed in detail by Bellou and colleagues).[292] It is also well established that cardiovascular risk factors such as hypertension are strong predictors of VaD and cognitive decline.[293] Studies in the general population have demonstrated associations between (midlife) vascular risk factors and dementia risk,[294,295] and because T2D (and prediabetes) are associated with an adverse vascular risk factor profile,[163] it has been hypothesised that these vascular consequences contribute to dementia risk in these individuals. It is also clear that patients with complications of a microvascular (e.g., diabetic retinopathy) or macrovascular nature (e.g., myocardial infarction, stroke) are more likely to have worse cognitive performance [296,297] and are at an increased dementia risk.[298,299] Other studies have identified IR, inflammation, and depression as potential risk factors for cognitive dysfunction in people with diabetes.[297]

1.10 Mechanisms that may underlie the relationship between T2D and brain pathology in dementia

There are several possible mechanistic pathways through which hyperglycaemia, and IR may have its effects on the brain. Some of these are discussed here.

1.10.1 Oxidative stress

Hyperglycaemia results in the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which contribute to oxidative stress. Previous evidence has shown that active oxidative products are associated with the pathogenesis of diabetes including its onset, progression and complications.[300] In animal models, hyperglycaemia has been found to reduce antioxidant levels in the brain and potentially contribute to cognitive deficits.

Hyperglycaemia can induce oxidative stress through various pathways. Elevated glucose levels can increase oxidative stress via the overproduction of superoxide radicals in the mitochondria.[301] This can impair endothelial function. For example, the induction of hyperglycaemia via the intraarterial injection of dextrose has been observed to disrupt endothelium-dependent vasodilation.[302] Oxidative stress caused by ROS overproduction also plays a key role in the activation of other pathogenic pathways involved in diabetic complications, including elevated polyol pathway flux, non-enzymatic glycation, and PKC levels, which in turn can lead to the development of microvascular complication and in some cases damage to the permeability of the BBB.[303,304] Glucose can also react with LDL phospholipids and apolipoprotein B (APOB) lysine groups to produce advanced glycation end products (AGEs) that facilitate lipid peroxidation.[305] In addition to their role in atherogenesis and macrovascular disease, markers of lipid peroxidation are elevated in brain tissues and bodily fluids of several neurodegenerative disorders such as AD and Parkinson's disease.[306–308]

The brain is highly sensitive to oxidative stress because it consumes about 20-30% of inspired oxygen and contains high levels of polysaturated fatty acids (PUFAs), making is an ideal target of free radical attack. ROS can also interact with both deoxyribonucleic acid (DNA) and proteins to cause cellular damage, especially targeting mitochondrial DNA, potentially establishing a vicious cycle of mitochondrial damage and ROS generation.

1.10.2 Inflammation

T2D diabetes is characterised by systemic and cerebrovascular inflammation which can have negative repercussions on brain health. Hyperglycaemia increases metabolism in the mitochondria which can result in the overproduction of ROS.[309,310] ROS may in turn induce damage to cellular components such as DNA. Hyperglycaemia may also result in the formation of AGEs.[311–313] This happens simultaneously and concurrently with the activation of the NF-κB pathway, a key transcription factor in inflammatory responses promoting the activation of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.[314] Some inflammatory markers and cytokines cause alteration or damage to the endothelium, and this may compromise the BBB contributing to brain damage. Concurrently, microglia may be activated to produce more pro-inflammatory cytokines, creating a feedback loop that perpetuates inflammation.[315] Furthermore, inflammatory and oxidative stress may cause excessive glutamate release which may result in neuronal death.[316] In addition, inflammation does not just result from hyperglycaemia but also exacerbates hyperglycaemia by impairing insulin signalling.[317]

Some studies have reported differences in systemic inflammation between individuals with T2D and healthy controls. Specifically, levels of a range of inflammatory markers including TNF-α, eotaxin, CRP, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1β, and monocyte chemoattractant protein (MCP)-1 were found to be significantly higher in individuals with T2D.[318] Conversely, interleukin (IL)-7 levels were lower in the T2D group suggesting a potential impairment in immune function. After adjusting for age and

BMI, the differences in TNF-α, eotaxin, and IL-7 remained significant. These findings suggest that individuals with T2D exhibit a state of low-grade systemic inflammation, some of which may be influenced by factors such as obesity and glycaemic control.

There is evidence that AD is associated with inflammatory processes.[319,320] At least two studies link elevated CRP with an increased risk of AD.[321,322] There is also some evidence that inflammatory cytokines accumulate at different rates in AD's patients compared with healthy control subjects;[323] the inflammatory cytokine IL-6 is present in senile plaques of AD patients[324] and elevated immunoreactivity to IL-6 is found in lumbar and ventricular CSF in patients with AD.[325]

In a population-based study, midlife systemic inflammation (as indexed by a composite score contructed from 5 markers) has been found to be associated with adverse health outcomes such as small volumes in AD signature regions, occipital lobe and hippocampus as well as increased ventricles.[326] However, they did not find a convincing association with total brain, frontal, temporal or parietal volumes. Some mixed findings on the inflammation-brain associations have also been reported by Jefferson and colleagues.[327]

1.10.3 Amylin

Amylin is an insoluble deposit of a misfolded protein in β pleated sheets found in the brain and pancreas of those with T2D.[328] Amylin was first identified in the pancreas of people with T2D.[329] The full range of pathophysiological functions of amylin is unclear, but they include being amyloidogenic and, in human but not rat models, neurotoxic.[330] Soluble amylin appears to be similar in its neurotoxicity to Aβ[331,332] and may share similar mechanisms of toxicity.[330] Amylin is elevated in obesity and in prediabetes/IR and may be a mechanism that underlies the oxidative and inflammatory stress seen in T2D.[333]

Multiple mechanistic pathways have been proposed to explain how amylin relates to cognitive decline and increased risk of AD pathology. One hypothesis is that, since amylin deposition is found in blood vessels and the parenchyma of people with AD, it

may compromise the BBB and diffuse into the brain.[328] This may then affect brain microstructural integrity and result in WM damage. Secondly, amylin burden is shown to be co-localized with Aβ in cerebral plaques.[328] Analysis from the Framingham Heart Study revealed an inverse association between plasma amylin concentrations and brain volume.[334] These observations suggest that the neurodegeneration and neuropathology that underlie dementia subtypes may be accounted for, at least in part, by abnormal amylin function.

1.10.4 Advanced glycation end-products

Advanced glycation end-products (AGEs) are products resulting from nonenzymatic chemical reactions between reduced sugars and proteins.[311] AGEs naturally increase during “normal” ageing, but accumulate faster in a state of hyperglycaemia, and are highly expressed in the CNS of people with diabetes.[311–313] AGEs are thought to be involved in the pathogenesis of AD.[335] Aβ is modified by AGEs and AGE-modification of Aβ exacerbates its toxicity.[336] AGEs are present in both neurofibrillary tangles and senile plaques of patients with AD and the receptor for AGE (RAGE) appears to be involved in the transport of amyloid peptides across the BBB.[337] In addition, AD patients with T2D seem to have more severe AD pathology and higher AGE levels in the brain compared with those with AD alone.[313] AGEs can be measured in plasma (circulating AGEs) or estimated in tissue using a relatively simple non-invasive measurement of skin autofluorescence (SAF), a method based on the fluorescent properties of some AGEs,[338] with some suggestions that this is reflective of tissue AGEs.[339,340] SAF has recently been shown to be associated with lower grey matter volume.[341] There is some evidence that AGEs might be associated with cognitive impairment and decline. Higher levels of serum AGEs were cross-sectionally associated with mild cognitive impairment in diabetes patients,[342] and higher SAF has also been associated with a higher likelihood of cognitive impairment in community-dwelling subjects.[343] Another study also showed that higher urinary pentosidine, a biomarker of AGEs, was associated with a greater 9-year cognitive decline in older people independent of diabetes status.[344]

1.10.5 Cerebral perfusion

Impaired cerebral perfusion has also been proposed as a mechanism of cerebrovascular disease that plays a role in the relationship between T2D and brain health. Since “normal” ageing is associated with CBF reduction of about ~20% at age 60 as compared to age 20,[345,346] further hyperglycaemia-linked hypoperfusion may induce additional damage. A study of 166 nondemented individuals from the Alzheimer’s Disease Initiative showed that lower CBF measured by Arterial Spin Labelled (ASL) in regions such as the medial temporal, inferior temporal gyrus and inferior parietal lobe was associated with a faster decline in everyday functioning.[347] Interestingly, brain hypoperfusion was found to predict this poor functional outcome independent of other neuropathology (e.g., amyloidosis and CSF tau). Since normal ageing is associated with some changes in CBF, any hyperglycaemia-related hypoperfusion is likely to have an additional burden on brain health outcomes.

A recent systematic review and meta-analysis of 13 studies consisting of 407 individuals with T2D and 443 control participants reported important differences between those two populations. This included CBF changes in a number of regions including the cerebral lobes, the right supplementary motor area and decreased CBF in the bilateral middle occipital gyrus and left caudate nucleus.[348] Inconsistencies across studies were noted, with some reporting no significant CBF changes and others highlighting different affected regions. Although some inconsistencies across studies were reported, these may be attributed to methodological differences in the ASL techniques used, the diversity of the sample considered, the analytical approaches taken as well as publication bias. Despite the mechanisms not being fully understood, such studies suggest that cerebral hypoperfusion may be one of the mechanisms through which T2D may potentially affect cognitive health and increase dementia risk.

1.11 The impact of T2D on brain tissue

As discussed in section 1.7, neuroimaging methods allow the comprehensive imaging of the brain. In the last two decades, they have been utilised to study brain structure,

and change in the context of T2D. The two common pathways often explored using brain imaging are: 1) neurocognitive disorders (or dementia) of AD aetiology (i.e., severe neurodegeneration, including hippocampal atrophy and β amyloid burden and 2) neurocognitive disorders (or dementia) vascular aetiology reflecting cerebrovascular disease (i.e., WMHs, lacunes, abnormal diffusion).

1.11.1 Microvascular pathology

The relationship between diabetes (and its markers) and WM damage has been explored primarily via three different imaging approaches. DTI, T2-weighted and FLAIR imaging. As previously discussed, DTI gives insight into the microstructural integrity of NAWM tissue, whereas conventional MRI sequences capture global and regional WM volumes, as well as SVD-related measures such as WMHs. Macroscopically, several studies using conventional MRI sequences and double inversion recovery sequences have shown that people with diabetes display a higher burden of WMH than controls.[349,350] Longitudinal studies have shown that diabetes was associated with faster WMH accumulation.[351] Prediabetes has also been found to be associated with larger volumes of WMHs suggesting that even the early stages of impaired glucose metabolism can have a detrimental effect on the brain small vessels.[351] Participants with diabetes and a HbA_{1c} ≥7.0% showed associated increased WMH burdens compared to diabetic individuals with HbA_{1c} <7.0%. Similarly, participants with longer duration of diabetes (≥10 years) had a higher burden of lacunes compared to those with a diabetes duration <10 years.[352] This suggests that hyperglycaemia and cumulative exposure to glycaemia may carry more of a risk on vascular brain health than just being diagnosed with diabetes.

It is important to acknowledge that not all studies show the same findings. For example, history of diabetes (as assessed by self-report and medication history) was found to be negatively associated with WMHV in both in a sample of participants of individuals with T2D or in a group of individuals who recently experienced an ischemic stroke.[353,354] De Bresser and colleagues also failed to find a difference in global WMHV between those with T2D and healthy controls.[355] However, they found that taking a

more nuanced approach to WMH pathology and looking at the shape, number and location of these lesions revealed differences between the two groups, with a higher number of lesions more non-punctuate WMHs, and a difference in shape (eccentricity) of punctuate deep WMHs found in those with T2D. This highlights that the characteristics of the sample considered, and the sensitivity of the brain imaging measures can impact possibility of detecting an association.

Micro-analysis of WM tissue using DTI has revealed that with T2D is associated with a decrease in FA, suggestive of microstructural disorganisation in the association tracts and the forceps minor.[356] Associations have been reported between impaired glucose metabolism and reduced FA in short association fibres, the ILF, the thalamic radiations, and the corpus callosum.[349] Reduction of FA in the right parts of corpus callosum as well as the right and left SLF have also been found in people with prediabetes.[357] There is some evidence suggesting that memory and executive function impairments in patients with T2D correlate with changes in the integrity of WM fibres such as those in the inferior fronto-occipital fascicle (IFOF) and ILF.[358] However, other studies failed to observe an association between T2D and WM microstructural integrity: for example, one study reported no differences in FA and MD between healthy controls and individuals who have T2D but with no peripheral microvascular complications.[359] This has raised the idea that microstructural abnormality of WM fibres in T2D is closely tied to peripheral microvascular complications. Other potential explanations for inconsistencies may once again likely to be due to the differences in the methodology approached used (e.g., confounder adjustments and attrition bias).

Some studies have studied NAWM using NODDI measures (previously outlined in the MRI methods found in section 1.7). They found that, when compared with healthy controls, people with T2D showed reduced FA and NDI and increased MD and ODI.[360]

1.11.2 Global and volumetric analysis

There is a consensus that diabetes is associated with lower whole brain volume (WBV). with some studies further indicating regional tissue loss. For example, a

recently published meta-analysis of over 14,000 journal entries, showed that T2D was associated with smaller total and regional brain volumes as well as greater atrophy over time.[361]

Because of its role in AD, numerous researchers have investigated whether diabetes is associated with structural abnormalities in the hippocampus. Results have been mixed with some cross-sectional studies reporting that T2D is associated with lower hippocampal volumes.[362,363] There is also evidence that hyperglycaemia (HbA_{1c} ≥7.0%) in individuals with diabetes (and duration of diabetes) is associated with smaller WBV, smaller regional brain volumes including frontal, temporal, occipital and parietal lobes and deep GM compared to individuals with hyperglycaemia who did not have diabetes.[352] Other population-based studies have failed to find similar associations.[364,365]

Findings from longitudinal studies have also been mixed, with some studies showing that T2D is associated with greater whole brain atrophy and increased ventricular volume.[366–368] Other longitudinal studies failed to observe these associations.[369,370] This may be explained by the variations in the methods used to quantify brain atrophy (thickness-based vs. volume-based) and the tissue considered (e.g., total brain tissue and GM, WM, and ventricular volume). It is also possible that differences between studies reflect differences in control of confounding, since not all studies apply extensive control for confounding, and by definition unmeasured or residual confounding cannot be accounted for.

It is nonetheless important to acknowledge the possibility that associations between diabetes and brain/cognitive health could occur as a consequence of reverse causality. Namely, poorer brain and cognitive health may drive behaviours (e.g., poor lifestyle) or biological mechanisms (e.g., autonomic dysfunction) that may drive hyperglycaemia and diabetes.

1.11.3 Amyloidosis

Amyloidogenesis is a central feature of both AD and T2D. In the former, there is an abnormal aggregation of Aβ peptide in the brain whereas for the latter, amylin can be found in the pancreas. Both of these can cause respective cell death and contribute to the pathogenesis of the diseases. There is also some evidence of overlapping pathophysiology. Mechanistically, it has been proposed that IR may promote the amyloidogenic processing of APP and Aβ42 formation. In mouse models, diet-induced IR was found to promote brain amyloidosis.[371] In human studies, the findings have been mixed. Some studies have found that HOMA-IR has been associated with brain amyloidosis[372,373] but others have failed to find a similar association when considering glycaemia traits. For example, results from the Baltimore Longitudinal Study of Aging cohort revealed no association between glucose and insulin measures on brain amyloid both in autopsy samples and in vivo imaging. Criticism about the sensitivity of the tracer Pittsburgh Compound B (^{11}C-PiB) has also been raised with some suggestions that recently developed tracers such as Florbetapir (^{18}F) with a longer half-life, may provide more sensitive imaging measures of β-amyloid accumulation.[374] How these relationships may vary by sex remains relatively unexplored.

1.12 T2D, cognition and cognitive decline

Several studies have explored the relationship between T2D, hyperglycaemia and cognitive health. The results have once again been mixed with some cross-sectional and longitudinal studies showing poorer cognitive outcomes,[375,376] whilst others failing to observe similar associations.[365,377–379] For example, diabetes has been associated with faster declines in attention[380] and global cognition,[375,381] poorer memory performance, lower verbal fluency,[376] poorer executive function,[382] and poor performance in semantic memory.[383] Other longitudinal studies have however failed to observe an association between diabetes and poorer cognitive outcomes.[384,385]

The mixed results reported by both cross-sectional and longitudinal studies may have many explanations. Firstly, there is considerable variability in the methods

implemented both during the assessments of the exposure (i.e., T2D) and the outcome (i.e., cognition). Ascertainment of diabetes status is often heterogeneous across studies with some using diagnostic tests such as OGT, HbA_{1c}, random blood glucose or fasting glucose. Others assessed diabetes status via self-report or self-reported use of glucose lowering medications. Such different approaches may have resulted in bias, including misclassification and underdiagnosis of participants. Similarly, the cognitive tests used varied from study-to-study with some using the MMSE and other studies using tests that evaluated a single cognitive domain. Few studies employed a composite measure derived from a battery of cognitive tests, which would have helped reduce floor and ceiling effects, which is a problem with a test like the MMSE.

Secondly the characteristics of the samples included in these studies varied considerably. For example, some studies consisted of participants aged ≥60 years (and even ≥75 years) whereas others included study members of age ≥40 years. The variability of selection methods, sampling frame, and in some cases explicit exclusion of some individuals, creates a considerable potential for bias. Results may also be subject to differences/inconsistencies in the use of confounders such as education, lifestyle factors, comorbid medical and neurological conditions. This can often be because confounder data was not measured during data collection. Even when recorded, it does not rule out errors in measurement. In some cases, variables considered in the models may not have been confounders of the relationship, either being on the putative causal pathway between the exposure and outcome (i.e., overadjustment bias) or being causal consequences of both the exposure and outcome (i.e., collider bias). For longitudinal studies, the follow-up period between cognitive assessments varied between studies, ranging from one year to twenty years with several studies having relatively long follow-up periods (≥10 years).[376,386–389] There were also inconsistencies in the focus between these studies – some studies looked at overall cognitive decline with others looking at decline prior to dementia. Consequently, the inclusion of cognitive impairment or incident dementia cases together with the wide age ranges may introduce a range of problems such as reverse causation or collider bias. It is also possible that findings may vary from study-to-study

or cohort-to-cohort due to attrition bias. Attrition bias describes a selection bias driven by systematic differences between those who continue to take part in the study and those who are lost throughout the process. This can potentially introduce a problem where associations are observed due to participants, often with characteristics that may be important, dropping out (e.g., younger participants may have higher mobility or different priorities that affect their study participation).

1.13 What is still unknown and general aims

This introductory chapter has given a brief perspective on T2D and dementia, and discussed the mechanisms through which these two conditions may be related. An overview of the techniques used to study this complex relationship was given and the existing evidence linking T2D to brain and cognitive health outcomes was discussed.

The nature of the relationship through which glycaemia, a defining component of diabetes, is associated with brain health in the general population is still poorly understood. This introductory chapter highlights that the majority of studies in this space have focused on clinical samples with T2D. However, there is value in looking at similar associations in population-based studies as that they allow analyses to go beyond the clinical thresholds of diabetes to: 1) look more generally at the mechanisms that may underlie this relationship across the spectrum of glycaemia, 2) be more nuanced about the subtleties of this complex relationship (e.g., look at non-linearity and sex differences) and 3) further combine different scientific approaches (e.g., genetics and time-sensitive medication analysis) to gain a more comprehensive insight into this relationship.

The introductory chapter also makes it clear that sex may be an important modifying risk factor for both diabetes and dementia. Despite this, most studies that explore diabetes-brain associations do not consider sex as potential effect modifier and do not stratify their analyses accordingly. In line with the growing evidence of sex differences in the context of metabolic and neurological health, there is important value in testing for sex interactions and/or proceeding with stratified analyses.

1.14 Research questions and the structure of the thesis:

In an attempt to better understand these relationships, two flagship UK-based population-based studies (the NSHD and UK Biobank samples) were used to examine the following research questions:

Chapter 3: To investigate whether glycaemia at different points in life is associated with a range of later-life brain health measures capturing AD-related pathology, SVD and cognitive health, and examine how these relationships differ by sex.

Chapter 4: Following on from the findings from Chapter 3, I aimed to examine whether other glycaemic traits were also associated with brain volumes differently in males and females. I further considered multiple volumetric measures of brain health to see whether any differences reflected preferential tissue loss.

Chapter 5: Following on from the findings from Chapter 3 and Chapter 4, I aimed to examine whether systemic inflammation mediated the glycaemia-volumetric associations observed in females.

Chapter 6: Following on from the findings observed in NSHD, I aimed to examine whether glycaemia also shows sex-specific associations with brain volume in the bigger UK Biobank sample. I aimed to use the increased power of the sample to look for evidence of non-linearity.

Chapter 7: To strengthen causal inference in the observational findings reported in Chapter 5, I aimed to examine whether genetic risk scores for glycaemia support the sex-specific associations observed with brain health outcomes.

2. General methods

The thesis required analysis of large multimodal data from two flagship UK-based population-based samples; thus, a brief methods chapter is included to introduce the data collection process and characteristics of the two key samples considered. The samples are the NSHD birth cohort (and its neuroimaging sub-study Insight 46) and UK Biobank (and its neuroimaging sub-study).

2.1 National Health Survey Of Health and Development

For Chapter 3-5, the sample used was NSHD and Insight 46. The studies conducted with this data are approved by the National Research Ethics Service Committee London (REC reference 14/LO/1173) and all participants provided written informed.

NSHD also known as the MRC 1946 birth cohort study, is a longitudinal study of a sample (n = 5362) of single births that occurred in the first week of March 1946 in Great Britain.[390–392] It was initiated to address pressing health and social policies in the UK prior to the establishment of the National Health Service (NHS) in 1948. These were the declining national fertility rate and distribution and use of obstetric and midwifery services.[391]

By 2020, the participants of the birth cohort had undergone 25 waves of data collection: starting with assessments approximately every 2 years during childhood and around 5-10 years during adulthood. Early NSHD data collection was more frequent in early life due to rapid growth and development. The focus began on ante-natal health, post-natal care, survival and socio-economic information of the family. As participants aged, the study expanded to further include health assessments by school doctors and teacher-administrated psychological and cognitive tests (measuring cognitive ability and conduct and behavioural problems). Educational attainment was quantified as highest level of educational qualification by age 26 years.

Between the ages of 32-53, NSHD researchers collected comprehensive health information on participants including those relating to respiratory, cardiovascular, metabolic, musculoskeletal and mental health. In addition, data on anthropometric measures and lifestyle factors were ascertained. In midlife, female participants were asked questions on menopausal status, symptoms, treatments, and monthly HRT history. Blood samples were conducted to measure various metabolic markers (i.e., HbA_{1c}, cholesterol, HDL, triglyceride and extracted DNA).

When the participants were aged 60-64, a more extensive array of biomarkers was measured to assess broader aspects of metabolic, cardiovascular, and general health. Fasting biological samples collected information on thyroid function, insulin, cholesterol and, inflammatory markers, and more.

At age 69, the data collection of NSHD participants focused on capturing detailed information on morbidity, functional limitations, and the use of health and social services. It repeated and expanded upon previous health and functional assessments, collected comprehensive data on common health symptoms, and included the collection of a third blood sample to further investigate biological markers of ageing. This follow-up aimed to enhance the understanding of the lifetime determinants and consequences of health and functional changes in older age.

NSHD has suffered notable attrition as participants got older. The most prominent overall attrition in the sample happened during the early adult years when changes of names and addresses were common. This was also when 5 out of the 7 sweeps of data collection were conducted via postal questionnaires. By age 53, 8.7% of the sample didn't participate because of death at infancy, 8.6% due to emigration and 2.2% due to living abroad.[391]

At the age 60-64 assessment, of the original 5362 participants, 957 (17.8%) had died, 620 (11.6%) had previously withdrawn from the study, 448 (8.3%) had emigrated and were no longer in contact with the study and 395 (7.4%) had been untraceable for more than 5 years. So, 2942 people were eligible and contacted. Of these 2453

(83.4%) completed a postal questionnaire.[393] At age 69, a home visit was performed for detailed phenotyping. Higher participation was associated with higher levels of prior contact and lower levels of recent health issues. This visit as well as previous sweeps provided a substantial part of the data used in this thesis.

It is important to note that the general trend of the sample suggests sex differences in participation rates. Up to age 69, the follow ups in adulthood indicated that males were less likely to take part. Of the 957 people who no longer took part in the study because they were deceased, 658 of them were males (20% of the overall sample).

2.2 Insight 46

Insight 46 is a neuroscience and clinical sub-study of NSHD.[118] It is a longitudinal (two-time points, with the third wave currently underway) detailed assessment of ~500 study members focused on acquiring information on clinical, neuropsychological, imaging and blood/urine biomarkers. To avoid potential bias in selecting those at risk of cognitive decline, participants were selected based on maximising the life course data available. From those who previously attended assessments at age 60-64, who had previously shown willingness to attend a clinical visit in London, and with data available during childhood and adulthood, 500 participants were selected at random. The participants undertook a series of neuropsychological tests including the MMSE, Choice Reaction time (inc. switching and inhibition measures), The Face Name Memory Exam (FNAME-12) and The Digit Symbol Substitution Test (DSST).

The inclusion criteria are further discussed in the Insight 46 protocol paper.[118]

Cognition		Neuroimaging	
Cognitive test used	Ability assessed	Imaging technique	Marker yielded:
WASI Matrix Reasoning	Non-verbal reasoning	T1 and T2 imaging	Global, tissue-specific and regional brain volumes
WMS-R Logical Memory	Free recall	PET (via PET/MRI)	Amyloid load
'What was where?' task	Visuo-spatial working memory	'Diffusion-weighted MRI	Measures of microstructural integrity and normal appearing white matter
FNAME-12	Associative memory	Arterial spin labelling (ASL)	Cerebral blood flow
Choice Reaction time (inc. switching and inhibition measures)	Task switching and response inhibition	Functional near-infrared stereoscopy (fNIRS)	Measures of haemodynamic response
WAIS-R Digit Symbol Substitution; Irrelevant Distractor (WAIS-R)	Attention and psychomotor speed	Resting brain imaging	Functional cortical connectivity (e.g., default mode network)
Mini Mental State Examination (MMSE)	Screening tool assessing multiple cognitive domains		

Table 2.1: Overview of neuropsychological tests and brain imaging conducted as part of Insight 46.
More details on these assessments are discussed in the Insight 46 protocol (further discussed by Lane and colleagues).[118]

The first stage of recruitment for Insight 46 involved contacting NSHD participants who had not previously withdrawn, died, or remained untraced from the main study by age 69. These participants were then asked if they were willing to undergo a neuroimaging study (yes = 40%), and if so, travel if this clinic was in London (yes = 70%).

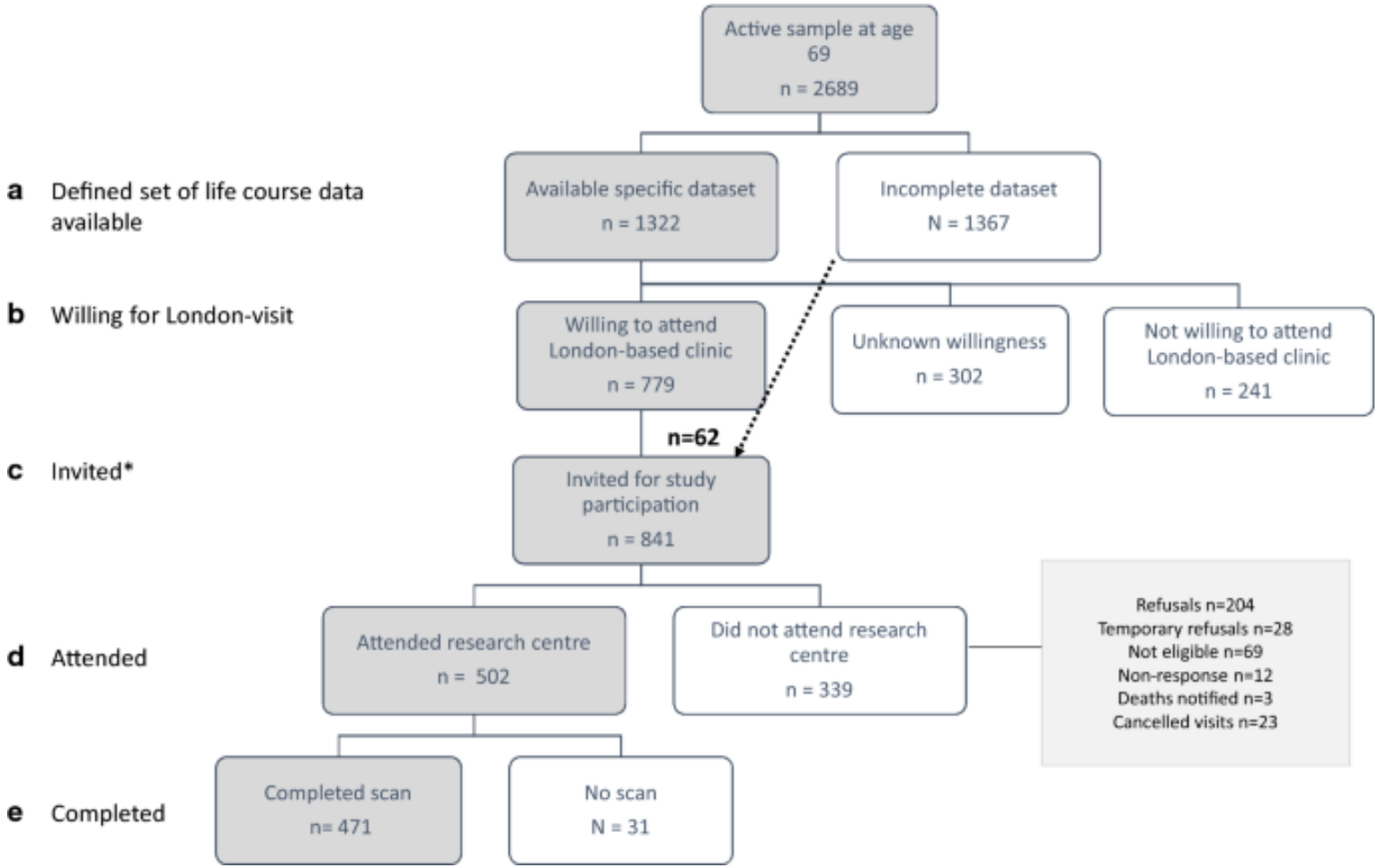


Figure 2.1: Overview of participant recruitment for phase 1 of Insight 46 (Source: published work by James and colleagues).[394]

2.3 Neuroimaging of Insight 46

The brain imaging data used in this thesis was derived and provided by the expert team at the Dementia Research Centre (DRC) at UCL, Queen's Square, Institute of Neurology. The DRC team provided me with data and advice of how to best use the key metrics for my analyses.

2.3.1 Imaging acquisition, preprocessing and quality control

In this thesis, the brain imaging markers from Insight 46 considered as outcomes in the analyses were: structural measures quantifying global and regional tissue volumes: (WBV, GM, WM and HV), SVD-related (WMHV), white matter-related (FA, MD, ODI, NDI) and a measure of amyloid burden (PET amyloid status).

WBV, GM, WM and HV, were derived following brain imaging using Biograph mMR 3T PET-MRI scanner (Siemens Healthcare, Erlangen), with simultaneous acquisition of dynamic PET and MRI data, including high resolution 3D (1·1 mm isotropic) T1-weighted and T2-weighted FLAIR scans. Following manual quality control, automated parcellation was performed using the Geodesic Information Flows (GIF) software[395] resulting in GM, WM and CSF tissue separation closely following the pipeline described by Eshagi and colleagues.[396] WBV was segmented using Multi-Atlas Propagation and Segmentation (MAPS) Similarity and Truth Estimation for Propagated Segmentations (STEPS) for hippocampal volumes.[397,398]

For WMHV, a validated, unsupervised, automated algorithm, Bayesian Model Selection (BaMoS)[136] was used to segment WMH jointly from 3D T1 and FLAIR images, followed by visual quality control. This generated a measure of WMHV including subcortical grey matter but excluding infratentorial regions.

For NAWM measures, the GIF software was used to automatically construct WM masks from the T1-weighted images.[395] Participant-specific masks were constructed by subtracting the BaMoS-WMH mask (see above) from the GIF-WM masks using NiftySeg (https://github.com/KCL-BMEIS/NiftySeg), before being eroded by 1 voxel. The full details including image correction and visual QC for the diffusion images are discussed.[148] Z-scores for each participants' diffusion map were generated (i.e., FA, MDI, NDI and ODI) through a comparison using a selected sample of 20 participants

with minimal evidence of WMHs (<1mL). Mean z-scores over the NAWM mask were then calculated for each diffusion metric. These standardised diffusion measures were then considered as outcomes in the regression analyses.

For amyloid status, a 3 Tesla PET MRI scanner was used for the simultaneous dynamic acquisition of PET-MRI (in addition to the T1-weighted and T2 weighted imaging). PET data was acquired following the injection of a radio tracer (^{18}F florbetapir) that acted as an imaging biomarker for AD. PET data was collected in list-mode pre- and post-injection to evaluate florbetapir uptake dynamics. The final assessment of amyloid burden was conducted over a 10-minute period, approximately 50 minutes post-injection. If participants were unable tolerate longer scan periods, the preceding 10-minute period was used instead. A Gaussian mixture modelling with two Gaussians using the 99th percentile of the lower distribution as the cut point (1.031, equivalent to 11.8 centiloids) was then used to determine Aβ positivity status.[399]

2.4 UK Biobank

2.4.1 Introduction to the sample

For Chapter 4 and Chapter 5, data from the UK Biobank was used. The UK Biobank received ethical approval from the North West Multicentre Research Ethics Committee and informed consent was obtained from all participants.

UK Biobank is a population-based, prospective cohort study of over 500,000 participants recruited between 2006-2010. The recruitment for the UK Biobank study recruited individuals aged between 40-69, registered to the NHS and living within 40 kilometres of one of the 22 assessment centres in England, Wales and Scotland. Overall, 9 million invitations were sent and 503,317 (5.45%) accepted the invitation to participate.[33,400] The sample consists of more females than males.

Participants recruited initially completed questionnaires, underwent computer-assisted interviews, were assessed on a range of physical measures and had blood and urine samples collected. Following initial baseline measurements, a subgroup of participants underwent (or are undergoing) further assessments including repeats of baseline assessments (n=20,000-25,000), diet questionnaires (n=210,000),

accelerometery (n=100,000) and multimodal imaging including a brain MRI (n=100,000).

Linkage to national datasets was made to acquire details on mortality, cancer incidence and hospital admission. With participant ages ranging between 40-69 years at baseline, the study provides a valuable open-access dataset to study risk factors for diseases in mid to late life.[401,402]

Although it is a very rich dataset with a large sample size, analysis of overall invitees versus participants indicates a healthy volunteer bias. The UK Biobank participants have been found to be different to the UK population across a number of sociodemographic, lifestyle and health-related measures.[33] Both males and females have been found to be less likely to smoke, drink and be obese. Linkage of their health records also indicates that that they a lower rate of all-cancer incidence (e.g., lung cancer), diabetes, chronic disease and respiratory disease compared to the general population of similar age.[33] For example, for diabetes, both males and females show lower self-reported health conditions at age 45-54 and 55-64 (see Table 2.2).

		UK Biobank	Health Survey for England 2008
Age 45-54	Males	4.5	8.1
	Females	2.4	3.5
Age 55-64	Males	7.8	10.5
	Females	6.3	8.0

Table 2.2: Self-reported diabetes prevalence (%) by age and sex in UK Biobank participants and Health Survey for England 2008.

UK Biobank is a large sample and participants have undergone genetic phenotyping which offers statistical power and also has (potentially) unbiased gene-outcome associations, despite its low response rate (5.5%) and the concerns over its non-representativeness. To mitigate biases, it is important to reinforce any findings from this sample with data from other sources, such as birth cohorts like NSHD, which provide detailed, longitudinal data from representative populations, enhancing the ability to identify causal relationships and control for confounding variables. This combined approach leverages the strengths of large-scale genetic data and detailed cohort studies, providing a comprehensive understanding that informs public health and policy more effectively.[403]

2.4.2 Neuroimaging of UK Biobank

Participants underwent brain imaging across four centres in Central, North, South-East and South-West England by a team responsible for training and monitoring quality assurance across all four centres, with all staff members having undergone extensive training by a MR physicist. Harmonisation of data across centres was assured by employing the same scanner models, software, adjustment and tuning techniques, coil types and protocols. In addition, a standardised training programme was provided for radiographers in each centre and standard operating procedures, alongside phantom measurements, servicing, and performance checks were conducted by a UK Biobank physicist. Qualitative and quantitative comparisons were performed by external imaging experts to confirm that images were of high quality and suitable for research Very briefly, the UK Biobank's brain MRI protocol uses a 3 Tesla Siemens Skyra scanner, taking approximately 35 minutes per session. It includes T1-weighted MRI for volumetric measures, T2 FLAIR for detecting inflammation or tissue damage, susceptibility-weighted MRI for iron content sensitivity, diffusion MRI for assessing WM integrity, and both resting and task fMRI for evaluating functional connectivity and brain responses to stimuli. Preprocessing involves converting images from DICOM to NIFTI format and correcting for artifacts. Postprocessing includes automated quality control, removing facial images for anonymity, and generating thousands of image-derived phenotypes for research, ensuring high-quality,

standardised data. This informed was derived from a paper published by Littlejohns and colleagues[404] where it is discussed in more details.

In line with this imaging protocol, the postprocessed measures (provided by the UK Biobank) used in this study included HV (cm^3) and total volume of WMHV (cm^3) both adjusted for total intracranial volume (TIV). WMH volume was log-transformed as it was positively skewed. Other measures included WBV, GM and WM (normalised for head size, cm^3). These same measures have been used in previous studies.[364,405]

2.4.3 Genetic measures

Blood samples were collected from participants during their initial assessment visit at UK Biobank centres. DNA was then extracted from these samples for genotyping. The genotyping and genetic analysis process in the UK Biobank involved several key steps to ensure accurate and comprehensive data. The two arrays used were Applied Biosciences UK BiLEVE Axiom Array (n=49,950) and the Applied Biosciences UK Biobank Axiom Array (n=438,427) capturing a wide range of genetic variation including SNPs and indels. DNA extracted from blood samples was automated to minimise bias. Genotype calling was conducted using a custom pipeline optimised for large-scale data and this was followed by rigorous QC to ensure data reliability (e.g., sample-based checks for high missing rates or extreme heterozygosity). Marker-based QC excluded unreliable markers, while sample-based QC flagged poor-quality samples. Principal Component Analysis (PCA) was performed to measure population structure revealing diverse ancestral backgrounds. Kinship analysis identified related individuals within the cohort (with 30% of participants found to be related to a third-degree or closer to another participant). Haplotype estimation and genotype imputation expanded the dataset to approximately 96 million variants, enhancing resolution. Finally, Human Leukocyte Antigen (HLA) alleles were imputed to study genetic associations with immune-related diseases. These steps collectively ensure high-quality genetic data, facilitating the discovery of new genetic associations and insights into complex traits.

2.4.4 Assessment of cognition

At first assessments, participants underwent a range of cognitive tests. These were administered via a computerised touchscreen interface.[406] The sample size for each of the 5 tests administered differed as some examinations were added part-way through the baseline assessment period, whilst others were removed due to time constraints. Following the original assessment, invitations were sent out by email to 103,514 participants to attend a repeat assessment visit between 2012-2013. 20,339 participants (20%) accepted the invitation to the repeat assessment.[406] Details about characteristics of individuals who accepted the invitation to the repeat assessment versus the overall cohort are provided on the UK Biobank website (https://www.ukBiobank.ac.uk/).

3. The relationship between glycaemia and markers of brain and cognitive health in a birth cohort

This chapter aims to examine whether there are associations between HbA_{1c} levels at different timepoints in adulthood (age 53, age 60-64 and age 69) and later life brain and cognitive health (at age ~70), using NSHD and its embedded neuroscience sub-study Insight 46 (introduced in Chapter 2.2 and 2.3). This chapter investigates whether: 1) poor glycaemic control and higher cumulative glycaemic exposure are associated with worse brain health in the National Survey of Health and Development birth cohort, 2) there is a sensitive period (e.g., midlife) where glycaemia is most damaging for later-life brain health and 3) glycaemia interacts with sex to affect brain health differently in males and females.

Some of the material in this chapter has been published in the Journal of Neurobiology of Ageing.[407] Further analyses have been conducted exclusively for the thesis using additional markers of brain and cognitive health. The aim of these additional analyses was to use more sensitive measures of cognition and microstructural integrity to further explore their potential relationships with HbA_{1c}.

3.1 Introduction

Past evidence has linked T2D with cerebral pathology.[408,409] This includes a higher burden of cerebral SVD such as lacunar infarcts and microbleeds, as well as more characteristic AD-related pathology affecting brain and hippocampal volumes.[362,363] There is also evidence (albeit inconsistent) that T2D is associated with poorer cognitive health (see Chapter 1.12). While growing evidence suggests that hyperglycaemia, a defining marker of T2D, may be damaging for the brain, the nature of this relationship across the population, i.e., the precise impact it has on the brain, whether there is a specific time window in which it affects the brain and whether any impact varies by sex remain poorly understood.[410] For example, identifying a "sensitive window" where poor glycaemic health, in a population sample, specifically carries more adverse effects on the brain is of research importance as it may guide time-sensitive preventative interventions. Some evidence has suggested that an earlier

exposure may reflect a greater cumulative burden and thus may pose a greater risk for later life brain health.[352,411,412] In addition, growing evidence suggesting sex differences in diabetes complications warrants further investigation in how these relationships manifest in males and females. Females with diabetes may show an increased risk of severe complications, when compared to males, as evidenced by their higher degree of cardiovascular, renal, and even hippocampal TOD.[187,413,414]

By considering various measures of brain (volumetric, diffusion and amyloid measures) and cognitive health (PACC and its subcomponents), this project aims to: examine whether: 1) higher HbA_{1c} and glycaemic burden (as indexed by A1 months) predict poorer brain and cognitive health, 2) there is a "sensitive window" for which this exposure (at 53, 60-64 or 69 years) may have its most adverse effects and 3) examine interactions between HbA_{1c} and sex on brain and cognitive health outcomes to see whether further investigation of differences between males and females is needed.

I hypothesised that higher HbA_{1c} across the different time-points (and greater A1 months) would be associated with more adverse brain health at age ~70, both when looking at markers of SVD (WMHs) and those more of AD pathology (hippocampal volume and amyloid burden). I also hypothesised that higher HbA_{1c} would be associated with poorer cognitive outcomes. I further hypothesised that the strength of these associations of HbA_{1c} with brain and cognitive outcomes would differ by sex.

3.2 Methods

3.2.1 Sample

The NSHD is a British birth cohort originally consisting of 5,362 males and females born in mainland Britain during the same week in 1946.[392] There have been 25 waves of data collections across childhood and adulthood with participants most recently assessed at age 68–69. Insight 46 is a neuroscience sub study of this cohort in which 502 participants underwent further assessments between May 2015 and January 2018. See Chapter 2.2 and 2.3 for additional information on the samples.

3.2.2 Investigations

Neuroimaging protocol

The neuroimaging protocol and postprocessed steps are described in detail in Chapter 2.4. The brain health measures considered in this analysis were structural brain imaging measures (WBV and HV), SVD-related measures including WMHV, microstructural integrity (FA, MD, ODI, NDI) and amyloid status.

Cognitive outcome measures

Cognitive function at age 69-71 was assessed using the Preclinical Alzheimer's Cognitive Composite (PACC). The PACC is a composite measure derived from the results of four cognitive assessments: total scores from the MMSE and FNAME-12, scores from the DSST and delayed recall scores from the Logical Memory test.[415] These have previously been described and used by Lu and colleagues.[416]

The MMSE is a 30-point composite device used to screen for cognitive impairment.[38] This test assesses a range of cognitive capacities including recall, language, registration and orientation, and typically lasts ~5-10 mins. A higher PACC score reflects a better performance.

The DSST is a neuropsychological test requiring participants to match symbols to numbers using a key.[417] It is considered a useful tool for assessing a range of cognitive capacities such as executive function, psychomotor speed, and visuo-perceptual functioning. It is scored based on the total number of items completed correctly within a period of 90 seconds.

The Logical Memory test assesses free recall of a short prose in which participants are asked to recall information following a delay of 20 minutes.[418]

The FNAME-12 is a cross-modal assessment of associative memory measuring the participant's ability to recall different unfamiliar pair associations (e.g., face-name and face-occupation).[419] Participants are first given time to learn different pairs and are then subsequently assessed numerous times for their ability to correctly match a face to the correct name and occupation. The total scores are based on the total number

of name and occupation pairs correctly recalled by the participant. This test and its reliance on associative memory, has been found to be especially sensitive to early stages of AD.[420]

Life course and clinical variables

HbA1c and Cumulative glycaemic exposure (or A1 months)

HbA_{1c} was measured in non-fasting blood samples at age 53, overnight fasting blood samples at age 60–64, and non-fasting blood samples at age 69-71 using high-performance liquid chromatography (HPLC) using a Tosoh A1c 2.2 analyser (Tosoh, Tokyo, Japan).

As a measure of cumulative glycaemic exposure, A1 months was calculated by multiplying the number of HbA_{1c} units above normal at each cycle by the number of months between the midpoints of the preceding and succeeding cycle intervals as per methods published by Orchard and colleagues.[421]

Type 2 diabetes status

T2D status was established based on usage of oral hypoglycaemic or injected insulin as measured in self-reported questionnaires at ages 36, 43, 53, 60-64 and 69-71 or a self-reported clinical diagnosis of diabetes during any home visit. Participant self-reported diabetes has been previously validated through a comparison with general practitioners' records.[422]

Confounders

Relevant confounders in these relationships were identified on the basis of a literature review exploring the associations between diabetes and brain outcomes and conceptualised through a directed cyclic graph (see Figure 3.1). Potential confounders were considered to be sex, childhood socio-economic position (SEP), adulthood SEP, childhood cognition, education, BMI, physical activity level and smoking status.

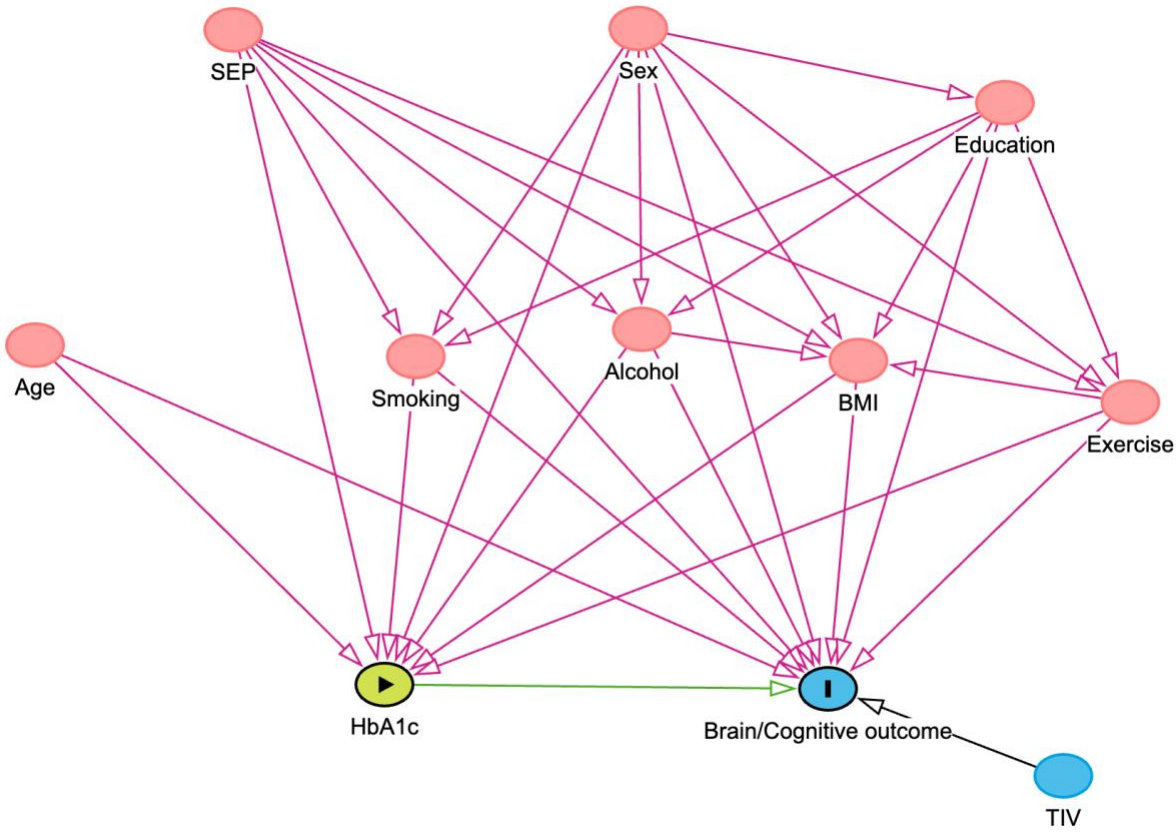


Figure 3.1: Directed acyclic graph constructed to model the relationship between HbA_{1c} (exposure) and brain/cognitive measures (outcomes).

These confounders were identified through an extensive literature search at the beginning of the thesis. The red circles in the directed acyclic graph (DAG) represent the confounder variables in these potential relationships with the red arrow pointing to their influence. Total intercranial volume (TIV) although not a confounder, was adjusted for to reduce variance in the outcome and to control for potential differences in the ratio of skull size to brain volumes between males and females. In addition, age at scanning was adjusted for in the models because although it is not a confounder, it is likely to be strongly associated with the relevant brain health outcomes.

Socioeconomic position

Childhood SEP was measured as father's occupational social class recorded at age 4-5 (or if missing, at age 11). Adult SEP was measured by the occupation of head of household at 53 years. These were coded according to the UK Registrar General's Standard Occupational Classification, then classified into 6 categories: unskilled, partly skilled, skilled manual, skilled nonmanual, intermediate, professional.

Educational attainment was represented as the highest educational or training qualification achieved by age 43, grouped into 5 categories: no qualification, below O-levels (vocational), O-levels and equivalents, A-levels and equivalents, higher education (degree and equivalents).

Smoking status

Smoking status was assessed through questionnaire-based self-report (available at ages 53, 60-64 and 69) and was classified into three groups: smokers, ex-smokers, and non-smokers at each age.

Physical activity

Physical activity level (available at ages 53, 60-64 and 69) was ascertained by self-report and classified into any physical activity (participants exercised at least once a week) or no physical activity (no or negligible physical activity in a week).

Body mass index

BMI was calculated as weight(kg)/height(m^2) using height and weight measurements collected by trained nurses during assessment at each time point to a standard protocol.

APOE status and APOE genotyping

APOE status classification was based on the two single SNPs rs439358 and rs7412. Individuals were subsequently categorised as *APOE*-ε4 carriers or non-carriers. Genotyping was carried out by KBioscience (www.lgcgenomics.com) on DNA extracted at the 60–64-year visit and was repeated for all participants with a sample collected at the Insight 46 visit to minimise missing data points, resulting in 500 individuals with known APOE status in Insight 46.

Childhood cognition

Childhood cognitive function was derived from four tests of verbal and non-verbal ability administered to the participants (at ages 8, 11 and 15) using tests created by the National Foundation for Educational Research.[423–425] These are shown in Table 3.1. At each age the four scores ascertained were standardised to a mean of 0 and a standard deviation (SD) of 1. These new scores were then re-summed and re-standardised to create a global measure of intelligence at each age. A summary variable of childhood cognition was derived using the average scores of the three ages (8, 11 and 15). If the data was only available for two ages, the summary variable was derived using this average.

Age	**Tests**			
Age 8	Word reading	Reading comprehension	Vocabulary	Picture intelligence test
Age 11	Word reading	Arithmetic assessment	Vocabulary	Alice Heim Group ability (80 items)
Age 15	A 47-item mathematics test	Reading Comprehension	The Watts-Vernon reading test.	Alice Heim Group ability (130 items)

Table 3.1: The different verbal and non-verbal assessments undertaken by the National Survey of Health and Development cohort at different ages during childhood to adolescence.

3.2.3 Statistical analysis

Statistical analyses were conducted in Stata version 15.1 and Stata version 17. For continuous variables that were normally distributed, means and SD were reported. For skewed data, the median and the range were reported. For categorical variables, frequency and percentages were reported.

A comparison of HbA_{1c} levels between the NSHD sample with available data at each time point and Insight 46 participants considered in this study for both males and females was made using t tests.

The associations between each HbA_{1c} measure (and A1 months) with brain imaging and cognitive outcomes were examined through a series of GLM models.

For each association a minimally confounder-adjusted model (Model 1) was first constructed by adjusting for sex and age at scan. For WBV, WMHV, HV and measures of NAWM (FA, MD, ODI and NDI), this simple model additionally adjusted for TIV. Model 2 further adjusted for social factors related to cognition including childhood cognition, childhood SEP, adulthood SEP and education levels. Model 3 was the fully confounder-adjusted model and further included lifestyle factors (BMI, physical exercise and smoking status as measured at each time point respectively).

Prior to running Model 1, interaction terms between each HbA_{1c} indicator and sex were examined. Models were subsequently sex-stratified if this term was statistically significant (likelihood ratio test, $p<0.05$). When the outcome was amyloid status, interaction terms between HbA_{1c} and APOE e4 were also examined. If any of the interaction terms were significant, the sample was stratified accordingly. Owing to its skewed distribution, WMHV was log-transformed prior to analysis as commonly performed.[426,427] One participant was excluded from the analysis for having a HbA_{1c} value of more than 3SD from the mean suggestive of a potential error in measurement.

To account for the effect of diabetic medication (including insulin) on blood glucose levels, a value 11 mmol/mol (or 1%) was added to the HbA_{1c} value of any participants receiving hypoglycaemic medication at a given time point. The 1% addition is consistent with a previous clinical review that reported the effectiveness of antidiabetic

drugs to range from 0.9-1%.[428] Moreover, the addition of 11mmol/mol, approximately 1SD reflects the approach used for blood pressure, where again a rough 1SD is added to the measured HbA_{1c}, reflecting longstanding exposure to elevated HbA_{1c}.

Multiple imputation for missing confounder and exposure data was performed using the Multivariate Imputation by Chained Equations (MICE) method by fully conditional specification (50 imputed datasets) under the assumption of missing at random (MAR). MICE results are presented below but were also checked for concordance with the complete case data. No auxiliary variables were included as the variables considered in the model fulfilled the MAR assumption.

A sensitivity analysis also was performed using measured HbA_{1c} whereby the corrections imposed for diabetes medication were omitted. As further sensitivity analyses, I re-ran key results using a complete case approach, excluding people with diabetes, and those with neurological conditions in separate analyses.

For all analyses, the conventional level of 5% was used to represent statistical significance.

The plots presented in the results section were generated using the avplot function from Stata. The avplot command used after regression analyses produces added-variables plots also known as partial-regression leverage plot, partial regression plot, or adjusted partial residual plot. It plots the residuals against the values of the predictor variable of interest (i.e., HbA_{1c} or A1 months) while holding confounders constant. This helps assess the influence of individual data points on the regression model and aids with the identification of outliers or points that may have a disproportionate impact on the regression coefficients and predictions. The E(Y) reported on the y-axis represents the predicted values of the response variable, while E(X|Xn) on the x-axis represents the conditional expected values of the predictor variable of interest.

3.3 Results

Following multiple imputation, the final analysis consisted of data for 454 participants. All 454 participants had available data for WBV, HV and WMHV and 446 (91%) had usable data for amyloid analysis (see Figure 3.2). Participant demographic and clinical characteristics for the complete case data are presented in Table 3.4.

Participants considered had a mean age of 70 years (SD = 0.7) and were more likely to be male (52%). 86 participants (~20%) met the threshold to be considered amyloid positive. HbA_{1c} levels were mostly similar across each sweep albeit suggestive of a small rising trend (see Table 3.4).

HbA_{1c} levels in the current sample (participants in Insight 46 with a HbA_{1c} value available) were compared to all participants in NSHD with HbA_{1c} results at each time point to see if glycaemic health varied between samples. The results showed differences in HbA_{1c} levels between NSHD and Insight 46 participants, whereby those who took part in the sub-sample had slightly lower mean HbA_{1c} (Table 3.2), which was exacerbated in males, suggesting a healthier bias towards males with lower HbA1c being part of the sub-sample.

	Variable	n	NSHD	n	Insight 46	p-value
Males	Age 53	1293	38.4 (7.2)	214	36.7 (4.7)	0.0002
Females	Age 53	1289	38.3 (7.7)	198	37.3 (4.7)	0.05
Males	Age 63	989	40.3 (7.1)	223	38.9 (5.1)	0.001
Females	Age 63	1056	40.5 (8.3)	216	39.9 (6.9)	0.3
Males	Age 69	923	40.1 (7.9)	213	38.6 (6.3)	0.001
Females	Age 69	994	40.1(7.1)	208	39.3 (6.9)	0.08

Table 3.2: Comparison of HbA_{1c} levels between the National Survey of Health and Development sample with available data at each time point and Insight 46 participants considered in this study.
The comparison is made for both males and females and p-values from t tests are presented.

Variable	Missing
Exposures	
HbA_{1c} at age 53	42
HbA_{1c} at 60-64	15
HbA_{1c} at 69	33
Confounders	
Childhood socioeconomic position	3
Adulthood socioeconomic position	0
Childhood cognition	0
Body mass index at age 53	0
Body mass index at age 60-64	0
Body mass index at age 69	0
Smoking status at age 69	8
APOE status	55
Outcomes	
Age at scanning	0
Whole brain volumes	0
Hippocampal volumes	0
White matter hyperintensity volumes	0
Amyloid status	8
Preclinical Alzheimer Cognitive Composite z-score	0
Logical Memory Delayed mean,	0
Digit symbol substitution test	0
Mini-mental state examination	0
Face Name Memory Exam -12	0
Fractional anisotropy	57
Medial diffusivity	57
Orientation dispersion index	70
Neurite Density Index	57

Table 3.3: Missingness numbers for the exposure, confounder and outcome data in the sample considered. To be considered, participants had to be part of Insight 46 and had to have volumetric imaging data available (n = 454).
Multiple imputation was only conducted for exposure and confounder data. APOE status was grouped in the confounder section but was only considered as a variable in relation to its role in interaction analyses with amyloid status.

