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An Exploration of Cardiovascular Risk Factors in People with Down Syndrome and Their Association with Dementia Onset and Blood-based Biomarkers of Alzheimer's Disease

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King's College London

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Title Page

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Aslam, A.; Baksh, R.A.; **Pape, S.E.**; Go-DS21 Consortium; Strydom, A.; Gulliford, M.C.; Chan, L.F.; Diabetes and Obesity in Down Syndrome Across the Lifespan: a Retrospective Cohort Study Using UK Electronic Health Records, *Diabetes Care*, Sept 30 2022.

Carmona-Iragui, M.; Alcolea, D.; Barroeta, I.; Videla, L.; Muñoz, L.; Van Pelt, K.L.; Schmitt, F.A.; Lightner, D.D.; Koehl, L.M.; Jicha, G.; Sacco, S.; Mircher, C.; **Pape, S.E.**; Hithersay, R.; Clare, I.C.H.; Holland, A.J.; Nübling, G.; Levin, J.; Zaman, S.; Strydom, A.; Rebillat, A-S.; Head, E.; Blesa, R.; Lleó, A.; Fortea, J. Prognostic Performance and Longitudinal Changes in

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Additional Publications

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Baksh, R.A.; **Pape, S.E.**; Smith, J.; Strydom, A. Understanding Inequalities in COVID-19 Outcomes Following Hospital Admission for People with Intellectual Disability Compared to the General Population, *BMJ Open*, 11(10), Oct 4 2021.

List of Abbreviations

General Terms

AC	Abdominal Circumference
AD	Alzheimer's Disease
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AVSD	Atrioventricular Septal Defects
Aβ	Amyloid Beta
Aβ40	Amyloid Beta 40
Aβ42	Amyloid Beta 42
BBB	Blood Brain Barrier
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
BP	Blood Pressure
C1inh	C1 Esterase Inhibitor
C1q	Complement Component C1q
C3	Complement Component 3
C4	Complement Component 4
CAA	Cerebral Amyloid Angiopathy
CAMCOG-DS	Cambridge Cognitive Examination adapted for individuals with Down Syndrome
CAMDEX-DS	Cambridge Examination for Mental Disorders of Older People with Down Syndrome and Others with Intellectual Disabilities
CANTAB	Cambridge Neuropsychological Tests Automated Battery
CBF	Cerebral Blood Flow
CHD	Congenital Heart Disease

CNS	Central Nervous System
CPRD	Clinical Practice Research Datalink
CR1	Complement Receptor 1
CRP	C-reactive protein
CSF	Cerebral Spinal Fluid
CVA	Cerebrovascular Accident
CVP	Cardiovascular Health Parameter
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
DS	Down Syndrome
DS-AD	Alzheimer's Disease in Down Syndrome
DS_{Cn}	DS Cognitively Normal (no dementia present)
DS_{dem}	Incident Dementia
DSQIID	Dementia Screening Questionnaire for Individuals with Intellectual Disability
DTB	Dementia Test Battery
EDTA	Ethylenediaminetetraacetic acid
FH	Factor H
FI	Factor I
GFAP	Glial Fibrillary Acidic Protein
GP	General Practitioner
HbA1c	Glycated Haemoglobin
HDL	High Density Lipoprotein
HTN	Hypertension
ID	Intellectual Disability
IFN	Interferon

IFNAR	Interferon alpha/beta receptor
IHD	Ischaemic Heart Disease
IL	Interleukin
IMT	Intima-media Thickness
LDL	Low Density Lipoprotein
MCI	Mild Cognitive Impairment
MOT	Motor Screening Task
MRI	Magnetic Resonance Imaging
NfL	Neurofilament Light
NGF	Nerve Growth Factor
NMDA	N-methyl-D-aspartate
p-tau	Phosphorylated Tau
PA	Physical Activity
PALFAMS	Paired Associate Learning First Attempt Memory Score
PET	Positron Emission Tomography
PP	Pulse Pressure
PVD	Peripheral Vascular Disease
ROS	Reactive Oxygen Species
SABS	Short Adaptive Behaviour Scale
SBP	Systolic Blood Pressure
SIMOA	Single Molecule Array
SOD	Superoxide Dismutase
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol

Tg	Triglycerides
TNF	Tumour Necrosis Factor
tPA	Tissue plasminogen activator
TSH	Thyroid Stimulating Hormone
UTS	Up-to-Standard Time
VEGF	Vascular Endothelial Growth Factors
VF	Verbal Fluency
WMH	White Matter Hyperintensities

Statistical Terms

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AUC	Area Under the Curve
CI	Confidence Interval
CV	Coefficient of Variation
HR	Hazard Ratio
IQR	Interquartile Range
IRR	Incident Rate Ratio
PCA	Principal Component Analysis
RR	Relative Risk
SD	Standard Deviation
SE	Standard Error
SMOR	Standardised Mortality Rate

Genetic Terms

ADAR	Adenosine Deaminase RNA
AGTR1	Angiotensin II Receptor Type 1
AIRE	Autoimmune Regulator
APOE	Apolipoprotein E
APP	Amyloid Precursor Protein
BACE	β -site APP cleaving enzyme
CRELD1	Cysteine Rich With EGF Like Domains 1
DSCR	Down Syndrome Critical Region
DYRK	Dual-Specificity Tyrosine Phosphorylated-Regulated Kinase
HEY2	Hairy/enhancer-of-split related with YRPW motif protein 2
HSA17	Human Chromosome 17
HSA21	Human Chromosome 21
PRMT2	Protein Arginine Methyltransferase 2
PSEN	Presenilin
RCAN	Regulator of Calcineurin
S100B	S100 calcium-binding protein B
TIAM1	T-cell lymphoma invasion and metastasis-inducing protein 1

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Abstract

BACKGROUND

People with Down syndrome (DS) are at ultra-high risk of developing Alzheimer's dementia (AD) as they get older. This is primarily due to the triplication of the amyloid precursor protein gene (*APP*) on chromosome 21. Triplication of *APP* induces dysregulation of amyloid processing pathways, leading to increased lifelong over-production of amyloid-beta ($A\beta$) protein which aggregates to form plaques in cerebral tissues. $A\beta$ can also be deposited in blood vessel walls leading to cerebral amyloid angiopathy (CAA). Plaque formation is accompanied by the development of neurofibrillary tangles consisting of tau proteins, neuroinflammation, synaptic injury and tissue atrophy. Over-time this contributes to progressive development of the clinical symptoms of dementia, particularly short-term memory difficulties, decline in functional skills, loss of independence, and eventually death.

Despite people with DS sharing similar genetic risks for the development of AD, there are large variations in the age of onset. Whilst the current average age at diagnosis is 55 years, diagnosis before age 40 and over 65 years have been reported. In sporadic AD, poor cardiovascular health has been associated with increased risk of developing vascular dementia and AD, particularly when the risks are present in mid-life. It has not been established whether the same risks apply to people with DS. It was hypothesised that cardiovascular health and lifestyle factors may affect dementia onset and progression in people with DS, modulating the genetic risks and pattern of neuropathology. The aims of the study were: 1) To establish the cardiovascular profile in people with DS - including blood pressure, body mass index, lipid and glucose measurements - throughout adulthood and understand whether this differs from the general population; 2) To determine whether cardiovascular health in mid-life affects incidence of dementia in people with DS; 3) To explore the relationship between cardiovascular health

parameters with cognitive outcomes and plasma biomarkers as a way to explore potential mechanistic pathways which link cardiovascular health and dementia pathology; 4) To consider targets for monitoring and therapeutic interventions which may help to modify the risk of dementia incidence in people with DS.

METHODS

Two complementary approaches were used in a synergistic manner. These were a population-based cohort study utilising data from UK-based primary care electronic health records, and a deeply phenotyped cohort study which collected health, cognitive and biomarker data from adults with Down syndrome approximately every 2 years (the LonDowns cohort). Data were extracted from the Clinical Practice Research Datalink (CPRD) for all people with DS identified in the dataset, alongside controls matched on age, sex and GP practice on a ratio up to 4:1. The incidence rate ratios of common cardiovascular co-morbidities were calculated between people with DS and controls. Direct measurements of nine cardiovascular parameters (CVPs) were used to analyse patterns across adulthood. These were systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), body mass index (BMI), total cholesterol (TC), high and low density lipoproteins (HDL and LDL), total triglycerides (Tg), and glycated haemoglobin (HbA1c). Results were presented graphically using scatterplots. Regression models were constructed to examine the effects of covariates (age, sex, level of intellectual disability). Multivariate analyses were used to compare differences between five-year age bands. Following this, survival curves were fitted using mid-life cardiovascular parameters as the predictor variables and incidence of dementia as the outcome. Age, sex, level of intellectual disability, history of congenital heart disease (CHD), cardiovascular disease diagnoses, hypothyroidism and diabetes (both type 1 and 2) were included as covariates in survival models.

Health, cognitive and plasma biomarker data from the LonDowns cohort were used to explore interactions between CVPs, cognitive outcomes, and markers of amyloid-beta and neuropathology. The focus was on blood pressure, BMI and exercise levels given their relationship with dementia incidence or symptomatology in earlier analyses. The cognitive outcomes used were paired associate learning (PAL) and semantic verbal fluency (VF) tasks alongside the CAMDEX-DS and short adaptative behaviour scale (SABS) informant questionnaires. Established plasma biomarkers relating to AD-neuropathology were analysed: A β 40, A β 42, A β 42/40 ratio, neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and phosphorylated-tau181 (p-tau181). Eight complement components were additionally examined (C3, C4, C1inh, C1q, CR1, clusterin, FH and FI). Two-sided Pearson correlations were calculated between the measures before the sample was split into an older and younger (below age 35 years) cohort. Regression models were constructed to evaluate the relationship between CVPs (dependent factors) and cognitive and biomarker values (independent factors), with adjustments for age, sex, and level of intellectual disability. These were performed cross-sectionally, and longitudinally in a sub-group of participants who had additional data available from approximately five years earlier. Comparisons were made between people with and without AD using two-way independent t-tests and regression models. Finally, principal component analysis was performed to assess the relationships between factors. Significance was set at $p < 0.05$ throughout.

RESULTS

6,424 adults with DS were identified in the CPRD, matched to 23,143 controls. People with DS had a distinct cardiovascular health profile throughout adulthood compared to controls. They had significantly lower SBP, DBP and PP values. Minimal increases with age were

observed. People with DS had higher BMI, and this was particularly in younger adulthood. A decline in BMI was observed after age 40 years in people with DS, which was steeper than that observed in controls. Women with DS had higher mean BP and BMI than men, whereas in controls men had higher average BP and BMI values. TC, LDL and HDL patterns were similar in both groups, but the DS cohort had lower mean TC and HDL. Tg and HbA1c patterns were different. Tg was more homogenous across ages compared to controls, with lower values observed in older people with DS. Trends of HbA1c with age were similar in people with diabetes in both DS and controls, although the DS cohort had slightly higher mean HbA1c values suggestive of poorer glycaemic control. In people without diabetes there was a plateauing of HbA1c values with age in DS but not in controls, and mean HbA1c values were lower in DS than controls.

Survival analyses showed increased incidence of AD in people with low PP (<40mmHg), Hazard Ratio (HR) 1.45; low DBP (<62mmHg), HR 1.77, and low SBP (<100mmHg), HR 1.83. High SBP (>140mmHg), HR 2.04 also increased risk but there were few people who experienced these higher values. Raised BMI >25kg/m² increased incidence dementia risk (HR 1.63) and the risk was the same whether someone was overweight (25-30kg/m²) or obese (BMI >30kg/m²). Hypothyroidism, history of CHD, and male sex were significant contributors in the survival models. There was a cumulative effect of risk. People with 1 or 2 risk factors had HRs of 3.15 compared to those with zero risks, whilst people with 3 or more cardiovascular risk factors had HRs of 5.69.

In the LonDowns cohort (*n*=92), NfL, p-tau181 and GFAP were negatively correlated with cognitive outcomes and positively correlated with dementia symptom burden. In the older cohort BMI was negatively related to GFAP and NfL, and positively with PAL scores. People

engaged in higher levels of exercise had lower CAMDEX-DS scores indicating less dementia symptomatology. Low SBP was associated with higher levels of NfL. People with greater numbers of comorbidities had higher NfL and GFAP levels, with lower A β 42/A β 40 ratios. This was alongside higher CAMDEX-DS scores and poorer PAL performance. In the younger cohort SBP was negatively associated with NfL and GFAP levels. PP was positively associated with p-tau181. People with increased numbers of cardiovascular co-morbidities had higher NfL and lower A β 40 levels. At a whole group level, BMI was positively associated with C3, FH and FI. People with dementia had higher GFAP, NfL, p-tau181, and C1q levels, with lower FI, clusterin and C4. In principal component analyses, NfL, GFAP and p-tau181 corresponded with CAMDEX-DS scores, and were good predictors of dementia status. Blood pressure measures grouped with A β measures but not NfL, GFAP or p-tau181. BMI did not correspond with the other CVPs, but was related to complement components and A β 42/40 ratio.

DISCUSSION

People with DS have a distinct cardiovascular profile and this is present throughout adulthood. Blood pressure, BMI, and glucose homeostasis appear to be particularly affected. Additional interactions were observed on lipid levels, with lower HDL and TC levels in people with DS. These differences may be attributable to intrinsic autonomic dysfunction, direct and downstream genetic effects, atherosclerotic mechanisms, alterations in metabolic pathways, pro-inflammatory states, or social and environmental factors. Due to the complex and variable nature of the DS phenotype, it is likely that interactions between numerous processes contribute to the cardiovascular profile observed.

The increased risk of incidence dementia related to low blood pressures was hypothesised to be secondary to chronic hypoperfusion, with consequential cerebrovascular compromise,

dysregulation of amyloid processing and clearance, ischaemia, neuroinflammation and neuronal injury. This was supported by the findings of the LonDowns study. In younger people low SBP was associated with higher levels of GFAP. GFAP is produced by astrocytes and is a marker of reactive gliosis. Increased levels have been observed following ischaemic events and traumatic brain injury. Similarly, low PP was associated with low levels of p-tau181. Disruption of tau processing pathways and reductions in p-tau have also been observed following cerebral hypoperfusion. Over-time this could lead to increased A β deposition and reduced clearance, alongside increased neuroinflammation which promotes further A β aggregation and neuropathology. This was evident in longitudinal analyses in the older adult cohort where lower SBP was associated with higher NfL values five years later.

Being overweight or obese was related to increased markers of inflammatory dysregulation measured via complement components. Increased incidence dementia risk may be related to the creation of a pro-inflammatory state which drives earlier progression of neuropathology. Raised BMI could also increase the risk of atherosclerotic or other cholesterol-mediated risks, despite the low incidence of these conditions in people with DS. In this way, BMI would increase the relative risk of cerebrovascular disease compared to other people with DS, which could potentiate AD pathology.

Cardiovascular multimorbidity was associated with increased incidence dementia risk and raised markers of neurodegeneration (NfL and GFAP) suggesting that risks may interact in a cumulative fashion. Reducing the number of risks by actively screening and managing medical co-morbidities in people with DS may therefore help delay the onset of AD. Multi-domain interventions may be particularly important. The results suggest a number of therapeutic targets and risk indicators, particularly low SBP and PP, and raised BMI. These should be routinely

measured and recorded in people with DS. Observational and interventional studies to understand the relationship between blood pressure, cerebral perfusion, and neuropathology staging will help to establish mechanisms underpinning the risks observed, and may identify new avenues for therapeutic interventions.

KEYWORDS: Down syndrome; Alzheimer's disease; plasma biomarkers; blood pressure; obesity; cardiovascular health.

Chapter I

Introduction and Background

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1.1. Thesis Overview

Cardiovascular health is affected by body weight, blood pressure, abnormalities in cholesterol and lipid pathways, comorbidities including diabetes mellitus, and lifestyle factors such as exercise, alcohol intake and tobacco smoking. Poor cardiovascular health is widely acknowledged as a leading cause of morbidity and mortality worldwide. It is implicated in almost all major life-limiting illness: coronary heart disease and myocardial infarction, stroke and cerebrovascular disease, depression, diabetes, kidney disease, respiratory illness, peripheral artery disease, and even cancers¹⁻⁶. Increased risk of dementia, encompassing both vascular and Alzheimer's disease, has also been explicitly associated with poor cardiovascular health in middle-age⁷⁻¹¹.

People with Down syndrome (DS) have an ultra-high risk of developing Alzheimer's disease (AD) as they get older, with estimated risks of 85% in those over 65 years¹²⁻¹⁴. Rates of obesity and diabetes are more commonly observed, especially in younger people with DS¹⁵⁻¹⁸. Despite this combination of factors, current research about the impact of cardiovascular health on dementia risk in people with Down syndrome is limited. Understanding how cardiovascular health affects dementia risk is vital for designing targeted interventions to delay dementia onset, and improve mortality and morbidity in people with Down syndrome, particularly whilst there is no existing curative treatment for AD. Investigating the relationship between cardiovascular risks and Alzheimer's pathology in people with Down syndrome also provides a unique opportunity to quantify risks in the context of what is considered to be a relatively pure amyloid-driven form of Alzheimer's disease. This may help to understand cardiovascular risks specific to AD and differentiate risks from those contributing to mixed AD-vascular pathologies that often arise in the general population.

This study strived to define cardiovascular profiles in people with DS to provide context for the development of cardiovascular disease and related risks. The relationships between cardiovascular health parameters (CVPs) and incident dementia risk were then determined. Firstly, population data was used to define cardiovascular profiles and establish hazard ratios for incident dementia related to mid-life cardiovascular health measures. This was replicated using data from a prospective cohort study with detailed phenotyping which allowed for quantification of the relationships between cardiovascular health and cognitive outcomes. Finally, cardiovascular risk factors were related to plasma biomarkers of AD-neuropathology to explore possible mechanisms of action. The inclusion of blood-based biomarkers may also help to identify objective measures that can be used to estimate risk and track the effects of interventions. The goal was to enhance our understanding of the impact of poor cardiovascular health on dementia onset in people with DS, and identify potential targets for risk-reduction interventions as well as measures that could be used to track them. Improving knowledge in this area will also help to quantify health-related risks to allow people with DS to make more informed decisions about their health.

1.2. Down Syndrome and Alzheimer's Disease

1.2.1. Down Syndrome Overview

1.2.1.1. Epidemiological and Clinical Features

Down syndrome is a genetic condition that affects between 1 in 800 to 1 in 1000 live births^{12,19,20}. In the UK there are an estimated 41,700 people with DS²¹. In the majority of cases DS is caused by complete triplication of chromosome 21 and is the most commonly occurring

aneuploidy in humans^{22,23}. In a small proportion of people (2-4%) Down syndrome is instead caused by Robertsonian translocation of the long-arm of chromosome 21. More rarely, in around 2% of cases, mosaicism can occur in which there are a percentage of cells carrying either two or three copies of chromosome 21^{24,25}.

Triplication of genetic material from chromosome 21 leads to a distinct clinical phenotype. An area identified as the Down syndrome critical region (DSCR) of chromosome 21 is reported to encompass many of the key genes which affect the phenotype²⁶. Whilst the DCSR appears necessary, it is debated whether triplication of this region in isolation is sufficient to replicate the entire phenotype, and complex gene interactions with other areas and chromosomes occur²⁷⁻³⁰.

People with Down syndrome have characteristic facial features including up-slanting palpebral fissures, epicanthal folds, brachycephaly, and small low-set ears. People with DS are generally of short stature and have low muscle tone, especially as babies and young children^{12,31,32}. Down syndrome is associated with intellectual disability, ranging from mild to severe, and a cognitive profile with particular weaknesses in processing verbal information (thought to be secondary to phonological loop deficits) and executive function, especially related to attention, processing speed, verbal working memory and set-shifting. Relative strengths are observed in non-verbal learning and memory³³⁻³⁵.

People with DS are more likely to experience a range of medical conditions, although the prevalence and severity of these varies significantly between individuals³⁶. Approximately 50% of people with DS will have congenital heart disease, most commonly atrioventricular septal defects (AVSD). This can lead to secondary complications such as pulmonary

hypertension³⁷. They can experience syncope due to low blood pressure and may become bradycardic. DS is associated with intestinal and digestive conditions, obesity, autoimmune conditions - particularly hypothyroidism – hearing difficulties, cataracts, increased risk of infection, and leukaemia. On the other hand there appears to be a lower risk of atherosclerosis and certain solid tumours^{12,31,38}. Better recognition and treatment of medical conditions have contributed to a significant increase in the life span of people with Down syndrome, with many people now living into their mid-60s³⁹. This is in contrast to the picture a few decades ago when people with Down syndrome often did not live long into adulthood⁴⁰.

As the lifespan of people with Down syndrome has increased it has been recognised that there is an ultra-high risk of developing Alzheimer's dementia in this population^{13,41}. Onset occurs at a younger age than in sporadic AD and the average age at diagnosis is currently 55 years^{14,42}. In the UK, dementia was the proximate cause of death in 70% of adults over the age of 35 with DS⁴³.

1.2.1.2. Body System Profiles

Trisomy 21 is associated with a number of systemic alterations. Specific immune, inflammatory and metabolic profiles are observed in DS, although this is not consistent between all individuals. Immune dysregulation may be related to the triplication of immune-related genes on chromosome 21 including *SI00B*, *IFNAR* and *TIAMI*⁴⁴. Intrinsic immune deficits are widely reported in DS, with deviations observed from birth⁴⁵. These include a smaller than average thymus gland, abnormalities in the number and function of white blood cells and disruptions in antibody expression, with attenuated antibody responses to some infections and vaccinations⁴⁶⁻⁴⁹.

Alongside this, people with DS are more likely to have a pro-inflammatory profile and over-expression of a number of inflammatory markers are reported. Higher levels of C-reactive protein (CRP), a general marker of inflammation, have been observed in people with DS compared to matched controls⁵⁰. A cross-sectional study across the lifespan of people with DS assessed the inflammatory profile at different ages. An early increase in inflammatory markers with an evolving neuroinflammatory phenotype was observed across the lifespan⁵¹. In a meta-analysis from 19 studies, 957 individuals with DS were compared to 541 controls. Inflammatory cytokines TNF α , IL-1 β , IFN- γ and neopterin were found to be significantly higher in DS, although there was a high degree of heterogeneity between studies⁵². These cytokines are involved in pro-inflammatory states and the results indicate dysregulation of inflammatory pathways in DS. Similarly, other studies found higher levels of IL-6 and IL-10 in DS compared to controls^{53,54}.

Metabolic alterations and dysregulation occur in DS. Children with DS have been found to have higher triglycerides, total cholesterol and low density lipoprotein compared to their siblings, with lower levels of high density lipoproteins⁵⁵. This is alongside differences in hormonal signals including insulin resistance and dysregulation of leptin and ghrelin levels, which help to regulate appetite and satiety, as well as higher adiposity⁵⁶. In adolescents with DS the altered metabolic profile has been associated with pro-inflammatory changes, for instance waist-to-height ratio has been found to have a positive association with levels of complement components C3, C4 and leptin which may further exacerbate the pro-inflammatory states observed⁵⁷.

In addition, DS is associated with autonomic dysfunction which can affect blood pressure and heart rate, often showing reduced compensatory responses to stress and exercise⁵⁸. This may be related to a blunted catecholamine response⁵⁹. People with Down syndrome appear to have lower blood pressure than age matched controls, and often experience a lower than average resting heart rate with attenuated increases in both blood pressure and heart rate in response to stressors^{60,61}.

1.2.2. Alzheimer's Disease Overview

It is estimated that over 47 million people worldwide are living with dementia⁶². Alzheimer's disease is the most commonly occurring cause of dementia. In the USA the overall prevalence is around 14%, and in the UK incidence rates of 1.6 per 1,000 person years have been calculated⁶³⁻⁶⁵. AD is associated with age, and risks increase in older adults. The most commonly observed type of AD is sporadic AD which generally occurs in older people (late-onset AD), but can also be found in younger adults (so called early onset AD). The exact causes of AD are not known, but there are genetic risk factors which predispose people in the general population to develop AD. The most common of these is the presence of $\epsilon 4$ alleles of the apolipoprotein E gene (*APOE*)⁶⁶. There are familial forms of AD which occur because of well-defined genetic mutations in the amyloid precursor protein (*APP*) and presenilin (*PSEN1* and *PSEN2*) genes located on chromosome 21, chromosome 14, and chromosome 1 respectively. This includes duplication and triplication of *APP*⁶⁷. Compared to sporadic AD, familial AD is associated with earlier onset, a close family history of the disease, and can have a more aggressive disease course⁶⁸.

Alzheimer's dementia is an irreversible, progressive neurodegenerative disease characterised by the accumulation of amyloid-beta protein (A β) forming plaques and neurofibrillary tangles consisting of hyperphosphorylated tau protein in brain tissue⁶⁹. Amyloid-beta is also deposited in blood vessels walls leading to cerebral amyloid angiopathy (CAA). Accompanying atrophy of subcortical structures fundamental to learning and memory processes occurs, including the hippocampus, thalamus and striatum^{70,71,72}. A cascade of other neuropathological changes arise including axonal demyelination, inflammation, synaptic dysfunction, neuronal death, metabolic dysregulation, tissue loss and vascular disruption⁷³. As the disease progresses there is a gradual loss of cognitive and functional skills, behaviour and personality changes, neurological symptoms and mood disturbance. Short-term memory loss is often the first recognised symptom, particularly episodic memory impairment⁷⁴. Over time there is gradual global cognitive decline, eventually leading to loss of independence and death.

The amyloid cascade hypothesis has been extremely influential in developing our understanding of the neuropathological processes driving AD⁷⁵. Imbalances in amyloid processing with abnormal accumulation of A β in the brain are identified as key instigating events. Extracellular A β forms into plaques within the brain parenchyma. Inflammatory and vascular responses incite further amyloid-beta deposition and the aggregation of tau in the form of neurofibrillary tangles. There is accompanying synaptic and neuronal injury which further perpetuates the pathology. Cognitive and functional impairments arise as a result, with progressive decline as pathology accumulates^{76,77}.

The genetics of familial AD support the key role of amyloid-beta in the pathogenesis of AD. *APP* and *PSEN* are involved in the processing of amyloid. APP is cleaved into A β proteins via sequential enzymatic reactions. There are two pathways, one referred to as the amyloidogenic

pathway instigated by β -site APP cleaving enzyme 1 (BACE1). The second pathway is the non-amyloidogenic pathway and is modulated by alpha-secretase. Both alpha and beta cleavage are followed by subsequent γ -secretase cleavage⁷⁸. PSEN forms a subunit of γ -secretase. In usual conditions the majority of APP is processed via alpha-secretase. In pathological conditions, BACE1 cleavage is induced with production of A β peptides including A β 40 and A β 42. These peptides, particularly A β 42, readily aggregate to form plaques and promote neurotoxic effects. Mutations in *APP* or *PSEN* genes promote amyloidogenic processing pathways leading to increased production of A β 42, aggregation and plaque formation, increased neurotoxicity, and clinical signs of dementia⁷⁹.

1.2.3. Down Syndrome and Alzheimer's Disease

Dementia risk in Down syndrome can be viewed as analogous to the risks observed in familial AD. The primary reason for the high risk of AD in people with DS is the triplication of *APP* located on chromosome 21^{80,81}. Rare individuals with DS who have incomplete trisomy and only two copies of *APP* (disomy) do not appear to have the same AD risk^{82,83}. Triplication of *APP* leads to increased deposition and accumulation of amyloid-beta throughout life in people with DS. Intra-neuronal amyloid-beta deposition starts as early as the first decade, with extracellular diffuse plaques observed in adolescents⁸¹. A β plaque formation in cerebral tissue found almost universally by age 40^{84,85}. Hence accumulation of amyloid-beta begins many years prior to overt neuropathological changes.

Triplication of *APP* may not be the only DS-associated genetic risk influencing the onset of Alzheimer's disease. Other genes on chromosome 21 which appear to increase A β deposition

independent of *APP* have also been implicated⁸⁶. These include genes involved in the immune response. Immune dysregulation observed in DS may therefore play a role in the pathogenesis of AD. Acute phase proteins, cytokines and the complement system have all been found to be disrupted in both DS and AD, although there is debate as to whether the immune responses are an instigating driver of AD or a reaction to the neuropathological changes^{40,87}. Other hypothesised contributors to AD in DS include systemic downstream effects as a result of aneuploidy and the role of oxidative stress pathways, and the dysregulation of microglial activity leading to impaired A β clearance⁸⁸. The interplay of these factors and the onset of AD in DS is not fully understood⁸⁹.

Despite the common genetic drivers for the development of AD in people with DS there are significant individual variations in age of onset, speed of progression and pattern of presentation^{85,90}. Quantifiable burden of disease pathology in the brain does not necessarily correlate with the onset or intensity of dementia symptoms. Those with the highest burden of amyloid-beta deposition or brain pathology do not inevitably exhibit the most severe cognitive and functional symptoms⁹¹. This indicates that additional factors distinct from chromosomal trisomy may contribute to the onset and progression of AD in Down syndrome, and could potentially be targets for intervention.

1.2.4. Health and Lifestyle Factors in Alzheimer's Disease and Down Syndrome

1.2.4.1. Health and Lifestyle Factors in Alzheimer's Disease

Knowledge gleaned from research in sporadic AD suggests a number of potential modulating factors in the risk for and progression of the disease. For example, *APOE*, a gene located on

chromosome 19, has three distinct alleles: $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. People with one or two copies of the *APOE* $\epsilon 4$ allele are at an increased risk of developing Alzheimer's disease than those without an $\epsilon 4$ allele⁹². In DS a similar risk has been found, and *APOE* $\epsilon 4$ carriers present with dementia at a younger age than non-carriers, show earlier cognitive decline and have higher mortality^{93,94}. This demonstrates that variables independent of the intrinsic risk related to trisomy 21 can affect the onset of Alzheimer's dementia in DS despite the genetic predisposition. Modulating the age of onset of AD and speed of decline in people with DS may therefore be possible.

It is not only genetic factors that appear to be relevant to dementia risk. Research in sporadic AD has highlighted the impact of health, social, lifestyle and environmental factors on the risk of developing dementia. In a population-based study *Norton et al* identified seven factors that increased the risk of dementia in the general population⁹⁵. These were being a tobacco smoker, being overweight or obese in mid-life, participating in low levels of physical activity, mid-life hypertension, depression, diabetes mellitus, and low educational attainment. The authors conclude that relative reductions of 10% per decade in the prevalence of each of these seven risk factors could reduce the worldwide prevalence of AD by 8.3% by the year 2050, with approximately one third of AD cases worldwide potentially attributable to modifiable risk factors. Other studies have identified factors including high alcohol intake, exposure to pollution, Western diets, low social activity, limited cognitive stimulation, poor sleep patterns, poor glycaemic control and high cholesterol as increasing the risk of developing Alzheimer's dementia^{8,96,97}.

The impact of lifestyle factors appears to be relevant both before and after disease onset. In a study of blood pressure changes in AD, increased systolic blood pressure variability was

present in people with confirmed AD and MCI compared to a cognitively stable group. There were no between-group differences in the level of AD-related neuropathology⁹⁸. This suggests that some modifiable factors may be relevant to the progression of clinically observed cognitive decline separate from their potential to modulate the development of underlying neuropathology.

A 2018 study reviewed the current standard of evidence to support the impact of lifestyle factors and the risk of cognitive decline or dementia in the general population⁹⁹. The authors highlight that the majority of evidence is based on observational studies often focused on a number of different lifestyle factors covering a wide set of domains including educational attainment, social circumstances, physical activity and health parameters. Randomised control trials investigating single domain interventions remain fairly uncommon, often involving small cohorts and being of short duration. It can therefore be difficult to unpick the potential impact of individual factors on the risk of dementia. However, targeted multi-domain lifestyle interventions such as the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) trial, have shown promise that these risks can be modified with positive effects. In this trial 1,260 older adults with risks for cognitive decline were enrolled in a two-year intervention including nutritional guidance, exercise and cognitive training, alongside regular monitoring and close management of metabolic and vascular risk factors. In comparison to the control group who only received general health advice there was less cognitive decline seen in the intervention group, with improvements observed in measures of executive functioning, processing speed and memory¹⁰⁰.

1.2.4.2. Health and Lifestyle Factors in People with Down Syndrome

Given the extremely high risk of Alzheimer's dementia in people with DS and the lack of treatments currently available it is pertinent to investigate whether health or lifestyle factors could help to delay onset and maintain functioning in this population, similar to the effects observed in sporadic AD.

Many lifestyle factors are particularly relevant for people with Down syndrome. These individuals can be more prone to obesity, with a higher body mass index (BMI) at a younger age compared to the general population^{16,101,102}. Like many people with intellectual disability (ID) they can be disadvantaged in terms of access to social and educational activities, especially as they get older¹⁰³. They may engage in less physical activity due to a combination of barriers to access, co-morbidities, and insufficient facilities being available^{104,105}. Down syndrome has been associated with depression which may be a risk factor for developing AD, as well as acting to compound difficulties in accessing health and social interventions^{106,107}.

Whilst life-expectancy for people with DS has increased significantly over recent decades, there remains a well-documented disparity in physical health care between people with ID and the general population which may further increase risk^{108,109}. On the other hand, many people with Down syndrome do not smoke, use recreational drugs or consume excess amounts of alcohol¹¹⁰. They are also less likely have high blood pressure, ischaemic heart disease or atherosclerotic complications^{38,111,112}.

1.2.4.3. Cardiovascular Risk in People with Down Syndrome

Out of these lifestyle factors, the most pertinent appear to be related to cardiovascular disease. Cardiovascular risk factors are of particular interest when considering the aetiology of AD in DS. There is increasing evidence that cerebrovascular changes impact on cognitive functioning and may contribute to the development of dementia-related pathology in AD¹⁰. Similar findings have been presented in DS. For example, low cerebral blood flow is observed in people with DS-AD secondary to AD pathology, and is associated with greater impairment on direct cognitive assessment and caregiver reports¹¹³. Poor cerebral blood flow is an indicator of cerebrovascular pathology, and is often related to the presence of other vascular risk factors including hypertension and high cholesterol. However, as mentioned earlier, people with DS may have lower rates of co-morbidities that are traditionally associated with vascular disease¹¹⁴. Indeed, people with DS appear more likely to have a blood pressure that is below established norms for the general population, rather than hypertension¹¹⁵. In DS it remains to be shown how the alterations in cerebral blood flow (CBF) and dementia risk may be exacerbated by other vascular factors and co-morbidities. It is also not clear whether low CBF contributes directly to the development of AD symptomatology or occurs as a secondary response in DS.

As noted above, DS can be associated with inflammatory dysregulation and deranged lipid profiles. Inflammation has been increasingly implicated in the pathogenesis and propagation of Alzheimer's disease. Integral components of inflammatory pathways including complement components and activated macrophages are found deposited around A β plaques in both DS and AD^{116,117}. It is not fully understood why modifying cardiovascular risk such as increasing physical activity or managing obesity may help to reduce the risk of AD, however one proposed

mechanism of action is that they reduce inflammation. Given the pro-inflammatory states often observed in DS, these pathways may be particularly relevant for reducing risk of AD in this population.

1.3. Cardiovascular Risk Factors and Cognition in Down Syndrome

Poor cardiovascular health is associated with increased cerebrovascular disease in the general population and contributes to the risk of AD¹¹. A β deposition is the primary risk factor for AD in DS, but cerebrovascular disease has also been established in the brains of people with DS-AD. White matter hyperintensities, enlarged perivascular spaces, microbleeds and infarcts are commonly observed, with an increased burden of these changes in people with mild cognitive impairment (MCI) or AD compared to cognitively normal individuals. Recently evidence has been put forward suggesting that vascular factors such as hypertension may interact with inflammatory and AD-specific pathways with each contributing to the onset and progression of clinical AD in people with DS rather than being separate age-related co-morbidities^{114,118}. At this stage the directionality of these associations are not clear, and further work is needed to understand how cardiovascular factors affect AD disease processes in DS.

Compared to research in sporadic AD there have been relatively few studies into the effects of cardiovascular factors on improving cognition or impacting dementia risk in DS. Of the relatively small number of studies the majority focus on the effects of physical activity (PA). A few studies have examined the effects of other cardiovascular risk factors. An overview of the current literature scrutinising cardiovascular health and cognition or dementia in Down syndrome is presented below.

1.3.1. Physical Activity and Cognition in Down Syndrome

1.3.1.1. Studies in Animal Models

Experiments using Ts65Dn mice, a model of DS, provided initial evidence that PA could play a role in enhancing cognition. For example, four weeks of voluntary wheel running was associated with superior performance on episodic, spatial and associative memory tasks compared to sedentary controls in male Ts65Dn mice. This was alongside increased neurogenesis and synaptic plasticity in hippocampal cells¹¹⁹. In female Ts65Dn mice, better motor performance on a cerebellar-based rota-rod task was observed in mice that ran regularly. The cohort of Ts65Dn mice that had access to wheel running immediately post-weaning showed significant improvements in a novel object exploration task which relies on hippocampal mediated processes. The same effect was not seen in Ts65Dn mice who only started running in adulthood¹²⁰. This suggests that there may be age-dependent and total exercise-dependent effects when considering the impact of PA on cognition. Running was found to modulate the levels of certain cognitive-related proteins found on human chromosome 21, including DYRK1A, APP, and SOD1. Effects of PA may therefore be mediated by influences on protein expression. On the other hand, in a separate study seven weeks of wheel running led to improved performance of male Ts65Dn mice on a Morris Water Maze task compared to sedentary controls, yet no corresponding neurogenesis or morphological changes in the hippocampus were identified¹²¹.

Whilst the hippocampus has been the focus of the majority of mouse model studies given its importance in learning, memory and cognitive deficits in AD and DS, there is evidence that other parts of the brain may also be affected by PA. An experiment investigating the effects of long-term voluntary wheel running on the cerebellar transcriptome found that both euploid and

Ts65Dn mice displayed modulation of gene expression in the cerebellum associated with wheel running. In the Ts65Dn mice, twenty biological pathways in the cerebellum were found to be affected by running: 18 upregulated and two downregulated. Upregulated pathways included those related to long-term potentiation and depression, cell proliferation, growth, migration and survival¹²². These findings suggest that the effects of PA may be mediated through the enhancement and modulation of complex underlying molecular mechanisms and pathways with wide-ranging functional and cognitive consequences throughout various brain structures. There are limitations in using mouse models to understand the effects of cardiovascular risk as they are imperfect models of disease. The Ts65Dn mouse for example is trisomic for only approximately two-thirds of genes orthologous to chromosome 21. Human studies are vital to translate pre-clinical findings identified in mice models.

1.3.1.2. Human Studies

In human studies emphasis has often been placed on immediate cognitive effects following relatively brief PA interventions. Often the trials are performed in small cohorts. One group studied the immediate effects of acute exercise interventions on a number of cognitive measures including reaction time, attention shifting, inhibitory control, and verbal fluency. In the first study, two groups of participants with DS aged between 15-30 years were assessed before and after a single 20 minute walking intervention classed as “moderate intensity” exercise. Improvements were seen in post-intervention measures of inhibition using the knock-tap test. Non-significant improvements were seen in measures of attention shifting (dimensional change card sort test), and choice reaction time when compared to controls¹²³.

Subsequent studies by the same group investigated the optimal intensity of exercise to promote cognitive improvement. Three groups were used: an attentional control, a moderate intensity exercise group, and a high intensity exercise group. Moderate intensity exercise was associated with immediate post-intervention improvements in reaction time and semantic verbal fluency, whereas both moderate and high intensity exercise groups performed better than controls in the knock-tap test. No group differences were found on the dimensional change card sorting test or in phonetic fluency^{124,125}. The authors proposed a quadratic relationship in which moderate intensity exercise may confer more benefit on reaction time measures and semantic fluency compared to high intensity exercise, although a linear relationship was found for inhibitory control.

Other studies analysed the effect of assisted versus voluntary exercise in DS. Assisted cycling, where pedalling speed was set up to 35% greater than the participant's preferred rate, was associated with immediate post-test benefits in dexterity and simple reaction time, and a non-significant improvement in cognitive planning¹²⁶. Perhaps surprisingly, voluntary exercise in this cohort was not associated with any benefit, despite the voluntary group experiencing higher average heart rates during the intervention. This suggests that not just engaging in PA, but also the type and consistency may be important to induce cerebral responses.

Longer-term studies using the same paradigm of assisted versus voluntary exercise reported benefits after engaging in a prolonged exercise intervention, although effect sizes were generally small¹²⁷⁻¹²⁹. Over eight weeks participants completed either 30 minutes of voluntary or assisted cycling three times a week. A convenience sample was used as a control. PA was associated with improvements in executive function in people with DS, with possible differential benefits depending on the type of intervention. For instance, both cycling

interventions were associated with improvements in manual dexterity and in semantic, but not phonetic, fluency. Voluntary cycling was associated with significant improvements in set shifting, whereas assisted cycling was associated with improvements in reaction time, cognitive inhibition and greater increases in cognitive planning abilities. Set-shifting improvement was observed in the assisted cycling group but did not reach significance. The authors speculated that assisted cycling may affect functioning via the sensorimotor cortex and basal ganglia thalamo-cortical circuit, and this supports the hypothesis that PA in DS may directly modulate cognitive pathways, rather than exert effects only via secondary cardiovascular and physical effects.

Two studies investigated the impact of a 12-week exercise interventions for adults with DS. *Ptomey et al* assessed 27 individuals who participated in video-led home exercise for one or two 30 minute sessions per week. A significant improvement in paired associate learning (PAL) scores were reported following the interventions, but no change in reaction time or attention-switching measures¹³⁰. *Sarlack et al* implemented three exercise sessions per week for 13 men with DS compared to a control group. The dementia screening questionnaire for individuals with intellectual disability (DSQIID) was used to identify pre-and post-changes. Ten of the participants in the intervention arm had at least one symptom related to dementia on pre-test measures. Following the intervention, eight of these showed some reduction in DSQIID score (suggesting an improvement), although this was limited to scores in the memory, confusion, and skills/social domains. No changes in score were observed in the control group¹³¹.

More recently a study of 61 adults with Down syndrome (without AD) found a positive association between the amount of time spent engaging in moderate-to-vigorous PA and

performance on cognitive outcome measures. Participants engaging in higher levels of exercise made fewer Stroop errors, performed better on block design and visual-motor integration tasks, and had lower scores on the Dementia Questionnaire for People with Learning Disabilities (DLD)¹³². Higher levels of exercise were also associated with higher composite memory scores¹³³.

The results from these experiments suggest that exercise interventions may have a positive impact on memory and functional skills in people with DS. However, it is difficult to rule out the potential confounding effects of aspects such as social interactions which are implicitly included in many of the study designs. In addition, the small sample sizes and heterogeneity of the samples and methods used in the current literature makes it difficult to generalise the results and draw firm conclusions. The studies above are also not directly investigating the link between PA and dementia risk in DS, but rather the general impact of PA on cognition.

1.3.2. Other Cardiovascular Risk Factors and Cognition in Down Syndrome

Limited literature studying the impact of other cardiovascular factors on cognition or dementia risk in DS was identified. One group performed a cross-sectional study to compare lifestyle factors in a cohort of individuals with DS and AD to a sample of adults with DS but no clinical signs of AD. The factors of interest were levels of PA, diet, weight, cognitive activity and educational attainment. No significant group differences were observed for any of the factors¹³⁴. The group sizes were small and there was missing data from the participants. It is also noted that this was a cross-sectional study which did not consider causal relationships.

1.3.2.1. Obesity

Two papers were identified that considered the effects of BMI on cognitive outcomes in people with DS. A cross-sectional study of 116 women between the ages of 40-60 years compared outcomes in pre-and post-menopausal participants. The impact of obesity on seven cognitive measures for each group were assessed. In post-menopausal women with DS being obese was significantly associated with better performance in an explicit memory test and higher scores on the Down Syndrome Mental Status Examination (DSMSE) compared to non-obese women. No differences were found in the pre-menopausal group. This suggests the potential for BMI to affect cognition and memory, although the effects are likely dependent on complex interactions with other endogenous and exogenous conditions¹³⁵.

The complexity of the relationship between BMI and dementia is reflected by a study of weight changes observed in 49 adults with DS between the ages of 41 and 60 years¹³⁶. Those with dementia were compared to those without dementia over a four-year period. Whilst both groups lost weight on average during the period, in the dementia group there was significantly greater loss of weight which was gradual and progressive throughout the period. In the non-demented group the weight of participants fluctuated. The rate of weight loss was significantly greater in the dementia group who lost an average of 3.89 kg per year, in contrast to the 0.93 kg loss observed in the non-demented group. Together these two studies suggest an interaction between obesity and cognition in DS, although the nature and direction of this relationship is not known. Obesity could be a protective factor against AD in older adults with DS; or having AD could lead to a reduction in BMI. Alternatively, declines in cognition and BMI could represent discrete outcomes from a common pathology as BMI interacts with other elements including an individual's age, health and lifestyle. In sporadic AD, being underweight, over-

weight or obese in mid-life has been identified as a risk factor for AD⁷. In DS the relationship between age, BMI and dementia risk is yet to be clearly elucidated.

1.3.2.2. Cholesterol

The role of cholesterol in the development of AD in DS has not been studied on a large scale, despite high cholesterol being an established risk factor for both AD and vascular dementia in the general population. In a study of 179 individuals with DS, serum cholesterol level was not significantly associated with dementia or amyloid-beta load. Higher cholesterol levels were associated with the presence of an APOE ϵ 4 allele, indicating a possible common pathway between cholesterol, *APOE* status and dementia¹³⁷. A longitudinal study with an average 5.5 year follow-up analysed the association between total cholesterol and dementia onset in DS. Here, participants classed as having higher total cholesterol were twice as likely to develop dementia than those with lower levels. Statins, a class of drugs which help to lower cholesterol, were found to have a protective effect in the high cholesterol group, but did not change outcomes for the group with cholesterol levels within the normal range¹³⁸. A pilot randomised control trial studying the feasibility of simvastatin for use in primary prevention of AD in DS measured cognitive change, health, adaptive skills and quality of life. The treatment showed promise, with results trending towards better outcomes in the group taking simvastatin, however small numbers impacted the power of the study with only 13 participants completing the full trial¹³⁹.

1.3.2.3. Blood Pressure and Cardiac Disease

Associations between blood pressure and dementia in DS have not been explored in depth. A brief description of blood pressure data from 80 participants collected as part of a five-year prospective longitudinal study in DS was reported in a letter to the editor of the British Medical Journal. In this study low average blood pressures were recorded in both the cognitively stable participants and those with DS-AD, with no significant group differences found. The author suggests that longstanding hypoperfusion secondary to low blood pressure throughout life could predispose people with DS to developing neuropathological changes related to dementia, however no direct analysis of blood pressure and cognition or AD symptomatology over time was performed¹⁴⁰.

A small retrospective pilot analysis investigated the association between heart disease and neurocognitive performance on a dementia test battery in people with DS. Lower DTB scores (indicating worse performance) were associated with a history of heart disease. The broadness of the “heart disease” category in this study and the small number of participants makes it difficult to assess the impact of blood pressure or hypertension specifically. In a descriptive analysis of extreme values the authors report that the three lowest scores on the DTB were from participants who had a history of hypertension, bradycardia and unspecified heart disease respectively¹⁴¹.

Physical health co-morbidities in people with Down syndrome in a cross-sectional analysis of 63 people with AD were compared to 61 people without. No significant differences between the groups was observed for prevalence of hypertension. However, only five people in the entire sample had a diagnosis of hypertension making it difficult to draw conclusions.

Hypotension was not recorded in this study¹⁴². In an analysis of clinical data extracted from community health records only epilepsy and severity of intellectual disability were significant health conditions associated with survival and age of AD diagnosis in people with DS⁴². Cardiovascular conditions including obesity were not explicitly examined in this study.

Further research is therefore warranted into the effect of cardiovascular health in DS and its association with onset of AD.

1.4. Diagnosis of Alzheimer's Disease in People with Down Syndrome

1.4.1. Investigation and Diagnosis of Dementia in People with Down syndrome

A complicating factor in the diagnosis, management and monitoring of dementia in people with Down syndrome is the presence of co-morbid ID. This means that care needs to be taken when differentiating between cognitive and functional difficulties related to a person's ID and those resulting from a dementing process. The severity of ID varies significantly within the DS population. Some people have a very mild disability whereas others have severe to profound disabilities and may be non-verbal¹⁸. This is particularly critical in the early stages of dementia when changes may be subtle and the impact of floor or ceiling effects in neuropsychological tests could lead to small declines being missed³³. It is difficult to design a diagnostic dementia tool that will meet the needs of all people with Down syndrome due to this variation in abilities^{143,89}. As a result a comprehensive assessment able to identify change in an individual is recommended to assess dementia in people with DS, rather than relying on single measures¹³.

Due to these challenges, AD in people with DS has traditionally been a clinical diagnosis based on cognitive and functional criteria, rather than relying purely on the results of quantitative tests¹⁴⁴. Diagnostic tools have been created to try and improve consistency and reliability of diagnoses. One of the most commonly used in the UK is the CAMDEX-DS¹⁴⁵. This consists of a face-to-face cognitive assessment alongside a semi-structured informant interview and has been shown to have good diagnostic validity¹⁴⁶. A benefit of this tool is that the informant portion gauges functioning based on change from a person's own baseline. The cognitive assessment is specifically designed to reduce floor effects with adapted tasks suitable for people with ID. Despite this, difficulties remain in accurately confirming a dementia diagnosis in people with DS. Direct cognitive assessment relies on repeat measures to mark objective change and this can raise concerns about the impact of practice effects over time. It is not recommended as a stand-alone diagnostic tool but rather an assessment to guide clinical decision making. Similarly, the reliance of the informant section to quantify change from baseline loses sensitivity if the person with DS is supported by a new or changing roster of staff who may be less able to identify changes in a person.

All of this creates complications in trying to establish timely and accurate dementia diagnoses in people with DS. On the one hand there is a potential risk of attributing longstanding cognitive or functional deficits to a dementing process, or alternate explanations such as depressive illness or hypothyroidism not being explored. On the other hand, diagnostic overshadowing or poor collateral information can lead to the opposite effect, with missed or late dementia diagnoses being given.

These diagnostic challenges present a substantial obstacle when considering how to understand and investigate the impact of cardiovascular health on dementia risk in people with DS.

Reliance on clinical symptoms in isolation can lead to the biases listed above, with the potential for premature or missed dementia diagnoses. The diagnosis would also depend heavily on the experience and competency of the assessor and the accuracy and extensiveness of the history provided. Whilst manuals such as the international classification of Disease (ICD) and Diagnostic and Statistical Manual (DSM) are designed to help promote diagnostic consistency between clinicians, studies have shown that in DS the judgement of an experienced clinician is often more accurate than relying on diagnostic criteria¹⁴⁷.

1.4.2. Biomarkers in Alzheimer's Disease and Down Syndrome

As a result of the challenges in making timely and accurate dementia diagnoses in DS there is now significant interest in the use of biomarkers to aid the diagnostic process. These biomarkers include neuroimaging signs (particularly MRI and PET imaging), cerebrospinal fluid (CSF) proteins, and peripheral blood markers. Such approaches have shown promise in improving diagnostic power in sporadic and familial AD.

Despite the substantial improvement in available resources and the increased use in routine clinical practice, brain imaging remains expensive and requires specialist facilities located in clinical centres. Although many people with Down syndrome manage the experience of brain imaging with relative ease, others struggle with the noise, the prolonged time in the scanner, and claustrophobia. Lumbar punctures require specialist clinical facilities and are not used routinely in dementia diagnostics in the UK. They can also be perceived as invasive and are associated with other considerations including the use of local anaesthesia and post-procedure monitoring. As an alternative, venepuncture is usually a familiar method, well tolerated by

patients, requires little specialised equipment, and is manageable in a community setting. Peripheral blood biomarkers rather than other modalities are considered here as these are the most likely to be acceptable to the greatest number of patients and are the most clinically feasible to be introduced into routine diagnostics.

1.4.2.1. Overview of Blood Biomarkers in Alzheimer's Disease

Blood biomarkers are now being incorporated into established diagnostic frameworks for AD in research settings. The most widely used of these frameworks is the A-T-N framework which requires evidence of A β , tau and neurodegeneration to inform an AD diagnosis¹⁴⁸. This is often used to stage participants in research and trials. Traditionally the presence of A β , tau and neurodegeneration required MRI-PET imaging and CSF samples to confirm. However, recent advances in blood biomarker analysis methods have shown that these can be used to identify the A-T-N stage and require much less time, equipment or expertise¹⁴⁹.

Blood biomarkers have been shown to be sensitive and specific for AD, and appear to have a strong correlation with each other and both CSF and neuroimaging biomarkers. In sporadic AD there is a significant amount of literature outlining the pattern of blood biomarkers that indicate risk or diagnosis of AD. Some of the most extensively researched and most promising are the Amyloid-beta 42/40 ratio (A β 42/40), phosphorylated tau (p-tau), total tau (t-tau) and neurofilament light (NfL)¹⁵⁰. For instance, having higher p-tau (both 181 and 217) levels and reduced A β 42/40 ratios have been associated with an increased risk of developing AD in the following years^{151,152}. An additional advantage of these markers is that they can be measured using Single Molecule Arrays (SIMOA) which, although limited in availability at present, can be easily and relatively cheaply be set up in clinical laboratories. The standardisation of assay

protocols with the introduction of automated SIMOA techniques have increased the comparability between studies and reduced the technical burden of analyses. Hence these plasma biomarkers are strong candidates for translation into clinical practice¹⁵³.

In a recent study in the USA, phosphorylated tau proteins p-tau181 and 217 were used to assign participants to one of four groups (p-tau +/- and symptomatic or asymptomatic). The four groups differed in terms of age, *APOE* ϵ 4 status, total tau, NfL and cortical thickness measured on MRI¹⁵⁴. Higher NfL was found in those who were symptomatic and/or had raised p-tau, when compared to the asymptomatic/low p-tau group. This suggests that NfL can be used to differentiate between people with clinical or neuropathological changes from those without. It also confirms that both p-tau levels and clinical symptoms are associated with some form of neurodegeneration and that plasma biomarkers such as NfL are robust proxy markers for AD neuropathology when there is a strong suspicion of AD in the context of adults with DS. The study was undertaken in a purposely diverse population in terms of race and ethnicity and findings suggest that these blood biomarkers do not vary by demographic factors.

Amyloid β Proteins and Tau

Both amyloid-beta and tau are recognised as fundamental components of AD neuropathology. However, the pattern of plasma A β 40 and A β 42 and dementia onset are at times conflicting, particularly the relationship between amyloid-beta levels and dementia stage. As yet no specific criteria have been universally adopted. Despite this the use of amyloid-beta blood markers have shown great potential when used in combination with other diagnostic modalities. Data were collated from thousands of biomarker studies in AD. The ratio of plasma A β 42/40 was one of the most consistently reported findings, with lower levels in amyloid-beta positive than amyloid-beta negative participants, as defined using amyloid-beta PET ligand

binding potentials. Newer techniques using mass spectrometry have increased the diagnostic and predictive value of A β 42/40 ratios in blood plasma, and have been suggested as part of screening in general populations prior to the use of other measures such as PET scans¹⁵⁵.

Plasma t-tau appears to have a robust association with AD. Higher levels are observed in those with dementia, although cross-over has been seen when compared to non-demented elderly people and t-tau has not been able to consistently distinguish between these groups¹⁵⁶. Phosphorylated tau, particularly p-tau181 and more recently p-tau217, have been shown to be better predictors of brain A β load and able to differentiate between AD and non-AD forms of neurodegeneration^{157,158}.

There is now a move to translate the results from these biomarker studies into clinical practice. P-tau has achieved recognition as a strong diagnostic marker for AD and for the monitoring of disease progression. However, its application in clinical settings and the collection of real world data is yet to be completed¹⁵⁹. It is noted that the majority of tau deposition occurs after amyloid-beta has begun aggregating into plaques. Tau markers may be better at distinguishing AD when the diagnosis is in question and may be less useful as a marker of neurodegenerative risk in people with DS where AD is the primary neurodegenerative condition. There is also a limited understanding of how A β and p-tau interact with covariates and how these may affect the interpretation of results¹⁵⁸.

Neurofilament Light

Neurofilament light is one of four subunits that make up neurofilament, an axonal cytoskeleton protein which contributes to the structural integrity of neurons¹⁶⁰. When a neuron is damaged, NfL is released into the CSF and blood stream. A robust correlation between CSF and blood

NfL levels has been demonstrated, meaning that peripheral blood levels of NfL appear to reflect changes within the central nervous system (CNS)¹⁶¹. NfL has shown promise in the diagnostics of AD, including in earlier disease stages¹⁶². Higher levels of NfL are seen in those with MCI and with AD, and it has been shown to discriminate between controls and people with AD¹⁶³. A negative correlation between NfL and measures of cognitive ability are also described¹⁶⁴. The downside of NfL as a biomarker is its non-specificity for AD, being a marker of general axonal degeneration which can be caused by a range of neuroinflammatory and neurodegenerative conditions¹⁶⁵. It also increases with age, independently of dementia status¹⁶⁶. However, work in familial AD has found that NfL may have particular benefit in identifying pre-symptomatic mutation carriers and has been found to be raised as early as 10 years before expected symptom onset, as well as being higher in those with AD¹⁶⁷. Given that DS is also a genetically driven form of AD, NfL may be similarly useful in this population at the pre-clinical dementia stages and for identifying people at increased risk of dementia.

Glial Fibrillary Acidic Protein

GFAP is a more recently identified biomarker for AD. GFAP is an intermediate filament protein expressed primarily by astrocytes in the CNS¹⁶⁸. It has roles in stabilising mechanical properties of astrocytes. It has been implicated in maintaining the structural integrity of the blood brain barrier (BBB) and myelination processes on neurones^{169,170}. Elevated plasma levels of GFAP have been identified following acute neuronal injury, in neuroinflammatory diseases, and as a marker of reactive gliosis¹⁷¹. Concentrations are increased in A β -positive patients, and plasma GFAP predicted the presence of A β positivity more strongly than CSF GFAP, with an AUC of 0.76. GFAP correlates with A β burden in the cortex but not tau pathology. In familial AD, levels of GFAP have been found to be raised in pre-symptomatic mutation carriers compared to non-carriers, with levels estimated to be 95% higher¹⁷². GFAP may therefore be

useful in identifying earlier disease stages of AD and changes in GFAP levels may be related to early amyloid-beta deposition rather than established disease¹⁷³.

Inflammatory Biomarkers

A less established but potentially fertile area to explore changes related to AD is through the complement system. This is part of the innate immune system that has a role in the body's defence against infection and is involved in the regulation of inflammatory responses. The complement system is formed of over 30 different proteins which interact and activate in a cascade in response to inflammatory signals and other cell and protein regulators. There are three interlinked pathways: the classical pathway, the alternative pathway, and the Mannose-binding lectin pathway. These pathways are activated in response to initiators including bacteria and viruses, dying cells, immune complexes, other proteins, and tumour cells¹⁷⁴.

The complement system has been implicated in the development and progression of AD¹⁷⁵. Complement is activated by amyloid-beta and tau depositions in brain tissue, and components of the complement system have been identified in and around amyloid-beta plaques and neurofibrillary tangles^{176,177}. In addition, mutations in regulatory proteins of the complement system have been identified in genome studies as risk factors for AD, specifically clusterin, complement receptor 1 and complement factor H^{178,179,180}. Clusterin, also known as APOJ, is closely related to APOE, another recognised risk factor for AD¹⁸¹. However, given the complex nature of the complement system and the number of proteins involved it has been challenging to unpick the potential pathways and roles in AD. Further difficulties are related to the synthesis of complement which can occur peripherally in the liver and blood leucocytes as well as in the CNS by microglia, astrocytes and neurons¹⁸². This means that peripheral blood samples do not

only reflect complement in the brain, but can also be affected by systemic changes in complement production.

In a meta-analysis of complement in AD, CRP levels, a non-specific marker of general inflammation, were significantly higher in people with AD compared to controls in peripheral blood samples, but there was no consistent difference between complement components found between the groups. There were trends towards certain complement proteins, for instance C9, C1q, and C4a, to be increased in AD patients but the results were inconsistent¹⁸³. Similarly some studies found that lower levels of C3 may predict a higher risk of developing AD, but this finding is not universal¹⁸⁴.

In a longitudinal study 1,001 blood proteins were compared in subjects with sporadic AD or MCI and stable controls¹⁸⁵. Proteins within the complement cascade were observed to change significantly in patients with rapidly progressing AD. These included proteins C2, C9, Mannose-binding protein C, and Factor H related protein and suggest that changes in complement components could be a potential biomarker to monitor disease advancement in AD patients.

It therefore appears that complement factors are involved during the progression of AD, but there are concerns that the variability seen in studies and their wide-ranging biological functions may limit their usefulness as potential biomarkers. At present amyloid-beta, tau and NfL remain the most prominent blood biomarkers studied in AD, however GFAP and complement may be more useful in identifying pre-clinical changes and earlier AD risk factors.

1.4.2.2. Blood Biomarkers in Down Syndrome and Alzheimer's Disease

Until recently there was more limited information about how plasma biomarkers of AD relate to the onset of dementia in people with Down syndrome. There have since been significant advances in the field, with evidence that a sequential pattern of biomarker changes, similar to that which is observed in sporadic AD, is also present in DS^{13,89,186}.

In a systematic review, 22 studies of blood-based AD biomarkers in DS were analysed. The results highlighted the disparate findings that have been reported and the heterogenicity of approaches¹⁸⁷. Despite this, some consensus results are observed. For example, in adults with DS levels of A β 42 are consistently elevated compared to controls, even in those without AD. The outcomes when comparing people with DS to those with DS-AD were more conflicted with different studies linking both higher and lower A β 42 plasma values to the development of AD. The A β 42/40 ratio has been reported to better differentiate DS-AD from DS, however both increases and decreases of A β 42/40 have been associated with DS-AD^{188,189}.

In a comprehensive evaluation of AD biomarkers in DS, higher plasma levels of A β 40 and lower A β 42/40 ratios were found in individuals with DS-AD compared to people with DS who were cognitively stable. However, these markers of A β had poor diagnostic performance and overlap between the groups. Likewise, t-tau levels were raised in the DS-AD group, but had limited diagnostic value due to overlap between groups. P-tau 181 (tau phosphorylated at residue 181) was increased in the DS-AD group but also the prodromal group¹⁹⁰. It showed good diagnostic accuracy to differentiate between DS-AD and the cognitively stable group, but was less able to differentiate between the prodromal and DS-AD group¹⁹¹.

When considering clinical utility, NfL appears to have significant promise to support dementia diagnosis in DS. In a multi-centre cohort study NfL had better discriminatory value in the diagnosis of prodromal and established AD in DS than A β or t-tau levels. This may be related to the near-universal presence of A β deposition in people with DS over 40 years old, meaning that A β levels are raised even in those who are cognitively stable. In this study plasma NfL levels had an accuracy of 0.88 in distinguishing asymptomatic individuals¹⁹². Similarly, in a cohort study NfL was shown to have a positive association with dementia in DS, with higher levels of NfL at baseline being predictive of conversion to dementia at follow-up¹⁹³. The potential utility of using NfL as a dementia diagnostic marker in DS has been confirmed in a recent validation study, with high accuracy in differentiating participants with DS from those with DS-AD¹⁵³.

It has been suggested that inflammatory markers may be more useful in AD risk prediction and diagnosis in DS, given the conflicting results from more typical AD markers such as A β and tau levels¹⁸⁷. These markers may be particularly pertinent in DS due to the inflammatory dysregulation commonly occurring. Indeed, in their 2021 review, *Montoliu-Gaya et al* suggested that IFN- γ , TNF- α , IL-6, IL-8 and IL-10 could be considered as non-ATN (amyloid-beta/tau/neurodegeneration) based biomarkers for AD in DS, alongside other inflammatory markers such as proNGF and tPA¹⁹¹. A positive association has also been found between IL-1 β and tau which may indicate an inflammatory role in neurodegeneration which could be measured via plasma markers¹⁹⁴. GFAP is associated with inflammatory changes and is a marker of reactive gliosis. It increases in people with DS and AD, and as noted, appears to track earlier neuropathological changes than tau¹⁹⁰.

Very little has been reported on the associations between other inflammatory pathways such as complement and AD in DS, despite the potential promise of these proteins being used to monitor disease progression in sporadic AD. It can be hypothesised that there may be less heterogeneity in the DS population with regards to complement levels given the common genetic background, and therefore, despite the concerns raised in using complement as a biomarker in sporadic AD, complement may be a more robust and clinically useful marker for AD risk and progression in DS.

1.5. Blood Biomarkers and Cardiovascular Risk Factors

At present, there has been relatively limited investigation into the association between blood-based biomarkers of AD and cardiovascular risk factors. The majority of literature has focused on CSF and brain imaging biomarkers. In one study an association between the presence of hypertension and higher levels of NfL at baseline was found, but there were no associations between NfL and other lifestyle factors such as education¹⁶⁶. In a longitudinal study, the associations between change in cognitive score, plasma A β 42/40 ratio, NfL and weight loss were analysed over a five year period. Combining clinical signs of weight loss with blood biomarkers levels helped predict risk of cognitive decline. For example, those with weight loss and low A β 42/40 ratios were more likely to experience cognitive decline over the next four years. Those with weight loss and higher levels of NfL similarly showed more cognitive decline compared to those without weight loss and lower NfL¹⁹⁵. Results such as these suggest that there is potential for the use of plasma biomarkers to understand and assess modifiable cardiovascular risks in AD, but further research is needed.

A pre-print of a recent study using data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) found evidence of a synergistic relationship between AD progression and vascular risk factors in the general population¹⁹⁶. In 503 cognitively unimpaired individuals there was an increased risk of cognitive decline in people with high vascular risk burden. The vascular risk factors included in the analysis were cardiovascular disease such as myocardial infarction or coronary artery disease; hypertension; diabetes mellitus; hyperlipidaemia; stroke; smoking status; atrial fibrillation; left ventricular hypertrophy. An interaction between vascular risk burden and AD pathophysiology was identified, specifically observed as increased plasma NfL levels. No associations with CSF A β 42 or p-tau181 measurements were found. An earlier study using the same dataset did not find associations between vascular risk and CSF A β 42 changes or hippocampal atrophy¹⁹⁷.

There is evidence that vascular risk factors may affect AD onset. This risk may be quantifiable using plasma biomarkers but the nature of these relationships and the optimum biomarkers to use has not been established. Given the paucity of literature, the DS population gives us a unique opportunity to further our understanding of how modifiable cardiovascular factors may contribute to the onset of AD and their relationship to blood-based biomarkers. It is hoped this will provide insight into common disease pathways and potential targets for intervention.

1.5.1. Cardiovascular Risk Factors and Alzheimer's Disease Biomarkers in Down

Syndrome

Few studies have considered how lifestyle and cardiovascular risk factors may be related to neuropathological changes in DS. A cohort of 65 adults with DS without clinical signs of AD participated in a brain imaging study to assess whether leisure activity was associated with

changes in cerebral A β load and episodic memory decline¹⁰³. No significant associations were found between the domains of leisure activity (which included PA) or total leisure activity and episodic memory at baseline, A β at baseline, or changes in A β between baseline and follow up. When stratified into two groups based on low (below the mean) or high overall leisure scores at baseline, an association was observed between levels of A β and decline in verbal episodic memory in the low activity group. Greater declines in cued recall intrusions and free and cued recall scores were associated with increased levels of A β . This held true for the social domain in terms of cued recall intrusions, but with no significant interactions found for the cognitively stimulating or physical leisure domains when analysed separately. No such associations were observed in the higher activity group. Whilst this study did not find any direct relationship between leisure activity and A β pathology, it suggests that lifestyle factors could have a moderating effect on the development of clinical symptoms of AD and it is possible that leisure activity could modulate some of the impact of AD neuropathology on cognition.

Fleming et al studied the association between physical activity and both cognitive and neuroimaging markers of AD in a group of 61 adults with DS prior to onset of dementia¹³². They reported a negative correlation between time spent in sedentary behaviour with cognitive functioning, but no association between level of physical activity and amyloid-beta or tau markers from PET imaging. They did however note a correlation between physical activity and white matter integrity in certain brain areas, namely the superior and inferior longitudinal fasciculus. A later study from the same group found a positive association between time spent engaging in physical activity and composite memory scores, but no associations between exercise and A β measures¹³³.

1.6. Chapter Summary

People with DS may have a distinct cardiovascular profile, but this has not been well-defined to date. The current literature is suggestive of a positive relationship between cardiovascular risk factors such as exercise and cognitive functioning in DS, however the mechanisms through which these effects act and their association with dementia onset is less clear. Existing studies have generally been performed in small cohorts with short-term interventions and heterogeneous methods and outcomes. Further large-scale research is therefore needed to better understand cardiovascular health in people with DS, and the associations with dementia onset. Plasma biomarkers represent a key methodology in which to unpick the mechanisms in which cardiovascular health interacts with dementia neuropathology, and there is good evidence that the plasma biomarkers A β , NfL, p-tau and GFAP represent AD-changes in people with DS, and that they may be affected by cardiovascular health status. Hence they can be used to help quantify and understand how cardiovascular health may modify AD neuropathology.

Chapter II

Study Overview

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2.1 Study Overview

2.2. Aims

2.3. Objectives

2.4. Hypotheses

2.1. Study Overview

This PhD project is focused on modifiable cardiovascular factors that are most pertinent to people with DS and which may differ from the general population. Particular focus has been put on factors that not only have particular relevance to the DS phenotype, but also have the potential to be modified. These are BMI, blood pressure, and exercise levels. Not only do these cardiovascular health factors correlate with risk factors for AD in the general population, they also represent parameters which are commonly altered in DS and have the potential to be modified with relatively simple interventions which can be readily implemented¹⁹⁸. Additional cardiovascular factors were explored to ensure BMI and blood pressure effects are understood in the context of the wider cardiovascular profile in DS.

In the general population there are guidelines in place for target blood pressure measurements, weight, and levels of physical activity to promote health and reduce the risk of dementia and other health conditions. At present no similar guidelines exist specifically for people with DS. It is possible that different values will be appropriate for people with DS as thresholds to

modulate incident dementia risk. More information is required about how cardiovascular parameters relate to risk of dementia across the lifespan so that tailored targets can be offered to people with DS. This may enhance healthcare for people with DS by improving identification of risks and allowing for timely and appropriate interventions.

It is recognised that a major difficulty faced by trials and studies into sporadic AD is the heterogeneity of the population and causes of AD in this diverse group. In Down syndrome, the common and predictable genetic driver for AD means that the impact of variations in co-morbidities and lifestyle factors may be more readily explored. Understanding the role of cardiovascular factors in DS and the development of DS-AD may also help to determine pathways of disease relevant to sporadic and familial AD by studying them within a more homogeneous population. This is particularly helpful for the interpretation of plasma biomarkers and their relationship to cardiovascular health which may be readily affected by other factors.