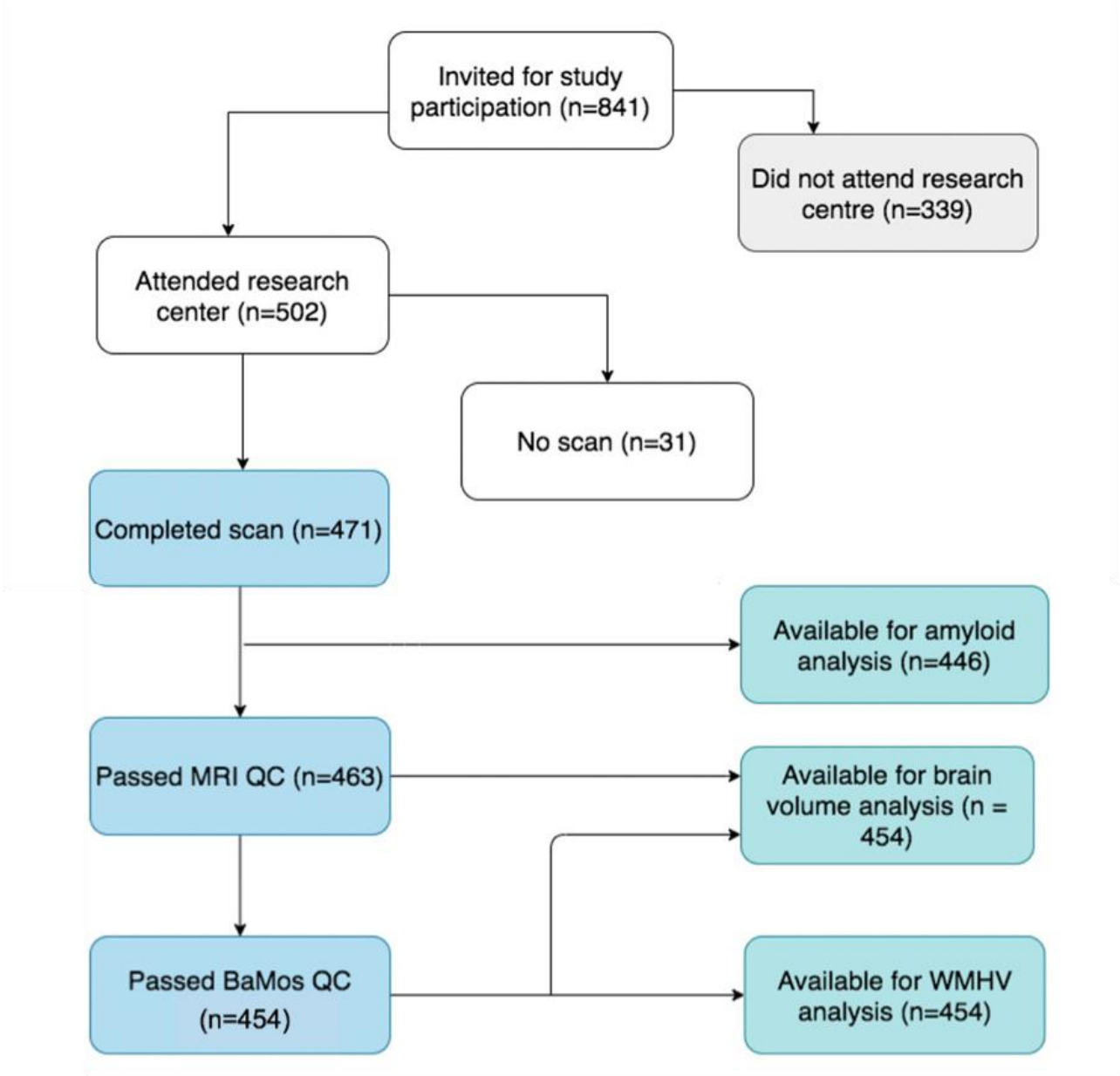


Figure 3.2: Flowchart providing an overview of Insight 46 recruitment and imaging of National Survey of Health and Development participants who were part of Insight 46. To be considered in the study, participants had to have been part of Insight 46 and have volumetric imaging data available, which amounted to 454 participants.
BaMoS, Bayesian Model Selection; MRI, magnetic resonance imaging; NSHD, National Survey of Health and Development; PET, positron emission tomography; QC, quality control; WMHV, white matter hyperintensity volume.

Participant characteristics		n	Pooled	n	Males	n	Females
Age at scanning in years		454	70·7 (0·7)	233	70·7 (0·7)	221	70·7 (0·7)
HbA_{1c}, mmol/mol	At 53 years	412	37.1 (4.7)	214	36.7 (4.5)	198	37.3 (4.7)
	At 60–64 years	439	37.4 (5.6)	223	38.9 (5.1)	216	39.9 (6)
	At 69 years	421	39 (6.6)	213	38.7 (6.4)	208	39.3 (6.9)
A1 months		400	206.4 (483)	206	197.9 (474.8)	194	215.8 (493.1)
Self-reported diagnosis of diabetes	At 53 years	454	2 (0.4%)	233	1 (0.2%)	221	1 (0.2%)
	At 60–64 years	454	20 (5%)	233	10 (4%)	221	10 (5%)
	At 69 years	454	28 (6%)	233	14 (6%)	221	14 (6%)
Diabetes medication use	At 53 years	448	2 (0.04%)	239	1 (0.5%)	229	1 (0.4%)
	At 60–64 years	448	16 (4%)	239	10 (2%)	229	6 (1%)
	At 69 years	444	27 (6%)	227	14 (3%)	217	13 (3%)
Smoking status at 70	Current Smokers	446	9 (2%)	231	4 (1.7%)	215	5 (2%)
	Ex-smokers		207 (46%)		120 (54%)		86 (40%)
	Never smoked		230 (52%)		107 (45.3%)		124 (58%)
Body-mass index, kg/m^2	At 53 years	454	27.3 (4.4)	233	27.1 (3.4)	221	26.7 (4.7)
	At 60–64 years	454	27.9 (4.5)	233	27.8 (3.5)	221	27.3 (4.7)
	At 69 years	454	27.9 (4.8)	233	27.7 (3.6)	221	27.2 (5.1)
Adult socioeconomic position	Non-manual	454	120 (26%)	233	28 (12%)	221	92 (
	Manual		334 (74%)		205 (88%)		129 (58%)
Childhood socioeconomic position	Non-manual	451	175 (39%)	234	87 (37%)	217	88 (40%)
	Manual		276 (61%)		147 (63%)		129 (60%)
APOE ε4 carrier (1 or 2 alleles)		399	116 (29%)	209	64 (30%)	190	50 (26%)
Childhood cognition		454	0.4 (0.8)	233	0.4 (0.8)	221	0.4 (0.8)
Amyloid positive status		446	86 (20%)	227	47 (54%)	219	39 (46%)
Whole brain volume (WBV), mL		454	1101·4 (99.5)	233	1152.4 (87.0)	221	1047.3 (82.2)

Hippocampal volume (HV), mL	454	6·1 (0·6)	233	6.5 (0·6)	221	6·0 (0·7)
White matter hyperintensity volume (WMHV), mL	454	1.15 (1)	233	4.7 (5.1)	221	5.5 (5.7)
Total intracranial volume (TIV), mL	454	1433·9 (133·4)	233	1519.8 (106·8)	221	1343·1 (92.6)
Preclinical Alzheimer Cognitive Composite z score (PACC)	454	-0.3 (0·7)	223	-0.18 (0·7)	221	0.14 (0·7)
Logical Memory Delayed mean, correct answers	454	11.5 (3.7)	223	10.7 (3.7)	221	12.3 (3.5)
Digital Symbol Substitution Test (DSST), correct answers	454	47.6 (10.4)	223	46.1 (10.4)	221	49.3 (10.1)
Mini Mental State Examination (MMSE), total score	454	29.3 (1)	223	29.2 (1)	221	29.3 (1)
12-item Face-Name test (FNAME-12) total score	454	65.2 (18.36)	223	60.9 (18.1)	221	69.7 (17.5)
Fractional anisotropy (FA)	397	-0.003 (0.2)	204	0.04 (0.2)	193	-0.05
Medial diffusivity (MD)	397	0.1 (0.3)	204	0.01 (0.3)	193	0.2 (0.3)
Orientation dispersion index (ODI)	384	-0.1 (0.1)	197	-0.2 (0.1)	187	-0.1 (0.1)
Neurite Density Index (NDI)	397	-0.2 (0.5)	204	-0.1 (0.5)	193	-0.2 (0.5)

Table 3.4: Sample characteristics for the participants considered in these analyses (n = 454).
Values presented are pre-imputation data: n (%), mean (SD) or median (IQR). % are calculated against the max data available for that specific measure for the pooled sample. As described above, the participants were considered if they were part of Insight 46 and have volumetric imaging data available which amounted to a max number of 454 participants of which 233 were males and 221 were females. SD: Standard deviation. IQR: Interquartile range.

Associations between HbA_{1c} (and A1 months) and whole brain volumes

Interaction analysis revealed an interaction between HbA_{1c} levels at all three ages and sex on WBV. All interaction terms ($p < 0.001$) suggested that the relationships between HbA_{1c} and WBV vary by sex. The results were subsequently stratified.

Higher HbA_{1c} at each time point was associated with lower mean WBV at age 69-71 in females (see Figures 3.3, 3.4, 3.5 and Table 3.5). In males, the associations suggested that higher HbA_{1c} was associated with high mean WBV. These associations were negligibly affected by adjustment for potential confounders and the magnitude of the associations was similar at the three time-points (see Table 3.5). Analysis of the associations between total A1 months and WBV showed essentially similar findings with a consistent negative association between cumulative HbA_{1c} in females, but a differential pattern in males when there is evidence of a positive but mainly non-significant association in males (see Table 3.5).

The exclusion of two males who appeared to be possible outliers also had negligible effects on the findings.

Associations between HbA_{1c} (and A1 months) and PET amyloid

As previously reported and as a positive check, APOE-amyloid associations were tested using logistic regression: APOE-e4 was found to be associated with a 4.6 OR (CI: 2-2-9.6, $p <0.001$) for being amyloid positive.

There were no significant associations between HbA_{1c} at any time-point investigated, or A1 months, on amyloid status at age 69-71 with or without adjustment for potential confounders (see Table 3.6). There was also no evidence of an interaction with either sex (p interaction for all >0.7) or APOE (p interaction for all > 0.5).

Associations between HbA_{1c} (and A1 months) and white matter hyperintensity volumes

There were no significant associations between HbA_{1c} or A1 months at any time-point investigated and WMHV at age 69-71 (see Figure 3.8). No associations were observed with or without associations for potential confounders (see Table 3.6). There was also no evidence of an interaction with sex (p interaction for all >0.7).

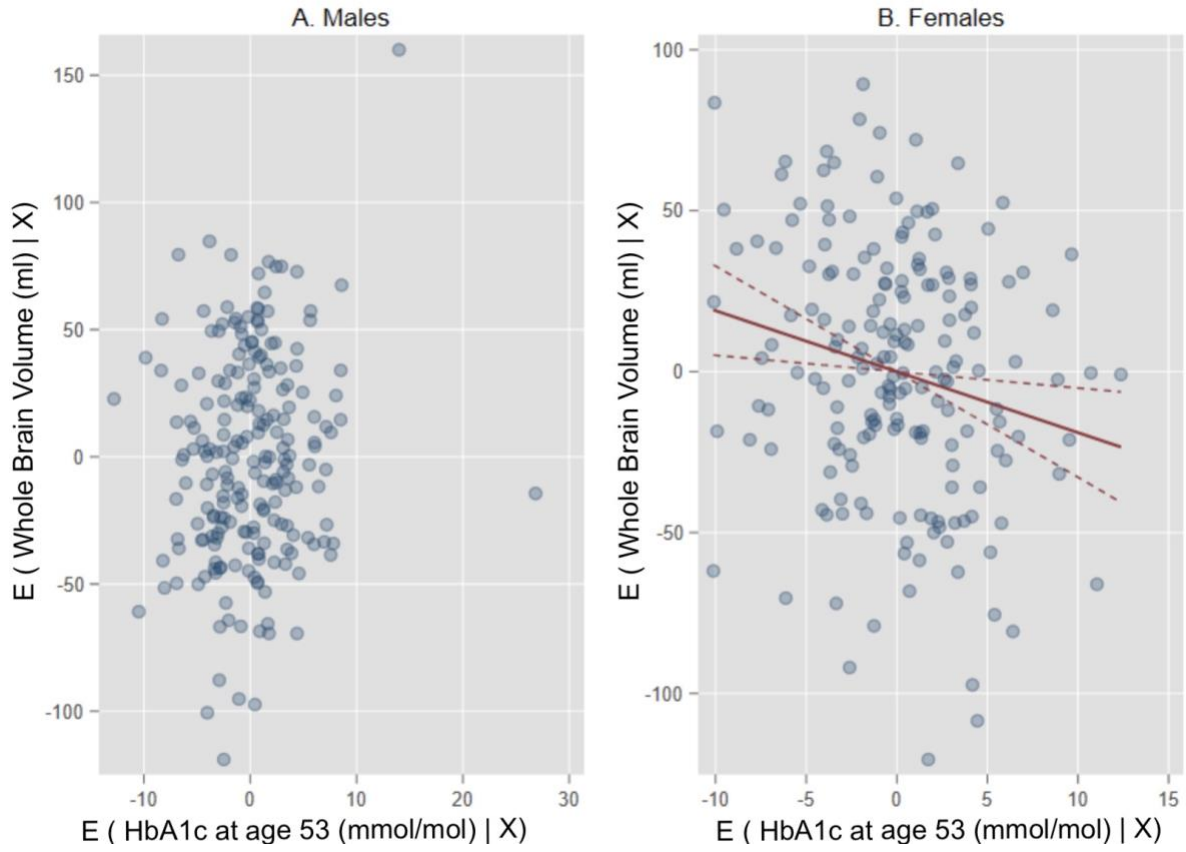


Figure 3.3: Partial regression plots showing associations of HbA_{1c} at age 53 with whole brain volumes (stratified by sex).
Plot A represents males and Plot B represents females. The regression models presented are for the fully confounder-adjusted models (adjusted for total intercranial volume, age at scan, childhood cognition, socio-economic position, body mass index, physical exercise, and smoking status). E(Y) on the y-axis represents the predicted values of the response variable, while E(X|Xn) on the x-axis represents the conditional expected values of the predictor variable of interest.

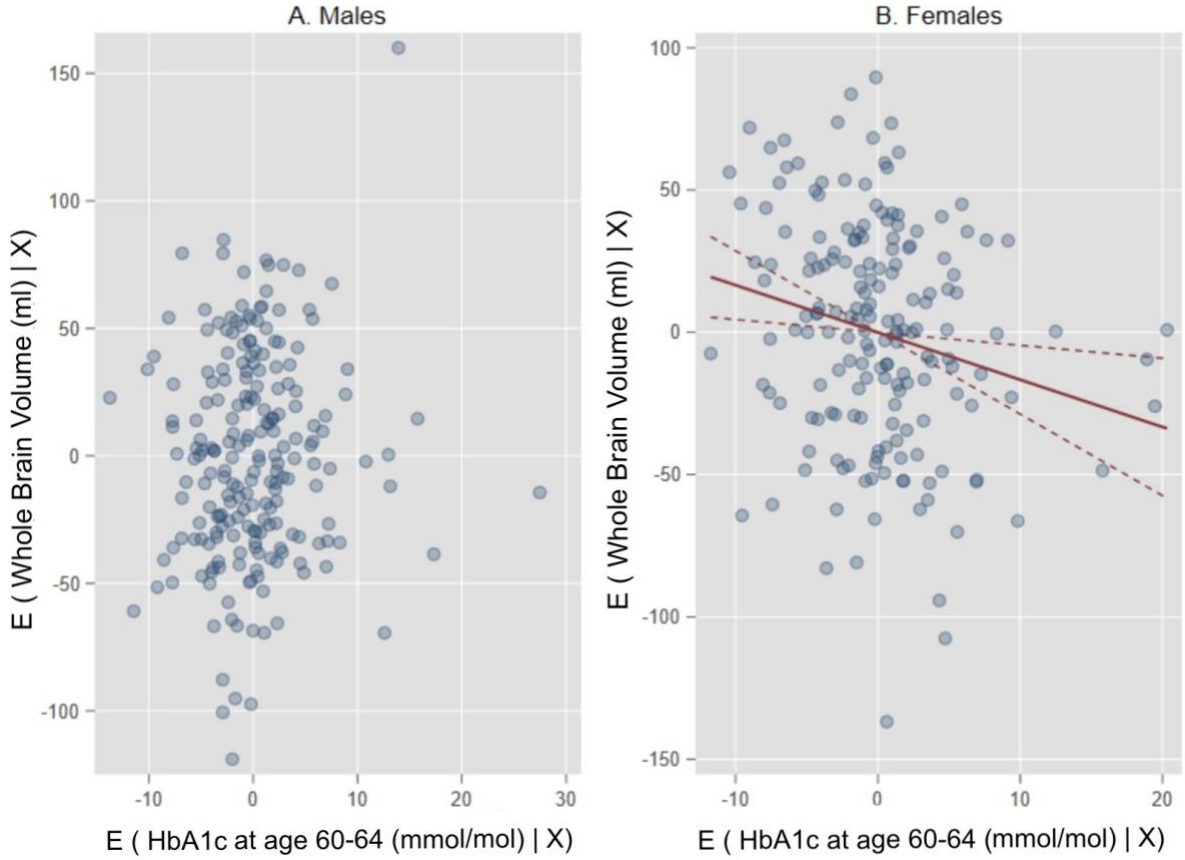


Figure 3.4: Partial regression plots showing associations of HbA_{1c} at age 60-64 years with whole brain volumes (stratified by sex).
Plot A represents males and Plot B represents females. The regression models presented are for the fully confounder-adjusted models (adjusted for total intercranial volume, age at scan, childhood cognition, socio-economic position, body mass index, physical exercise, and smoking status). As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

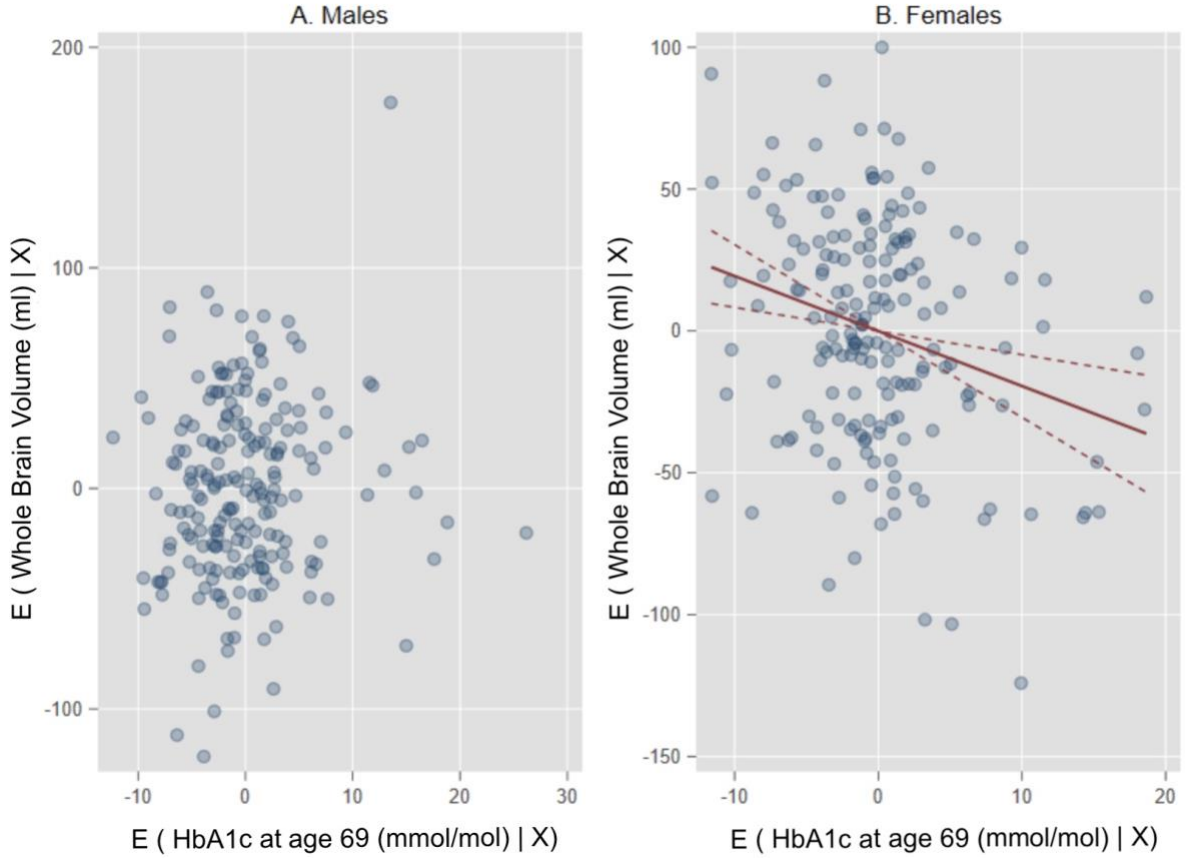


Figure 3.5: Partial regression plots showing associations of HbA_{1c} at age 69 years with whole brain volumes (stratified by sex).
Plot A represents males and Plot B represents females. The regression models presented are for the fully confounder-adjusted models (adjusted for total intercranial volume, age at scan, childhood cognition, socio-economic position, body mass index, physical exercise, and smoking status). As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

		Whole brain volumes (WBV)							
		Males				Females			
		β	95% CI		p	β	95% CI		p
Age 53	M1	0.82	-0.48	2.12	0.2	-1.58	-2.88	-0.28	0.02
	M2	0.88	-0.452	2.21	0.2	-1.67	-3.08	-0.25	0.02
	M3	0.92	-0.46	2.31	0.2	-1.61	-3.04	-0.18	0.03
Age 60-64	M1	0.41	-0.72	1.54	0.5	-1.38	-2.53	-0.23	0.02
	M2	0.4	-0.75	1.55	0.5	-1.34	-2.61	-0.16	0.03
	M3	0.49	-0.65	1.63	0.4	-1.4	-2.62	-0.17	0.03
Age 69	M1	0.45	-0.6	1.51	0.4	-1.55	-2.58	-0.53	p < 0.001
	M2	0.49	-0.58	1.56	0.4	-1.57	-2.66	-0.49	0.01
	M3	0.72	-0.37	1.81	0.2	-1.66	-2.78	-0.54	p < 0.001
A1 months	M1	0.004	-0.008	0.02	0.5	-0.01	-0.020	-0.001	0.03
	M2	0.004	-0.008	0.02	0.5	-0.01	-0.022	0.000	0.05
	M3	0.005	-0.006	0.02	0.4	-0.01	-0.023	-0.001	0.04

Table 3.5: Regression analyses output of associations between HbA_{1c} (mmol/mol) at each time point and cumulative glycaemic exposure (A1 months) on cognitive and whole brain volumes stratified by sex.
The values presented are the standardised coefficients (β and CI) and p values. The models were as follows: Model 1: minimally confounder-adjusted model for total intercranial volume and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education, and childhood cognition. Model 3: Model 2 + further adjustments for body mass index, physical activity, and smoking status.

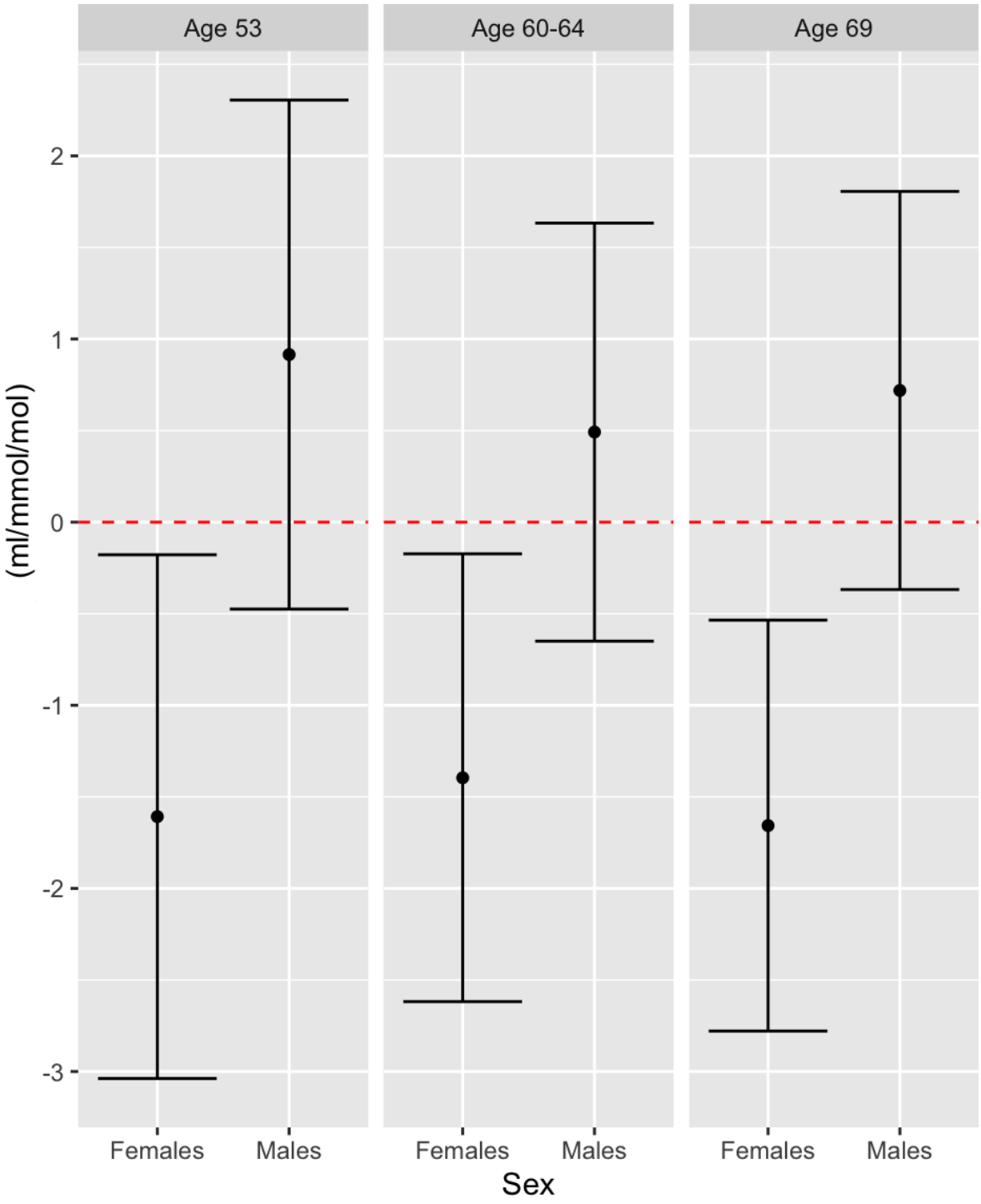


Figure 3.6: Forest plots representing the associations between HbA_{1c} levels across all ages and whole brain volume at age 69-71 stratified by sex for fully adjusted models.
The estimates presented are for the fully confounder-adjusted models (adjusted for total intercranial volume, age at scan, childhood cognition, socio-economic positions, body mass index, physical exercise, and smoking status).

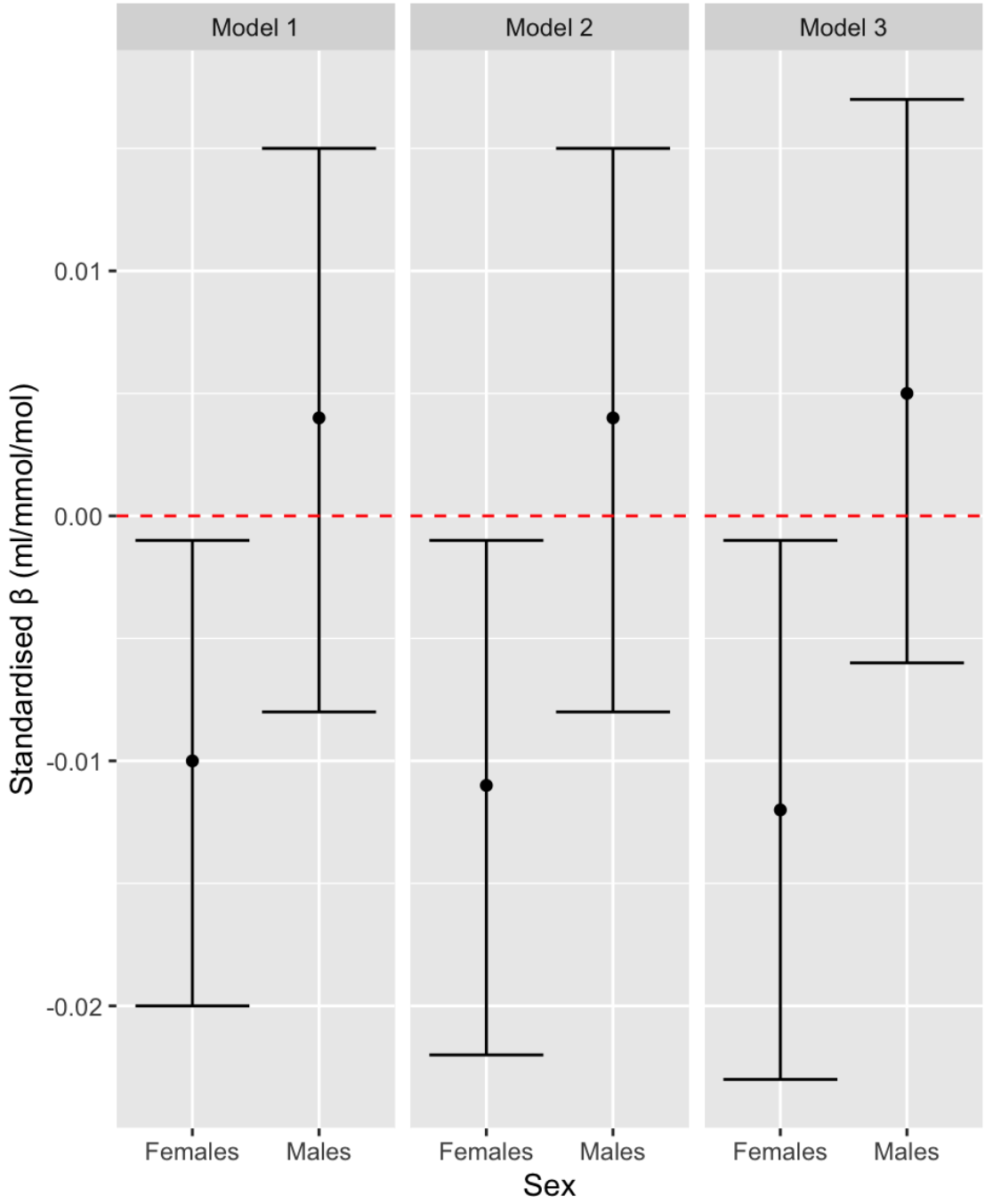


Figure 3.7: Forest plots representing the associations between A1 months (cumulative glycaemic burden) and whole brain volume at 69–71 years of age for all three models.
Model 1: minimally adjusted model for total intercranial volume and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education, and childhood cognition. Model 3: Model 2 + further adjustments for body mass index, physical activity, and smoking status. The standardised coefficients are presented here. Standardised coefficients are presented here to facilitate comparison.

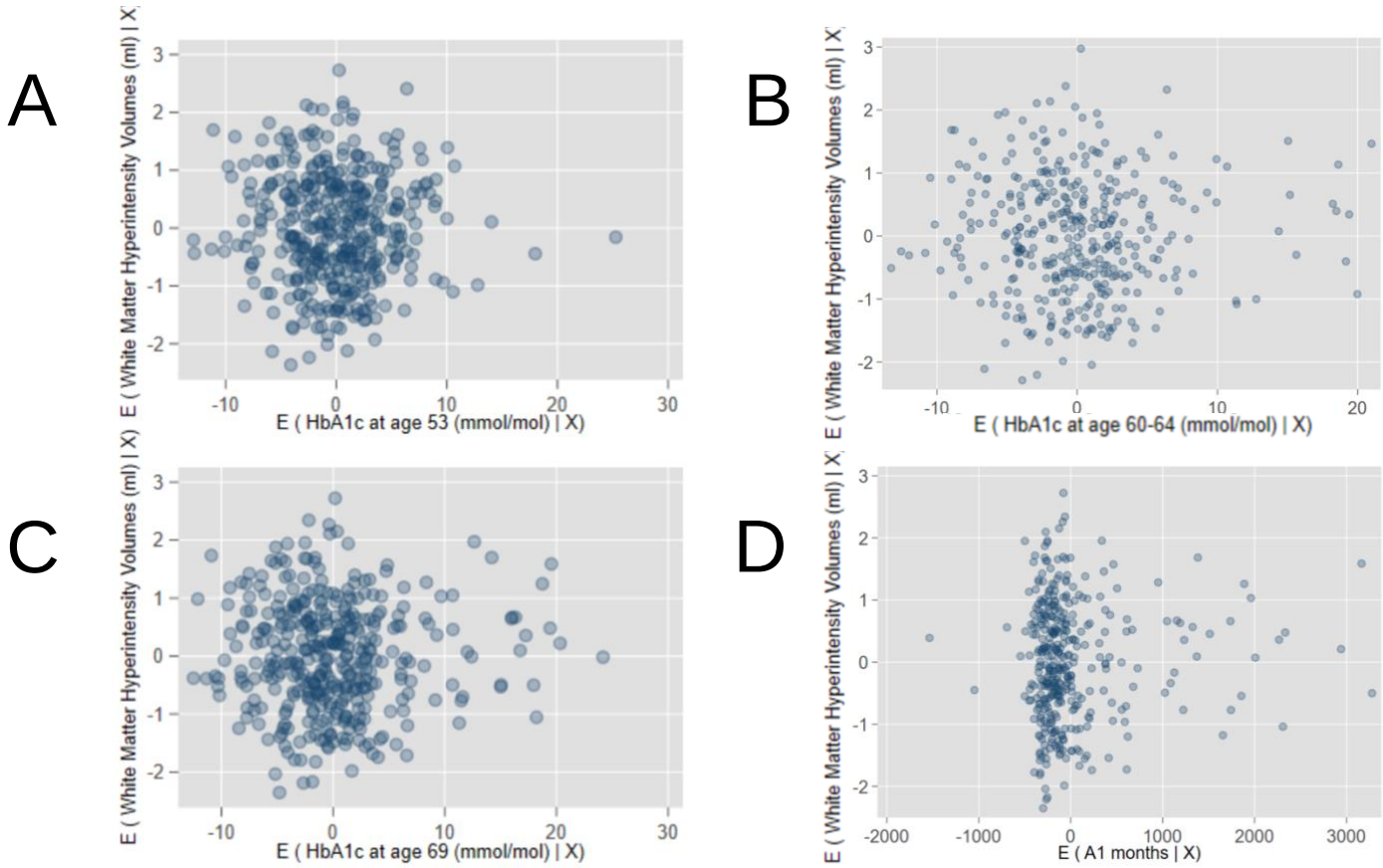


Figure 3.8: Partial regression plots showing associations between expected E(HbA_{1c}) at all three time points (and E (A1 months)) with expected E(white matter hyperintensities volume). A) HbA_{1c} mmol/mol at age 53. B) HbA_{1c} mmol/mol at age 60-64 C) HbA_{1c} mmol/mol at age 69. D) A1 months

The regression models presented are for the fully adjusted model (adjusted for total intercranial volume, age at scan, sex, childhood cognition, child, and adulthood socio-economic position; and BMI, physical exercise, and smoking status at the time of the exposure). White matter hyperintensities volumes was log-transformed. As per the plots for whole brain volumes, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

Associations between HbA_{1c} (and A1 months) and hippocampal volumes

There were no convvincing associations between HbA_{1c} at any time-point (or A1 months) and HV at age 69-71 with or without adjustment for potential confounders (see Table 3.5 and Figure 3.9). There was no evidence of a sex interaction (all interaction P values>0.1). The associations remained essentially unchanged with confounder adjustment.

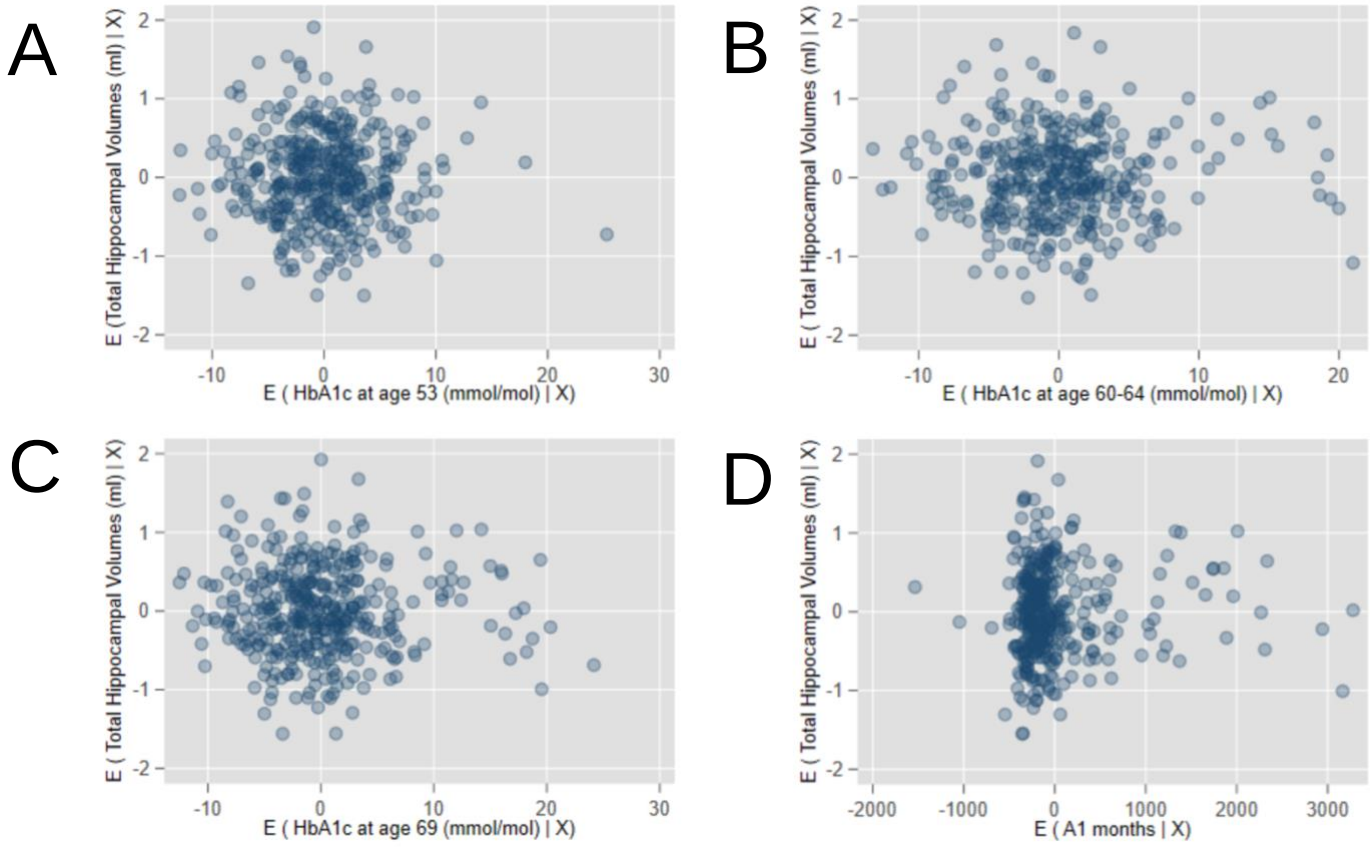


Figure 3.9: Partial regression plots showing associations between expected E(HbA_{1c}) at all three time points (and E (A1 months)) with expected E(hippocampal volumes). A) HbA_{1c} mmol/mol at age 53. B) HbA_{1c} mmol/mol at age 60-64 C) HbA_{1c} mmol/mol at age 69. D) A1 months
The regression models presented are for the fully confounder-adjusted model (adjusted for total intercranial volume, age at scan, sex, childhood cognition, child, and adulthood socio-economic position; and BMI, physical exercise, and smoking status at the time of the exposure). White matter hyperintensities volumes was log-transformed. As per the plots for whole brain volumes, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

Associations between HbA_{1c} (and A1 months) and cognitive outcomes

There were no associations between HbA_{1c} at any time-point investigated or A1 months and the PACC scores at age 69-71 (see Table 3.6 and Figure 3.10). There was no evidence of a sex interaction (all interaction P values>0.8). The associations remained unchanged with confounder adjustments (Table 3.6). Further analyses were conducted to see whether HbA_{1c} was associated with any of the subcomponents of the PACC (MMSE, FNAME-12, Delayed Memory and DSST). The analysis did not reveal any significant associations between HbA_{1c} at any of the time points (and A1 months) with these cognitive markers (see Table 3.7). There was some weak evidence suggestive of an association between HbA_{1c} in midlife (age 60-64) as well as A1 months with DSST, but confounder adjustments made these relationships non-significant. There was once again no evidence of a sex interaction (all interaction p values > 0.2).

Associations between HbA_{1c} (and A1 months) and normal appearing white matter measures

There were no associations between HbA_{1c} at any time-point investigated or A1 months and measures of NAWM at age 69-71. There was no evidence of a sex interaction (all interaction p values>0.7). The associations remained largely unchanged with confounder adjustments. Findings are presented in Table 3.8.

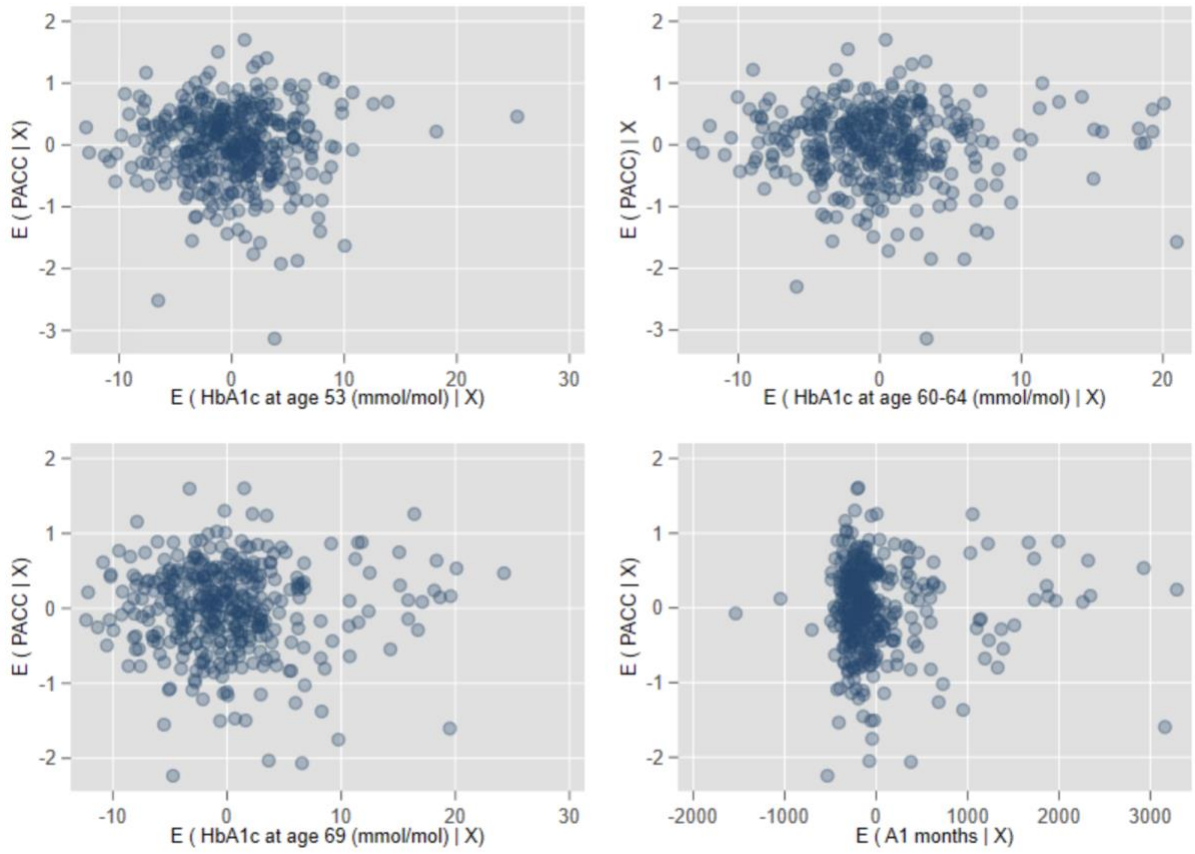


Figure 3.10: Partial regression plots showing associations between expected E(HbA_{1c}) at all three time points (and E (A1 months)) with expected E(Preclinical Alzheimer Composite score). A) HbA_{1c} mmol/mol at age 53. B) HbA_{1c} mmol/mol at age 60-64 C) HbA_{1c} mmol/mol at age 69. D) A1 months
The regression models presented are for the fully confounder-adjusted model (adjusted for age at scan, sex, childhood cognition, child, and adulthood socio-economic position; and BMI, physical exercise, and smoking status at the time of the exposure). White matter hyperintensities volumes was log-transformed. As per the plots for whole brain volumes, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

		Amyloid status				WMHV (μL/(mol/mol)				HV (μL/(mol/mol)				PACC			
		OR	Lower CI	Upper CI	p	β	Lower CI	Upper CI	p	β	Lower CI	Upper CI	p	β	Lower CI	Upper CI	p
Age 53	M1	1.2	0.7	2.2	0.5	-2.8	-23.2	17.6	0.8	-1.5	-13.1	10.2	0.8	-0.001	-0.01	0.013	0.94
	M2	1.2	0.7	2.2	0.5	-1.8	-22.9	19.3	0.8	-2.0	-13.9	9.9	0.7	0.0001	-0.01	0.013	0.97
	M3	1.2	0.7	2.2	0.5	-2.8	-24.6	18.9	0.8	-2.1	-14.2	10.1	0.7	0.001	-0.01	0.013	0.97
Age 60-64	M1	1.2	0.7	1.9	0.6	1.2	-16.6	19.1	0.9	-0.4	-8.6	9.9	0.9	-0.002	-0.014	0.011	0.80
	M2	1.2	0.7	1.9	0.6	1.8	-16.5	20.1	0.8	-0.4	-9.4	9.4	0.9	0.0001	-0.011	0.011	0.98
	M3	1.2	0.7	1.9	0.5	0.1	-18.5	18.7	0.9	-0.7	-11.4	8.0	0.8	0.001	-0.011	0.012	0.94
Age 69	M1	1.0	0.7	1.4	>0.9	4.1	-12.4	20.5	0.6	-0.6	-12.1	2.8	0.2	-0.001	-0.012	0.010	0.82
	M2	1.0	0.7	1.4	>0.9	4.8	-12.1	21.6	0.6	-0.1	-12.0	3.3	0.3	-0.001	-0.011	0.010	0.92
	M3	1.1	0.8	1.5	>0.8	2.7	-14.7	20.0	0.7	-1.7	-11.5	4.1	0.3	0.001	-0.010	0.011	0.91
A1 months	M1	1.0	0.9	1.3	0.7	0.1	-0.1	0.3	0.3	-5.6	-14.5	3.4	0.2	-0.016	-0.15	0.12	0.82
	M2	1.0	0.9	1.3	0.7	0.3	-0.1	0.3	0.3	-5.2	-14.4	4.0	0.3	-0.006	-0.13	0.12	0.92
	M3	1.0	0.9	1.3	0.7	0.1	-0.1	0.3	0.4	-4.4	-13.8	5.0	0.4	0.007	-0.12	0.13	0.91

Table 3.6: Regression analyses output of the relationships between HbA_{1c} at each time point and cumulative glycaemic exposure (or A1 months) on cognitive and brain imaging outcomes. The outcomes considered are amyloid status, white matter hyperintensities volume, hippocampal volume and Preclinical Alzheimer Cognitive Composite. Except for amyloid status where odds ratio are shown). For white matter hyperintensities volume and hippocampal volume, the models were constructed as follows: Model 1: minimally adjusted model for total intercranial volume, sex and age at scanning. Model 2: Model 1 +further adjustments for childhood socio-economic position, adulthood socio-economic position, education and childhood cognition. Model 3: Model 2 + further adjustments for body mass index, physical activity, and smoking status. For amyloid status and Preclinical Alzheimer Cognitive Composite. Model 1: minimally adjusted model for sex and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education and childhood cognition. Model 3: Model 2 + further adjustments for BMI, physical activity, and smoking status. Estimates for white matter hyperintensities volume and hippocampal volume were reported in μL (β*1000) to generate bigger coefficients. OR: Odds ratio. WMHV: white matter hyperintensities volume, HV: hippocampal volume, PACC: Preclinical Alzheimer Cognitive Composite, CI; Confidence intervals.

		MMSE				FNAME-12				Delayed Memory				DSST			
		β	**Lower CI**	**Upper CI**	**p**	**β**	**Lower CI**	**Upper CI**	**p**	**β**	**Lower CI**	**Upper CI**	**p**	**β**	**Lower CI**	**Upper CI**	**p**
Age 53	M1	-0.3	-0.26	0.2	0.8	-0.05	-0.3	0.2	0.6	0.09	-0.13	0.3	0.4	-0.2	-0.4	0.1	0.07
	M2	-0.002	-0.2	0.2	0.9	0.001	-0.21	0.21	0.9	0.1	-0.1	0.3	0.2	-0.17	-0.4	0.05	0.1
	M3	-0.001	-02	0.2	0.9	0.001	-0.2	0.22	0.9	0.1	-0.1	0.3	0.3	-0.17	-0.4	0.05	0.1
Age 60-64	M1	-0.03	-0.2	0.1	0.7	0.002	-0.17	0.17	0.9	0.06	-0.1	0.2	0.2	-0.18	-0.4	-0.01	0.04
	M2	-0.01	-0.2	0.2	0.9	0.05	-0.1	0.2	0.5	0.1	-0.7	0.3	0.3	-0.14	-0.3	0.02	0.08
	M3	0.03	-0.2	0.2	0.7	0.05	-0.1	0.2	0.5	0.1	-0.1	0.3	0.2	-0.14	-0.3	0.04	0.1
Age 69	M1	0.02	-0.1	0.2	0.8	0.02	-0.13	0.16	0.8	0.1	-0.1	0.2	0.5	-0.07	-0.2	0.07	0.3
	M2	0.3	-0.1	0.2	0.7	0.05	-0.1	0.2	0.5	0.1	-0.9	0.3	0.2	-0.06	-0.2	0.1	0.4
	M3	0.3	-0.2	0.2	0.7	0.05	-0.1	0.2	0.5	0.1	-0.1	0.3	0.3	-0.06	-0.2	0.08	0.4
A1 months	M1	-0.0004	-0.002	0.002	0.7	-0.0003	-0.002	0.002	0.7	0.001	-0.001	0.002	0.6	-0.002	-0.004	-0.0002	0.03
	M2	-0.0001	-0.002	0.002	0.9	0.0003	-0.001	0.002	0.7	0.001	-0.001	0.003	0.3	-0.002	-0.004	-0.0001	0.05
	M3	0.0002	-0.002	0.002	0.8	0.0002	-0.002	0.002	0.8	0.001	-0.001	0.003	0.4	-0.002	-0.004	0.0002	0.07

Table 3.7: Regression output of the relationships between HbA_{1c} at each time point and cumulative glycaemic exposure (or A1 months) on additional cognitive outcomes.
The outcomes considered are the MMSE, FNAME-12, Delayed memory and DSST. β, CI and p-values are presented. For these cognitive outcomes, the models are constructed as follows: Model 1: minimally adjusted model for sex and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education and childhood cognition. Model 3: Model 2 + further adjustments for body mass index, physical activity, and smoking status. MMSE: Mini-mental state examination, FNAME-12: Face-Name Associative Memory Exam, Delayed memory and DSST: Digital Symbol Substitute Test.

		Fractional Anisotropy (FA)				Mean Diffusivity (MD)				Neurite Density Index (NDI)				Orientation Dispersion index (ODI)			
		β	Lower CI	Upper CI	p	β	Lower CI	Upper CI	p	β	Lower CI	Upper CI	p	β	Lower CI	Upper CI	p
Age 53	M1	-0.0003	-0.006	0.006	0.9	-0.001	-0.01	0.007	0.7	-0.002	-0.01	0.01	0.7	0.0004	-0.003	0.04	0.7
	M2	-0.0006	-0.007	0.005	0.8	-0.001	-0.01	0.008	0.8	-0.04	-0.02	0.01	0.5	0.001	-0.003	0.003	0.9
	M3	-0.0005	-0.006	0.005	0.9	-0.001	-0.01	0.008	0.8	-0.04	-0.2	0.02	0.5	-0.0001	-0.003	0.003	0.9
Age 60-64	M1	0.0006	-0.004	0.005	0.8	-0.003	-0.01	0.004	0.4	-0.002	-0.01	0.01	0.6	0.0002	-0.002	0.003	0.9
	M2	0.0004	-0.005	0.005	0.8	-0.003	-0.01	0.004	0.4	-0.003	-0.01	0.01	0.5	0.0001	-0.003	0.003	0.9
	M3	0.001	-0.004	0.006	0.6	-0.004	-0.01	0.003	0.3	-0.002	-0.01	0.01	0.7	-0.0003	-0.003	0.002	0.8
Age 69	M1	0.0001	-0.003	0.005	0.7	-0.003	-0.01	0.003	0.3	-0.003	-0.01	0.01	0.5	0.001	-0.002	0.003	0.6
	M2	0.0001	-0.004	0.005	0.7	-0.003	-0.01	0.003	0.3	-0.003	-0.01	0.01	0.5	0.001	-0.002	0.003	0.6
	M3	0.001	-0.003	0.006	0.5	-0.003	-0.01	0.003	0.3	-0.002	-0.01	0.01	0.6	0.001	-0.002	0.003	0.8
A1 months	M1	0.0002	-0.0004	0.0007	0.5	-0.001	-0.001	0.0002	0.2	-0.00004	-0.001	0.001	0.9	0.0001	-0.002	0.0004	0.5
	M2	0.0002	-0.0004	0.0007	0.5	-0.001	-0.001	0.0002	0.2	-0.0002	-0.001	0.001	0.8	0.0001	-0.002	0.004	0.6
	M3	0.0002	-0.0004	0.0007	0.4	0.0001	-0.001	0.0002	0.1	-0.00006	-0.001	0.001	0.9	0.00004	-0.0003	0.0003	0.7

Table 3.8: Regression output of the relationships between HbA_{1c} at each time point and cumulative glycaemic exposure (or A1 months) on measures of normal appearing white matter.
The outcomes considered are fractional anisotropy, mean diffusivity, neurite density index and orientation dispersion. β, CI and p-values are presented. For these cognitive outcomes, the models are constructed as follows: Model 1: minimally adjusted model for sex and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education and childhood cognition. Model 3: Model 2 + further adjustments for body mass index, physical activity, and smoking status.

Sensitivity analyses

Sensitivity analyses were conducted in an attempt to see whether the results would change when excluding people with diabetes (age 53: n=2, age 60-64: n=18, age 69: n= 27). This did not materially change the results and nor my interpretations of the findings (see Table 3.9). Similarly, a complete case analysis did not yield output substantially different to the results from the multiple imputation models (see Table 3.10). Excluding participants with neurological disorders (n = 50) also did not materially alter the results.

		Whole Brain Volumes (WBV)							
		Males				Females			
		β	95% CI		p	β	95% CI		p
Age 53	M1	0.9	-0.4	2.1	0.2	-1.3	-2.7	-0.03	0.05
	M2	0.9	-0.4	2.2	0.2	-1.5	-2.9	-0.08	0.04
	M3	1.0	-0.4	2.3	0.2	-1.5	-2.9	-0.06	0.04
Age 60-64	M1	0.4	-0.7	1.5	0.4	-1.2	-2.4	0.03	0.05
	M2	0.4	-0.7	1.5	0.5	-1.3	-2.5	-0.02	0.04
	M3	0.6	-0.6	1.7	0.3	-1.4	-2.6	-0.1	0.03
Age 69	M1	0.5	-0.6	1.5	0.4	-1.4	-2.5	-0.37	0.008
	M2	0.5	-0.5	1.6	0.3	-1.5	-2.6	-0.42	0.007
	M3	0.8	-0.3	1.8	0.2	-1.6	-2.7	-0.43	0.007
A1 months	M1	0.004	-0.007	0.02	0.48	-0.008	-0.02	0.003	0.14
	M2	0.004	-0.007	0.02	0.49	-0.009	-0.02	0.002	0.12
	M3	0.006	-0.006	0.02	0.31	-0.01	-0.02	0.002	0.09

Table 3.9: Regression analyses output of associations between HbA_{1c} (mmol/mol) at each time point and cumulative glycaemic exposure (A1 months) on whole brain volumes stratified by sex (excluding participants with diabetes at each respective point.
β, Cl and p-values are presented). The models were as follows: Model 1: minimally adjusted model for total intercranial volume and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education, and childhood cognition. Model 3: Model 2 + further adjustments for body mass index, physical activity, and smoking status. The coefficients are unstandardised.

Table 3.10: Regression analyses output of associations between HbA_{1c} (mmol/mol)

		Whole Brain Volumes (WBV)							
		Males				Females			
		β	95% CI		p	β	95% CI		p
Age 53	M1	0.97	-0.27	2.2	0.12	-1.97	-3.21	-0.74	p < 0.001
	M2	1.1	-0.21	2.35	0.10	-2.09	-3.46	-0.72	0.003
	M3	1.2	-0.14	2.49	0.08	-1.89	-3.28	-0.51	0.008
Age 60-64	M1	0.51	-0.57	1.58	0.35	1.73	-1.76	-0.63	0.002
	M2	0.51	-0.61	1.63	0.37	-1.76	-2.95	-0.58	0.004
	M3	0.76	-0.49	2.02	0.23	-1.66	-2.87	-0.45	0.007
Age 69	M1	0.54	-0.48	1.55	0.30	-1.94	-2.91	-0.97	0.000
	M2	0.59	-0.45	1.64	0.26	-1.97	-3.003	-0.93	p < 0.001
	M3	0.77	-0.31	1.85	0.16	-1.94	-3.05	-0.83	p < 0.001
A1 months	M1	0.006	-0.005	0.02	0.28	-0.01	-0.02	-0.004	0.006
	M2	0.006	-0.006	0.02	0.36	-0.02	-0.03	-0.005	0.006
	M3	0.007	-0.006	0.02	0.27	-0.02	-0.03	-0.004	0.006

at each time point and cumulative glycaemic exposure (A1 months) on whole brain volumes stratified by sex (using complete case data).
The models were as follows: Model 1: minimally adjusted model for total intercranial volume and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education, and childhood cognition. Model 3: Model 2 + further adjustments for body mass index, physical activity, and smoking status. The coefficients are unstandardised.

3.4 Discussion

Summary of findings

High HbA_{1c} in late midlife and early late life (and higher cumulative glycaemic exposure) were associated with lower WBV at age ~70 in females but not in males. In females the associations between glycaemic levels and smaller WBV were consistent across all three time points with no temporal period standing out in strength. To put the size of this decrement in perspective, a 10 mmol/mol increase in HbA_{1c} at age 60-64 was equivalent to a reduction of WBV corresponding to 2 years of normal ageing (UK Biobank used as a reference). There was no convincing evidence of an association between HbA_{1c} levels or A1 months and markers typical of AD pathology (PET β amyloid status, and lower HV), or SVD (higher WMH burden), microstructural integrity (altered NAWM metrics), nor those of cognitive performance (PACC and its subcomponents). For these null results, there was no evidence of effect modification by sex.

Specific findings and associations with the literature

A recent meta-analysis summarising past evidence has shown that T2D is associated with smaller total brain volumes.[361] The results from this study add to the existing evidence by showing that: 1) poorer glycaemic health in a population-based sample is also associated with lower WBV and 2) there are sex differences in these associations since the effects were exclusively observed in females.

Previous evidence has shown that females with T2D have lower cortical thickness and WBV.[413,429] However, the findings from this study show that, in a population-based sample, higher HbA_{1c} levels are associated with poorer brain health, more specifically smaller overall brain size. Following the publication of my findings, a newly published cross-sectional study from the UK Biobank also reported associations between HbA_{1c} and smaller brain volumes in a pooled sample of males and females.[365] In my study, the standardised coefficients for the fully confounder-adjusted analysis of HbA_{1c} at age 60-64 for females were -0.9 (CI: -0.2-0.3) in line with the -0.07 estimates of those from the UK Biobank analyses (CI not reported). However, since the findings presented in this chapter considered multiple time exposures and the analyses were sex-stratified,

they provide evidence of sex-specific associations between poorer glycaemic control at different times, throughout midlife and early late life and later-life lower WBV.

There is precedent for sex differences in the susceptibility of other TOD in the context of T2D. Evidence has shown that females with T2D have a higher degree of vascular and renal TOD.[187,413,414] Since A1 months was also associated with smaller brains, it further suggests that exposure to glycaemia and cumulative exposure to glycaemia is potentially damaging for female brain health. Overall, these findings suggest that the brain may also be a structure susceptive to sex-specific TOD.

No particular time point when HbA_{1c} was measured, from midlife to early life, appeared to be more strongly associated with lower WBV in females. The size of the associations between HbA_{1c} at age 53, 60-64 and 69 and WBV in later life were mostly similar. This finding does not support the idea that midlife is a sensitive temporal window when hyperglycaemia may exert more damaging effects on brain health. This contrasts with some studies that have found that an earlier onset of T2D and/or longer duration of diabetes predicts a higher risk for dementia and poorer brain health outcomes.[352,411,430] There are multiple possible reasons behind this apparent conflict in results. The first is that the analysis in this thesis only considered glycaemia-related markers (as indexed by HbA_{1c}), one of several metabolic pathophysiological mechanisms that underlie T2D. Thus, it is possible that hyperglycaemia in midlife, on its own, may not be sufficiently impactful and it is the aggregation of multiple correlates of T2D (i.e. β cell dysfunction, IR, and hyperglycaemia) that may contribute to the midlife sensitive window. However, these measures are often closely related, and hyperglycaemia arguably should be a good proxy for all. The second is that since the study sample was population-based, the levels of hyperglycaemia were lower than other studies which explore similar associations in people with T2D. Perhaps this is a reflection that population-based studies such as NSHD can be helpful correctives to clinical studies, which may over-estimate association strengths. In line with this, it is also possible that this increased hyperglycaemia-driven midlife vulnerability becomes more apparent when both a sufficiently high threshold and duration of hyperglycaemia is encountered. Previous studies have shown that as the duration of diabetes increases, the difference in GM volumes between individuals with and without diabetes also increases. For example, a recent meta-analysis reported that for every additional

year beyond the average duration of diabetes (which is 10.5 years) in individuals aged 60 or older, there was an 8.8% difference in GM volumes between those with diabetes and those without.[361] This being said, it is important to consider that those with a longer exposure to HbA_{1c} are also likely to have a poorer overall glycaemic state. This is often neglected in the literature which highlights the value of using a measure of cumulative glycaemic burden such as A1 months which captures both the magnitude and duration of glycaemia outside the normal range. Although higher HbA_{1c} in midlife may not be associated with increased susceptibility to poorer brain health, glycaemic burden above the "normal range" over the three time points, was predictive of smaller brain volumes in females.

There are many potential reasons why hyperglycaemia may exert its effects differently on female brains. It is possible that these associations are stronger in females due to differences in hormonal health. This is particularly important because most, if not all, female participants in this sample would have gone through the menopause or be perimenopausal by the time HbA_{1c} was measured at age 53, considering the average age for the menopause in the UK is 51 (NHS data). This stage of hormonal transformation in a woman's life has been associated with a reduction in the all-encompassing neuroprotective hormone oestrogen which has consequences on body fat composition, inflammatory health and even brain metabolism.[431,432] Previous studies have also observed sex differences in chronic inflammation in the context of T2D.[433] Females with and without T2D have been shown to have higher levels of systemic inflammation compared to their male counterparts.[413,434] This may be important since inflammatory markers have been inversely associated with a range of brain outcomes, including global and tissue specific measures.[326,435,436] Since inflammation is raised during the menopause and post-menopause, and in the context of T2D, it is possible that hyperglycaemia may affect brain volumes preferentially in females via inflammatory pathways once oestrogen's anti-inflammatory properties may no longer be exerted. Alternatively, hyperglycaemia may drive neurodegeneration via increased production of ROS and AGES which are known to aggravate neuronal injury and promote neurodegeneration.[310,437,438] Other menopausal (and perimenopausal) related potential mechanisms that may drive poorer brain health include disrupted sleep and reductions in CBF.[439] These are all possible explanations,

but the data presented in this chapter does not provide an answer into why females appear to be more susceptible to glycaemia.

It must be acknowledged that these differences may also be explained by issues more specific to the study itself, including residual confounding from confounders not considered such as diet or from those crudely measured such as physical exercise. Bias may also arise from selective mortality. In the entire NSHD cohort, out of the 957 participants that were deceased by the sweep when they were aged ~70, 60% (or 568) were males. This raises the possibility that males with both poorer glycaemic health and brain atrophy died earlier.[392] Attrition may also play a role as historically males have been less likely to participate compared to females, raising the possibility that the remaining males are healthier and/or keener in taking part in the study. This is evidenced by the "healthy bias" that comes with participation into Insight 46 since it has been shown to be biased towards people of higher SEP, education, cognitive function, and better general health.[394]

Other brain health markers

Although my findings showed that higher HbA_{1c} predicted lower WBV in females, a similar pattern was not observed for HV. Previous studies have suggested that individuals with T2D show a noticeable decline in HV.[361] One study previously reported sex differences in the association between HV and T2D, with females found to have smaller hippocampi despite having better glucose control.[365] However, my null finding is consistent with results from previous population-based studies that also did not find an association between HbA_{1c} levels and HV.[364,365] My standardised estimates at age 53 suggest a -0.04 (CI -0.2 0.08, p = 0.3) decrease in HV consistent with the -0.01 (p= 0.1) decrease observed by Ranglani and colleagues in their fully confounder-adjusted model (CI not reported).[365] Once again, many possible interpretations can be made about this null finding. For example, rather than having a regional or localised influence, glycaemia may have a more diffuse effect on brain tissue. Future studies should examine these glycaemic associations with different tissue types (GM vs WM) and other regions at higher resolution (e.g., 7 Tesla).

There was no evidence of an association between hyperglycaemia at any three of the time points (and A1 months) and amyloid status nor an interaction between APOE

genotype and hyperglycaemia. These findings are consistent with previous studies that have also failed to observe an association between HbA_{1c} levels and the accumulation of amyloid in the brain.[362] This perhaps suggests that that hyperglycaemia might not be associated with the early neuropathological markers of AD. However, how glycaemia may be associated with tau or amyloid progression and how these interact with cerebrovascular disease still needs to be examined. Despite my null findings, a recent population-based study found that IR in midlife, as indexed by HOMA-IR, was associated with amyloid accumulation in some regions of the brain, such as the prefrontal cortex, parietal lobe and precuneus.[373] These findings suggest that IR in midlife, a marker closely related to glycaemia, is associated with increased amyloid burden. However, the study considered multiple measures of amyloid burden including regional measures that may be more sensitive to amyloid accumulation. Thus, the association between hyperglycaemia and amyloidosis should be re-examined using more sensitive measures of this neuropathological marker.

One of the hypotheses investigated in this chapter was that HbA_{1c} throughout life and cumulative glycaemic burden would be associated with later-life SVD. There were no convincing associations between HbA_{1c} at any time point (and A1 months) and WMH burden. Although some cross-sectional studies have previously shown statistically significant, but weak associations between high fasting blood glucose concentration and WMHV, other studies have also reported no diabetes-SVD associations.[354,440] When exploring my findings in relation to the published literature, the size of the standardised estimates in my analysis are consistent with previous evidence. In my study a 1SD increase in HbA_{1c} at age 60-64 was associated with 0.01 (Cl: -0.1; 0.2 p = 0.8) cm^3 increase in WMHV for the fully confounder-adjusted model. This estimate is similar to the 0.0099 (p = 0.1 [Cl not reported]) increase observed by Ranglani and colleagues in the UK Biobank.[365] Overall, inconsistency in the existing literature may be explained by variations in the methodological approach taken. Firstly, it is important to note that certain studies may report findings that pass the threshold for significance due to the large size of their sample, but their coefficients remain consistent with my findings. Secondly, studies vary in the sample they consider, with some exploring these associations in subgroups of patients with a history of cardiovascular disease (CVD), [354] whereas others look at these in healthy subjects.[365] Naturally this could introduce inconsistencies in the associations between T2D-related

pathology and WMH burden examined. A wide range of imaging markers have also been used to quantify WM burden. It has been found that some participants with hyperglycaemia do not show associations with WMH globally but instead have more compartmentalised and regional WM pathology, as previously shown.[441] One study found that although people with T2D did not differ from controls in regards to the traditional WMHV measure (akin to the one used in this study), they displayed more non-punctuate WMH and a difference in shape (eccentricity) of punctuate deep WMH.[355] This indicates how the sensitivity of the marker considered can affect the results observed and the value of going beyond the traditional WMH assessments as this may help uncover more subtle cerebral SVD.

One may also argue that the null finding could also be due to the participants being too young or insufficiently burdened by WMHs when they underwent imaging at age 70. However, age of participants, despite its low range in this birth cohort, was associated with higher WMHV suggesting that even quite small relationships of SVD burden are detectable in the sample. It may also be possible that poorer glycaemic control may specifically relate to more subtle microstructural damage that precedes 'full blown' WMH. Despite using more sensitive measures, supposedly reflective of early-stage degeneration of vessels, no HbA_{1c}-NAWM associations were observed. Findings were similar when exploring the impact of cumulative glycaemic burden on these measures of microstructural integrity. The absence of a detectable association between hyperglycaemia and different markers of brain SVD and microstructural integrity does not however preclude the possibility that SVD may be occurring elsewhere in the body. Research on the organ-specific effects of hyperglycaemia highlight the nuanced impact it can have on different tissues. Hyperglycaemia can exert diverse effects on small vessels in different organs and tissues, and the response may vary due to the unique microenvironments and regulatory mechanisms of each structure. There is ample evidence linking T2D with retinopathy, nephropathy and microvascular diseases which are not addressed here.[442,443] Once again this is a population-representative cohort not a clinical sample. In people with more severe cases of glycaemic and longer exposure to glycaemia, these associations may still exist.

Associations with cognitive outcomes

There were no convincing associations between HbA_{1c} at any time point and cognition as assessed by the PACC. Evidence on the relationship between hyperglycaemia (or T2D) and cognitive health has been mixed[409]: Some studies report that poorer glycaemic control, as measured by higher HbA_{1c} levels, is associated with worse cognitive function, while others report no convincing associations.[384,385,444] Thus, this finding may be considered to fall within the scope of what is expected from the literature. Discrepancies between studies could be explained once again by the different population studied (i.e., clinical sample vs population cohort) and the wide range of different glycaemic indices considered in many of these studies with some focusing on HbA_{1c}, while others considered other biomarkers, such as fasting and post-load glucose. Each of these biomarkers, although related, capture a different metabolic pathology. Heterogeneity in the cognitive measures considered or the age of the participants studied may also explain the inconsistencies reported in the literature. The outcome measures used range from composite scores for global cognition[445] to single tests that assess a specific cognitive domain.[446] This null finding in my study is also not surprising considering that the PACC is sensitive to early AD, and that I also failed to find an association between hyperglycaemia and amyloidosis, a hallmark of AD thought to be an important driver of cognitive decline.

As additional analyses, subcomponents of the PACC such as the MMSE, DSST, FNAME-12 and logical delayed memory were explored. Once again, no convincing associations were observed between HbA_{1c} at any time point and these measures of cognition. Previous studies have found that T2D is associated with impaired memory, attention, psychomotor speed, and executive function.[447] In this study, there were some suggestions that late midlife hyperglycaemia and cumulative glycaemic exposure could negatively impact performance in the DSST, an assessment of motor speed, attention and executive function. However, the strength of the associations was attenuated with confounder adjustment and there is a possibility that these were chance findings due to multiple testing. Nonetheless, it is possible that there is a latent period or sufficient glycaemic exposure required for the effects of hyperglycaemia to manifest into cognitive impairments. It is also possible that the relationships between high HbA_{1c} (and cumulative glycaemic burden) and smaller WBV did not translate to

cognitive impairment due to Insight 46 participants possessing cognitive reserve. Cognitive reserve describes the brain's ability to circumvent neuropathological damage via compensatory mechanisms such as the recruitment of alternative brain networks.[448] Education is one of the key components that underlies cognitive reserve and previous evidence shows that participation in Insight 46 is biased towards those who are more educated.[394]

It is also possible that variability in glycaemia plays an important role in brain health. In this study, HbA_{1c} at three time points and cumulative glycaemia were considered. Measures of day-to-day or weekly variations in glycaemia were not explored. Growing evidence suggests that fluctuations in glycaemia may be pathological and have an effect on brain health outcomes.[449] Similarly, another key component of T2D, IR, was not considered. This is particularly important considering previous studies have linked the mechanism behind IR with impaired amyloid clearance (thus amyloidosis).

It remains however important to acknowledge that null findings could also be driven by characteristics of this birth cohort. The Insight 46 sub-sample consists of 502 participants who were selected on the basis of having previously attended a clinic visit along with other criteria. Participation into Insight 46 has been found to be associated with a bias towards people with a higher SEP, education, cognitive function, and better health.[394] This may also explain some of the negative findings observed as the Insight 46 is "cognitively normal" and healthier than the general population. In addition, differential survival, and attrition of less healthy individuals from the birth cohort may have also biased the results toward the null. Despite benefiting from the deep phenotyping of the participants, restricting this analysis to clinic attenders with antecedent data may have introduced a selection bias that has affected the results. In addition, although many important factors influencing cognitive function were adjusted for in the analyses, some confounders including non-APOE genetic predisposition, are likely to have been omitted or only partially accounted for resulting in bias.

Strengths and weaknesses

A major strength of this study is the uniqueness of this birth cohort especially regarding its rich longitudinal phenotyping conducted over decades of the participants' lives. This includes the combination of data on cognitive function and brain imaging as well as

comprehensive longitudinal data on metabolic and vascular risk factor clusters. To further maximise the richness of this dataset and account for the missingness, multiple imputation models were conducted. This technique allowed the use of all the available data while also preserving the size and statistical power of the sample; under some assumptions this will reduce bias. A sensitivity analysis found that sex differences in findings with respect to WBV were not different to complete case analysis, which excludes individuals with missing values.

I acknowledge that since the participants considered are from a birth cohort, the findings may be affected by secular effects. This post-World War II cohort is likely to have characteristics and exposures that are different to modern day cohorts, especially in terms of lifestyle and diet. This may make it difficult to know the extent to which findings from this study apply to other younger cohorts. Another important limitation is that this sample consists exclusively of white British participants, and thus may not be representative of other populations. This is particularly important since both rates of T2D and all cause-dementia have been shown to vary based on ethnicity.[170,450,451]

Future work

Further work should explore whether these associations suggestive of a sex-specific relationship between poor glycaemic control and smaller brains can be replicated in a larger sample with different characteristics to NSHD (such as UK Biobank). Mechanistically, it may be interesting to see whether operationalising glycaemia using a marker that captures a shorter temporal window (e.g., fasting glucose or random glucose) or looking at other diabetes-related markers (IR and β cell function) will also show similar associations with brain pathology. Further exploring the nature of how hyperglycaemia affects brain volumes is of interest, particularly whether these reflect specific tissue type vulnerability (i.e., whether poor glycaemia affects glial cells or GM volumes preferentially).

3.5 Conclusions

The findings show sex-specific associations between hyperglycaemia and whole brain volumes, with higher HbA_{1c} being associated with smaller brains particularly in females. This association remained fairly consistent even if glycaemia was measured at different time points (i.e., age 53, 60-64 and 69) suggesting that there is no evident specific temporal window where hyperglycaemia exerts a stronger impact on brain health. There was no convincing evidence of associations between HbA_{1c} and other markers of brain health such as those relating to small vessel disease, Alzheimer's disease-related pathology, and cognitive outcomes. The null findings in regard to hippocampal volumes and amyloidosis may suggest that hyperglycaemia might affect the brain through pathways independent from those typically affected in Alzheimer's disease. Overall, the findings suggest that high glycaemia, even in a normal population sample, may still be associated with adverse brain volumes, although repercussions on cognition may not appear by age 70.

4. Sex-stratified analyses of glycaemic traits and brain volume outcomes in NSHD

I previously reported that elevated HbA_{1c} levels in mid- and later-life were associated with lower whole brain volumes in older females but not males (Chapter 3). To provide further mechanistic insight into this relationship, I investigated whether similar sex differences were apparent for other glycaemic measures including fasting glucose, insulin resistance (HOMA2-IR) and β-cell function (HOMA-%B). I also investigated whether glycaemic traits were associated with preferential lower white matter or grey matter volumes. A manuscript for this chapter was sent to European Journal of Endocrinology in June 2024.

4.1 Introduction

The relationship between T2D and brain volume has been discussed extensively (Chapter 1.12). Most research, primarily focused on combined sex samples, has shown that individuals with T2D exhibit smaller WBV.[408,452] Furthermore, there is emerging research suggesting sex-specific differences in brain health outcomes in people with T2D.[407,413,429] Sex differences have also been observed in relation to glucose metabolism, insulin sensitivity and β cell function.[180,255–257]

Although fasting glucose and HbA_{1c} both reflect the combined influence of IR and pancreatic β cell dysfunction, studies only show a moderate correlation between these measures, and there is evidence of differential genetic influences on fasting glucose and HbA_{1c}. It has been argued that each biomarker provides a different pathophysiological insight into diabetes risk.[453] In addition, insulin is important for brain health: its receptors are highly expressed in the brain, and it is thought to support neuronal and glial cell growth and survival.[454]

In previous work in a population-based British birth cohort (Chapter 3), I demonstrated that elevated HbA_{1c} through mid- and later-life (age 53, 60-64 and 69) was associated with smaller WBV at age ~70 only in females.[407] However, it is valuable from a research perspective to explore whether abnormality of other glycaemia-related pathophysiological markers: 1) are also related to poorer later-life brain volume, 2) affect females preferentially and 3) predicts preferential tissue loss. The latter is

particularly relevant since there is some evidence of sex-specific differences in WM and GM volumes during ageing.[18–20]

Harnessing the unique dataset NSHD and Insight 46, I aim to expand on my previous findings and assess whether there are sex differences in associations between a range of glycaemia-related markers measured at age 60-64 (HbA_{1c}, glucose, HOMA2-IR and HOMA%B) and global and tissue-specific brain volumes at age ~70.

4.2 Methods

4.2.1 Sample

The National Survey of Health and Development (NHSD) is a British birth cohort originally made up of 5,362 males and females born across mainland Britain during the same week in 1946.[392] In 2006, the study members (aged 60-64 at the time) received postal questionnaires and were invited to attend a clinic visit. Between 2015-2018, a subset of NSHD were enrolled into the Insight 46 sub-study to undergo neuroimaging and further assessments. More details in the sample are discussed in Chapter 2.2.

4.2.2 Investigations

The brain imaging measures considered in these analyses were the volumetric measures WBV, GM and WM. The collection of the neuroimaging data and the postprocessing steps were discussed in Chapter 2.4.

Blood measures

A fasting blood sample was collected at age 60–64. HbA_{1c} was measured by ion exchange HPLC on a Tosoh analyzer (Tosoh Bioscience, Tessenderlo, Belgium). Glucose was measured by enzymatic assay using hexokinase coupled to glucose 6-phosphate dehydrogenase, using a Siemens Dimension Xpand analyzer, Siemens Medical Solutions, Erlangen, Germany. Insulin was measured by fluoroimmunoassay using a PerkinElmer AutoDELFIA analyzer, PerkinElmer, Waltham, MA, USA. Due to delays in analysing blood samples for insulin within the specified one-hour window, only 62% of samples collected for insulin measurement were considered valid. HOMA2-IR and HOMA-%B were calculated using insulin and glucose values using the

validated HOMA2 calculator from the University of Oxford (https://www.dtu.ox.ac.uk/homacalculator).[254]

Demographics and other measures

Confounders were identified through a review of the literature exploring the association between hyperglycaemia (or T2D) and volumetric brain measures and depicted using a directed acyclic graph (see Figure 3.1). Potential confounders considered were childhood SEP adult SEP, childhood cognition, education, waist-to-hip ratio (WHR), physical activity level, alcohol consumption and smoking status at the time of glycaemic measurement, and age at neuroimaging scan. Sex was only included as a confounder for the pooled analyses that were conducted for completeness but were the focus of this study.

Childhood SEP was measured as father's occupational social class recorded at age 4 (or if missing, at age 11) and categorized into manual or non-manual according to the UK Registrar General's Standard's Occupation Classification. Adult SEP was based on head of household occupation at age 53 years. These were coded according to the UK Registrar General's Standard Occupational Classification, then grouped as follows: I (professional), II (managerial and technical), IIIN (skilled non-manual), IIIM (skilled manual), IV (partly skilled), and V (unskilled).

Childhood cognitive function was derived from four tests of verbal and non-verbal ability administered to the participants at ages 8, 11 and 15.[423] Cognitive ability at age 15 was used. Educational attainment was represented as the highest educational or training qualification achieved by age 43, grouped into 5 categories: no qualification, below O-levels (vocational), O-levels and equivalents, A-levels and equivalents, higher education (degree and equivalents). More details on this are discussed in Chapter 3.

WHR at the time of glycaemic measurement (age 60-64) was obtained by calculating the ratio of the circumference of the waist to that of the hips. Based on self-reported engagement over the preceding 4 week before the assessment at age 60-64, physical activity was categorized as inactive, moderately active, and most active. Alcohol consumption at age 60-64 was categorised according to UK guidelines into none, ≤ 14 units per week, or > 14 units per week assuming that one drink was equivalent to

1 unit. Smoking status at age 60-64 was assessed by self-report and was classified into three groups: current smokers, ex-smokers, and never-smokers.

4.2.3 Statistical analysis

Statistical analyses were conducted in Stata version 16.1. Normally distributed continuous variables describing the sample were summarised as means and SD For skewed data, the median and interquartile range were reported. For categorical variables, frequency and percentages were reported.

Pairwise linear correlations between HbA_{1c}, fasting glucose, HOMA2-IR and HOMA-%B were summarized using Pearson's correlation coefficient when assumptions of linearity were satisfied, and Spearman's if not. Correlation coefficients were reported in a correlation matrix separately for males and females.

Based on evidence suggesting sex-specific differences in the association between HbA_{1c} and whole brain volume,[407] regression models for each association between glycaemic traits and brain volume tested were sex stratified *a priori*. However, interaction terms between sex and glycaemic markers on brain health were reported for completeness.

Associations between HbA_{1c}, glucose, HOMA2-IR and HOMA-%B with brain volume measures were quantified using multivariable linear regression models. Associations were presented as mean regression coefficients (beta β) with 95% confidence intervals (CI). To allow comparison of the strengths of association for different glycaemic traits, regression coefficients were also standardised (standardised β*).

For each association, a minimally confounder-adjusted model (Model 1) adjusted for TIV and age at neuroimaging scan was first constructed. Model 2 included Model 1 plus further adjustments for demographic factors related to cognition (childhood SEP, adulthood SEP, childhood cognition and education). Model 3 was the fully confounder-adjusted model and included Model 2 plus further adjustment for lifestyle factors (WHR, physical activity, alcohol consumption and smoking status measured at age 60-64). Model coefficients are presented in Table 4.2.

Multiple imputation for missing confounder data was performed using the MICE method by fully conditional specification (50 imputed datasets) under the assumption of MAR. Data were combined using Rubin's rules. Results were checked for concordance with the complete case data and in all cases the pattern of effects were similar (see Table 4.3). For completeness, pooled analyses were also conducted but these were not the focus of this chapter.

4.3 Results

Data were available for a maximum of 453 participants based on availability of neuroimaging data (see Figure 4.1). Demographic and clinical characteristics for the participants considered in this sample are presented in Table 4.1. Around half of the participants were males and the mean age of the imaging cohort was 70. On average, participants had relatively normal insulin sensitivity and evidence of mildly reduced pancreatic β cell function.

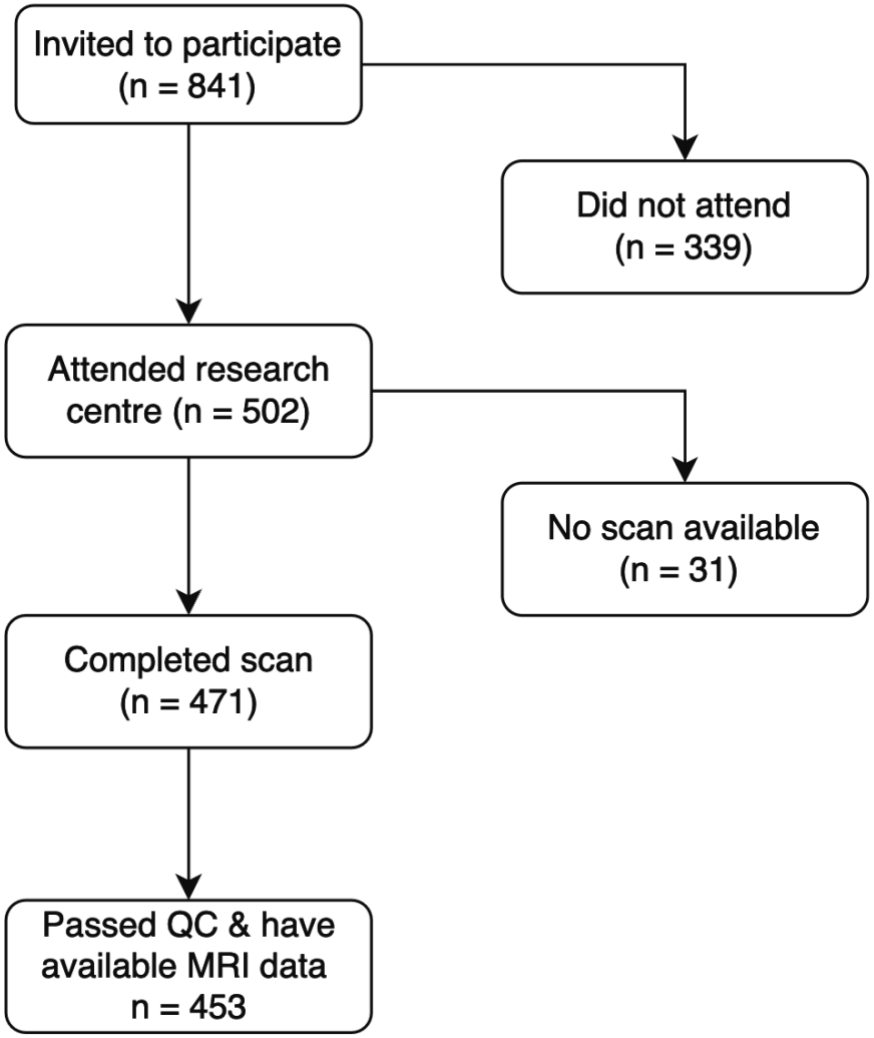


Figure 4.1: Flowchart providing an overview of Insight 46 recruitment and imaging of National Survey of Health and Development participants who undertook imaging. Although 471 participants completed the scan, 18 did not pass quality control. To be considered in the study, participants had to have been part of Insight 46 and have volumetric imaging data available, which amounted to 453 participants.

Participant characteristics	n	Males	n	Females
Standardised childhood cognition score	219	0.36 (0·73)	212	0·44 (0·74)
Education		231		221
No qualifications		24 (10%)		31 (14%)
Below O-levels (vocational)		17 (7%)		17 (8%)
O-levels and equivalents		38 (17%)		56 (25%)
A-levels and equivalents		83 (36%)		79 (36%)
Degree or higher		69 (30%)		38 (17%)
Adult socioeconomic position		231		222
Non-manual (Class I–IIIN)		193 (84%)		193 (87%)
Manual (Class IIIM-V)		38 (16%)		29 (13%)
Childhood socioeconomic position		231		218
Non-manual (Class I–IIIN)		142 (61%)		120 (55%)
Manual (Class IIIM-V)		89 (39%)		98 (45%)
Characteristics, age 60-64				
HbA_{1c}, %	215	5.71 (0.47)	209	5.8 (0.55)
HbA_{1c}, mmol/mol	215	39.91 (5.15)	215	38.8 (6)
Fasting glucose, mmol/L	227	5.9 (0.9)	212	5.5 (1.1)
Fasting insulin	135	44 (42)	137	35 (24)
HOMA2-IR	135	1.1 (0.8)	137	0.9 (0.5)
HOMA-%B,	135	68.1 (28.9)	137	67.2 (31.4)
Diabetes medication use	231	10 (4%)	222	6 (2.7%)
Waist-hip ratio	231	0.96 (0.06)	222	0.86 (0.06)

Smoking status		231		215
Current Smokers		4 (2 %)		5 (2%)
Ex-smokers		120 (52%)		86 (40%)
Never smoker		107 (46%)		124 (58%)
Alcohol (units/week)		231		222
≤ 14		181 (78%)		203 (91%)
> 14		50 (22%)		19 (9%)
Exercise levels		229		219
Inactive		125 (55%)		107 (49%)
Moderately active		38 (17%)		47 (21%)
Most Active		66 (28%)		65 (30%)
Brain imaging markers measured at age ~70				
Mean age at scanning, years	231	70.7 (0.7)	222	70.7 (0.7)
Whole brain volume (WBV), mL	231	1152.4 (87.0)	222	1047.3 (82.1)
White matter volume (WM), mL	231	439.6 (2.8)	222	394.3 (2.8)
Grey matter volume (GM), mL	231	649.6 (3.4)	222	602.6 (3)
Total intracranial volume (TIV), mL	231	1519.8 (106.8)	222	1343.1 (92.6)

Table 4.1: Sample characteristics for the participants considered in these analyses (n = 453).
Values presented are pre-imputation data: n (%), mean (SD) or median (IQR). % are calculated against the max data available for that specific measure for the pooled sample. As described above, the number of participants considered had to have been part of Insight 46 and have volumetric imaging data available which amounted to 453 participants of which 231 were males and 222 were females. Whole brain volume, white matter volume and grey matter volume measurements reported are unadjusted for total intercranial volume for these descriptions. Values are n (%) or mean (SD).SD: Standard deviation. HOMA2-IR: Homeostatic Model Assessment for Insulin Resistance. HOMA-%B: Homeostatic Model Assessment for β cell function.

Pairwise correlations of glycaemic traits at age 60-64

Overall, the correlation analysis revealed a positive relationship between HbA_{1c}, glucose and HOMA2-IR across both sexes, with a slightly stronger association demonstrated in males (for example, the correlation between glucose and HbA_{1c} was r=0.60 in males vs r=0.54 in females) (Figure 4.2). The positive correlation between HOMA2-IR and HOMA%B was stronger in males ($r=0.79_{male}$, $r=0.55_{female}$), but the negative correlation between glucose and HOMA%B was stronger in females (-0.17_{male}, -0.37_{female}).

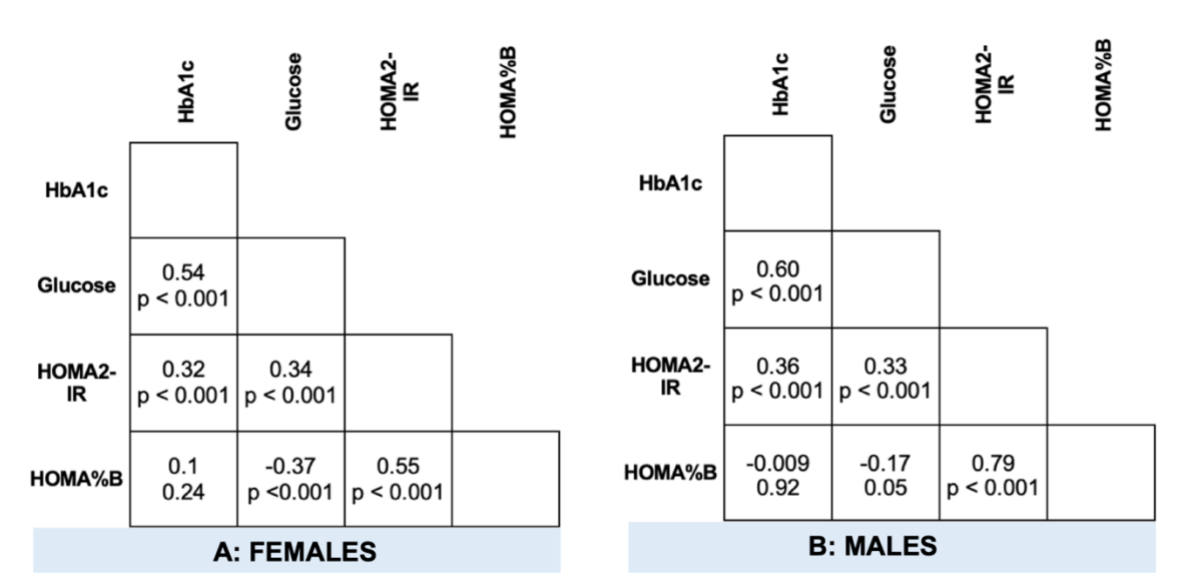


Figure 4.2: Correlation matrix displaying the correlation between the glycaemic markers HbA_{1c}, glucose, HOMA2-IR and HOMA%B.
On the first row, the r value represents the direction of correlation (from a Pearson's correlation). In the second row, the p value represents the strength of any association. A provides the correlation matrix for the variables in females and B provides the correlation matrix for the variables in males.

Associations between glycaemic traits at age 60-64 and whole brain volume at age 70

Plots reporting the standardised coefficients by sex and confidence intervals are displayed in Figure 4.3.

Glucose

There was a significant association between higher glucose and WBV in females (β*=-0.07 [95%CI: -0.13, -0.01] p=0.02), but not in males (β*= -0.05 [-0.14, 0.03] p=0.2) (Figure 4.3, Table 4.2). There was no strong evidence of an interaction by sex (p = 0.5).

HbA_{1c}

I have previously published the results between HbA_{1c} and discussed them in chapter 3. [407] They demonstrated a significant association between higher HbA_{1c} at age 60-64 and smaller WBV in females only.

Beta cell function (HOMA-%B)

There were no statistically significant associations between HOMA-%B and WBV in either males (β*= -0.02 [95%CI: -0.12, 0.09] p=0.8), or females (β*= -0.01 [-0.09, 0.07] p=0.8) (Figure 4.3, Table 4.2). There was no evidence of an interaction by sex (p = 0.7).

Insulin resistance (HOMA2-IR)

The association between greater HOMA2-IR and smaller WBV was statistically significant in females (β*= -0.12 [-0.2, -0.002] p=0.04), but not in males (β*= -0.06 [-0.16, 0.05] p=0.31) (Figure 4.3 and Figure 4.2). This represents a ~50% difference between males and females in the strength of association between HOMA2-IR at age 60-64 and WBV at age 70.

Adjustments for confounders did not materially change the pattern of effects for WBV (see Figure 4.3, Table 4.2). There was marginal evidence of an interaction by sex (p = 0.05).

Overall, in females, effect sizes of the relationship between significant glycaemic traits at age 60-64 and WBV were ordered as follows: HOMA2-IR (β=-0.12), HbA_{1c} (β= -

0.09), and glucose (β=-0.07)**.** Whereas in males, none of these relationships were statistically significant.

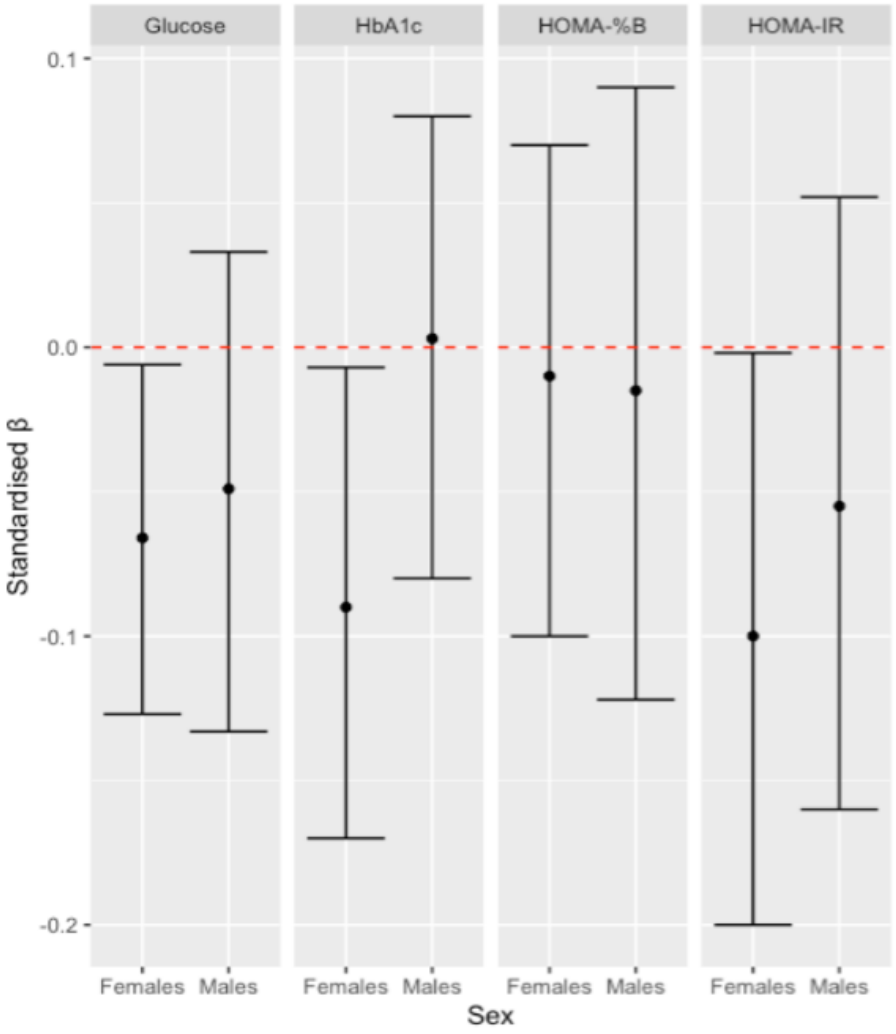


Figure 4.3: Forest plots displaying the associations between glycaemic traits at age 60-64 (glucose, HbA_{1c}, HOMA-%B, HOMA-IR) with whole brain volumes at age 69-71, stratified by sex.
The estimates presented are standardised regression coefficients for the fully confounder-adjusted models (adjusted for total intercranial volume, age at scan, cognitive measures, socio-economic position, waist-to-hip ratio, physical exercise, alcohol status and smoking status). Standardised coefficients are presented here to facilitate comparison.

Associations between glycaemic traits and brain tissue type

Plots reporting the standardised coefficients and confidence intervals are displayed in Figure 4.8 and Table 4.2.

Glucose

In females there was a significant association between higher glucose with smaller GM (β*= -0.04 [-0.08, -0.002] p=0.04) and WM (β*= -0.06 [-0.1, -0.02] p=0.02) volumes, with a slightly stronger coefficient observed for WM volumes (Figure 4.4, Figure 4.8, Table 4.2). In males, no significant associations between glucose and GM or WM volume emerged (p>0.05). There was no evidence of an interaction by sex for either GM (p = 0.7) or WM (p = 0.3).

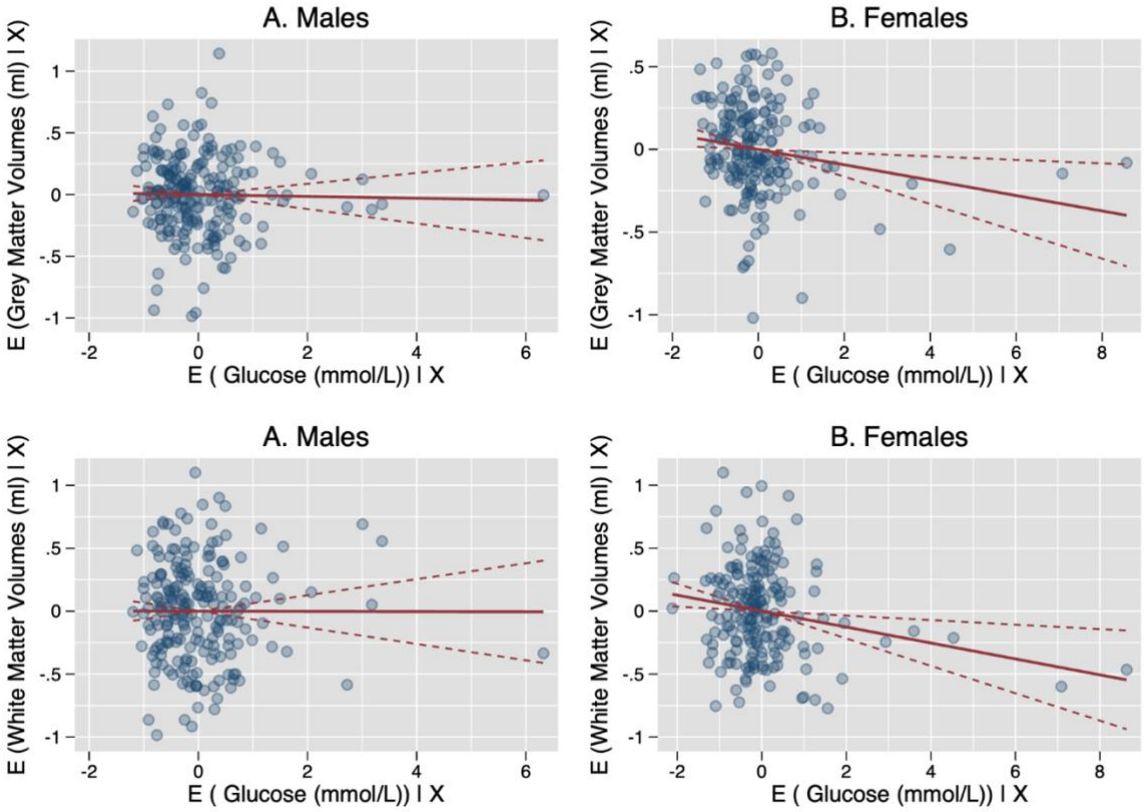


Figure 4.4: Partial regression plots showing associations between expected E (glucose) and expected E (Grey Matter volumes) and expected E (White Matter volumes).
A represents the figures for males. B represent the figures in females. The coefficients presented are for the fully confounder adjusted model (adjusted for total intercranial volume, age at scan, childhood cognition, child, and adulthood socio-economic position, waist-to-hip ratio, physical exercise levels, and alcohol status and smoking status at the time of the exposure. E(Y) on the y-axis represents the predicted values of the response variable, while E(X|Xn) on the x-axis represents the conditional expected values of the predictor variable of interest.

HbA$_{1c}$

In females there was a significant association between higher HbA$_{1c}$ and smaller WM (β*=-0.06 [-0.12, -0.004] p=0.04), but not between HbA$_{1c}$ and GM volume (β*= -0.03 [-0.07, 0.006] p=0.1) (Figure 4.5, Figure 4.8, Table 4.2), although the confidence intervals for the GM and WM volume relationships in females largely overlap, suggesting that the difference may not be substantial. In males, no significant associations between glucose and GM or WM volume emerged (p>0.05). There was some evidence of an interaction by sex for GM (p = 0.08) but not WM (p = 0.4).

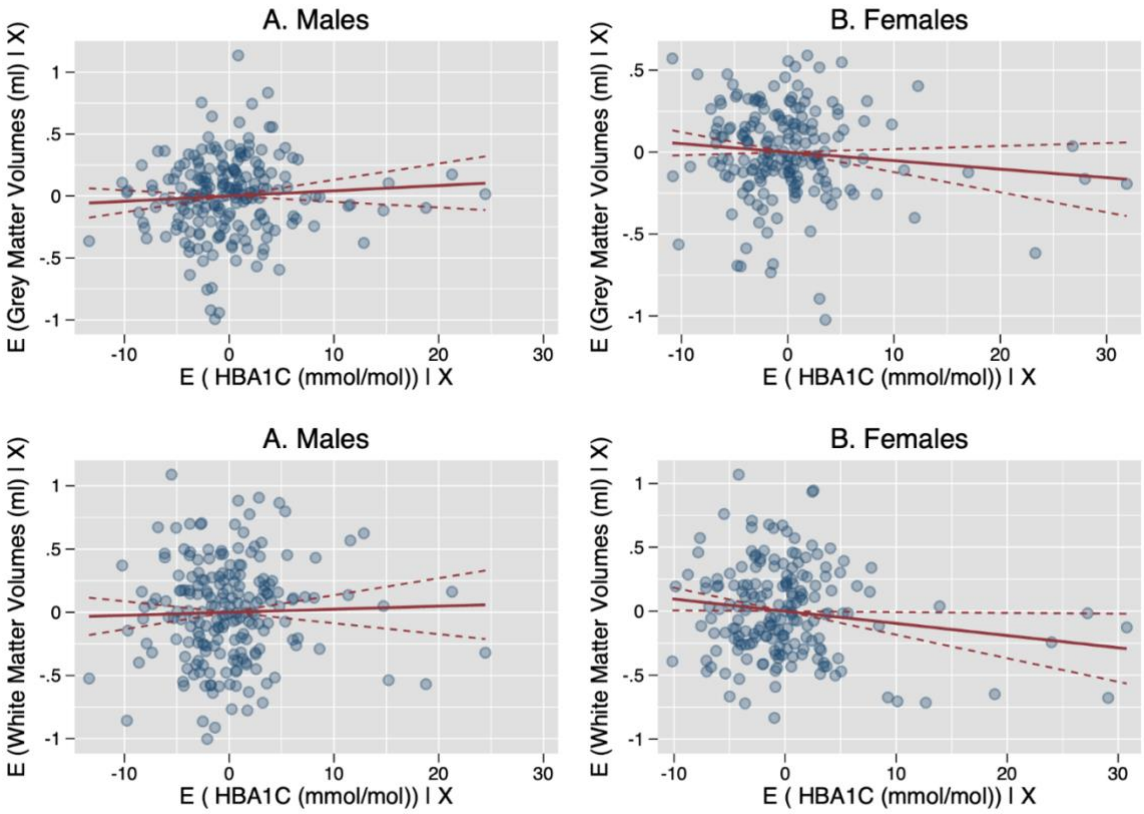


Figure 4.5: Partial regression plots showing associations between expected E (HbA$_{1c}$) and expected E (Grey Matter volumes) and expected E (White Matter volumes).
A represents the figures for males. B represent the figures in females. The coefficients presented are for the fully confounder-adjusted model (adjusted for total intercranial volume, age at scan, childhood cognition, childhood, and adulthood socio-economic position, waist-to-hip ratio, physical exercise levels, and alcohol status and smoking status at the time of the exposure. As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

Beta cell function (HOMA-%B)

No significant associations emerged between HOMA-%B and GM or WM volume, in either sex (p>0.05) (Figure 4.6, Figure 4.8, Table 4.2). There was no evidence of an interaction by sex or both GM (p = 0.5) and WM (p = 0.4) volumes.

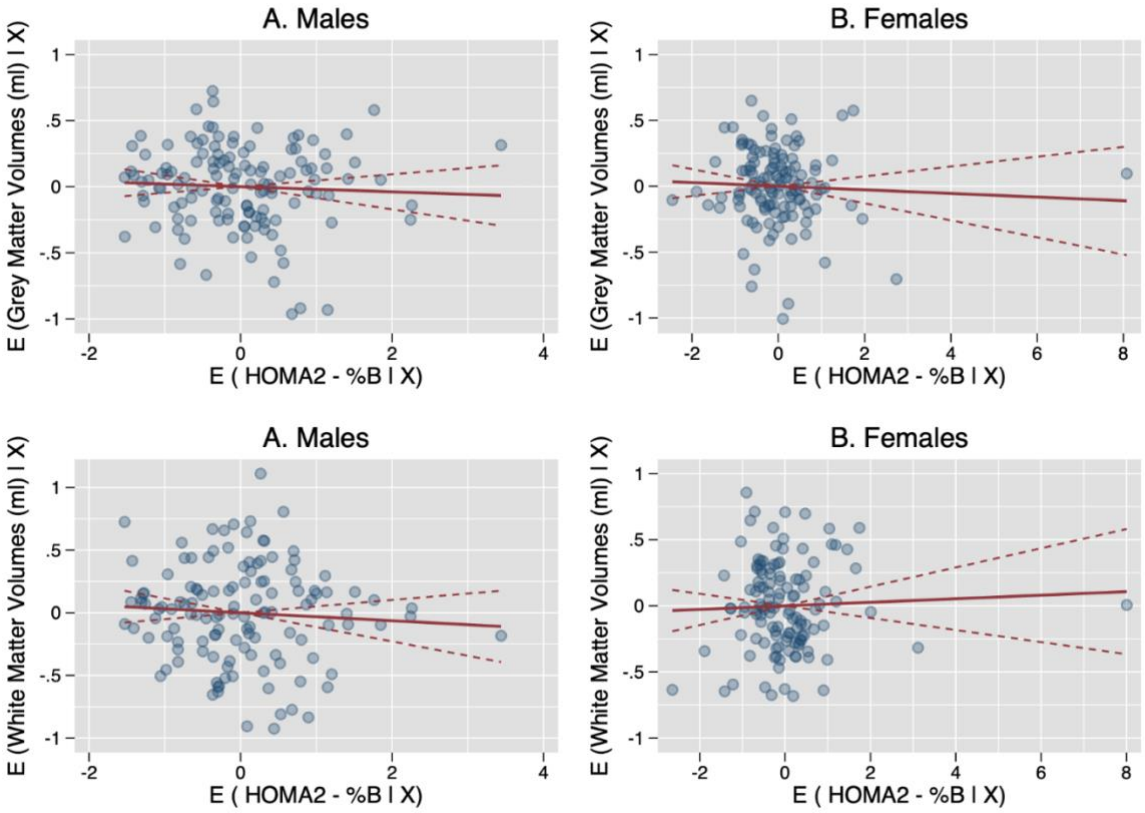


Figure 4.6: Partial regression plots showing associations between expected E(HOMA%B) and expected E (Grey Matter volumes) and expected E (White Matter volumes). A represents the figures for males. B represent the figures in females.
The coefficients presented are for the fully confounder-adjusted model (adjusted for total intercranial volume, age at scan, childhood cognition, childhood, and adulthood socio-economic position, waist-to-hip ratio, physical exercise levels, and alcohol status and smoking status. As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

Insulin resistance (HOMA2-IR)

In females there was a significant association between higher HOMA2-IR and smaller GM volumes in females (β*=-0.05 [-0.1, -0.007], p=0.04), but not WM volumes, despite a similar effect size (β*= -0.05 [-0.13, 0.04] p=0.27). In males, no significant associations between HOMA2-IR and GM or WM volume emerged (p>0.05). There was evidence of an interaction by sex for GM (p = 0.02) but not WM (p = 0.4) volumes.

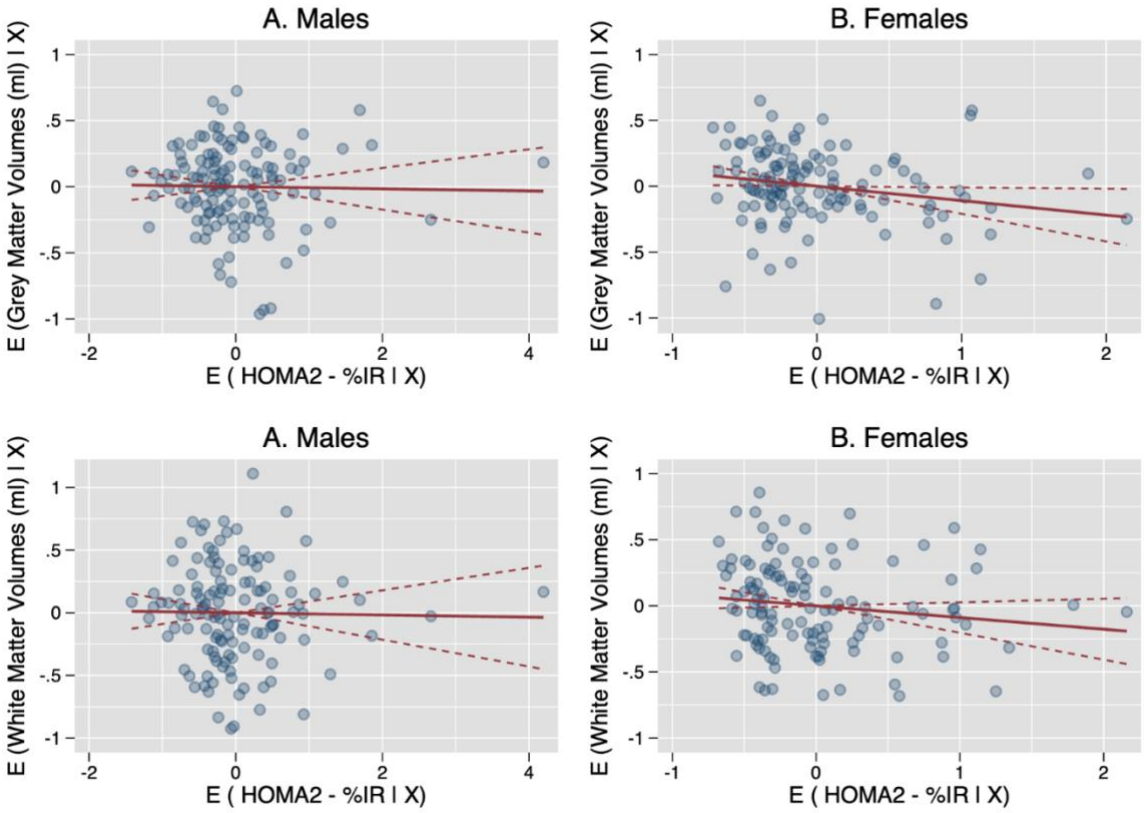


Figure 4.7: partial regression plots showing associations between expected E (HOMA2 %IR) and expected E(Grey Matter volumes) and expected E(White Matter volumes).
A represents the figures for males. B represent the figures in females. The coefficients presented are for the fully confounder-adjusted model (adjusted for total intercranial volume, age at scan, childhood cognition, childhood, and adulthood socio-economic position, and waist-to-hip ratio, physical exercise levels, and alcohol status and smoking status at the time of the exposure. As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

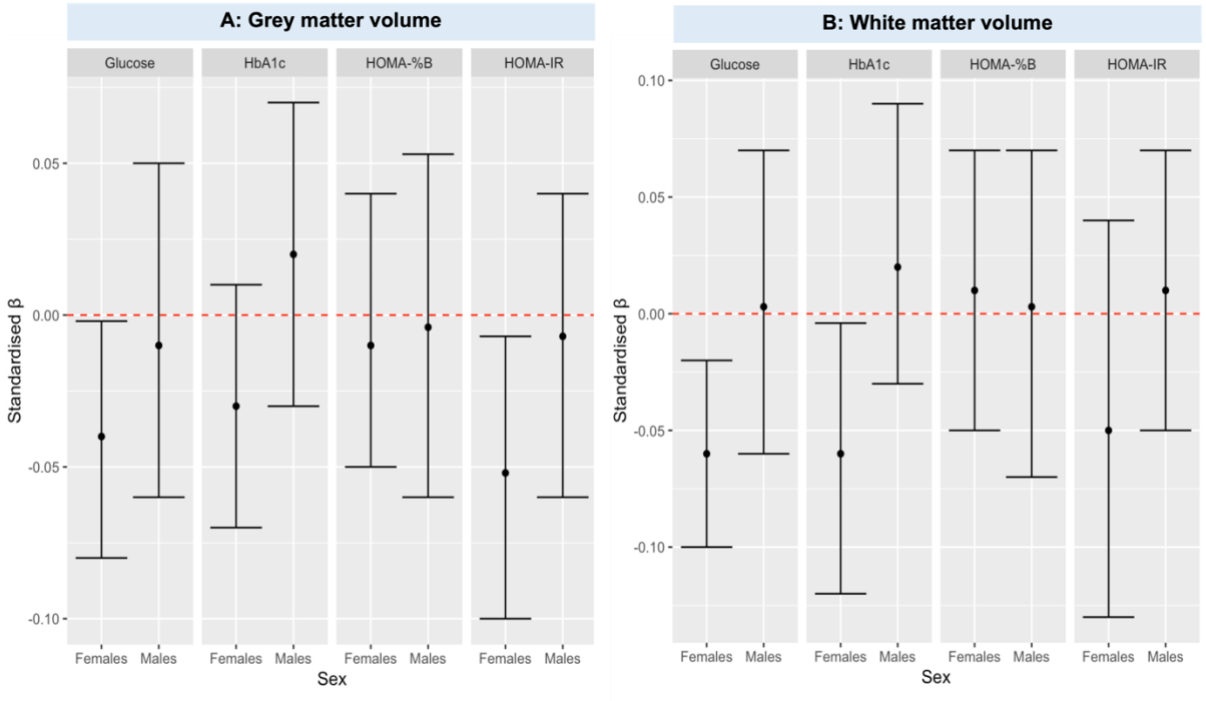


Figure 4.8: Forest plots showing the associations between glycaemic traits at age 60-64 (glucose, HbA_{1c}, HOMA-%B, HOMA-IR) with GM and WM at age 70, stratified by sex.
The coefficients presented are for the fully confounder-adjusted model (adjusted for total intercranial volume, age at scan, childhood cognition, childhood, and adulthood socio-economic position, and waist-to-hip ratio, physical exercise levels, and alcohol status and smoking status at the time of the exposure. The associations between HbA_{1c} and WBV were previously published by Fatih and colleagues,[407] but are included in this model for the sake of comparison.

Overall, adjustments for confounders did not materially change the pattern of effects (Table 4.2). The analyses were also repeated with the non-imputed data and had no discernible effect on the findings (Table 4.6).