2.2. Aims

The overall study aims were:

- To establish the cardiovascular profile of people with Down syndrome and understand whether this differs from the general population.
- To explore associations between cardiovascular health parameters in earlier adulthood and dementia onset in people with Down syndrome at a population level and consider

whether cardiovascular health factors modulate the age of dementia onset in this population.

- To investigate the relationship between cardiovascular risk and dementia-related changes, considering both neuropathological changes represented by plasma biomarkers and symptoms of cognitive decline.
- To identify health and plasma biomarkers that are associated with cardiovascular risk status and may indicate increased risk of incident dementia in people with Down syndrome. This will help guide interventions and monitoring that may modulate these risks.

2.3. Objectives

1. To determine the pattern of key cardiovascular health parameters across adulthood in people with Down syndrome using a large dataset of electronic health records and compare this to matched controls from the general population.
2. To establish the relationship between cardiovascular health in earlier adulthood and risk of incident dementia in people with Down syndrome through the construction of survival models.
3. To outline key cardiovascular parameters which indicate an increased risk of incident dementia and that can be identified in earlier adulthood in people with Down syndrome. Potential interventions to modulate the effects of identified risks will be considered.

4. To analyse the association between cardiovascular health parameters and Alzheimer's disease pathology in people with Down syndrome using a deeply phenotyped cohort with mixed cognitive, functional and plasma biomarker outcomes.
5. To explore potential mechanistic pathways in which cardiovascular health parameters influence ageing and cognitive decline in people with Down syndrome by evaluating the relationships between cardiovascular health parameters and plasma biomarkers representative of amyloid-beta and tau pathology, neurodegeneration and inflammation.

2.4. Hypotheses

1. There are variations in the values and distribution of cardiovascular health parameters in the Down syndrome population compared to the general population throughout adulthood.
2. Indicators of cardiovascular health, including those associated with increased dementia risk in the general population (e.g. obesity, blood pressure control and level of physical activity) can affect the age of onset and progression of Alzheimer's dementia in Down syndrome. Those with higher risks will have earlier ages of dementia onset.
3. Cardiovascular health parameters including body mass index, levels of physical activity and blood pressure interact with pathways of neurodegeneration related to Alzheimer's

disease. Differences in blood-based biomarkers of Alzheimer's disease will be detectable in people with Down syndrome who have different cardiovascular risks. Those with higher risks will have higher levels of neurodegeneration and neuronal injury at an earlier age compared to those with lower risks.

4. Cardiovascular health affects inflammatory pathways implicated in Alzheimer's disease in people with Down syndrome, including the complement system. Those with higher risks will have altered inflammatory profiles compared to those with lower risks.
5. Specific clinical targets for cardiovascular health parameters in mid-life may be beneficial in delaying onset of Alzheimer's disease in people with Down syndrome. The target thresholds for these may differ from guidance for the general population.

Chapter III

Methods

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3.1. Summary of Study Background and Rationale

In the literature for sporadic AD there is convincing evidence that mid-life cardiovascular risk is related to later dementia onset. For instance, having high blood pressure or BMI in mid-life appears to be associated with an increased risk of dementia in later life^{199,200}. People with DS are at an ultra-high risk of developing AD due to known genetic drivers associated with triplication of chromosome 21. It is not known if mid-life cardiovascular risks also contribute to dementia onset in people with Down syndrome in the context of amyloid-beta overproduction associated with triplication of *APP*.

Although the literature remains relatively sparse, there is increasing interest in moderating AD progression in DS by better management of health factors, given the positive results from trials of such strategies in the general population. Positive effects of exercise on cognition in DS have been suggested as an important component of a multi-domain intervention, although the results are inconsistent and a clear mechanism of effect has not been established. The relationships between other cardiovascular risks and dementia in people with DS are debated. BMI appears related to dementia onset, with studies observing a decline in BMI just prior to diagnosis of AD²⁰¹. In other studies it is suggested that a high BMI in later life may be protective against cognitive decline¹³⁵.

To date there has not been a comprehensive analysis of the cardiovascular profiles present across the lifespan in people with DS and potential cardiovascular risks across the life-course have not been quantified. The aim of this thesis was to understand the cardiovascular profiles of adults with DS, determine the incident dementia risk associated with mid-life cardiovascular

risks, and link this with detailed individual health, cognitive and biomarker profiles to explore possible disease mechanisms and consider potential therapeutic targets.

3.2. General Methodological Approaches

A two-stage complementary research approach was employed. A population cohort study was conducted first, with the results of these analyses then applied to data collected in a deeply phenotyped prospective cohort study. The advantage of this approach is the ability to link detailed inter-individual factors, including cognitive and health parameters as well plasma biomarkers, to observed population-level disease patterns and risks. There have been well-documented difficulties in translating findings at a population level into risk predications at an individual level^{202,203}. The two-part synergistic design of this study was intended to bridge this gap. The goal was to add additional insight to the results of population analyses, particularly to consider mechanisms of interactions between cardiovascular health parameters (CVPs), AD and DS, and examine the potential impact of interventions targeting CVPs for an individual with DS. Mixed outcomes including direct cognitive assessment, informant questionnaires, and plasma biomarkers were selected to help generate new hypotheses regarding mechanistic pathways.

Understanding how population-based patterns are replicated in individuals is essential to inform clinical care and to contextualise risk²⁰⁴. In DS, the absolute risk of developing AD is high in older people, with an estimated lifetime risk of approximately 90%⁹⁰. Therefore what appear to be small relative risks could have a substantial overall impact. Studies using detailed

data from individuals are vital to understand the impact of such relative risks and explore potential mechanistic explanations for risk profiles observed at a population level.

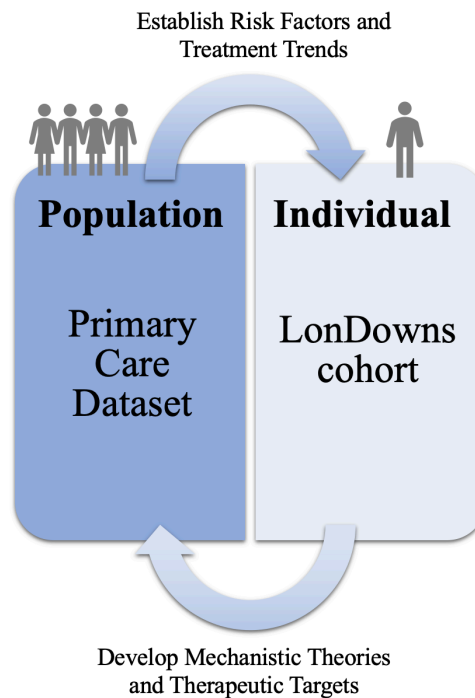


Figure 1. Theoretical model representing the integrated approach of the research project, using both population and individual cohorts in a synergistic fashion. The primary care dataset used was the CPRD, based on electronic records from UK general practices. The LonDowns cohort is a longitudinal prospective cohort study following adults with Down syndrome. Health and lifestyle data, a comprehensive neurocognitive battery and blood samples are collected approximately every 2 years.

Confirming dementia diagnoses in people with DS remains challenging at a clinical level, particularly in the early stages of DS-AD. Use of clinical tools alone may miss earlier, more subtle cognitive changes²⁰⁵. Plasma biomarkers represent a potentially more sensitive measure of AD compared to cognitive testing which could aid diagnostic accuracy and identify changes occurring in younger people, years before overt dementia neuropathology occurs. This is

especially pertinent when trying to understand the impact of risk factors in younger adulthood. The relationships with plasma biomarkers can therefore help to shed light on underlying neuropathological mechanisms driving dementia-risks associated with CVPs. The primary biomarker of interest was neurofilament light (NfL), a cytoskeleton protein whose levels are increased in neurodegenerative disorders including mild cognitive impairment and AD^{206,207}.

In DS higher baseline plasma NfL has been associated with an increased risk of dementia at follow-up without association with premorbid level of intellectual disability²⁰⁸. A further study reported 90% sensitivity and 92% specificity for NfL in distinguishing between people with prodromal AD and asymptomatic individuals with DS²⁰⁹. NfL can therefore be considered a reliable marker of neuropathological change in people with DS which can be used to estimate associations between CVPs and dementia-related neurodegeneration, representing the final step in the ATN model of AD (amyloid-beta/tau/neurodegeneration).

Comparisons with additional established plasma biomarkers of AD were included to explore disease pathways and relationships between factors. These were amyloid-beta protein (A β) 40 and 42, alongside a calculated A β 42/40 ratio and p-tau181 as indicators of the initiating amyloid-beta pathology and subsequent tau deposition, in keeping with the ATN model. Since I hypothesised that CVPs may act indirectly of amyloid-beta and tau deposition to modulate neurodegeneration, I included an additional marker of neuroinflammation and reactive gliosis: GFAP¹⁹⁰. This was alongside markers of inflammation represented by complement components. Complement is an integral part of the innate immune system, and is involved in inducing and modulating inflammatory responses²¹⁰.

The structure of the study is outlined in figure 2. The methodological techniques used in each complementary part of the study are detailed below. These are a population cohort study based on electronic health records and a prospective study in a deeply-phenotyped cohort following adults with Down syndrome.

Statistical analysis was performed using SPSS statistic software package (version 28.0.1.1, IBM SPSS Statistics for Mac, version 28. Armonk, NY: IBM); R studio (version 1.3.959, RStudio); and Prism 9 (Prism 9 for macOS, version 9.4.1 (458), GraphPad Software LLC).

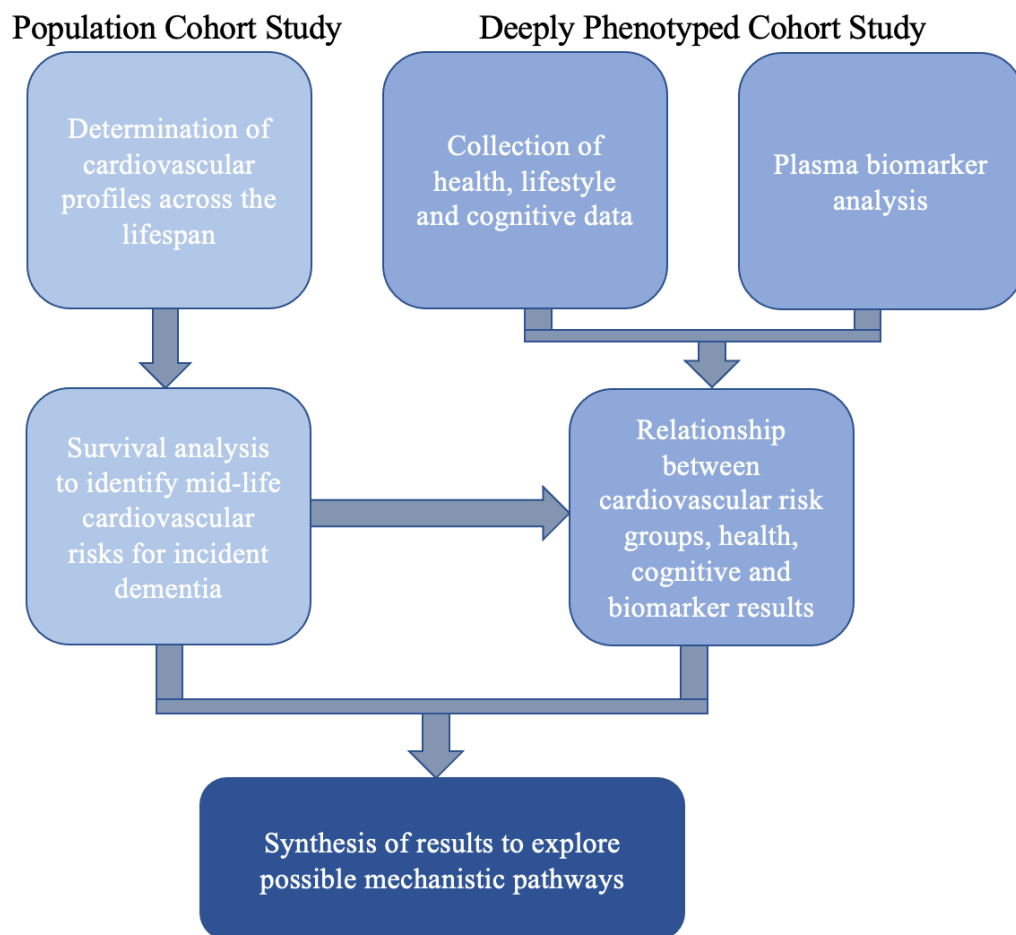


Figure 2. Overview of study design.

3.3. Population Based Cohort Study Using Electronic Health Records

A population-based cohort study was conducted using routinely-collected data extracted from primary care electronic health records in the UK. The methodology was designed to meet standards outlined in the RECORD statement for use in this type of health data²¹¹. The study was conducted in two parts. First I conducted a cross-sectional analysis, calculating incident rate ratios of common co-morbidities and CVP values across the life-span, comparing adults with DS to general population controls. Second, I undertook an analysis of longitudinal data using Cox regression techniques to compare hazard ratios for incident dementia between people with DS who have cardiovascular risk markers and those who do not. Full details of the analyses are outlined below.

3.3.1. Data Source and Extraction

3.3.1.1. Overview of the Clinical Practice Research Datalink

Data were extracted from the Clinical Practice Research Datalink (CPRD). CPRD is a UK-based resource providing real-world clinical data collected from a network of over 1000 general practices in England, Scotland, Wales and Northern Ireland, representing more than one in ten GP practices in these UK countries.²¹² CPRD collects de-identified fully coded electronic patient health records based on routinely collected health data. This includes demographic data, diagnoses, symptoms and signs, referrals, immunisations, prescriptions and ordered tests. Studies have found that the data available from CPRD is broadly representative of the UK population in terms of age, sex and ethnicity²¹³. Median follow up time for patients is currently around 10 years.

Access to CPRD data is regulated, and until June 2021 access was only granted following protocol approval by the Independent Scientific Advisory Committee (ISAC), a non-statutory expert advisory body. ISAC provided scientific advice on research requests from applicants wishing to use CPRD data in their research and vetted any proposals prior to access being granted. A full licence agreement is also required to be in place before use. The research governance processes are designed to ensure investigators have methodologically robust and viable research plans in order to be given permission to use CPRD data.

There are greater than 63,000,000 patients available in the CPRD, with 16,390,255 currently registered with research acceptable data. However, within the UK there are differences between GP practices in terms of the software systems used to record patient data. GP practices registered with CPRD may use either Vision® or EMIS® software systems which have differences in their structure and clinical coding. Data are therefore available as two separate datasets. These are referred to as CPRD GOLD and CPRD Aurum. I used the CPRD GOLD dataset. This has 20,945,522 patients with available research acceptable data, including 3,090,188 who have a current active registration with a GP practice. This database was selected as it has been used for the longest period and its reliability and quality standard has been well documented²¹⁴. At the time of application to CPRD there were 11,928 individuals with Down syndrome identified across all years in CPRD GOLD. Individuals contribute data to their GP health records throughout life, and therefore each person may have data across multiple years. These 11,928 individuals represent 71,688 person years of data.

3.3.1.2. Ethical Approvals

The initial application to use CPRD data was approved in 2020 by ISAC and sponsored by King's College London: ISAC reference number 20_048; personal ISAC CV reference number 177_20. The ISAC were established in 2006 by the Secretary of State for Health as a body to review the scientific merit of research proposals using data from the CPRD as well as the Yellow Card Scheme database. The ISAC is affiliated with the Medicines and Healthcare products Regulatory Agency (MHRA). The application was supported by Professor Martin Guilford, a member of the Department of Primary Care and Public Health Sciences at King's College London who has extensive experience and expertise in accessing and analysing CPRD data.

3.3.1.3. Data Quality Considerations

As the CPRD consists of real-world health data there can be variations in the quality of recording within the dataset, although initiatives such as the Quality and Outcomes Framework have helped to incentive GPs to record key data items at each patient consultation²¹⁵. Researchers using CPRD data are required to make data quality checks before proceeding with data analysis. CPRD has defined a metric called the "Up to Standard Time" (UTS) for GP practices to help identify research-quality data. This is a practice-based quality metric which assesses the stability of recording over a period for a given GP practice and provides dates between which a practice is deemed to be meeting minimum criteria for quality²¹³.

For this study only data collected within UTS windows were used for analysis. The 1st January 1990 was used as the earliest acceptable start date for the CPRD period given variability in the

accuracy and frequency of electronic records prior to this time. Additional data cleaning was undertaken to minimise errors, outlined in more detail below.

3.3.1.4. Study Population

Down Syndrome Cohort

The population of interest were people with Down syndrome registered at a CPRD-linked GP practice in the UK. Cohort entry was defined as the date the person registered at a practice with UTS status, the time that a practice where the person was already registered met UTS requirements, or 1st January 1990, whichever was latest. Cohort exit was defined as date of death, date the person left the practice, date that UTS criteria were no longer met, or date of data extraction (1st July 2020), whichever occurred first. For the cross-sectional analysis the following inclusion criteria were applied: over 18 years old at cohort entry; minimum of 12 months of data available; for the study population there must be at least one recorded Down syndrome code in the patient record. Exclusion criteria were: below 18 years old at cohort entry; less than 12 months of data available.

For the survival analyses additional inclusion and exclusion criteria were used. First a determination of the age-group of interest for risk profiling was made. There were challenges in interpreting the age when cardiovascular risks may be most pertinent, given the difference in life-expectancy and early aging effects observed in many people with DS²¹⁶. In the general population in the UK, mid-life is generally classified as between 40-64 years old, with an average life-expectancy of 81 years. At present people with DS have an average life expectancy around 60 and average age of dementia onset of around 55 years^{85,217,218,219}. A different age

range may therefore be required to capture a similar pre-dementia period for people with DS, and it was necessary to define a time period which could capture similar adulthood risks.

CVPs measured between the years of 25-34 years old (inclusive) were used as predictors. The age bracket of 25-34 was chosen as a period in which to identify cardiovascular risks prior to significant amyloid-beta related brain pathology developing based on our current understanding from neuroimaging and neuropathological studies. This was to isolate these risks from dementia-related changes. Whilst amyloid-beta accumulates throughout life, the age of 40 years has been identified in numerous biomarker and pathology studies as a key age when amyloid-beta deposition and brain pathology appears to significantly increase in people with DS, therefore CVP values were required before this age^{220,186,221}. The age bracket of 25-34 years old was also selected to represent approximately 20 years prior to dementia onset currently estimated as 55 years in DS⁴², which is a similar time distance between the ages of cardiovascular health assessment and average dementia onset in sporadic AD studies. Most commonly the effects of cardiovascular health status between the ages of 45-60 years are analysed in association with an average dementia onset between 75-80 years. People younger than 25 were excluded from the longitudinal analysis to distinguish between developmental or pubertal changes and adult-onset related risks.

For the longitudinal population study all of the following inclusion criteria were required to be met: at least one DS code recorded in CPRD; age 35 or over at cohort entry; valid CVP measurement available between ages 25-34; and a minimum of 12 months of available data. Exclusion criteria were: having a pre-existing diagnosis of dementia, epilepsy or stroke at cohort entry; no available CVP measurements recorded between age 25-34 years old. Cohort entry was calculated at year of 35th birthday, UTS date, or registration date, whichever was

latest. Cohort exit was classified as date of outcome event, de-registration, end of UTS period, or date of extraction, whichever was earliest. The outcomes for the longitudinal analysis are defined in detail later in this section. Data for all identified individuals with at least one recorded Down syndrome code in their patient record were extracted.

Controls

For comparison patients with DS were matched up to a 1:4 ratio to a general population control sample allowing for 280,000 person years in the comparison group. Control participants were sampled from the list of all registered patients in the database without Down syndrome. Controls were exactly matched with participants with DS on family practice, sex, year of birth, and calendar date of record start to within 1.5 years. Up to four controls were randomly sampled for each Down syndrome participant using the ‘sample’ function in the R statistical program²²².

Power Calculations

Using the populations defined above, for an outcome with an incidence of 1 per 100 person years there would be more than 90% power to detect relative risks of 1.2, while for an outcome of 1 per 1,000 person years there would be 80% power to detect relative risks of 1.4. Cardiovascular disease is common, with recent estimates of over 7.6 million people living with cardiovascular disease in the UK²²³. Therefore this sample size should provide sufficient power for the analysis.

3.3.1.5. Code List Generation

The CPRD database consists of coded healthcare records. This means that a diagnosis such as “Down syndrome” has a corresponding “read code” which represents the diagnosis. Usually

there are a number of different read codes for each condition and a list of relevant codes therefore needs to be generated before data can be extracted. There are published code lists available for certain conditions. Where these were available the published lists were reviewed by a medically qualified researcher (SP) in the first instance to assess the diagnostic terms for relevance, comprehensiveness, and accuracy. Additional codes were added using the methods below if the lists were felt to be inadequate. For this analysis only one of the code lists (for Down syndrome diagnosis) was based completely on an existing list, accessed via the OpenSAFELY open source software platform²²⁴.

For the remainder of the diagnostic terms I generated new lists. I used a clinical terminology browser to perform a systematic search for relevant diagnostic terms based on hierarchical medical classifications and clinical knowledge. Additional codes were identified via keyword search. The lists were manually cross-checked to ensure major diagnoses were included. The identified terms were then matched onto the relevant read codes to generate an initial list for each diagnosis of interest. Two clinicians then reviewed the lists for relevance of included diagnostic terms and to consider if there were any omissions. A consensus meeting was held to agree the final lists. In the event that a consensus agreement could not be reached between the two clinicians an external specialist in the area was consulted. Similar approaches to code list generation have been applied previously^{225,226}.

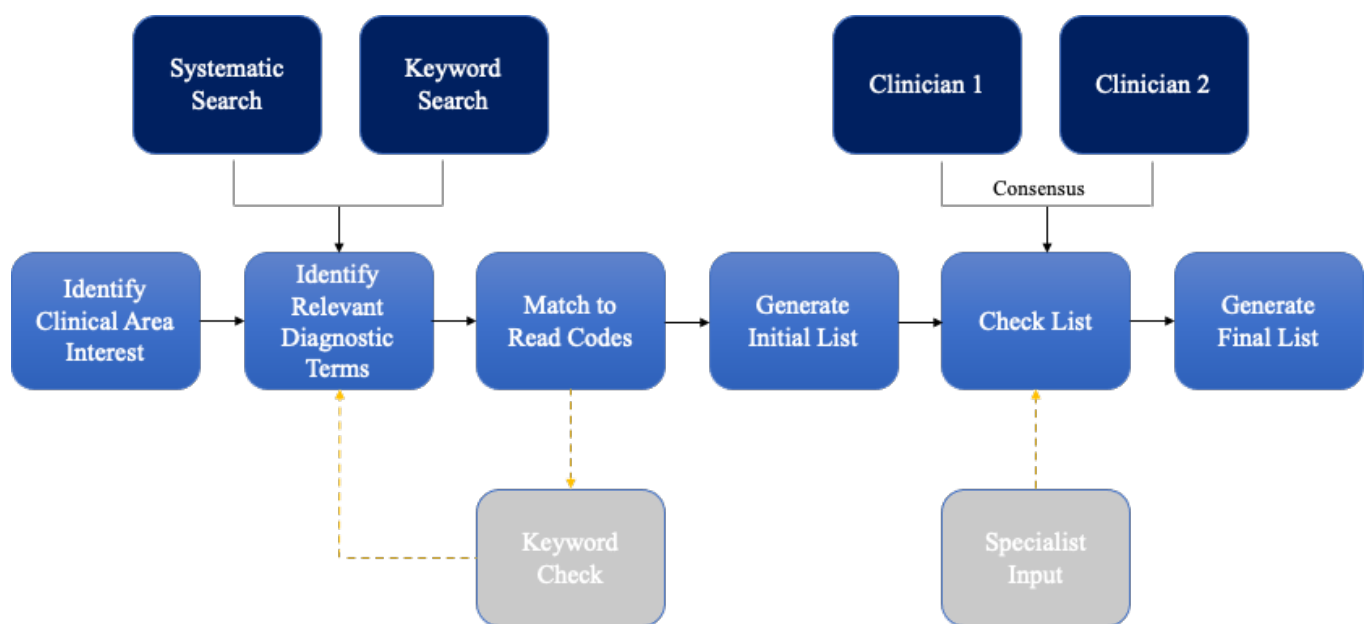


Figure 3. Flowchart outlining the process for code list generation.

Code lists were generated for the following conditions: Down syndrome, ischaemic heart disease (IHD), obesity, peripheral vascular disease (PVD), diabetes mellitus (DM), hypertension (HTN), hypercholesterolaemia, smoking status, congenital heart disease (CHD), hypothyroidism, AD, stroke, and epilepsy or seizures. These were selected for one of three reasons:

1. They represent relevant common cardiovascular conditions that have been identified in Alzheimer's disease literature as potentially affecting risk of dementia in the general population (ischaemic heart disease, obesity, peripheral vascular disease, diabetes, hypertension, high cholesterol, smoking).

2. They represent common co-morbidities in Down syndrome (congenital heart disease, hypothyroidism, diabetes, obesity) and may be associated with cardiovascular disease and dementia risk.
3. They are to be used as outcome measures of incident risk in the longitudinal analysis, and are commonly occurring in Down syndrome (dementia, late-onset epilepsy or seizures, stroke).

To address the potential inaccuracy of dementia diagnosis and recording, two additional surrogate end-points were included in the definition of an incident dementia event. This is because dementia can be challenging to diagnose in people with DS due to a combination of factors including the presence of ID, lack of specialised assessment, and large variations in an individual's baseline presentation^{143,144}. The additional clinical outcomes were new onset of epilepsy or seizures over the age of 35 years in people with no previous diagnosis of epilepsy, and first incident of stroke in people with no previous history of stroke. Both new onset epilepsy and stroke are highly correlated with dementia onset in people with DS and may precede the recording of a clinical dementia diagnosis^{227,228,229,85,230}.

In addition to the diagnostic data, relevant clinical observations were extracted to provide raw values for eight cardiovascular parameters (CVPs): Body Mass Index (BMI) in kg/m²; Systolic Blood Pressure (SBP) in mmHg; Diastolic Blood Pressure (DBP) in mmHg; Total Cholesterol (TC) in mmol/L; High Density Lipoproteins (HDL) in mmol/L; Low Density Lipoproteins (LDL) in mmol/L; Triglycerides (Tg) in mmol/L; and Glycated Haemoglobin (HbA1c) in mmol/mol. If BMI data were not directly available then this was calculated using the formula: $BMI = weight(kg)/height(m)^2$. An additional parameter, pulse pressure (PP), was calculated using the formula $PP = SBP - DBP$.

These measurements were selected as they are routinely performed in general practice and are linked to cardiovascular health status. In addition, BMI, SBP, HDL and TC are fundamental components of the QRISK3 score, a risk prediction algorithm for future cardiovascular disease²³¹.

Condition	Function	Number of Read Codes
Down Syndrome	Study population identifier	10
Ischaemic Heart Disease	Cardiovascular risk factor	274
Obesity	Cardiovascular risk factor	76
Peripheral Vascular Disease	Cardiovascular risk factor	58
Diabetes Mellitus	Cardiovascular risk factor	605
Hypertension	Cardiovascular risk factor	88
Hypercholesterolaemia	Cardiovascular risk factor	99
Smoking Status	Cardiovascular risk factor	125
Congenital Heart Disease	Common comorbidity in DS	449
Hypothyroidism	Common comorbidity in DS	52
Dementia	Primary outcome measure	90
Stroke	Surrogate outcome measure	158
Epilepsy/Seizures	Surrogate outcome measure	190

Table 1. Summary of diagnostic code lists created for the analysis.

3.3.1.6. Data Extraction and Computation

In line with the data access requirements of CPRD, data were extracted by Professor Martin Guilford who has been approved and licensed to access the CPRD database. The date of extraction was 1st July 2020. Basic demographic data (year of birth, sex, and practice ID) were also extracted in addition to the diagnoses and clinical observations listed above. Exact date of birth for patients is not available to ensure patient anonymity is preserved. In addition to the inclusion and exclusion criteria listed above, analysis was restricted to people aged between 18-75 years. An upper limit of 75 years was selected as very few individuals with DS live into their 70s, and therefore diagnosis of DS in significantly older individuals is likely to represent an error in the clinical records²³².

Further data cleaning was performed to identify extreme values for the continuous variables under study. Values that fell outside clinically feasible limits were excluded as they were liable to be the results of an error in data collection or in data entry, including inaccurate value input and incorrect entering of units of measurements. Post-cleaning data checks were made to ensure variables fell within viable ranges. Any duplicate cases were identified based on patient ID and discarded.

Person years were calculated using the period for each patient in which data was available within the UTS time, with censoring at the point of data extraction, death, de-registration or failure of the practice to meet UTS, whichever occurred soonest.

For each continuous variable (BMI, HbA1c, SBP, DBP, TC, HDL, LDL, Tg) exact values were extracted from the CPRD dataset for both the DS and the control groups. Age at variable

measurement was calculated by subtracting the year of birth from the date of the investigation.

Data were counted as available if at least one recorded value for that variable was present in the patient's record within the UTS time frame.

Variable	Units	Lowest Limit Applied	Highest Limit Applied
Age	Years	18	75
Body Mass Index	kg/m ²	5	100
HbA1c	mmol/mol	1	150
Systolic Blood Pressure	mmHg	50	295
Diastolic Blood Pressure	mmHg	20	200
Total Cholesterol	mmol/L	0.01	20
High Density Lipoprotein	mmol/L	0.01	20
Low Density Lipoprotein	mmol/L	0.01	20
Triglycerides	mmol/L	0.01	35.8

Table 2. A-priori limits applied to cardiovascular health parameters based on clinically feasible ranges.

3.3.2. Statistical Analysis

3.3.2.1. Cross-Sectional Analysis Methods

Data Preparation

Data were screened prior to analysis based on data screening checklists outlined by *Tabachnick et al* (p. 125)²³³. Univariate descriptive statistics were evaluated for accuracy of input, ensuring means and standard deviations were plausible and identifying any potential out of range values or outliers. Normality of distribution for each variable was assessed graphically using

histograms and probability plots, and on assessment of values for skewness and kurtosis, with skewness between -2 and +2 and kurtosis values between -7 and +7 deemed acceptable^{234,233}. The significance of the Kolmogorov-Smirnov statistic was not relied upon as in very large samples it is likely to report statistical significance for small deviations that do not affect overall distribution or interpretation of results²³⁵.

In the event that data did not meet criteria for normality, either non-parametric tests were used for comparisons, or data was converted into low, medium and high value groups to allow for comparisons between levels using logistic regression techniques. Transformation of data, for instance using log values, was avoided due to complications that transformations bring to interpreting large-scale clinical data. Detection of multivariate outliers was performed by calculating Mahalanobis distance following linear regression with degrees of freedom equal to number of predictor variables (*Tabachnick et al*, p.10 and 133²³³). Presence of multicollinearity was assessed using a correlation matrix to check for any variables that are significantly correlated (Pearson correlation coefficient values >0.8)²³⁶.

Once these checks were completed the final sample size for the Down syndrome cohort and control group were confirmed. Controls that no longer had a corresponding matched case in the sample were removed using a right censoring strategy.

Clinical Data Definitions

Whole group demographics and key values (sex, year of birth, age range, frequency of GP practice, CPRD record length) were reported using median (IQR) for date variables, mean (SD) for continuous variables, and count (%) for categorical variables. For each categorical variable (diagnostic term) prevalence was calculated if there was any code for the diagnosis ever

recorded in a patient's health notes, both prior to cohort entry and as a combined measure of pre-existing and new incidence during the registration period. If a new diagnosis was made during the UTS CPRD period then the date of the first recorded entry from the code list was listed as the incident date.

For tobacco smoking status a person was assigned to one of three groups: smoker, ex-smoker, never smoked. This was defined based on the last recorded smoking status event. To compare groups, the "ex-smoker" and "smoker" groups were then collapsed into a single "ever smoked" group. If no record of smoking status was identified in a patient's record this was counted as missing data.

For the values of the nine continuous CVPs, the mean of each variable per year of age across the sample was calculated by aggregating the data on age at CVP measurement. Results were presented graphically on scatterplots of age (x-axis) by mean value (y-axis) with error bars representing 95% confidence intervals, stratified by Down syndrome status, to illustrate trends in values across different ages. The mean of each variable per person per age was calculated by aggregating the data on both unique patient identification code and age at measurement.

Group Comparisons

Data were initially examined cross-sectionally. Pearson chi square tests were used to compare frequencies for each diagnosis between the DS and control group, with p-values based on 2-sided asymptotic significance. Mean age and standard deviation (SD) for incidence of each variable was calculated and visualised graphically as frequency histograms. Two-way independent sample t-tests were used to compare means between DS and controls at a whole group level. Two-sided Pearson correlations were calculated with 95% confidence intervals to

compare the relationships between CVPs in people with DS and controls. Significance for analyses was set at $p < 0.05$. Incident rates per 1000 person years were estimated for each CVP. Incident rate ratios with 95% confidence intervals derived from the Poisson distribution were calculated to allow comparison between the DS and control group.

Regression Models and Covariate Analysis

Regression models were fitted to examine the relationship between cohort group and continuous CVP value. Each CVP was inputted as the dependent variable. Down syndrome status alongside the covariates age at time of measurement and sex were independent variables. Regression was performed using the enter method to identify factors contributing significantly to the model, with significance set at $P < 0.05$. Overall model goodness of fit was assessed using R^2 . The contribution of individual predictors was assessed using the t-statistic of the coefficient to assess effect size.

To further explore trends across age groups the sample was stratified into five year age bands from age 20-75 years. Those between the ages of 18-19 years were grouped together as the first age band. Multivariate regression models were constructed to compare CVP values between the DS and control group at each age band, with Bonferroni post-hoc corrections applied. Sex was included as a covariate. Within-group comparisons between age brackets were made to assess changes in CVP with age using one-way ANCOVA controlling for sex.

3.3.2.2. Survival Analyses

For each CVP, all measurements from the age period 25-34 years were aggregated on unique identification code with mean and SD values calculated. Mean age at measurement was also

calculated. Continuous variables were grouped into low, moderate and high categories for relative risk comparisons. In the general population there are recommended targets for cardiovascular risk factors, and these were taken from the UK health guidelines (see table 41 in chapter V). It is not known whether these targets are appropriate for people with DS given the reported differences in co-morbidity patterns in this population. For example, a study examining the association between body fat levels and BMI in young people with DS suggested that 95% percentiles for BMI rather than the usual 85% had better predictive values²³⁷. Longitudinal analyses were therefore performed with grouping based on standardised criteria, and repeated as exploratory work using values derived from the 10th and 90th percentiles for the Down syndrome population for each age group, derived from the earlier cross-sectional analysis. Those with values below the 10th centile were classed as low, those above the 90th centile were classed as high.

The outcome of interest was new onset of dementia, epilepsy or stroke in people with Down syndrome over 35 years old. Those with incident dementia (or secondary outcome) were compared to those without using two-way independent t-tests and Pearson Chi square analyses for continuous and categorical variables respectively.

Time to event analyses were performed using Kaplan-Meier methods to calculate average age at event and mean survival times for each CVP group. The time variable was calculated based on time from cohort entry to either time at incident event or cohort exit. Incident dementia, including the surrogate end-points of new-onset epilepsy or new stroke diagnosis, was the outcome event. Cox proportional hazards regression models were then constructed for each CVP to adjust for the effect of covariates: sex, age, and registration period. Additional relevant co-morbidities were selected based on clinical relevance, specifically history of cardiovascular

disease (congenital heart defect (CHD), ischaemic heart disease (IHD), peripheral vascular disease (PVD), hypertension (HTN) or hypercholesterolaemia). The presence of diabetes mellitus and hypothyroidism were included due to their links with cognitive changes and communality in Down syndrome. Any covariates with less than 10 records were excluded or combined with another variable to reduce the risk of model overfitting^{238,239}. Survival curves were fitted with group comparisons based on whether the person was in the low, moderate or high value category for each variable with estimation of hazard ratios. Unless specified the middle value CVP group was used as the reference indicator, as was male sex.