		Whole Brain Volumes (WBV)								Gray Matter volumes (GM)								White Matter volumes (WM)							
		Males				Females				Males				Females				Males				Females			
		β*	95% CI		p	β*	95% CI		p	β*	95% CI		p	β*	95% CI		p	β*	95% CI		p	β*	95% CI		p
HbA_{1c}	M1	Previously shown in Chapter 3				Previously shown in Chapter 3				0.015	-0.03	0.06	0.51	-0.04	-0.08	-0.001	0.05	0.01	-0.05	0.07	0.76	-0.03	-0.08	0.16	0.19
	M2									0.02	-0.03	0.07	0.38	-0.04	-0.08	-0.002	0.04	0.02	-0.05	0.08	0.61	-0.05	-0.1	0.002	0.06
	M3									0.02	-0.02	0.07	0.33	-0.03	-0.07	0.006	0.1	0.02	-0.03	0.08	0.4	-0.06	-0.12	-0.004	0.04
Glucose	M1	-0.06	-0.13	0.01	0.1	-0.08	-0.13	-0.02	0.005	-0.02	-0.07	0.03	0.45	-0.05	-0.08	-0.009	0.016	-0.02	-0.08	0.04	0.41	-0.06	-0.1	-0.01	0.02
	M2	-0.06	-0.13	0.02	0.2	-0.08	-0.14	-0.02	0.007	-0.01	-0.06	0.04	0.705	-0.05	-0.09	-0.01	0.01	-0.01	-0.08	0.06	0.78	-0.07	-0.1	-0.21	0.005
	M3	-0.05	-0.13	0.03	0.2	-0.07	-0.13	-0.01	0.02	-0.008	-0.06	0.046	0.77	-0.04	-0.08	-0.002	0.04	0.003	-0.06	0.07	0.92	-0.06	-0.1	-0.02	0.02
HOMA-%B	M1	0.004	-0.77	0.89	0.92	-0.002	-0.07	0.07	0.97	-0.003	-0.05	0.047	0.904	-0.15	-0.6	0.03	0.51	-0.008	-0.07	0.06	0.81	0.01	-0.05	0.07	0.77
	M2	-0.01	-0.09	0.08	0.90	0.001	-0.07	0.08	0.90	-0.007	-0.06	0.047	0.081	-0.01	-0.05	0.04	0.88	-0.016	-0.08	0.05	0.63	0.01	-0.05	0.08	0.67
	M3	-0.02	-0.12	0.09	0.77	-0.01	-0.09	0.07	0.84	-0.004	-0.06	0.053	0.89	-0.01	-0.05	0.04	0.88	0.003	-0.07	0.07	0.93	0.01	-0.05	0.07	0.68
HOMA2-IR	M1	-0.007	-0.07	0.05	0.81	-0.08	-0.17	-0.03	0.04	-0.01	-0.05	0.03	0.66	-0.08	-0.14	-0.023	0.006	-0.001	-0.06	0.04	0.72	-0.04	-0.12	0.03	0.27
	M2	-0.03	-0.09	0.04	0.43	-0.09	-0.18	0.004	0.06	-0.01	-0.05	0.03	0.67	-0.07	-0.13	-0.012	0.02	-0.1	-0.07	0.04	0.68	-0.05	-0.13	0.03	0.21
	M3	-0.06	-0.16	0.05	0.31	-0.12	-0.2	0.002	0.04	-0.007	-0.06	0.042	0.77	-0.06	-0.12	-0.004	0.04	0.01	-0.05	0.07	0.72	-0.05	-0.13	0.04	0.27

Table 4.2: Linear regression analyses of imputed data exploring the relationship between HbA_{1c}, glucose, HOMA2-IR and HOMA%-β on brain imaging outcomes for the whole sample.
The outcomes considered are whole brain, grey matter and white matter volumes. Models were constructed as follows: Model 1: minimally adjusted model for total intercranial volume, sex and age at scanning. Model 2: Model 1 +further adjustments for childhood socio-economic position, adulthood socio-economic position, education and childhood cognition. Model 3: Model 2 + further adjustments for waist-to-hip ratio, physical activity levels, and alcohol and smoking status.

		Whole Brain Volumes (WBV)								Gray Matter volumes (GM)								White Matter volumes (WM)							
		Males				Females				Males				Females				Males				Females			
		β*	95% CI			p	β*	95% CI			p	β*	95% CI			p	β*	95% CI			p			
HbA_{1c}	M1	Previously shown in Chapter 3				Previously shown in Chapter 3				0.01	-0.03	0.06	0.54	-0.04	-0.07	0.002	0.07	-0.004	-0.06	0.05	0.89	-0.028	-0.76	0.02	0.26
	M2									0.02	-0.03	0.07	0.4	-0.04	-0.07	0.003	0.07	0.02	-0.06	0.065	0.93	-0.045	-0.095	0.005	0.07
	M3									0.02	-0.03	0.07	0.34	-0.03	-0.07	0.01	0.1	0.01	-0.05	0.076	0.67	-0.06	-0.12	-0.004	0.04
Glucose	M1	-0.06	-0.13	0.01	0.1	-0.08	-0.13	-0.02	0.005	-0.02	-0.07	0.03	0.4	-0.05	-0.08	-0.01	0.02	-0.03	-0.1	0.03	0.36	-0.06	-0.1	-0.01	0.017
	M2	-0.06	-0.13	0.02	0.2	-0.08	-0.14	-0.02	0.007	-0.01	-0.06	0.04	0.7	-0.05	-0.08	-0.007	0.02	-0.01	-0.08	0.05	0.69	-0.07	-0.12	-0.02	0.004
	M3	-0.05	-0.13	0.03	0.2	-0.07	-0.12	-0.006	0.02	-0.01	-0.06	0.05	0.78	-0.04	-0.08	0.0003	0.05	-0.001	-0.07	0.07	0.98	-0.07	-0.1	-0.02	0.007
HOMA-%B	M1	0.02	-0.05	0.09	0.06	0.01	-0.06	0.08	0.78	-0.012	-0.07	0.047	0.68	-0.02	-0.07	0.03	0.42	-0.03	-0.11	0.04	0.37	0.02	-0.4	0.08	0.47
	M2	-0.0003	-0.08	0.08	0.99	0.004	-0.07	0.08	0.92	-0.021	-0.08	0.04	0.49	-0.11	-0.06	0.04	0.68	-0.05	-0.13	0.026	0.199	0.01	-0.05	0.07	0.65
	M3	-0.004	-0.1	0.09	0.93	0.02	-0.06	0.1	0.63	-0.02	-0.09	0.047	0.56	-0.01	-0.06	0.037	0.59	-0.03	-0.11	0.05	0.45	0.007	-0.05	0.066	0.8
HOMA2-IR	M1	-0.007	-0.07	0.05	0.81	-0.08	-0.17	-0.03	0.04	-0.006	-0.05	0.04	0.8	-0.1	-0.16	-0.03	0.006	-0.02	-0.08	0.04	0.6	-0.04	-0.12	0.04	0.35
	M2	-0.03	-0.09	0.04	0.43	-0.09	-0.18	0.004	0.06	-0.008	-0.06	0.04	0.76	-0.08	-0.15	-0.01	0.03	-0.02	-0.085	0.037	0.44	-0.06	-0.1	0.02	0.13
	M3	-0.04	-0.13	0.05	0.37	-0.1	-0.2	0.002	0.04	-0.006	-0.06	0.05	0.84	-0.08	-0.15	-0.007	0.03	-0.006	-0.076	0.06	0.86	-0.07	-0.15	0.03	0.1

Table 4.3: Linear regression analyses of the raw, complete case data exploring the relationship between HbA_{1c}, glucose, HOMA2-IR and HOMA%-β on brain imaging outcomes for the whole sample.
The outcomes considered are whole brain, grey matter and white matter volumes. Models were constructed as follows: Model 1: minimally adjusted model for total intercranial volume, sex and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education and childhood cognition. Model 3: Model 2 + further adjustments for waist-to-hip ratio, physical activity levels, and alcohol and smoking status.

		Whole Brain Volumes (WBV)				Gray Matter volumes (GM)				White Matter volumes (WM)			
		β*	**95% CI**		**p**	**β***	**95% CI**		**p**	**β***	**95% CI**		**p**
HbA_{1c}	M1	Previously shown in Chapter 3				-0.1	-0.04	0.02	0.4	-0.02	-0.05	-0.02	0.32
	M2					-0.01	-0.04	0.02	0.6	-0.02	-0.06	-0.01	0.21
	M3					-0.004	-0.04	0.03	0.8	-0.03	-0.07	-0.006	0.04
Glucose	M1	-0.07	-0.11	-0.03	0.001	-0.03	-0.06	0.004	0.07	-0.05	-0.1	-0.15	0.005
	M2	-0.08	-0.12	-0.03	0.001	-0.04	-0.07	-0.01	0.03	-0.06	-0.1	-0.18	0.004
	M3	-0.07	-0.12	-0.03	0.002	-0.04	-0.07	-0.01	0.01	-0.04	-0.08	-0.005	0.02
HOMA-%B	M1	-0.03	-0.08	0.03	0.29	-0.04	-0.08	-0.006	0.02	-0.03	-0.07	0.02	0.27
	M2	-0.05	-0.1	0.02	0.19	-0.04	-0.08	0.002	0.06	-0.03	-0.08	0.01	0.16
	M3	-0.1	-0.2	-0.002	0.05	-0.02	-0.06	0.02	0.34	-0.03	-0.08	0.02	0.28
HOMA2-IR	M1	-0.01	-0.04	0.06	0.71	-0.02	-0.06	0.02	0.27	-0.03	-0.05	0.04	0.89
	M2	0.001	-0.06	0.06	0.98	-0.02	-0.06	0.02	0.26	-0.1	-0.06	0.03	0.56
	M3	0.01	-0.05	0.07	0.84	-0.01	-0.05	0.03	0.57	-0.1	-0.05	0.03	0.7

Table 4.4: Linear regression analyses for the whole sample exploring the relationship between HbA_{1c}, glucose, HOMA2-IR and HOMA%-β on brain imaging outcomes for the whole sample.
The outcomes considered are whole brain, grey matter and white matter volumes. Models were constructed as follows: Model 1: minimally adjusted model for total intercranial volume, sex and age at scanning. Model 2: Model 1 + further adjustments for childhood socio-economic position, adulthood socio-economic position, education and childhood cognition. Model 3: Model 2 + further adjustments for waist-to-hip ratio, physical activity levels and alcohol and smoking status. These pooled analyses were not the focus of the study but are shared for completeness.

4.4 Discussion

Summary of findings

In a sample from a population-based birth cohort of people born in the same week, measures of hyperglycaemia (glucose, HbA_{1c}) and IR (HOMA2-IR) at age 60-64 were associated with smaller brain volumes ~10 years later in females only, with limited evidence of preferential tissue type. There was no convincing evidence of one specific glycaemic marker being more strongly associated with poorer brain health. Generally, the associations were fairly weak. No convincing associations emerged between glycaemic traits and brain volume for males, or for pancreatic β cell function (HOMA %B) and brain volume in either sex. Overall, this suggests there may be a stronger association between hyperglycaemia and IR and later-life structural brain volume in females.

Specific findings and associations with the literature

I previously reported that HbA_{1c} across midlife was associated with smaller whole brain volume at age ~70 in females but not in males (Chapter 3 and published work).[407] I now expand on this work by demonstrating that other related, but mechanistically distinct, glycaemic traits (i.e., glucose, and IR) also follow this sex-specific trend. Few population-based studies have had the availability of multiple glycaemic markers to begin disentangling the complex mechanistic traits of diabetes in the population. These results are consistent with the growing evidence demonstrating that poor glycaemic health (in the presence and absence of diabetes) is associated with poorer brain health, with some findings suggesting an increased vulnerability in females.[407,413,429]

There are many possible factors that may explain sex-specific vulnerability to poor glycaemic health. One important mechanism may be related to the critical changes in hormonal health in midlife, specifically the rapid decline of oestrogen in post-menopausal females. Oestrogen has consistently been found to be neuroprotective working via multiple pathways to decrease inflammation, oxidative stress, and vascular reactivity,[455] and it is conceivable that its withdrawal increases vulnerability to hyperglycaemia. Other sex differences, for example the location of adiposity could also play a modifying role since higher abdominal adiposity has been associated with

poorer brain health.[456] Other more gender-related factors related to caregiving could contribute, as females with diabetes with caregiving roles are less likely to attain glycaemic targets and be screened for long-term complications.[457]

Interestingly, in females, the correlations between HbA_{1c} and fasting glucose ($r=54_{females}$), and HbA_{1c} and IR ($r=30_{females}$), were only of moderate strength, consistent with a previous study.[458] It therefore seems plausible that associations between glycaemic traits and smaller brain volume in females could reflect temporal and mechanistic differences between the measures. Fasting glucose represents a single 'snapshot' of glycaemia in a state when insulin requirements are low, whereas HbA_{1c} provides an integrated measure of glycaemic load over a preceding period of 2-3 months. Each marker has been argued to provide different and complementary information on diabetes risk[459] and there is evidence that fasting glucose, HbA_{1c} and IR are subject to different genetic influences.[453] Although I acknowledge that they are related markers, the inclusion of each marker accounted for a higher total variance of the relationship explained (e.g., 72% of WBV variance was explained by the glucose and covariate model, which increased to $r^2=76\%$ when HOMA-IR was included). Other issues may also be important in sex differences: these include variation in glycosylation rates,[460,461] differences in erythrocyte environments[214] and heterogeneity in erythrocyte lifespan.[215]

There were no associations between HOMA-%B and brain volume metrics in either sex. HOMA-%B is a measure of β cell response or insulin secretion in the pancreas, which may not necessarily reflect glycaemia.[462] The relationship between β cell dysfunction and IR are complex, with both mechanisms having been shown to contribute to the development of T2D. Consistent with it being a population-based sample, NSHD only had a small proportion of participants with diabetes. Although in some cases, β cell dysfunction is the primary mechanism underlying diabetes, in many others, this feature appears in the latter stages of the condition. The small degree of variance in HOMA-%B in NSHD may have limited the ability to detect a relationship between HOMA-%B and brain outcomes.[463]

Importance of sub-clinical glycaemic traits

While previous studies have shown that diabetes is associated with smaller WBV,[413,442,452] this study goes beyond examining its relationship to a clinical diagnoses of diabetes, and instead assesses associations with the underlying glycaemic traits. These findings show that a negative relationship between glycaemic traits and smaller brain volume is present in females, even in a population-based sample with only mildly abnormal glycaemic traits. This is consistent with other studies reporting that higher glucose in the normal range (3.2-6.1 mmol/L) and higher glucose independent of diabetes status are associated with lower brain volumes.[410,464] Recent population-based studies using UK Biobank also reported associations between sub-clinical (prediabetes), HbA_{1c} and poorer brain health outcomes including brain volume, hippocampal volume and cognitive decline, independently of diabetes status.[364,365] Together, my findings emphasise the need to manage blood glucose levels in the population, which in turn may help maintenance of optimal brain health in later life.

Preferential tissue

Since diabetes (and hyperglycaemia) are states of abnormal glucose metabolism, I aimed to investigate whether this would be associated with reductions in GM and WM tissue differently, since the former consists primarily of neuronal cells and the latter of supportive glial cells. Previous studies addressing whether diabetes is associated with preferential tissue loss have yielded inconsistent results.[20,410] In this study there is no convincing evidence of an association between glycaemic traits and preferential GM or WM tissue loss. No associations with smaller brain tissue volume emerged in males. In females, glucose was related to both GM and WM volumes; while HbA_{1c} was slightly more related to WM volume; and HOMA2-IR was more strongly related to GM volume, but since the CI between tissue types in females were largely overlapping, there was no robust evidence of a preferential association of tissue type. More recently, a paper explored the relationships between HbA_{1c} and a range of brain volumetric measures in the UK Biobank imaging sample.[365] Their standardised coefficients for their fully confounder-adjusted models for GM (β -0.03) and WM (β = -0.01) volumes were largely consistent with my estimates for WM (β*=-0.06 [-0.12, -0.004]) and GM (β*= -0.03 [-0.07, 0.006]) volumes.

Strengths and weaknesses

By harnessing data from a national birth cohort of deeply phenotyped people who have been studied for over 7 decades since their birth in 1946, I was able to characterise the relationship between a range of glycaemic traits measured at age 60-64 with brain volume metrics, whilst adjusting for an extensive set of key confounders including childhood cognition, education and social class, in addition to important lifestyle factors such as anthropometric body composition, alcohol and exercise use. This enabled me to better characterise the observed relationship between glycaemic traits and smaller brain volume in females. Another strength is that the study followed people born across the UK in the same week, limiting the confounding effect of age in these relationships.

Ascertainment bias is a potential weakness to consider, similar to other longitudinal studies, where individuals who participated in the neuroimaging sub-study were more likely to be educated, from a higher SEP, and have with better overall health.[394] In addition, differential survival rates in this cohort, especially amongst unhealthy males, may have biased the results and contributed to these sex-specific differences.[392] There were also fewer people with available HOMA measures, and this will have reduced the power to detect associations for these exposures.

Overall, the findings add to the growing evidence that female brains may have greater susceptibility to poorer glycaemic health, even with regard to only mildly abnormal glycaemic traits. The effect sizes are quite small; taking the relationship between fasting glucose and WBV as an example, (β*=-0.07 [95%CI: -0.13, -0.01]), cm^3 per 1 SD increase of fasting glucose (1.1mmol/L) in females is equivalent to around 6 months of WBV ageing (1 SD in age = -0.13ml and 1SD in glucose = -0.07) as measured in UK Biobank. This may be important, however, as although the effect reported is small, small effects within large populations could add up to have a significant impact on public health. These findings are also consistent with emerging evidence suggestive of a sex-specific effect of diabetes (and its traits) on brain health [407,413,429] warranting further research.

Future research

Future research should aim to elucidate potential mechanistic mediators of the relationship between poor glycaemic health and poor brain health in females. Potential factors may include inflammation, CVD or kidney health since these have been shown to be impacted by hyperglycaemia which itself has previously been found to be predictive of poorer brain health.[326,465,466]

In addition, future research should investigate whether the sex-specific vulnerability in females with poor glycaemic health is influenced by menopausal status, particularly whether the drop of neuroprotective hormones such as oestrogen/oestradiol around that stage, plays a role in explaining the vulnerability. It may also be of value to further explore whether the relationship between poor glycaemic health and smaller brain volume in females reflects tissue loss across a single specific or multiple brain regions.

4.5 Conclusions

The findings in this chapter indicate that, similarly to HbA_{1c}, there are sex-specific associations between other glycaemic measures in midlife (i.e., fasting glucose and Insulin resistance) and lower brain volume later in life, with greater susceptibility in females. There was no compelling evidence of preferential tissue loss associated with the glycaemic markers in either sex. Overall, this may suggest a greater susceptibility in females to adverse effects of hyperglycaemia and insulin resistance in midlife on later-life structural total brain volume.

5. Examining the role of inflammation as a mediator of the glycaemia-brain health associations in females

Chapter 3 and Chapter 4 revealed evidence of associations between poorer glycaemia and its related traits and lower brain volume in female participants of the National Survey of Health and Development birth cohort. In line with this, this mediation analysis aims to examine whether these associations in females are mediated by inflammation.

5.1 Introduction

Previous analyses in Chapter 3 and Chapter 4 revealed sex-specific associations between glycaemia (indexed by HbA_{1c} and glucose) in midlife and brain volume at age ~70 exclusively in females (also published by Fatih and colleagues).[407] An important next step that follows from these findings is to examine the possible mechanisms that may mediate these sex-specific associations.

One potential mediating factor of this relationship could be inflammation since there is growing evidence of: 1) higher systemic inflammation in females,[434] 2) evidence that inflammation is a central feature of T2D[437] and 3) associations between systemic inflammatory markers such as IL-6 and brain health outcomes both in animals and humans.[28,326,436] Sex differences in inflammation levels may particularly become more prominent and influential following the decline of oestrogen's neuroprotective effect during the perimenopause and following the menopause.[467,468] Oestrogen is a multifunctional hormone with important anti-inflammatory effects. Furthermore, a sex-specific transcriptomic analysis of human myeloid cells in relation to AD also showed that female immune cells, particularly microglia in the brain, exhibit higher activity of inflammation-related genes and AD risk genes compared to males.[469] Thus, systemic inflammation may mediate the sex-specific associations between midlife glycaemia, and volumetric brain health exclusively observed in female participants of the NSHD sample.

As such, the research question investigated here is whether the relationship between glycaemic markers (HbA_{1c} and glucose) at age 60-64 and the different volumetric

measures considered (WBV, WM, and GM) in females at age ~70 is mediated by systemic inflammation (as indexed by IL-6, CRP and GlycA).

5.2 Methods

5.2.1 Sample

The participants were from the NSHD cohort who undertook further assessments as part of Insight 46. The recruitment process has been discussed in Chapter 2. In this analysis, the participants were included if they had a HbA_{1c} or glucose measure at age 60-64 and have volumetric data available. Since my previous findings found these relevant associations only in females, male participants were excluded from the analyses.

5.2.2 Investigations

Neuroimaging

The brain imaging measures considered in this analysis were the volumetric measures WBV, GM volumes and WM volumes. The neuroimaging protocol performed was described in detail in Chapter 2.

Variables and confounding variables

Exposure

HbA_{1c}: HbA_{1c} was measured in a fasting blood sample collected at age 60–64. HbA_{1c} was measured by ion exchange HPLC on a Tosoh analyzer (Tosoh Bioscience, Tessenderlo, Belgium). Additional details for these measures are discussed in Chapter 4.

Glucose: Glucose was also measured using a fasting blood sample collected at age 60-64. It was measured by enzymatic assay using hexokinase coupled to glucose 6-phosphate dehydrogenase, using a Siemens Dimension Xpand analyzer, Siemens Medical Solutions, Erlangen, Germany. Additional details for these measures are discussed in Chapter 4.

Mediators

IL-6: IL-6 was measured on serum samples derived from overnight fasting blood samples taken during clinic or home visits. These samples were initially processed at

the Clinical Research Facility (CRFs) laboratories, where aliquots were frozen and stored before being transferred to the MRC Human Nutrition Research Laboratory in Cambridge for long-term storage. Analyses of IL-6, along with other inflammatory markers, were conducted by the British Heart Foundation Research Centre in Glasgow using plasma and serum aliquots stored at -70°C using Enzyme-linked immunosorbent assay (ELISA) at inter-assay coefficients of variation (CV) of 6.5%. Units are reported in pg/L.

CRP: CRP was collected via blood samples taken at clinic or during a home visit and taken to the MRC Human Nutrition Research laboratory in Cambridge to be stored at -80°C. It was assayed using Particle-enhanced immunoturbidimetric. This assay was chosen for its high sensitivity, which is crucial for accurately measuring CRP levels in blood samples. The inter-assay CV for CRP and the detection limit (sensitivity) were reported as 6.28% and 1ng/ml respectively, indicating the assay's specificity and the minimum level at which CRP could be reliably detected. Units were reported in mg/L.

Glycoprotein acetyls: Alpha1-acid glycoprotein (glycA) was measured using metabolomic analyses performed on serum collected at ages 60–64. All blood samples were collected after an overnight fast and were not subjected to any free-thaw cycles prior to metabolomics. Serum metabolites were assayed using a high-throughput NMR metabolomics platform (by Nightingale Health using Bruker AVANCE III 500 MHz and Bruker AVANCE III HD 600 MHz spectrometers) able to quantify up to 233 metabolite measures and ratios representing a broad molecular signature of systemic metabolism. For the majority of the metabolic biomarkers, the inter-assay CVs across spectrometers were below 5%. More details have been discussed by Soininen and colleagues. [470]

Confounders

Confounders were identified based on prior knowledge of associations between hyperglycaemia and inflammation, and inflammation and brain health, which were then represented through a DAG (see Figure 5.1B). Confounders were considered for both the exposure-mediator relationships and the mediator-outcome relationships. In this analysis, the confounders considered were:

Socioeconomic position: Childhood SEP was measured as father's occupational social class recorded at age 4 (or if missing, at age 11) and categorised into manual or non-manual according to the UK Registrar General's Standard's Occupation Classification. Adult SEP was based on head of household occupation at age 53 years. These were coded according to the UK Registrar General's Standard Occupational Classification, then grouped as follows: I (professional), II (managerial and technical), IIIN (skilled non-manual), IIIM (skilled manual), IV (partly skilled), and V (unskilled).

Education: The highest educational attainment or training qualification achieved by 26 years was classified according to the Burnham scale[471] and grouped into the following: no qualification; below ordinary secondary qualifications (e.g., vocational qualifications); ordinary level qualifications ('O' levels or their training equivalents); advanced level qualifications ('A' levels or their equivalents); or higher education (degree or equivalent).

Alcohol: Information on alcohol consumption over the previous 7 days was obtained by a self-completed questionnaire that participants completed between the ages of 60-64. Questions asked about drinking choices more specifically the consumption of: 1) spirits or liqueurs (number of measures), 2) wine, sherry, martini, or port (number of glasses), and 3) beer, lager, cider, or stout (number of half-pints). Responses to these three items were totalled to provide an approximate measure of drinks per week, where a drink (or unit in UK terminology) contains ~9.0 g of alcohol. Participants were then categorised into two categories: those who drunk under 14 units of alcohol per week and those who drunk over 14 units of alcohol per week.

Smoking: Smoking status at age 60-64 was assessed by self-report and was classified into three groups: current smokers, ex-smokers, and never-smokers.

Physical activity: Physical activity was collected at age 60–64 using the EPIC physical Activity questionnaire-2, originally derived from Minnesota leisure time physical activity questionnaire.[472] This assessed how often participants had had taken part in any sports, vigorous leisure activities or exercise in the previous 4 weeks. Similarly to previous work in the sample, at each age, responses were categorised into: 1) not active (no participation in physical activity/month), 2) moderately active (participated 1–4 times/month) and 3) most active (participated 5 or more times/month) as previously described by Black and colleagues.[473] Previous research within the NSHD cohort revealed a consistency between patterns of variation obtained through self-reported and objective measures of physical activity.[474]

Body mass index: body mass index was calculated using the following equation: (weight(kg)/height(m^2)).

Arthritis: Arthritis status was assessed through questionnaires asking whether participants had taken non-steroid medication at age 60-64 using a postal questionnaire.

COPD: COPD categorisation was made based on the presence of airflow obstruction defined by the ratio of FEV_1/FVC of less than the lower limit of normal or 0.7.[475]

5.2.3 Statistical analyses

Statistical analyses were conducted using Stata 17 (StataCorp, College Station, TX, USA). Two-component mediation analysis was conducted using the 'sem' package (StataCorp). This approach, which is analogous to path analysis, decomposes the total 'effect' (i.e., association between exposure and outcome) into a direct, and an indirect (or mediated) effect.[476,477] While a three-component mediation analysis which also included a mediated interactive effect was contemplated, for reasons that will become apparent from the results, this was not performed. Note that in this analysis framework the term effect is used to describe the relationships between exposure, mediator and outcome. Such relationships or associations can only be interpreted as causal under very strong assumptions that are arguably never (or perhaps almost never) satisfied. Some eschew the term 'effect' in all epidemiological studies for this reason, I use the term here since it is standard terminology but emphasise that casual interpretations should not be applied to the term effect in this context. This is discussed by Hernan and colleagues.[478,479]

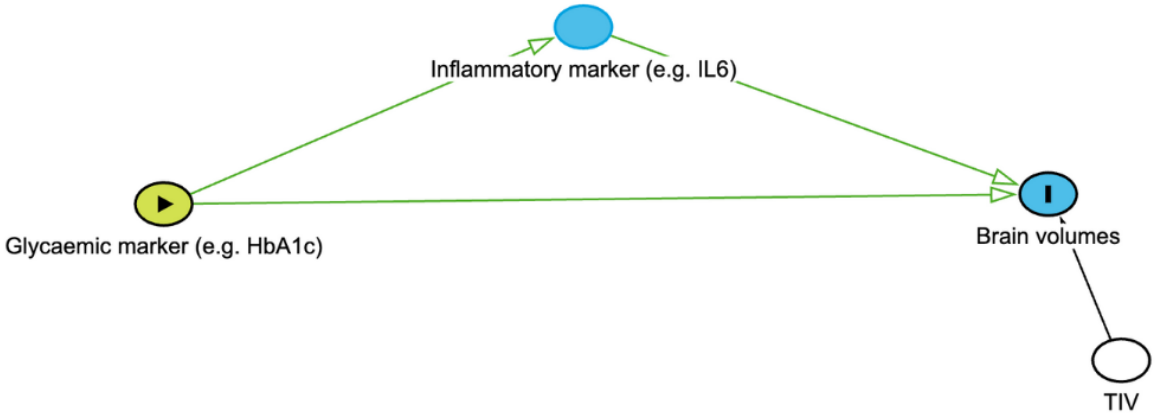
All models were estimated using full information maximum likelihood, which assumes linear relationships and multivariate normality but allows for missing data under a MAR assumption and was therefore considered preferable to estimation based on complete case data.

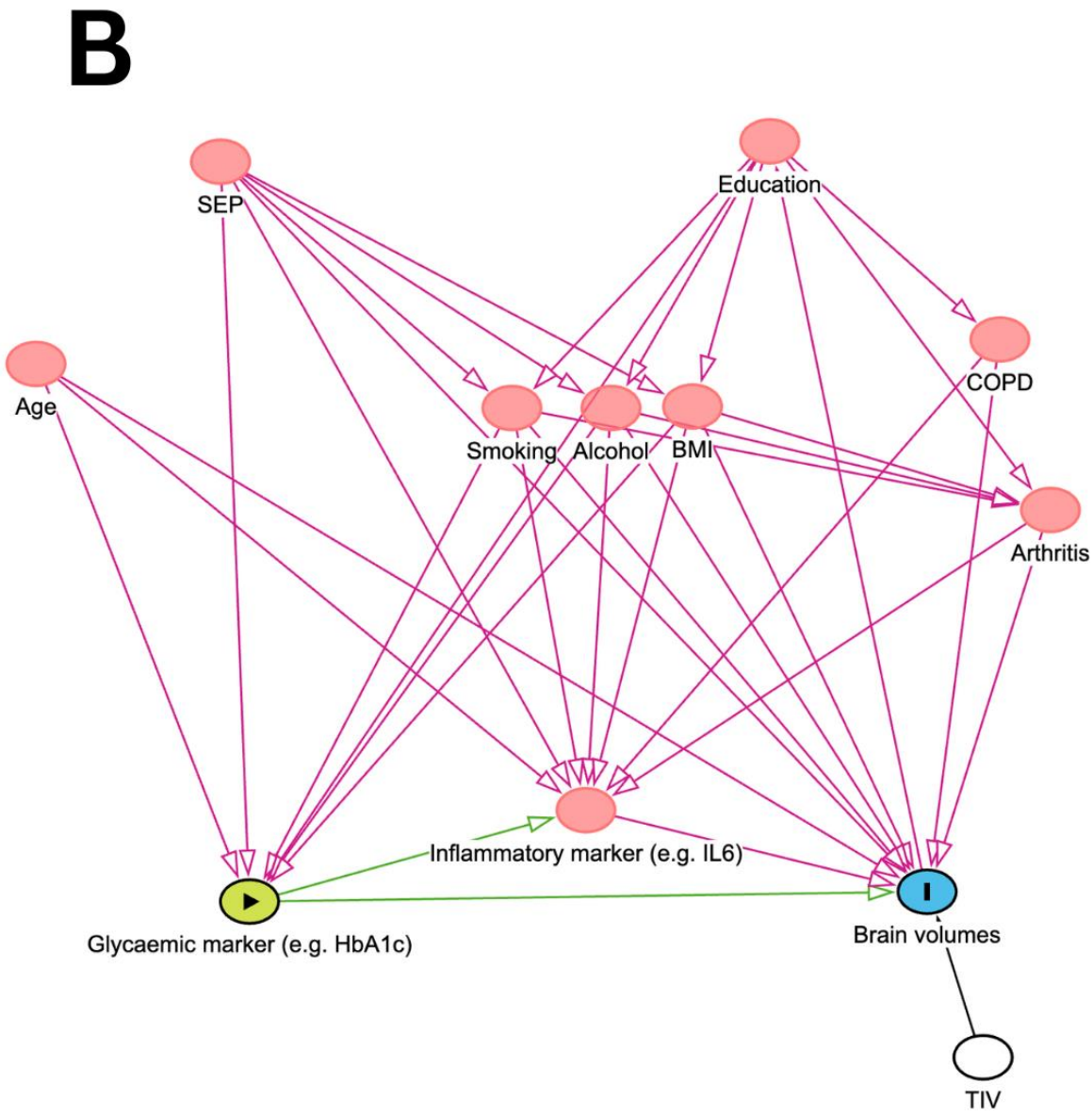
The exposure variable was HbA_{1c} at age 60-64. The putative mediator variables were IL-6, CRP and GlycA measured at age 60-64 (Figure 5.1C). The outcome variables were WBV, GM and WM volumes. Due to their skewed distribution, IL-6 and CRP were log-transformed to conform with the multivariate normal assumptions of structural equation modelling. In addition, participants with CRP values below the limit of detection (LOD), (i.e., under the value of 1mg/L) were assigned a value of 1 mg/L.

The first sets of path analyses were conducted to model the relationship between the glycaemic markers and each inflammatory marker (IL-6, CRP and GlycA) and outcome (WBV, WM volume and GM volume). This mediation model was initially created as a minimally confounder-adjusted model (Figure 5.1B) but then built into a fully confounder-adjusted model (Figure 5.1B).

The other path analysis approach used to investigate the indirect effect of glycaemic markers on brain health outcomes operationalised inflammation as a latent variable using IL-6, CRP, and GlycA. As per the model above, the latent variable model was initially built as a minimally confounder-adjusted model but then constructed with full adjustments for confounders. For the model with a latent variable, a number of fit indices were then explored to test model adequacy, more specifically the Chi-square test, root mean square error of approximation (RMSEA) and the comparative fit index (CFI).The confounders used were SEP, education, BMI, alcohol, smoking status, physical activity, arthritis disease, and COPD. In addition, TIV and age at scan were adjusted for.

A




B
SEP
Education
Age
COPD
Smoking
Alcohol
BMI
Arthritis
Inflammatory marker (e.g. IL6)
Glycaemic marker (e.g. HbA1c)
Brain volumes
TIV

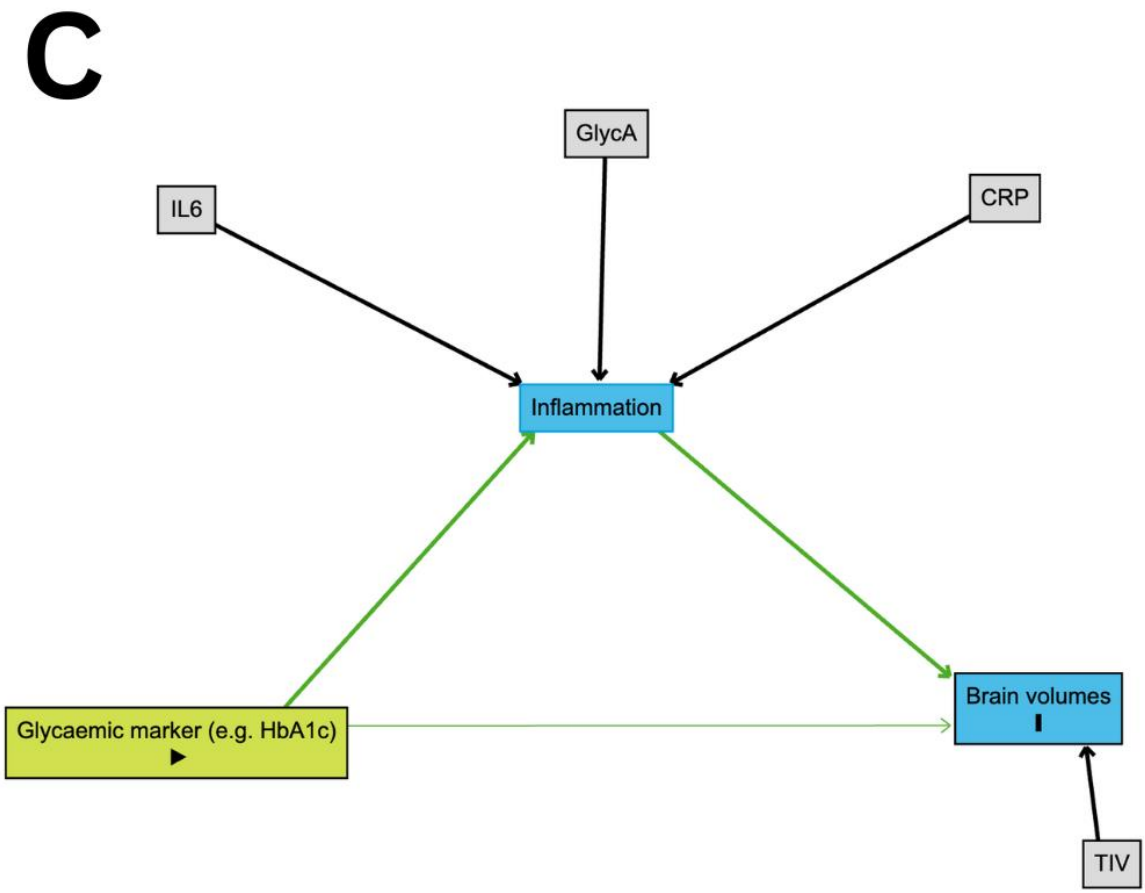


Figure 5.1: Path analysis models conducted in the analyses
A) Simple mediation model as a first step of model building. B) Fully confounder-adjusted model. Adjustments were made for both exposure-mediator and mediator-outcome relationships. C) Conceptual latent variable model considering each inflammatory marker together (i.e., IL-6, glycA and CRP) to get a more comprehensive measure of systemic inflammation. Although not displayed to enhance visibility, the same confounder-adjusted approach as diagram B was taken for the latent model.

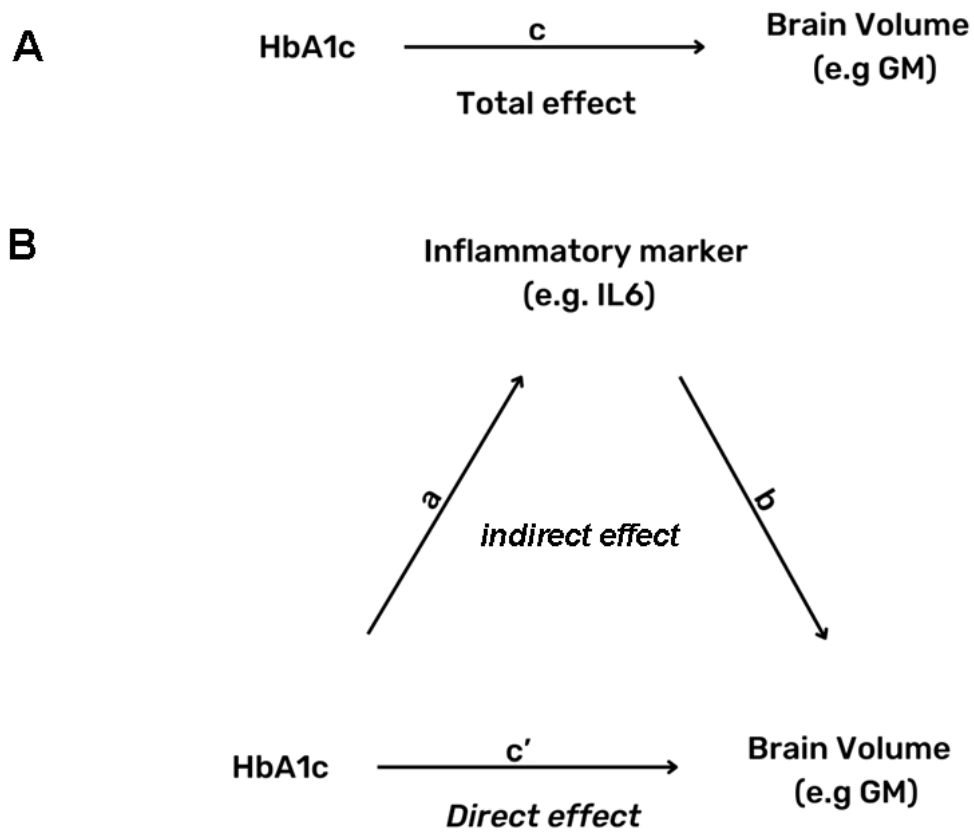


Figure 5.2: A theoretical analysis of the mediation models considered.
A) shows the assumed (total) effect, c. B) the total effect decomposed into a direct, c' and indirect (mediated via a and b) effect.

Sensitivity analyses

Sensitivity analyses were performed, in which CRP values below the LOD were replaced with a value of 0mg/L or with a value 1mg/L.

5.3 Results

A flowchart of the participants considered is presented in Figure 5.3 and sample characteristics are shown on Table 5.1.

43 out of 216 participants had a CRP below the LOD of 1mg/L. Assigning these to a value of 1mg/L or 0mg/L had negligible effect on the results obtained and hence it was assumed that the analysis was insensitive to the values assigned below the LOD.

For IL-6 analyses, 10 participants with IL-6 levels above the maximum level reliably quantified by the current methods (10pg/mL) were not considered.

CRP and IL-6 were both skewed. As a result, quantile-normality plots were run to see which function would best fit the data. In both cases, a log transformation was the best solution. Figure 5.4 and Figure 5.5 show the plots and respective transformation of IL-6.

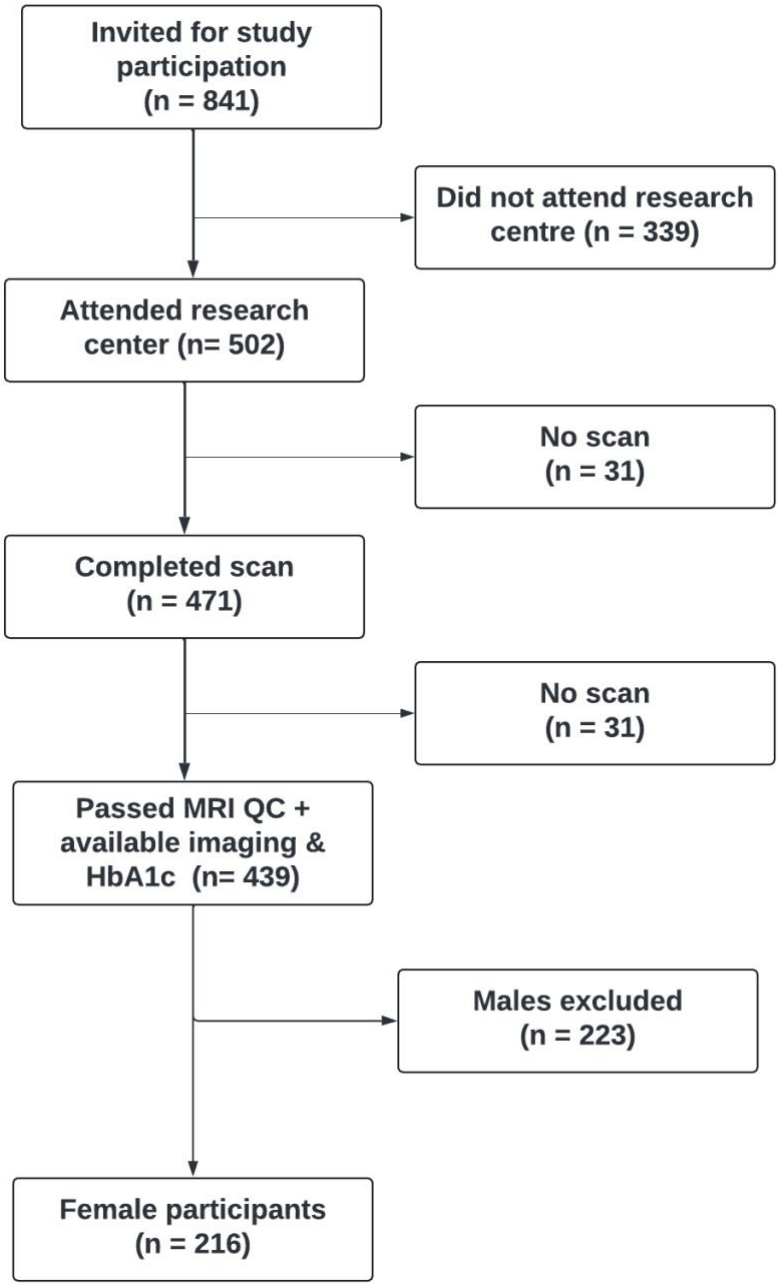


Figure 5.3: Flowchart providing an overview of Insight 46 recruitment of National Survey of Health and Development participants who undertook imaging and were part of my study. To be considered in this study, participants had to have available volumetric imaging data, HbA_{1c} data at age 60-64 and be a female. This amounted to 216 participants being included in the study.

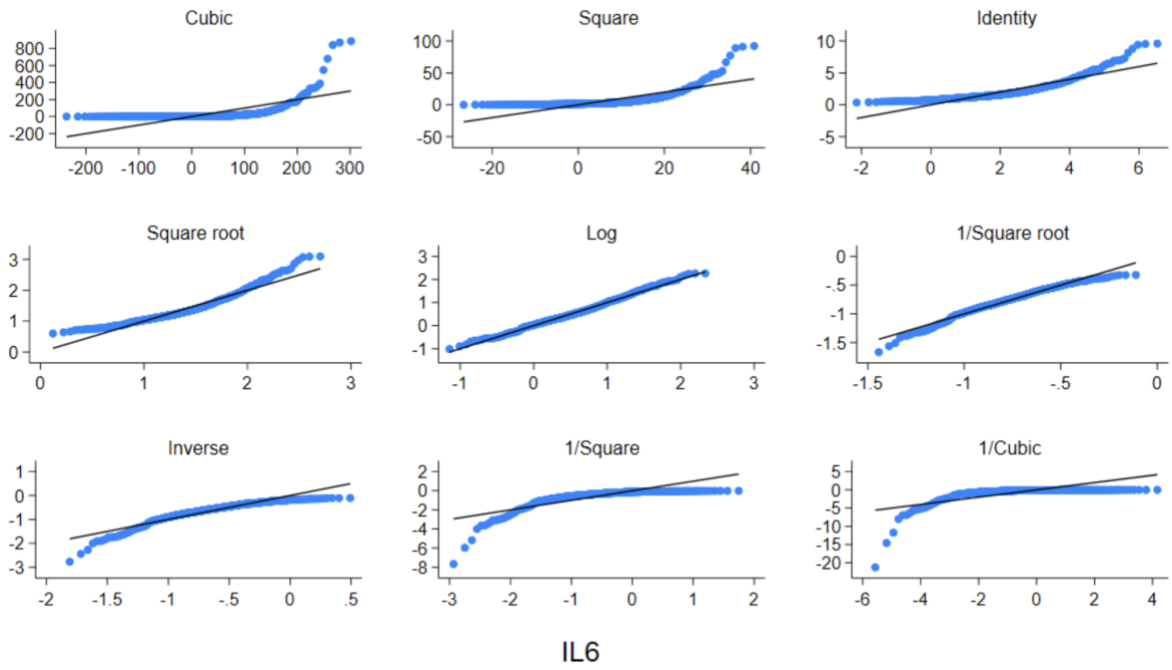


Figure 5.4: Quantile-normal plots showing the different possible transformations for interleukin-6 (with the limit of detection assigned value of 1mg/L).
The log-transformation appeared to offer the best fit.

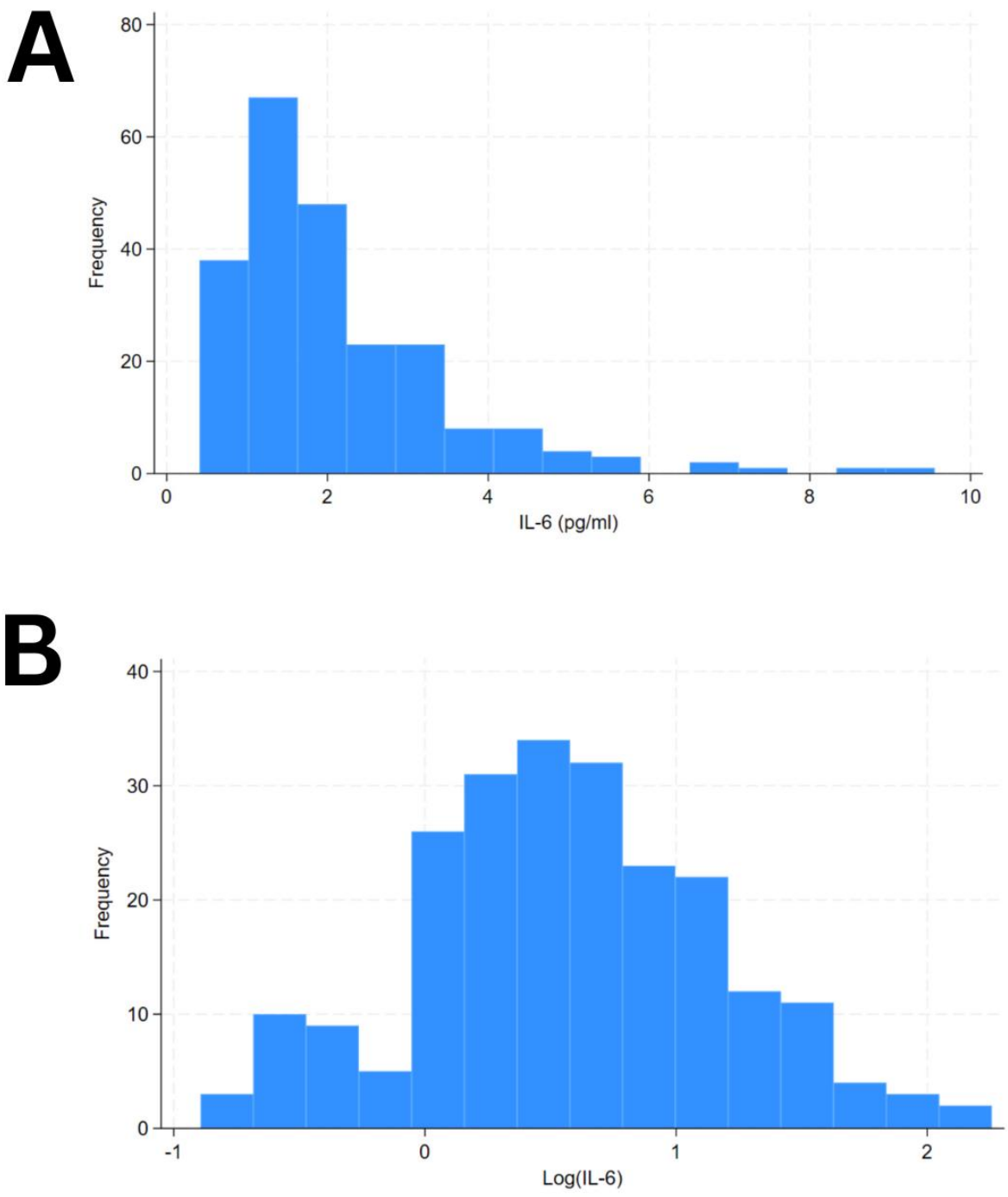


Figure 5.5: Transformation of interleukin-6.
Plot A represents the raw measure of interleukin-6 (pg/ml) and Plot B represents interleukin-6 after log transformation.

Participant characteristics		n	
Standardised childhood cognition score		212	0·44 (0·74)
Education			
	No qualifications		30 (14%)
	Below O-levels (vocational)		18 (8%)
	O-levels and equivalents		53 (25%)
	A-levels and equivalents		80 (37%)
	Degree or higher		35 (16%)
Adult socioeconomic position			216
	Non-manual (Class I–IIIN)		186 (87%)
	Manual (Class IIIM-V)		30 (13%)
Childhood socioeconomic position			212
	Non-manual (Class I–IIIN)		114 (55%)
	Manual (Class IIIM-V)		98 (45%)
HbA_{1c}, %,		216	5.8 (0.55)
HbA_{1c}, mmol/mol		216	38.8 (6)
Interleukin-6 (IL-6) pg/mL		204	2.1 (1.4)
C-reactive protein (CRP) (mg/L)		212	3.5 (6.6)
GlycA (mg/L)		202	1.1 (0.3)
Diabetes medication use		216	6 (2.7%)
BMI kg/m2		216	27.5 (4.9)
Smoking status			
	Current Smokers		10 (5%)
Smoking status	Ex-smokers		70 (34%)
	Never smoker		125 (61%)
Alcohol (units/week)			216

	≤ 14		198 (91%)
	> 14		18 (9%)
Exercise levels			219
	Inactive		107 (49%)
	Moderately active		47 (21%)
	Most Active		65 (30%)
Neuroimaging metrics, age 69-71			
Mean age at scanning, years		216	70.7 (0.7)
Whole brain volume (WBV), mL		216	1046.7 (82.4)
White matter volumes (WM), mL		216	394.3 (2.8)
Grey matter volumes (GM), mL		216	602.6 (3)
Total intracranial volume (TIV)		216	1342.4 (91.8)

Table 5.1: Sample characteristics for the participants considered in the analysis (n = 216).
Values are n (%), mean (SD) and median (IQR). Whole brain, grey matter and white matter volume measurements reported are unadjusted for total intercranial volumes for these descriptions. % are calculated against the max data available for that specific measure for the pooled sample. SD: Standard deviation. As described above, to be considered in the study, participants had to have available volumetric imaging data, HbA1c data at age 60-64 and be a female which amounted to 216 participants.

Linear correlation between inflammatory markers

	IL6	GlycA	CRP
IL6			
GlycA	0.1 (0.01)		
CRP	0.4 p<0.001	0.2 (0.004)	

Table 5.2: Correlation matrix displaying the correlation between the different inflammatory markers: interleukin-6, glycoprotein-A and c-reactive protein.
On the first row, the r value represents the direction of correlation (from a Pearson's correlation). In the second row, the p value represents the strength of any association.

The correlation matrix shows weak associations between the different inflammatory markers. The associations were strongest between CRP and IL-6 and least strong between GlycA and CRP.

Path analysis of HbA_{1c} and brain health outcomes

The results presented here are the path analysis for the fully confounder-adjusted models. Prior to these, minimally adjusted models were run for model construction purposes but were not included here.

Whole brain volumes

There was a total effect of HbA_{1c} on WBV for all the models that included an inflammatory marker (see Table 5.3).

In the mediator model, a direct effect of HbA_{1c} on WBV was also observed for CRP (β = -2.0, [3.3, -0.7], p = 0.002), IL-6 (β = -1.9, [-3.2, -0.5], p = 0.005), and GlycA (β = -1.8, [-3.2, -0.5], p = 0.006). However, there was no indirect effect of HbA_{1c} on WBV through any of the inflammatory pathways: CRP (β = -0.01, [-0.2, 0.2], p = 0.8), IL-6 (β = -0.1, [-0.3, 0.1], p = 0.28), or GlycA (β = -0.08, [-0.3, 0.1], p = 0.4). The glycaemia-inflammation pathway was not significant for CRP (β = 0.01, [-0.1, 0.1], p = 0.9) but was significant for IL-6 (β = 0.05, CI = 0.02, 0.01, p = 0.001) and GlycA (β = 0.02, [0.01, 0.02], p = 0.0001). The inflammatory marker-brain pathway showed no convincing effect for any of the three markers: CRP (β = -1.9, [-3.8, 0.1], p = 0.07), IL-6 (β = -3.1, [-7.7, 1.5], p = 0.2), or GlycA (β = -10.5, CI = -31.4, 10.2, p = 0.3).

Grey matter volumes

There was a total effect of HbA_{1c} on GM volumes for all of the models that included an inflammatory marker (see Table 5.3). Similarly, a direct effect of HbA_{1c} on GM volumes was also observed for CRP (β = -0.6, [-1.1, -0.2], p = 0.004), IL-6 (β = -0.6, [-1.0, -0.2], p = 0.006), and GlycA (β = -0.6, [-1.0, -0.2], p = 0.007). However, there was no indirect effect of HbA_{1c} on GM volumes through the inflammatory pathways: CRP (β = -0.003, [-0.1. 0.05], p = 0.9), IL-6 (β = -0.1, [-1.1, 0.3], p = 0.2), or GlycA (β = 0.0003, [-0.06,0.3], p = 0.9). The glycaemia-inflammation pathway was not significant for CRP (β = 0.01, [-0.1, 0.1], p = 0.9) but was significant for IL-6 (β = 0.05, [0.02, 0.01], p = 0.001) and GlycA (β = 0.02, [0.01, 0.02], p = 0.0001). The inflammatory marker-brain

pathway showed no convincing effect for any of the three markers: CRP (β = -0.5, [-1.1, 0.2], p = 0.1), IL-6 (β = -0.3, [-1.8, 1.2], p = 0.7), or GlycA (β = 0.04, [6.9, 7.0], p = 0.9).

White matter volumes

Since there was no total effect of HbA_{1c} on WM volumes, the results for these analyses were not decomposed into direct and indirect effects. For completeness, the tables are shown in Table 5.3.

Whole brain volumes (WBV)												
		c-reactive protein (CRP)				interleukin-6 (IL-6)				glycoprotein-A (GlycA)			
	Path	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**
Total effect	c	-2.1	-3.4	-0.8	0.002	-2.0	-3.3	-0.7	0.003	-1.9	-3.2	-0.6	0.004
Direct effect	c'	-2.0	-3.3	-0.7	0.002	-1.9	-3.2	-0.5	0.005	-1.8	-3.2	-0.5	0.006
Indirect effect		-0.01	-0.2	0.2	0.8	-0.1	-0.3	0.1	0.28	-0.08	-0.3	0.1	0.4
Exposure-mediator	a	0.01	-0.1	0.1	0.9	0.05	0.02	0.01	0.001	0.02	0.01	0.02	0.0001
Mediator-outcome	b	-1.9	-3.8	0.1	0.07-	-3.1	-7.7	1.5	0.2	-10.5	-31.4	10.2	0.3
Grey matter volumes (GM)													
		c-reactive protein (CRP)				interleukin-6 (IL-6)				glycoprotein-A (GlycA)			
	Path	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**
Total effect	c	-0.6	-1.1	-0.2	0.008	-0.6	-1.0	-0.2	0.008	-0.6	-1.0	-0.2	0.008
Direct effect	c'	-0.6	-1.1	-0.2	0.004	-0.6	-1.0	-0.2	0.006	-0.6	-1.0	-0.2	0.007
Indirect effect		-0.003	-0.1	0.05	0.9	-0.1	-1.1	0.3	0.2	0.0003	-0.06	0.3	0.9
Exposure-mediator	a	0.01	-0.1	0.1	0.9	0.05	0.02	0.01	0.001	0.02	0.01	0.02	0.0001
Mediator-outcome	b	-0.5	-1.1	0.2	0.1	-0.3	-1.8	1.2	0.7	0.04	-6.9	7.0	0.9
White matter volumes (WM)													
		c-reactive protein (CRP)				interleukin-6 (IL-6)				glycoprotein-A (GlycA)			
	Path	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**
Total effect	c	-0.4	-0.9	0.2	0.18	-0.3	-0.8	0.2	0.2	-0.3	-0.8	0.2	0.2
Direct effect	c'	-0.4	-0.9	0.2	0.19	-0.3	-0.8	0.2	0.3	-0.3	-0.8	0.2	0.3
Indirect effect		-0.01	-0.1	0.1	0.8	-0.3	-0.1	0.05	0.5	-0.1	-0.1	0.03	0.2
Exposure-mediator	a	0.01	-0.1	0.1	0.9	0.05	0.02	0.01	0.001	0.02	0.01	0.02	0.0001
Mediator-outcome	b	-0.6	-1.4	0.2	0.2	-0.7	-2.5	1.2	0.4	-0.3	-0.8	0.3	0.3

Table 5.3: Path analysis of the fully confounder-adjusted models of the HbA_{1c} -brain associations (whole brain, grey matter and white matter volumes) via inflammation (c-reactive protein, interleukin-6 and glycoprotein-A) The table presents the β coefficients, confidence intervals and p values.
Path a is the effect of HbA_{1c} on the respective inflammatory mediator.
Path b is the effect of the respective inflammatory on the respective brain volume.
Path c' is the direct effect of HbA_{1c} on the respective brain volume.
Path c is the total effect of HbA_{1c} on the respective brain volume

HbA_{1c}-glucose brain outcomes

The findings for glucose on WBV, GM and WM volumes were similar to those for HbA_{1c} (fully confounder-adjusted models). For WBV, GM and WM volumes, there was a total effect of glucose on WBV for CRP, IL-6, and GlycA, with significant direct effects observed across these markers. However, there were no significant indirect effects through the inflammatory pathways. The exposure-mediator pathway was not significant for CRP but was significant for IL-6 and GlycA. The mediator-outcome pathway showed no significant effect for any of the markers.

Overall, the trends observed for glucose were consistent with those seen for HbA_{1c}, suggesting the role of both glycaemic measures in influencing brain volumes through direct effects rather than through indirect inflammatory pathways. These are presented in Table 5.4.

Whole brain volumes (WBV)												
		c-reactive protein (CRP)				interleukin-6 (IL-6)				glycoprotein-A (GlycA)			
	Path	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**
Total effect	c	-6.5	-11.4	-1.6	0.009	-6.5	-11.4	-1.6	0.01	-6.4	-11.3	-1.5	0.01
Direct effect	c'	-6.4	11.3	-1.5	0.01	-6.1	-11.0	-1.2	0.01	-6.0	-11.0	-1.0	0.02
Indirect effect		-0.1	-0.8	0.5	0.7	-0.4	-1.1	0.3	0.3	-0.4	-1.5	0.6	0.4
Exposure-mediator	a	0.1	-0.3	0.5	0.7	0.1	-0.04	0.3	0.1	0.05	0.01	0.1	0.005
Mediator-outcome	b	-2.0	-3.5	0.4	0.1	-3.4	-8.1	1.3	0.1	-9.4	-30.6	11.6	0.38
Grey matter volumes (GM)													
		c-reactive protein (CRP)				interleukin-6 (IL-6)				glycoprotein-A (GlycA)			
	Path	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**
Total effect	c	-1.7	-3.4	-0.09	0.04	-1.7	-3.4	-0.05	0.04	-1.7	-3.4	-0.04	0.04
Direct effect	c'	-1.7	-3.4	-0.06	0.04	-1.6	-3.3	-0.04	0.05	-1.7	-3.4	-0.02	0.04
Indirect effect		-0.03	-0.2	0.1	0.7	-0.05	-0.2	0.1	0.6	-0.001	-0.3	0.3	0.9
Exposure-mediator	a	0.1	-0.3	0.5	0.7	0.1	-0.04	0.3	0.1	0.05	0.01	0.08	0.005
Mediator-outcome	b	-0.4	-1.0	0.2	0.2	-0.4	-1.9	1.0	0.5	-0.03	-7.1	7.0	0.9
White matter volumes (WM)													
		c-reactive protein (CRP)				interleukin-6 (IL-6)				glycoprotein-A (GlycA)			
	Path	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**
Total effect	c	-2.3	-4.3	-0.3	0.02	-2.2	-4.2	-0.2	0.03	-2.2	-4.2	-0.2	0.03
Direct effect	c'	-2.2	-4.2	-0.3	0.03	-2.2	-4.2	-0.1	0.04	-2.0	-4.0	-0.3	0.05
Indirect effect		-0.05	-0.03	0.2	0.7	-0.01	-0.3	0.2	0.5	-0.2	-0.7	0.2	0.3
Exposure-mediator	a	0.1	-0.3	0.5	0.7	0.1	-0.04	0.3	0.1	0.05	0.01	0.08	0.006
Mediator-outcome	b	-0.6	-1.4	0.2	0.1	-0.7	-2.5	1.1	0.4	-5.3	-13.7	3.1	0.2

Table 5.4: Path analysis of the fully confounder-adjusted models of the fasting glucose-brain associations (whole brain, grey matter and white matter volumes) via inflammation (c-reactive protein, interleukin-6 and glycoprotein-A).
The table presents the β coefficients, confidence intervals and p values.

Latent variable analysis

For the latent model with HbA_{1c}, there was a significant total effect on WBV, but no total or direct effect on GM or WM volumes (see Table 5.5). There were no significant indirect effects observed for WBV, GM, or WM volumes. The exposure-mediator pathway was not significant for any of the volumes, and the mediator-outcome pathways were constrained.

For the latent model with glucose, there was a significant total effect on WBV and WM volumes, but not on GM volumes. The direct effect was significant for WBV but not for GM or WM volumes. There were no significant indirect effects observed for any of the associations. The exposure-mediator pathway was not significant for any of the volumes, and the mediator-outcome pathways were constrained (see Table 5.5).

Model checks using several fit indices were used to determine the adequacy of the latent variable model. The Chi-square test was significant ($p = 0.003$), indicating that the model was misspecified to some extent. This result is not unexpected, given the sensitivity of the Chi-square test to sample size. The RMSEA value was 0.08, which is at the upper limit of what is considered a “reasonable fit”, suggesting that there is some error in approximation, but it is not excessively poor. The CFI value was 0.9, which falls within the range of acceptable fit (0.90 and 0.95 are deemed acceptable). In summary, these fit indices present a mixed picture of the model's adequacy: the significant Chi-square test and the RMSEA value suggest some limitations in model fit, while the CFI indicates an acceptable fit.

			Whole brain volumes (WBV)				Grey matter volumes (GM)				White matter volumes (WM)			
		Path	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**
HbA_{1c}	Total effect	c	-1.1	-2.1	-1.0	0.03	-0.2	-0.7	0.3	0.3	-0.2	-0.6	0.2	0.3
	Direct effect	c'	-0.5	-1.7	0.6	0.3	-0.2	-0.7	0.3	0.3	0.01	-0.5	0.5	0.9
	Indirect effect		-0.5	-1.3	0.1	0.1	-0.02	-0.1	0.1	0.7	-0.2	-0.5	0.08	0.1
	Exposure-mediator	a	-0.5	-1.3	0.1	0.1	-0.02	-0.1	0.1	0.7	-0.2	-0.5	0.08	0.1
	Mediator-outcome	b	1 Constrained				1 Constrained				1 Constrained			
		Path	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**
Glucose	Total effect	c	-6.5	-11.4	-1.6	0.009	-0.1	-0.3	1.2	0.3	-2.3	-4.2	-0.3	0.03
	Direct effect	c'	-5.3	-10.3	-0.02	0.04	-0.1	-3.2	1.4	0.4	-1.9	-3.9	0.2	0.08
	Indirect effect		-1.2	-3.1	0.7	0.2	-0.07	-1.4	1.3	0.9	-0.4	-1.2	0.3	0.2
	Exposure-mediator	a	-1.2	-3.1	0.7	0.2	-0.07	-1.4	1.3	0.9	-0.4	-1.2	0.3	0.2
	Mediator-outcome	b	1 Constrained				1 Constrained				1 Constrained			

Table 5.5: Path analysis output of the latent model for the HbA_{1c} brain associations (whole brain, grey matter and white matter volumes) via the latent variable of inflammation (constructed using c-reactive protein, interleukin-6 and glycoprotein-a).
The total effects, direct, indirect effects, exposure-mediator are presented. β, CI and p-values are presented.

5.4 Discussion

Summary of findings

The aim of this mediation analysis was to get a better insight into an important potential mechanism linking hyperglycaemia with smaller brain volumes observed in female participants part of the NSHD cohort (reported in Chapter 3 and Chapter 4). In line with the literature, the potential role of systemic inflammation as a mediator of these relationships was tested.

The path analyses confirmed (as shown previously) that there was: 1) a total effect of glycaemic markers on the brain imaging measures, 2) hyperglycaemia was associated with higher inflammation (for IL-6 and GlycA) but 3) there were no credible associations between the inflammatory markers and volumetric brain measures. Hence overall there was no indirect effect of glycaemia on brain outcomes via the inflammation path. Considering the inflammatory markers as a latent variable for inflammation had little influence on the results. Considering the fasting glucose measure instead of HbA_{1c} as the exposure did not materially change the findings.

Thus, the findings suggest that while both high glucose and increased inflammation are related, inflammation, as measured by serum biomarkers, does not mediate the relationship between glucose levels and brain volume reduction in this sample.

Specific findings and associations with the literature

The study was motivated by previous research suggesting that individuals with T2D show elevated levels of inflammatory markers compared to healthy controls (regardless of disease duration).[318] Similarly, females with T2D showed higher inflammation TNF-α, IL-6 and CRP compared to females without the condition.[433,480] In line with this, there was evidence that inflammatory markers were associated with poorer brain health and that cytokines accumulate at different rates in AD patients compared with healthy control subjects.[323,326,436]

My observation that poorer glycaemic control was associated with higher inflammation, is in line with previous evidence in a mixed sample of individuals with T2D and healthy controls.[481] This is consistent with the known effect of

hyperglycaemia on NF-κB–dependent inflammatory cytokine production and other mechanisms linked to the activation of inflammatory pathways.[437,482]

Evidence linking inflammation to brain health is less consistent. Previous analyses from population-based studies have revealed mixed findings in regard to the association between markers of inflammation and brain health outcomes. For example, recent findings from the ARIC study failed to find an association with WBV (a 1 SD change in an inflammation composite score was associated with -1.9 cm^3 ([CI: -6.5, 2.5], p = 0.4) cm^3 decrease in total brain volume.[326] Although null, their 95% CI for the inflammation-WBV relationship were consistent with the results from my NSHD analyses where a 1 SD increase in IL-6 was associated with a -3.4 (CI: -8.1, 1.2, p = 0.1) cm^3 decrease in WBV. In contrast, the Framingham study found that most, but not all, of the inflammatory markers they considered were associated with lower total brain volume.[327] Reasons for these differences are not known. More generally, it is possible that subtle inflammatory processes within the brain microenvironment, such as glial activation or neuronal damage are not captured by systemic markers. It would be worth revisiting this question in a few years to assess whether a potential mediating role of inflammation emerges with increased age. Also, the on-going study of Insight 46 (wave 3) will have biomarkers measured in CSF which would allow investigation of this question using more direct measures of brain inflammation.

Few population-based studies have had the availability of multiple inflammatory markers. IL-6, CRP, and GlycA, each measure providing unique and complementary insights into the inflammatory process. IL-6 is a cytokine that plays a central role in initiating and sustaining inflammatory responses, serving as an early indicator of inflammation. CRP, an acute phase protein, reflects the immediate inflammatory response and is sensitive to short-term changes, although it exhibits high intra-individual variability. GlycA, a novel composite biomarker offers stability and less variability over time by capturing a broader spectrum of markers of systemic inflammation via the measurement of glycan complexity and acute phase protein levels. This multi-marker approach should enhance the accuracy and depth of inflammation assessments, particularly in relation to hyperglycaemia and cardiometabolic health.

Interestingly, I observed an association between GlycA and IL-6 with glycaemia but not for CRP. Mechanistically, studies have shown that IL-6 and CRP are closely related, with IL-6 being the major factor that triggers the hepatic synthesis of CRP.[483,484] However, although inflammatory markers are usually linked, recent studies have found them to diverge in certain contexts. For example, differing concentrations of IL-6 and CRP have been found in relation to HRT use.[485] Recent clinical findings have demonstrated that the different effects of HRT on IL-6, CRP, and TNF-α, may be due to direct hepatic stimulation of CRP by HRT.[464] Similarly, divergent levels of these inflammatory markers have been found in relation to other clinical factors such as alcohol use and exercise.[485] Thus, although CRP and IL-6 are biologically linked, their levels can diverge under certain conditions which may account for the weak correlation observed in this analysis.

Considering inflammation as a latent variable offered no additional insight into these associations. When designing this study, I made the decision to construct a latent variable for inflammation using IL-6, CRP and GlycA. The idea was that combining multiple measures may give a more comprehensive insight into systemic inflammation by capturing the common variance amongst them and reducing measurement error. However, the general lack of correlation between the inflammatory variables may have limited the potential utility of this tool. I also ran some model checks using several fit indices to determine the adequacy of the latent variable model for inflammation. The fit indices used presented a mixed picture of the model's adequacy. The Chi-square test and the RMSEA value suggest that are there some limitations in model fit, while CFI indicates an acceptable fit. Overall, while the current model provides a reasonably good starting point, there is room for improvement to achieve a better fit with the data.

It is possible that the relationship between hyperglycaemia and brain health is mediated by factors other than inflammation. Certainly, while I found no evidence that inflammation directly mediates the relationship between HbA_{1c} and WBV, other possible mediators to consider may be oxidative stress, SVD and CVD. Oxidative stress was not considered due to the current lack of available measures in NSHD that can capture it.

Potential factors not considered in these analyses, but particularly important around late midlife include hormonal health such as oestrogen levels. The neuroprotective

oestrogen reduces during the perimenopause and the post menopause. This change in hormonal health can influence brain structure and function in females.[431] Additionally, non-biological factors in social roles and responsibilities, including caregiving duties and family responsibilities, may mediate these sex-specific associations found in females. These factors could impose chronic stress and time constraints, which might affect brain health. Therefore, future research should consider important biological and social factors specific to females to achieve a comprehensive understanding of the underlying mechanisms of the glycaemia-brain health pathways.

In this study, I considered that hyperglycaemia precedes inflammation. This is based on previous studies suggesting that hyperglycaemia and abnormal glucose metabolism can result in the production of ROS contributing to oxidative stress.[437] Hyperglycaemia may also result in the formation of AGES which, with ROS, triggers pathways that regulate the inflammatory response resulting in the increase of pro-inflammatory cytokines (e.g., IL-6). However, it is also worth acknowledging that these cytokines produced by adipose tissue and macrophage may also result in a state of IR thus contributing to the pathophysiology of T2D. For example, IL-6 and CRP have previously been found to be significant predictors of T2D in a group of middle-aged females in the Females' Health Study.[266] This was found even when adjustments were made for inflammatory-related confounders (e.g., smoking, exercise, and BMI). In an attempt to reduce the likelihood of infection-driven hyperglycaemia, participants with an IL-6 value of > 10 pg/mL were excluded prior to my analyses.

Strengths and weaknesses

The study has multiple strengths. First, it considered multiple markers of inflammation. Similarly, the sample is data-rich with both exposure data (glycaemia), confounders (e.g., social and lifestyle factors) and outcome data (later-life measures of brain volumes). The confounder data includes those for the exposure-mediator and the mediator-outcome paths, which are known problems with causal mediation studies.[479] Furthermore, an important strength of this study is that it considered samples of females of the same age. The homogeneity of age ensured age-specific brain changes do not confound the results, providing a clearer picture of the relationship between HbA_{1c} and brain health.

It is worth acknowledging the potential for reverse causation, where changes in brain volume could influence metabolic parameters, rather than the reverse. This highlights the need for caution in interpreting the directionality of the observed relationships. Additionally, other unmeasured variables not considered in this analysis, such as genetic factors or specific dietary components, might influence the relationships explored. Some of these data are available in NSHD, but analyses of them were not possible due to the time constraints of this thesis.

5.5 Conclusions

These findings reveal that for the females in National Survey of Health and Development cohort, the relationship between glycaemia in midlife and later life smaller brains were not mediated by systemic inflammation as measured by selected blood markers. As per the findings I reported in my previous chapters, poorer glycaemia was directly associated with smaller brains but there was no indirect path of this relationship through inflammation. Future studies could investigate these associations in a different sample (e.g., UK Biobank) and also consider the role of other potential metabolic markers.

6. Exploring the sex-stratified analyses between glycaemia and brain and cognitive health in UK Biobank

I previously found that HbA_{1c} during adult life was associated with poorer brain outcomes at age ~70 in females in a birth cohort. To further elaborate on these findings, I performed similar analyses in UK Biobank to validate the previous results and used the increased power of the sample to explore the possibility that the glycaemia-brain associations were non-linear.

6.1 Introduction

Chapter 3 and Chapter 4 examined the sex-specific associations between markers of glycaemic health in midlife and later-life brain health in the oldest British birth cohort, the NSHD. The results revealed that glycaemic traits (HbA_{1c}, higher fasting glucose and IR) at age ~60 were associated with smaller volumetric brain tissue measures at age ~70 exclusively in females. The studies had multiple strengths such as considering participants of homogenous age, having multiple measurements of glycaemia, as well as detailed imaging data of volumetric and biomarkers of the brain.

The larger sample of UK Biobank, although not population representative, offers much more statistical power: it gives the opportunity to undertake a replication of the previous analyses to see whether they are generalisable to a different and larger sample. The aim of this replication is facilitated by the similar, in-depth, structural neuroimaging and deep phenotyping of participants across the relevant biomarkers of interest including confounders (i.e., socioeconomic position, BMI, and smoking status) of UK Biobank participants. Furthermore, the UK Biobank sample allows the opportunity to take a more nuanced perspective on the complex glycaemia-brain relationships. For example, the sample size of UK Biobank may allow the identification of a non-linear relationship which might appear as a linear relationship in a smaller sample with a more restricted range.

Previous studies using the UK Biobank sample have found that HbA_{1c} is associated with poorer brain health for some outcomes.[364,365] But these have focused on sex-pooled samples (aggregating males and females) and/or have assumed linear relationships. HbA_{1c} has previously been found to exhibit non-linear relationships with

some outcomes; notably it has been observed to have a J shaped relationship with cardiovascular events.[486] A non-linear approach to modelling these glycaemia-brain relationships may therefore offer important benefits: it may shed light on a possible threshold effect where both low and high HbA_{1c} (and glucose) are associated with poorer brain health, albeit perhaps through different mechanisms. It may help understand interactions between variables such as HbA_{1c} and sex on brain health outcomes. It also offers the opportunity to re-explore some of the findings from the NSHD analysis that may require a more nuanced lens and benefit from the greater precision provided by the large sample size of the UK Biobank. For example, previously, no convincing associations were found between HbA_{1c} with WMHV and HV.

The overall aims of this chapter are to better understand the relationships between markers of glycaemia to those of brain and cognitive health. Based on the previous findings in NSHD, and in order to expand on the growing body of work in the UK Biobank, the analyses will adopt: 1) a sex-stratified approach, hypothesising that poorer glycaemic health will be associated with worse brain health outcomes in females and 2) a non-linear approach, hypothesising that glycaemic state will show a non-linear relationship with brain health outcomes.

6.2 Methods

6.2.1 Sample

The source sample consisted of participants enrolled to the UK Biobank study. The UK Biobank cohort comprises around 500,000 people (94% of self-reported European ancestry) aged 40 to 69 at baseline. Participants were recruited between 2006 and 2010 and attended various assessment centres throughout the UK. Upon recruitment, participants completed questionnaires, a computer-assisted interview, and underwent data collection for blood, saliva, and urine samples. Other assessments included mental and lifestyle measures, as well as linkage to routinely collected data. Since 2014, a subsample of these participants was re-invited to 4 assessment centres for brain imaging scanning (the ‘first imaging visit’). More details on this sample are discussed in Chapter 2.5.2.

Within this analysis, the number of participants considered were those with imaging data available at the time of data analysis.

6.2.2 Investigations

Measures

Glycaemic markers: HbA_{1c} assays were performed on whole blood using five Bio-Rad Variant II Turbo analysers, manufactured by Bio-Rad Laboratories, Inc., and employed a HPLC method. The analysers underwent a multi-instrument comparison to ensure that they were in agreement.[487] More details are outlined in the UK Biobank HbA_{1c} protocol.[487] Random glucose was analysed on serum and measured by hexokinase analysis on a Beckman Coulter AU5800.

Anthropometrics: Data on height and weight were collected at baseline when participants attended the assessment centre. Height was measured in whole centimetres (cm) with a Seca 202 device. Weight was measured to the nearest 0.1 kilograms (kg). These measurements were made at the time when blood samples were collected.

Neuroimaging protocol: The neuroimaging protocol for UK Biobank was described in Chapter 2.5.2.

The measures considered in the analyses were WBV, GM, WM (normalised for head size cm^3) and HV and WMHV (adjusted for intercranial volume). These same measures have been used in previous studies.[364,405]

Cognitive markers: Two cognitive markers representing distinct cognitive domains were selected based on the rich availability of this data in participants at baseline: reaction time (RT) and visual memory (VM). RT (measured in milliseconds) assessed how long participants took to successfully identify a correct match from trials of matching symbol pairs. The longer participants took, the higher the RT. VM assessed spatial recall of 6 pairs of cards with participants instructed to recall the position of each. The number of incorrect attempts were recorded with the higher number of these indicating a poorer performance in the task (and poorer VM). In line with previous studies, RT was log-transformed due to being skewed.[364,406]

Confounders: Confounders were defined based on background knowledge and depicted using the DAG presented in Chapter 3 (Figure 3.1). The chosen confounders were closely aligned with those used in NSHD analyses in Chapter 3 and Chapter 4.

Very briefly, these were age, socioeconomic deprivation (derived from a self-rated questionnaire and operationalised into quintiles of Townsend deprivation index, from 'least deprived' to 'most deprived'), educational attainment (recorded by questionnaire and operationalised as years of full-time education completed, as per qualifications based on coding from the International Standard Classification of Education), self-reported smoking status (never, current smoker and ex-smoker) and BMI.

6.2.3 Statistical analysis

Analyses were performed in Stata 18.0 using the fp and mfp packages. Plots were produced using the fp plot and fracplot commands.

Fractional polynomials

Fractional polynomials (fp) were used to model potential non-linear relationships between the glycaemic markers (HbA_{1c} and glucose) and the different brain imaging and cognitive measures.

Fp modelling is a useful flexible parametric approach that aims to represent a (non)linear relationship that 'best' fits the data. This is an improvement over low order polynomials which produce a limited number of shapes or high order polynomials which are flexible but often fit poorly at extremes. They are an alternative to cubic splines and have the advantage that they are based on simple equations for prediction. Fps were first proposed by Royston and Altman.[488] Fps differ from standard polynomials by permitting the use of non-integer powers, logarithms and repeated powers allowing for a wider range of shapes to be constructed.

For example, rather than a simple quadratic term to model a non-linear relationship for age such as in:

$$y = b_0 + b_1 x_i + b_2 age_i + b_3 age_i^2 + u_i$$

A fractional polynomial function of age could be used:

$$y = b_0 + b_1 x_i + b_2 age_i + b_3 age_i^{(p)} + u_i$$

where *p*, the fractional polynomial is a vector of powers of degree, m (for example p + -0.5, 2 would be a 2nd degree polynomial). The round bracket around p, indicates the Box-Tidwell transformation.

$$X^p = \begin{cases} X^p & \text{if } p \neq 0, \\ \ln X & \text{if } p = 0, \end{cases}$$

Typically, models are chosen by including m powers from a predefined set {−2, −1, −0.5, 0, 0.5, 1, 2, 3} aiming to capture a wide number of functional forms in X that are useful in regression models of real data (note that as mentioned above *p = 0* denotes ln *X*).

The procedure involves fitting all possible combinations of powers from the set. A deviance statistic between the set of models is generated to select the best fitting model for each degree of fractional polynomial. This can be used to test whether the inclusion of an additional fractional polynomial term significantly improves the model fit using a partial F test or a likelihood-ratio test. Each model uses a different pair of powers for transformation, for example (-2, -2), (-2, -1) or (3, 3). To select the best-fitting second-order fractional polynomial (p=2), it fits 8 first-order fractional polynomial models and 32 second-order fractional polynomial models.

In Stata, the fp function outputs a model comparison table showing the best fractional polynomial model of HbA_{1c} for each examined degree, m. In practice, fractional polynomial functions with $m \leq 2$ are fitted. There are different ways to choose the 'best' model, some choose the model with the lowest deviance, I chose the most efficient (parsimonious) model, i.e., the lowest degree model that is not significantly ($p<0.05$) different from the degree 2 model which is what was recommended by Royston.[489]

Modelling

In this analysis, a series of sex-stratified fractional polynomials were used to model the relationships between glycaemic exposures and brain imaging outcomes. Simple and fully confounder-adjusted models were conducted. The confounders considered were based on previous analyses.[407] Minimally adjusted: adjustment made for age and TIV (if appropriate). Fully confounder adjusted models included age, TIV (if

appropriate), socioeconomic factors (education and deprivation), and lifestyle factors (smoking and BMI). There is little or no literature available on how to compare fractional polynomial models across factors (e.g., sex), therefore I investigated whether there were sex differences in the non-linear relationships by constraining the parameters of the fit in females to those in males and performing a likelihood ratio test (LRT). Whether this is an optimal strategy is uncertain.

Since the interpretation of fractional polynomial generated coefficients can be difficult (due to the complexity of the function), graphs were produced to examine the average curve for the most efficient polynomial (example see Figure 6.2).

Previous studies have dealt with the skewed distribution of the VM variable by log transforming it. Evidence shows that transformation (e.g., log) of count variables may not always be the best approach due to the trade-off between linearity and homoscedasticity, the difficulty with dealing with zero values in log transformation and the introduction of negative numbers.[490] In order to deal with this, a robust Poisson regression was conducted. This approach provides more robust parameter estimates and standard errors, which can be helpful in situations where the assumptions of traditional Poisson regression are violated.

As part of my sensitivity analyses, each analysis was repeated by: 1) excluding those on medication (in case any associations in the low range are driven by hypoglycaemic agents) and 2) excluding people with diabetes to explore whether any associations are driven only by those diagnosed as having diabetes.

6.3 Results

A total of 36,321 participants were included in the analyses (consisting of 47% males and a mean age of ~55). A visual representation of those considered in the sample is shown in Figure 6.1. Males had higher HbA_{1c} and random glucose levels than females, and were more likely to be current or ex-smokers and to have diabetes. A more comprehensive representation of sample characteristics stratified by sex is presented in Table 6.1. A visual layout of the participants considered is shown in Figure 6.1.

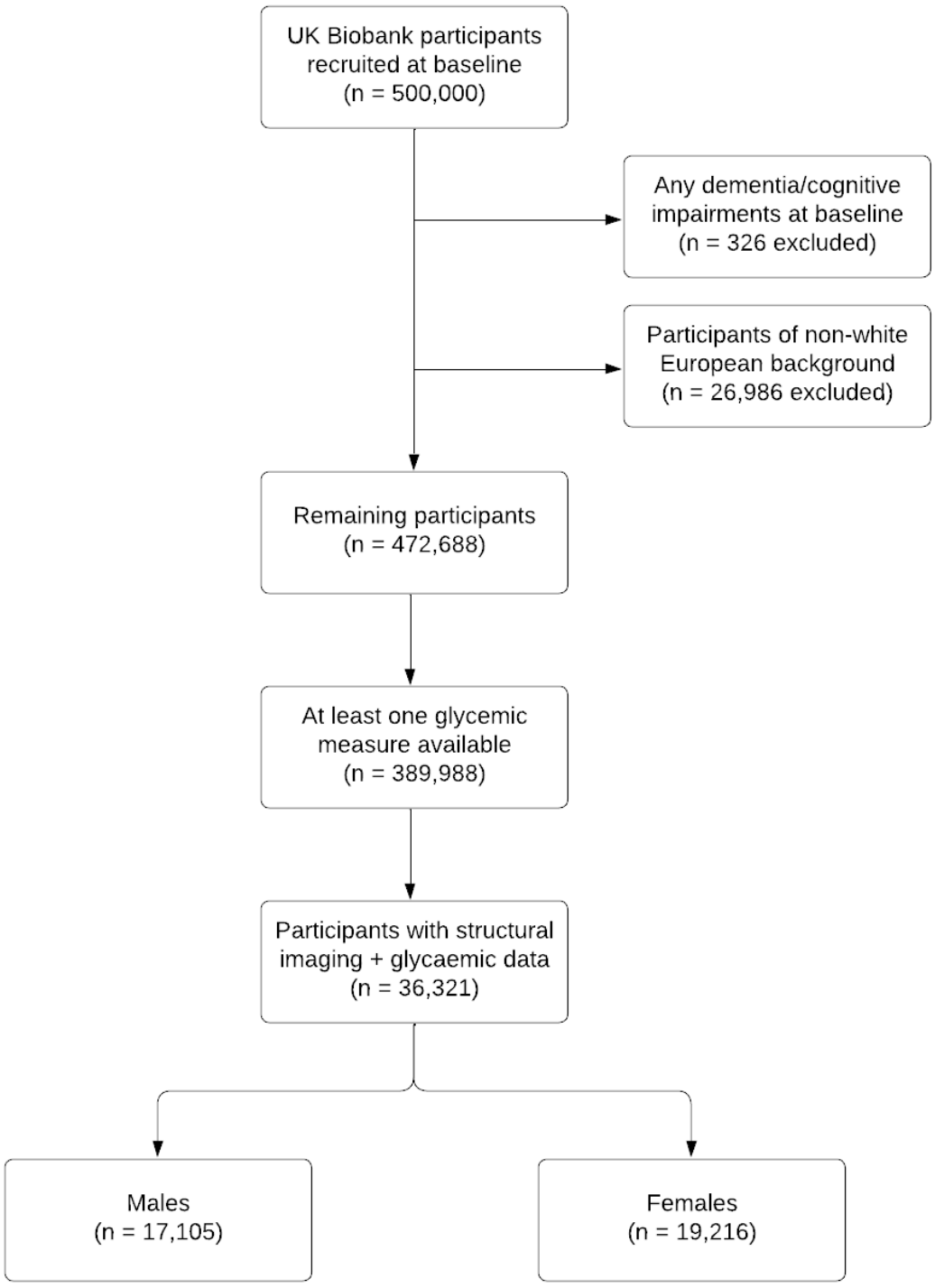


Figure 6.1: Flowchart displaying participants considered in this study. UK Biobank participants had to have structural imaging data and at least one measure of glycaemia to be considered (n= 36,321).

Sample Characteristics			Males = 17,105	n	Females = 19,216
Age, years:			55.6 (7.5)		54.2 (7.2)
Deprivation	Least deprived	17,095	4238 (24%)	19,196	4518(25%)
	Second least deprived		4067 (24%)		4485 (23%)
	Median deprivation level		3547 (21%)		4036 (21%)
	Second most deprived		3078 (18%)		3614 (18%)
	Most deprived		2165 (13%)		2543 (13%)
Smoking	Never smoker	17,090	11,619 (68%)	19,201	14,616 (76%)
	Current Smoker		1,242 (7%)		995 (5%)
	Ex-smoker		4,229 (25%)		3,590 (19%)
BMI, kg/m^2		17,083	27.1 (3.7)	19,192	26.1 (4.5)
HbA_{1c}, mmol/mol		16,505	35.2 (5.5)	18,506	34.7 (4.5)
HbA_{1c}, mmol/mol, range		16,505	16-122.6	18,506	15.3-91.1
Glucose, mmol/L mean		13,215	5 (1.1)	15,081	4.9 (0.8)
Glucose, mmol/L, range		13,215	1.9-26.6	15,081	1.78-24.1
Diabetes medication		17,105	365 (2.1%)	19,216	222 (1.2%)
Diabetes diagnosis		17,105	519 (3%)	19,216	233 (1.2%)
Brain imaging and cognitive markers					
Whole brain volume (WBV) cm^3		17,105	1480.7 (70.8)	19,216	1505.3 (73.0)
Grey matter volume (GM) cm^3		17,105	775.6 (930.2)	19,216	807.1 (458.4)
White matter volume (WM) cm^3		17,105	705.3 (407.6)	19,216	698.2 (403.7)
Hippocampal volume (HV) cm^3		17,105	3.8 (0.12)	19,216	3.8 (0.1)
White matter hyperintensity volume (WMHV) cm^3		17,105	8.1 (1)	19,216	7.9 (1)

Total intracranial volume (TIV) cm^3	17,105	1644.2 (131.1)	19,216	1468.4 (115.7)
Whole brain volume unadjusted cm^3	17,105	1225.3 (98.7)	19,216	1107.1 (89.9)
Reaction time (ms)	17,070	195.4 (96.6)	19,180	209.8 (98.4)
Visual memory (incorrect matches)	16,191	3.8 (3)	18,201	3.7 (2.9)

Table 6.1: Sample characteristics for the male and female participants considered in this study (n = 36,321).
As described above, participants had to have structural imaging and data on least one measure of glycaemia to be considered amounting to 36,321 participants of which 17,105 were males and 19,216 were females. Values presented are: n (%), mean (SD) or median (IQR). % are calculated against the max data available for that specific measure for the respective sample. Whole brain, grey matter and white matter volume measurements reported were already normalised for head size by the UK Biobank. SD: Standard deviation. IQR: Interquartile range.

Results from fractional polynomials:

Overall, the fp analyses showed that the model with two fp components best fitted the relationships between the glycaemic markers and the brain imaging outcomes for both males and females. The first figure of these relationships (Figure 6.2) shows a fully confounder-adjusted partial regression plot for the glycaemia and WBV relationship with each dot representing a participant for males and females. The remaining plots are fully confounder-adjusted partial regression plots of the fp models that best fitted the relationship between the glycaemic markers and the different brain imaging outcomes with individual data points being omitted for clarity.

There was no additional utility of using a non-linear fp model to a standard linear model when modelling the relationship between the glycaemic markers and the two cognitive outcomes, so these were modelled as linear relationships.

Very briefly, the table shows a comparison of different model fits for the HbA_{1c}-WBV relationship in males. The row labelled "omitted" describes the null model, which entirely omits HbA_{1c}. A separate row is provided for the model with a linear function of HbA_{1c} because it is often the default when including a predictor in the model. The model deviance, defined as twice the negative log likelihood, is given in the Deviance column. The Deviance Diff. column reports the difference in deviance compared with the model of the exposure-outcome relationship with the lowest deviance, which is always the model with the highest-degree fp. Based on the model-comparison table, the model without HbA_{1c} and the linear model can be rejected and models with m=2 which includes HbA_{1c} and with powers of -2 and -0.5 offer the best fit (see Table 6.2).

Model	Test df	Deviance	Deviance Diff.	p	Powers
Omitted	4	181408.4	52.062	p <0.001	
Linear	3	181370.7	14.353	0.002	1
m = 1	2	181367.3	11.028	0.004	2
m = 2	0	181356.3	0.00	N/A	-2 -0.5

Table 6.2: Model comparison table for the fully adjusted fractional polynomial models for HbA_{1c} and whole brain volumes for males.
It shows the best fractional polynomial model of weight for each examined degree, m, which is obtained by searching through all possible power combinations. The m=2 model (powers -2, -0.5) has lower deviance, and the fit is superior to the other models (indicated by p<0.05 for these models). df: degrees of freedom. Deviance Diff: deviance difference. p: p-value.

Model	Test df	Deviance	Deviance Diff	p	Powers
Omitted	4	165592.91	59.842	p <0.001	
Linear	3	165570.07	59.799	p <0.001	1
m = 1	2	165557.44	59.773	0.04	3
m = 2	0	165551.24	0.00	N/A	-2 -2

Table 6.3: Model comparison table for the fully confounder-adjusted fractional polynomial models for the relationship between HbA_{1c} and whole brain volumes for females.
The most parsimonious model was the fractional polynomial model with powers -2 and -2. df: degrees of freedom. Deviance Diff: deviance difference. p: p-value.

The relationship between glycaemic markers and WBV

HbA_{1c}

The model that best fitted the data for both males and females had 2 components and was not monotonic (see Figure 6.2). Both lower HbA_{1c} and higher HbA_{1c} were associated with smaller WBV (with ~35 mmol/mol being most optimal). There was no evidence of sex differences in the relationship between HbA_{1c} and WBV in either the minimally adjusted or fully adjusted models based on the LRT. The plots also show that WBV are higher in females at the lowest range of HbA_{1c}. As evidenced by their different means, the partial regression plots show that females tend to have higher WBV for the same HbA_{1c} levels. However, the overlapping of the confidence differences make it difficult to assert confidence in these differences at high levels of HbA_{1c}.

Glucose

The relationship that best fitted the data for both males and females had 2 components and was not monotonic (Figure 6.2). Both lower and higher glucose were associated with smaller WBV (with ~5 mmol/L being most optimal). There was no evidence of sex interactions between glucose and WBV in either the minimally or fully adjusted models. Despite this, above 5 mmol/L of glucose, the decline in WBV in females appeared to be steeper than in males. However, the overlapping of the confidence differences make it difficult to assert confidence in these potential sex differences.

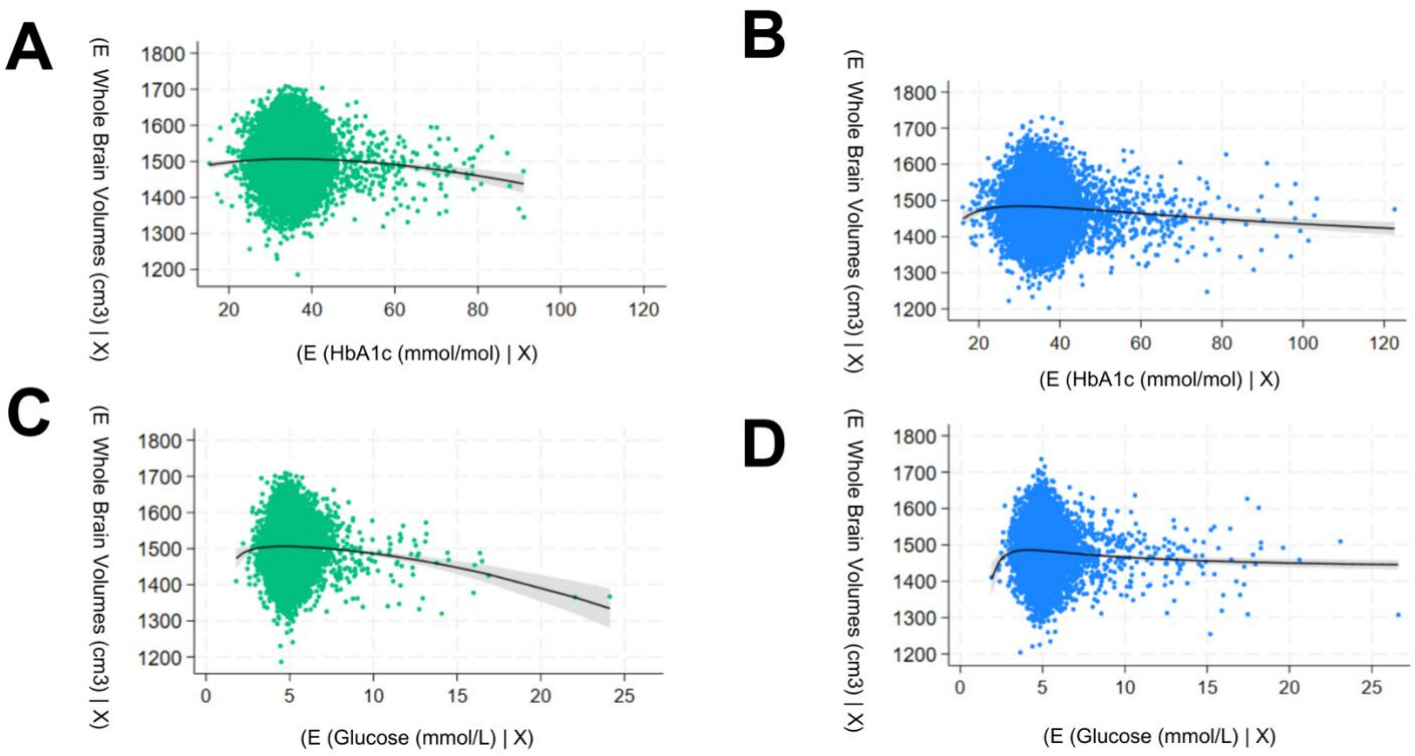


Figure 6.2: Multivariable fractional polynomial models with 95% confidence limits of the relationship between HbA_{1c} (A and B) and glucose (C and D). and whole brain volumes.
Females are represented in green (A and C) and males in blue (B and D). E(Y) on the y-axis represents the predicted values of the response variable, while E(X|Xn) on the x-axis represents the conditional expected values of the predictor variable of interest.

The relationship between glycaemic markers and: GM and WM

HbA_{1c}

The relationship that best fitted the HbA_{1c}-GM relationship for both males and females had 2 components and was not monotonic (see Figure 6.3). For GM, both low and high HbA_{1c} were associated with a smaller brain volume in males and females (with ~38 mmol/mol being most optimal, i.e., the highest level of brain volume). As HbA_{1c} increased, the decline appeared visually steeper in females, but the precision of the estimates reduced. As evidenced by their different means, the partial regression plots show that females tend to have higher GM for the same HbA_{1c} levels. However, the overlapping of the confidence differences make it difficult to assert confidence in these potential sex differences at high levels of HbA_{1c}.

The relationship that best fitted the HbA_{1c}-WM relationship for both males and females had 2 components and was not monotonic (see Figure 6.3). For WM, the estimates largely overlapped across the range of HbA_{1c}.

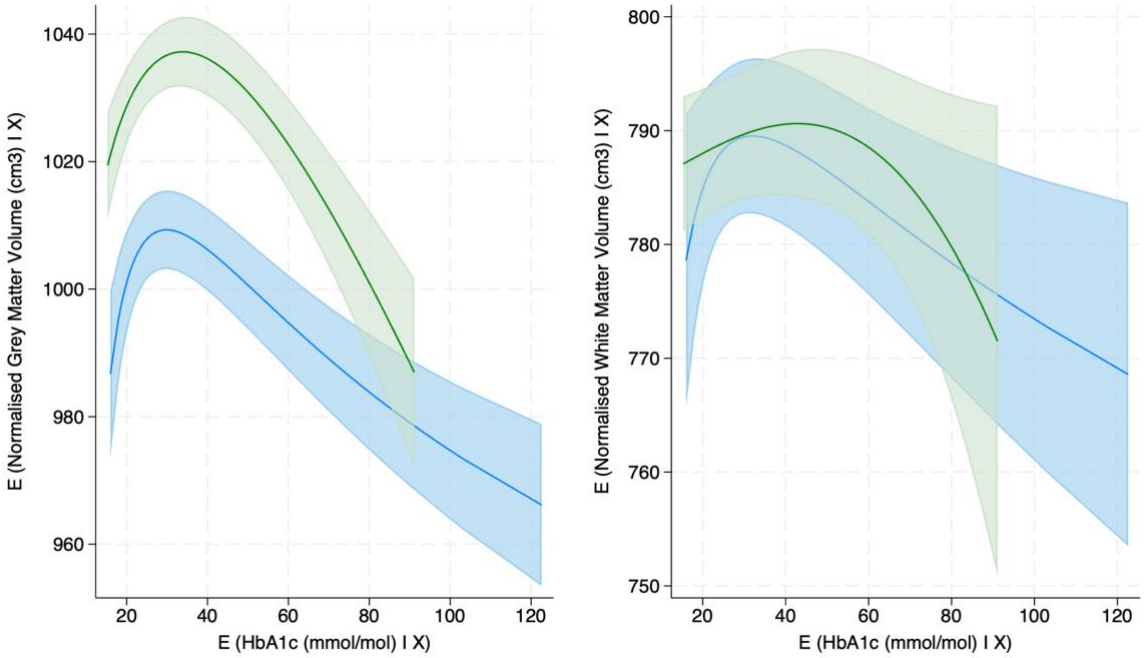


Figure 6.3: The partial regression plots of the fractional polynomial models that best fitted the relationship between HbA_{1c} and grey matter and white matter volumes. These relationships for females are represented in green and for the males in blue. Confidence limits are represented by the shading surrounding the line. The models presented are the fully-confounder adjusted models. As per described in the caption of Figure 6.2 above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

Glucose

The relationship that best fitted the glucose-GM relationship for both males and females had 2 components and was not monotonic (see Figure 6.4). For GM, both low and high glucose were associated with a smaller brain volume in males and females (with ~5 mmol/L being most optimal). As glucose increased, the decline appeared visually steeper in females, but the precision of the estimates reduced. As evidenced by their different means, the partial regression plots show that females tend to have higher GM for the same glucose levels. However, the overlapping of the confidence differences make it difficult once again to assert confidence in these potential sex differences at high levels of glucose.

The relationship that best fitted the glucose-WM relationship for both males and females had 2 components and was not monotonic (see Figure 6.4). For WM, the estimates largely overlapped across the range of glucose.

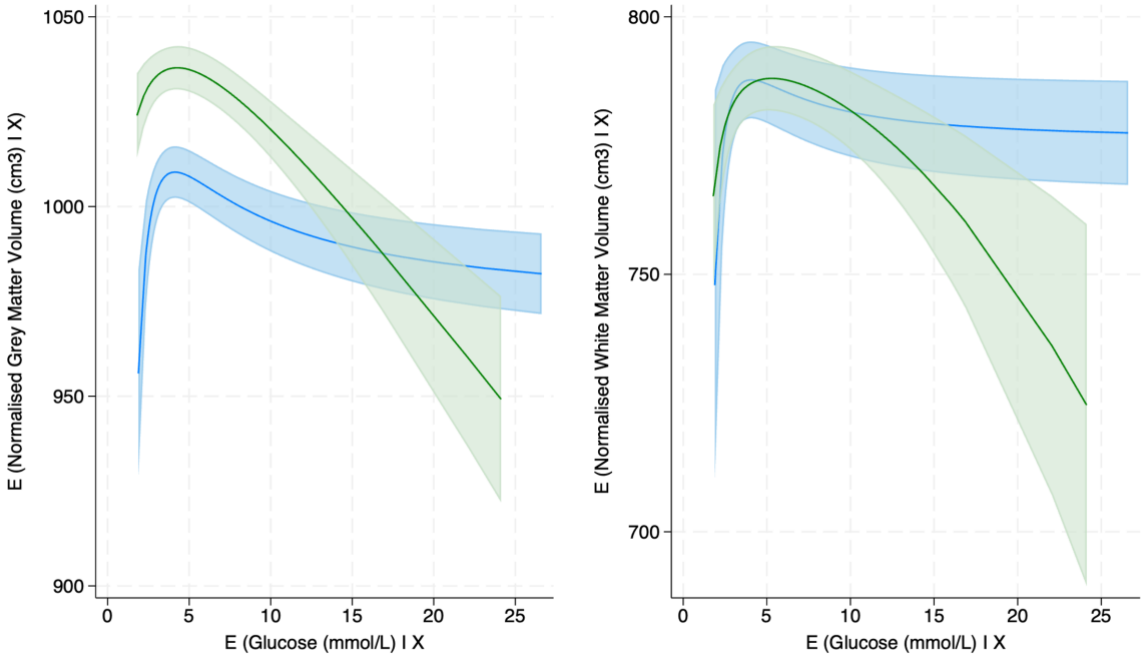


Figure 6.4: The partial regression plot of the fractional polynomial models that best fitted the relationship between glucose and grey matter and white matter volumes. These relationships for females are presented in green and for the males in blue. Confidence limits are represented by the shade surrounding the line. The models presented are the fully confounder-adjusted models. As per plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

The relationship between glycaemic markers with hippocampal volumes

HbA_{1c}

The relationship that best fitted the HbA_{1c}-HV relationship for both males and females had 2 components and was not monotonic (see Figure 6.5). For HV, the estimates by sex largely overlapped across the range of HbA_{1c}.

Glucose

The relationship that best fitted the glucose-HV relationship for both males and females had 2 components and was not monotonic (see Figure 6.5). Both low and high glucose was associated with a smaller HV in males and females (with ~5 mmol/L being most optimal). As glucose increased, the decline appeared visually steeper in females, but the precision of the estimates reduced.

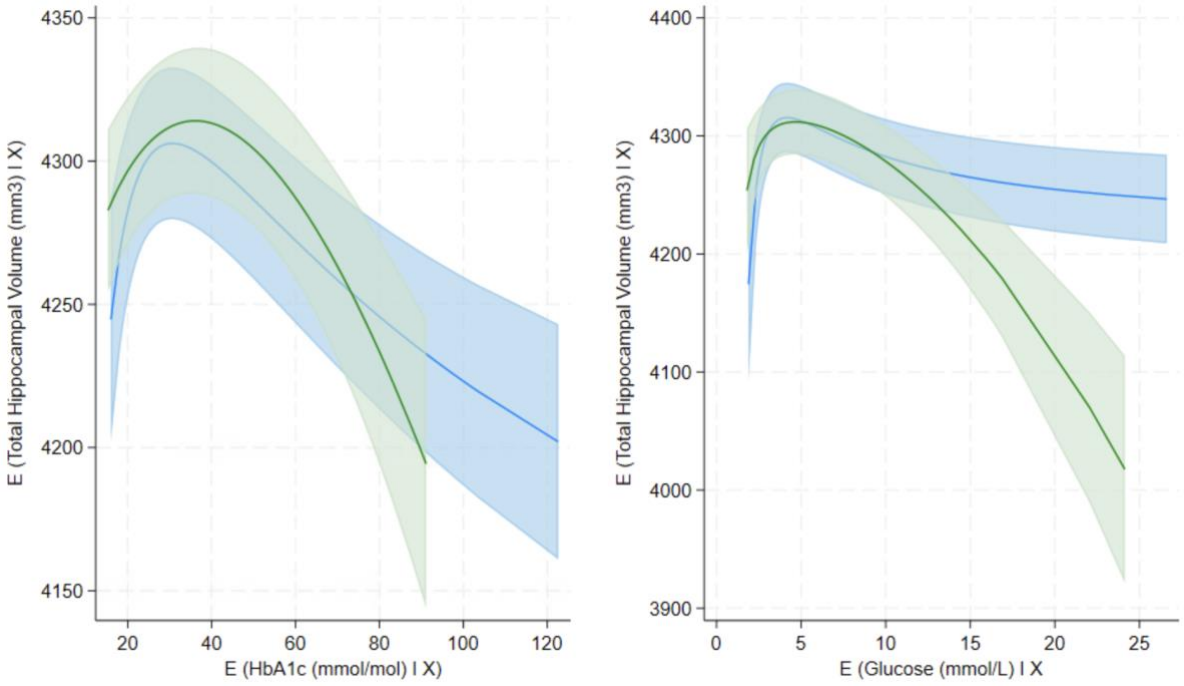


Figure 6.5: The partial regression plots of the fractional polynomial models that best fitted the relationship between the glycaemic markers and HV.
These relationships for females are presented in green and for the males in blue. Confidence limits are represented by the shade surrounding the line. The models presented are the fully confounder-adjusted models. As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

Glycaemic markers and white matter hyperintensity volume

HbA_{1c}

The relationship that best fitted the HbA_{1c}-WMHV relationship for both males and females had 2 components and was not monotonic (see Figure 6.6). For GM, both low and high HbA_{1c} were associated with higher WMHV (with ~30 mmol/L being most optimal). As HbA_{1c} increased, the decline appeared visually steeper in females, but the precision of the estimates reduced. As evidenced by their different means, the partial regression plots show that females tend to have higher WMHV for the same HbA_{1c} levels. However, the overlapping of the confidence differences make it difficult to assert confidence in these potential sex differences.

Glucose

The model that best fitted the glucose-WMHV relationship for both males and females had 2 components and was not monotonic (see Figure 6.6). The estimates largely overlapped across the range of glucose and the imprecision makes it difficult to interpret the associations.

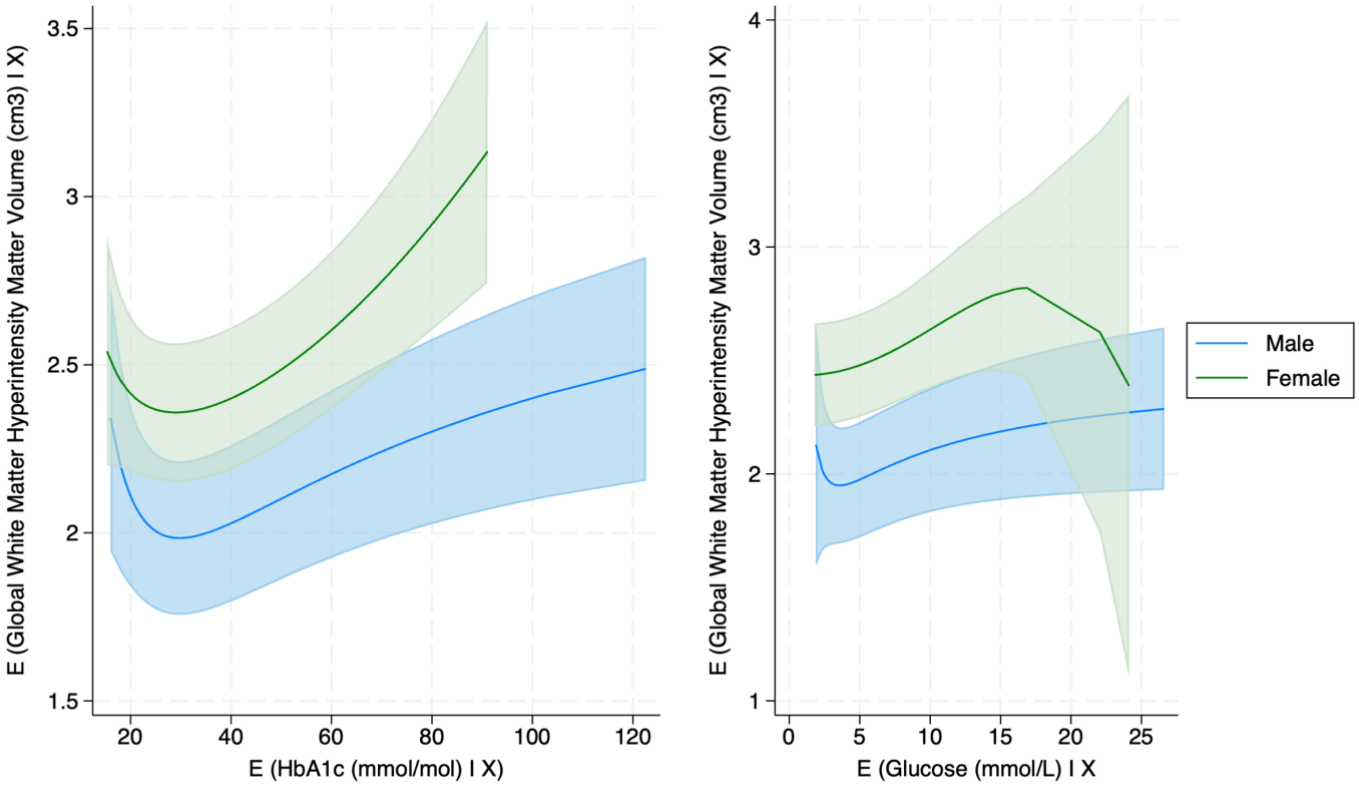


Figure 6.6: The partial regression plot of the predicted model that best fitted the relationship between HbA_{1c} and glucose on white matter hyperintensity volumes. These relationships for females are presented in green and for the males in blue. Confidence limits are represented by the shade surrounding the line. The models presented are the fully confounder-adjusted models. As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

The relationship between glycaemic markers with cognition: reaction time and visual memory

Based on Table 6.4, there is no evidence that any model fits the data. Since no model fits best, the most parsimonious (in this case, the linear) was used to get an idea of the uncertainty in the estimates (95% CI) recognising that there is no evidence of association.

Model	Test df	Deviance	Deviance Diff	p	Powers
Omitted	4	93.774	5.495	0.241	
Linear	3	93.761	0.707	0.872	1
m = 1	2	60.760	0.481	0.787	0.5
m = 2	0	60.762	N/A	N/A	-2 -1

Table 6.4: Model comparison table for the fully adjusted fractional polynomial models for glucose and reaction time for males.
It shows the best fractional polynomial model of weight for each examined degree, m, which is obtained by searching through all possible power combinations with no evidence that any models fitted the data. df: degrees of freedom. Deviance Diff: Deviance difference. p: p value.

Glycaemic markers and visual memory

Due to the skewed distribution of this count variable, a robust Poisson was used.
For both males and females, there were no convincing associations between the glycaemic markers and VM (see Table 6.5) albeit higher glucose was associated with lower number of errors in the VM in males.

Glycaemic markers and reaction time

For females, higher HbA_{1c} was associated with longer RT in females (see Table 6.5). The associations were in a similar direction for glucose but were of smaller magnitude. For males, higher glucose was weakly associated with a shorter RT. The associations were in a similar direction for HbA_{1c} but were once again of smaller magnitude.

Visual Memory (VM)					
		β	**95% CI**		p
HbA_{1c}	Males	-0.001	-0.002	0.001	0.1
	Females	-0.001	-0.003	0.004	0.1
Glucose	Males	-0.01	-0.02	-0.001	0.02
	Females	-0.001	-0.01	0.01	0.7
Reaction Time (RT)					
		β	**95% CI**		p
HbA_{1c}	Males	-0.03	-0.3	0.2	0.8
	Females	0.4	0.05	0.6	0.02
Glucose	Males	-1.6	-3.1	-1.3	0.04
	Females	0.9	-0.9	2.8	0.3

Table 6.5: Table representing the output from robust Poisson regression (for visual memory) and the linear regression (for reaction time) when modelling the relationship between markers of glycaemia and those of cognition.
These are presented both for males and females. The β coefficients, confidence intervals and p-values are presented.

Sensitivity analyses

Sensitivity analyses were conducted excluding individuals who were either on diabetes medication or had diabetes (see Figure 6.7 and Figure 6.8). Examples are only shown for the relationship between glycaemic markers and some of the brain imaging measures (WBV and GM volume). These analyses demonstrated that the exclusion of the participants did not materially change the findings nor my interpretations. Similarly, analyses were conducted this time considering the cognitive markers as the outcomes. The exclusion of people on diabetes medication attenuated the previously weak finding that higher glucose in men was associated with better VM and that higher HbA1c was associated with slower RT (see Table 6.6).