A minimum size of 10 events per predictor group was used as a guide to perform predicted power calculations for the survival analyses^{239,240}. First a determination of the expected rate of dementia for each CVP was used, with setting of the HR of the two groups. The number of events was estimated using the formula: $Events = (Z_{\alpha} + Z_{\beta})^2 / (\log(RH))^2 q_0 q_1$, where α is the type I error rate, β is the type II error rate, q_0 and q_1 are the proportion of subjects in the unexposed and exposed groups. RH is the relative hazard for the exposed compared to unexposed group.

Model fit was assessed using chi square change and examination of residuals (Cox-Snell, Schoenfeld and Martingale residuals). Proportional hazards assumptions were checked using a hierarchical regression strategy and graphically by plotting the partial residuals and checking for trends over time. In the event that proportional hazards assumptions were not met a time-dependent interaction term was included in the model²⁴¹. Post-hoc checking of validity of the proportional hazards assumption were made using log-minus-log plots to ensure no group intersection occurred.

Crude incident rates per 1000 person years were calculated with 95% confidence intervals derived from the Poisson distribution using the formula $CI = \left(\frac{1000}{n}\right)(d \pm (1.96 * \sqrt{d}))$ where d is the number of events. Similar methodologies have been employed in previous analyses of the impact of health parameters on dementia and mortality in people with Down syndrome and to examine the effect of blood pressure on dementia outcomes^{242,243}.

3.4. LonDowns Cohort Study

3.4.1. Background for the LonDowns Study

The LonDowns study is an ongoing prospective cohort study following adults with DS to determine changes as they grow older. On average participants undergo a detailed assessment every two years, although in younger participants the time scale for follow-up can be longer. Additional factors, most significantly the recent Covid-19 pandemic, have affected follow-up periods and recruitment due to the cessation of face-to-face work and necessary risk precautions in order to maintain the safety and well-being of participants.

The LonDowns study has a number of components. At each assessment participants complete a comprehensive battery of neurocognitive assessments alongside a physical examination. Informants, usually a family member, family carer, or support worker, answer validated questionnaires designed to capture a person's adaptive skills, cognitive abilities and any symptoms related to dementia onset. A semi-structured interview with the informant provides additional information about medical history, development, family history, lifestyle and major life events. For those participants able and willing to provide a blood sample, venepuncture is performed on the same day as the neurocognitive assessment²⁴⁴.

3.4.2. *Ethical Approvals and Consent Procedures*

Ethical approval for the LonDowns study was granted from the North West Wales Research Ethics Committee (13/WA/0194). Written informed consent was obtained from individuals with capacity to consent after a full explanation of the study was provided. In the event that individuals lacked capacity to provide informed consent, a consultee indicated the decision regarding the individual's inclusion based on their knowledge of the individual's wishes. The consultee was asked about the participant's likely views about participating in the study. If the consultee considered that the individual would like to take part in the study, they were asked to sign a form to confirm this.

Capacity was assessed following the guidelines within the UK Mental Capacity Act 2005^{245,246}. The study was explained to participants, supported with accessible information sheets and communication aids as appropriate. This may include Makaton signs and object cues. A family member or carer who was experienced in communicating with the participant was present during the capacity assessment to provide further support. Participants who had capacity to consent to the study were asked to sign and date the consent form. If a participant had difficulty with writing this was noted on the form and a witness and the researcher signed the form to confirm that verbal consent was given. If a participant was able to consent to part of the assessment they were asked to sign the sections for which they had capacity. The consultee would indicate wishes for the sections which the participant lacked capacity.

Information was stored in accordance with UK general data protection regulations (GDPR) and Health Research Authority guidance and the study was registered with the local research governance team. Participant data was anonymised using assigned ID codes. Anonymised data

were collected and managed using REDCap electronic data capture tools hosted by King's College London²⁴⁷. Confidential information was stored separately in an encrypted and password protected location.

3.4.3. Assessment Battery and Data Collection Protocols

3.4.3.1. Initial Work Establishing the Impact of Physical Activity on Dementia-related Symptoms in People with Down Syndrome

To establish the exercise-related risk groups, a preliminary study using previously collected LonDowns data was performed to assess the relationship between levels of regular physical activity and dementia-related symptoms (prior to dementia onset) in 214 adults with DS. The results have been published in The Journal of Clinical Medicine (*Pape et al, 2021*) and a pre-print of the paper is included in appendix 1²⁴⁸.

This approach was adapted from the methods used in *Kenshole et al* and *Laurin et al*^{134,249}. The method was selected to understand the effects of exercise in everyday life, rather than in a highly regulated research-based setting. The approach also ensured that every type of physical activity was captured which may be missed when using screening questionnaires. Due to the diversity of activities, coding was necessary to allow comparison between individuals.

The criteria were used to define exercise level are detailed below:

- High intensity. Exercise of an intensity greater than walking at least three times a week, or for at least three hours a week.

- Moderate intensity. Exercise at an intensity equal to that of walking at least three times a week, or exercise less than three hours a week/three times a week of an intensity greater than walking, or at least two hours a week of exercise where intensity or type was not specified, or a combination of walking and other exercise when duration or intensity not specified.
- Low intensity. All exercise not meeting the above thresholds, including individuals who do not engage in any exercise.

In brief, the results showed that people who engaged in moderate intensity physical activity had a 47% reduced risk of showing decline in everyday skills domains of the CAMDEX-DS, compared to those engaged in low levels of physical activity (Relative Risk=0.53 (95% CI 0.26–0.95), $p=0.03$). High intensity physical activity was associated with a 62% reduced risk of decline in personality and behaviour domains (RR = 0.38 (0.11–0.92), $p=0.04$). There were also longitudinal benefits of exercise. Moderate exercise levels at baseline were associated with a 62% reduced risk of decline in memory and orientation domains at T1 (RR = 0.38 (0.10–0.97), $p=0.047$). High exercise levels at baseline was associated with an 87% reduced risk of decline in personality and behaviour domains at follow up (RR = 0.13 (0.005–0.83), $p=0.04$).

This study established proof of principle for the coding of exercise groups in people with DS using the grouping system outlined above. It also demonstrated a meaningful relationship between everyday levels of physical activity and dementia-related symptoms in people with DS, which could be captured by CAMDEX-DS measures.

3.4.3.2. Selection of Cardiovascular Risk Groups

The following measurements of cardiovascular health were collected at each assessment: height (m), weight (kg), abdominal circumference (cm), blood pressure (mmHg), and pulse (bpm). BMI was calculated using the formula: $BMI = weight(kg)/(height(m))^2$. Height was measured using a telescopic measuring rod. Abdominal circumference (AC) was measured at the level of the iliac crest using a flexible tape measure. Both were recorded to the nearest 0.1cm. Weight was measured to the nearest 0.1kg using digital scales with shoes removed. Blood pressure was measured using an digital sphygmomanometer²⁵⁰. The participant was seated a rest for at least three minutes prior to measurement with the arm lightly supported during the procedure in line with best practices²⁵¹. Pulse was measured concurrently. In the case that blood pressure measurement failed or the participant declined, pulse was manually palpated from the radial pulse.

Additional demographic and lifestyle data about alcohol consumption and smoking were collected. Relevant medical history included history of congenital heart disease, ischaemic heart disease, hypertension, hypercholesterolaemia, peripheral vascular disease, hypothyroidism, autoimmune disease, diabetes, epilepsy or seizures, stroke, and dementia status. Level of intellectual disability was determined using diagnostic criteria from the International Classification of Diseases 10th edition²⁵². Records of regular physical activity level were collected in a semi-structured interview with the informant. Participants' current exercise levels were coded into three categories: low, moderate or high intensity as defined above.

The results of both the population-based analysis presented in chapters 4-5 and the pilot work outlined above were used to inform the creation of risk groups for comparison of people with higher and lower risks for each relevant CVP.

The CVP risk groups selected based on the survival analyses were: SBP <100mmHg or >140mmHg; DBP <62mmHg or >80mmHg; PP <40mmHg; BMI >25kg/m². It was predicted that there would be low numbers of people in the extreme SBP and DBP risk groups if the LonDowns cohort mirrored the population blood pressure parameters. Low exercise was included as a risk group, compared to moderate or high intensity exercise. History of CHD or a diagnosis of hypothyroidism were considered as additional risk groups due to their significance in the survival models.

3.4.4. Cognitive Outcome Measures

Screening Tests

Prior to neurocognitive assessment a participant's vision and hearing were screened to ensure they did not have impairments in these areas that would affect their ability to complete the assessments. Both tests were completed whilst the person was wearing any corrective aids they required. The whisper hearing test, adapted for people with intellectual disability, was used to detect hearing loss and has been shown to be a simple and accurate screening tool for gross hearing impairment^{253,254}. Vision screening was performed using the Kay vision test, which is a picture-based vision screen. A minimum threshold of 3/19 for participation was applied, below which indicates moderate-severe vision loss²⁵⁵.

In addition, before the completion of the PAL task (described in detail below), participants were familiarised with the touch screen device using the motor screening task (MOT), a short test of response-speed (CANTAB® [CANTAB MOT]. Cambridge Cognition (2019). All rights reserved)²⁵⁶. Participants were required to touch a cross which appears on the screen in different locations in a set time period. Participants unable to interact with the touch screen adequately were not asked to complete the PAL task.

Cognitive Outcomes

Two direct cognitive outcomes were selected for this analysis. The CANTAB – Paired Associates Learning (PAL) task and a semantic verbal fluency task²⁵⁶. Both of these tasks have been validated for use in adults with DS and are sensitive measures of executive function, visual episodic memory and hippocampal functioning^{244,257-259}. Using events-based modelling techniques the PAL and verbal fluency have been identified as early markers of cognitive change in DS, and should therefore be sufficiently sensitive to assess subtle cognitive variations²⁶⁰.

Data from direct neurocognitive assessments was complemented with information collected in a semi-structured informant interview using validated screening questionnaires and the Cambridge Examination for Mental Disorders of Older People with Down Syndrome and Others with Intellectual Disabilities (CAMDEX-DS). The short adaptive behaviour scale (SABS) was included alongside the CAMDEX interview to provide information about a participant's functional abilities. Both the SABS and the CAMDEX-DS have been validated for use in people with intellectual disabilities including DS^{146,261}. The benefit of including these is two-fold. One, they allow for information to be collected for people who may be unable to complete the neurocognitive tasks due to their level of ID, physical requirements, or who choose not to participate in the tasks. Two, they provide collateral information about a person's

functioning and behaviour globally, whereas the direct tasks only provide a snapshot about a specific cognitive domain.

CANTAB PAL

The PAL task from the Cambridge Neuropsychological Tests Automated Battery was used (CANTAB® [CANTAB PAL]. Cambridge Cognition (2019). All rights reserved)²⁶². This task has been extensively validated, with discriminative power to differentiate between people with dementia and those with depression, as well as identifying people with AD²⁶³⁻²⁶⁵. It has been included in existing neuropsychological batteries in adults with Down syndrome^{244,258,260}. Poorer performance on a PAL task has been correlated to amyloid-beta positivity²⁶⁶.

In this task the participant is required to recall the position of a pattern from a number of identical boxes on the screen. The task progressively increases in difficulty. In the adapted version used for this study the first two stages require one pattern to be recalled with six possible options. The 3rd and 4th stage have two patterns and six boxes; the 5th and 6th stages have three patterns and six boxes. The 7th stage has six patterns and six boxes and the final 8th stage has eight patterns and eight boxes. At each stage the participant is allowed a maximum of ten trials to make the correct response. The task is terminated if ten trials are made unsuccessfully or if the participant completes all eight stages. The main outcome of interest was the “First Attempt Memory Score” (PALFAMS) which is a measure of the number of patterns correctly located in the first trial of each attempted stage. Higher scores for PALFAMS indicate better performance.

The PAL task is a measure of episodic memory which is dependent on hippocampal neuronal pathways²⁶⁷. Reducing hippocampal synaptic plasticity via blockade of NMDA receptors or compromising hippocampal neuronal activity via blockage of AMPA receptors have been implicated in poorer encoding and retrieval in PAL tasks²⁶⁸.

Semantic Verbal Fluency

Semantic verbal fluency was assessed using a category naming task. The participant was asked to name as many different animals as they are able in one minute. Age and sex variations of the same animal were counted as unique. For example, puppy and dog would count as two animals, as would cow and bull. The main outcome is the number of unique animals named, which was converted into an adapted score based on the validated frontal assessment battery scoring matrix²⁶⁹. Higher scores indicate better performance. Verbal fluency measures are commonly used in neuropsychological test batteries, including the Cambridge Cognitive Examination adapted for individuals with Down Syndrome (CAMCOG-DS), and have been included in test batteries for adults and children with DS^{146,270,271,272}.

Verbal fluency is a measure of verbal ability and executive function, particularly inhibitory control, monitoring, and set-shifting²⁷³. Verbal fluency tasks have been shown to distinguish between patients with dementia and cognitively intact controls²⁷⁴.

Short Adaptive Behaviour Scale

The SABS is a 24-item questionnaire adapted from the longer Adaptive Behaviour Scale: Residential and Community (part 1)²⁷⁵. It has good internal reliability and is highly correlated to the original scale²⁷⁶. It assesses everyday skills and abilities across three domains: personal self-sufficiency, community self-proficiency, and personal-social responsibility. The total combined score was used as this has the lowest floor and ceiling effects compared to using the domains individually in people with DS²⁴⁴. Informants are required to answer two types of statement. In one they state the highest level of a task the participant usually performs. For example for bathing the top level is “prepares and completes bathing unaided”, the lowest level is “makes no attempt to wash or dry self”. In other questions a binary “yes/no” is required,

for example “Hangs up clothes without prompting”. Higher scores on the SABS are indicative of stronger adaptive abilities.

CAMDEX-DS

The CAMDEX-DS is administered as an interview with an informant with close knowledge of the participant and has been well-validated for use in the DS population^{146,261}. It identifies change across five domains which correlate to symptoms associated with dementia onset: everyday skills; memory and orientation; other cognitive skills (general mental functioning, language, perception, praxis, and executive functions); personality and behaviour; and self-care. For each question the informant is asked whether a change has been observed from the person’s “best ever” level of functioning. Any deteriorations are then further classified as either a “great” or “slight” deterioration. The CAMDEX-DS is primarily a clinical tool used in partnership with clinical assessment to identify dementia in people with DS. There is no threshold score which is diagnostic for dementia based on the interview alone, however higher scores indicate cognitive, functional and behavioural changes which may be consistent with dementia. For this analysis the presence of dementia-related changes were identified and coded. A “great decline” scored two points and a “slight decline” scored one point. This approach provided an estimate of dementia symptom load for each participant at the time of assessment compared to their own baseline abilities. In contrast to the other measures used in this analysis, higher CAMDEX-DS scores are indicative of increased AD symptomology and poorer function.

3.4.4. Biomarker Analysis

3.4.4.1. Plasma Sample Collection and Storage Procedures

Venepuncture of the antecubital fossa was performed on the same day as neurocognitive assessment. Plasma samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes using a closed Vacutainer system with a butterfly needle. EMLA cream was provided at participant request. After blood draw, vacutainers were inverted 8-10 times prior to labelling and packaging. Samples were centrifuged at $2000 \times g$ at 4°C for 10 min, aliquoted into 250ul cryotubes and stored at the Social, Genetic and Developmental Psychiatry (SGDP) Centre, King's College London at -80°C until analysis. No samples were exposed to additional freeze-thaw cycles. All reasonable efforts were made to ensure samples were processed within a target of approximately three hours to reduce degradation although studies have shown these biomarkers to be a relatively stable in blood samples^{277,278}.

3.4.4.2. Biomarker Outcome Measures

Plasma biomarkers were used in exploratory analyses. Established plasma biomarkers related to cognition and AD were selected based on their potential associations with cardiovascular health and their relationships with AD pathology. A focus was on those with potential clinical utility. This was determined by whether they could be reliably measured using commercially-available automated SIMOA methods. The primary biomarkers analysed were neurofilament light (NfL), Glial Fibrillary Acidic Protein (GFAP), tau phosphorylated at threonine 181 (p-tau181) and the amyloid-beta peptides: A β 40, A β 42, with calculated A β 42/40 ratios. Amyloid-beta, tau and NfL form the core "ATN" pathology framework that is recommended for use in AD diagnostics¹⁵⁹. Additional analyses using eight components of the complement system

were performed to consider the relationship between inflammatory pathways, cardiovascular risk and AD in people with DS. Descriptions of the biomarkers and rationales for their inclusion as outcome measures are outlined below.

Neurofilament Light

NfL is one of four subunits that make up neurofilament, an axonal cytoskeleton protein which contributes to the structural integrity of neurons¹⁶⁰. When a neuron is damaged, NfL is released into the CSF and blood stream. In a meta-analysis based on studies of AD in the general population, plasma and CSF NfL measures were found to be well correlated ($r=0.6$, 95% CI 0.5 – 0.7)²⁷⁹. In the same study, NfL was shown to negatively correlate with MMSE score ($r=-0.3$, 95% CI -0.4 - -0.2). NfL has been correlated with markers of cerebral hypometabolism, particularly in people with existing amyloid-beta deposition, and is accepted as a proxy measurement of neurodegeneration²⁸⁰. Work in familial AD suggest that NfL may have particular benefit in identifying pre-symptomatic mutation carriers and has been found to be raised as early as 10 years before expected age of symptom onset, as well as being higher in those with AD^{167,281}.

Similar findings have been replicated in people with DS, and NfL measurements have been reported as having both diagnostic and prognostic value. In a multi-centre validation study, NfL was found to differentiate between DS-AD and cognitively intact participants. Sensitivity was reported as 100% with 71% specificity in distinguishing the two groups¹⁵³. In a study of 24 adults with DS compared to 24 controls, NfL measured using SIMOA techniques was significantly higher in people with DS across age groups. Plasma NfL was not correlated with cross-sectional functional assessment outcomes in this small sample, but higher levels were associated with greater declines in adaptive functioning scores repeated 12 months later²⁸². In

work leading up to this thesis, the correlation between NfL and clinical dementia diagnosis in the LonDowns cohort was established (*see Pape et al, 2021*)²⁸³.

In summary, NfL is a non-specific marker of neurodegeneration and can provide information about disease and health states that may promote neuronal injury.

Glial Fibrillary Acidic Protein

GFAP is an intermediate filament protein expressed primarily by astrocytes in the CNS¹⁶⁸. It is coded by the *GFAP* gene on chromosome 17. It has roles in stabilising mechanical properties of astrocytes. GFAP is most widely expressed in the medulla and hippocampus of the brain. It has been implicated in maintaining the structural integrity of the blood brain barrier (BBB) and potentially myelination processes on neurones^{169,170}. Elevated plasma levels of GFAP have been identified following acute neuronal injury, in neuroinflammatory diseases, and as a marker of reactive gliosis¹⁷¹. Increased numbers of GFAP positive cells have been identified in foetuses with DS²⁸⁴. In people with DS-AD, GFAP has been identified as a potential AD biomarker alongside NfL and A β proteins, increasing with age and disease state¹⁹⁰.

GFAP is therefore a protein of interest when defining hippocampal-mediated memory pathways, considering the impact of vessel integrity in the CNS, identifying neuronal degeneration and inflammation, and processes specific to Down syndrome.

Amyloid-beta Peptides 40 and 42.

A β is a key protein implicated in the pathogenesis of Alzheimer's dementia. It is strongly associated with Down syndrome due to the triplication of *APP*, a gene located on chromosome 21⁸⁰. Higher levels of plasma levels of A β have been identified in people with DS compared to

controls and plasma A β levels have been associated with cognitive performance in people with DS, independent of dementia status²⁸⁵. Changes in plasma A β levels have been associated with dementia in the general population and in people with DS²⁸⁶. There are two primary A β proteins considered in AD, A β 40 and A β 42. These are of different fragment length and formation is based on cleavage of APP. In healthy states APP processing predominantly produces the peptide A β 40. Altered metabolism of APP with a shift towards longer peptides and increased A β 42 production has been suggested as an important mechanism in the progression of AD, promoting amyloid-beta aggregation and deposition. The relationship between plasma A β proteins and dementia status remains poorly defined with conflicting results presented²⁸⁷. A 26% selective increase in A β 42 levels, but not A β 40, have been found in DS-AD compared to cognitively intact individuals²⁸⁸. Higher values of plasma A β 40 and A β 42 have been associated with estimates of over two-fold increased risk of incident dementia in DS²⁸⁹. Raised levels of plasma A β 40 and A β 42 may therefore be indicative of increased dementia risk, and be associated with poorer cognitive performance in people with DS. On the other hand, reductions in A β 42 levels have also been detected in people with DS with age, whereas markers such as NfL have been found to increase²⁹⁰. A β 42 levels may also vary dependent on cerebral amyloid angiopathy (CAA) status, with moderately lower levels of A β 42 associated with the presence of a moderate level of CAA compared to mild or absent CAA²⁹¹. Measures of amyloid-beta have shown mixed correlations with neuropsychological tests, despite the associations between amyloid-beta deposition and progression of AD^{91,292,293}.

Relative ratios between amyloid-beta peptides may provide more robust prognostic and diagnostic markers of amyloid-beta pathology and AD. Values of the A β 42/40 ratio decrease in people with AD, and an area under the curve (AUC) of 61% for A β 42/40 in distinguishing people with high AD-related neuropathological changes from people with low-changes has

been estimated. In familial AD caused by *PSEN1* mutations, a combined ratio based on the full spectrum of A β peptides (shorter peptides 37, 38 and 40 to longer peptides 42 and 43) has shown a strong linear correlation with age of AD onset²⁹⁴. However there are concerns that current analytical techniques to measure plasma A β may not be sensitive enough. In a recent cross-sectional review of biomarkers in DS-AD a reduction in CSF A β 42/40 was observed from around age 28-30 years which was not observed in plasma until age 40 years¹⁹⁰.

Measuring A β levels is a way to determine amyloid-beta load and detect potential amyloid processing deficits in individuals, including younger people, despite being less clearly correlated to clinical symptoms of dementia. This is useful for the current study when the focus is on exploring mechanistic pathways that may link cardiovascular health status and risk, rather than using biomarkers to diagnose dementia. Using this combination of biomarkers may therefore provide information about amyloid-beta status and cognitive health across adulthood.

Phosphorylated Tau

Tau is protein associated with microtubules. It forms the core component of neurofibrillary tangles (NFTs), a hallmark of AD. Tau activity is regulated via phosphorylation: hyperphosphorylation reduces its activity²⁹⁵. Tau is involved in maintaining the structural integrity of microtubules. In AD there is a much greater burden of cortical p-tau which polymerises into paired helical filaments leading to the formation of neurofibrillary tangles. The deposition of A β promotes this abnormal hyperphosphorylation, and increased tau sequestration in NFTs occurs later in the pathophysiological progression of AD^{296,297}. Markers of phosphorylated tau have shown to discriminate between AD and other forms of neurodegeneration. Plasma p-tau181 in particular has been identified as a robust marker of AD in the general population²⁹⁸. In DS it has been found to correlate with markers of cerebral

atrophy and hypoperfusion²⁹⁹. Raised plasma p-tau181 levels are associated with clinical symptoms of dementia, but the increases are observed later in the disease progression than other markers^{300,301}.

The relationship between p-tau and DS prior to dementia onset has not been established. Total tau appears to decrease from childhood into young adulthood before increasing again in later years, but patterns of p-tau were not reported²⁹⁰. P-tau 181 can therefore be considered a marker of dementia severity, with higher levels in older adults reflecting greater neuropathological burden of disease.

Complement Components

Inflammatory processes have been suggested to interact with neurodegeneration and AD in people with DS. For example, plasma IL-6, a pro-inflammatory cytokine, has been found in higher concentrations in DS, with a positive correlation to t-tau levels¹⁹⁴. Complement components represent a key factor of inflammatory pathways and have been identified as dysregulated in both DS and AD. Autopsy-based studies have located the complement components C1q and C3 alongside amyloid-beta plaques and hippocampal neurones in the brains of people with dementia providing evidence of classical complement cascade activation^{116,117}. Inflammation has been implicated in the dementia pathway and this appears to be both through amyloid-independent pathways and by exacerbating amyloid-beta deposition and neuropathology^{302,303,304}. Similarly CVPs interact with inflammatory processes. Obesity has been linked to systemic inflammation, while physical activity has been found to modulate inflammatory pathways³⁰⁵. In recent work we showed that complement is significantly dysregulated in people with DS (*Veteleanu, Pape et al, 2022*)³⁰⁶. There were changes related to AD in people with DS. Lower C3 and FI were detected in people with DS

and dementia, with trends towards lower clusterin and increased C1q components. Measuring complement can hence provide further information about how CVPs, inflammation and AD pathology interact in people with DS.

Eight complement components were measured: C3, C4, clusterin, FH, FI, CR1, C1q, and C1inh. These were selected to represent key components of the complement system that have been implicated with AD pathology. They are broadly grouped into complement components (C3, C4, C1q) and regulators (FH, FI, Clusterin, C1inh and CR1). The complement components analysed, their functions, and relationships with Alzheimer's disease are summarised in table 3.

Component	Role in Inflammation	Relationship to AD
C1q	Activator of classical complement pathway	Increased in AD. Can be co-localised with amyloid-beta plaques and hippocampal neurones in people with DS-AD, associated with reactive astrocytes and activated microglia ^{116,183} .
C3	Complement protein integral to classical, alternative, and Mannose-Lectin binding pathways. Activation leads to triggering of opsonisation of pathogens, phagocytosis and formation of TCC.	Increased expression and activation in brains and CSF of people with AD ³⁰⁷ . Has been identified localised with amyloid-beta plaques. C3 deficiency has been suggested as protective against age-related synaptic loss ³⁰⁸ . Deficiency is linked to accelerated amyloid-beta deposition and neurodegeneration ^{184,309} . Plasma C3 is reduced in AD compared to MCI ³¹⁰ .
C4	Complement protein activated early in the cascade by C1q. Combines with C2 to form C3 convertase on membrane surfaces.	May be co-located with amyloid-beta plaques in brains of people with AD ¹¹⁷ .
FH	Regulator protein that works as a cofactor for FI to promote the decay of C3 convertase and inhibit complement activation, protecting human cells.	Has been found to be predictive of conversion from MCI to AD in adjusted models ³¹⁰ . Increased in MCI compared to controls, decreased in AD compared to MCI ³¹⁰ .

FI	Regulator protein found on host cell surfaces. Interacts with C3 to deactivate it and prevent host-cell injury from complement cascade.	Levels have been linked to predication of brain atrophy. Decreased in AD compared to MCI.
C1inh	Inhibits initial activation of classical and Mannose-binding leptin pathways. Reduces the consequences of complement activation.	Reduced stimulation in response to pro-inflammatory cytokines in amyloid-beta plaques with disassociation between complement regulators and activators in plaques ^{311,312} .
CR1	Membrane receptor for activated C3 (C3b). Involved in clearance of immune complexes. Inhibits the activation of classical and alternative pathways.	GWAS studies have implicated isoforms of CR1 gene with AD risk ^{313,314} .
Clusterin	Multi-functional protein. Interacts with TCC to inactivate it and reduce lytic activity (regulator of terminal complement pathway)	GWAS studied have implicated gene in AD risk (third most commonly associated risk gene for late onset AD) ^{314,315} . Predictive of AD when used in model with relevant comorbidities (higher in AD) and of progression to MCI in a multivariate model ³⁰³ . Higher levels have been linked to AD ³¹⁶ . Has been detected co-located with amyloid-beta plaques in DS ¹¹⁷ .

Table 3. Roles of complement components and their associations with Alzheimer's disease.

3.4.4.3. SIMOA Techniques and Assay Protocols

SIMOA Background

Single-molecule array (SIMOA) is a two-step digital immunoassay. It is ultra-sensitive with the ability to detect proteins with very low concentrations in the blood. Sensitivity to proteins at concentrations of 10fg/ml – 1pg/ml are reported, a much greater degree of sensitivity compared to other commonly used immunoassay techniques³¹⁷⁻³¹⁹. For NfL the lower limit of detection using SIMOA is 0.038 pg/ml and the lower limit of quantification is 0.174 pg/mL. Within-run reproducibility precision coefficient of variation (CV) is estimated to be 6.9%, with repeatability precision of 6.1%. Analytical sensitivity has been calculated as 0.62 pg/mL for SIMOA, compared to 78.0 pg/mL for enzyme-linked immunosorbent assay (ELISA)³¹⁹.

In brief, microscopic paramagnetic carboxylated beads coated in antibodies are incubated with samples and detector reagent. Molecules of interest in the sample are captured by the antibody-coated beads, binding simultaneously with the detector reagent. The immunocomplex is washed and then incubated with reagent which binds to the anti-protein antibodies in the detector reagent and results in enzyme labelling of molecules. This is followed by washing and addition of further reagent before transfer to the SIMOA disc. At this stage the beads and immunocomplexes are dispersed into microwell reaction chambers, with beads sealed into individual microwells. In the event that a molecule is captured and labelled the reagent hydrolyses and fluoresces. The fluorescence is measured using a fully-automated fluorescent microscope. As each chamber holds only one bead, the number of wells detected is proportional to the number of molecules in the sample. A calibration curve using average enzyme per bead (AED) values from calibrators provided with the SIMOA kits is derived and used to extrapolate the concentration of protein in the samples.

SIMOA has been used extensively for the measurement of neurofilament light, GFAP, p-tau, and A β proteins in a range of conditions, including Alzheimer's disease³²⁰⁻³²³. The intra and inter-assay precision of SIMOA has been well-reported, with good reproducibility of results.

NfL Assay Protocols

I performed in-house immunoassays using a SIMOA® NF-Light™ Advantage Kit (Quanterix™) on a HD-1 analyser at the Maurice Wohl Clinical Neuroscience Institute, King's College London. A standardised protocol was followed. Samples and calibrators were equilibrated to room temperature for 1 hour. Samples were subject to a 1:4 at bench dilution, using the provided sample diluent and shaken. 200ul of diluted sample was added to a 96 well SIMOA plate. The RGB vial was placed in a heated shaker for 30 minutes at 800rpm. Beads were spun for 30 seconds. Beads, SBG, RBG and detector vials were added to the analyser, followed by the plate. Calibrators were run on the same plate (182ul per well per calibrator), with the analysis completed in a single run. Both samples and calibrators were run in duplicate. The plate was sealed to reduce sample evaporation.

Amyloid-beta Proteins, p-tau181 and GFAP Analyses

Amyloid-beta proteins, p-tau181 and GFAP were measured using equivalent automated SIMOA methods (Neuro 4-Plex-E Assay Kit by Quanterix™) by collaborators at the neurochemistry lab, University of Gothenburg. The same EDTA plasma samples were used for all assays. Additional NfL samples were analysed in the same run, with duplication of samples between sites to allow for comparative analyses to be made.

Genome Analysis

Trisomy of chromosome 21 was confirmed genetically using blood or saliva samples. After DNA extraction, genome-wide SNP genotyping was performed using an Illumina

OmniExpressExome array v1.2-1.4 at University College London Genomics. *APOE* status was determined using TaqMan assays for rs7412 and rs429358 (ThermoFisher Scientific). Participants were classified as $\epsilon 4$ carriers if they had $\epsilon 3:\epsilon 4$ or $\epsilon 4:\epsilon 4$ genotype.

Complement Processing

Frozen plasma EDTA samples from a subset of participants were sent on dry ice to Professor Paul Morgan at the University of Cardiff. Complement measurement was performed by collaborators in his lab using enzyme-linked immunosorbent assays. The techniques have been validated in a comparison study between people with DS and adults from a general population sample. The processing methods used by the collaborators are outlined in *Veteleanu, Pape et al, 2022*³⁰⁶.

3.4.4.4. Data Calibration and Quality Considerations for Biomarker Assays

To reduce potential bias during sample processing the original participant identifiers were randomly assigned to a unique sample identification code, and these codes were randomly distributed around the sample preparation plate. All researchers performing the immunoassays were blind to participant details during the processing of the samples. After completion of the immunoassay the sample identification codes were matched back to their original participant identifiers to allow linkage with demographic, medical, neurocognitive and physical health data.

Automated SIMOA assays are robust and there is good reproducibility of measurements between different sites and instruments. NfL samples were run across two different sites. Five samples were processed in both runs to allow for comparisons to be made between SIMOA

machines to ensure consistency. Correlations between these duplicated values were calculated, and a correction applied to the samples if there was a consistent difference in output. This approach has been validated in numerous comparative studies, including a study of NfL levels in people with DS, with samples processed at the same two sites¹⁵³.

Data Quality Checks

Following processing, sample values were checked to ensure they were above the lower limit of quantification for SIMOA. Calibration curves were plotted and intra-assay CV calculated using the formula: $CV = \left(\frac{SD}{\bar{x}} \right) * 100$. Acceptable CV was set at $\leq 10\%$ variation. Samples found to have a CV of greater than 10% were assessed against the mean and the duplicate value with the smallest variation was included in the analysis.

Data were checked for normality by applying Shapiro-Wilks normality tests and graphically on P-P plots alongside measures of skewness and kurtosis. Non-parametric tests and mathematical transformations using natural log were applied for variables not meeting criteria for normality as appropriate. Outliers were identified as values ± 3 SD from the mean. These data points were individually assessed for possible bias.

Sample Size Calculations

To estimate sample size for regression models using CVP factors, exploratory analysis using existing LonDowns data was performed. Effect size was estimated using correlations for blood pressure (SBP and DBP), BMI, physical activity and NfL levels and inputted into the G Power software tool. A sample size of $n=80$ would have power of approximately 0.90 and a sample size of $n=99$ participants would yield power of 0.95 for linear regression using these variables.

To estimate group sizes for biomarker analyses, NfL changes were used as a guide as there is the most consistent research in NfL changes in people with DS. Data available from *Mengel et al* provided exact serum NfL values for a sample of 100 people with DS²⁹⁰. Mean NfL was 10.316 pg/ml (SD 9.31). Considering estimates for the minimum expected change in serum NfL between groups, *Fortea et al* measured median plasma NfL as 5.88 pg/ml in asymptomatic people with DS, 15.39 pg/ml in preclinical dementia, and 23.04 pg/ml in AD (average of 8.58 pg/ml between groups)²⁰⁹. *Strydom et al* found a greater difference: median levels of 19.96 pg/ml in those without dementia and 63.76 pg/ml in those with dementia at baseline²⁰⁸.

There is limited data available looking at the effect of modifiable factors on biomarker levels to provide information about expected change between groups. *Evers et al* used NfL as a predictor of pre-eclampsia and found an average difference of 10.5 pg/mL between people with pre-eclampsia and controls, and this could be a guide for considering the impact of blood pressure on NfL³²⁴. A-priori power calculations were made to estimate minimum sample sizes using the G Power software tool^{325,326}. The smallest of the above values from the literature was inputted, with a minimum expected change of 8.58 pg/ml between groups used in the calculation. With significance level set at 5% and 80% power, the minimum group size would be $n=19$ to adequately power the analyses.

3.4.5. Statistical Analysis

Cross-sectional analysis was performed to determine the relationship between age, cognitive outcomes (PALFAMS, VF, SABS and CAMDEX scores), CVPs (SBP, DBP, PP, BMI, AC) and plasma biomarkers (NfL, GFAP, p-tau181, A β 40, A β 42, A β 42/40) using Pearson

correlations with two-way significance set at $p < 0.05$. Chi square comparisons were performed for categorical variables (exercise levels and diagnostic terms). Sex, age and exercise group differences were compared using two-tailed independent t-tests. Patterns of CVPs with age were visualised graphically using scatter plots, with lines of best fit and 95% confidence intervals plotted. Multivariate analysis models were constructed to compare CVPs against age.

Initially the whole sample was analysed together. The sample was then stratified into those below 35 years and those 35 years and older. This was to study the associations present prior to significant amyloid-beta deposition, in comparison to older individuals. Poisson regression models were constructed with each CVP value as a continuous primary predictor variable; cognitive outcomes and plasma biomarkers were inputted as the dependent variables. Age, sex, and level of ID were included as covariates. Binary logistic analyses were used to compare cognitive and biomarker outcome measures between binary risk groups identified in the earlier analyses (the groups compared were based on CHD, exercise level and hypothyroidism). Age, sex, and level of ID were used as covariates.

Associations between CVPs, blood based biomarkers of neurodegeneration (NfL), amyloid-beta ($A\beta_{40}$ and $A\beta_{42}$), AD pathology (p-tau181), neuroinflammation (GFAP), and cognition were investigated. The aim was to explore possible mechanistic pathways to explain any relationship between CVPs and incident dementia risks identified in the preceding population analyses. A combined risk group was created based on the number of risk factors present and inputted separately into the models. Effect size was assessed using partial eta squared values.

In the whole sample and older cohort analyses both cognitive and biomarkers were assessed. In the younger cohort the focus was on biomarker measures as outcomes only. Cognitive

outcomes were not included for the younger adults due to the lack of expected decline in cognitive measures in younger cohorts and possible confounding effects between experience-related improvements in cognition with age, and ageing-related declines. Plasma biomarkers hence represent a more objective measure of neuropathology in this younger group and may provide information about pathological processes prior to the onset of cognitive changes.

Longitudinal analyses were then undertaken in a subgroup of participants who had baseline CVP and cognitive data available. The historical assessment was referred to as the baseline assessment. The most recent assessment (which was used in the cross sectional analysis above) was referred to as T1. The CVP values at baseline were used to evaluate the longer-term impact of cardiovascular health on cognitive and biomarker outcomes at T1 using predictive regression models. A combined CVP-associated risk group was created to determine cumulative effects of multiple-morbidity on cognitive outcomes between time points. Age, time between assessments, level of ID, sex, *APOE*, and presence of dementia were entered as covariates with CVP risk group at baseline as the predictor variable. Effects of CVP on change in cognitive outcome between the assessments was assessed based on the F statistic with effect size considered using partial eta squared measures. Post-hoc Bonferroni corrections were applied to assess between group differences. *APOE* status was included due to the association between presence of $\epsilon 4$ alleles and earlier dementia onset in the general population and people with DS⁹³.

In a small subgroup ($n=17$), NfL measurements from additional follow-up assessments were available. Repeated measures ANCOVAs were constructed to identify the association between risk group at baseline and change in NfL. Greater increases in NfL would suggest greater neurodegeneration over the time period.

Exploratory principal component analysis (PCA) was then performed at a whole sample level to support hypothesis generation for underlying mechanisms of dementia risk associated with CVPs. PCA was selected due to the large number of variables and multicollinearity in the analyses. The data included were the primary CVPs (SBP, DBP, PP, BMI), the plasma biomarkers (NfL, GFAP, p-tau181, A β 40, A β 42, A β 42/40), and the cognitive outcome measures (PALFAMS, VF, SABS, CAMDEX). PCA was based on a correlation matrix with standardisation and orthogonal rotation³²⁷. The number of principal components were selected based on an Eigenvalue >1 and a minimum cumulative proportion of variance of 75%. PC scores were visualised on a score plot colour-coded on dementia status, alongside a loading plot for the first two PCs. Variables with loadings >0.5 were considered significant contributors to a PCA^{233,328}. Logistic regression using the derived principal components was performed to assess predictive power of variables on dementia status.

Lastly complement proteins were evaluated in an exploratory analysis of the relationship between CVPs and inflammatory pathways, repeating the above group comparisons and PCA methods.

3.5. Reporting of Results

Results were presented in accordance with the RECORD Statement (*Reporting of studies Conducted using Observational Routinely-collected health Data*). This guideline has been adapted from the STROBE statement (*Strengthening the Reporting of Observational Studies in Epidemiology*). RECORD was created to outline the information required for research

reports using routinely collected clinical data to improve transparency and reduce inaccuracies or missing data in such research²¹¹.

Chapter IV

Analysis of Cardiovascular Health Markers in People with Down Syndrome and Their Relationship to Incident Dementia Risk Using Electronic Primary Care Records

Part A. Comparison of Cardiovascular Profiles Between People with Down Syndrome and General Population Controls

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4.1. Summary of Methods and Aims

In the general population, the presence of cardiovascular risk factors including hypertension, obesity, diabetes mellitus and high cholesterol are associated with increased dementia risk, both for vascular dementia and Alzheimer's disease (AD)²⁰⁰. In addition it is estimated that up to 84% of people with Alzheimer's disease also have cerebrovascular disease, with mixed AD and vascular pathologies increasingly recognised as being a common neuropathological finding in dementia³²⁹. In contrast, in Down syndrome (DS) the development of AD is amyloid-driven, primarily through the triplication of the *APP* gene on chromosome 21 which promotes increased amyloid-beta protein production and deposition^{12,13,80}. People with DS often lack other traditional vascular risk factors, and in particular have low levels of atherosclerosis and experience hypertension less commonly^{60,111}. Defining the relationship between cardiovascular risk factors and dementia in people with DS is therefore an opportunity to understand how these factors influence dementia onset in a relatively "pure" amyloid model, as well as a way to identify potentially modifiable risk factors for dementia onset in people with DS. This in turn can help to elucidate mechanisms of Alzheimer's dementia pathology, consider how cardiovascular risks interact with amyloid-beta pathology, and improve risk management strategies for people with DS.

People with Down syndrome are reported to have certain cardiovascular characteristics. They tend to have relatively low blood pressure with blunted autonomic responses, higher BMIs, and deranged blood lipids^{55,58,330,331}. Despite these observations, detailed information about the average cardiovascular profile in people with Down syndrome and its pattern over the adult lifespan is lacking, with most work having been carried out in children and adolescents. My

hypothesis to be tested in this chapter is that adults with DS have a unique cardiovascular profile that is distinct from the one described in the general population.

In order to explore the associations between cardiovascular health profile and dementia in Down syndrome it is vital to first understand the cardiovascular profile in people with DS. A population-based cross-sectional analysis using electronic UK primary care health records extracted from the Clinical Practice Research Datalink (CPRD) was performed to define the cardiovascular profile of people with DS and compare this to a general population control sample. The incidence of relevant cardiovascular comorbidities were calculated for both the Down syndrome cohort and control group. Raw values for eight cardiovascular health parameters (CVP) were then used to map the mean values across the lifespan, with additional analyses to establish the effects of age and sex in each population.

4.2. Sample Description

4.2.1. Data Extraction

In accordance with the definitions proposed in the RECORD document, the source population was made up of all patients accessing general practices in the UK; the database population consisted of all those individuals included within the CPRD GOLD database: the sample population was made up of those patients identified based on the relevant read codes²¹¹.

At the time of data extraction there were available records for 19,018,786 individuals who met data quality standards for inclusion. This represents approximately 4.8% of the UK population. The median available follow up time was 5.61 years (IQR 2 – 13). The total number of GP practices contributing data was 927 between England, Scotland, Wales and Northern Ireland.

10,204 of these patients were identified as having Down syndrome, based on diagnostic read codes. These were matched up to a 4:1 ratio to 39,814 controls based on sex, year of birth and GP practice (see figure 4).

Children, classified as people below 18 years old at cohort entry, were excluded from the sample. This left an adult cohort of 6,424 people with Down syndrome and 23,143 controls across 859 GP practices. Year of birth ranged between 1926 and 2001 for both groups. There were 51,044 person years of follow up in the Down syndrome group, with length of registration ranging between 1 and 30 years, median 8 years (IQR 4-15). For the control group there were 247,062 person years of follow up, with registration period also ranging between 1-30 years, median 11 years (IQR 5-17).

The Down syndrome cohort consisted of 2,847 males (44.3%) and 3,577 females. The control cohort had 10,294 males (44.4%) and 12,849 females. The majority of patients with Down syndrome were coded with read code “Down’s syndrome – trisomy 21”. “Down’s syndrome NOS” and “Trisomy 21” were the next most commonly used (see table 4).

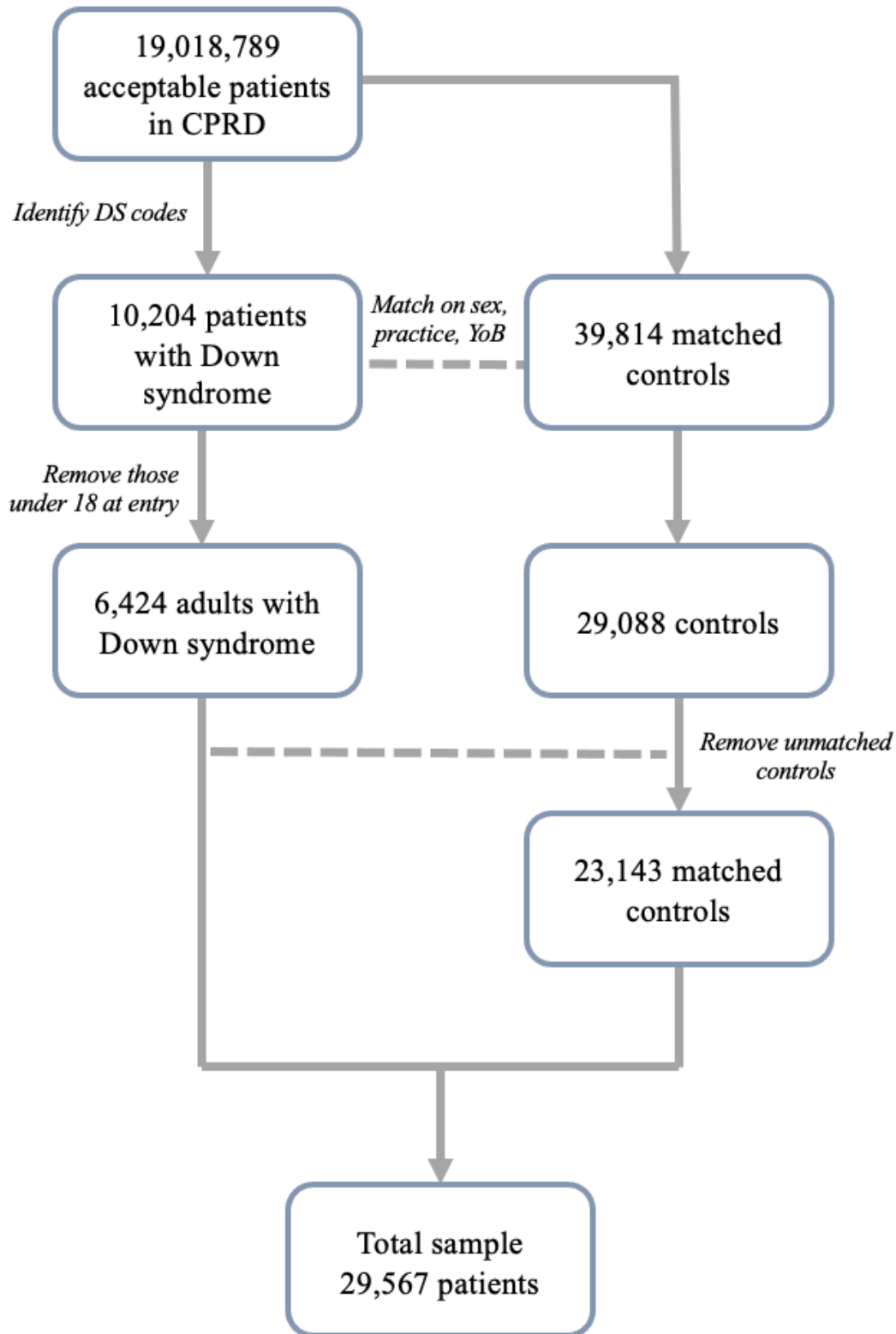


Figure 4. Flowchart describing sample selection for the population analysis.
YoB = year of birth.

Read Code	Frequency	Percent
Down's Syndrome – Trisomy 21	5475	85.2
Down's Syndrome NOS	720	11.2
Trisomy 21	103	1.6
Mongolism	86	1.3
Trisomy 21 NOS	18	0.3
Trisomy 21, Mosaicism	11	0.2
Trisomy 21, Translocation	5	0.1
Partial Trisomy 21 in Down's Syndrome	3	0.05
Trisomy 21, Meiotic Nondisjunction	3	0.05
<i>Total</i>	<i>6424</i>	<i>100</i>

Table 4. Frequency of Down syndrome diagnostic codes used to identify the DS cohort.

4.2.2. Available Measurements for Cardiovascular Health Parameters

Values of eight variables associated with cardiovascular health status were extracted from the CPRD data set. PP was calculated from SBP and DBP values. The CVPs used were: systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), body mass index (BMI), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (Tg) and glycated haemoglobin (HbA1c). Frequency counts and percentage of total sample were calculated for each CVP (see table 5 and figure 5).

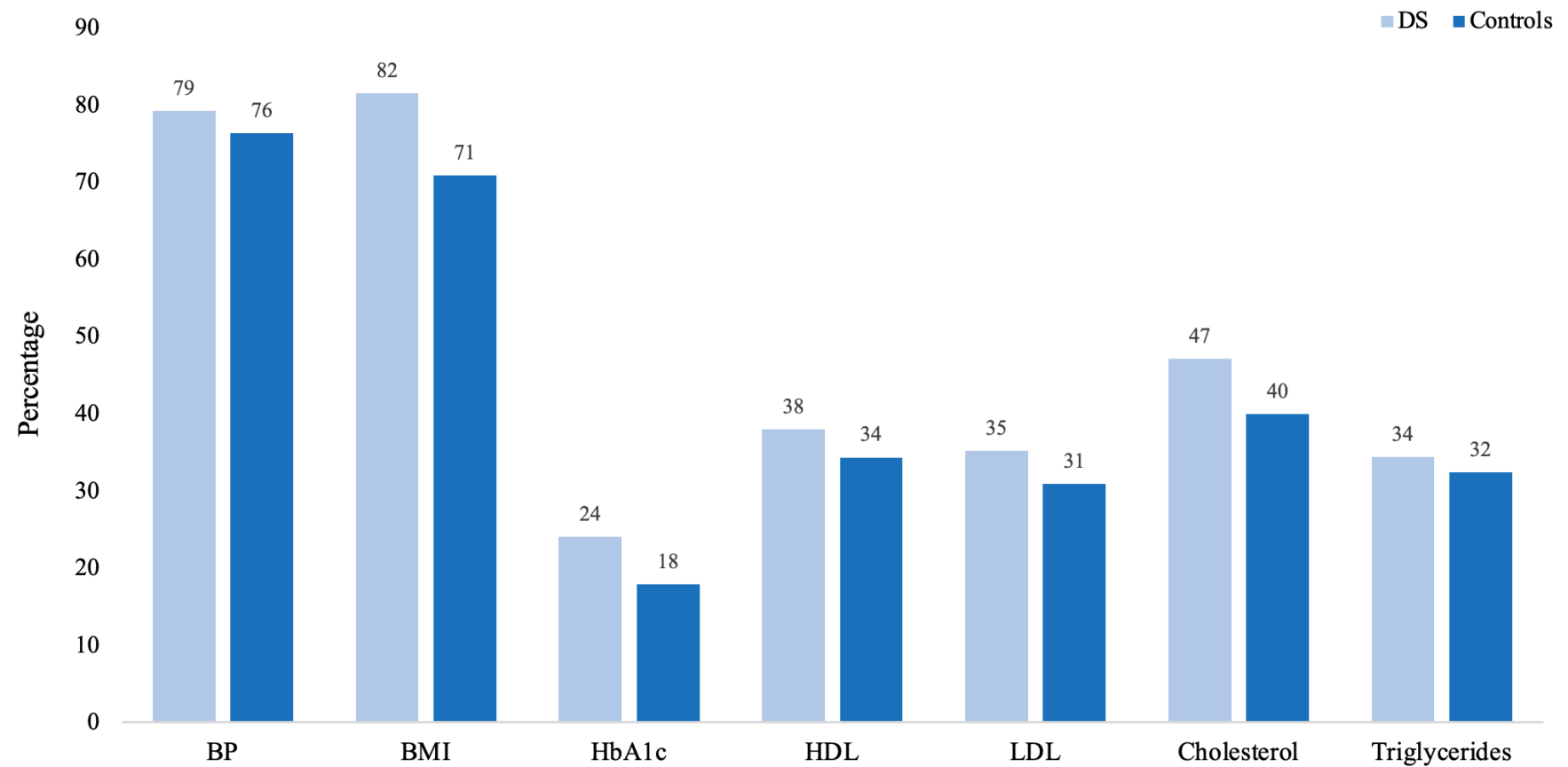


Figure 5. Clustered bar chart showing percentage of sample with available measurements for each cardiovascular health marker

	DS Cohort, <i>n</i> (% DS sample)	Control Cohort, <i>n</i> (% control sample)
Total	10204	39814
Adult	6424	23143
GP Practice	859	859
Male	2847 (44.3)	10294 (44.5)
Female	3577 (55.7)	12849 (55.5)
BP Available	5087 (79.2)	17660 (76.3)
BMI Available	5236 (81.5)	16392 (70.8)
HbA1c Available	1540 (24.0)	4073 (17.6)
HDL Available	2435 (37.9)	7952 (34.4)
LDL Available	2062 (32.1)	6904 (29.8)
Cholesterol Available	3023 (47.1)	9229 (39.9)
Triglycerides Available	2207 (34.4)	7489 (32.4)

Table 5. Frequency count and percentage of patients with available measurements for each cardiovascular health parameter

4.2.3. Data Quality Checks

Following data extraction the a-priori exclusion criteria outlined in chapter 3 (table 2) were applied for each variable. The values were then re-examined to identify any remaining recordings that were likely to represent errors, extreme outliers or clinically unfeasible results. In the examination of BP, BMI and LDL data, additional extreme values were identified and excluded as outlined below. Table 6 describes the available sample for each variable, and table 7 describes the skew of the data.

Blood Pressure

After application of exclusion criteria the data was examined for outliers and unrealistic values. Four cases were identified for review, one from the DS cohort and three from the control group. In each of these cases it was found that the systolic blood pressure was lower than the diastolic blood pressure which is not within expected physiological norms. These values were excluded from the analysis due to likely data input error.

Body Mass Index

Cases with extreme BMI (<12 or >70 kg/m²) were examined for possible error as these represent clinically uncommon results. Recorded heights and weights of extreme BMIs were checked to confirm the results. There were eleven cases with very low BMI (<12 kg/m²) which were missing a corresponding height or weight value to confirm the low BMI, two from the DS cohort and nine from controls. These were excluded from the analysis due to potential recording error. Similarly there were five cases with BMI >70 kg/m² with either height or weight missing to confirm BMI value and these were also excluded (two with Down syndrome and three controls).

Low Density Lipoprotein

On examination of the data there was significant kurtosis (80.157) in the distribution which was identified to be the result of a small number of extreme outliers. Thirty-six cases with outlying values (greater than three standard deviations from the mean) were identified. These values were likely due to incorrect recording of units (for example some of the values appear to have been erroneously recorded in the units mmol/L when values were within normal range if the older mg/dl units were applied). These 36 values were therefore removed from the analysis. The mean and median were unchanged after the outliers were removed and kurtosis reduced to within acceptable limits.

	Number of measurements	Identified outliers or unfeasible values	Final aggregated sample (DS)	Final aggregated sample (controls)
BP	325,198	4	33,706	135,447
BMI	465,908	16	27,489	86,618
TC	77,113	0	11,227	47,299
HDL	56,667	0	8,277	36,647
LDL	50,100	36	6,447	29,950
Tg	51,948	0	7,500	34,480
HbA1c	39,709	0	4,869	16,687

Table 6. Sample size for each variable in CPRD.

	Down Syndrome Cohort		Controls	
	Skewness (SE)	Kurtosis (SE)	Skewness (SE)	Kurtosis (SE)
SBP	0.462 (0.013)	0.994 (0.027)	0.446 (0.007)	0.6 (0.013)
DBP	0.228 (0.013)	0.773 (0.027)	0.134 (0.007)	0.420 (0.013)
PP	0.551 (0.013)	1.437 (0.027)	0.596 (0.007)	1.113 (0.013)
BMI	0.956 (0.015)	2.965 (0.30)	1.236 (0.008)	3.378 (0.017)
TC	0.628 (0.021)	4.000 (0.041)	0.515 (0.10)	1.597 (0.020)
HDL	1.36 (0.027))	5.59 (0.054)	1.333 (0.013)	3.836 (0.026)
LDL	0.578 (0.030)	1.133 (0.060)	0.458 (0.014)	0.684 (0.028)
Tg	0.351 (0.228)	1.121 (0.453)	1.254 (0.20)	4.243 (0.40)
HbA1c	0.171 (0.163)	1.776 (0.324)	0.278 (0.145)	1.569 (0.289)

Table 7. Normality checks for each variable in CPRD.

Group Comparisons of Recorded Measurements

Given the nature of the data available from CPRD which is real-world clinical data, not every person in a cohort will undergo the same tests or have them at the same time. This is because clinical tests are arranged based on need rather than performed as part of a pre-determined research protocol. To understand any differences between people who had recorded measures and those who didn't, the groups were compared on two key demographics: year of birth and sex. Two-way independent t-tests were used for continuous variables and Pearson's chi square statistic for categorical variables. Significance was set at $p < 0.05$.

In both the DS cohort and controls, those who did not have recorded measurements tended to have a later year of birth which indicates a younger overall group. This is consistent with an increase in health checks occurring as people get older. In the control group this was for every variable. In the DS cohort this was for every variable apart from BMI where year of birth was not significantly different based on whether or not there was a BMI recorded. This may reflect

the fact that BMI measurements are recommended as part of learning disability health checks from the age of 14 years and so take place across adult ages for people with Down syndrome³³².

In terms of sex differences, in the DS cohort there was a greater proportion of females who had records of BP, BMI and LDL measurements and a smaller proportion of females who had records of HbA1c, TC and HDL compared to those who did not have a recording of these variables (table 8). For the control group there was a greater proportion of females with recorded measurements for BP, BMI, HbA1c and TC. There was no difference between the groups for HDL, LDL and Tg (table 9). The sex variation present may be related to recommended checks around pregnancy, childbirth, and menopause. There is also a potential survival effect: women have a longer average life expectancy than men which means they may be more likely to have measurements during their lives and during the GP registration period. Previous research in the general population has found similar sex differences, with men having a 32% lower crude consultation rate in primary care than women. This difference was not accounted for solely by increased morbidity in women or reproductive concerns³³³.

Year of Birth						Sex - Female		
	Measurements		No Measurements		t	Measurements	No Measurements	χ^2
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)		n (%)	n (%)	
BP	1968.2 (14.2)	1967 (20)	1971.8 (19.0)	1972 (32)	7.01*	3415 (56.4)	767 (48.1)	34.85*
BMI	1969.1 (14.4)	1968 (12)	1968.4 (18.1)	1967 (16)	-1.77	3176 (55.6)	1006 (51.9)	7.805*
HbA1c	1970.4 (13.7)	1968 (20)	1968.5 (15.9)	1967 (24)	-5.11*	920 (50.2)	3262 (56.1)	19.74*
TC	1967.6 (13.1)	1966 (17)	1970.1 (17.1)	1970 (27)	7.21*	1884 (45.1)	1716 (49.5)	14.94*
HDL	1968.0 (12.9)	1966 (18)	1969.6 (16.7)	1969 (26)	4.62*	1501 (51.8)	2681 (56.4)	15.94*
LDL	1968.3 (12.9)	1967 (17)	1969.3 (16.5)	1968 (26)	2.70*	1283 (51.5)	1208 (48.5)	14.90*
Tg	1967.9 (12.9)	1966 (17)	1969.5 (16.5)	1969 (26)	4.45*	1355 (51.6)	2827 (56.3)	15.59*

Table 8. Average year of birth and sex distribution for each cardiovascular health parameter in the DS cohort. Independent sample two way t -tests used to compare continuous variables, Pearson's chi square used for categorical variables.

*Significant at $p < 0.05$.

Year of Birth						Sex - Female		
	Measurements		No Measurements		<i>t</i>	Measurements	No Measurements	χ^2
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)		<i>n</i> (%)	<i>n</i> (%)	
BP	1965.7 (14.4)	1965.0 (20)	1977.5 (15.2)	1979.0 (24)	57.32*	13086 (59.0)	2543 (36.9)	1031.87*
BMI	1965 (13.8)	1964 (19)	1976.9 (15.7)	1980 (25)	60.47*	12387 (60.4)	3242 (37.8)	1244.47*
HbA1c	1961.0 (15.2)	1960 (17)	1970.1 (15.2)	1969 (22)	41.85*	2856 (55.8)	12773 (53.3)	10.41*
TC	1960.0 (12.4)	1960 (16)	1974.1 (14.6)	1974 (22)	87.89*	6283 (54.7)	9346 (53.1)	6.86*
HDL	1959.5 (12.1)	1959 (16)	1973.2 (14.8)	1973 (22)	84.79*	5412 (54.3)	10217 (53.4)	1.94
LDL	1959.4 (12.1)	1959 (15)	1972.4 (15.0)	1972 (22)	77.56*	4715 (54.3)	10914 (53.5)	1.88
Tg	1959.3 (12.2)	1959 (16)	1972.9 (14.9)	1972 (22)	82.54*	5037 (53.8)	10592 (53.7)	0.01

Table 9. Average year of birth and sex distribution for each cardiovascular health parameter in the control cohort. Independent sample two way *t*-tests used to compare continuous variables, Pearson's chi square used for categorical variables.

*Significant at $p < 0.05$.

4.3. Cross-sectional Analysis of Cardiovascular Health Markers

4.3.1. Incidence of Cardiovascular Disease Comorbidities

The frequencies of relevant cardiovascular comorbidities were calculated for both the Down syndrome cohort and control group. Mean age at first recorded diagnosis was calculated for each comorbidity. Group comparisons were performed using two-way independent t-tests. Incident rate ratios (IRR) were calculated after removing prevalent cases. Results are presented per 1000 person years with 95% confidence intervals, see table 10.

People with DS had markedly increased incidence of dementia (IRR 37.01 (95% CI 29.00 – 47.24)), as well as epilepsy and stroke which are commonly associated with dementia onset and progression in people with DS (IRR 16.21 (13.77 – 19.08) for epilepsy; 1.77 (95% CI 1.45 – 2.16) for stroke). The significantly increased incidence of dementia, epilepsy and stroke in people with DS is consistent with the known ultra-high risk of Alzheimer's dementia in this population which has an average onset at 55 years^{227,42}. The DS cohort also had high incidence of hypothyroidism (IRR 6.07 (95% CI 5.48 – 6.72)) which is a frequent comorbidity associated with DS³³⁴. These results illustrate the fidelity of the CPRD dataset for research in this population and confirms that the DS sample used in this analysis appears to represent the clinical population in the UK.

Comparing incidence of cardiovascular related disease between the two groups, people with DS had reduced incidence of IHD, hypercholesterolaemia and hypertension over the age of 18 compared to controls (IRR 0.51 (95% CI 0.40 – 0.65) for IHD; 0.55 (95% CI 0.47 – 0.64) for hypercholesterolaemia; 0.24 (95% CI 0.20 – 0.29) for hypertension). There was a similar incidence rate for diabetes (IRR 0.92 (95% CI 0.80 – 1.06)). There was increased incidence of

peripheral vascular disease and obesity in the DS cohort (IRR 1.40 (95% CI 1.11 – 1.76) for PVD; 1.77 (95% CI 1.58 – 2.00) for obesity).

A significantly greater percentage of people with Down syndrome never smoked tobacco compared to controls, with over 80% having never smoked at any point in their lives. This is in contrast to approximately 50% of controls. Similarly only 8.4% of people with Down syndrome were recorded as current smokers compared to 25.5% of controls.

As presented in table 11, apart from epilepsy or seizures, people with DS had a lower average age of first diagnosis for all the examined comorbidities. The average age of first diagnosis of epilepsy in controls was significantly younger than in DS (23.76 (18.6) years compared to 40.73 (18.1) years, t 20.2, $p < 0.001$). This is likely driven by the bimodal onset seen in DS, with an increase in epilepsy incidence in older age as well as in children²²⁷.

These results provide strong evidence that people with Down syndrome have a different cardiovascular morbidity profile compared to the general population. People with DS appear to be at reduced risk of being diagnosed with the common cardiovascular conditions of ischaemic heart disease, hypercholesterolaemia or hypertension, and may be less likely to engage in certain risk behaviours such as smoking. On the other hand people with DS had increased incidence of obesity and peripheral vascular disease.

	Down Syndrome			Controls			Incident rate ratio (95% CI)
	Incident Cases	Person Years	Incident Rate	Incident Cases	Person Years	Incident Rate	
IHD	70	50 874	1.38	662	244 482	2.71	0.51 (0.40 – 0.65)*
Hypercholesterolaemia	193	50 346	3.83	1696	243 083	6.98	0.55 (0.47 – 0.64)*
Hypertension	118	50 401	2.34	2274	234 806	9.68	0.24 (0.20 – 0.29)*
Diabetes	230	49 717	4.63	1225	243 009	5.04	0.92 (0.80 – 1.06)
Hypothyroid	775	43 272	17.91	719	243 600	2.95	6.07 (5.48 – 6.72)*
Obesity	375	48 412	7.75	1052	240 971	4.37	1.77 (1.58 – 2.00)*
PVD	94	50 721	1.85	326	245 580	1.32	1.40 (1.11 – 1.76)*
Stroke	134	50 660	2.65	368	246 115	1.50	1.77 (1.45 – 2.16)*
Epilepsy/Seizures	592	46 199	12.81	191	241 656	0.79	16.21 (13.77 – 19.08)*
Dementia	558	51 021	10.94	73	247 062	0.30	37.01 (29.00 – 47.24)*

Table 10. Incident rate ratios for adults with DS compared to controls. Incident rates are presented per 1000 person years.

*indicates significance of $p < 0.05$ for difference in rates between DS and control cohorts.

IHD = Ischaemic Heart Disease; PVD = Peripheral Vascular Disease.

	Mean Age at Diagnosis in years (SD)					
	DS	Controls	t	Mean Difference	95% CI	p
IHD	46.81 (12.32)	54.26 (9.95)	-7.311	-7.449	-9.45 - -5.45	<0.001
Hypercholesterolaemia	44.89 (11.12)	53.06 (10.13)	-12.003	-8.170	-9.51 – -6.83	<0.001
Hypertension	42.04 (13.33)	50.26 (10.91)	-9.144	-8.216	-9.99 - -9.45	<0.001
Diabetes	39.68 (14.13)	50.26 (13.83)	-14.830	-10.581	-11.98 - -9.18	<0.001
Hypothyroid	37.34 (14.01)	46.74 (12.16)	-19.483	-9.399	-10.34 - -8.45	<0.001
Obesity	35.72 (12.40)	43.25 (13.34)	-13.48	-7.522	-8.62 – -6.43	<0.001
PVD	46.73 (13.34)	49.44 (13.96)	-2.120	-2.711	-5.22 - -0.20	0.034
Stroke	48.03 (13.15)	54.47 (12.92)	-6.261	-6.436	-8.45 - -4.42	<0.001
Epilepsy/Seizures	40.73 (18.10)	23.79 (18.63)	20.393	16.933	15.30 – 18.56	<0.001
Dementia	54.70 (6.30)	67.70 (9.72)	-11.259	-12.999	-15.30 – 10.70	<0.001

Table 11. Average age at diagnosis for comorbidities. Group comparisons performed using 2-way independent t-tests.

IHD = Ischaemic Heart Disease; PVD = Peripheral Vascular Disease.

4.3.2. Comparison of Cardiovascular Parameter Measures Across the Adult Lifespan

4.3.2.1. Whole Group and Sex Comparisons

Two-way independent t-tests were performed to compare values at a whole group level for each cardiovascular parameter (CVP). Male to female comparisons were made for the DS and control groups separately to identify any sex differences in the mean values. As outlined in table 12, at a whole group level the Down syndrome cohort differed to controls for all CVPs. People with DS had lower mean values of SBP, DBP, PP, TC, HDL, Tg, total HbA1c, and HbA1c in people without diabetes mellitus compared to controls. They had higher mean values for BMI, LDL and HbA1c in those with comorbid diabetes. The differences for all groups were significant.

Sex differences were present in both the DS cohort and controls. In the DS group, males had significantly lower mean values of SBP, DBP, BMI, TC, HDL and LDL. Males had higher mean levels of Tg compared to females and borderline higher PP levels. There were no sex differences in HbA1c values in people with diabetes mellitus. In those without comorbid diabetes, males had slightly higher mean HbA1c levels (table 13). In controls, males had higher mean SBP, DBP, BMI, Tg and HbA1c values compared to females and this was not changed with the presence of diabetes. Males had lower mean TC, HDL and LDL values. The sex differences for PP were not significant (table 14).

People with DS appeared to show similar patterns of sex differences in cholesterol and lipid markers (TC, HDL, LDL and Tg) as well as HbA1c levels in people without diabetes compared to controls. On the other hand, the DS cohort showed opposite effects of sex related to blood pressure measurements and BMI when compared to controls.

	DS	Controls	<i>t</i>	Mean Difference	95% CI	p
SBP (mmHg)	116.19 (14.32)	128.21 (15.22)	-5.209	-10.527	-14.51 - -6.55	<0.001
DBP (mmHg)	71.84 (9.54)	77.91 (10.03)	-4.307	-5.366	-7.82 – -2.91	<0.001
PP (mmHg)	47.71 (10.81)	52.86 (13.80)	-2.863	-5.161	-8.71 - -1.61	0.005
BMI (kg/m²)	29.75 (7.30)	27.83 (6.48)	6.455	1.919	1.83 – 2.01	<0.001
TC (mmol/L)	4.98 (1.12)	5.13 (1.15)	-12.159	-0.144	-0.17 - -0.12	<0.001
HDL (mmol/L)	1.35 (0.36)	1.40 (0.43)	-11.37	-0.051	-0.06 - -0.04	0.001
LDL (mmol/L)	3.09 (0.94)	3.05 (1.03)	3.050	0.040	0.01 – 0.07	0.002
Tg (mmol/L)	1.46 (0.86)	1.73 (1.23)	-22.058	-0.264	-0.29 - -0.24	<0.001
HbA1c – total (mmol/mol)	46.36 (18.39)	51.58 (18.56)	-17.439	-5.238	-5.83 - -4.65	<0.001
HbA1c – DM (mmol/mol)	68.30 (16.93)	66.68 (16.33)	3.449	1.616	0.70 – 2.53	<0.001
HbA1c - no DM (mmol/mol)	36.14 (5.80)	38.50 (6.07)	-19.736	-2.357	-2.59 – 2.12	<0.001

Table 12. Group comparisons of cardiovascular health parameters between DS and control cohorts using two way independent sample t-tests. Results are presented as mean (SD).