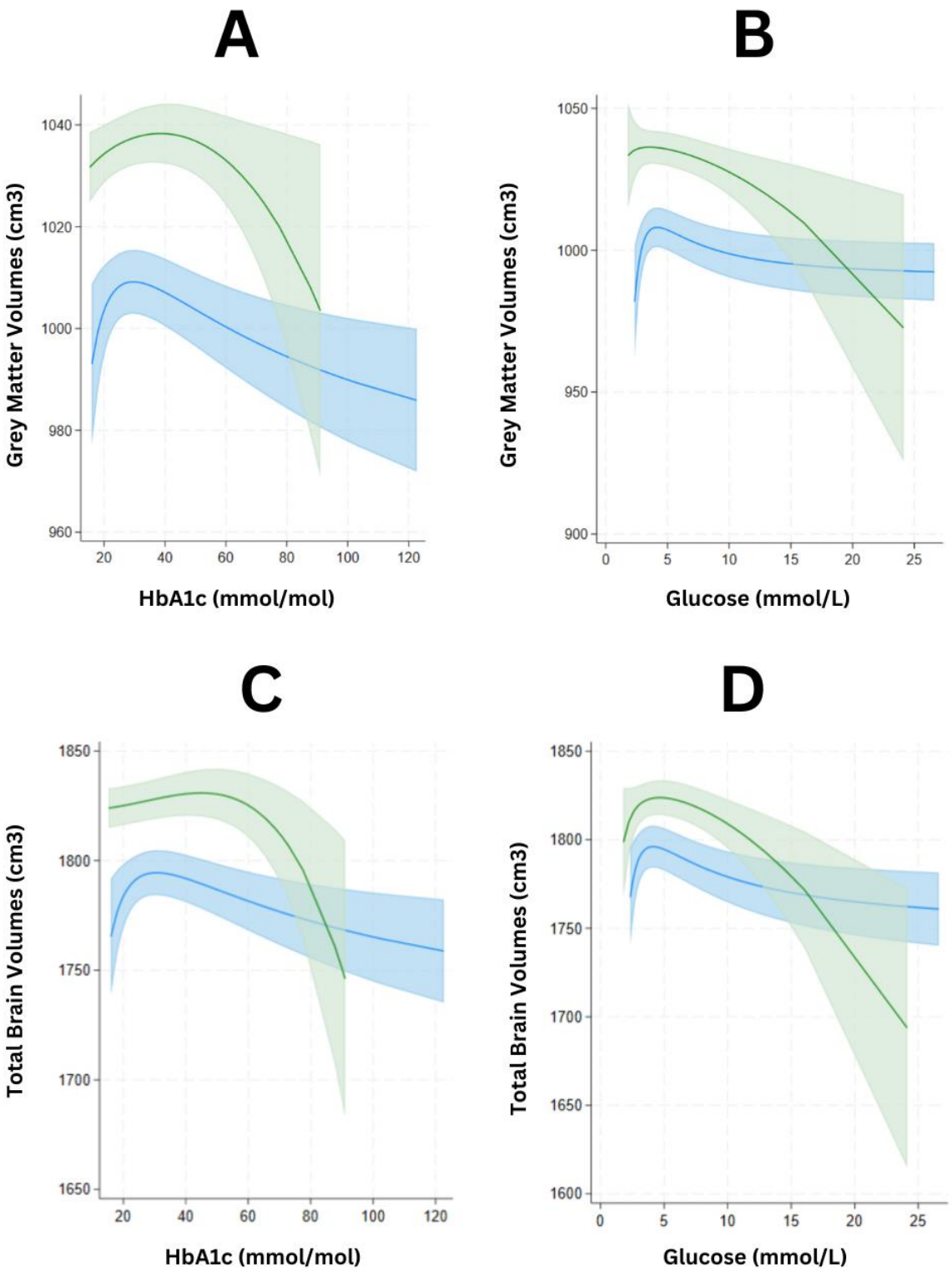


Figure 6.7: The partial regression plot of the fractional polynomial models that best fitted the relationship between HbA_{1c} and glucose on grey matter (A and B) and whole brain volumes (C and D). The models presented are the fully confounder-adjusted models excluding those on diabetes medication.
These relationships for females are presented in green and for the males in blue. Confidence limits are represented by the shade surrounding the line. As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

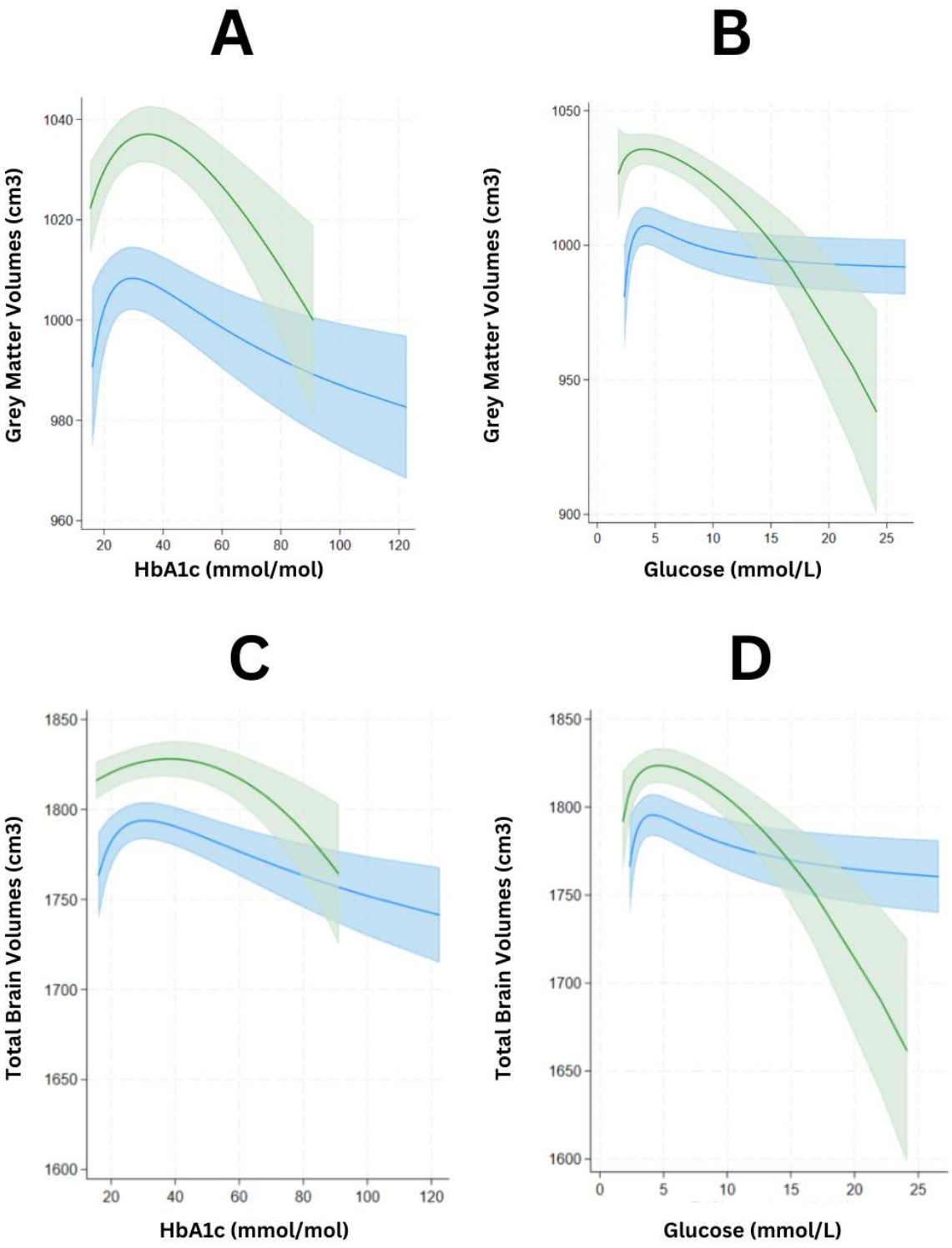


Figure 6.8: The partial regression plot of the fractional polynomial models that best fitted the relationship HbA_{1c} and glucose on grey matter (A and B) and whole brain volumes (C and D). The models presented are the fully confounder-adjusted models excluding participants with diabetes.
The β coefficients, confidence intervals and p-values are presented. These relationships for females are presented in green and for the males in blue. Confidence limits are represented by the shade surrounding the line. As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

		Visual Memory (VM)			
		β	**95% CI**		**p**
HbA_{1c}	Males	-0.001	-0.002	0.001	0.3
	Females	-0.001	-0.003	0.001	0.3
Glucose	Males	-0.01	-0.02	-0.001	0.2
	Females	-0.001	-0.01	0.01	0.9
		Reaction Time (RT)			
		β	**95% CI**		**p**
HbA_{1c}	Males	-0.07	-0.3	0.5	0.7
	Females	0.1	-0.2	0.5	0.5
Glucose	Males	-2.1	-3.9	-0.3	0.03
	Females	-0.7	-3.1	1.6	0.5

Table 6.6: Table representing the output from robust Poisson regression analyses (for visual memory) and the linear regression (for reaction time) when modelling the sex-stratified relationships between markers of glycaemia and those of cognition.
These analyses exclude participants on diabetes medication. These analyses are sex-stratified presenting them for males and females separately. The β coefficients, confidence intervals and p-values are presented.

Minimally confounder-adjusted models

The results presented above were for the fully confounder-adjusted models. As a preliminary step, a number of minimally confounder-adjusted fp models adjusted only for age of scanning (and TIV, if appropriate) were conducted. These plots for WMHV, GM and WM volumes are shared here for completeness (see Figure 6.9 and 6.10). Overall, the findings were mostly in line with the results described above with some slight differences (e.g., HbA_{1c}-WMHV relationships being more imprecise).

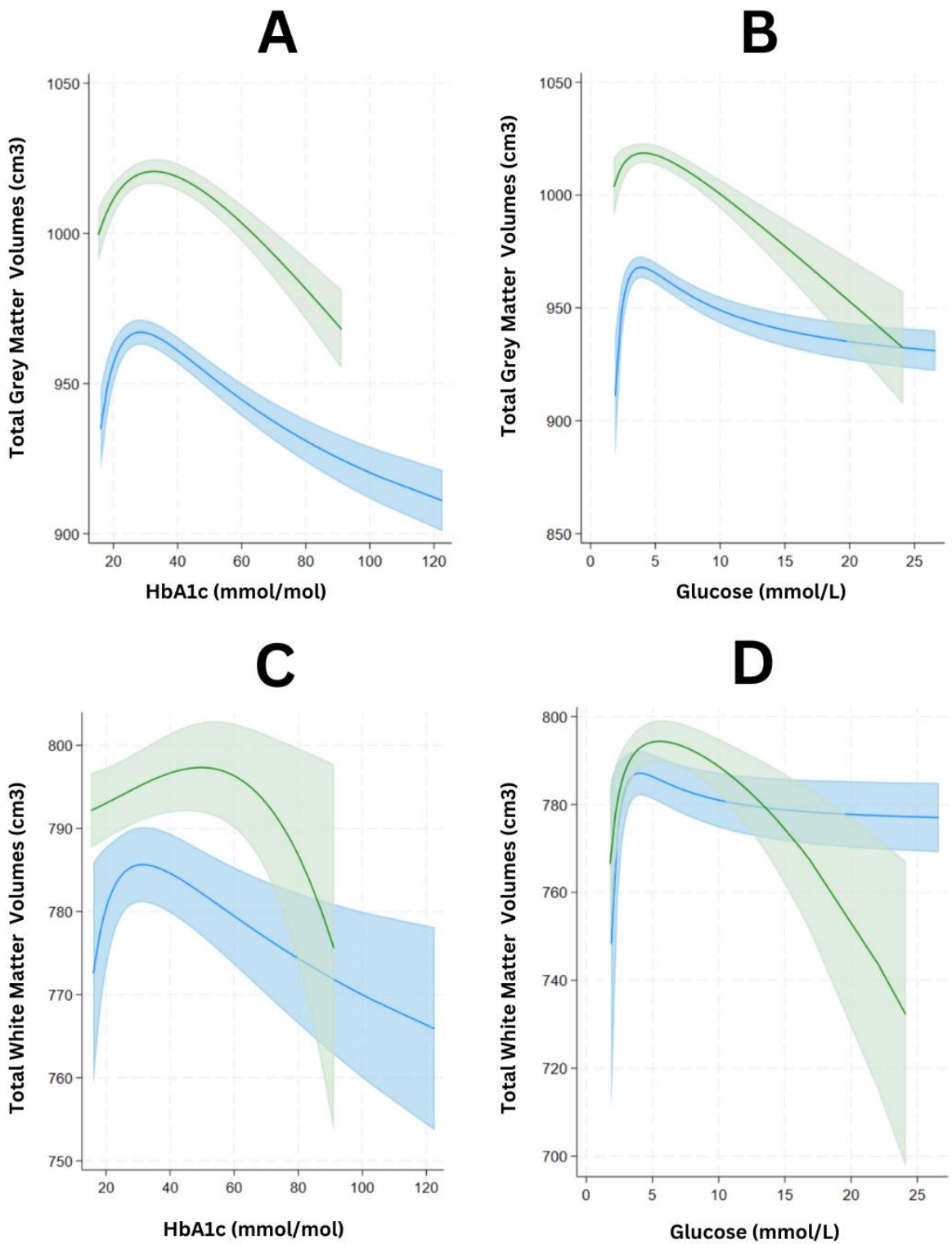


Figure 6.9: The partial regression plots of the fractional polynomial models that best fitted the relationship between the glycaemic markers on grey matter (A and B) and white matter volumes (C and D).
The models presented are for minimally confounder-adjusted models. These relationships for females are presented in green and for the males in blue. Confidence limits are represented by the shade surrounding the line. As per the previous plots, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

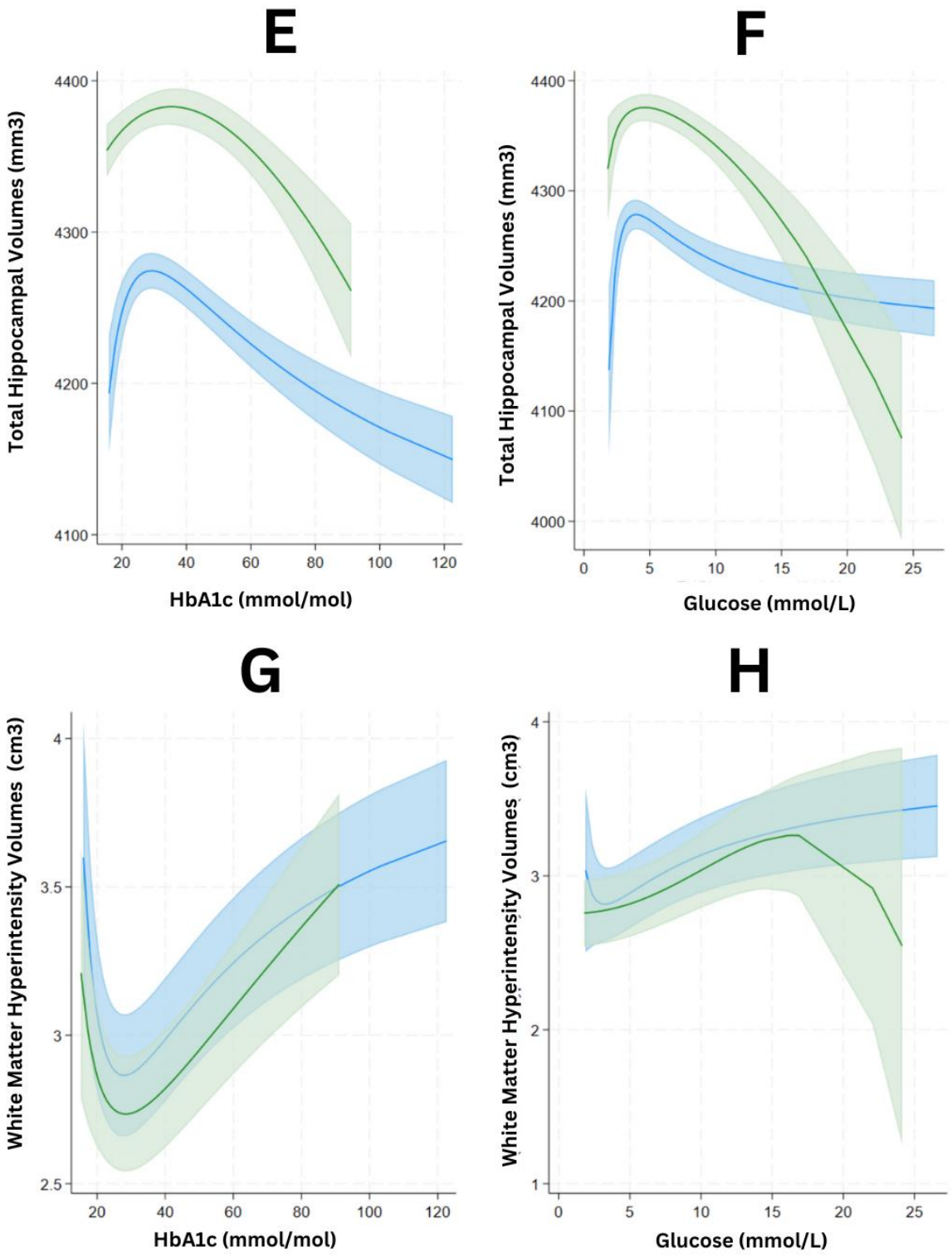


Figure 6.10: The partial regression plots of the fractional polynomial models that best fitted the relationship HbA_{1c} and glucose on hippocampal volumes (E and F) and white matter hyperintensitity volumes (G and H).
The models presented are for minimally confounder-adjusted models. These relationships for females are presented in green and for the males in blue. Confidence limits are represented by the shade surrounding the line. As per the plot above, the predicted values of the response variable and the conditional expected values of the predictor variable of interest are displayed.

6.4 Discussion

Summary of findings

Overall, the results from this large sample provide evidence of non-linear associations between the glycaemic markers (HbA_{1c} and glucose) and some of the brain health measures. The lower and upper ranges of glycaemia were mostly associated with poorer brain health outcomes especially for some of the volumetric tissue measures such as lower GM and WBV (and less convincingly for HV).

Across the different associations explored, the 2-degree fp models were generally the most efficient in capturing these nuanced relationships. The only exceptions to this were the relationships between glycaemia and cognition where linear models were not inferior to fp models. There were some visual distinctions in the shape of the glycaemia-brain outcome associations between males and females although generally the imprecision of the estimates makes it difficult to be confident about possible sex differences. Across the various relationships investigated, HbA_{1c} and glucose behaved mostly consistently with each other in their relationship with the brain health outcomes. Analyses taking participant diabetes status or medication use into account had some, although negligible effects on the results.

Specific findings and associations with the literature

Non-linear results in context

The findings suggest that glycaemic health (as indexed by HbA_{1c} and glucose) has a non-linear, J shaped, association with some of the brain health markers: specifically, both low and high HbA_{1c} were associated with lower WBV, GM volumes and to some extent HV.

The finding that HbA_{1c} has a non-linear (or J-shaped) association with health outcomes has previously been reported in the context of CVD.[486] In a sample of over 100,000 participants, researchers observed that both low and high HbA_{1c} levels were associated with a high incidence of CVD (i.e., stroke and coronary heart disease). Another study of the UK Biobank reported a statistically nonsignificant non-linear relationship between HbA_{1c} variability and incidence of dementia and HV in adults without diabetes.[491] Although nonsignificant, these findings are consistent with mine

by suggesting that that glycaemic traits (i.e., HbA_{1c} variability, HbA_{1c} and glucose) at the lower and upper range may be associated with adverse brain health outcomes. I mention these study findings while acknowledging that reverse causality cannot be ruled out when discussing such J-shaped relationships (e.g., CVD driving the drop in HbA_{1c} levels). I similarly observed a non-linear association between HbA_{1c} and adverse brain health. Although important confounding variables (e.g., BMI) were controlled for and a sensitivity analysis was conducted excluding those with medication (in case that was driving hypoglycaemia), I acknowledge that reverse causality cannot be ruled out in my study. It is plausible that the associations in the low glycaemic range were driven by changes in brain health outcomes in regions important for glucose metabolism. Similarly, it is possible that participants with poorer brain health may have nutritional deficiencies, demonstrate dietary inadequacies or possess metabolic or endocrine disorders which may then have manifested in these associations observed in the lower range of glycaemia.

More specifically, I observed evidence of non-linear associations between glucose and HV without clear differences between males and females, although estimates were imprecise. A previous study in the UK Biobank found that stratifying HbA_{1c} at different ranges, had different relationships with HV: Low HbA_{1c} (under 35 mmol/mol) was associated with higher HV but prediabetes (42 ≤ 48 mmol/mol), undiagnosed diabetes and known diabetes (≥48 mmol/mol) were associated with lower HV (although clinical criteria use 47 mmol/mol as the upper limit for prediabetes).[364] It may be argued that these findings, particularly the associations observed in the upper range, are consistent with the findings from the HbA_{1c} models reported here since both sets of results suggest that as HbA_{1c} increases, HV decreases.

The observation that HbA_{1c} in the higher range (or hyperglycaemia) is associated with smaller brains has previously been reported both in a population-based sample and in those with T2D.[352,407,410] Hyperglycaemia has previously been linked to poorer cognition and brain health via multiple potential mechanisms (e.g., oxidative stress, cerebrovascular health, impaired neuronal signalling, amyloid metabolism, and inflammation).[437] In regard to sex differences, higher HbA_{1c} has previously been associated with smaller WBV in females,[407] as discussed in Chapter 3. In this study there was some modest evidence of sex differences in the relationship between

glycaemia and brain volume associations. HbA_{1c} and glucose in the upper ranges were associated with smaller WBV (and WM volumes) both in males and females, although the steepness of the decline in those above the normoglycaemia range appeared to be more prominent in females. However, once again the imprecision of the estimates warrants exercising caution when interpreting the results. Potential mechanisms that may explain the differential brain changes related to sex in the context of hyperglycaemia include differences in inflammatory or hormonal states related to oestrogen's neuroprotective effect. For example, one study found that hippocampal atrophy in females with T2D could be potentially explained by sex differences in markers of low-grade inflammation such as fibrinogen and CRP.[413]

Analyses into preferential tissue loss

There were some suggestive sex differences in the associations with the tissue measures. HbA_{1c} and glucose, particularly in the upper ranges, were associated with smaller GM volumes. These were convincing when examining the associations with WM volumes. This is consistent with previous studies, which have found that HbA_{1c} and T2D were associated with lower WBV and GM but not WM volumes.[365,452] The findings from my study demonstrate that poorer glycaemic health across the population spectrum is associated with reduced GM volume while also highlighting potential sex differences in these associations. The precise reasons why the glycaemic markers may be more robustly associated with lower GM but not WM volumes are still poorly understood. It has been proposed that neurons found in abundance in GM tissue may be more susceptible to oxidative injury due to their high metabolic activity and energy demands. Hyperglycaemia can exacerbate oxidative stress in neurons, resulting in direct oxidation of cellular components such as proteins, lipids, and DNA.[437] This oxidative damage can contribute to neurodegenerative processes and affect GM volume. On the other hand, there is some evidence that glial cells such as oligodendrocytes, responsible for producing myelin in WM tracts, are also vulnerable to oxidative injury but may possess greater resistance when compared to neurons.[492] Astrocytes and microglia, which provide support and immune surveillance in the CNS, have also been proposed to be more resistant to oxidative stress and may play protective roles in WM regions.[492] This being said, chronic hyperglycaemia can still disrupt myelin integrity and WM connectivity, albeit perhaps to a lesser extent than GM regions. Although my findings from Chapter 4 failed to find

convincing sex differences between different glycaemic indices and GM volumes, the estimates were consistent with previously published work.[365] I do however acknowledge that the confidence limits once again suggest applying caution due to the uncertainty of the estimates. Based on the confidence intervals of these estimates, any sex difference in these relationships is likely to be small. Future studies are required to examine whether poorer glycaemia is indeed predictive of preferential tissue loss.

There is a similar case for the associations between the glycaemic markers and WMHV. The modelling of both glycaemic markers with WMHV imply a very weak non-linear association with considerable imprecision of the estimates. For HbA_{1c} the non-linear findings appear slightly more convincing (i.e., low, and higher HbA_{1c} associated with the higher burden of WMHV) and thus perhaps in line with the WBV/GM volume findings, with both hypoglycaemia and hyperglycaemia appearing to be associated with poor brain health outcomes, especially in females. A previous study of 1904 Japanese participants also reported non-linear associations between HbA_{1c} and WMHV in females.[449] My previous analysis reported in Chapter 3 did not find convincing evidence of a linear association between HbA_{1c} and WMHV in the pooled sample (also published),[407] and potential explanations such as those relating to the sensitivity of the measure considered in the analyses were given. In this chapter, although weak associations were reported, abnormal glycaemic health has mechanistically been associated with damage to small vessels.[493] In terms of sex-specific findings, there is some suggestion of increased vulnerability in females, but this once again must be interpreted with caution in the context of the imprecision of the estimates and the non-linear model. Previous evidence has also suggested that females generally have a higher WMHV despite having a lower prevalence of diabetes than males.[133,494] Since WMHs may mediate some of the associations between diabetes and cognitive health, a lower prevalence of diabetes being associated with a higher burden of WMHV may suggest an increased susceptibility to cerebral small vessel damage in females. Future studies are required to further examine these sex-specific associations using more sensitive measures of SVD (e.g., location and shape of lesions).[355]

In regard to the effects observed in the hypoglycaemic range, previous studies have shown that hypoglycaemic events in those with T2D can be detrimental to the brain increasing the risk for dementia and cognitive decline,[260–262] although these studies often include states of considerably lower glucose. Hypoglycaemia can be associated with a range of negative neurophysiological consequences such as oxidative stress, microgliosis, impaired synaptic plasticity and neuronal death.[495–497] One potential mechanistic explanation of low glycaemic states being associated with lower brain volumes may be related to the very high and sensitive metabolic demands of neuronal cells. My findings suggest that low glycaemia, even without being in the extreme lower range, may still be associated with poorer brain health. Once again, the possibility that these effects observed in this range may be a consequence of reverse causality must be acknowledged.

The cognition findings

Unlike the glycaemia-brain volume associations, modelling of the relationship of HbA_{1c} and glucose to the cognitive outcomes did not support non-linear models. Thus, a series of robust Poisson (for VM) and linear regressions (for RT) were used. Broadly, the findings suggested some weak evidence of sex-specific associations between the glycaemic markers and the cognitive outcomes: poorer glycaemic health suggested slower RT in females (mainly for HbA_{1c}). Previous evidence suggested that increasing HbA_{1c} is associated with slower RT as well as poorer performance across a range of other cognitive measures.[365] Prediabetes (categorised as HbA_{1c} between 42 and 48 mmol/mol) was associated with a 1% slower RT.[365] The findings from this analysis go further by suggesting that continuous HbA_{1c} is associated with slower RT in females, but this has to be considered in the context of multiple testing. For VM, there was some evidence that higher glucose in males predicted better performance, although these associations were attenuated when adjustments were made for T2D medication. Previous studies have reported mixed findings in the associations between glycaemic markers and VM performance. For example, Garfield and colleagues found no association in those with low-normal HbA_{1c} and those with undiagnosed diabetes, but surprisingly those with T2D showed better VM making fewer errors than their peers with normal glycamia.[364]

The incongruence between the glycaemia-brain and glycaemia-cognition findings may be explained in many ways. Firstly, even in the case of AD, the pathological onset of imaging biomarkers of disease has been shown to precede the onset of the symptoms by decades.[8] It is thus possible that the time between the measurements of the glycaemic markers and those of cognitive outcomes is too short for the deficits to become apparent. To maximise data availability, only two measures of cognition were considered in this study. However, these are unlikely to assess the full complexity of the cognitive spectrum. It is also worth acknowledging that the discrepancy between brain volume outcomes and cognition may be due to the participant's cognitive reserve. Cognitive reserve denotes the capacity to withstand brain pathology either due to structural benefit, compensatory mechanisms or functional reorganisation which may involve changes in neural activation patterns, synaptic connectivity, or neurotransmitter systems.[448] This may particularly be of relevance in a sample such as UK Biobank which has been found to be non-representative and considerably healthier than the general population.[33]

The value of considering different glycaemia markers

Overall, my results suggest that HbA_{1c} and glucose were mostly similar in their associations with the brain health outcomes. This is important since HbA_{1c} and glucose are markers representing different aspects of glucose metabolism.[459] HbA_{1c} is formed when haemoglobin, the protein in a non-enzymatic blood cell that carries oxygen, becomes glycated through a non-enzymatic reaction with glucose in the bloodstream. The concentration of HbA_{1c} reflects the average plasma glucose concentration over the lifespan of red blood cells, which is typically around 120 days. Blood glucose provides a snapshot of immediate glucose levels at the time of measurement. It is important to note that HbA_{1c} levels can be affected by medical conditions that influence erythrocyte turnover, as well as genetic hereditary anaemia and iron storage disorders.[214,215] But glucose is unaffected by this issue. The drawback of using random glucose is that each participant may be at a different postprandial state which may influence their glucose serum levels measured. Thus, the consideration of both HbA_{1c} and glucose is complimentary offering a more comprehensive capture of the glycaemia-brain health associations. Since both measures mostly behave similarly in relation to the outcomes, confidence in the

findings is reinforced while also suggesting that both short and long-term glycaemia are associated with poorer brain health.

My sensitivity analyses did not reveal that the poorer glycaemic health in the upper range was driven exclusively by participants with diabetes nor those with medication. Naturally it may be expected that excluding individuals with more extreme hyperglycaemia such as those with diabetes or on diabetes medication will tend to attenuate relationships, but these sensitivity analyses did not change my interpretation of the findings.

Strengths and weaknesses

The study possesses some important strengths. First, the UK Biobank sample is one of the largest studies to have both glycaemic markers and neuroimaging measures. It also has a breadth of lifestyle and metabolic variables that can be used to reduce confounding in these relationships. And perhaps most important to this study, is that its sample size offers sufficient power to explore the possibility of non-linear relationships between the glycaemic markers and those of brain health. Furthermore, UK Biobank is a community-based sample this may have advantages over a clinical case-control sample (e.g., selecting people with diabetes and 'controls'). However, an important limitation is that the UK Biobank is not population representative. For example, there is an important healthy bias in the participation in this study.[33]

A benefit of fp models is that they offer more flexibility for modelling and potentially provide a better fit to the observed data. But at the same time, the interpretation of the fp models is complex as the coefficients outputted are transformed variables which do not have straightforward interpretation in the original units of the predictor (in this case glycaemia). It is also important to note that the lower and upper ranges of glycaemia had fewer participants which resulted in wide CI.

6.5 Conclusions

The findings in this chapter revealed a non-linear association between markers of glycaemia and some brain health outcomes in UK Biobank. Both low and high HbA_{1c} and glucose were associated with lower whole brain volumes and grey matter volumes. Evidence for associations between markers of glycaemia and white matter

hyperintensity volumes and white matter volumes were less convincing and there were no convincing associations with hippocampal volumes and cognitive outcomes. There were some suggestions that these associations differed by sex, although the lack of precisions in the estimates make it difficult to claim this with confidence.

7. Use genetic tools to strengthen causal inferences in the glycaemia-brain volume associations

In order to strengthen causal inference in the observational findings reported in Chapter 6, the aim of this study is to examine whether genetic risk scores for glycaemia (HbA_{1c} and glucose) support the sex-specific associations observed with brain health outcomes and the differences between people in the low and high glucose strata.

7.1 Introduction

HbA_{1c} and blood glucose show considerable heritability: for HbA_{1c} levels, heritability estimates range between 47% to 59% whereas for fasting glucose it is around 35%.[453] The past decade of research has identified common genetic variants, SNPs, associated with these glycaemic measures. An individual SNP accounts for only a small proportion of increased risk conferred by one's genetic background. However the aggregation of these SNPs (i.e., by constructing a PRS) can produce a summary effect of these multiple variants, which can then be used to predict the glycaemic measures of interest. Recent studies have identified 60 and 139 SNPs for HbA_{1c} explaining between 2.8% and 5.8% of total variance,[206,364] although other studies do not report the variance explained by their glycaemia/diabetes-related PRS.[365,498] Thus, PRSs can be considered useful quantitative measures of genetic susceptibility for glycaemia. The utility of these predictive tools can further be reinforced by changing the parameters around their construction (i.e., constructing multiple PRS instruments), allowing a more comprehensive investigation of their possible contribution in predicting health outcomes. PRSs are particularly useful since they are unconfounded: they rely on the random allocation alleles at conception and independent assortment, which ensures that genetic associations are free from confounding factors.

Some studies have suggested that PRSs for T2D have predictive utility for brain health outcomes such as dementia risk. In a recent population-based study, they were predictive of all-cause dementia, mixed dementia, and vascular dementia.[499] With this in mind, it is of further interest to examine whether this predictive utility can also be applied when looking more specifically at markers of brain pathology (such as volumetric brain measures or WMHV). Following from the observational findings of the

previous chapters, it is also of value to examine whether an unconfounded genetic tool supports the previous the sex- and strata-specific associations described. To be more specific, Chapter 6 showed non-linear associations between glycaemia and some of the volumetric brain health outcomes in UK Biobank participants (since both lower and higher levels of glycaemia were associated with smaller WBV, GM and WM volumes).

Thus, the aims of this project are to use genetic tools to strengthen causal inference in the observational findings reported in Chapter 3, Chapter 4, and Chapter 6. More precisely, examine whether the PRSs show: 1) sex-specific associations with the brain health outcomes and 2) differences between individuals in low and high glucose strata.

7.2 Methods

7.2.1 Sample

The sample included participants from the UK Biobank, a prospective cohort study of approximately 500,000 individuals, aged 40 to 69 years at baseline, recruited from the general UK population. Participants underwent physical examinations, completed questionnaires on sociodemographics, lifestyle and health history, and provided blood, urine, and saliva samples. The sample was previously introduced in more detail in Chapter 2.4.1.

7.2.2 Investigations

HbA_{1c} and glucose

HbA_{1c} assays were performed using five Bio-Rad Variant II Turbo analysers, manufactured by Bio-Rad Laboratories, Inc., and employed a HPLC method.[487] More details are given in Chapter 6. Random serum glucose was analysed and measured by hexokinase analysis on a Beckman Coulter AU5800. These were previously used in published work.[364,500]

Confounders

Although adjustments for confounders have previously been made in studies that consider genetic tools for hyperglycaemia, this approach was not implemented in this study. This is because including these covariates may introduce bias through opening a path between the PRS, and the outcome trait via an unmeasured common genetic

or environmental cause of the covariate and the outcome trait (collider bias). The complexity and pitfalls of adjusting for confounders in genetic studies is discussed by Aschard and colleagues.[501] The exceptions to this were age, which was considered a covariate in the model on the basis that it substantially reduces variance in the outcomes of interest and thereby increases the precision of the estimates and principal components (PC) to ensure that the results were not affected by population stratification. Adjustment for 10 PC has previously been done in similar studies and its importance in genetic studies is discussed here in more details.[502,503]

Outcomes

The neuroimaging protocols are the same as those listed in Chapter 6.2.

7.2.3 Genotyping in UK Biobank

The following subsections describe the steps taken to construct and validate the genetic tools. The same genetic data was used in previous publications.[364,502,504]

The full details of the genotyping as well as the QC steps conducted were described in Bycroft and colleagues and on the Biobank website.[505] In brief, 488,377 participants were initially genotyped (after QC). The first 50,000 participants in the sample were genotyped using two arrays. The UK Biobank then used to a combination of the UK10K, 1000 Genomes Phase 3, and the Haplotype Reference Consortium (HRC) reference panels to conduct imputation.[505] More details are discussed in Chapter 2.5.3.

A number of variant- and individual-level QC steps were conducted. For the former, this included, checking for minor allele frequency <1% and a missing call rate of more than 5%. Individual-level QC was also applied resulting in the exclusion of extreme or minimal heterozygosity rates, of those with more than 10 putative third-degree relatives, of participants who did not consent to their DNA being extracted, of sex mismatches between self-reported and genetic-inferred sex, absent QC information and non-European ancestry (based on how individuals had self-reported their ancestry and the similarity with their genetic ancestry, as per a PC analysis of their genotype). More details of the QC steps conducted are once again described by Bycroft and colleagues.[505]

The construction of PRS tools

PRS instruments for HbA_{1c} and fasting glucose were constructed using GWAS summary statistics from the recent MAGIC Consortium.[506] For consistency with the NSHD analyses, and based on the availability of MAGIC consortium genetic data, only the summary data of White Europeans were used. The study used GWAS summary statistics aggregated from 281,416 individuals without diabetes, of whom approximately 70% were of European ancestry. This meant that the HbA_{1c} PRSs were generated in a sample different to UK Biobank. The calculation of the PRS tools only included SNPs that showed a GWAS association p-value below a specified threshold (e.g., $p < 1 \times 10^{-8}$).

Prior to construction, a few QC assessments were made in line with previous studies [502] using a previously published pipeline.[507] Checks were made for duplicates SNPs, but none were found. SNPs were checked for strand alignment. Once these QC steps were taken, the PRS tools were constructed with β coefficients being used as external weights. The datasets were also checked to see if they were genotyped using the DNA strand conventions (i.e., one to the forward strand and the other to the backward strand). In the case of any inconsistencies, the strands were flipped in PLINK2.

Controlling for Linkage disequilibrium

Linkage disequilibrium (LD) among SNPs was accounted for by carrying a two-step clumping procedure on PLINK 2. The SNPs were clumped to ensure that only those with the locus with the smallest GWAS p-values were used, i.e., those largely independent from each other. LD clumping ensures that each PRS comprises of independent genetic variants, enhancing the overall predictive power and allowing for the exploration of potential genetic heterogeneity across populations or subgroups. This was combined with thresholding. Thresholding is conducted by removing SNPs with a p-value larger than a certain threshold to reduce noise in the score generated. PRS tools were created by choosing different r^2 values within a 250kb range. Here I used *a priori* selected r^2 of <0.01, <0.1 and <0.2 for each glycaemic marker. These PRS tools were labelled as PRS1, PRS2 and PRS3 respectively.

Genetic scoring was conducted on PLINK2 using the –score command. This generated a score for each participant by summing across the number of risk alleles

for each of the SNPs, weighted by the effect size. This generated 6 PRSs, 3 for HbA_{1c} and 3 for fasting glucose. Each PRS was standardised and centred, so that the mean was zero and a 1-unit increase is equal to 1 SD. This facilitates the interpretation of the associations observed.[507]

7.2.4 Statistical analysis

The validity of all assumptions was assessed by visually inspecting the normality and homoscedasticity of the data. Variables displaying non-normal distributions underwent log transformation. WMHV was log-transformed as per the previous Chapters in this thesis. Brain MRI volumes were standardised to z-scores for analysis.

Validation of the PRS tools

The different PRS tools were regressed against observed HbA_{1c} and glucose respectively in the UK Biobank sample. The PRS-glycaemia associations were examined both for the entire UK Biobank sample, as well as the subset of participants who only underwent brain imaging. The analyses were sex-stratified and β coefficients, CI, p values and variance explained (r^2) were reported.

PRS-outcome sex-stratified analyses

For the PRS-brain associations examined, each PRS was regressed against the brain imaging (WBV, WMHV, HV, GM and WM) and cognitive outcomes (RT and VM) in males and females separately to further examine differences and maintain consistency with the rest of the thesis. As described in the methods, for each model, the only adjustments made were for age and 10 PCs.

Stratified analysis

Participants were stratified into groups based on their observed HbA_{1c} and glucose. For the HbA_{1c} analyses, participants were split into the following categories: low HbA_{1c} (< 35 mmol/mol) and hyperglycaemia (≥ 42 mmol/mol) based on criteria published in previous papers.[364,508] This stratification was consistent with the shape of the non-linear associations reported in Chapter 6. For the glucose analyses, participants were categorised as: low glucose (< 6 mmol/L) and high glucose (≥ 7 mmol/L).

Statistical Software and Packages

All data management and analyses were performed using R Studio version 4.0.2 and Stata 17.

7.3 Results

Sample characteristics

Genotype data was available for 407,869 participants. 389,988 had at least one glycaemic marker, and of those 36,321 also had structural imaging data (see Figure 7.1). These were the same participants considered in the analyses described in Chapter 6.

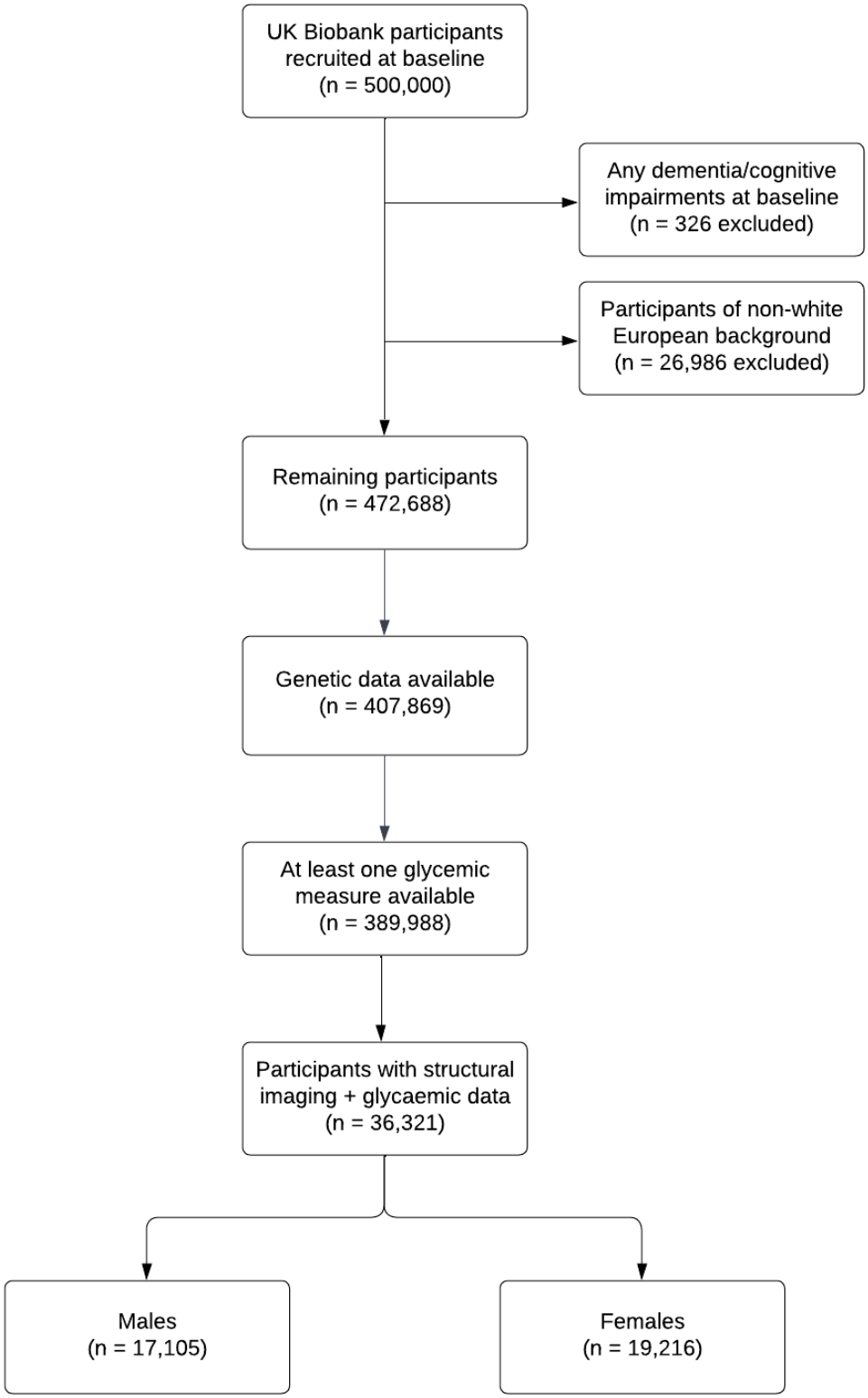


Figure 7.1: Flowchart displaying participants considered in this study. UK Biobank participants had to have structural imaging data and at least one measure of glycaemia to be considered (n= 36,321).
This means that the participants were the same as those considered in Chapter 6.

Sample Characteristics			Males = 17,105	n	Females = 19,216
Age, years:			55.6 (7.5)		54.2 (7.2)
Deprivation	Least deprived	17,095	4238 (24%)	19,196	4518(25%)
	Second least deprived		4067 (24%)		4485 (23%)
	Median deprivation level		3547 (21%)		4036 (21%)
	Second most deprived		3078 (18%)		3614 (18%)
	Most deprived		2165 (13%)		2543 (13%)
Smoking	Never smoker	17,090	11,619 (68%)	19,201	14,616 (76%)
	Current Smoker		1,242 (7%)		995 (5%)
	Ex-smoker		4,229 (25%)		3,590 (19%)
BMI, kg/m^2		17,083	27.1 (3.7)	19,192	26.1 (4.5)
HbA_{1c}, mmol/mol		16,505	35.2 (5.5)	18,506	34.7 (4.5)
HbA_{1c}, mmol/mol, range		16,505	16-122.6	18,506	15.3-91.1
Glucose, mmol/L mean		13,215	5 (1.1)	15,081	4.9 (0.8)
Glucose, mmol/L, range		13,215	1.9-26.6	15,081	1.78-24.1
Diabetes medication		17,105	365 (2.1%)	19,216	222 (1.2%)
Diabetes diagnosis		17,105	519 (3%)	19,216	233 (1.2%)
Brain imaging and cognitive markers					
Whole brain volume (WBV) cm^3		17,105	1480.7 (70.8)	19,216	1505.3 (73.0)
Grey matter volume (GM) cm^3		17,105	775.6 (930.2)	19,216	807.1 (458.4)
White matter volume (WM) cm^3		17,105	705.3 (407.6)	19,216	698.2 (403.7)
Hippocampal volume (HV) cm^3		17,105	3.8 (0.12)	19,216	3.8 (0.1)
White matter hyperintensity volume (WMHV) cm^3		17,105	8.1 (1)	19,216	7.9 (1)
Total intracranial volume (TIV) cm^3		17,105	1644.2 (131.1)	19,216	1468.4 (115.7)

Whole brain volume unadjusted cm^3	17,105	1225.3 (98.7)	19,216	1107.1 (89.9)
Reaction time (ms)	17,070	195.4 (96.6)	19,180	209.8 (98.4)
Visual memory (incorrect matches)	16,191	3.8 (3)	18,201	3.7 (2.9)

Table 7.1: Sample characteristics for the male and female participants considered in this study (n = 36,321).
As described above, participants had to have genetic, structural imaging and data on least one measure of glycaemia to be considering amounting to 36,321 participants of which 17,105 were males and 19,216 were females. Values presented are: n (%), mean (SD) or median (IQR). % are calculated against the max data available for that specific measure for the respective sample. Whole brain, grey matter and white matter volume measurements reported were already normalised for head size by the UK Biobank. SD: Standard deviation. IQR: Interquartile range.

Analysis 1: Description and validation of the PRS

For HbA_{1c}, the three different PRSs included 92, 143 and 210 SNPs respectively (see Appendix, Supplementary Table 1 and Supplementary Table 2). The results of the sex-stratified validation are reported in Table 7.2. Briefly, there was minimal evidence that the PRS instruments predicted observed HbA_{1c} in either the whole UK Biobank sample or the neuroimaging sub-sample in either sex. The size of the r^2 suggested that the PRS had miniscule predictive power in explaining observed HbA_{1c}. Nonetheless, the coefficients and direction suggested that an increase in genetic score for glycaemia (as indexed by the HbA_{1c} PRS) was weakly related to higher measured HbA_{1c}, especially when looking at the whole UK Biobank sample. This being said, the size of the coefficients did not materially change between looking at the whole sample or only at the neuroimaging sub-sample.

For glucose, the three different PRSs included 84, 148 and 190 SNPs respectively (see Table 7.3). Results were very similar for those of HbA_{1c} yet there was some slightly stronger evidence that the PRS-glucose instruments predicted measured glucose in the whole UK Biobank sample although once again not convincingly in the imaging sub-sample. As per the HbA_{1c} PRS tools, the explained r^2 was small.

As a positive control, logistic regressions were conducted to see if the PRS instruments predicted T2D diagnoses (see Table 7.4). There was some evidence that the glucose PRSs were associated with an increased risk of T2D in the whole sample: for PRS3, the OR was 1.02 (CI: 1.01-1.02, p = 0.003). This, however, was not observed for the HbA_{1c} genetic instruments since all three PRSs had an OR of 1.01 (CI: 0.9, 1.02), p = 0.2).

Whole Biobank Sample (n = 389, 988)								
		PRS	SNPs	β	95% CI		p	r^2
Males	179,170	PRS1	92	0.03	0.001	0.010	0.04	<1%
		PRS2	143	0.01	-0.02	0.05	0.3	
		PRS3	210	0.02	-0.001	0.008	0.2	
Females	210,818	PRS1	92	0.002	-0.002	0.005	0.4	
		PRS2	143	0.002	-0.001	0.006	0.2	
		PRS3	210	0.002	-0.001	0.006	0.1	
Pooled	389, 988	PRS1	92	0.02	0.001	0.04	0.04	
		PRS2	143	0.02	-0.005	0.04	0.05	
		PRS3	210	0.02	-0.01	0.04	0.06	
Neuroimaging sample (n = 36,321)								
		PRS	SNPs	β	95% CI		p	r^2
Males	17,105	PRS1	92	0.04	-0.007	0.021	0.34	<1%
		PRS2	143	0.02	-0.011	0.017	0.69	
		PRS3	210	0.03	-0.009	0.018	0.54	
Females	19,216	PRS1	92	-0.003	-0.011	0.010	0.93	
		PRS2	143	-0.01	-0.01	0.012	0.84	
		PRS3	210	0.01	-0.009	0.012	0.77	
Pooled	36,321	PRS1	92	0.02	-0.04	0.08	0.50	
		PRS2	143	0.01	-0.04	0.07	0.60	
		PRS3	210	0.02	-0.03	0.07	0.50	

Table 7.2: Validation analyses regressing each polygenic risk score for HbA_{1c} against observed HbA_{1c} measured in: 1) the whole UK Biobank sample and 2) its neuroimaging sub-sample.
r^2 column describes total variance explained by the genetic tools. β, confidence intervals and p-values for males, females and pooled are presented. Units presented are standardised.

Whole sample (n = 345, 865)								
	n =	PRS	SNPs	β	95% CI		p	r^2
Males	159,754	PRS1	84	0.008	0.002	0.013	0.01	<1%
		PRS2	148	0.006	0.001	0.011	0.03	
		PRS3	190	0.006	0.001	0.012	0.03	
Females	186,111	PRS1	84	0.007	0.003	0.011	0.001	
		PRS2	148	0.007	0.003	0.011	<0.001	
		PRS3	190	0.007	0.003	0.011	0.001	
Pooled	345, 865	PRS1	84	0.008	0.005	0.013	<0.001	
		PRS2	148	0.008	0.003	0.011	<0.001	
		PRS3	190	0.008	0.003	0.01	<0.001	
Neuroimaging sample (n = 36,321)								
		PRS	SNPs	β	95% CI		p	r^2
Males	17,105	PRS1	84	0.005	-0.011	0.02	0.57	<1%
		PRS2	148	0.004	-0.012	0.02	0.59	
		PRS3	190	0.005	-0.011	0.02	0.58	
Females	19,216	PRS1	84	0.004	-0.007	0.015	0.45	
		PRS2	148	0.004	-0.007	0.015	0.49	
		PRS3	190	0.005	-0.006	0.016	0.42	
Pooled	36,321	PRS1	84	0.005	-0.01	0.02	0.5	
		PRS2	148	0.005	-0.009	0.02	0.5	
		PRS3	190	0.005	-0.007	0.02	0.4	

Table 7.3: Validation analyses regressing each polygenic risk score for glucose against observed glucose measured in the 1) whole UK Biobank sample and 2) its neuroimaging sub-sample.
r^2 column describes total variance explained by the genetic tools. β, confidence intervals and p-values for males, females and the pooled sample are presented. Units presented are standardised.

	Whole sample (n = 389, 988)					
	n =	PRS	OR	95% CI		p
HbA_{1c}	389. 988	PRS1	1.01	0.9	1.02	0.2
		PRS2	1.01	0.9	1.02	0.2
		PRS3	1.01	0.9	1.02	0.2
	Neuroimaging sample (n = 36,321)					
	n =	PRS	β	95% CI		p
	36,321	PRS1	1.0	0.9	1.07	0.3
		PRS2	1.0	0.9	1.04	0.3
		PRS3	1.0	0.9	1.03	0.3
	Whole sample (n = 345, 865)					
	n =	PRS	β	95% CI		p
Glucose	345, 865	PRS1	1.02	1.01	1.03	0.02
		PRS2	1.02	1.01	1.04	0.005
		PRS3	1.02	1.01	1.04	0.003
	Neuroimaging sample (n = 36,321)					
	n =	PRS	β	95% CI		p
	36,321	PRS1	1.0	0.9	1.04	0.3
		PRS2	1.0	0.9	1.05	0.3
		PRS3	0.9	0.9	1.02	0.3

Table 7.4: Regression of the HbA_{1c} and glucose polygenic risk scores on Type 2 diabetes diagnosed in the pooled (males + females) 1) whole UK Biobank sample and 2) its neuroimaging sub-sample.
β, confidence intervals and p-values are presented. Units presented are standardised.

Analysis 2: sex stratified PRS-outcome relationships

Glycaemia PRS and WBV associations

Overall, there were no convincing associations between PRS-HbA_{1c} and WBV in either males or females (see Table 7.5). Some coefficients were larger in females (e.g., the size of the coefficient for PRS2 was triple that of males), but this could easily be a chance finding since the CI were wide and compatible with no relationship. The estimates from the different PRS instruments also did not substantially vary between them.

There were also no associations between PRSs for glucose and WBV in either males or females (see Table 7.5). The estimates from the different PRSs did not vary between them, although there may be some suggestions that the size of the coefficients increased as more SNPs were considered (i.e., PRS3 had the biggest coefficient, followed by PRS2 then PRS1).

Glycaemia PRS and GM and WM volume associations

Overall, there were no convincing associations between PRS-HbA_{1c} and GM in either males or females in any models (see Table 7.5). The association between PRS-HbA_{1c} and WM suggest some small associations in females but not in males. PRS2 and PRS3 in females weakly predicted smaller WM suggesting that the addition of more SNPs increased the size of the estimates for these associations, however this needs to be considered in the context of multiple testing. Neither of the PRSs predicted WM in males.

There were no convincing associations between PRS-glucose and GM or WM in either males or females in any models, albeit some weak evidence of PRS3 predicting smaller GM volumes in males (see Table 7.5).

Glycaemia PRS and WMHV

Overall, there were no convincing associations between PRS-HbA_{1c} and WMHs in either males or females (see Table 7.5).

There were also no convincing associations between PRS-glucose and WMHs in either males or females (see Table 7.5). Some evidence for an association between

PRS1 and WMHs in males were found but were considered dubious due to multiple testing undertaken.

Glycaemia PRS and HV

Overall, there were no convincing associations between PRS-HbA_{1c} and PRS-glucose instruments and HV in either males or females (see Table 7.5).

		Whole brain volumes (WBV)							
	PRS	Males				Females			
		β	**95 CI**		**p**	**β**	**95% CI**		**p**
HbA_{1c}	PRS1	0.001	-0.013	0.014	0.93	-0.003	-0.016	0.009	0.58
	PRS2	-0.003	-0.017	0.010	0.61	-0.011	-0.024	0.001	0.07
	PRS3	-0.004	-0.017	0.009	0.56	-0.009	-0.021	0.003	0.15
Glucose	PRS1	-0.003	-0.016	0.010	0.66	-0.001	-0.014	0.011	0.81
	PRS2	-0.008	0.243	0.005	0.24	0.004	-0.009	-0.009	0.57
	PRS3	-0.011	-0.024	-0.024	0.11	0.001	0.001	0.001	0.82
		White matter hyperintensity volumes (WMHV)							
	PRS	Males				Females			
		β	**95% CI**		**p**	**β**	**95% CI**		**p**
HbA_{1c}	PRS1	-0.002	-0.016	0.013	0.84	-0.004	-0.016	0.009	0.57
	PRS2	-0.001	-0.016	0.013	0.85	0.001	-0.012	0.014	0.89
	PRS3	-0.008	-0.022	0.006	0.25	-0.004	-0.017	0.009	0.56
Glucose	PRS1	0.013	-0.001	0.027	0.08	0.004	-0.008	0.017	0.49
	PRS2	0.008	-0.006	0.022	0.28	0.278	-0.009	0.017	0.53
	PRS3	0.008	-0.007	0.022	0.29	0.002	-0.011	0.014	0.79
		Hippocampal volumes (HV)							
	PRS	Males				Females			
		β	**95% CI**		**p**	**β**	**95% CI**		**p**
HbA_{1c}	PRS1	0.001	-0.015	0.017	0.91	-0.003	-0.011	0.01	0.83
	PRS2	0.001	-0.015	0.016	0.94	0.004	-0.01	0.02	0.62
	PRS3	-0.001	-0.017	0.015	0.90	0.004	-0.01	0.02	0.77
Glucose	PRS1	-0.003	-0.019	0.012	0.68	0.008	-0.005	0.02	0.25
	PRS2	-0.008	-0.023	0.008	0.36	0.010	-0.003	0.023	0.11

	PRS3	-0.004	0.012	0.002	0.6	0.008	-0.005	0.020	0.25
		Grey matter (GM) volumes							
	PRS	Males				Females			
		β	**95% CI**		**p**	**β**	**95% CI**		**p**
HbA1c	PRS1	0.0001	-0.012	0.012	0.96	0.0001	-0.011	0.011	0.96
	PRS2	-0.001	-0.013	0.011	0.84	-0.002	-0.013	0.009	0.68
	PRS3	-0.001	-0.012	0.011	0.91	0.000	-0.011	-0.011	0.94
Glucose	PRS1	-0.003	-0.014	0.009	0.66	-0.005	-0.016	0.006	0.39
	PRS2	-0.006	-0.018	0.006	0.31	0.001	-0.010	0.012	0.86
	PRS3	-0.011	-0.023	0.001	0.07	-0.002	-0.013	0.009	0.72
		White matter (WM) volumes							
	PRS	Males				Females			
		β	**95% CI**		**p**	**β**	**95% CI**		**p**
HbA1c	PRS1	0.003	-0.012	0.018	0.72	-0.004	-0.018	0.010	0.60
	PRS2	-0.002	-0.017	0.013	0.76	-0.015	-0.029	-0.001	0.03
	PRS3	-0.004	-0.019	0.011	0.58	-0.014	-0.028	0.000	0.05
Glucose	PRS1	0.001	-0.014	-0.014	0.95	0.002	-0.012	-0.012	0.8
	PRS2	-0.005	-0.020	0.010	0.53	0.002	-0.012	-0.012	0.77
	PRS3	0.002	-0.012	0.016	0.77	0.003	-0.010	0.017	0.63

Table 7.5: Results from the regression analyses between the different glycaemic polygenic risk scores and the brain imaging outcomes stratified by sex.
Adjustments were only made for age and principal components as described in the methods. The values presented are standardised β coefficients, confidence intervals, and p values.

Analysis 3: Stratified analyses exploring associations between PRSs in the low glycaemia and high glycaemia groups

There was some weak evidence that the PRS tools for HbA_{1c} were associated with smaller WBV and WM in the low glycaemia strata. There was no other convincing evidence that PRS tools for HbA_{1c} or glucose were associated with brain health measured in either the high or low glycaemic strata (Table 7.6).

In all of my analyses for this study, I adjusted for 10 PCs which meant that the findings presented are unlikely to suffer from issues related to population stratification.