Variable	Males (DS)	Females (DS)	<i>t</i>	Mean Difference	95% CI	p
SBP (mmHg)	117.61 (15.66)	120.10 (18.81)	-4.117	-3.421	-5.07 - -1.77	<0.001
DBP (mmHg)	70.98 (11.76)	73.47 (10.20)	-3.131	-2.125	-3.48 - -0.77	0.003
PP (mmHg)	48.80 (15.08)	46.64 (15.33)	-2.025	-1.297	-2.57 - -0.03	0.045
BMI (kg/m²)	29.04 (6.31)	30.30 (7.95)	-4.166	-1.126	-1.42 - -1.09	<0.001
TC (mmol/L)	4.93 (1.12)	5.05 (1.12)	-5.516	-0.116	-0.16 – -0.07	<0.001
HDL (mmol/L)	1.26 (0.31)	1.44 (0.38)	-22.20	-0.170	-0.19 – -0.16	<0.001
LDL (mmol/L)	3.04 (0.94)	3.14 (0.94)	-4.475	-0.103	-0.15 – -0.06	<0.001
Tg (mmol/L)	1.51 (0.85)	1.40 (0.88)	5.866	0.117	0.08 – 0.16	<0.001
HbA1c – total (mmol/mol)	46.84 (18.43)	45.81 (18.34)	1.955	1.032	-0.01 – 2.07	0.051
HbA1c – DM (mmol/mol)	68.54 (16.95)	68.02 (16.91)	0.594	0.514	-1.18 – 2.21	0.552
HbA1c - no DM (mmol/mol)	36.47 (5.66)	35.77 (5.94)	3.463	0.202	0.30 – 1.09	<0.001

Table 13. Sex comparisons of cardiovascular health parameters in the DS cohort using two way independent sample t-tests. Results are presented as mean (SD).

Variable	Males (Con)	Females (Con)	t	Mean Difference	95% CI	p
SBP (mmHg)	131.30 (15.81)	127.50 (16.31)	2.398	3.312	0.58 - 6.04	0.018
DBP (mmHg)	80.71 (9.86)	76.50 (9.81)	2.745	2.119	0.59 – 3.65	0.007
PP (mmHg)	56.14 (12.06)	54.92 (11.77)	1.007	1.223	-1.18 – 3.63	0.316
BMI (kg/m²)	28.46 (5.73)	27.50 (6.82)	1.187	0.955	0.87 – 1.04	<0.001
TC (mmol/L)	4.99 (1.16)	5.27 (1.12)	-26.875	-0.282	-0.30 - -0.26	<0.001
HDL (mmol/L)	1.26 (.37)	1.53 (0.45)	-64.604	-0.274	-0.28 - -0.27	0.001
LDL (mmol/L)	2.94 (1.03)	3.15 (1.01)	-18.023	-0.213	-0.24 - -0.19	<0.001
Tg (mmol/L)	1.91 (1.41)	1.55 (0.98)	26.886	0.352	0.33 – 0.38	<0.001
HbA1c – Total (mmol/mol)	53.56 (18.85)	49.65 (17.84)	13.728	3.902	3.34 – 4.46	<0.001
HbA1c – DM (mmol/mol)	67.01 (16.50)	66.27 (16.06)	1.981	0.737	0.01 – 1.47	0.048
HbA1c - no DM (mmol/mol)	39.03 (6.22)	38.06 (5.91)	7.511	0.967	0.71 – 1.22	<0.001

Table 14. Sex comparisons of cardiovascular health parameters in the control cohort using two way independent sample t-tests. Results are presented as mean (SD).

4.3.2.2. Correlations Between Cardiovascular Parameters

Differences were observed in how the CVPs related to each other in the DS and control group, as indicated using two-tailed Pearson correlations (tables 15 and 16). SBP had noticeably different correlations. In the DS group SBP was positively correlated with DBP (correlation 0.77, 95% CI 0.69 – 0.84, $p<0.001$), PP (correlation 0.87, 95% CI 0.82 – 0.91, $p<0.001$) and HDL (correlation 0.56, 95% CI 0.41 – 0.67, $p<0.001$). In controls SBP was positively correlated with all CVPs apart from HDL (SBP: DBP correlation 0.78, 95% CI 0.70 – 0.84, $p<0.001$; SBP: PP correlation 0.88, 95% CI 0.83 – 0.91, $p<0.001$; SBP: BMI correlation 0.88, 95% CI 0.84 – 0.92, $p<0.001$; SBP: TC correlation 0.31, 95% CI 0.14 – 0.47, $p<0.001$; SBP: LDL correlation 0.20, 95% CI 0.01 – 0.37, $p=0.04$; SBP: Tg correlation 0.70, 95% CI 0.59 – 0.78, $p<0.001$; SBP: HbA1c correlation 0.28, 95% CI 0.11 – 0.44, $p=0.002$).

DBP values were correlated with HDL as well as BMI, TC, LDL and Tg in DS, despite the lack of correlation they had with SBP (DBP: BMI correlation 0.46, 95% CI 0.30 – 0.59, $p<0.001$; DBP: TC correlation 0.35, 95% CI 0.17 – 0.50, $p<0.001$; DBP: HDL correlation 0.54, 95% CI 0.39 – 0.66, $p<0.001$; DBP: LDL correlation 0.47, 95% CI 0.31 – 0.60, $p<0.001$; DBP: Tg correlation 0.22, 95% CI 0.04 – 0.39, $p=0.02$). In controls there was a correlation between DBP and BMI, TC, LDL and Tg but not HDL. DBP was correlated with HbA1c in controls but not in DS (DBP: HbA1c correlation in controls 0.42, 95% CI 0.25 – 0.56, $p<0.001$; DBP: HbA1c correlation in DS 0.16, 95% CI -0.03 – 0.34, $p=0.09$).

PP was positively correlated with BMI in controls, but negatively correlated in DS (PP: BMI correlation in controls 0.67, 95% CI 0.55 – 0.76, $p<0.001$; PP: BMI correlation in DS -0.45,

95% CI -0.59 - -0.29, $p<0.001$). PP was correlated with Tg and negatively correlated with LDL in controls, whereas in DS it was positively correlated with HDL.

Patterns of correlations for TC were similar between the DS group and controls (apart from with SBP which was absent in DS). On the other hand HDL correlations were different. In people with DS, HDL was positively correlated with SBP, DBP, PP, and LDL (HDL:PP correlation 0.40, 95% CI 0.23 – 0.54, $p<0.001$; HDL:LDL correlation 0.37, 95% CI 0.20 – 0.52, $p<0.001$). In controls there were no correlations between HDL and blood pressure parameters, but there was a borderline positive correlation with BMI and a negative correlation with HbA1c (HDL:BMI correlation 0.19, 95% CI 0.003 – 0.358, $p=0.047$; HDL:HbA1c correlation -0.60, 95% CI -0.71 - -0.47, $p<0.001$). Both groups showed a positive correlation between TC and HDL, and a negative correlation with Tg. Similarly, in DS only DBP from the blood pressure parameters was correlated with Tg, and this was a relatively weak correlation. In controls Tg was strongly correlated with SBP, DBP and to a lesser extent PP (Tg:SBP correlation 0.70, 95% CI 0.59 – 0.78, $p<0.001$; Tg:DBP correlation 0.83, 95% CI 0.76 – 0.88, $p<0.001$; Tg:PP correlation 0.39, 95% CI 0.23 – 0.54, $p<0.001$).

HbA1c showed different relationships. Whilst in both groups there was a positive correlation with Tg (HbA1c:Tg correlations 0.38, 95% CI 0.20 – 0.53, $p<0.001$ and 0.44, 95% CI 0.28 – 0.57, $p<0.001$), in the DS group HbA1c was also positively correlated with BMI and LDL (HbA1c:BMI correlation 0.23, 95% CI 0.05 – 0.40, $p=0.01$; HbA1c:LDL correlation 0.24, 95% CI 0.05 – 0.40, $p=0.01$). In controls HbA1c was positively correlated with SBP and DBP and negatively with HDL.

These correlations reveal a different pattern of CVPs in people with DS compared to controls. In DS, SBP was not strongly related to metabolic markers including BMI, TC, LDL, Tg and HbA1c, but in controls this relationship was observed. On the other hand DBP was associated with these measures suggesting a link between DBP but not SBP and wider cardiovascular health in DS. PP had an inverse relationship with BMI in DS compared to controls, with lower PP values associated with greater BMI. In controls BMI was not associated with HbA1c levels but was with blood pressure values. In DS HbA1c was related to BMI but not to blood pressure.

<i>DS</i>	Age	SBP	DBP	PP	BMI	TC	HDL	LDL	Tg	HbA1c
Age		0.638**	0.255*	0.743**	-0.486**	0.014	0.586**	-0.003	-0.006	-0.126
SBP	0.638**		0.777**	0.869**	-0.062	0.067	0.555**	0.170	0.171	0.118
DBP	0.255**	0.777**		0.363**	0.457**	0.345**	0.540**	0.466**	0.222*	0.160
PP	0.743**	0.869**	0.363**		-0.450**	-0.172	0.397**	-0.114	0.078	0.049
BMI	-0.486**	-0.062	0.457**	-0.450**		0.480**	0.121	0.474**	0.098	0.232*
TC	0.014	0.067	0.345**	-0.172	0.480**		0.465**	0.781**	0.300**	0.096
HDL	0.586**	0.555**	0.540**	0.397**	0.121	0.465**		0.371**	-0.207*	-0.071
LDL	-0.003	0.170	0.466**	-0.114	0.474**	0.781**	0.371**		0.471**	0.235*
Tg	-0.006	0.171	0.222*	0.078	0.098	0.300**	-0.207*	0.471**		0.376**
HbA1c	-0.126	0.118	0.160	0.049	0.232*	0.096	-0.071	0.235*	0.376**	

<i>Controls</i>	Age	SBP	DBP	PP	BMI	TC	HDL	LDL	Tg	HbA1c
Age		0.872**	0.485**	0.913**	0.847**	0.141	0.292**	-0.006	0.378**	-0.065
SBP	0.872**		0.780**	0.876**	0.884**	0.314**	0.002	0.197*	0.696**	0.284**
DBP	0.485**	0.780**		0.382**	0.824**	0.672**	-0.174	0.624**	0.828**	0.416**
PP	0.913**	0.876**	0.382**		0.669**	-0.054	0.134	-0.190*	0.393**	0.102
BMI	0.847**	0.884**	0.824**	0.669**		0.529**	0.187*	0.418**	0.609**	0.078
TC	0.141	0.314**	0.672**	-0.054	0.529**		0.292**	0.937**	0.502**	-0.002
HDL	0.292**	0.002	-0.174	0.134	0.187*	0.292**		0.156	-0.391**	-0.602**
LDL	-0.006	0.197*	0.824**	-0.190*	0.418**	0.937**	0.156		0.475**	0.067
Tg	0.378**	0.696**	0.828**	0.393**	0.609**	0.502**	-0.391**	0.475**		0.437**
HbA1c	-0.065	0.284**	0.416**	0.102	0.078	-0.002	-0.602**	0.067	0.437**	

Tables 15 and 16. Two-tailed Pearson correlations between CVPs for the DS cohort (table 15) and control cohort (table 16).

Significant correlations are indicated. *significance at $p < 0.05$; ** significance at $p < 0.01$.

4.3.3. Patterns of Cardiovascular Health Markers with Age

The mean values of each CVP per year of age were presented graphically using scatterplots comparing the DS cohort and controls. Values were aggregated on DS status, sex, and age at measurement. Lines of best fit and 95% confidence intervals were plotted.

4.3.3.1. Blood Pressure

In whole-group comparisons the DS cohort had lower average values of SBP than controls (mean SBP 116.2mmHg (SD 14.3) in DS vs 128.2mmHg (SD 15.2) in controls). There were also marked differences between the DS and control group in patterns of SBP with age. The DS cohort had consistently lower SBP values than controls, and this was present at every age. People with DS showed limited increases in SBP with age. This is in contrast to the control group when there was marked increases in SBP with age, with mean SBP values increasing rapidly between ages 40 – 60 years. The maximum mean SBP in DS was 125.43mmHg (SD 19.65), whereas in controls it was 137.75mmHg (SD 14.57).

Differences were also observed in DBP values, with the DS cohort having consistently lower DBP values than controls in both the overall group comparison (mean DBP 71.8mmHg (SD 9.5) in the DS group vs 77.9mmHg (SD 10.0) in controls) and at each age. In the literature DBP values in general population samples show a characteristic inverted u-shape pattern, increasing until middle age and then decreasing again with advancing age³³⁵. This inverted u-shape pattern was apparent in the control cohort in this analysis, but not in the DS group who showed very little change in DBP with age. In the DS group the highest mean DBPs was observed in the 39 year old group (73.67mmHg, SD 9.58) and the 74 year old group

(73.34mmHg, SD 13.27). In controls the highest values were in 54 and 55 year olds (81.89mmHg, SD 9.51 and 82.06mmHg, SD 9.35 respectively).

Consistent with the SBP and DBP findings, PP was lower in the DS group compared to controls (mean PP 47.7 mmHg (SD 10.8) in the DS cohort versus 52.9 mmHg (SD 13.8) in controls). PP showed a different pattern with age. In people with DS, PP was relatively stable over the lifespan, with increases seen only in those over 60 years old. In controls, PP increased steadily with age as a result of a widening gap between SBP and DBP. Between age 18-60 years, the range for mean PP values in those with DS was only 3.76mmHg whereas the difference was 11.11mmHg in controls.

As displayed in figure 6, these results suggest that people with DS have significantly different blood pressure profiles compared to controls, and that this difference remains throughout adulthood. Notably people with DS have lower blood pressures than controls and do not experience the same magnitude of age-related changes in BP that have been described in the general population.

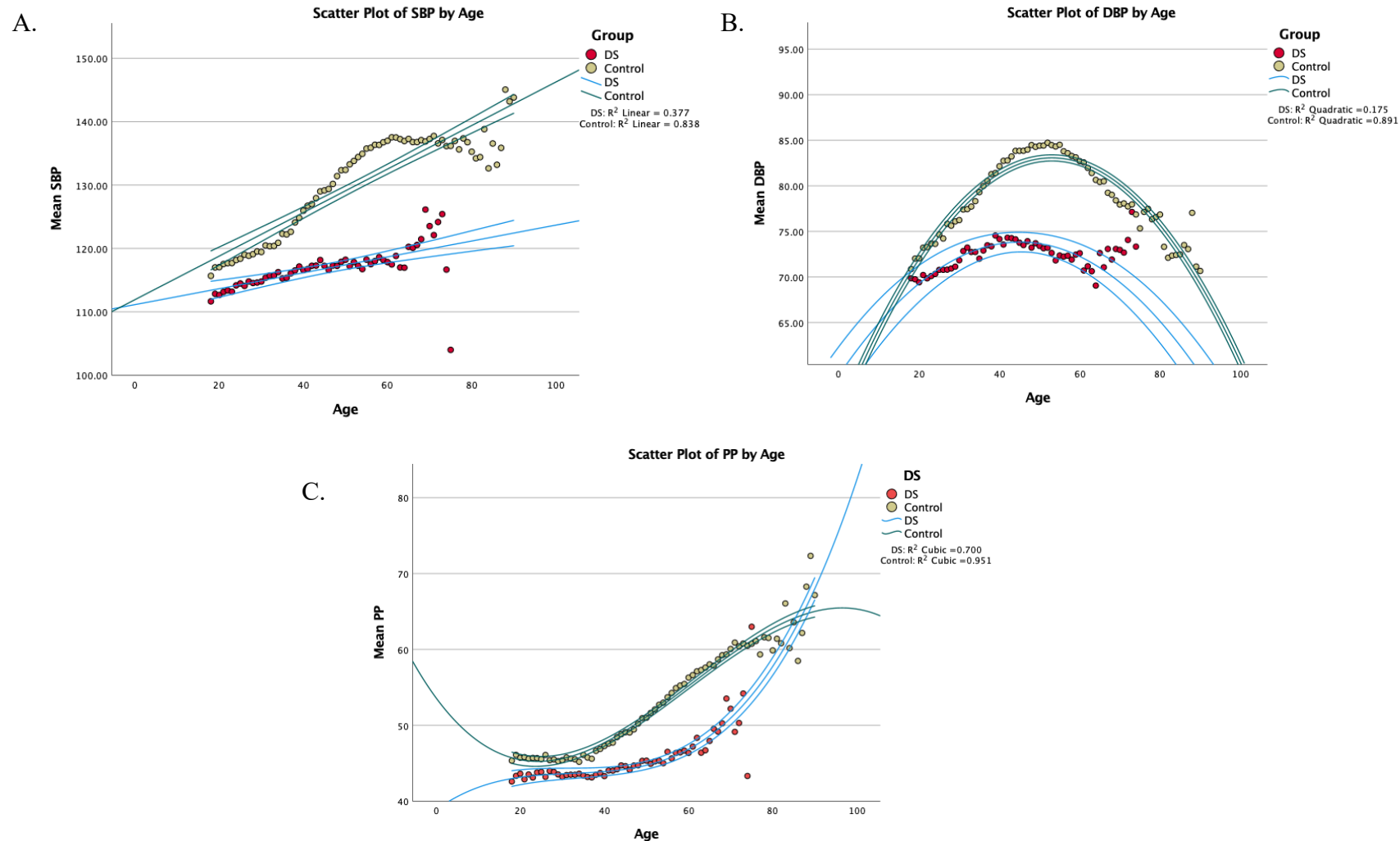


Figure 6. Scatter plots displaying mean BP values (in mmHg) per year of age in DS cohort and controls. Error bars represent 95% CI. A) Systolic Blood Pressure (SBP); B) Diastolic Blood Pressure (DBP); C) Pulse Pressure (PP).

4.3.3.2. *Body Mass Index*

In group comparisons, the DS population had a higher average BMI than controls (29.8 kg/m², SD 7.3, versus 27.8kg/m², SD 6.5). There were prominent differences in the peak values of BMI as well as the pattern of BMI with age, see figure 7. In the DS cohort the highest BMI values occurred around the ages of 30-40 years and then decreased at older ages. In DS the highest mean value of 31.36kg/m² occurred in the 39 year old group. In controls the peak values occurred later, at around 50 years old, and there was a lower maximum BMI value. In this group the maximum mean value was 29.78kg/m² which occurred at age 54 years. Controls also showed a less steep decline in BMI with age. Between ages 40 -75 years, the greatest difference in mean BMI was 3.78 in the DS group but only 0.23 in controls. Overall therefore, people with DS had higher BMIs than controls, but this relationship was age-dependent. At younger ages people with DS tended to have higher BMIs than controls, but this reversed in older ages when controls were more likely to have a higher BMI than someone with DS.

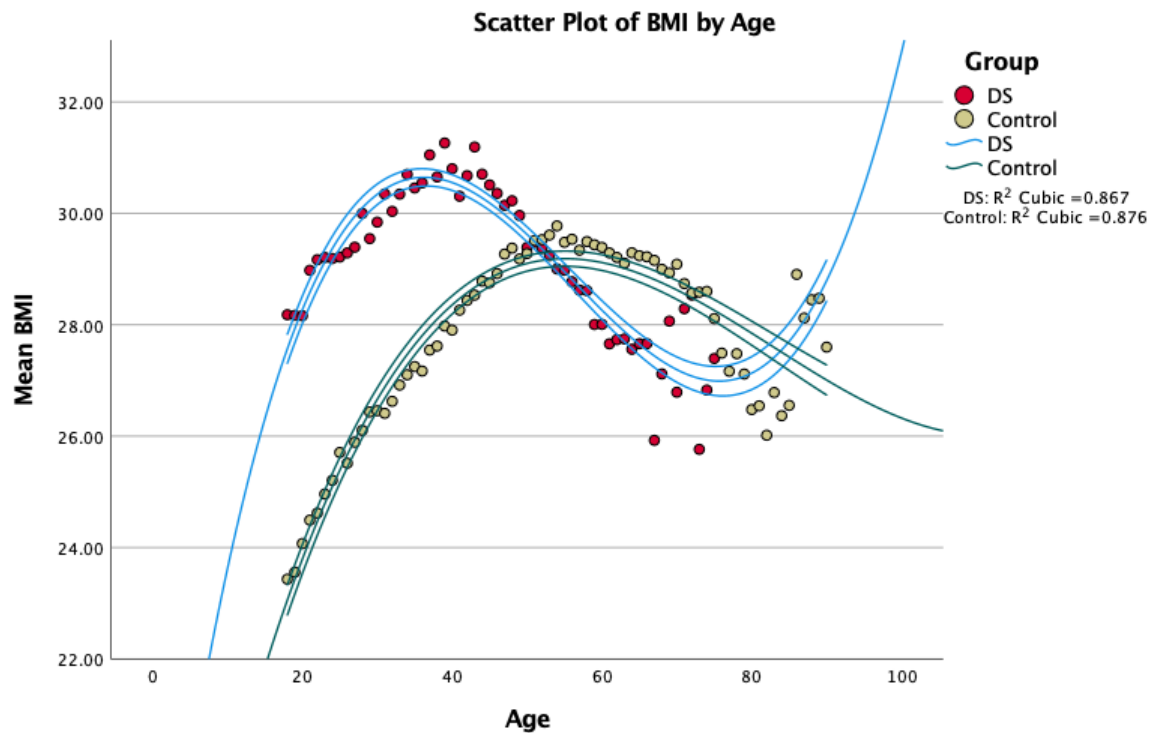


Figure 7. Scatter plot displaying mean Body Mass Index (BMI) value (in kg/m²) per year of age in the DS cohort and controls. Error bars represent 95% CI.

4.3.3.3. Lipid Profiles

See figure 8 for graphical representation of TC, HDL, LDL and Tg values in the DS and control populations.

Total Cholesterol

The DS group had slightly lower average cholesterol levels than controls at a group level (mean TC 4.98 mmol/L, SD 1.12 in DS and 5.13 mmol/L, SD 1.15 in controls). This was consistent from approximately 20 years old. Despite the lower values of TC observed in DS, both the DS and control cohorts demonstrated a similar pattern of cholesterol change with age. Values

showed an inverted-u shaped relationship with age, increasing towards middle age and then decreasing at older years. The peak mean value was higher in controls, but occurred at approximately the same age in both groups (peak mean TC in DS was 5.21mmol/L occurring at age 50; in controls the peak value was 5.37mmol/L occurring at age 51). Between 18-20 years the DS cohort had similar or higher mean cholesterol levels to controls.

High Density and Low Density Lipoproteins

In the whole group comparison, the DS cohort had slightly lower mean HDL levels and slightly higher LDL levels compared to controls (mean HDL 1.35mmol/L, SD 0.36 in DS vs 1.40mmol/L, SD 0.43 in controls; mean LDL 3.09mmol/L, SD 0.94 in DS vs 3.05mmol/L, SD 1.03 in controls). When comparing HDL levels at different ages, younger people with DS tended to have lower mean HDL than controls. Values of HDL were higher in older people with DS, peaking at similar or slightly greater values than controls. For example, at age 18 years mean HDL in DS was 1.14mmol/L (SD 0.25) compared to 1.27mmol/L (SD 0.28) in controls. At age 40 there was little difference between the two groups, with mean values of 1.31mmol/L (SD 0.36) in DS and 1.34mmol/L (SD 0.42) in controls. In the DS cohort the peak value occurred at age 61 (1.48 mmol/L, SD 0.35) and in controls it occurred at age 68 (1.44 mmol/L, SD 0.44). After age 60 years HDL values appeared to decline in the DS cohort whereas they remained higher in controls. The difference between lowest and highest values was greater in the DS cohort (0.25 in controls vs 0.34 in DS).

The values and pattern of LDL levels with age were similar between the DS cohort and controls, showing an inverted-u shape pattern with age. Peak mean values were similar in both groups, but occurred at an earlier age in controls. Peak mean LDL in DS was 3.30mmol/L (SD 1.09) recorded in the 50 year old group; in controls this was 3.31 (SD 1.00) recorded at 38

years old. The range between minimum and maximum values was 0.76 in DS and 0.96 in controls.

Triglycerides

At a whole group level average Tg levels were lower in the DS group compared to controls (Mean Tg 1.46 mmol/L, SD 0.86 in DS compared to 1.73mmol/L, SD 1.23 in controls). The DS cohort had lower mean Tg levels compared to controls at all ages apart from at the youngest ages (18-20 years). For example, at age 18 years, mean Tg was 1.48mmol/L (SD 0.13) in DS compared to 1.06mmol/L (SD 0.08) in controls. On the other hand, at age 30 mean Tg was 1.48mmol/L (SD 0.10) in DS versus 1.58 (SD 0.09) in controls. There were also differences in the pattern of Tg values with age. In controls an increase with age was observed until approximately age 40 years, followed by a plateauing. In the DS cohort Tg values appeared to be relatively stable between years, with little change across ages. The highest Tg value was observed in the 37 year old and 65 year old groups in DS (mean Tg 1.55mmol/L (SD 0.07) and 1.57mmol/L (SD 0.10) at 37 and 65 years respectively). In controls this occurred at age 47 (mean Tg 1.73mmol/L, SD 0.09). The difference in maximum and minimum values was lower in the DS group (0.47 vs 0.67) reflecting the reduced variation in values in DS.

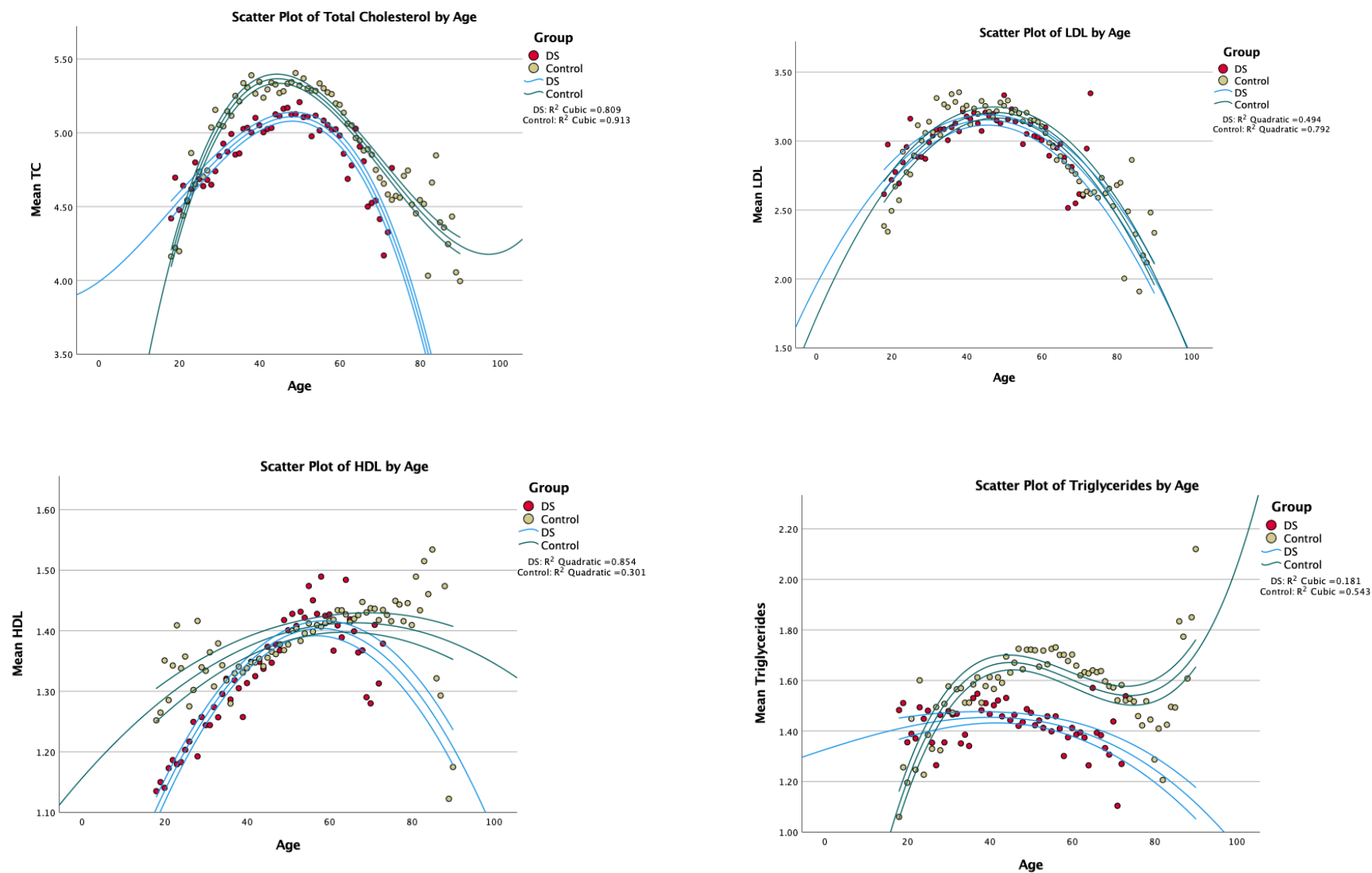


Figure 8. Scatter plots displaying mean Total Cholesterol (TC), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), and Triglyceride (Tg) values (in mmol/L) per year of age in DS cohort and controls. Error bars represent 95% CI.

3.3.4 Glycated Haemoglobin (HbA1c)

In whole group comparisons HbA1c values in the combined group formed of both diabetic and non-diabetic patients were lower in the DS compared to the control group (mean HbA1c 46.4mmol/mol, SD 18.4 in DS and 51.6mmol/mol, SD 18.6 in controls). However there was little difference between HbA1c values nor pattern of change with age between the DS and control group.

In the sub-set of patients with diabetes, HbA1c values were higher in DS than controls (mean HbA1c 68.3 mmol/mol (SD 16.9) in DS vs 66.8 mmol/mol (SD 16.3) in controls). The two groups showed similar changes with age, and there was an overall pattern of slightly lower HbA1c values with advancing age, although the control group had a steeper gradient. The highest value observed in DS was 75.9mmol/mol (SD 19.4) at age 35 years. In controls this was 81.4mmol/mol, (SD 21.3) at age 18. The range of values was slightly greater in controls than in DS (15.8 in DS vs 20.8 in controls). See figure 9.

In the sub-group without diabetes a difference was observed between the DS and control group. People with DS had lower average HbA1c levels (mean HbA1c 36.1 mmol/mol (SD 5.8) in DS vs 38.5 mmol/mol (SD 6.1) in controls). There were differences in age-related patterns observed between the groups. At younger ages both groups had similar HbA1c values and showed a pattern of increasing HbA1c with increasing age. After around 40 years old the control group continued to follow a pattern of increasing HbA1c with age whereas in the DS group the HbA1c values plateaued. In DS the highest value occurred at age 50 (mean 38.1mmol/mol, SD 3.8). In controls this was at age 74 (mean HbA1c 40.1 mmol/mol, SD 3.68). The peak HbA1c value was therefore higher in controls than in DS and occurred at a later age.

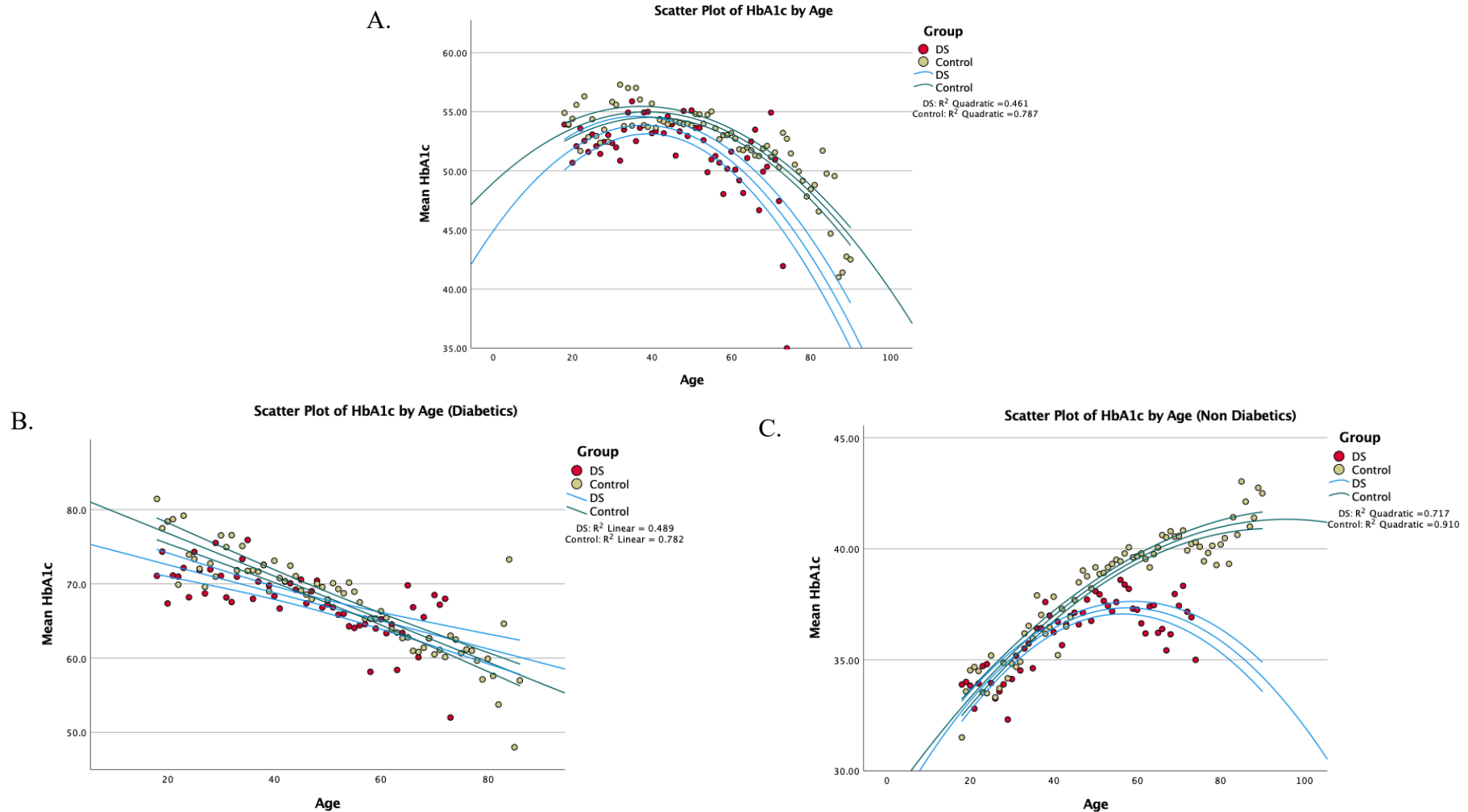


Figure 9. Scatter plots displaying mean HbA1c values per year of age in DS cohort and controls. Graphs represent mean HbA1c in mmol/mol. A) Combined group; B) Diabetic group; C) People without diabetes. Error bars represent 95% CI.

4.3.4. Effects of DS Status, Age and Sex on Cardiovascular Health Parameters

To further quantify the pattern of CVPs across adulthood the relationships between CVP, group membership (Down syndrome vs control group), age at time of measurement, and sex were determined by constructing linear regression models. Each CVP was included as a dependent variable. Multi-collinearity checks were performed with no significant correlations between variables in each model ($R < 0.8$ for all examined variables). Curve estimation procedures were used to determine the best-fit model relationship for each variable, considering R^2 increase and statistical significance. Quadratic or cubic terms were included in models when indicated based on the nature of the relationship.

The sample was then split into five-year age bands from age 20 years. Values for the 18-19 year olds were grouped separately. Changes in each cardiovascular health marker between age groups over time were compared using multivariate regression models with sex as a covariate alongside all main and interaction effects. Sex: Male; and Group: Control, were used as indicators for simple contrasts. Bonferroni post-hoc corrections were applied for multiple comparisons.

Blood Pressure

As illustrated above, when comparing the DS and control group there was a significant difference in average BP values, with the DS cohort having lower mean values. There was also a difference in the sex pattern between DS and controls. In people with DS, females tended to have higher average SBP and DBP values (mean SBP 117.6mmHg (SD 15.7) in males versus 120.10mmHg (SD 18.9) in females; mean DBP 71.0mmHg (SD 11.8) in males versus 73.5mmHg (SD 10.2) in females). In controls females tended to have lower values (mean SBP 131.3mmHg (SD 15.8) in males versus 127.5mmHg (SD 16.3) in females; mean DBP

80.7mmHg (SD 9.9) in males versus 76.5mmHg (SD 9.8) in females). In addition, sex differences observed for PP between males and females in the DS group were of borderline significance, with higher levels in males (mean PP 48.8mmHg (SD 15.1) in males, compared to 46.6 mmHg (SD 15.3) in females). In the control cohort there were no significant differences in PP values between the sexes. People with DS therefore had a contrasting relationship between blood pressure values and sex compared to controls.

Regression models confirmed significant relationships between group membership, age and blood pressure for SBP, DBP and PP and the results are presented in tables 17-19. In all models, sex differences were not significant when DS status and age were included. For SBP, R^2 for the final model was 0.871, with the model explaining approximately 87% of the variation. For DBP, R^2 of the final model was 0.803. For PP, R^2 of the final model was 0.848. DS status was hence a significantly important predictor of blood pressure alongside age.

SBP	B	SE	beta	(95% CI for B)
Step 1				
Constant	102.672	1.45		
Group	15.70	0.887	0.740**	13.95 – 17.44
Step 2				
Constant	93.114	1.242		
Group	13.686	0.664	0.646**	12.38 – 14.99
Age	0.251	0.017	0.468**	0.28 – 0.29
Step 3				
Constant	93.823	1.579		
Group	13.681	0.664	0.645**	12.37 – 14.99
Age	0.252	0.017	0.469**	0.22 – 0.29
Sex	-0.471	0.646	-0.022	-1.74 – 0.801

**P<0.001

Table 17. Regression model for SBP using CPRD. Note: $R^2=0.548$ for Step 1; $\Delta R^2 = 0.210$ for Step 2; $\Delta R^2 =0.001$ for Step 3 (step 1 and 2 $p<0.001$, step 3 $p>0.05$).

DBP	B	SE B	beta	(95% CI for B)
Step 1				
Constant	65.584	0.896		
Group	6.483	0.547	0.594**	5.41 – 7.56
Step 2				
Constant	46.157	1.577		
Group	7.762	0.424	0.711**	6.93 – 8.60
Age	0.807	0.058	2.919**	0.69 – 0.92
Quadratic	-0.008	0.001	-3.030**	-0.009 - -0.007
Step 3				
Constant	46.50	1.695		
Group	7.759	0.424	0.711**	6.92 – 8.60
Age	0.807	0.058	2.919**	0.69 – 0.92
Quadratic	-0.008	0.001	-3.029**	-0.009 - -0.007
Sex	-0.226	0.405	-0.021	-1.01 – 0.57

**P<0.001

Table 18. Regression model for DBP using CPRD. Note: $R^2 = 0.594$ for Step 1; $\Delta R^2 = 0.292$ for Step 2; $\Delta R^2 = 0.00$ for Step 3 (step 1 and 2 $p < 0.001$, step 3 $p > 0.05$)

PP	B	SE B	beta	(95% CI for beta)
Step 1				
Constant	37.093	1.221		
Group	9.220	0.745	0.610**	7.75 – 10.69
Step 2				
Constant	36.661	1.429		
Group	6.619	0.384	0.438**	5.86 – 7.38
Age	-0.089	0.052	-0.233	-0.19 – 0.01
Quadratic	0.003	0.00	0.939**	0.002 – 0.004
Step 3				
Constant	36.034	1.535		
Group	6.616	0.384	0.438**	5.86 – 7.36
Age	-0.089	0.052	-0.234	-0.19 – 0.01
Quadratic	0.003	0.00	0.939**	0.002 – 0.004
Sex	-0.245	0.366	-0.016	-0.97 – 0.48

**P<0.001

Table 19. Regression model for PP using CPRD. Note: $R^2 = 0.372$ for Step 1; $\Delta R^2 = 0.475$ for Step 2; $\Delta R^2 = 0.00$ for Step 3 (step 1 and 2 $p < 0.001$, step 3 $p > 0.05$)

The sample was then split into five-year age bands. Overall the Down syndrome group showed significant homogeneity in blood pressure values with age. Whilst there was an increase in mean SBP with age, the magnitude of change was only small between age bands, and post-hoc group-wise comparison confirmed that there were no significant differences. On the other hand, the control group experienced increasing values of SBP from age 18 to age 60 years at which point the values plateaued. This is in keeping with the literature which describes positive correlations between SBP values and age in the general population. The overall change between the lowest and highest group in SBP was only 11.08mmHg for the Down syndrome cohort but almost double that in controls (20.7mmHg).

Similar to the observations for SBP, the DS group showed homogeneity in DBP values with little variance between age bands. In contrast, controls showed significant differences between age groups. The difference between the highest and lowest mean DBP values was 3.86mmHg in the Down syndrome cohort and 11.05mmHg in controls. As expected based on the SBP and DBP results, PP also did not vary significantly between age groups in the DS cohort. On the other hand, the control group showed significant increases from age 35 which reflected the increase with SBP and decrease in DBP observed with age in the general population.

The differences between the DS group and control group were significant at almost every age bracket for SBP, DBP and PP with marked differences in blood pressure profile between the DS and control groups (tables 20-22). The only group in which no difference was found was between groups in the 18-20 year old age band for DBP.

Age Group	<i>Down syndrome</i>		<i>Control group</i>		Mean		
	<i>n</i>	SBP	<i>n</i>	SBP	Difference	95% CI	<i>p</i>
18-20	937	113.49 (0.17)	3987	119.18 (4.43)	5.59	2.02 – 9.36	0.003
20-25	3501	114.15 (0.79)	11278	120.85 (4.52)	6.69	4.37 – 9.01	<0.001
25-30	4308	115.07 (0.94)	12121	123.08 (5.54)	8.01	5.68 – 10.33	<0.001
30-35	4302	116.01 (0.90)	12445	124.88 (6.08)	8.23	5.91 – 10.55	<0.001
35-40	4134	117.35 (2.12)	12603	128.39 (5.10)	11.04	8.72 – 13.37	<0.001
40-45	4268	118.59 (3.22)	13964	132.63 (4.62)	14.04	11.72 – 16.36	<0.001
45-50	4225	118.94 (15.1)	16064	135.65 (3.20)	16.71	14.39 – 19.04	<0.001
50-55	3578	118.77 (3.64)	16216	138.40 (2.15)	19.63	17.31 – 21.95	<0.001
55-60	2425	119.19 (3.97)	14138	140.45 (0.97)	21.56	18.94 – 23.58	<0.001
60-65	1364	118.59 (2.71)	11134	140.86 (0.77)	22.28	19.96 – 24.60	<0.001
65-70	560	123.27 (2.69)	7534	140.36 (0.53)	17.09	14.77 – 19.41	<0.001
70+	120	123.42 (10.6)	3660	140.03 (1.15)	16.86	14.61 – 19.11	<0.001

Table 20. Mean SBP for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. SBP values are presented as mean (SD) in mmHg.

Age Group	<i>Down syndrome</i>		<i>Control group</i>		Mean difference	95% CI	<i>p</i>
	<i>n</i>	DBP	<i>n</i>	DBP			
18-20	937	69.80 (0.50)	3987	71.45 (1.55)	1.65	2.07 – 5.37	0.383
20-25	3501	69.99 (0.42)	11269	73.17 (1.95)	3.19	0.84 – 5.54	0.008
25-30	4308	70.89 (0.88)	12112	75.29 (2.72)	4.40	2.05 – 6.75	<0.001
30-35	4296	72.68 (0.61)	12435	77.43 (3.53)	4.75	2.40 -7.10	<0.001
35-40	4127	73.26 (1.80)	12593	80.51 (3.30)	7.24	4.89 – 9.60	<0.001
40-45	4267	74.09 (2.33)	13956	82.95 (2.93)	8.86	6.51 – 11.22	<0.001
45-50	4224	73.63 (2.14)	16055	84.09 (2.14)	10.46	8.11 – 12.81	<0.001
50-55	3577	72.85 (2.52)	16208	84.47 (1.59)	11.63	9.28 – 13.98	<0.001
55-60	2425	72.26 (2.12)	14135	83.66 (0.97)	11.40	9.05 – 13.75	<0.001
60-65	1363	69.70 (1.59)	11133	81.87 (1.09)	11.02	8.67 – 13.38	<0.001
65-70	560	72.36 (1.56)	7534	79.52 (0.95)	7.17	4.82 – 9.52	<0.001
70+	120	70.81 (11.4)	3660	77.32 (1.12)	6.46	4.18 – 9.52	<0.001

Table 21. Mean DBP for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. DBP values are presented as mean (SD) in mmHg.

<i>Down syndrome</i>		<i>Control group</i>					
Age					Mean		
Group	<i>n</i>	PP	<i>n</i>	PP	Difference	95% CI	p
18-20	937	43.69 (0.58)	3987	47.74 (2.95)	4.05	1.91 – 6.19	<0.001
20-25	3501	44.17 (0.71)	11269	47.75 (2.69)	3.58	2.23 – 4.94	<0.001
25-30	4308	44.20 (0.53)	12112	47.85 (3.05)	3.66	2.30 – 5.01	<0.001
30-35	4296	44.01 (0.61)	12435	47.49 (2.66)	3.49	2.13 – 4.84	<0.001
35-40	4127	44.14 (0.89)	12593	47.93 (1.89)	3.79	2.44 – 5.15	<0.001
40-45	4267	44.51 (1.08)	13956	49.72 (1.72)	5.20	3.85 – 6.56	<0.001
45-50	4224	45.31 (1.05)	16055	51.58 (1.18)	6.27	4.91 – 7.62	<0.001
50-55	3577	45.94 (1.15)	16208	53.94 (0.92)	7.99	6.64 – 9.35	<0.001
55-60	2425	46.93 (2.04)	14135	56.79 (0.77)	9.86	8.51 – 11.22	<0.001
60-65	1363	47.76 (1.41)	11133	58.99 (0.72)	11.23	9.88 – 12.59	<0.001
65-70	560	50.91 (2.17)	7534	60.84 (0.79)	9.93	8.57 – 11.28	<0.001
70+	120	52.61 (5.91)	3660	62.70 (0.81)	10.40	9.09 – 11.71	<0.001

Table 22. Mean PP for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. PP values are presented as mean (SD) in mmHg.

Given the sex differences observed during whole group comparison, interaction effects were added to the models. There was an effect of cohort group, age group and sex, with Group*Age*Sex interactions being significant: $F(47, 11) = 13.29, p < 0.001$ for SBP; $F(47, 11) = 14.54, p < 0.001$ for DBP; and $F(47, 11) = 6.16, p < 0.001$ for PP. Estimated marginal means plots revealed group and sex differences between the Down syndrome cohort and controls. As expected, group differences were present with the DS cohort having lower overall BP values. With regards to sex effects, the Down syndrome group showed reduced variation between males and females. The DS group also had increased average BP values in females compared to males, which is opposite to the sex effects observed in the control group (see figure 10 for sex comparisons).

Overall these results demonstrate that people with Down syndrome have different blood pressure profiles compared to controls. People with DS tended to have lower magnitude BP readings and this occurred across all age groups. In addition, people with Down syndrome did not show the expected changes in blood pressure with age, and particularly did not have increasing systolic blood pressures nor increasing pulse pressures as they got older.

There were different interactions between sex and blood pressure in people with Down syndrome compared to controls. Sex differences within the DS cohort were of smaller magnitudes. This may suggest that genetic effects related to trisomy 21 exert more influence over blood pressure - and perhaps other health measures - than genetic effects related to sex. Further investigation is warranted to quantify how sex effects relate to cardiovascular health and dementia onset in people with Down syndrome.

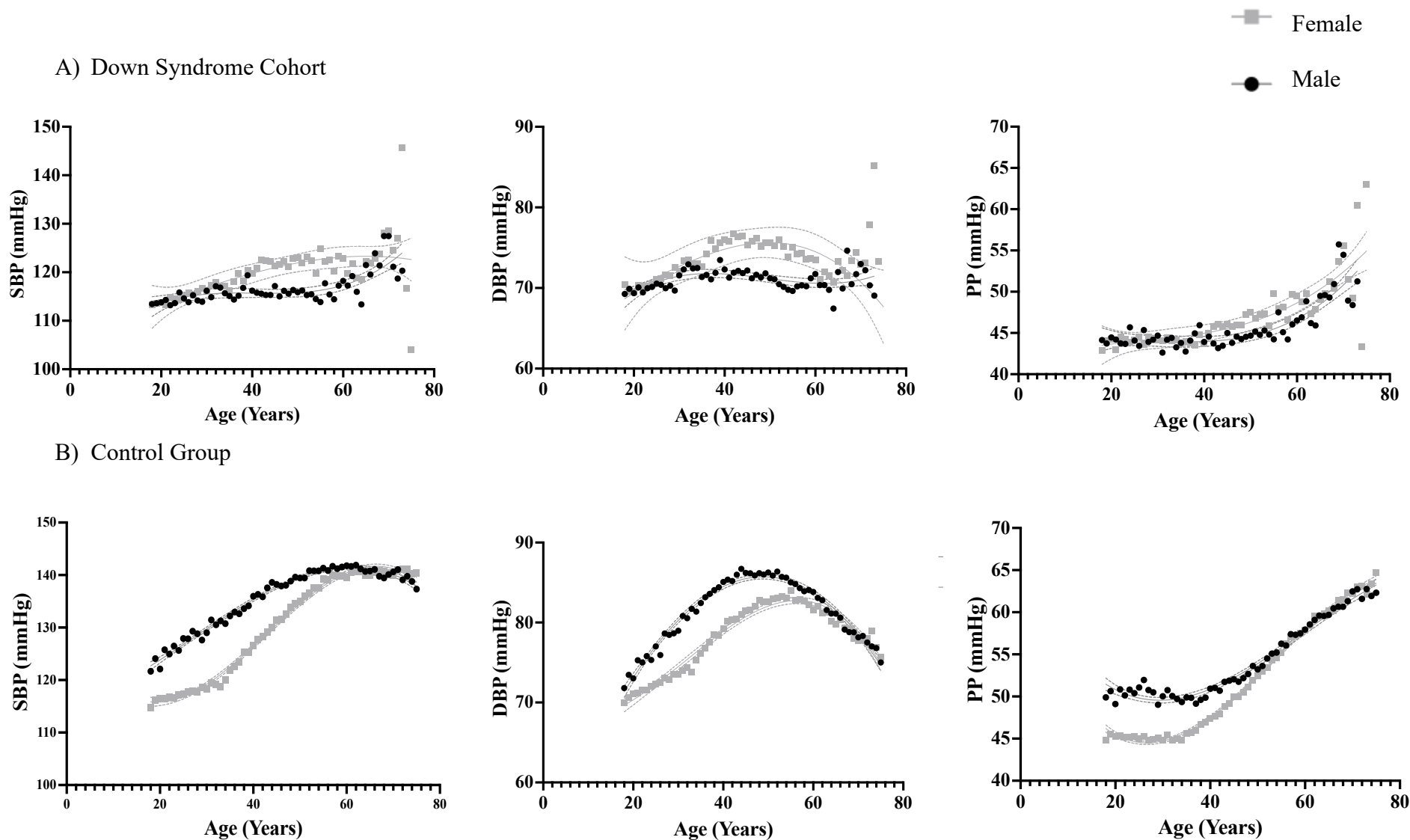


Figure 10. Scatterplots displaying mean values in mmHg for SBP, DBP and PP by age, with groupings by sex. Error bars represent 95% confidence intervals. A) Down syndrome cohort – top row; B) Control groups – bottom row.

Body Mass Index

BMI values were first classified into groups according to the standardised criteria for BMI to compare crude group rates for the DS and control cohorts: underweight: $<18.5 \text{ kg/m}^2$; normal weight: $18.5 - 25 \text{ kg/m}^2$; overweight: $25-30 \text{ kg/m}^2$; obese: $>30 \text{ kg/m}^2$.

There were proportionally more people with Down syndrome classed as obese (43.1%) compared to controls (30.7%), and fewer categorised as having a BMI within “normal” limits (26.2% of the DS cohort compared to 35.4% of controls). Contrasting relationships between sex and BMI were observed. In the DS cohort males had lower mean BMI compared to females (29.0 kg/m^2 , SD 6.3 versus 30.3 kg/m^2 , SD 8.0). In controls the opposite association was observed with mean BMI in males being higher than females (28.5 kg/m^2 , SD 5.7 versus 27.5 kg/m^2 , SD 6.8).

Exact BMI values were then used as the dependent variable in a stepwise regression model. There were effects of both cohort group and age at time of measurement on BMI. Addition of sex did not improve the model and R^2 change was non-significant at step 3. Overall model R^2 was 0.42 showing that the variables only accounted for approximately 42% of the model variance (table 23).

	B	SE	beta	(95% CI for B)
Step 1				
Constant	30.407	0.338		
Group	-1.322	0.207	-0.368**	-1.73 - -0.92
Step 2				
Constant	16.809	1.443		
Group	-1.214	0.179	-0.338**	-1.57 - -0.86
Age	0.810	0.095	8.912	0.62 – 1.00
Quadratic	-0.014	0.002	-16.625	-0.02 - -0.01
Cubic	7.76e ⁻⁵	0.00	7.962**	0.00 – 0.00
Step 3				
Constant	16.335	1.458		
Group	-1.214	0.178	-0.338**	-1.57 - -0.86
Age	0.81	0.095	8.912	0.62 – 1.00
Quadratic	-0.014	0.002	-16.63	-0.02 - -0.01
Cubic	7.76e ⁻⁵	0.00	7.962**	0.00 – 0.00
Sex	0.316	0.17	0.089	-0.02 – 0.65

**P<0.001

Table 23. Regression model for BMI using CPRD. Note: $R^2 = 0.136$ for Step 1; $\Delta R^2 = 0.276$ for Step 2; $\Delta R^2 = 0.008$ for Step 3 (step 1 and 2 $p < 0.001$, step 3 $p > 0.05$).

When comparing five-year age bands for BMI, the DS cohort showed more homogeneity across age groups than controls, specifically in the younger groups between 18 and 55 years old (see table 24). BMIs in the Down syndrome groups were higher than controls until around age 40 when values began to decline. The highest mean value was observed in the 35-40 year old group (30.88 kg/m²). The control group also showed a decline in BMI in older age groups, however the decline started at older ages. The highest mean value was observed in the 50-55 year old group (29.65 kg/m²). Whilst the DS cohort had higher peak BMI values than controls, there was less variation in values in the DS group. The difference between the highest mean BMI by age bracket and the lowest in people with Down syndrome was 3.66 kg/m² compared to 5.97 kg/m² in controls. The difference between the DS and control group was significant at

all ages apart from in the 50-54 age bracket where BMI was similar between groups. Before this point BMI was higher in DS, after this point BMI in the DS group was lower than controls.

Age Group	<i>Down syndrome</i>		<i>Control group</i>		Mean		
	<i>n</i>	BMI	<i>n</i>	BMI	difference	95% CI	<i>p</i>
18-20	889	28.30 (6.8)	2439	23.55 (5.1)	4.68	3.90 – 5.46	<0.001
20-25	2894	29.03 (7.2)	7288	24.68 (5.42)	4.27	3.78 – 4.76	<0.001
25-30	3440	29.53 (7.5)	8122	25.80 (5.8)	3.56	3.07 – 4.05	<0.001
30-35	3391	30.23 (7.7)	8378	26.53 (6.2)	3.55	3.06 – 4.04	<0.001
35-40	3335	30.88 (7.7)	8492	27.45 (6.4)	3.28	2.79 – 3.77	<0.001
40-45	3544	30.82 (7.4)	9176	28.32 (6.7)	2.35	1.86 – 2.84	<0.001
45-50	3494	30.29 (7.1)	9919	29.04 (6.7)	1.14	0.65 – 1.63	<0.001
50-55	3009	29.29 (6.8)	9791	29.52 (6.8)	0.24	-0.74 – 0.25	0.33
55-60	1912	28.60 (6.7)	8388	29.45 (6.6)	-0.86	-0.37 – -1.35	<0.001
60-65	1024	27.76 (6.1)	6662	29.26 (6.0)	-1.52	-2.01 - -1.03	<0.001
65-70	426	27.22 (6.1)	4752	29.13 (5.9)	-1.82	-2.31 - -1.33	<0.001
70+	131	27.22 (5.7)	2556	28.70 (5.6)	-1.48	-1.97 - -0.98	<0.001

Table 24. Mean BMI for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. BMI values are presented as mean (SD) in kg/m².

Despite the relatively small R^2 in the regression model, when age and sex interactions were added there was a significant effect of Group*Age*Sex: $F(47, 11) = 5.221, p < 0.001$ in addition to the main effects. In the DS cohort females had higher mean BMIs than males, whereas in the control group the sex difference was much smaller, and females had slightly lower average

BMI than males. This is in contrast to the effects observed in BP where controls showed greater sex differences than the DS group (figure 11). Complex interactions between sex effects, genetic effects related to trisomy 21, and cardiovascular health markers are apparent.

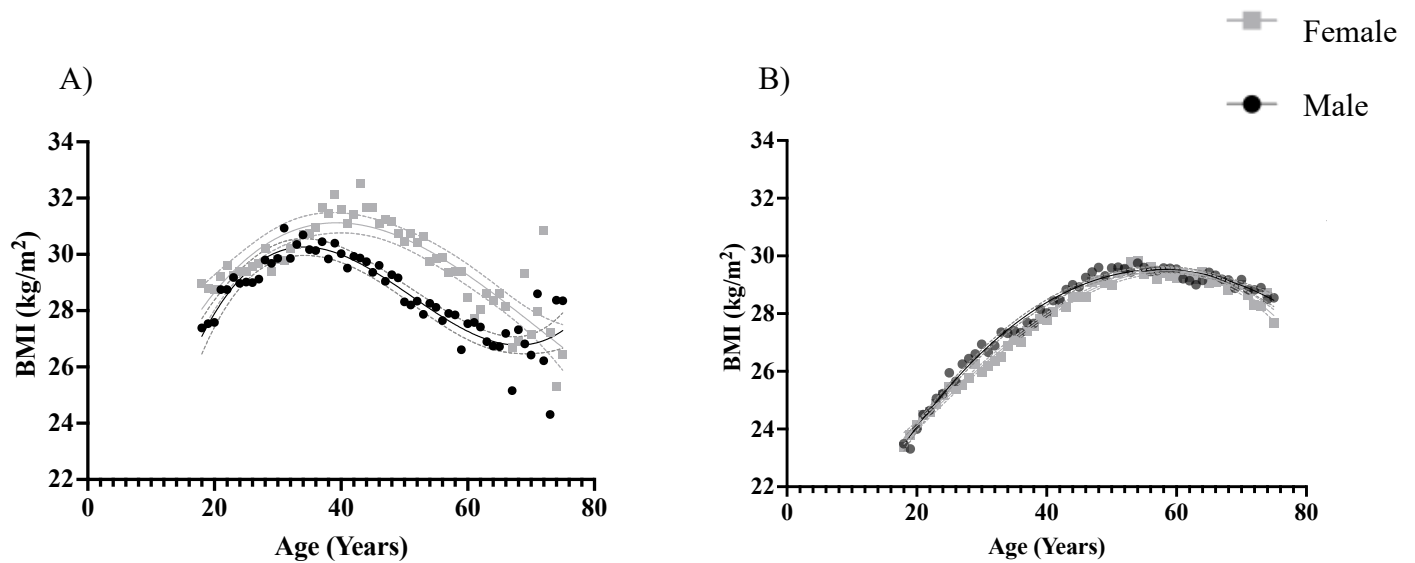


Figure 11. Scatterplots displaying mean values for Body Mass Index (BMI) in kg/m² by age. A) Down syndrome cohort. B) Controls. Error bars represent 95% confidence intervals.

Total Cholesterol

In the overall group comparison the mean TC values were lower in the DS compared to the control group. Both groups showed a similar sex difference with males having lower average TC values than females (mean TC in controls was 4.99 mmol/L, SD 1.16 for males versus 5.27, SD 1.12 for females; mean TC in DS was 4.93, SD 1.12 in males and 5.05, SD 1.12 in females).

Inputting TC values in a stepwise regression model there was no significant group effect when entered alone (table 25). This is consistent with the whole group analysis where only a small difference in average TC values were observed between the DS and control groups. However, on visualisation of the data using scatterplots there appeared to be an age effect. This was confirmed in the regression analysis. When age was added into the model there were both significant effects of age and cohort group. Sex improved the model further and highlights additional sex differences in cholesterol levels. The overall model R^2 was 0.626.

When comparing values between five year age bands, values in the DS cohort were more homogeneous than the control group (table 26). Younger people with Down syndrome tended to have higher TC values than controls, however above age 25 years this pattern was reversed and the control group maintained higher average values across all other age groups. Both the DS and control groups showed limited variation in mean TC values between age groups with the most marked changes being observed in the control group between the ages of 40 and 55 years, after which point a decline in values occurred. A similar decline was detected in the older Down syndrome group but did not reach significance. The control group had higher maximum values of TC compared to the Down syndrome group (maximum value of 5.15mmol/L in Down syndrome and 5.32mmol/L in controls) as well as a greater variance. The range of values in controls was 1.1mmol/L and 0.76mmol/L in the DS group. Both groups

show a similar pattern of change, with increases in TC values until age 45-50, and a decline after this time. TC levels were higher in DS in the youngest age group (18-20 years) and the values were comparable in the 20-24 age group. In all the other age groups the control group had significantly higher TC values than the DS cohort.

When age and sex were added as covariates there was a significant effect of DS*Age*Sex as well as main effects: $F(47, 11) = 4.357, p < 0.001$. All lower order interactions were also significant apart from sex*DS ($p = 0.580$).

	B	SE	beta	(95% CI for B)
Step 1				
Constant	4.818	0.087		
Group	0.040	0.053	0.47	-0.07 – 0.15
Step 2				
Constant	1.795	0.303		
Group	0.185	0.038	0.218**	0.11 – 0.26
Age	0.165	0.020	7.653**	0.13 – 0.21
Quadratic	-0.003	0.00	-12.917**	-0.003 - -0.002
Cubic	1.180e ⁻⁵	0.00	4.666**	0.00 – 0.00
Step 3				
Constant	1.437	0.280		
Group	0.185	0.034	0.218**	0.12 – 0.25
Age	0.165	0.018	7.653**	0.13 – 0.20
Quadratic	-0.003	0.00	-12.917**	-0.003 - -0.002
Cubic	1.18e ⁻⁵	0.00	5.088**	0.00 – 0.00
Sex	0.238	0.033	0.282**	0.17 – 0.30

** $P < 0.01$.

Table 25. Regression model for total cholesterol using CPRD. Note: $R^2 = 0.002$ for Step 1; $\Delta R^2 = 0.544$ for Step 2; $\Delta R^2 = 0.080$, (R^2 at step 1, $p > 0.05$; all other steps $p < 0.001$).

<i>Down syndrome</i>			<i>Control group</i>				
Age					Mean		
Group	<i>n</i>	TC	<i>n</i>	TC	difference	95% CI	<i>p</i>
18-20	123	4.53 (0.9)	167	4.22 (0.9)	-0.37	-0.58 - -0.16	<0.001
20-25	614	4.64 (1.1)	535	4.52 (1.0)	-0.08	-0.21 – 0.06	0.255
25-30	816	4.67 (0.9)	791	4.86 (1.0)	0.20	0.07 – 0.33	0.003
30-35	920	4.90 (1.1)	1295	5.08 (1.1)	0.23	0.10 – 0.36	<0.001
35-40	1226	5.04 (1.09)	2198	5.21 (1.1)	0.30	0.17 – 0.43	<0.001
40-45	1663	5.04 (1.09)	4092	5.26 (1.1)	0.26	0.13 – 0.39	<0.001
45-50	1945	5.15 (1.1)	6148	5.32 (1.1)	0.19	0.06 – 0.32	0.005
50-55	1760	5.13 (1.1)	7680	5.31 (1.1)	0.21	0.08 – 0.34	0.002
55-60	1173	5.06 (1.2)	8028	5.27 (1.2)	0.24	0.11 – 0.37	<0.001
60-65	661	4.89 (1.2)	7309	5.09 (1.2)	0.21	0.08 – 0.34	0.001
65-70	269	4.73 (1.2)	5336	4.87 (1.2)	0.21	0.08 – 0.33	0.002
70+	57	4.39 (0.8)	2956	4.63 (1.1)	0.19	0.06 – 0.33	0.006

Table 26. Mean TC for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. TC values are presented as mean (SD) in mmol/L.

Visualising the data using scatterplots grouped by sex confirm a similar relationship between TC and sex in both the DS and control groups which is in keeping with the lack of significance for the DS*Sex interaction. This is in contrast to BP and BMI in which the sex effects in the DS group differed from those in controls. For TC, the sex effects mirror that of controls, with lower values seen in males and higher values in females. The magnitude of this sex difference was approximately equal in the DS cohort and controls (figure 12).

High Density Lipoprotein

In the whole group comparison people with DS had slightly lower mean HDL values compared to controls. Both groups showed similar sex differences with females having higher average levels than males (mean HDLs for controls were 1.26 mmol/L (SD 0.37) in males versus 1.53 mmol/L (SD 0.45) in females; mean HDL in DS was 1.26 mmol/L (SD 0.31) in males versus 1.44 mmol/L (SD 0.38) in females).

In the stepwise regression model, cohort group did contribute significantly to the model although the R^2 was small (0.028 at step 1). Inclusion of age improved the model significantly but again the effect was relatively small (model R^2 0.177 at step 2). Sex further improved the model and contributed the largest effects. Adding sex to the model improved R^2 by 0.537 signifying a strong sex-related effect on HDL values in both the DS and control group. The overall R^2 was 0.709 (table 27).

	B	SE B	beta	95% CI for B
Step 1				
Constant	1.274	0.032		
Group	0.052	0.019	0.167**	0.01 – 0.09
Step 2				
Constant	0.928	0.069		
Group	0.048	0.019	0.152*	0.01 – 0.08
Age	0.013	0.002	1.642***	0.01 – 0.02
Age ²	0.00	0.00	-1.371***	0.00 – 0.00
Step 3				
Constant	0.588	0.043		
Group	0.048	0.011	0.152***	0.03 – 0.07
Age	0.013	0.001	1.642***	0.01 – 0.02
Age ²	0.00	0.00	-1.371***	0.00 – 0.00
Sex	0.227	0.010	0.733***	0.21 – 0.25

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 27. Regression model for HDL using CPRD. Note: $R^2 = 0.028$ for Step 1; $\Delta R^2 = 0.149$ for Step 2; $\Delta R^2 = 0.537$ for Step 3 (step 1 $p = 0.007$; step 2 and 3 $p < 0.001$).

When comparing between age bands the levels of HDL were relatively stable over the lifespan in both the DS and control groups (table 28). Significant between age-group differences were only observed between 35-40 and 45-50 years in people with DS. In controls there were no step-wise age-group differences. The highest average value was similar in both DS and controls (1.45 and 1.43 mmol/L respectively), but in DS this occurred in a younger age group (55-60 years compared to 70+ group in controls). HDL was lower in younger people with DS (18 – 35 year age groups) with significantly different values to controls. Between 35 – 55 years there were no group differences in HDL values observed between DS and controls. At 50-60 years the DS group had higher HDL values, although the difference was only significant in the 55-59 group. In the oldest groups, controls again had higher HDL values than people with DS. Given the relative stability of HDL over time it is difficult to interpret whether these age pattern differences are meaningful between the groups.

There was a significant interaction effect of DS*Age*Sex as well as main effects: $F(47, 11) = 0.482, p < 0.001$. All lower order interactions were significant, with the greatest effects seen from sex: DS*Sex: $F(47, 1) = 20.947, p < 0.001$. In both groups females had higher average HDL values. Sex differences were more pronounced in the control group compared to the DS group. Males with DS had higher levels of HDL compared to males without DS, whereas females with DS appeared to have lower levels of HDL than females without DS. Sex affects HDL values in both the control and DS cohort, with sex effects exerting significant influence on HDL values. The presence of trisomy 21 appears to modulate some of these sex effects: whilst males and females with DS have differences in HDL values, the sex difference was less marked than in controls (figure 12).

Age Group	<i>Down syndrome</i>		<i>Control group</i>		Mean		
	<i>n</i>	HDL	<i>n</i>	HDL	difference	95% CI	<i>p</i>
18-20	67	1.15 (.25)	84	1.27 (.29)	-0.12	-0.17 - -0.06	<0.001
20-25	430	1.16 (.28)	280	1.35 (.46)	-0.17	-0.21 - -0.14	<0.001
25-30	559	1.21 (.28)	466	1.34 (.40)	-0.11	-0.15 - -0.08	<0.001
30-35	619	1.25 (.28)	730	1.35 (.45)	-0.08	-0.12 - -0.05	<0.001
35-40	825	1.29 (.34)	1304	1.33 (.41)	-0.02	-0.06 – 0.01	0.23
40-45	1222	1.34 (.35)	2894	1.36 (.42)	-0.01	-0.05 – 0.03	0.55
45-50	1508	1.38 (.37)	4629	1.38 (.43)	0.01	-0.03 – 0.05	0.54
50-55	1385	1.42 (.37)	6073	1.40 (.44)	0.03	-0.01 – 0.06	0.16
55-60	884	1.45 (.39)	6621	1.41 (.43)	0.05	0.01 – 0.08	0.014
60-65	515	1.41 (.35)	6212	1.42 (.43)	-0.01	-0.05 – 0.03	0.56
65-70	217	1.37 (.36)	4711	1.42 (.44)	-0.06	-0.10 - -0.02	0.001
70+	46	1.32 (.38)	2643	1.43 (.44)	-0.09	-0.12 - -0.05	<0.001

Table 28. Mean HDL for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. HDL values are presented as mean (SD) in mmol/L.

Low Density Lipoprotein

In the whole group comparison people with DS had slightly higher mean LDL values compared to controls. As in HDL, both groups showed sex differences with females having higher average levels than males (mean LDL in controls was 2.94 mmol/L (SD 1.03) in males vs 3.15 mmol/L (SD 1.01) in females; mean LDL in DS was 3.04 mmol/L (SD 0.94) in males and 3.14 mmol/L (SD 0.94) in females).

In the stepwise regression model cohort group had a small relationship to LDL value (beta - 2.470, $p < 0.05$), however this became insignificant when age and sex were added to the model.

Age contributed the most weight to the model, with sex exerting smaller but significant effects (table 29). The overall R^2 of the model was 0.537. When cohort group was removed, model fit improved (R^2 0.543, $p < 0.001$) indicating that DS status was not significantly associated with LDL values when age and sex were controlled for.