		PRS	WBV				WMHV				HV				GM				WM			
			β	**95% CI**		**p**	**β**	**95% CI**		**p**	**β**	**95% CI**		**p**	**B**	**95% CI**		**p**	**β**	**95% CI**		**p**
HbA_{1c}	**Hypoglycaemia** (< 35 mmol/mol)	PRS1	-0.007	-0.019	0.006	0.30	0.000	-0.013	0.014	0.94	-0.003	-0.018	0.011	0.65	-0.006	-0.018	0.006	0.33	-0.006	-0.020	0.008	0.41
		PRS2	-0.013	-0.026	-0.001	0.04	0.007	-0.006	0.020	0.29	-0.003	-0.017	0.012	0.74	-0.010	-0.022	0.002	0.09	-0.012	-0.026	0.002	0.09
		PRS3	-0.012	-0.025	0.000	0.06	-0.002	-0.015	0.011	0.74	-0.005	-0.020	0.009	0.49	-0.006	-0.018	0.006	0.32	-0.014	-0.028	0.000	0.06
	Hyperglycaemia (≥ 42 mmol/mol)	PRS1	0.003	-0.030	0.036	0.87	-0.007	-0.042	0.028	0.70	-0.002	-0.040	0.037	0.94	-0.003	-0.035	0.029	0.840	0.009	-0.029	0.046	0.64
		PRS2	-0.001	-0.034	0.031	0.94	-0.001	-0.036	0.033	0.94	0.010	-0.028	0.048	0.61	-0.005	-0.037	0.027	0.77	0.005	-0.032	0.043	0.77
		PRS3	-0.001	-0.034	0.031	0.93	-0.001	-0.035	0.034	0.97	0.006	-0.032	0.043	0.77	-0.005	-0.037	0.026	0.74	0.002	-0.035	0.039	0.91
Glucose	**Hypoglycaemia** (< 6 mmol/L)	PRS1	-0.001	-0.011	0.009	0.86	0.006	-0.005	0.017	0.25	-0.003	-0.015	0.009	0.59	-0.004	-0.014	0.005	0.38	0.005	-0.007	0.016	0.45
		PRS2	-0.002	-0.013	0.008	0.69	0.004	-0.007	0.015	0.49	-0.006	-0.018	0.006	0.33	-0.001	-0.011	0.009	0.86	-0.003	-0.014	0.009	0.67
		PRS3	-0.004	-0.014	0.006	0.45	0.003	-0.008	0.013	0.64	-0.004	-0.016	0.008	0.51	-0.004	-0.014	0.006	0.44	-0.001	-0.013	0.011	0.85
	Hyperglycaemia (≥ 7 mmol/L)	PRS1	-0.006	-0.025	0.013	0.52	0.015	-0.005	0.035	0.14	0.023	0.001	0.045	0.04	-0.003	-0.021	0.015	0.74	-0.007	-0.029	0.014	0.49
		PRS2	0.004	-0.015	0.023	0.71	0.011	-0.009	0.032	0.28	0.021	-0.001	0.043	0.06	0.003	-0.015	0.020	0.78	0.000	-0.021	0.022	0.97
		PRS3	-0.001	-0.020	0.018	0.96	0.003	-0.008	0.013	0.64	0.014	-0.008	0.035	0.22	-0.002	-0.020	0.016	0.82	-0.002	-0.023	0.020	0.86

Table 7.6: Results from the regression analyses between the different glycaemic polygenic risk scores and the brain imaging outcomes in the low and high glucose strata (pooled sample). Adjustments were only made for age and principal components as described in the methods. The values presented are standardised β coefficients, confidence intervals, and p values. WBV: Whole brain volumes. WMH: white matter hyperintensity volumes. HV: hippocampal volumes. GM: grey matter volumes. WM: white matter volumes.

7.4 Discussion

Summary of findings

The aims of this project were to investigate whether PRS instruments for HbA_{1c} and fasting glucose showed: 1) sex-specific associations with brain health outcomes and 2) accounted for differences between people in low and high glucose strata.

The three fasting PRS-glucose instruments weakly predicted a modest increase in observed glucose in both sexes in the whole cohort (n ≈ 400,000). The estimates for the PRS-HbA_{1c} associations suggested that the genetic instruments predicted higher measured HbA_{1c}, but the estimates were very weak and there were imprecisions in the predictions made. When examining these associations in the smaller imaging sub-sample of UK Biobank (n = 36,321), the size of the associations remained the same, but their precision reduced (i.e., CI widened).

While there were some small and modest associations observed with the outcomes (e.g., two of the HbA_{1c} PRS tools predicted smaller WM volumes in females), considering the number of statistical comparisons made, there is limited confidence in the relationship between the glycaemic genetic tools and brain health outcomes in either males or females. There were also no convincing associations of the PRS tools explaining brain health vulnerabilities in those part of the low or high glucose strata.

Specific findings and associations with the literature

Validation of the genetic tool

The PRSs did not predict as much variance in glycaemic measures as expected. However, the associations with observed HbA_{1c} and glucose were in the expected direction (i.e., higher genetic predisposition to the glycaemic measures was associated with higher fasting glucose measured in life). Each PRS explained less than 1% of the total variance for its respective glycaemic marker. Previous studies have estimated that their HbA_{1c} or T2D PRSs explained between ~2-5% of total variance.[206,502] However, there is limited capacity to make direct comparisons to previous work because: 1) many published articles do not share the variance explained by their genetic tool and 2) it has not always been made clear whether the explained variance described refers to the entire variance explained by a confounder-

adjusted model that includes the PRS, or one solely accounted by the genetic tool. Running sensitivity analyses revealed that SNPs used in this analysis were independent of important confounders such as BMI. As an exploratory check, an adjustment for BMI in my validation model increased the explained variance to 12%. Although, it is never possible to exclude horizontal pleiotropy when using genetic instruments, this finding suggests that pleiotropic effects via BMI are unlikely.

As a positive control, the PRSs were also regressed against lifetime T2D diagnosis. The glucose PRS was more convincingly associated with an increased risk, but for both glycaemic markers, the predictive power of the tool was small as evidenced by the size of the OR. A new recently published study argues that a HbA_{1c} PRS instrument with fewer SNPs may offer the strongest association with observed HbA_{1c} and is less likely to suffer from weak instrument bias.[509] They found that although the PRS instruments with the 16 glycaemic or 19 erythrocytic SNPs explained slightly less of the total variance for HbA_{1c}, they showed a higher F statistic (187.5 and 184.3) than a PRS with more 157 SNPs (27.43 and 27.9). This perhaps suggests that future studies should use fewer SNPs for a better signal-to-noise ratio and reduced risk of pleiotropy and weak instrument bias.

As discussed below, despite its limitations, the associations between the PRS and the brain health outcomes are mostly consistent with previous published findings.

Sex-stratified findings between PRS and the brain imaging outcomes

Overall, there were no convincing associations between the HbA_{1c} PRS and glucose-PRS tools with any of the different brain health outcomes in either males or females. There was no evidence that the different constructed PRS tools offered different and meaningful associations to any of the brain health outcomes in either sex groups.

Out of all the analyses, some revealed small and modest associations between the PRS tools and the brain health outcomes; for example, two of the HbA_{1c} PRS tools were associated with smaller WM volumes and one with smaller HV in females. But these were small effects that may reflect a false discovery due to the total number of analyses conducted. Null associations between genetic tools for T2D and brain health outcomes have previously been reported in an mendelian randomisation (MR) study.[502] A recently published study of the Biobank sample also failed to find an

association between a HbA_{1c} PRS and a range of brain imaging outcomes including WMHV, diffusion metrics, HV, and WM volumes.[365] Since the analyses presented in this chapter are mostly sex-stratified (as motivated by previous results), they further add to past published work by showing that genetic predisposition for glycaemia may not predict sex-specific vulnerabilities in tissue types. But the stratification by sex further reduces the strength of an already weak tool. Thus, the previously reported power issues commonly characteristic of PRS tools are magnified.

Despite all those nulls, Ranglani and colleagues reported that in their fully adjusted models, their HbA_{1c} PRS predicted smaller GM volumes.[365] Although this may differ from the findings reported here, some small associations between the PRS tools and some of the brain health outcomes were also found in my study as listed above. A direct comparison may also be challenging, as my study did not consider confounder adjustment. In my analysis of the association between PRS instruments for glycaemia and brain volumes, I did not adjust for other variables due to concern about collider (such as those raised by Aschard and colleagues).[501] In causal terms the genetic tool 'precedes' any observed data and so should be inherently unconfounded. Mistakenly adjusting for a collider could create a spurious association between the PRS and brain volume, compromising the validity of these findings.

My findings suggest that, based on this PRS, genetic predisposition to hyperglycaemia may not strongly predict brain health outcomes. Since a unit change in HbA_{1c} itself only predicts a small decrease in brain tissue in observational studies, the weak PRS-outcome associations may be within the range of what is expected from a tool of such minimal power. This being said, a more robust genetic instrument for T2D, explaining around 2.8% variance, also failed to find any convincing associations with brain health outcomes in both sexes combined.[502] It is established that the regulation of HbA_{1c} and glucose is complex – it is influenced by a multitude of factors beyond genetics, including environmental factors, lifestyle choices, medication and physiological processes. Thus, other factors which are not captured by the PRS may be contributing significantly to the observed variance of a genetic tool for glycaemia.

Some of the sex-stratified analyses suggested associations between the PRS and the brain health outcomes. For example, for HbA_{1c}, the two PRS tools with the most SNPs were also associated with lower WM volumes in females. However, since 3 PRS tools

for two glycaemic markers were each regressed against 5 different outcomes separately for males and females, the possible consequences of multiple testing have to be considered, i.e., increased risk of Type I error (false positives).

Stratified results

In Chapter 3, the findings showed that low and high HbA_{1c} (<35 mmol/mol & ≥42 mmol/mol) and low and high fasting glucose (<6 mmol/L & ≥7 mmol/L) predicted smaller WBV and GM volumes. The aim of this analysis was to test whether increased genetic vulnerability for glycaemia predicted poorer brain health outcomes in those in the lower and upper tiers of recorded glycaemia in life. There were no convincing associations observed for any of the PRS and brain health investigations. A few models suggested significant associations (e.g., in those with low HbA_{1c}, two of the PRS tools predicted smaller brains) but the findings were not convincing and were found in both directions. One of the drawbacks of this analysis is that it further suffers from power issues; the sample size was reduced upon stratification with the majority of participants excluded in the normoglycaemic range (i, e. ≥35 mmol/mol & <42 mmol/mol). This exclusion reduces the predictive power of an already weak instrument. Thus, although the aim of introducing genetic tools to strengthen causal inferences in my previous observational non-linear results was interesting, it became a methodological challenge to address it in this study.

Although genetic tools were used aiming to strengthen causal inferences in non-linear findings reported in Chapter 6, non-linear MR methods were not considered when modelling these associations. This is due to the growing evidence highlighting the important flaws in their methodology. For instance, studies have shown that non-linear MR can introduce biases such as non-constant genetic effects and collider bias, which can distort the true relationship between variables.[510] For example, in the case of BMI and mortality, non-linear MR has yielded biologically implausible J-shaped associations. Furthermore, negative control studies have revealed that non-linear methods can produce nonsensical results such as impossible causal associations between BMI and assigned sex. Thus, in line with the current field's stance doubting the reliability of non-linear MR, this method was not used to model the previous non-linear observational findings of Chapter 6.

Strengths and weaknesses

The PRSs were weighted and generated using published GWAS estimates for HbA_{1c} and fasting glucose. This two-sample method reduces bias in the results since the UK Biobank, the target dataset in this analysis, was not part of the summary statistics used to derive the PRSs. In addition, the analyses involved constructing PRS instruments for two different glycaemic markers (fasting glucose and HbA_{1c}). Although HbA_{1c} and fasting glucose are both markers of glucose metabolism, they are influenced by different mechanisms and differ to some degree with regard to heritability.[459] Fasting glucose values represent glucose tolerance and IR whereas HbA_{1c} captures levels of glycaemia over time and is influenced by factors such as IR, β-cell dysfunction, hepatic glucose production and red cell lifespan. Another strength of this analysis is that it takes a novel approach by looking at the relationship between genetic tools for glycaemia and brain health outcomes through a sex-stratified lens. Traditionally, analyses have looked at the relationship in pooled samples of males and females.[365,502]

One limitation is that the PRS instruments generated were developed and tested predominantly in a highly selected white British population and thus may not apply to other ethnic groups. It has been argued that the UK Biobank cohort is not representative of the UK population for a number of sociodemographic, physical, lifestyle, and health-related characteristics (e.g., participants have been found to be less likely to drink, smoke and be obese).[33] Participants who were willing to undergo scanning (time, inconvenience etc.) differ from those who did the less onerous UK Biobank study. Further sampling bias is related to scanning. Those with MRI contraindications such as pacemakers, metallic intraocular foreign bodies, cochlear implants, drug infusion pumps etc. did not take part. This selection may produce bias and an underrepresentation of specific demographic groups, leading to limited generalisability of study findings. Another weakness is that this study uses genetic risk scores as a proxy for lifetime exposure to higher HbA_{1c}. Additionally, the PRS construction did not distinguish between the different mode of signals (i.e., erythrocytic vs glycaemic SNPs vs other).

Future work:

Future work should aim to replicate these analyses in other Biobank samples (e.g. China Kadoorie Biobank, Shanghai Zhangjiang Biobank and the new emerging Biobanks in the Middle East). This will shed important light on whether genetic vulnerability for glycaemia is useful in predicting lifetime glycaemic health in other ethnic group and whether has predictive value in relation to brain health and dementia outcomes. Future work should also follow up similar PRS analyses to explore the pathways through which genetic risk for hyperglycamia may exert its effects (e.g. insulin signalling pathway, neurotrophic signalling and glucose transport pathway).

7.5 Conclusions

The aim of this chapter was to strengthen causal inference in the observational findings reported in Chapter 6. This project examined whether genetic risk scores for glycaemic markers supported the different sex-specific associations between glycaemia and brain health outcomes and differences between people in the low and high glucose strata. There was little evidence of the genetic tools predicting brain health outcomes in males or females as well as those in the low and higher glucose strata.

8. General discussion

8.1 Aims

With growing evidence suggesting that Type 2 diabetes is a risk factor for poorer brain health, there is an important need to better understand the precise nature of this complex relationship.[1] To take a more nuanced perspective on these relationships, associations between its underlying mechanisms, primarily glycaemia, with different measures that encapsulate brain health were examined in the National Survey of Health and Development. In line with the growing evidence suggesting that there are important sex differences in metabolic and neurological outcomes, these associations were examined separately in males and females. The analyses were then further extended to the UK Biobank sample to test whether glycaemia has a non-linear relationship with brain health outcomes and whether genetic instrumental variables could be used to confirm the observational associations, with less risk of confounding or reverse causality. Finally, in an attempt to begin unravelling the mechanisms behind this relationship, the mediating role of inflammation was considered in the glycaemia-brain volume association in female participants of NSHD.

8.2 Summary of findings

The findings from NSHD and Insight 46 show that poor glycaemic health (Chapter 3) and related metabolic markers, such as those of insulin resistance (Chapter 4), are associated with lower volumes of different brain measures in females compared to males. This suggests that there are sex-specific differences in the relationship between glycaemia-related metabolic health and brain health in a population-based sample. Further examination of these relationships revealed that the glycaemia-brain associations observed in females did not appear to be mediated by inflammatory pathways (Chapter 5).

In the larger UK Biobank sample, the relationship between the markers of glycaemia and brain health appeared to be non-linear with both low and high strata of glycaemia being associated with smaller brain volumes (Chapter 6). There were some suggestions of increased susceptibility in these relationships for females, although analyses were complicated by the non-linear nature of those associations. Polygenic

risk scores for glycaemia were not convincingly associated with the different brain imaging outcomes, despite the associations being mostly consistent with previously published work (Chapter 7). This being said, the weakness of the genetic instrument limited the conclusions that could be drawn from the findings.

8.3 The value of population-based research

The recent Lancet Commission on Dementia Prevention, Intervention and Care identified diabetes as a risk factor for dementia.[1] Type 2 diabetes is traditionally diagnosed in those with an HbA_{1c} of ≥ 48 mmol/mol. However, an advantage of population-based studies, such as those considered in this thesis, is that they allow researchers to go beyond the clinical thresholds for diabetes to: 1) look more precisely at the mechanisms that may underlie this relationship and 2) be more nuanced about the subtleties of this complex relationship (e.g., look at non-linearity and examine sex differences). Although thresholds are valuable for clinicians, considering exposures as continuous and graded allows for more flexibility in examining the underlying mechanistic relationship between glycaemia and brain health. The findings revealed a consistent association between several glycaemic traits and brain health in females. These were observed independently of medication and diabetes status, suggesting that high glycaemia in a sample with population characteristics is associated with measures of poorer brain health. But it is worth noting that the size of the associations was small. To put the size of this decrement in perspective, a 10 mmol/mol increase in HbA_{1c} during midlife (as reported in Chapter 3) was equivalent to a reduction of whole brain volumes in late life corresponding to 2 years of normal ageing. This is still valuable knowledge as small effects across an entire population can have important repercussions on public health. However, we should not discount the possibility that these associations are due to reverse causation (i.e., small brain volumes indicative of brain atrophy, resulting in alterations in glucose metabolism and insulin sensitivity) or residual confounding.

Interestingly in the UK Biobank sample, non-linear associations were observed with both low and high HbA_{1c} (and glucose) being associated with smaller whole brain, hippocampal, and grey matter volumes. As expected from the NSHD, higher HbA_{1c} (> 42 mmol/mol) was predictive of poorer brain health. Although this is consistent with the traditional threshold for glycaemia, the findings that participants with low HbA_{1c} (<35 mmol/mol) showed poorer brain outcomes from those in the “normoglycaemic” range between 35 and 42 mmol/mol further demonstrate the value of examining HbA_{1c} across the whole range. These findings highlight the complexity of the associations between glycaemia and brain health outcomes.

8.4 Sex differences

An important finding of this thesis is that sex moderates the associations between glycaemia and brain health outcomes. Poorer glycaemic health as assessed by different traits such as HbA_{1c}, glucose and insulin resistance were found to be associated with smaller brains in female but not in male participants of the NSHD sample. In UK Biobank, the non-linear models revealed some visual suggestions of a difference in the glycaemia-brain relationship between males and females.

This adds to the growing evidence suggestive of sex differences in metabolic and neurological outcomes (e.g., dementia being more prevalent in females). I believe that moving forward, it may be a necessity for all future research studies that explore similar associations, to present sex-stratified results, or at the very least, test for sex interactions, as the impact of glycaemia and its related metabolic factors on brain health can evidently vary between males and females.

In regard to what factors drive these relationships, the analyses in NSHD (Chapter 5) suggest that glycaemia may not impact the brain through inflammatory pathways. Future studies should, I think, examine other potential mediating factors in this relationship, particularly those relating to sex hormones (e.g., ovarian functions) and gender-related psychosocial stressors (e.g., caregiving responsibilities and workplace discrimination).

Beyond this, it remains important to acknowledge potential methodological flaws that come with these analyses. Some of these may emerge as possible issues during the process of adjusting for important confounders related to the sex-specific analyses. For example, I considered body mass index or waist-to-hip ratio as two anthropometric indicators playing a potential confounding role in my glycaemia-brain analyses. It is established that body fat distribution is not well captured by body mass index, and only to a limited extent by waist-to-hip ratio, such as that any imprecisions in measurement of these (or any other confounder) potentially result in residual confounding. This is particularly relevant to my sex-specific findings as during unique events in a female's life (e.g. the menopause), HbA_{1c} has been found to increase in parallel with unfavourable changes in body fat distribution which has important repercussions on glucose metabolism in females.[180]

8.5 Mismatch between brain imaging and cognition

The thesis revealed associations between glycaemia and some of the brain health measures, but not cognition. This was the case whether a composite cognitive score sensitive for Alzheimer's disease, or single cognitive capacities were considered. There are many possible explanations for this.

The first possibility is there may be a lag between brain pathology from poor glycaemia and the manifestations of the respective cognitive deficits. This is commonly discussed in the Alzheimer's disease literature where the pathological changes can precede the cognitive presentation by many years.[8] It may be that the effect of poor glycaemia on cognitive outcomes becomes more prominent when corresponding age-related impairments become noticeable. Insight 46's third wave of cognitive phenotyping should shed light on this question. It may also be possible that the participants do not show cognitive impairment as they have built cognitive reserve throughout their earlier life. Cognitive reserve describes the brain's ability to circumvent damage by finding novel or compensatory systems to support cognitive function.[448] This may particularly be relevant to the participants recruited to the UK Biobank or to those who remain involved in longitudinal assessment of Insight 46 as they have previously been shown to be healthier than the general population, and the entire NSHD sample from which they were drawn respectively. Thus, they are likely to have been exposed to more protective lifestyle factors, such as higher education, that are crucial in building cognitive reserve explaining the null cognition findings.[33,394]

Hyperglycaemia did not appear to predict small vessel-related disease. This was the case irrespective of the different proxy measures of cerebral small vessel disease considered. Mechanistically, hyperglycaemia has been found to be damaging to small vessels by causing endothelial dysfunction, blood-brain barrier disruption, vascular structural changes and oxidative stress.[437] Considering the mixed findings in the literature, the results from this thesis are not inconsistent with past evidence.[409] As previously mentioned, it is plausible that that the effects of glycaemia on vessels may become more prominent as participants get older, or manifest when they co-exist with other comorbidities such as those related to cardiovascular health or neurological pathology such as tau burden. Other studies have also begun to show that diabetes-related small vessel disease may only become more apparent when more sensitive

measures, looking at the shape and location of these lesions are considered.[355] Future studies that examine glycaemia-small vessel disease associations should also aim to consider these more subtle measures.

8.6 Future directions

The findings from the thesis revealed associations between glycaemia and its related metabolic markers and some, but not all of the brain imaging outcomes. It is thus possible that these mechanisms may follow a specific temporal manifestation where structural gross brain imaging changes precede small vessel disease, amyloidosis and cognitive pathology. More recently, Insight 46 participants are midway through a third round of scanning at age 75. Longitudinal brain imaging and cognitive assessments may shed light on the trajectory of these associations; more specifically on whether glycaemia continues to possibly exert its effect on the brain by resulting in a decline in brain volumes (and if so, quantify the size of it). In line with this, the longitudinal measures will give an insight on whether small vessel disease pathology, usually characteristic of hyperglycaemia, becomes more prominent as people age. This would address the question of whether the effects of poorer glycaemia on brain health accelerates with age. Similar questions can also be asked about cognition.

Future research should investigate whether the structural vulnerabilities observed in females reflect specific regional damage: i.e., does it reflect vulnerability in specific areas of the brain such as the frontal lobes. In line with this, considering data from functional neuroimaging may uncover the possible compensatory mechanisms that could explain the mismatch between the structural brain imaging results and the null cognitive findings. As mentioned above, there is a possibility that NSHD and UK Biobank participants with poorer glycaemic health may show preserved cognitive capacities due to cognitive reserve, mechanisms through which different circuits of the brain compensate for previous damage through engaging novel pathways.

The findings from Chapter 6 revealed non-linear associations between the glycaemia and brain health outcomes. Future research should aim to elucidate the underlying mechanisms through which both low and high blood glucose levels exert their detrimental effects on the brain. This may involve investigating the cellular and

molecular pathways that underlie glucose metabolism, oxidative stress, neuroinflammation, synaptic dysfunction, and neurovascular coupling.

Future research should consider additional factors that may mediate the relationships between poorer glycaemia and smaller brains in females. Chapter 5 began to examine this question by investigating whether inflammation may mediate smaller brain volume. I found that although HbA_{1c} was directly associated with brain volume and increased inflammation, there was no indirect path through which it affected brain health through this mediator. Other potential candidates may include cerebrovascular disease and small vessel disease (although both of these were not found to be associated with glycaemia).

Overall, the findings from the thesis highlight the importance of continuing the study of the relationship between glycaemia and brain health given the escalating burdens of obesity, diabetes, and cognitive impairment in society. In line with the findings of this thesis and the other growing evidence suggestive of prominent sex differences in the relationship between metabolic health and brain health, future studies should further examine how these relationships manifest in males and females in an attempt to better understand the pathophysiological mechanisms through which glycaemic traits may damage the brain and contribute to dementia.

9. References

1. Livingston, G. *et al.* Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *The Lancet* **396**, 413–446 (2020).
2. Noto, S. Perspectives on Aging and Quality of Life. *Healthcare* **11**, 2131 (2023).
3. Hou, Y. *et al.* Ageing as a risk factor for neurodegenerative disease. *Nat Rev Neurol* **15**, 565–581 (2019).
4. Hugo, J. & Ganguli, M. Dementia and cognitive impairment: epidemiology, diagnosis, and treatment. *Clin Geriatr Med* **30**, 421–442 (2014).
5. Regier, D. A., Kuhl, E. A. & Kupfer, D. J. The DSM-5: Classification and criteria changes. *World Psychiatry* **12**, 92–98 (2013).
6. Nichols, E. *et al.* Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health* **7**, e105–e125 (2022).
7. Jansen, W. J. *et al.* Association of Cerebral Amyloid-β Aggregation With Cognitive Functioning in Persons Without Dementia. *JAMA Psychiatry* **75**, 84 (2018).
8. Tondelli, M. *et al.* Structural MRI changes detectable up to ten years before clinical Alzheimer's disease. *Neurobiology of Aging* **33**, 825.e25-825.e36 (2012).
9. Swaddiwudhipong, N. *et al.* Pre-diagnostic cognitive and functional impairment in multiple sporadic neurodegenerative diseases. *Alzheimer's & Dementia* **19**, 1752–1763 (2023).
10. Chatterjee, S. *et al.* Type 2 Diabetes as a Risk Factor for Dementia in Women Compared With Men: A Pooled Analysis of 2.3 Million People Comprising More Than 100,000 Cases of Dementia. *Diabetes Care* **39**, 300–307 (2016).
11. Hedman, A. M., Van Haren, N. E. M., Schnack, H. G., Kahn, R. S. & Hulshoff Pol, H. E. Human brain changes across the life span: A review of 56 longitudinal

magnetic resonance imaging studies. *Human Brain Mapping* **33**, 1987–2002 (2012).

12. Scahill, R. I. *et al.* A Longitudinal Study of Brain Volume Changes in Normal Aging Using Serial Registered Magnetic Resonance Imaging. *Arch Neurol* **60**, 989 (2003).
13. Elobeid, A., Libard, S., Leino, M., Popova, S. N. & Alafuzoff, I. Altered Proteins in the Aging Brain. *J Neuropathol Exp Neurol* **75**, 316–325 (2016).
14. Raz, N. *et al.* Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb Cortex* **15**, 1676–1689 (2005).
15. Resnick, S. M., Pham, D. L., Kraut, M. A., Zonderman, A. B. & Davatzikos, C. Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. *J Neurosci* **23**, 3295–3301 (2003).
16. Cabeza, R. & Dennis, N. A. Frontal Lobes and Aging. in *Principles of Frontal Lobe Function* (eds. Stuss, D. T. & Knight, R. T.) 628–652 (Oxford University Press, 2013). doi:10.1093/med/9780199837755.003.0044.
17. Bethlehem, R. A. I. *et al.* Brain charts for the human lifespan. *Nature* **604**, 525–533 (2022).
18. Allen, J. S., Damasio, H., Grabowski, T. J., Bruss, J. & Zhang, W. Sexual dimorphism and asymmetries in the gray–white composition of the human cerebrum. *NeuroImage* **18**, 880–894 (2003).
19. Taki, Y. *et al.* Correlations among Brain Gray Matter Volumes, Age, Gender, and Hemisphere in Healthy Individuals. *PLoS ONE* **6**, e22734 (2011).
20. Crivello, F., Tzourio-Mazoyer, N., Tzourio, C. & Mazoyer, B. Longitudinal Assessment of Global and Regional Rate of Grey Matter Atrophy in 1,172 Healthy Older Adults: Modulation by Sex and Age. *PLoS ONE* **9**, e114478 (2014).

21. Bullitt, E. *et al.* The effects of healthy aging on intracerebral blood vessels visualized by magnetic resonance angiography. *Neurobiology of Aging* **31**, 290–300 (2010).
22. Longstreth, W. T. *et al.* Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. *Stroke* **27**, 1274–1282 (1996).
23. Habes, M. *et al.* White matter hyperintensities and imaging patterns of brain ageing in the general population. *Brain* **139**, 1164–1179 (2016).
24. Cohen, A. D. *et al.* Fluid and PET biomarkers for amyloid pathology in Alzheimer's disease. *Mol Cell Neurosci* **97**, 3–17 (2019).
25. Xu, X. *et al.* Age-related Impairment of Vascular Structure and Functions. *Aging and disease* **8**, 590 (2017).
26. Salthouse, T. A. Selective review of cognitive aging. *J Int Neuropsychol Soc* **16**, 754–760 (2010).
27. Horn, J. L. & Cattell, R. B. Age differences in fluid and crystallized intelligence. *Acta Psychologica* **26**, 107–129 (1967).
28. Singh-Manoux, A. *et al.* Timing of onset of cognitive decline: results from Whitehall II prospective cohort study. *BMJ* **344**, d7622–d7622 (2012).
29. Salthouse, T. A. When does age-related cognitive decline begin? *Neurobiol Aging* **30**, 507–514 (2009).
30. Harada, C. N., Natelson Love, M. C. & Triebel, K. L. Normal cognitive aging. *Clin Geriatr Med* **29**, 737–752 (2013).
31. Salthouse, T. & Davis, H. Organization of cognitive abilities and neuropsychological variables across the lifespan. *Developmental Review* **26**, 31–54 (2006).

32. Davis, D. *et al.* Decline in Search Speed and Verbal Memory Over 26 Years of Midlife in a British Birth Cohort. *Neuroepidemiology* **49**, 121–128 (2017).
33. Fry, A. *et al.* Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. *American Journal of Epidemiology* **186**, 1026–1034 (2017).
34. Grundman, M. Mild Cognitive Impairment Can Be Distinguished From Alzheimer Disease and Normal Aging for Clinical Trials. *Arch Neurol* **61**, 59 (2004).
35. DeCarli, C. Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. *The Lancet Neurology* **2**, 15–21 (2003).
36. Nettiksimmons, J., DeCarli, C., Landau, S., Beckett, L., & Alzheimer's Disease Neuroimaging Initiative. Biological heterogeneity in ADNI amnestic mild cognitive impairment. *Alzheimer's & Dementia* **10**, 511 (2014).
37. Cooper, S. The clinical assessment of the patient with early dementia. *Journal of Neurology, Neurosurgery & Psychiatry* **76**, v15–v24 (2005).
38. Folstein, M. F., Folstein, S. E. & McHugh, P. R. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189–198 (1975).
39. Skrobot, O. A. *et al.* Vascular cognitive impairment neuropathology guidelines (VCING): the contribution of cerebrovascular pathology to cognitive impairment. *Brain* **139**, 2957–2969 (2016).
40. Reitz, C. & Mayeux, R. Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochemical Pharmacology* **88**, 640–651 (2014).
41. Jorm, A. F. & Jolley, D. The incidence of dementia: A meta-analysis. *Neurology* **51**, 728–733 (1998).

42. Gao, S., Hendrie, H. C., Hall, K. S. & Hui, S. The Relationships Between Age, Sex, and the Incidence of Dementia and Alzheimer Disease: A Meta-analysis. *Arch Gen Psychiatry* **55**, 809 (1998).
43. Bretsky, P. M. *et al.* Evidence for an Interaction between Apolipoprotein E Genotype, Gender, and Alzheimer Disease. *Alzheimer Disease & Associated Disorders* **13**, (1999).
44. Corder, E. H. *et al.* Gene Dose of Apolipoprotein E Type 4 Allele and the Risk of Alzheimer's Disease in Late Onset Families. *Science* **261**, 921–923 (1993).
45. Mahley, R. W. & Rall, S. C. Apolipoprotein E: Far More Than a Lipid Transport Protein. *Annu. Rev. Genom. Hum. Genet.* **1**, 507–537 (2000).
46. Bu, G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat Rev Neurosci* **10**, 333–344 (2009).
47. Farrer, L. A. *et al.* Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* **278**, 1349–1356 (1997).
48. Sando, S. B. *et al.* APOE epsilon 4 lowers age at onset and is a high risk factor for Alzheimer's disease; a case control study from central Norway. *BMC Neurol* **8**, 9 (2008).
49. Serrano-Pozo, A., Das, S. & Hyman, B. T. APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. *The Lancet Neurology* **20**, 68–80 (2021).
50. Goldberg, T. E., Huey, E. D. & Devanand, D. P. Association of APOE e2 genotype with Alzheimer's and non-Alzheimer's neurodegenerative pathologies. *Nat Commun* **11**, 4727 (2020).

51. Neu, S. C. *et al.* Apolipoprotein E Genotype and Sex Risk Factors for Alzheimer Disease: A Meta-analysis. *JAMA Neurol* **74**, 1178–1189 (2017).
52. Corder, E. H. *et al.* The Biphasic Relationship between Regional Brain Senile Plaque and Neurofibrillary Tangle Distributions: Modification by Age, Sex, and *APOE* Polymorphism. *Annals of the New York Academy of Sciences* **1019**, 24–28 (2004).
53. Shiekh, S. I. *et al.* Ethnic Differences in Dementia Risk: A Systematic Review and Meta-Analysis. *JAD* **80**, 337–355 (2021).
54. Foubert-Samier, A. *et al.* Education, occupation, leisure activities, and brain reserve: a population-based study. *Neurobiology of Aging* **33**, 423.e15-423.e25 (2012).
55. Sabia, S. *et al.* Association of ideal cardiovascular health at age 50 with incidence of dementia: 25 year follow-up of Whitehall II cohort study. *BMJ* l4414 (2019) doi:10.1136/bmj.l4414.
56. Hamer, M. & Chida, Y. Physical activity and risk of neurodegenerative disease: a systematic review of prospective evidence. *Psychol. Med.* **39**, 3–11 (2009).
57. Erickson, K. I. *et al.* Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A* **108**, 3017–3022 (2011).
58. Zhong, G., Wang, Y., Zhang, Y., Guo, J. J. & Zhao, Y. Smoking Is Associated with an Increased Risk of Dementia: A Meta-Analysis of Prospective Cohort Studies with Investigation of Potential Effect Modifiers. *PLoS ONE* **10**, e0118333 (2015).
59. Niu, H., Álvarez-Álvarez, I., Guillén-Grima, F. & Aguinaga-Ontoso, I. Prevalence and incidence of Alzheimer's disease in Europe: A meta-analysis. *Neurología (English Edition)* **32**, 523–532 (2017).

60. Gong, J. *et al.* Sex differences in dementia risk and risk factors: Individual-participant data analysis using 21 cohorts across six continents from the COSMIC consortium. *Alzheimer's & Dementia* **19**, 3365–3378 (2023).
61. Huque, H. *et al.* Could Country-Level Factors Explain Sex Differences in Dementia Incidence and Prevalence? A Systematic Review and Meta-Analysis. *J Alzheimers Dis* **91**, 1231–1241 (2023).
62. Alzheimer's Association. 2016 Alzheimer's disease facts and figures. *Alzheimer's & Dementia* **12**, 459–509 (2016).
63. Dekaban, A. S. & Sadowsky, D. Changes in brain weights during the span of human life: Relation of brain weights to body heights and body weights. *Annals of Neurology* **4**, 345–356 (1978).
64. Ritchie, S. J. *et al.* Sex Differences in the Adult Human Brain: Evidence from 5216 UK Biobank Participants. *Cerebral Cortex* **28**, 2959–2975 (2018).
65. Maurer, K., Volk, S. & Gerbaldo, H. Auguste D and Alzheimer's disease. *The Lancet* **349**, 1546–1549 (1997).
66. Haaksma, M. L. *et al.* Survival time tool to guide care planning in people with dementia. *Neurology* **94**, e538–e548 (2020).
67. Helzner, E. P. *et al.* Survival in Alzheimer disease: A multiethnic, population-based study of incident cases. *Neurology* **71**, 1489–1495 (2008).
68. Jahn, H. Memory loss in Alzheimer's disease. *Dialogues in Clinical Neuroscience* **15**, 445–454 (2013).
69. Braak, H. & Braak, E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* **82**, 239–259 (1991).
70. Cronin-Golomb, A., Corkin, S. & Growdon, J. H. Visual dysfunction predicts cognitive deficits in Alzheimer's disease. *Optom Vis Sci* **72**, 168–176 (1995).

71. Aronoff, J. M. *et al.* Information content versus relational knowledge: semantic deficits in patients with Alzheimer's disease. *Neuropsychologia* **44**, 21–35 (2006).
72. Perl, D. P. Neuropathology of Alzheimer's Disease. *Mount Sinai J Medicine* **77**, 32–42 (2010).
73. Vijayakumar, A. & Vijayakumar, A. Comparison of hippocampal volume in dementia subtypes. *ISRN Radiol* **2013**, 174524 (2013).
74. Barnes, J. *et al.* A meta-analysis of hippocampal atrophy rates in Alzheimer's disease. *Neurobiol Aging* **30**, 1711–1723 (2009).
75. McDonald, C. R. *et al.* Relationship between regional atrophy rates and cognitive decline in mild cognitive impairment. *Neurobiol Aging* **33**, 242–253 (2012).
76. Sarazin, M. *et al.* The Amnestic Syndrome of Hippocampal type in Alzheimer's Disease: An MRI Study. *JAD* **22**, 285–294 (2010).
77. Bastos-Leite, A. J. *et al.* The contribution of medial temporal lobe atrophy and vascular pathology to cognitive impairment in vascular dementia. *Stroke* **38**, 3182–3185 (2007).
78. Laakso, M. P. *et al.* Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: An MRI study. *Neurology* **46**, 678–681 (1996).
79. Chan, D. *et al.* Patterns of temporal lobe atrophy in semantic dementia and Alzheimer's disease. *Ann Neurol* **49**, 433–442 (2001).
80. Van De Pol, L. A. Hippocampal atrophy on MRI in frontotemporal lobar degeneration and Alzheimer's disease. *Journal of Neurology, Neurosurgery & Psychiatry* **77**, 439–442 (2006).

81. Rami, L. *et al.* Distinct Functional Activity of the Precuneus and Posterior Cingulate Cortex During Encoding in the Preclinical Stage of Alzheimer's Disease. *JAD* **31**, 517–526 (2012).
82. Apostolova, L. G. *et al.* Hippocampal Atrophy and Ventricular Enlargement in Normal Aging, Mild Cognitive Impairment (MCI), and Alzheimer Disease. *Alzheimer Disease & Associated Disorders* **26**, 17–27 (2012).
83. Serrano-Pozo, A., Frosch, M. P., Masliah, E. & Hyman, B. T. Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med* **1**, a006189 (2011).
84. Seeley, W. W., Crawford, R. K., Zhou, J., Miller, B. L. & Greicius, M. D. Neurodegenerative Diseases Target Large-Scale Human Brain Networks. *Neuron* **62**, 42–52 (2009).
85. Mattson, M. P. Pathways towards and away from Alzheimer's disease. *Nature* **430**, 631–639 (2004).
86. Braak, H. & Braak, E. Frequency of Stages of Alzheimer-Related Lesions in Different Age Categories. *Neurobiology of Aging* **18**, 351–357 (1997).
87. Zhang, K. *et al.* PET imaging of neural activity, β-amyloid, and tau in normal brain aging. *Eur J Nucl Med Mol Imaging* **48**, 3859–3871 (2021).
88. Andrade-Moraes, C. H. *et al.* Cell number changes in Alzheimer's disease relate to dementia, not to plaques and tangles. *Brain* **136**, 3738–3752 (2013).
89. Grundke-Iqbal, I. *et al.* Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem* **261**, 6084–6089 (1986).
90. Mietelska-Porowska, A., Wasik, U., Goras, M., Filipek, A. & Niewiadomska, G. Tau Protein Modifications and Interactions: Their Role in Function and Dysfunction. *IJMS* **15**, 4671–4713 (2014).

91. DeTure, M. A. & Dickson, D. W. The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegeneration* **14**, 32 (2019).
92. Malpetti, M., Joie, R. L. & Rabinovici, G. D. Tau Beats Amyloid in Predicting Brain Atrophy in Alzheimer Disease: Implications for Prognosis and Clinical Trials. *J Nucl Med* **63**, 830–832 (2022).
93. Bejanin, A. *et al.* Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer's disease. *Brain* **140**, 3286–3300 (2017).
94. Bierer, L. M. *et al.* Neocortical Neurofibrillary Tangles Correlate With Dementia Severity in Alzheimer's Disease. *Archives of Neurology* **52**, 81–88 (1995).
95. Jack, C. R. *et al.* Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol* **12**, 207–216 (2013).
96. Jack, C. R. *et al.* A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* **87**, 539–547 (2016).
97. McVeigh, C. & Passmore, P. Vascular dementia: prevention and treatment. *Clinical Interventions in Aging* **1**, 229–235 (2006).
98. Barbay, M. *et al.* Vascular cognitive impairment: Advances and trends. *Revue Neurologique* **173**, 473–480 (2017).
99. Iadecola, C. The Pathobiology of Vascular Dementia. *Neuron* **80**, 844–866 (2013).
100. Looi, J. C. L. & Sachdev, P. S. Differentiation of vascular dementia from AD on neuropsychological tests. *Neurology* **53**, 670–670 (1999).
101. Podcasy, J. L. & Epperson, C. N. Considering sex and gender in Alzheimer disease and other dementias. *Dialogues Clin Neurosci* **18**, 437–446 (2016).

102. Grodstein, F., Leurgans, S. E., Capuano, A. W., Schneider, J. A. & Bennett, D. A. Trends in Postmortem Neurodegenerative and Cerebrovascular Neuropathologies Over 25 Years. *JAMA Neurol* **80**, 370–376 (2023).
103. Schneider, J. A., Arvanitakis, Z., Bang, W. & Bennett, D. A. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* **69**, 2197–2204 (2007).
104. Esiri, M. M. *et al.* Cerebral Subcortical Small Vessel Disease in Subjects With Pathologically Confirmed Alzheimer Disease: A Clinicopathologic Study in the Oxford Project to Investigate Memory and Ageing (OPTIMA). *Alzheimer Disease & Associated Disorders* **28**, 30–35 (2014).
105. on behalf of the LeARN project *et al.* White Matter Hyperintensities Potentiate Hippocampal Volume Reduction in Non-Demented Older Individuals with Abnormal Amyloid-β. *JAD* **55**, 333–342 (2016).
106. Kril, J. J. Patients with vascular dementia due to microvascular pathology have significant hippocampal neuronal loss. *Journal of Neurology, Neurosurgery & Psychiatry* **72**, 747–751 (2002).
107. Nitkunan, A., Lanfranconi, S., Charlton, R. A., Barrick, T. R. & Markus, H. S. Brain Atrophy and Cerebral Small Vessel Disease: A Prospective Follow-Up Study. *Stroke* **42**, 133–138 (2011).
108. Snowdon, D. A. *et al.* Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *JAMA* **277**, 813–817 (1997).
109. Vemuri, P. *et al.* Vascular and amyloid pathologies are independent predictors of cognitive decline in normal elderly. *Brain* **138**, 761–771 (2015).

110. Schneider, J. A., Wilson, R. S., Bienias, J. L., Evans, D. A. & Bennett, D. A. Cerebral infarctions and the likelihood of dementia from Alzheimer disease pathology. *Neurology* **62**, 1148–1155 (2004).
111. Launer, L. J., Hughes, T. M. & White, L. R. Microinfarcts, brain atrophy, and cognitive function: The Honolulu Asia Aging Study Autopsy Study. *Annals of Neurology* **70**, 774–780 (2011).
112. Toledo, J. B. *et al.* Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. *Brain* **136**, 2697–2706 (2013).
113. Matthews, F. E. *et al.* Epidemiological Pathology of Dementia: Attributable-Risks at Death in the Medical Research Council Cognitive Function and Ageing Study. *PLoS Med* **6**, e1000180 (2009).
114. Arvanitakis, Z. *et al.* Association of white matter hyperintensities and gray matter volume with cognition in older individuals without cognitive impairment. *Brain Struct Funct* **221**, 2135–2146 (2016).
115. Villeneuve, S. *et al.* Vascular risk and Aβ interact to reduce cortical thickness in AD vulnerable brain regions. *Neurology* **83**, 40–47 (2014).
116. Grover, V. P. B. *et al.* Magnetic Resonance Imaging: Principles and Techniques: Lessons for Clinicians. *Journal of Clinical and Experimental Hepatology* **5**, 246–255 (2015).
117. Vemuri, P. & Jack, C. R. Role of structural MRI in Alzheimer's disease. *Alz Res Therapy* **2**, 23 (2010).
118. Lane, C. A. *et al.* Study protocol: Insight 46 - a neuroscience sub-study of the MRC National Survey of Health and Development. *BMC Neurol* **17**, 75 (2017).
119. MRI Basics. https://case.edu/med/neurology/NR/MRI%20Basics.htm.

120. Wardlaw, J. M. *et al.* Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* **12**, 822–838 (2013).
121. Jagust, W. J. *et al.* Neuropathological basis of magnetic resonance images in aging and dementia. *Annals of Neurology* **63**, 72–80 (2008).
122. Baare, W. F. C. Quantitative Genetic Modeling of Variation in Human Brain Morphology. *Cerebral Cortex* **11**, 816–824 (2001).
123. Mills, J. D. *et al.* Unique Transcriptome Patterns of the White and Grey Matter Corroborate Structural and Functional Heterogeneity in the Human Frontal Lobe. *PLoS ONE* **8**, e78480 (2013).
124. He, F. & Sun, Y. E. Glial cells more than support cells? *The International Journal of Biochemistry & Cell Biology* **39**, 661–665 (2007).
125. Kim, Y. S., Jung, H. M. & Yoon, B.-E. Exploring glia to better understand Alzheimer's disease. *Animal Cells and Systems* **22**, 213–218 (2018).
126. Wardlaw, J. M., Valdés Hernández, M. C. & Muñoz-Maniega, S. What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. *J Am Heart Assoc* **4**, 001140 (2015).
127. Ylikoski, A. *et al.* White Matter Hyperintensities on MRI in the Neurologically Nondiseased Elderly: Analysis of Cohorts of Consecutive Subjects Aged 55 to 85 Years Living at Home. *Stroke* **26**, 1171–1177 (1995).
128. Garde, E., Mortensen, E. L., Krabbe, K., Rostrup, E. & Larsson, H. B. Relation between age-related decline in intelligence and cerebral white-matter hyperintensities in healthy octogenarians: a longitudinal study. *Lancet* **356**, 628–634 (2000).

129. Gouw, A. A. *et al.* Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. *Journal of Neurology, Neurosurgery & Psychiatry* **82**, 126–135 (2011).
130. Prins, N. D. & Scheltens, P. White matter hyperintensities, cognitive impairment and dementia: an update. *Nat Rev Neurol* **11**, 157–165 (2015).
131. Inzitari, D. *et al.* Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ* **339**, b2477–b2477 (2009).
132. Morrison, C., Dadar, M., Collins, D. L., & for the Alzheimer's Disease Neuroimaging Initiative. Sex differences in risk factors, burden, and outcomes of cerebrovascular disease in Alzheimer's disease populations. *Alzheimer's & Dementia* **20**, 34–46 (2024).
133. Sachdev, P. S., Parslow, R., Wen, W., Anstey, K. J. & Easteal, S. Sex differences in the causes and consequences of white matter hyperintensities. *Neurobiol Aging* **30**, 946–956 (2009).
134. Scheltens, P. *et al.* A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. *J Neurol Sci* **114**, 7–12 (1993).
135. Wahlund, L. O. *et al.* A New Rating Scale for Age-Related White Matter Changes Applicable to MRI and CT. *Stroke* **32**, 1318–1322 (2001).
136. Sudre, C. H. *et al.* Bayesian Model Selection for Pathological Neuroimaging Data Applied to White Matter Lesion Segmentation. *IEEE Trans. Med. Imaging* **34**, 2079–2102 (2015).
137. Brickman, A. M. *et al.* Regional white matter hyperintensity volume, not hippocampal atrophy, predicts incident Alzheimer disease in the community. *Arch Neurol* **69**, 1621–1627 (2012).

138. Ma, M. Role of calpains in the injury-induced dysfunction and degeneration of the mammalian axon. *Neurobiology of Disease* **60**, 61–79 (2013).
139. Basser, P. J. & Jones, D. K. Diffusion-tensor MRI: theory, experimental design and data analysis – a technical review. *NMR in Biomedicine* **15**, 456–467 (2002).
140. Beaulieu, C. The basis of anisotropic water diffusion in the nervous system – a technical review. *NMR in Biomedicine* **15**, 435–455 (2002).
141. Ukmar, M. *et al.* Evaluation of white matter damage in patients with Alzheimer's disease and in patients with mild cognitive impairment by using diffusion tensor imaging. *Radiol med* **113**, 915–922 (2008).
142. Stricker, N. H. *et al.* Decreased white matter integrity in late-myelinating fiber pathways in Alzheimer's disease supports retrogenesis. *Neuroimage* **45**, 10–16 (2009).
143. Mielke, M. M. *et al.* Regionally-specific diffusion tensor imaging in mild cognitive impairment and Alzheimer's disease. *Neuroimage* **46**, 47–55 (2009).
144. Liu, J., Rutten-Jacobs, L., Liu, M., Markus, H. S. & Traylor, M. Causal Impact of Type 2 Diabetes Mellitus on Cerebral Small Vessel Disease: A Mendelian Randomization Analysis. *Stroke* **49**, 1325–1331 (2018).
145. Nir, T. M. *et al.* Effectiveness of regional DTI measures in distinguishing Alzheimer's disease, MCI, and normal aging. *Neuroimage Clin* **3**, 180–195 (2013).
146. Zhang, H., Schneider, T., Wheeler-Kingshott, C. A. & Alexander, D. C. NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain. *Neuroimage* **61**, 1000–1016 (2012).
147. Kamiya, K., Hori, M. & Aoki, S. NODDI in clinical research. *J Neurosci Methods* **346**, 108908 (2020).

148. James, S.-N. *et al.* Neuroimaging, clinical and life course correlates of normal-appearing white matter integrity in 70-year-olds. *Brain Communications* **5**, fcad225 (2023).

149. Shukla, A. & Kumar, U. Positron emission tomography: An overview. *J Med Phys* **31**, 13 (2006).

150. Kapoor, V., McCook, B. M. & Torok, F. S. An introduction to PET-CT imaging. *Radiographics* **24**, 523–543 (2004).

151. Klunk, W. E. *et al.* Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Annals of Neurology* **55**, 306–319 (2004).

152. Clark, C. M. Use of Florbetapir-PET for Imaging β-Amyloid Pathology. *JAMA* **305**, 275 (2011).

153. Jack, C. R. *et al.* Age, Sex, and *APOE* ε4 Effects on Memory, Brain Structure, and β-Amyloid Across the Adult Life Span. *JAMA Neurol* **72**, 511 (2015).

154. Morris, J. C. *et al.* APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Ann Neurol* **67**, 122–131 (2010).

155. Nordberg, A., Rinne, J. O., Kadir, A. & Långström, B. The use of PET in Alzheimer disease. *Nat Rev Neurol* **6**, 78–87 (2010).

156. Dickson, D. W. *et al.* Immunocytochemistry of neurofibrillary tangles with antibodies to subregions of tau protein: identification of hidden and cleaved tau epitopes and a new phosphorylation site. *Acta Neuropathol* **84**, 596–605 (1992).

157. Leuzy, A. *et al.* Tau PET imaging in neurodegenerative tauopathies—still a challenge. *Mol Psychiatry* **24**, 1112–1134 (2019).

158. Sheikh-Bahaei, N., Sajjadi, S. A., Manavaki, R. & Gillard, J. H. Imaging Biomarkers in Alzheimer's Disease: A Practical Guide for Clinicians. *J Alzheimers Dis Rep* **1**, 71–88 (2017).

159. Glover, G. H. Overview of Functional Magnetic Resonance Imaging. *Neurosurgery Clinics of North America* **22**, 133–139 (2011).

160. Lv, H. *et al.* Resting-State Functional MRI: Everything That Nonexperts Have Always Wanted to Know. *AJNR Am J Neuroradiol* ajnr;ajnr.A5527v1 (2018) doi:10.3174/ajnr.A5527.

161. Iwatsubo, T., Mann, D. M. A., Odaka, A., Suzuki, N. & Ihara, Y. Amyloid β protein (Aβ) deposition: Aβ42(43) precedes Aβ40 in down Syndrome. *Annals of Neurology* **37**, 294–299 (1995).

162. Halban, P. A. *et al.* β-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. *J Clin Endocrinol Metab* **99**, 1983–1992 (2014).

163. Carracher, A. M., Marathe, P. H. & Close, K. L. International Diabetes Federation 2017. *Journal of Diabetes* **10**, 353–356 (2018).

164. Romao, I. & Roth, J. Genetic and Environmental Interactions in Obesity and Type 2 Diabetes. *Journal of the American Dietetic Association* **108**, S24–S28 (2008).

165. Weyer, C., Bogardus, C., Mott, D. M. & Pratley, R. E. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* **104**, 787–794 (1999).

166. Stumvoll, M., Goldstein, B. J. & van Haeften, T. W. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* **365**, 1333–1346 (2005).

167. Cornelis, M. C. & Hu, F. B. Gene-Environment Interactions in the Development of Type 2 Diabetes: Recent Progress and Continuing Challenges. *Annu. Rev. Nutr.* **32**, 245–259 (2012).

168. Ong, K. L. *et al.* Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet* **402**, 203–234 (2023).
169. Tillin, T. *et al.* Insulin resistance and truncal obesity as important determinants of the greater incidence of diabetes in Indian Asians and African Caribbeans compared with Europeans: the Southall And Brent REvisited (SABRE) cohort. *Diabetes Care* **36**, 383–393 (2013).
170. Signorello, L. B. *et al.* Comparing diabetes prevalence between African Americans and Whites of similar socioeconomic status. *Am J Public Health* **97**, 2260–2267 (2007).
171. Casqueiro, J., Casqueiro, J. & Alves, C. Infections in patients with diabetes mellitus: A review of pathogenesis. *Indian J Endocr Metab* **16**, 27 (2012).
172. Farmaki, P. *et al.* Complications of the Type 2 Diabetes Mellitus. *CCR* **16**, 249–251 (2021).
173. Fazeli, P. K., Lee, H. & Steinhauser, M. L. Aging Is a Powerful Risk Factor for Type 2 Diabetes Mellitus Independent of Body Mass Index. *Gerontology* **66**, 209–210 (2020).
174. Annis, A. M., Caulder, M. S., Cook, M. L. & Duquette, D. Family history, diabetes, and other demographic and risk factors among participants of the National Health and Nutrition Examination Survey 1999-2002. *Prev Chronic Dis* **2**, A19 (2005).
175. Pikhartova, J., Blane, D. & Netuveli, G. The role of childhood social position in adult type 2 diabetes: evidence from the English Longitudinal Study of Ageing. *BMC Public Health* **14**, 505 (2014).
176. Uusitupa, M. *et al.* Prevention of Type 2 Diabetes by Lifestyle Changes: A Systematic Review and Meta-Analysis. *Nutrients* **11**, 2611 (2019).

177. Magliano, D. J., Boyko, E. J., & IDF Diabetes Atlas 10th edition scientific committee. *IDF DIABETES ATLAS*. (International Diabetes Federation, Brussels, 2021).

178. Cho, N. H. *et al.* IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice* **138**, 271–281 (2018).

179. The DECODE Study Group. Age- and Sex-Specific Prevalences of Diabetes and Impaired Glucose Regulation in 13 European Cohorts. *Diabetes Care* **26**, 61–69 (2003).

180. Kautzky-Willer, A., Harreiter, J. & Pacini, G. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. *Endocrine Reviews* **37**, 278–316 (2016).

181. Mauvais-Jarvis, F. *et al.* Sex and gender: modifiers of health, disease, and medicine. *The Lancet* **396**, 565–582 (2020).

182. Goossens, G. H., Jocken, J. W. E. & Blaak, E. E. Sexual dimorphism in cardiometabolic health: the role of adipose tissue, muscle and liver. *Nat Rev Endocrinol* **17**, 47–66 (2021).

183. Xu, W., Morford, J. & Mauvais-Jarvis, F. Emerging role of testosterone in pancreatic β cell function and insulin secretion. *Journal of Endocrinology* **240**, R97–R105 (2019).

184. Silva-Costa, A. *et al.* Lifetime night work exposure and the risk of type 2 diabetes: results from the longitudinal study of adult health (ELSA-Brasil). *Chronobiology International* **37**, 1344–1347 (2020).

185. Tomic, D. *et al.* Lifetime risk, life expectancy, and years of life lost to type 2 diabetes in 23 high-income jurisdictions: a multinational, population-based study. *The Lancet Diabetes & Endocrinology* **10**, 795–803 (2022).
186. Kautzky-Willer, A., Dorner, T., Jensby, A. & Rieder, A. Women show a closer association between educational level and hypertension or diabetes mellitus than males: a secondary analysis from the Austrian HIS. *BMC Public Health* **12**, 392 (2012).
187. Gómez-Marcos, M. Á. *et al.* Cardio-ankle vascular index is associated with cardiovascular target organ damage and vascular structure and function in patients with diabetes or metabolic syndrome, LOD-DIABETES study: a case series report. *Cardiovasc Diabetol* **14**, 7 (2015).
188. de Ritter, R. *et al.* Adverse differences in cardiometabolic risk factor levels between individuals with pre-diabetes and normal glucose metabolism are more pronounced in women than in men: the Maastricht Study. *BMJ Open Diabetes Res Care* **7**, e000787 (2019).
189. Shen, Y. *et al.* Diabetes mellitus as a risk factor for incident chronic kidney disease and end-stage renal disease in women compared with men: a systematic review and meta-analysis. *Endocrine* **55**, 66–76 (2017).
190. Retnakaran, R. *et al.* Risk Factors for Renal Dysfunction in Type 2 Diabetes. *Diabetes* **55**, 1832–1839 (2006).
191. Guthrie, D. The History of Diabetes Mellitus, 2nd ed., by N. S. Papaspyros, Stuttgart, G. Thieme Verlag, 1964, pp. 104, 10 plates, revised and supplemented, DM. 19.50. *Med. Hist.* **9**, 198–198 (1965).

192. Cusi, K., Consoli, A. & DeFronzo, R. A. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. *The Journal of Clinical Endocrinology & Metabolism* **81**, 4059–4067 (1996).
193. Turner, R. C. Glycemic Control With Diet, Sulfonylurea, Metformin, or Insulin in Patients With Type 2 Diabetes MellitusProgressive Requirement for Multiple Therapies (UKPDS 49). *JAMA* **281**, 2005 (1999).
194. Seo-Mayer, P. W. *et al.* Preactivation of AMPK by metformin may ameliorate the epithelial cell damage caused by renal ischemia. *American Journal of Physiology-Renal Physiology* **301**, F1346–F1357 (2011).
195. Horakova, O. *et al.* Metformin acutely lowers blood glucose levels by inhibition of intestinal glucose transport. *Sci Rep* **9**, 6156 (2019).
196. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *The Lancet* **352**, 854–865 (1998).
197. Prattichizzo, F. *et al.* Glucose-lowering therapies in patients with type 2 diabetes and cardiovascular diseases. *Eur J Prev Cardiolog* **26**, 73–80 (2019).
198. Dunn, C. J. & Peters, D. H. Metformin: A Review of its Pharmacological Properties and Therapeutic Use in Non-Insulin-Dependent Diabetes Mellitus. *Drugs* **49**, 721–749 (1995).
199. Drucker, D. J., Habener, J. F. & Holst, J. J. Discovery, characterization, and clinical development of the glucagon-like peptides. *Journal of Clinical Investigation* **127**, 4217–4227 (2017).
200. Drucker, D. J. Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. *Cell Metabolism* **27**, 740–756 (2018).

201. Holst, J. J., Gasbjerg, L. S. & Rosenkilde, M. M. The Role of Incretins on Insulin Function and Glucose Homeostasis. *Endocrinology* **162**, bqab065 (2021).
202. DeFronzo, R. A., Triplitt, C. L., Abdul-Ghani, M. & Cersosimo, E. Novel Agents for the Treatment of Type 2 Diabetes. *Diabetes Spectrum* **27**, 100–112 (2014).
203. Abdul-Ghani, M. & DeFronzo, R. A. Is It Time to Change the Type 2 Diabetes Treatment Paradigm? Yes! GLP-1 RAs Should Replace Metformin in the Type 2 Diabetes Algorithm. *Diabetes Care* **40**, 1121–1127 (2017).
204. Gentilella, R., Pechtner, V., Corcos, A. & Consoli, A. Glucagon-like peptide-1 receptor agonists in type 2 diabetes treatment: are they all the same? *Diabetes Metabolism Res* **35**, e3070 (2019).
205. Nauck, M. A. & Meier, J. J. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. *The Lancet Diabetes & Endocrinology* **4**, 525–536 (2016).
206. Wheeler, E. *et al.* Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: A transethnic genome-wide meta-analysis. *PLoS Med* **14**, e1002383 (2017).
207. Diagnostic criteria for diabetes. *Diabetes UK* https://www.diabetes.org.uk/for-professionals/improving-care/clinical-recommendations-for-professionals/diagnosis-ongoing-management-monitoring/new_diagnostic_criteria_for_diabetes.
208. Herman, W. H. & Fajans, S. S. Hemoglobin A1c for the diagnosis of diabetes: practical considerations. *Pol Arch Med Wewn* **120**, 37–40 (2010).
209. Christy, A. L., Manjrekar, P. A., Babu, R. P., Hegde, A. & Rukmini, M. S. Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. *Iran Biomed J* **18**, 88–93 (2014).

210. Dubowitz, N. *et al.* Aging is associated with increased HbA $_{1c}$ levels, independently of glucose levels and insulin resistance, and also with decreased HbA $_{1c}$ diagnostic specificity. *Diabet. Med.* **31**, 927–935 (2014).
211. Inada, S. & Koga, M. HbA1c and Glycated Albumin Levels Are High in Gastrectomized Subjects with Iron-Deficiency Anemia. *Ann Clin Lab Sci* **47**, 52–57 (2017).
212. Bloomgarden, Z. & Handelsman, Y. How does CKD affect HbA1c?: CKD affect HbA1c? *Journal of Diabetes* **10**, 270–270 (2018).
213. Vlassopoulos, A., Lean, M. E. J. & Combet, E. Influence of smoking and diet on glycated haemoglobin and 'pre-diabetes' categorisation: a cross-sectional analysis. *BMC Public Health* **13**, 1013 (2013).
214. Khera, P. K. *et al.* Evidence for Interindividual Heterogeneity in the Glucose Gradient Across the Human Red Blood Cell Membrane and Its Relationship to Hemoglobin Glycation. *Diabetes* **57**, 2445–2452 (2008).
215. Cohen, R. M. *et al.* Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. *Blood* **112**, 4284–4291 (2008).
216. English, E. *et al.* The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. *Diabetologia* **58**, 1409–1421 (2015).
217. McCane, D. R. *et al.* Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *BMJ* **308**, 1323–8 (1994).
218. Engelgau, M. M. *et al.* Comparison of Fasting and 2-Hour Glucose and HbA1c Levels for Diagnosing Diabetes: Diagnostic criteria and performance revisited. *Diabetes Care* **20**, 785–791 (1997).

219. Ting, D. S. W., Cheung, G. C. M. & Wong, T. Y. Diabetic retinopathy: global prevalence, major risk factors, screening practices and public health challenges: a review. *Clinical Exper Ophthalmology* **44**, 260–277 (2016).

220. Wong, T. Y. *et al.* Relation between fasting glucose and retinopathy for diagnosis of diabetes: three population-based cross-sectional studies. *The Lancet* **371**, 736–743 (2008).

221. Khaw, K.-T. *et al.* Association of Hemoglobin A $_{1c}$ with Cardiovascular Disease and Mortality in Adults: The European Prospective Investigation into Cancer in Norfolk. *Ann Intern Med* **141**, 413 (2004).

222. Bernier, F., Ohno, K., Katsumata, N., Shimizu, T. & Xiao, J. Association of Plasma Hemoglobin A1c with Improvement of Cognitive Functions by Probiotic Bifidobacterium breve Supplementation in Healthy Adults with Mild Cognitive Impairment. *JAD* **81**, 493–497 (2021).

223. Tabák, A. G., Herder, C., Rathmann, W., Brunner, E. J. & Kivimäki, M. Prediabetes: a high-risk state for diabetes development. *The Lancet* **379**, 2279–2290 (2012).

224. Schlesinger, S. *et al.* Prediabetes and risk of mortality, diabetes-related complications and comorbidities: umbrella review of meta-analyses of prospective studies. *Diabetologia* **65**, 275–285 (2022).

225. Gerstein, H. C. *et al.* Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: A systematic overview and meta-analysis of prospective studies. *Diabetes Research and Clinical Practice* **78**, 305–312 (2007).

226. Tuso, P. Prediabetes and Lifestyle Modification: Time to Prevent a Preventable Disease. *TPJ* **18**, 88–93 (2014).

227. Bryan, S. *et al. NHSR 158. National Health and Nutrition Examination Survey 2017–March 2020 Pre-Pandemic Data Files*. https://stacks.cdc.gov/view/cdc/106273 (2021) doi:10.15620/cdc:106273.

228. Haeusler, R. A., McGraw, T. E. & Accili, D. Biochemical and cellular properties of insulin receptor signalling. *Nat Rev Mol Cell Biol* **19**, 31–44 (2018).

229. Saltiel, A. R. & Kahn, C. R. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414**, 799–806 (2001).

230. Batista, T. M., Haider, N. & Kahn, C. R. Defining the underlying defect in insulin action in type 2 diabetes. *Diabetologia* **64**, 994–1006 (2021).

231. Marks, J. L., Porte, D., Stahl, W. L. & Baskin, D. G. Localization of insulin receptor mRNA in rat brain by in situ hybridization. *Endocrinology* **127**, 3234–3236 (1990).

232. Unger, J. W. & Betz, M. Insulin receptors and signal transduction proteins in the hypothalamo-hypophyseal system: a review on morphological findings and functional implications. *Histology and Histopathology* 1215–1224 (1998) doi:10.14670/HH-13.1215.

233. Werther, G. A. *et al.* Localization and Characterization of Insulin Receptors in Rat Brain and Pituitary Gland Using *in Vitro* Autoradiography and Computerized Densitometry*. *Endocrinology* **121**, 1562–1570 (1987).

234. Zhao, W. *et al.* Brain Insulin Receptors and Spatial Memory. *Journal of Biological Chemistry* **274**, 34893–34902 (1999).

235. Deacon, C. F. Physiology and Pharmacology of DPP-4 in Glucose Homeostasis and the Treatment of Type 2 Diabetes. *Front. Endocrinol.* **10**, 80 (2019).

236. Polonsky, K. S. *et al.* Abnormal Patterns of Insulin Secretion in Non-Insulin-Dependent Diabetes Mellitus. *N Engl J Med* **318**, 1231–1239 (1988).

237. Pick, A. *et al.* Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes* **47**, 358–364 (1998).
238. Cerf, M. E. Beta Cell Dysfunction and Insulin Resistance. *Front. Endocrinol.* **4**, (2013).
239. Banday, M. Z., Sameer, A. S. & Nissar, S. Pathophysiology of diabetes: An overview. *Avicenna J Med* **10**, 174–188 (2020).
240. Kanat, M. *et al.* The Relationship Between β-Cell Function and Glycated Hemoglobin. *Diabetes Care* **34**, 1006–1010 (2011).
241. Rossetti, L., Giaccari, A. & DeFronzo, R. A. Glucose Toxicity. *Diabetes Care* **13**, 610–630 (1990).
242. Kurowski, T. G. *et al.* Hyperglycemia inhibits insulin activation of Akt/protein kinase B but not phosphatidylinositol 3-kinase in rat skeletal muscle. *Diabetes* **48**, 658–663 (1999).
243. Gunasekaran, U. & Gannon, M. Type 2 Diabetes and the Aging Pancreatic Beta Cell. *Aging* **3**, 565–575 (2011).
244. Bunn, H. F., Haney, D. N., Kamin, S., Gabbay, K. H. & Gallop, P. M. The biosynthesis of human hemoglobin A1c. Slow glycosylation of hemoglobin in vivo. *J. Clin. Invest.* **57**, 1652–1659 (1976).
245. Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A. & Sakharkar, M. K. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. *Biomark Insights* **11**, BMI.S38440 (2016).
246. Lai, Y.-R. *et al.* HbA1C Variability Is Strongly Associated With the Severity of Peripheral Neuropathy in Patients With Type 2 Diabetes. *Front. Neurosci.* **13**, 90 (2019).

247. Lind, M. *et al.* The relationship between glycaemic control and heart failure in 83,021 patients with type 2 diabetes. *Diabetologia* **55**, 2946–2953 (2012).
248. Selvin, E. *et al.* Meta-Analysis: Glycosylated Hemoglobin and Cardiovascular Disease in Diabetes Mellitus. *Ann Intern Med* **141**, 421 (2004).
249. Kim, J., Bachmann, R. A. & Chen, J. Chapter 21 Interleukin-6 and Insulin Resistance. in *Vitamins & Hormones* vol. 80 613–633 (Elsevier, 2009).
250. Tam, C. S. *et al.* Defining Insulin Resistance From Hyperinsulinemic-Euglycemic Clamps. *Diabetes Care* **35**, 1605–1610 (2012).
251. Singh, B. Surrogate markers of insulin resistance: A review. *WJD* **1**, 36 (2010).
252. Zaletel, J., Pongrac Barlovic, D. & Prezelj, J. Adiponectin-leptin ratio: A useful estimate of insulin resistance in patients with Type 2 diabetes. *J Endocrinol Invest* **33**, 514–518 (2010).
253. Chauhan, A., Singhal, A. & Goyal, P. TG/HDL Ratio: A marker for insulin resistance and atherosclerosis in prediabetics or not? *J Family Med Prim Care* **10**, 3700 (2021).
254. Levy, J. C., Matthews, D. R. & Hermans, M. P. Correct Homeostasis Model Assessment (HOMA) Evaluation Uses the Computer Program. *Diabetes Care* **21**, 2191–2192 (1998).
255. Sicree, R. A. *et al.* Differences in height explain gender differences in the response to the oral glucose tolerance test— the AusDiab study. *Diabetic Medicine* **25**, 296–302 (2008).
256. Nuutila, P. *et al.* Gender and Insulin Sensitivity in the Heart and in Skeletal Muscles: Studies Using Positron Emission Tomography. *Diabetes* **44**, 31–36 (1995).

257. Gannon, M., Kulkarni, R. N., Tse, H. M. & Mauvais-Jarvis, F. Sex differences underlying pancreatic islet biology and its dysfunction. *Molecular Metabolism* **15**, 82–91 (2018).

258. Schopman, J. E. *et al.* The incidence of mild and severe hypoglycaemia in patients with type 2 diabetes mellitus treated with sulfonylureas: a systematic review and meta-analysis. *Diabetes Metabolism Res* **30**, 11–22 (2014).

259. Christou, M. A., Christou, P. A., Kyriakopoulos, C., Christou, G. A. & Tigas, S. Effects of Hypoglycemia on Cardiovascular Function in Patients with Diabetes. *IJMS* **24**, 9357 (2023).

260. Graveling, A. J., Deary, I. J. & Frier, B. M. Acute Hypoglycemia Impairs Executive Cognitive Function in Adults With and Without Type 1 Diabetes. *Diabetes Care* **36**, 3240–3246 (2013).

261. Yaffe, K. *et al.* Association between hypoglycemia and dementia in a biracial cohort of older adults with diabetes mellitus. *JAMA Intern Med* **173**, 1300–1306 (2013).

262. Bruce, D. G. *et al.* Severe hypoglycaemia and cognitive impairment in older patients with diabetes: the Fremantle Diabetes Study. *Diabetologia* **52**, 1808–1815 (2009).

263. Hotamisligil, G. S., Shargill, N. S. & Spiegelman, B. M. Adipose Expression of Tumor Necrosis Factor-α: Direct Role in Obesity-Linked Insulin Resistance. *Science* **259**, 87–91 (1993).

264. Schmidt, M. I. *et al.* Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *The Lancet* **353**, 1649–1652 (1999).

265. Duncan, B. B. *et al.* Low-Grade Systemic Inflammation and the Development of Type 2 Diabetes. *Diabetes* **52**, 1799–1805 (2003).

266. Pradhan, A. D. C-Reactive Protein, Interleukin 6, and Risk of Developing Type 2 Diabetes Mellitus. *JAMA* **286**, 327 (2001).

267. Barzilay, J. I. *et al.* The Relation of Markers of Inflammation to the Development of Glucose Disorders in the Elderly. *Diabetes* **50**, 2384–2389 (2001).

268. Vozarova, B. *et al.* High Alanine Aminotransferase Is Associated With Decreased Hepatic Insulin Sensitivity and Predicts the Development of Type 2 Diabetes. *Diabetes* **51**, 1889–1895 (2002).

269. Sun, Q., Li, J. & Gao, F. New insights into insulin: The anti-inflammatory effect and its clinical relevance. *WJD* **5**, 89 (2014).

270. Li, W. *et al.* Adaptive Cerebral Neovascularization in a Model of Type 2 Diabetes. *Diabetes* **59**, 228–235 (2010).

271. Harris, A. K. *et al.* Type 2 Diabetes Causes Remodeling of Cerebrovasculature via Differential Regulation of Matrix Metalloproteinases and Collagen Synthesis. *Diabetes* **54**, 2638–2644 (2005).

272. Abdelsaid, M., Kaczmarek, J., Coucha, M. & Ergul, A. Dual endothelin receptor antagonism with bosentan reverses established vascular remodeling and dysfunctional angiogenesis in diabetic rats: Relevance to glycemic control. *Life Sciences* **118**, 268–273 (2014).

273. Ergul, A., Kelly-Cobbs, A., Abdalla, M. & C. Fagan, S. Cerebrovascular Complications of Diabetes: Focus on Stroke. *EMIDDT* **12**, 148–158 (2012).

274. Dorner, G. T. *et al.* Hyperglycemia affects flicker-induced vasodilation in the retina of healthy subjects. *Vision Research* **43**, 1495–1500 (2003).

275. Jarajapu, Y. P. R., Guberski, D. L., Grant, M. B. & Knot, H. J. Myogenic tone and reactivity of cerebral arteries in Type II diabetic BBZDR/Wor rat. *European Journal of Pharmacology* **579**, 298–307 (2008).
276. Kelly-Cobbs, A. I. *et al.* Cerebral Myogenic Reactivity and Blood Flow in Type 2 Diabetic Rats: Role of Peroxynitrite in Hypoxia-Mediated Loss of Myogenic Tone. *J Pharmacol Exp Ther* **342**, 407–415 (2012).
277. Gudala, K., Bansal, D., Schifano, F. & Bhansali, A. Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. *J of Diabetes Invest* **4**, 640–650 (2013).
278. Newman, B. *et al.* Concordance for Type 2 (non-insulin-dependent) diabetes mellitus in male twins. *Diabetologia* **30**, (1987).
279. Kaprio, J. *et al.* Concordance for Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia* **35**, 1060–1067 (1992).
280. Tillil, H. & Köbberling, J. Age-corrected empirical genetic risk estimates for first-degree relatives of IDDM patients. *Diabetes* **36**, 93–99 (1987).
281. Fajans, S. S., Bell, G. I. & Polonsky, K. S. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* **345**, 971–980 (2001).
282. Horikawa, Y. *et al.* Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* **26**, 163–175 (2000).
283. Reynisdottir, I. *et al.* Localization of a Susceptibility Gene for Type 2 Diabetes to Chromosome 5q34–q35.2. *The American Journal of Human Genetics* **73**, 323–335 (2003).

284. Shastry, B. S. SNPs: Impact on Gene Function and Phenotype. in *Single Nucleotide Polymorphisms* (ed. Komar, A. A.) vol. 578 3–22 (Humana Press, Totowa, NJ, 2009).
285. Sladek, R. *et al.* A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* **445**, 881–885 (2007).
286. Xue, A. *et al.* Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. *Nat Commun* **9**, 2941 (2018).
287. Thomsen, S. K. & Gloyn, A. L. The pancreatic β cell: recent insights from human genetics. *Trends in Endocrinology & Metabolism* **25**, 425–434 (2014).
288. Povel, C. M. *et al.* Single nucleotide polymorphisms (SNPs) involved in insulin resistance, weight regulation, lipid metabolism and inflammation in relation to metabolic syndrome: an epidemiological study. *Cardiovasc Diabetol* **11**, 133 (2012).
289. Huang, Q. Genetic Study of Complex Diseases in the Post-GWAS Era. *Journal of Genetics and Genomics* **42**, 87–98 (2015).
290. Ashenhurst, J. R. *et al.* A Polygenic Score for Type 2 Diabetes Improves Risk Stratification Beyond Current Clinical Screening Factors in an Ancestrally Diverse Sample. *Front. Genet.* **13**, 871260 (2022).
291. Chen, D. W., Shi, J. K., Li, Y., Yang, Y. & Ren, S. P. Association between ApoE Polymorphism and Type 2 Diabetes: A Meta-Analysis of 59 Studies. *Biomed Environ Sci* **32**, 823–838 (2019).
292. Bellou, V., Belbasis, L., Tzoulaki, I. & Evangelou, E. Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. *PLoS ONE* **13**, e0194127 (2018).

293. Launer, L. J. *et al.* Midlife blood pressure and dementia: the Honolulu–Asia aging study☆. *Neurobiology of Aging* **21**, 49–55 (2000).

294. Norton, S., Matthews, F. E., Barnes, D. E., Yaffe, K. & Brayne, C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *The Lancet Neurology* **13**, 788–794 (2014).

295. De Bruijn, R. F. A. G. *et al.* Subclinical cardiac dysfunction increases the risk of stroke and dementia: The Rotterdam Study. *Neurology* **84**, 833–840 (2015).

296. Biessels, G. J., Strachan, M. W. J., Visseren, F. L. J., Kappelle, L. J. & Whitmer, R. A. Dementia and cognitive decline in type 2 diabetes and prediabetic stages: towards targeted interventions. *The Lancet Diabetes & Endocrinology* **2**, 246–255 (2014).

297. Feinkohl, I., Price, J. F., Strachan, M. W. J. & Frier, B. M. The impact of diabetes on cognitive decline: potential vascular, metabolic, and psychosocial risk factors. *Alz Res Therapy* **7**, 46 (2015).

298. Haroon, N. N. *et al.* Risk of Dementia in Seniors With Newly Diagnosed Diabetes: A Population-Based Study. *Diabetes Care* **38**, 1868–1875 (2015).

299. Exalto, L. G. *et al.* Risk score for prediction of 10 year dementia risk in individuals with type 2 diabetes: a cohort study. *The Lancet Diabetes & Endocrinology* **1**, 183–190 (2013).

300. Rains, J. L. & Jain, S. K. Oxidative stress, insulin signaling, and diabetes. *Free Radical Biology and Medicine* **50**, 567–575 (2011).

301. Brownlee, M. A radical explanation for glucose-induced β cell dysfunction. *J. Clin. Invest.* **112**, 1788–1790 (2003).

302. Beckman, J. A., Goldfine, A. B., Gordon, M. B. & Creager, M. A. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. *Circulation* **103**, 1618–1623 (2001).

303. Chawla, A., Chawla, R. & Jaggi, S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian J Endocr Metab* **20**, 546 (2016).

304. Barrett, E. J. *et al.* Diabetic Microvascular Disease: An Endocrine Society Scientific Statement. *The Journal of Clinical Endocrinology & Metabolism* **102**, 4343–4410 (2017).

305. Bucala, R., Makita, Z., Koschinsky, T., Cerami, A. & Vlassara, H. Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6434–6438 (1993).

306. Gianazza, E. *et al.* Lipid Peroxidation in Atherosclerotic Cardiovascular Diseases. *Antioxidants & Redox Signaling* **34**, 49–98 (2021).

307. Butterfield, D. A. *et al.* In vivo oxidative stress in brain of Alzheimer disease transgenic mice: Requirement for methionine 35 in amyloid β-peptide of APP. *Free Radical Biology and Medicine* **48**, 136–144 (2010).

308. Ruipérez, V., Darios, F. & Davletov, B. Alpha-synuclein, lipids and Parkinson's disease. *Progress in Lipid Research* **49**, 420–428 (2010).

309. Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**, 813–820 (2001).

310. S. Roriz-Filho, J. *et al.* (Pre)diabetes, brain aging, and cognition. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1792**, 432–443 (2009).

311. Brownlee, M.D, M. ADVANCED PROTEIN GLYCOSYLATION IN DIABETES AND AGING. *Annu. Rev. Med.* **46**, 223–234 (1995).

312. Schalkwijk, C., Brouwers, O. & Stehouwer, C. Modulation of Insulin Action by Advanced Glycation Endproducts: A New Player in the Field. *Horm Metab Res* **40**, 614–619 (2008).

313. Valente, T., Gella, A., Fernàndez-Busquets, X., Unzeta, M. & Durany, N. Immunohistochemical analysis of human brain suggests pathological synergism of Alzheimer's disease and diabetes mellitus. *Neurobiology of Disease* **37**, 67–76 (2010).

314. Mosquera-Sulbarán, J. A. & Hernández-Fonseca, J. P. Advanced Glycation End Products in Diabetes. in *Biomarkers in Diabetes* (eds. Patel, V. B. & Preedy, V. R.) 171–194 (Springer International Publishing, Cham, 2023). doi:10.1007/978-3-031-08014-2_8.

315. Woodburn, S. C., Bollinger, J. L. & Wohleb, E. S. The semantics of microglia activation: neuroinflammation, homeostasis, and stress. *J Neuroinflammation* **18**, 258 (2021).

316. Duchen, M. R. Mitochondria and calcium: from cell signalling to cell death. *J Physiol* **529 Pt 1**, 57–68 (2000).

317. Ormazabal, V. *et al.* Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc Diabetol* **17**, 122 (2018).

318. Okdahl, T. *et al.* Low-grade inflammation in type 2 diabetes: a cross-sectional study from a Danish diabetes outpatient clinic. *BMJ Open* **12**, e062188 (2022).

319. Xie, J., Van Hoecke, L. & Vandenbroucke, R. E. The Impact of Systemic Inflammation on Alzheimer's Disease Pathology. *Front. Immunol.* **12**, 796867 (2022).

320. Kinney, J. W. *et al.* Inflammation as a central mechanism in Alzheimer's disease. *A&D Transl Res & Clin Interv* **4**, 575–590 (2018).

321. Schmidt, R. *et al.* Early inflammation and dementia: A 25-year follow-up of the Honolulu-Asia aging study. *Annals of Neurology* **52**, 168–174 (2002).
322. Engelhart, M. J. *et al.* Inflammatory Proteins in Plasma and the Risk of Dementia: The Rotterdam Study. *Arch Neurol* **61**, 668 (2004).
323. Lue, L. Modeling microglial activation in Alzheimer's disease with human postmortem microglial cultures. *Neurobiology of Aging* **22**, 945–956 (2001).
324. Hull, M., Strauss, S., Berger, M., Volk, B. & Bauer, J. The participation of interleukin-6, a stress-inducible cytokine, in the pathogenesis of Alzheimer's disease. *Behavioural Brain Research* **78**, 37–41 (1996).
325. Rösler, N., Wichart, I. & Jellinger, K. A. *Intra vitam* lumbar and *post mortem* ventricular cerebrospinal fluid immunoreactive interleukin-6 in Alzheimer's disease patients: CSF interleukin-6 in Alzheimer's disease. *Acta Neurologica Scandinavica* **103**, 126–130 (2001).
326. Walker, K. A. *et al.* Midlife systemic inflammatory markers are associated with late-life brain volume: The ARIC study. *Neurology* **89**, 2262–2270 (2017).
327. Jefferson, A. L. *et al.* Inflammatory biomarkers are associated with total brain volume: The Framingham Heart Study. *Neurology* **68**, 1032–1038 (2007).
328. Jackson, K. *et al.* Amylin deposition in the brain: A second amyloid in Alzheimer disease? *Annals of Neurology* **74**, 517–526 (2013).
329. Cooper, G. J. *et al.* Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7763–7766 (1988).
330. Fu, W., Patel, A. & Jhamandas, J. H. Amylin Receptor: A Common Pathophysiological Target in Alzheimer's Disease and Diabetes Mellitus. *Front. Aging Neurosci.* **5**, (2013).

331. Lim, Y.-A., Ittner, L. M., Lim, Y. L. & Götz, J. Human but not rat amylin shares neurotoxic properties with Aβ42 in long-term hippocampal and cortical cultures. *FEBS Letters* **582**, 2188–2194 (2008).
332. Lim, Y. *et al.* Aβ and human amylin share a common toxicity pathway *via* mitochondrial dysfunction. *Proteomics* **10**, 1621–1633 (2010).
333. Despa, F. & DeCarli, C. Amylin: what might be its role in Alzheimer's disease and how could this affect therapy? *Expert Review of Proteomics* **10**, 403–405 (2013).
334. Zhu, H. *et al.* Association of Plasma Amylin Concentration With Alzheimer Disease and Brain Structure in Older Adults. *JAMA Netw Open* **2**, e199826 (2019).
335. Ko, S.-Y. *et al.* The Possible Mechanism of Advanced Glycation End Products (AGEs) for Alzheimer's Disease. *PLoS ONE* **10**, e0143345 (2015).
336. Li, X.-H. *et al.* Glycation exacerbates the neuronal toxicity of β-amyloid. *Cell Death Dis* **4**, e673–e673 (2013).
337. Candela, P. *et al.* Apical-to-Basolateral Transport of Amyloid-β Peptides through Blood-Brain Barrier Cells is Mediated by the Receptor for Advanced Glycation End-Products and is Restricted by P-Glycoprotein. *JAD* **22**, 849–859 (2010).
338. Meerwaldt, R. *et al.* Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* **47**, 1324–1330 (2004).
339. Smit, A. J. & Gerrits, E. G. Skin autofluorescence as a measure of advanced glycation endproduct deposition: a novel risk marker in chronic kidney disease: *Current Opinion in Nephrology and Hypertension* **19**, 527–533 (2010).
340. Bos, D. C., De Ranitz-Greven, W. L. & De Valk, H. W. Advanced Glycation End Products, Measured as Skin Autofluorescence and Diabetes Complications: A Systematic Review. *Diabetes Technology & Therapeutics* **13**, 773–779 (2011).

341. Moran, C. *et al.* Type 2 Diabetes, Skin Autofluorescence, and Brain Atrophy. *Diabetes* **64**, 279–283 (2015).

342. Chen, G. *et al.* Serum Level of Endogenous Secretory Receptor for Advanced Glycation End Products and Other Factors in Type 2 Diabetic Patients With Mild Cognitive Impairment. *Diabetes Care* **34**, 2586–2590 (2011).

343. Igase, M. *et al.* Skin Autofluorescence Examination as a Diagnostic Tool for Mild Cognitive Impairment in Healthy People. *JAD* **55**, 1481–1487 (2016).

344. Yaffe, K. *et al.* Advanced glycation end product level, diabetes, and accelerated cognitive aging. *Neurology* **77**, 1351–1356 (2011).

345. Leenders, K. L. *et al.* CEREBRAL BLOOD FLOW, BLOOD VOLUME AND OXYGEN UTILIZATION: NORMAL VALUES AND EFFECT OF AGE. *Brain* **113**, 27–47 (1990).

346. Heo, S. *et al.* Resting hippocampal blood flow, spatial memory and aging. *Brain Research* **1315**, 119–127 (2010).

347. for the Alzheimer's Disease Neuroimaging Initiative *et al.* Regional Hypoperfusion Predicts Decline in Everyday Functioning at Three-Year Follow-Up in Older Adults without Dementia. *JAD* **77**, 1291–1304 (2020).

348. Liu, J. *et al.* Cerebral Blood Flow Alterations in Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Arterial Spin Labeling Studies. *Front. Aging Neurosci.* **14**, 847218 (2022).

349. Weinstein, G. *et al.* Glucose indices are associated with cognitive and structural brain measures in young adults. *Neurology* **84**, 2329–2337 (2015).

350. Raffield, L. M. *et al.* Analysis of the relationships between type 2 diabetes status, glycemic control, and neuroimaging measures in the Diabetes Heart Study Mind. *Acta Diabetol* **53**, 439–447 (2016).

351. Van Agtmaal, M. J. M. *et al.* Prediabetes Is Associated With Structural Brain Abnormalities: The Maastricht Study. *Diabetes Care* **41**, 2535–2543 (2018).

352. Schneider, A. L. C. *et al.* Diabetes, Prediabetes, and Brain Volumes and Subclinical Cerebrovascular Disease on MRI: The Atherosclerosis Risk in Communities Neurocognitive Study (ARIC-NCS). *Diabetes Care* **40**, 1514–1521 (2017).

353. Bryan, R. N. *et al.* Effect of Diabetes on Brain Structure: The Action to Control Cardiovascular Risk in Diabetes MR Imaging Baseline Data. *Radiology* **272**, 210–216 (2014).

354. Rist, P. M., Buring, J. E., Rexrode, K. M., Cook, N. R. & Rost, N. S. Prospectively collected lifestyle and health information as risk factors for white matter hyperintensity volume in stroke patients. *Eur J Epidemiol* **34**, 957–965 (2019).

355. de Bresser, J. *et al.* White matter hyperintensity shape and location feature analysis on brain MRI; proof of principle study in patients with diabetes. *Sci Rep* **8**, 1893 (2018).

356. De Groot, M. *et al.* Tract-specific white matter degeneration in aging: The Rotterdam Study. *Alzheimer's & Dementia* **11**, 321–330 (2015).

357. Liang, M. *et al.* Diffusion tensor imaging of white matter in patients with prediabetes by trace-based spatial statistics. *Magnetic Resonance Imaging* **49**, 1105–1112 (2019).

358. Xiong, Y. *et al.* A Diffusion Tensor Imaging Study on White Matter Abnormalities in Patients with Type 2 Diabetes Using Tract-Based Spatial Statistics. *American Journal of Neuroradiology* **37**, 1462–1469 (2016).

359. Zhuo, Y. *et al.* White matter impairment in type 2 diabetes mellitus with and without microvascular disease. *NeuroImage: Clinical* **24**, 101945 (2019).

360. Alotaibi, A. *et al.* White Matter Microstructural Alteration in Type 2 Diabetes: A Combined UK Biobank Study of Diffusion Tensor Imaging and Neurite Orientation Dispersion and Density Imaging. Preprint at https://doi.org/10.1101/2022.07.09.22277453 (2022).
361. Zhang, T., Shaw, M. & Cherbuin, N. Association between Type 2 Diabetes Mellitus and Brain Atrophy: A Meta-Analysis. *Diabetes Metab J* **46**, 781–802 (2022).
362. Roberts, R. O. *et al.* Association of type 2 diabetes with brain atrophy and cognitive impairment. *Neurology* **82**, 1132–1141 (2014).
363. Monereo-Sánchez, J. *et al.* The association of prediabetes and type 2 diabetes with hippocampal subfields volume: The Maastricht study. *NeuroImage: Clinical* **39**, 103455 (2023).
364. Garfield, V. *et al.* HbA1c and brain health across the entire glycaemic spectrum. *Diabetes Obes Metab* **23**, 1140–1149 (2021).
365. Ranglani, S., Ward, J., Sattar, N., Strawbridge, R. J. & Lyall, D. M. Testing for associations between HbA1c levels, polygenic risk and brain health in UK Biobank (N = 39 283). *Diabetes Obes Metab* (2023) doi:10.1111/dom.15207.
366. Kooistra, M. *et al.* Diabetes mellitus and progression of vascular brain lesions and brain atrophy in patients with symptomatic atherosclerotic disease. The SMART-MR study. *Journal of the Neurological Sciences* **332**, 69–74 (2013).
367. Van Elderen, S. G. C. *et al.* Progression of brain atrophy and cognitive decline in diabetes mellitus: A 3-year follow-up. *Neurology* **75**, 997–1002 (2010).
368. De Bresser, J. *et al.* Microvascular Determinants of Cognitive Decline and Brain Volume Change in Elderly Patients with Type 2 Diabetes. *Dement Geriatr Cogn Disord* **30**, 381–386 (2010).

369. Biessels, G. J., Deary, I. J. & Ryan, C. M. Cognition and diabetes: a lifespan perspective. *The Lancet Neurology* **7**, 184–190 (2008).

370. Reitz, C. *et al.* Relation of Dysglycemia to Structural Brain Changes in a Multiethnic Elderly Cohort. *J American Geriatrics Society* **65**, 277–285 (2017).

371. Ho, L. *et al.* Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. *FASEB j.* **18**, 902–904 (2004).

372. Toppala, S. *et al.* Association of Early β-Amyloid Accumulation and Neuroinflammation Measured With [^{11} C]PBR28 in Elderly Individuals Without Dementia. *Neurology* **96**, (2021).

373. Ekblad, L. L. *et al.* Midlife insulin resistance, APOE genotype, and late-life brain amyloid accumulation. *Neurology* **90**, e1150–e1157 (2018).

374. Su, Y. *et al.* Comparison of Pittsburgh compound B and florbetapir in cross-sectional and longitudinal studies. *Alz & Dem Diag Ass & Dis Mo* **11**, 180–190 (2019).

375. Rajan, K. B. *et al.* Cognitive decline following incident and preexisting diabetes mellitus in a population sample. *Neurology* **87**, 1681–1687 (2016).

376. Mayeda, E. R., Whitmer, R. A. & Yaffe, K. Diabetes and Cognition. *Clinics in Geriatric Medicine* **31**, 101–115 (2015).

377. Cukierman-Yaffe, T. *et al.* Effects of basal insulin glargine and omega-3 fatty acid on cognitive decline and probable cognitive impairment in people with dysglycaemia: a substudy of the ORIGIN trial. *The Lancet Diabetes & Endocrinology* **2**, 562–572 (2014).

378. Christman, A. L., Selvin, E., Margolis, D. J., Lazarus, G. S. & Garza, L. A. Hemoglobin A1c predicts healing rate in diabetic wounds. *J Invest Dermatol* **131**, 2121–2127 (2011).

379. Saczynski, J. S. *et al.* Cognitive Impairment: An Increasingly Important Complication of Type 2 Diabetes: The Age, Gene/Environment Susceptibility-Reykjavik Study. *American Journal of Epidemiology* **168**, 1132–1139 (2008).

380. Palta, P. *et al.* Diabetes and Cognitive Decline in Older Adults: The Ginkgo Evaluation of Memory Study. *The Journals of Gerontology: Series A* **73**, 123–130 (2018).

381. Okereke, O. I. *et al.* Type 2 Diabetes Mellitus and Cognitive Decline in Two Large Cohorts of Community-Dwelling Older Adults. *J American Geriatrics Society* **56**, 1028–1036 (2008).

382. Sano, M., Zhu, C. W., Grossman, H. & Schimming, C. Longitudinal Cognitive Profiles in Diabetes: Results From the National Alzheimer's Coordinating Center's Uniform Data. *J American Geriatrics Society* **65**, 2198–2204 (2017).

383. Arvanitakis, Z., Tatavarthy, M. & Bennett, D. A. The Relation of Diabetes to Memory Function. *Curr Neurol Neurosci Rep* **20**, 64 (2020).

384. Wu, J. H. *et al.* Impact of diabetes on cognitive function among older Latinos. *Journal of Clinical Epidemiology* **56**, 686–693 (2003).

385. Van Den Berg, E., De Craen, A. J. M., Biessels, G. J., Gussekloo, J. & Westendorp, R. G. J. The impact of diabetes mellitus on cognitive decline in the oldest of the old: a prospective population-based study. *Diabetologia* **49**, 2015–2023 (2006).

386. Tuligenga, R. H. *et al.* Midlife type 2 diabetes and poor glycaemic control as risk factors for cognitive decline in early old age: a post-hoc analysis of the Whitehall II cohort study. *The Lancet Diabetes & Endocrinology* **2**, 228–235 (2014).

387. Demakakos, P., Muniz-Terrera, G. & Nouwen, A. Type 2 diabetes, depressive symptoms and trajectories of cognitive decline in a national sample of community-dwellers: A prospective cohort study. *PLoS ONE* **12**, e0175827 (2017).
388. Rawlings, A. M. *et al.* Diabetes in Midlife and Cognitive Change Over 20 Years: A Cohort Study. *Ann Intern Med* **161**, 785 (2014).
389. Spauwen, P. J. J., Köhler, S., Verhey, F. R. J., Stehouwer, C. D. A. & Van Boxtel, M. P. J. Effects of Type 2 Diabetes on 12-Year Cognitive Change. *Diabetes Care* **36**, 1554–1561 (2013).
390. Kuh, D. *et al.* Cohort Profile: Updating the cohort profile for the MRC National Survey of Health and Development: a new clinic-based data collection for ageing research. *International Journal of Epidemiology* **40**, e1–e9 (2011).
391. Wadsworth, M., Kuh, D., Richards, M. & Hardy, R. Cohort Profile: The 1946 National Birth Cohort (MRC National Survey of Health and Development). *International Journal of Epidemiology* **35**, 49–54 (2006).
392. Kuh, D. *et al.* The MRC National Survey of Health and Development reaches age 70: maintaining participation at older ages in a birth cohort study. *Eur J Epidemiol* **31**, 1135–1147 (2016).
393. Stafford, M. *et al.* Using a birth cohort to study ageing: representativeness and response rates in the National Survey of Health and Development. *Eur J Ageing* **10**, 145–157 (2013).
394. James, S.-N. *et al.* Using a birth cohort to study brain health and preclinical dementia: recruitment and participation rates in Insight 46. *BMC Res Notes* **11**, 885 (2018).

395. Cardoso, M. J. *et al.* Geodesic Information Flows: Spatially-Variant Graphs and Their Application to Segmentation and Fusion. *IEEE Trans Med Imaging* **34**, 1976–1988 (2015).

396. Eshaghi, A. *et al.* Applying causal models to explore the mechanism of action of simvastatin in progressive multiple sclerosis. *Proc Natl Acad Sci U S A* **116**, 11020–11027 (2019).

397. Leung, K. K. *et al.* Brain MAPS: an automated, accurate and robust brain extraction technique using a template library. *Neuroimage* **55**, 1091–1108 (2011).

398. Jorge Cardoso, M. *et al.* STEPS: Similarity and Truth Estimation for Propagated Segmentations and its application to hippocampal segmentation and brain parcelation. *Medical Image Analysis* **17**, 671–684 (2013).

399. Coath, W. *et al.* Operationalising the Centiloid Scale for [^{18} F]florbetapir PET Studies on PET/MR. Preprint at https://doi.org/10.1101/2022.02.11.22270590 (2022).

400. Sudlow, C. *et al.* UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLoS Med* **12**, e1001779 (2015).

401. Hodel, A. S. *et al.* Duration of early adversity and structural brain development in post-institutionalized adolescents. *NeuroImage* **105**, 112–119 (2015).

402. Rannikmäe, K. *et al.* Accuracy of identifying incident stroke cases from linked health care data in UK Biobank. *Neurology* **95**, (2020).

403. Ebrahim, S. & Davey Smith, G. Commentary: Should we always deliberately be non-representative? *International Journal of Epidemiology* **42**, 1022–1026 (2013).

404. Littlejohns, T. J. *et al.* The UK Biobank imaging enhancement of 100,000 participants: rationale, data collection, management and future directions. *Nat Commun* **11**, 2624 (2020).

405. Paz, V., Dashti, H. S. & Garfield, V. Is there an association between daytime napping, cognitive function, and brain volume? A Mendelian randomization study in the UK Biobank. *Sleep Health* **9**, 786–793 (2023).

406. Lyall, D. M. *et al.* Cognitive Test Scores in UK Biobank: Data Reduction in 480,416 Participants and Longitudinal Stability in 20,346 Participants. *PLoS ONE* **11**, e0154222 (2016).

407. Fatih, N. *et al.* Sex-related differences in whole brain volumes at age 70 in association with hyperglycemia during adult life. *Neurobiology of Aging* **112**, 161–169 (2022).

408. Zhang, Y. *et al.* Metformin and the risk of neurodegenerative diseases in patients with diabetes: A meta-analysis of population-based cohort studies. *Diabet Med* **39**, e14821 (2022).

409. Geijselaers, S. L. C., Sep, S. J. S., Stehouwer, C. D. A. & Biessels, G. J. Glucose regulation, cognition, and brain MRI in type 2 diabetes: a systematic review. *The Lancet Diabetes & Endocrinology* **3**, 75–89 (2015).

410. Mortby, M. E., Janke, A. L., Anstey, K. J., Sachdev, P. S. & Cherbuin, N. High “Normal” Blood Glucose Is Associated with Decreased Brain Volume and Cognitive Performance in the 60s: The PATH through Life Study. *PLoS ONE* **8**, e73697 (2013).

411. Xu, W. *et al.* Mid- and Late-Life Diabetes in Relation to the Risk of Dementia. *Diabetes* **58**, 71–77 (2009).

412. Reynolds, E. L. *et al.* Association between brain health outcomes and metabolic risk factors in persons with diabetes. *Ann Clin Transl Neurol* **10**, 1891–1898 (2023).
413. Hempel, R., Onopa, R. & Convit, A. Type 2 diabetes affects hippocampus volume differentially in men and women. *Diabetes Metab. Res. Rev.* **28**, 76–83 (2012).
414. De Ritter, R. *et al.* Sex differences in the risk of vascular disease associated with diabetes. *Biol Sex Differ* **11**, 1 (2020).
415. Donohue, M. C. *et al.* The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. *JAMA Neurol* **71**, 961–970 (2014).
416. Lu, K. *et al.* Cognition at age 70: Life course predictors and associations with brain pathologies. *Neurology* **93**, (2019).
417. Wechsler, D. WAIS-R: Manual: Wechsler adult intelligence scale-revised. *(No Title)* (1981).
418. Wechsler, D. *WMS-R: Wechsler Memory Scale--Revised : Manual*. (Psychological Corporation, 1987).
419. Papp, K. V. *et al.* Development of a Psychometrically Equivalent Short Form of the Face–Name Associative Memory Exam for use Along the Early Alzheimer's Disease Trajectory. *The Clinical Neuropsychologist* **28**, 771–785 (2014).
420. Blackwell, A. D. *et al.* Detecting dementia: novel neuropsychological markers of preclinical Alzheimer's disease. *Dement Geriatr Cogn Disord* **17**, 42–48 (2004).
421. Orchard, T. J., Forrest, K. Y., Ellis, D. & Becker, D. J. Cumulative glycemic exposure and microvascular complications in insulin-dependent diabetes mellitus. The glycemic threshold revisited. *Arch Intern Med* **157**, 1851–1856 (1997).

422. Pastorino, S. *et al.* Validation of self-reported diagnosis of diabetes in the 1946 British birth cohort. *Prim Care Diabetes* **9**, 397–400 (2015).
423. Pigeon, D. Tests used in the 1954 and 1957 surveys. *The home and the school* 129–132 (1964).
424. Pigeon, D., Douglas, J., Ross, J. & Simpson, H. All our future. (1968).
425. Heim, A. The AH4 group test of intelligence. *Windsor: NFER-Nelson* **758**, (1970).
426. Garnier-Crussard, A. *et al.* White matter hyperintensities across the adult lifespan: relation to age, Aβ load, and cognition. *Alzheimer's Research & Therapy* **12**, 127 (2020).
427. Lane, C. A. *et al.* Associations between blood pressure across adulthood and late-life brain structure and pathology in the neuroscience substudy of the 1946 British birth cohort (Insight 46): an epidemiological study. *The Lancet Neurology* **18**, 942–952 (2019).
428. Chaudhury, A. *et al.* Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. *Front. Endocrinol.* **8**, (2017).
429. Thomas, E. G. *et al.* Sex-Specific Associations of Diabetes With Brain Structure and Function in a Geriatric Population. *Front Aging Neurosci* **14**, 885787 (2022).
430. Reynolds, E. L. *et al.* Association between brain health outcomes and metabolic risk factors in persons with diabetes. *Ann Clin Transl Neurol* **10**, 1891–1898 (2023).
431. Mosconi, L. *et al.* Menopause impacts human brain structure, connectivity, energy metabolism, and amyloid-beta deposition. *Sci Rep* **11**, 10867 (2021).
432. Russell, J. K., Jones, C. K. & Newhouse, P. A. The Role of Estrogen in Brain and Cognitive Aging. *Neurotherapeutics* **16**, 649–665 (2019).

433. Hu, F. B., Meigs, J. B., Li, T. Y., Rifai, N. & Manson, J. E. Inflammatory Markers and Risk of Developing Type 2 Diabetes in Women. *Diabetes* **53**, 693–700 (2004).

434. Cartier, A. *et al.* Sex differences in inflammatory markers: what is the contribution of visceral adiposity? *The American Journal of Clinical Nutrition* **89**, 1307–1314 (2009).

435. Taki, Y. *et al.* Correlation between high-sensitivity C-reactive protein and brain gray matter volume in healthy elderly subjects. *Hum Brain Mapp* **34**, 2418–2424 (2013).

436. Marsland, A. L., Gianaros, P. J., Abramowitch, S. M., Manuck, S. B. & Hariri, A. R. Interleukin-6 Covaries Inversely with Hippocampal Grey Matter Volume in Middle-Aged Adults. *Biological Psychiatry* **64**, 484–490 (2008).

437. Gupta, M., Pandey, S., Rumman, M., Singh, B. & Mahdi, A. A. Molecular mechanisms underlying hyperglycemia associated cognitive decline. *IBRO Neuroscience Reports* **14**, 57–63 (2023).

438. Sims-Robinson, C., Kim, B., Rosko, A. & Feldman, E. L. How does diabetes accelerate Alzheimer disease pathology? *Nat Rev Neurol* **6**, 551–559 (2010).

439. Namsrai, T., Ambikairajah, A. & Cherbuin, N. Poorer sleep impairs brain health at midlife. *Sci Rep* **13**, 1874 (2023).

440. Bryan, R. N. *et al.* Effect of diabetes on brain structure: the action to control cardiovascular risk in diabetes MR imaging baseline data. *Radiology* **272**, 210–216 (2014).

441. Last, D. *et al.* Global and Regional Effects of Type 2 Diabetes on Brain Tissue Volumes and Cerebral Vasoreactivity. *Diabetes Care* **30**, 1193–1199 (2007).

442. Moran, C. *et al.* Brain Atrophy in Type 2 Diabetes. *Diabetes Care* **36**, 4036–4042 (2013).

443. van Wijngaarden, R. P. T. *et al.* Relation Between Different Measures of Glycemic Exposure and Microvascular and Macrovascular Complications in Patients with Type 2 Diabetes Mellitus: An Observational Cohort Study. *Diabetes Ther* **8**, 1097–1109 (2017).

444. Christman, A. L. *et al.* Glycated haemoglobin and cognitive decline: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetologia* **54**, 1645–1652 (2011).

445. van Harten, B. *et al.* Cognitive impairment and MRI correlates in the elderly patients with type 2 diabetes mellitus. *Age Ageing* **36**, 164–170 (2007).

446. Cosway, R., Strachan, M. W., Dougall, A., Frier, B. M. & Deary, I. J. Cognitive function and information processing in type 2 diabetes. *Diabet Med* **18**, 803–810 (2001).

447. Teixeira, M. M. *et al.* Association between diabetes and cognitive function at baseline in the Brazilian Longitudinal Study of Adult Health (ELSA- Brasil). *Sci Rep* **10**, 1596 (2020).

448. Pettigrew, C. & Soldan, A. Defining Cognitive Reserve and Implications for Cognitive Aging. *Curr Neurol Neurosci Rep* **19**, 1 (2019).

449. Li, H., Yu, J. & Guo, S. Sex Differences in the Association between Hemoglobin A1c and Cerebral White Matter Lesions in the General Japanese Population. *Acta Neurologica Scandinavica* **2023**, 1–10 (2023).

450. Mehta, K. M. *et al.* Race/ethnic differences in AD survival in US Alzheimer's Disease Centers. *Neurology* **70**, 1163–1170 (2008).

451. Tillin, T., Forouhi, N. G., McKeigue, P. M. & Chaturvedi, N. Southall And Brent REvisited: Cohort profile of SABRE, a UK population-based comparison of cardiovascular disease and diabetes in people of European, Indian Asian and African Caribbean origins. *Int. J. Epidemiol.* **41**, 33–42 (2012).
452. Espeland, M. A. *et al.* Influence of type 2 diabetes on brain volumes and changes in brain volumes: results from the Women's Health Initiative Magnetic Resonance Imaging studies. *Diabetes Care* **36**, 90–97 (2013).
453. Simonis-Bik, A. M. C. *et al.* The Heritability of HbA1c and Fasting Blood Glucose in Different Measurement Settings. *Twin Res Hum Genet* **11**, 597–602 (2008).
454. Lee, S.-H., Zabolotny, J. M., Huang, H., Lee, H. & Kim, Y.-B. Insulin in the nervous system and the mind: Functions in metabolism, memory, and mood. *Molecular Metabolism* **5**, 589–601 (2016).
455. Sato, K., Takayama, K. & Inoue, S. Expression and function of estrogen receptors and estrogen-related receptors in the brain and their association with Alzheimer's disease. *Front. Endocrinol.* **14**, 1220150 (2023).
456. Yamashiro, K. *et al.* Visceral fat accumulation is associated with cerebral small vessel disease. *Euro J of Neurology* **21**, 667–673 (2014).
457. The Lancet Diabetes & Endocrinology. Sex disparities in diabetes: bridging the gap. *The Lancet Diabetes & Endocrinology* **5**, 839 (2017).
458. Sikaris, K. The Correlation of Hemoglobin A1c to Blood Glucose. *J Diabetes Sci Technol* **3**, 429–438 (2009).
459. Ho-Pham, L. T., Nguyen, U. D. T., Tran, T. X. & Nguyen, T. V. Discordance in the diagnosis of diabetes: Comparison between HbA1c and fasting plasma glucose. *PLoS ONE* **12**, e0182192 (2017).

460. Modan, M. *et al.* Significance of High HbA1 Levels in Normal Glucose Tolerance. *Diabetes Care* **11**, 422–428 (1988).
461. Cohen, R. M. & Smith, E. P. Frequency of HbA1c discordance in estimating blood glucose control: *Current Opinion in Clinical Nutrition and Metabolic Care* **11**, 512–517 (2008).
462. Matthews, D. R. *et al.* Homeostasis model assessment: insulin resistance and ?-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412–419 (1985).
463. Weir, G. C. & Bonner-Weir, S. Five stages of evolving beta-cell dysfunction during progression to diabetes. *Diabetes* **53 Suppl 3**, S16-21 (2004).
464. Walsh, E. I., Shaw, M., Sachdev, P., Anstey, K. J. & Cherbuin, N. The impact of type 2 diabetes and body mass index on cerebral structure is modulated by brain reserve. *Eur J Neurol* **26**, 121–127 (2019).
465. Cavero-Redondo, I., Peleteiro, B., Álvarez-Bueno, C., Rodriguez-Artalejo, F. & Martínez-Vizcaíno, V. Glycated haemoglobin A1c as a risk factor of cardiovascular outcomes and all-cause mortality in diabetic and non-diabetic populations: a systematic review and meta-analysis. *BMJ Open* **7**, e015949 (2017).
466. Qiao, Y.-C. *et al.* Changes of Regulatory T Cells and of Proinflammatory and Immunosuppressive Cytokines in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. *J Diabetes Res* **2016**, 3694957 (2016).
467. Cioffi, M. *et al.* Cytokine pattern in postmenopause. *Maturitas* **41**, 187–192 (2002).
468. Su, H. I. & Freeman, E. W. Hormone changes associated with the menopausal transition. *Minerva Ginecol* **61**, 483–489 (2009).

469. Coales, I. *et al.* Alzheimer's disease-related transcriptional sex differences in myeloid cells. *J Neuroinflammation* **19**, 247 (2022).

470. Soininen, P., Kangas, A. J., Würtz, P., Suna, T. & Ala-Korpela, M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. *Circ Cardiovasc Genet* **8**, 192–206 (2015).

471. Hatch, S. L., Mishra, G., Hotopf, M., Jones, P. B. & Kuh, D. Appraisals of stressors and common mental disorder from early to mid-adulthood in the 1946 British birth cohort. *Journal of Affective Disorders* **119**, 66–75 (2009).

472. Richardson, M. T., Leon, A. S., Jacobs, D. R., Ainsworth, B. E. & Serfass, R. Comprehensive evaluation of the Minnesota leisure time physical activity questionnaire. *Journal of Clinical Epidemiology* **47**, 271–281 (1994).

473. Black, S. V. *et al.* Physical Activity and Mental Well-being in a Cohort Aged 60–64 Years. *American Journal of Preventive Medicine* **49**, 172–180 (2015).

474. on behalf of the ProActive study group *et al.* Physical activity, sedentary time and gain in overall and central body fat: 7-year follow-up of the ProActive trial cohort. *Int J Obes* **39**, 142–148 (2015).

475. Torén, K. *et al.* The ratio FEV $_1$ /FVC and its association to respiratory symptoms—A Swedish general population study. *Clin Physio Funct Imaging* **41**, 181–191 (2021).

476. VanderWeele, T. J. Subtleties of explanatory language: what is meant by "mediation"? *Eur J Epidemiol* **26**, 343–346 (2011).

477. VanderWeele, T. J. A Three-way Decomposition of a Total Effect into Direct, Indirect, and Interactive Effects: *Epidemiology* **24**, 224–232 (2013).

478. Hernán, M. A. The C-Word: Scientific Euphemisms Do Not Improve Causal Inference From Observational Data. *Am J Public Health* **108**, 616–619 (2018).

479. Cole, S. R. & Hernán, M. A. Fallibility in estimating direct effects. *International Journal of Epidemiology* **31**, 163–165 (2002).
480. Liu, S. A Prospective Study of Inflammatory Cytokines and Diabetes Mellitus in a Multiethnic Cohort of Postmenopausal Women. *Arch Intern Med* **167**, 1676 (2007).
481. Gautam, D., Adhikari, S., Thapa, R. & Kharel, L. Study to determine between HbA1C and C-reactive protein in Diabetes mellitus. *J Pathol Nep* **13**, 1979–1982 (2023).
482. Shukla, V., Shakya, A. K., Perez-Pinzon, M. A. & Dave, K. R. Cerebral ischemic damage in diabetes: an inflammatory perspective. *J Neuroinflammation* **14**, 21 (2017).
483. Sproston, N. R. & Ashworth, J. J. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front. Immunol.* **9**, 754 (2018).
484. Boras, E. *et al.* Monomeric C-reactive protein and Notch-3 co-operatively increase angiogenesis through PI3K signalling pathway. *Cytokine* **69**, 165–179 (2014).
485. Bermudez, E. A., Rifai, N., Buring, J., Manson, J. E. & Ridker, P. M. Interrelationships Among Circulating Interleukin-6, C-Reactive Protein, and Traditional Cardiovascular Risk Factors in Women. *ATVB* **22**, 1668–1673 (2002).
486. Di Angelantonio, E. *et al.* Glycated Hemoglobin Measurement and Prediction of Cardiovascular Disease. *JAMA* **311**, 1225 (2014).
487. Anna Tierney, Fry, D., Almond, R., Gordon, M. & Moffat, S. UK Biobank Biomarker Enhancement Project Companion Document to Accompany HbA1c Biomarker Data.

488. Royston, P. & Altman, D. G. Regression Using Fractional Polynomials of Continuous Covariates: Parsimonious Parametric Modelling. *Applied Statistics* **43**, 429 (1994).
489. Royston, P. Model Selection for Univariable Fractional Polynomials. *The Stata Journal* **17**, 619–629 (2017).
490. O'Hara, R. B. & Kotze, D. J. Do not log-transform count data. *Methods Ecol Evol* **1**, 118–122 (2010).
491. Li, C. *et al.* Visit-to-visit HbA1c variability, dementia, and hippocampal atrophy among adults without diabetes. *Experimental Gerontology* **178**, 112225 (2023).
492. Lassmann, H. & Van Horssen, J. Oxidative stress and its impact on neurons and glia in multiple sclerosis lesions. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1862**, 506–510 (2016).
493. D, A. & Ej, R. How hyperglycemia promotes atherosclerosis: molecular mechanisms. *Cardiovascular diabetology* **1**, (2002).
494. Fatemi, F. *et al.* Sex differences in cerebrovascular pathologies on FLAIR in cognitively unimpaired elderly. *Neurology* **90**, (2018).
495. Bree, A. J., Puente, E. C., Daphna-Iken, D. & Fisher, S. J. Diabetes increases brain damage caused by severe hypoglycemia. *American Journal of Physiology-Endocrinology and Metabolism* **297**, E194–E201 (2009).
496. Won, S. J. *et al.* Prevention of hypoglycemia-induced neuronal death by minocycline. *J Neuroinflammation* **9**, 225 (2012).
497. Suh, S. W., Hamby, A. M. & Swanson, R. A. Hypoglycemia, brain energetics, and hypoglycemic neuronal death. *Glia* **55**, 1280–1286 (2007).

498. Frangou, S. *et al.* Insulin resistance: Genetic associations with depression and cognition in population based cohorts. *Experimental Neurology* **316**, 20–26 (2019).
499. Dybjer, E. *et al.* Polygenic risk of type 2 diabetes is associated with incident vascular dementia: a prospective cohort study. *Brain Commun* **5**, fcad054 (2023).
500. Young, K. G. *et al.* The impact of population-level HbA1c screening on reducing diabetes diagnostic delay in middle-aged adults: a UK Biobank analysis. *Diabetologia* **66**, 300–309 (2023).
501. Aschard, H., Vilhjálmsson, B. J., Joshi, A. D., Price, A. L. & Kraft, P. Adjusting for Heritable Covariates Can Bias Effect Estimates in Genome-Wide Association Studies. *The American Journal of Human Genetics* **96**, 329–339 (2015).
502. Garfield, V. *et al.* Relationship Between Glycemia and Cognitive Function, Structural Brain Outcomes, and Dementia: A Mendelian Randomization Study in the UK Biobank. *Diabetes* **70**, 2313–2321 (2021).
503. Price, A. L. *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* **38**, 904–909 (2006).
504. Henry, A. *et al.* The relationship between sleep duration, cognition and dementia: a Mendelian randomization study. *International Journal of Epidemiology* **48**, 849–860 (2019).
505. Bycroft, C. *et al.* The UK Biobank resource with deep phenotyping and genomic data. *Nature* **562**, 203–209 (2018).
506. Chen, J. *et al.* The trans-ancestral genomic architecture of glycemic traits. *Nat Genet* **53**, 840–860 (2021).
507. Choi, S. W., Mak, T. S.-H. & O'Reilly, P. F. Tutorial: a guide to performing polygenic risk score analyses. *Nat Protoc* **15**, 2759–2772 (2020).

508. Ginde, A. A., Cagliero, E., Nathan, D. M. & Camargo, C. A. Value of Risk Stratification to Increase the Predictive Validity of HbA1c in Screening for Undiagnosed Diabetes in the US Population. *J GEN INTERN MED* **23**, 1346–1353 (2008).
509. Garfield, V., Salzmann, A., Burgess, S. & Chaturvedi, N. A Guide for Selection of Genetic Instruments in Mendelian Randomization Studies of Type 2 Diabetes and HbA1c: Toward an Integrated Approach. *Diabetes* **72**, 175–183 (2023).
510. Wade, K. H. *et al.* Challenges in undertaking nonlinear Mendelian randomization. *Obesity* **31**, 2887–2890 (2023).