	B	SE	beta	95% CI for B
Step 1				
Constant	3.115	0.075		
Group	-0.112	0.045	-2.470*	-0.20 - -0.23
Step 2				
Constant	1.795	0.126		
Group	0.032	0.034	0.044	-0.04 – 0.10
Age	0.057	0.005	3.073**	0.05 – 0.07
Quadratic	-0.001	0.00	-3.468***	-0.001 - -0.001
Step 3				
Constant	1.559	0.129		
Group	0.032	0.033	0.044	-0.03 – 0.10
Age	0.057	0.004	3.073**	0.049 – 0.066
Quadratic	-0.001	0.00	-3.468***	-0.001 - -0.001
Sex	0.157	0.031	0.216***	0.10 – 0.22

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 29. Regression model for LDL using CPRD. Note: $R^2 = 0.023$ for Step 1; $\Delta R^2 = 0.475$ for Step 2; $\Delta R^2 = 0.047$ for Step 3 (step $p = 0.014$ for step 1; step 2 and 3 $p < 0.001$).

In both the DS group and controls there was a parabolic relationship with age. LDL values were lower in controls at younger ages, but in both groups these increased with age until 50-55 years (table 30). The average peak value in both groups was 3.21mmol/L although the range was slightly higher in controls (0.58 vs 0.84mmol/L in the DS group and controls respectively). This confirms the similarity between cohort groups in LDL values. The values in the youngest age group (18-19 years) and oldest group (70+) were significantly higher in DS than in controls.

The only other significant difference was in the 35 – 40 year bracket when values were lower in DS. At all other age points there were no significant differences between DS and control groups, with few differences observed in LDL. This is in contrast to other cardiovascular variables where there are significant differences between the DS and control groups with age. It suggests that the metabolic dysregulation observed in DS may be restricted to certain pathways and not be universal. It also suggests that cholesterol differences may be more marked in childhood compared to adulthood in DS.

There was a significant effect of Age*Sex: $F(47, 11) = 18.58, p < .001$; and Age*Group: $F(47, 11) = 2.246, p = 0.01$ interactions. Neither Age*Sex*Group, nor Sex*Group interactions were significant ($p = 0.191$ and 0.453). DS status was not significant ($p = 0.484$), but age and sex retained significance in the final model (both $p < 0.001$).

Scatterplots confirmed the similarities in sex and age patterns between the DS group and controls. Sex and age effects are therefore important modulators of LDL and do not appear to be significantly affected by the presence of trisomy 21 which is in contrast to the effects of other markers analysed (see figure 12).

<i>Down syndrome</i>		<i>Control group</i>					
Age					Mean		
Group	<i>n</i>	LDL	<i>n</i>	LDL	difference	95% CI	<i>p</i>
18-20	55	2.80 (.87)	66	2.37 (.70)	0.43	0.23 – 0.64	<0.001
20-25	353	2.79 (.83)	240	2.69 (.89)	0.11	-0.02 – 0.24	0.09
25-30	449	2.93 (.82)	394	2.91 (.96)	-0.03	-0.16 – 0.10	0.67
30-35	518	3.07 (.86)	572	3.16 (1.02)	-0.11	-0.24 – 0.02	0.099
35-40	650	3.12 (.88)	1061	3.25 (1.02)	-0.19	-0.32 - -0.06	0.004
40-45	983	3.12 (.92)	2281	3.20 (.93)	-0.07	-0.20 – 0.06	0.275
45-50	1221	3.20 (.96)	3738	3.21 (.96)	-0.06	-0.18 – 0.07	0.400
50-55	1128	3.21 (.99)	4884	3.21 (1.02)	-0.02	-0.15 – 0.11	0.713
55-60	688	3.06 (1.02)	5319	3.15 (1.07)	-0.11	-0.24 – 0.02	0.08
60-65	401	2.98 (1.00)	4950	2.97 (1.04)	-0.01	-0.14 – 0.12	0.869
65-70	165	2.78 (.88)	3775	2.81 (1.00)	-0.07	-0.20 – 0.06	0.262
70+	36	2.87 (.05)	2103	2.64 (.05)	0.24	0.10 – 0.37	<0.001

Table 30. Mean LDL for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. LDL values are presented as mean (SD) in mmol/L.

Triglycerides

Overall the control group had a higher mean Tg level than those with Down syndrome. In both groups males had higher Tg values than females, although the difference between the sexes appeared larger in controls (mean Tg values in controls were 1.91 mmol/L (SD 1.41) in males versus 1.55 mmol/L (SD 0.98) in females; mean Tg values in DS were 1.51 mmol/L (SD 0.85) in males versus 1.40 mmol/L (SD 0.88) in females). In the step-wise regression model, cohort group, age and sex were all significant contributors although each with a small overall effect size (table 31). The R^2 of the final model was only 0.312, explaining just 31% of the variance observed.

In the DS cohort there were no significant differences between age groups when comparing mean values of Tg, with homogeneity in values across the age span (table 32). For example, values in people with DS aged 18-20 years were not significantly different to the values observed in any of the other age groups. In the control group there were significant differences between the younger age groups and older age groups, although on a group-by-group step-wise comparison the differences did not reach significance. In controls, people aged 18-30 years had significantly lower scores than those aged above 30 years when an increase in Tg values was observed (Bonferroni corrections, $p < 0.001$ comparing groups 18-20, 20-25, 25-30 years against older age groups). Therefore people with Down syndrome do not appear to have the same age related Tg increases that are observed in the general population. The peak mean values in the general population were higher than in DS (1.71 vs 1.50mmol/L) and the difference between the highest and lowest value was smaller in DS than controls (0.14 vs 0.55mmol/L).

	B	SE	beta	(95% CI for B)
Step 1				
Constant	1.258	0.051		
Group	0.163	0.031	0.312***	0.10 – 0.22
Step 2				
Constant	-0.028	0.244		
Group	0.138	0.031	0.265***	0.08 – 0.20
Age	0.084	0.016	6.346***	0.05 – 0.12
Age ²	-0.002	0.00	-12.928***	-0.002 - -0.001
Age ³	9.834e ⁻⁵	0.00	6.943***	0.00 – 0.00
Step 3				
Constant	0.218	0.232		
Group	0.138	0.029	0.265***	0.08 – 0.19
Age	0.084	0.015	6.346***	0.05 – 0.11
Age ²	-0.002	0.00	-12.928***	-0.002 - -0.001
Age ³	9.834e ⁻⁵	0.00	6.943***	0.00 – 0.00
Sex	-0.164	0.027	-6.082***	-0.22 - -0.11

***p<0.001

Table 31. Regression model for Tg using CPRD. Note: $R^2=0.09$ for Step 1; $\Delta R^2 = 0.113$ for Step 2; $\Delta R^2 = 0.101$ for Step 3 (all $p<0.001$).

Across the age bands Tg levels were significantly lower in the DS group compared to controls. This was apart from in the youngest age group where levels were higher in DS, and between 20 – 30 where there were no between group differences. When age and sex were added as covariates there was a significant effect of Group*Age*Sex interaction: $F(47, 11) = 4.463$, $p<0.001$, as well as all lower order effects (all $p<0.001$). People with Down syndrome showed significant differences to the general population in terms of age-related changes, sex effects and overall values of Tg, with increased homogeneity in Tg values between ages and sexes in Down syndrome. This is reflected in the scatterplots which demonstrate similar sex differences in DS and control groups, but with lower magnitudes of change in the DS cohort (figure 12).

<i>Down syndrome</i>			<i>Control group</i>				
Age					Mean		
Group	<i>n</i>	Tg	<i>n</i>	Tg	difference	95% CI	<i>p</i>
18-20	68	1.50 (.71)	89	1.16 (.63)	-0.35	0.21 – 0.49	<0.001
20-25	402	1.42 (.68)	318	1.34 (.75)	-0.04	-0.13 – 0.05	0.364
25-30	515	1.38 (.72)	496	1.42 (.90)	0.07	-0.02 – 0.15	0.149
30-35	611	1.43 (.75)	763	1.54 (.95)	0.17	0.08 – 0.26	<0.001
35-40	793	1.49 (.81)	1352	1.56 (.90)	0.12	-0.20 - -0.03	0.010
40-45	1112	1.50 (.81)	2759	1.62 (.95)	0.15	0.07 – 0.24	<0.001
45-50	1327	1.45 (.74)	4301	1.68 (.96)	0.26	0.17 – 0.35	<0.001
50-55	1221	1.44 (.70)	5521	1.69 (.97)	0.25	0.17 – 0.34	<0.001
55-60	765	1.39 (.71)	5949	1.71 (.91)	0.32	0.23 – 0.40	<0.001
60-65	442	1.37 (.58)	5532	1.65 (.86)	0.27	0.19 – 0.36	<0.001
65-70	182	1.43 (.62)	4118	1.62 (.81)	0.22	0.13 – 0.31	<0.001
70+	38	1.36 (.42)	2244	1.55 (.74)	0.10	0.01 – 0.19	0.039

Table 32. Mean Tg for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. Tg values are presented as mean (SD) in mmol/L.

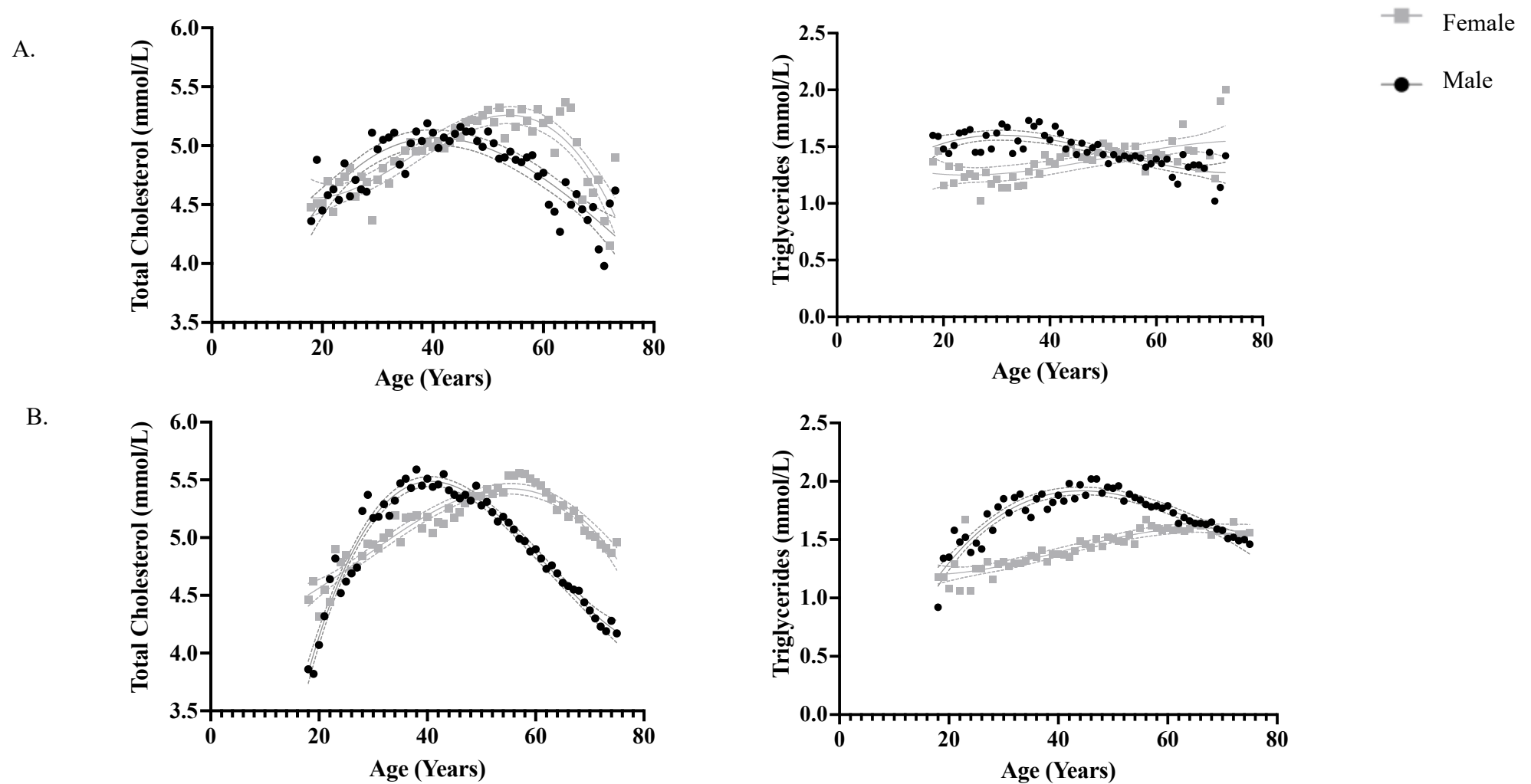


Figure 12a. Scatterplots displaying mean values in mmol/L for Total Cholesterol and Triglycerides by age, with groupings by sex. Error bars represent 95% confidence intervals.

A) Down syndrome cohort – top row. B). Control groups – bottom row.

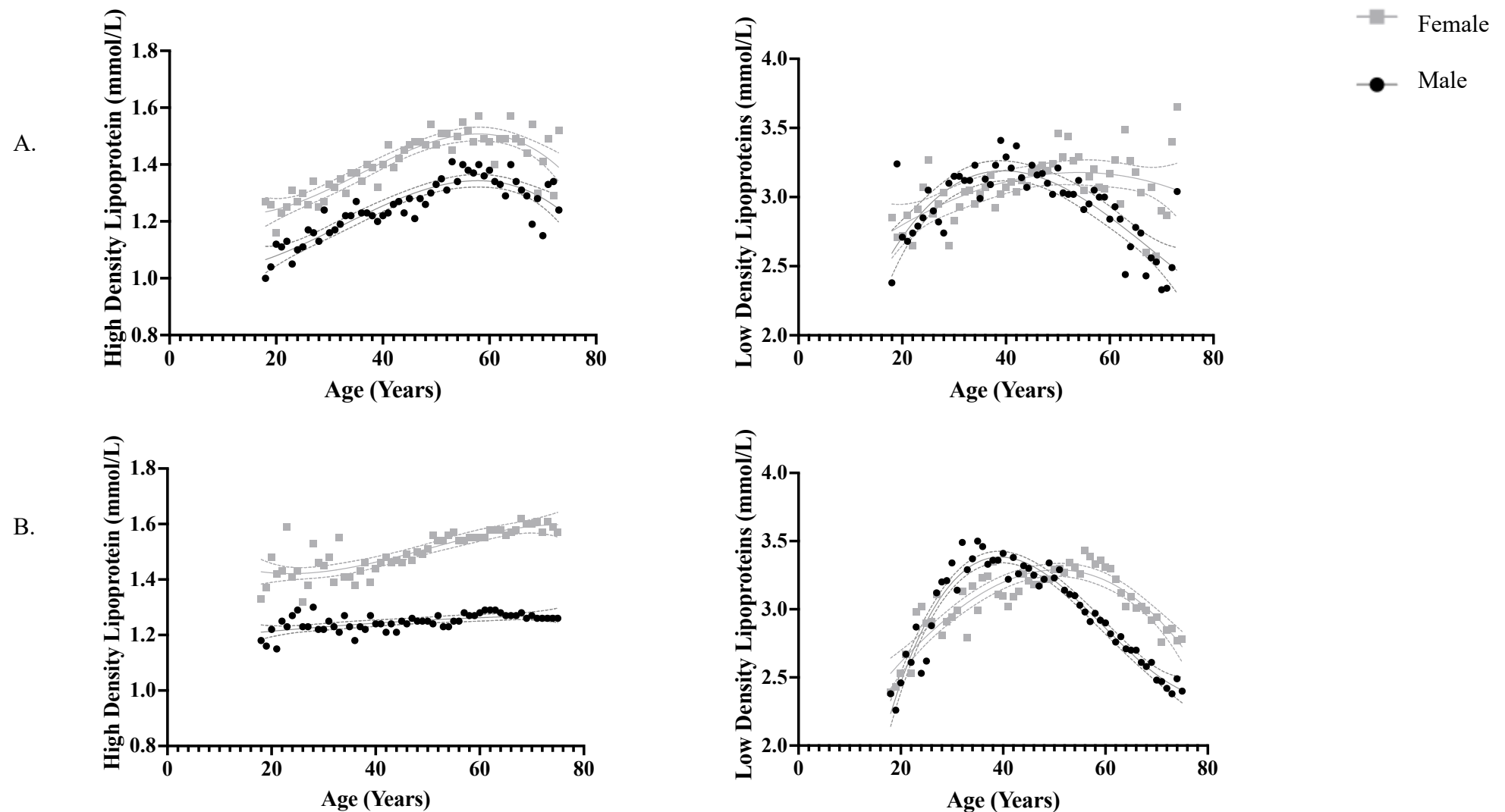


Figure 12b. Scatterplots displaying mean values in mmol/L for High Density Lipoproteins and Low Density Lipoproteins by age, with groupings by sex. Error bars represent 95% confidence intervals.

A) Down syndrome cohort – top row. B). Control groups – bottom row.

Glycated Haemoglobin

Overall the Down syndrome group had lower mean HbA1c values compared to controls in the whole group comparison in those without diabetes. In people with diabetes those with Down syndrome had higher HbA1c values than controls. There were only significant sex differences for mean HbA1c values in the DS group who did not have co-morbid diabetes: females had lower values (mean HbA1c 36.5 mmol/mol (SD 5.7) in males versus 35.8 mmol/mol (SD 5.9) in females with DS). In controls males had higher values of HbA1c and this remained true when the groups were split on diabetes status. In the control group, males with diabetes had a mean HbA1c of 67.0 mmol/mol (SD 16.5) compared to 66.3 mmol/mol (SD 16.1) in women. Males without diabetes had a mean HbA1c of 39.0 mmol/mol (SD 6.2). In females this was 38.1 mmol/mol (SD 5.9).

In the initial regression model there were no significant effects of cohort group, sex or age on mean HbA1c values (with both diabetic and non-diabetic groups included). Adding age improved the R^2 of the model slightly, but the effects of age did not reach significance at either Step 2 or Step 3 (both $p > 0.088$). The R^2 of the final model was only 0.136 (table 33). As expected, adding diabetes status improved the model significantly with an R^2 of 0.913 (beta 0.948, $p < 0.001$). In this model DS status also gained significance (beta 0.045, $p < 0.05$) suggesting an interaction between Down syndrome, diabetes and HbA1c value (table 34).

When those with diabetes were analysed separately, DS status alone did not significantly contribute to the model and at step 1 R^2 was 0.01 ($p > 0.05$). When age was added at step 2, both age and DS status were significant contributors to the model, and R^2 improved to 0.487 (ΔR^2 $p < 0.01$). Adding sex did not improve the model, with a final R^2 of 0.488 (table 35).

In the non-diabetic group, DS status was significant at step 1 (beta 0.393, $p < 0.001$) and retained significance at step 2 and step 3 when age and sex were added to the model (table 36). Both age and sex also contributed significantly at each step. The final model had an R^2 of 0.724. These findings show a differential effect of sex on HbA1c values and interactions with DS and age in people without diabetes compared to those who do, with sex differences more prominent in people without diabetes. In people with diabetes, DS group and age were significant predictors, and people with DS appeared to have poorer glycaemic control.

	B	SE	beta	(95% CI for B)
Step 1				
Constant	51.508	2.376		
Group	0.549	1.452	0.017	-2.30 – 3.40
Step 2				
Constant	46.728	5.662		
Group	1.751	1.497	0.054	-1.19 – 4.69
Age	0.259	0.211	0.304	-0.16 – 0.67
Quadratic	-0.004	0.002	-0.428	-0.008 – 0.001
Step 3				
Constant	46.747	6.071		
Group	1.751	1.499	0.054	-1.19 – 4.70
Age	0.259	0.211	0.304	-0.16 – 0.67
Quadratic	-0.004	0.002	-1.710	-0.008 – 0.001
Cubic	-0.013	1.434	0.000	-2.83 – 2.80

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 33. Regression model for HbA1c (whole sample). Note: $R^2 = 0.00$ for Step 1 ($p > 0.05$); $\Delta R^2 = 0.013$ for Step 2 ($p = 0.01$); $\Delta R^2 = 0.011$ for Step 3 ($p > 0.05$).

	B	SE	beta	(95% CI for B)
Constant	36.167	1.819		
Group	1.452	0.448	0.045*	0.57 – 2.33
Age	0.035	0.063	0.041	-0.09 – 0.159
Quadratic	-0.001	0.001	-0.113	-0.002 – 0.000
Sex	-0.094	0.428	-0.003	-0.936 – 0.747
Diabetes	30.703	0.430	0.948***	29.86 – 31.55

*p<0.05; **p<0.01; ***p<0.001

Table 34. Regression model for HbA1c with inclusion of diabetic status. Note: $R^2 = 0.913$, $p < 0.001$.

	B	SE	beta	(95% CI for B)
Step 1				
Constant	68.795	1.368		
Group	-0.399	0.839	-0.030	-2.05 – 1.26
Step 2				
Constant	78.473	1.172		
Group	1.239	0.613	0.094*	0.03 – 2.45
Age	-0.251	0.017	-0.708***	-0.28 - -0.22
Step 3				
Constant	77.840	1.481		
Group	1.243	0.613	0.095*	0.04 – 2.45
Age	-0.252	0.017	-0.708***	-0.28 - -0.22
Sex	0.420	0.600	0.032	-0.76 – 1.60

*p<0.05; **p<0.01; ***p<0.001

Table 35. Regression model for HbA1c (diabetic sample). Note: $R^2 = 0.01$ for Step 1 ($p > 0.05$); $\Delta R^2 = 0.486$ for Step 2 ($p < 0.001$); $\Delta R^2 = 0.001$ for Step 3 ($p > 0.05$).

	B	SE	beta	(95% CI for B)
Step 1				
Constant	34.035	0.507		
Group	2.116	0.309	0.393***	1.51 – 2.73
Step 2				
Constant	27.680	0.699		
Group	1.423	0.188	0.264***	1.05 – 1.79
Age	0.206	0.025	1.507***	0.16 – 0.26
Quadratic	-0.001	0.000	-0.774***	-0.001 - -0.001
Step 3				
Constant	28.506	0.737		
Group	1.420	0.185	0.263***	1.06 – 1.77
Age	0.206	0.025	1.506***	0.16 – 0.26
Quadratic	-0.001	0.000	-0.773***	-0.001 - -0.001
Sex	-0.547	0.176	-0.102**	-0.89 - -0.20

*p<0.05; **p<0.01; ***p<0.001.

Table 36. Regression model for HbA1c (non-diabetic sample). Note: $R^2 = 0.154$ for Step 1; $\Delta R^2 = 0.559$ for Step 2 both ($p < 0.001$); $\Delta R^2 = 0.010$ for Step 3 ($p < 0.01$).

When comparing between age bands, in people with DS and diabetes there were no significant step-wise differences between age groups although there was a downward trend in values with increasing age. The control group showed a similar pattern but with significant decline between 50-55 and 55-60 years, as well as between 60-65 and 65-70 years. This suggests more homogeneity across ages in the DS group compared to controls. The control group showed more variation in their HbA1c values with a range of 15.19 mmol/mol compared to 9.86 mmol/mol in the DS group. There were similarities in HbA1c values between DS and controls in the majority of age brackets, although values in DS trended towards being lower than controls. Between 20-24, 30-35, and 55-60 years the differences were significant (table 37).

<i>Down syndrome</i>		<i>Control group</i>					
Age		HbA1c		HbA1c	Mean		
Group	<i>n</i>	(DM)	<i>n</i>	(DM)	difference	95% CI	<i>p</i>
18-20	37	72.75 (18.45)	81	76.35 (19.52)	-2.43	-7.84 – 2.98	0.376
20-25	102	70.07 (16.93)	206	73.05 (16.76)	-4.01	-7.43 – 0.59	0.022
25-30	127	72.42 (15.72)	248	72.09 (15.58)	0.579	-2.84 – 4.00	0.739
30-35	114	70.30 (15.72)	292	73.20 (18.03)	-5.95	-9.37 - -2.53	<0.001
35-40	169	71.17 (18.30)	429	71.19 (18.50)	-0.69	-4.12 – 2.73	0.689
40-45	271	68.89 (18.40)	784	70.62 (16.99)	-0.92	-4.34 – 2.50	0.596
45-50	226	68.86 (17.04)	1059	69.03 (16.89)	-0.133	-3.55 – 3.29	0.939
50-55	211	66.00 (15.72)	1477	68.81 (17.11)	-1.49	-4.92 – 1.93	0.390
55-60	148	63.17 (14.87)	1472	66.21 (16.55)	-4.83	-8.25 - -1.41	0.006
60-65	87	62.89 (15.53)	1434	64.27 (14.96)	-1.95	-5.37 – 1.47	0.263
65-70	42	65.20 (14.50)	1108	61.83 (13.55)	1.92	-1.51 – 5.34	0.271
70+	13	66.79 (15.50)	712	61.16 (11.98)	0.143	-3.96 – 4.25	0.945

Table 37. Mean HbA1c (diabetic group) for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. HbA1c values are presented as mean (SD) in mmol/mol.

In those without diabetes HbA1c values were lower at all ages bands compared to those with diabetes. In controls the highest value was 39.82 mmol/mol which was observed in the 70+ group, whereas in the DS cohort the highest value was 37.64mmol/mol which was observed in 55-60 year olds (table 38). In the DS group a significant increase between age groups was only observed between the 30-35 and 35-40 age groups. In the control group there were significant increases at this time, as well as between 40-45 and 45-50; 50-55 and 55-60; 60-65 and 65-70 years. This suggests less variance in the DS cohort across age groups compared to controls. The DS and control groups had similar HbA1c values across age brackets until age 45, following which the DS group had consistently lower HbA1c values than controls.

<i>Down syndrome</i>		<i>Control group</i>					
Age		HbA1c		HbA1c	Mean		
Group	<i>n</i>	(no DM)	<i>n</i>	(no DM)	difference	95% CI	<i>p</i>
18-20	51	33.94 (6.66)	107	32.54 (6.91)	-1.40	-2.90 – 0.09	0.065
20-25	236	34.02 (5.90)	445	34.15 (5.73)	0.13	-0.82 – 1.07	0.789
25-30	268	33.53 (6.05)	514	33.72 (6.05)	0.85	-0.10 – 1.78	0.077
30-35	303	34.86 (6.54)	539	34.90 (6.49)	0.41	-0.53 – 1.36	0.391
35-40	349	36.36 (5.89)	664	36.09 (6.43)	0.30	-0.65 – 1.24	0.536
40-45	368	36.14 (5.54)	905	36.41 (6.31)	0.31	-0.64 – 1.26	0.520
45-50	477	36.70 (5.75)	1466	37.60 (5.89)	1.38	0.43 – 2.32	0.005
50-55	552	37.34 (4.87)	1929	38.22 (5.52)	1.42	0.48 – 2.37	0.003
55-60	392	37.64 (4.76)	1839	38.89 (5.33)	1.67	0.72 – 2.61	<0.001
60-65	211	36.59 (6.62)	1513	38.86 (6.43)	2.59	1.65 – 3.54	<0.001
65-70	94	36.70 (4.98)	1297	39.82 (5.82)	4.08	3.13 – 5.02	<0.001
70+	21	37.17 (4.42)	1036	39.96 (4.59)	3.30	2.33 – 4.28	<0.001

Table 38. Mean HbA1c (non-diabetic group) for each five-year age band in DS cohort and controls. Results are reported from multivariate regressions comparing the DS and control cohort at each age-band. HbA1c values are recorded as mean (SD) in mmol/mol.

For people with diabetes, there were significant effects of Group*Age interaction: $F(47, 11) = 1.913$, $p=0.033$. Group and age individually were also significant ($p=0.016$ and $p<0.001$ respectively). No other interactions or main effects were significant. Therefore, in people with diabetes, those with Down syndrome exhibit different age effects compared to controls, but with limited sex effects.

For non-diabetics, there were significant effects of the DS*Age interaction: $F(47, 11) = 5.113$, $p<0.001$; and Age*Sex: $F(47, 11) = 73.466$, $p=0.013$. Neither Group*Age*Sex nor Group*Sex interactions were significant (both $p>0.05$). People with Down syndrome showed different

patterns of HbA1c with age compared to controls, as well as age-related sex-effects on glycaemic control in the absence of diabetes.

Scatterplots confirmed a difference between people with Down syndrome and controls for those without diabetes (see figure 13). In those with diabetes, the most prominent effects were related to age. Group status had effects when age was taken into account, but did not contribute significantly when considered independently. Similarly there were no significant effects of sex on HbA1c status, even when group and age were controlled for in the diabetic cohort.

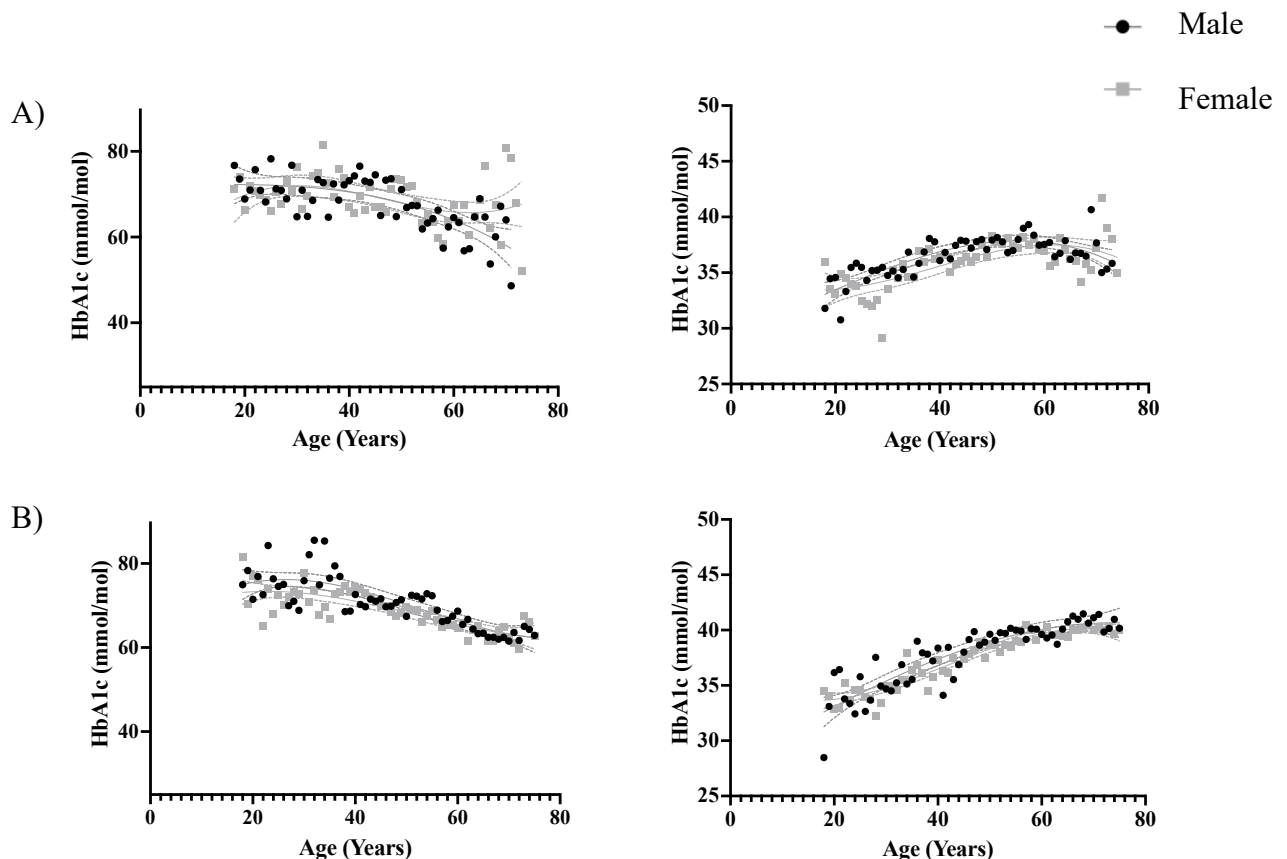


Figure 13. Scatterplots displaying mean values for HbA1c in mmol/mol by age, with groupings by sex. Values for those with diabetes presented in the first column. Values for those without diabetes presented in the second column. Error bars represent 95% confidence intervals.

A) Down syndrome cohort – top row. B). Control groups – bottom row.

4.3.5. Interactions Between BMI and Blood Pressure

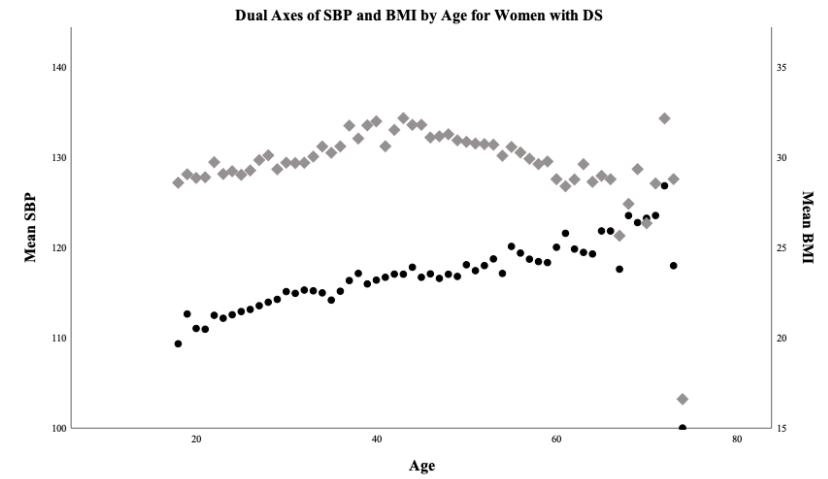
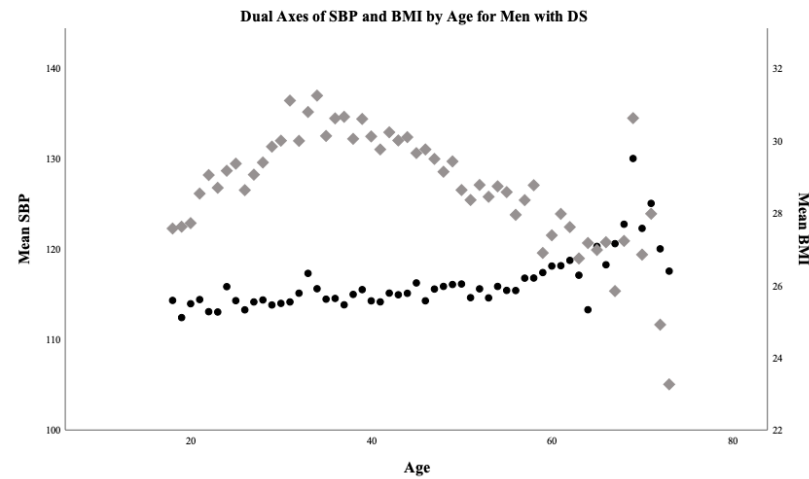
The effects of BMI on blood pressure were considered as a possible explanation for the sex-effects outlined above. Given that women with DS had both higher BMI and higher blood pressures, it is possible that BMI was driving the effects. BMI was therefore added as a predictor into the regression models for SBP, DBP and PP alongside DS status, age and sex. BMI exerted a small but significant positive relationship in the blood pressure models with the strongest effect observed in the SBP model. The beta values for BMI were 0.525 (SE 0.007), 95% CI 0.51 - 0.54 for SBP; 0.354 (0.005), 95% CI 0.35 - 0.36 for DBP; and 0.170 (0.005), 95% CI 0.16 – 0.18 for PP.

For all three models (SBP, DBP and PP), adding BMI did not affect the significance or effect size of DS group or age, showing that these predictors have a significant relationship to BP value even when adjusted for BMI. On the other hand, sex effects were modulated by the inclusion of BMI. In the earlier models sex was not a significant predictor, but became significant with the inclusion of BMI. In the fully adjusted SBP regression model, the beta value for sex increased from -0.471 (SD 0.65), 95% CI -1.01 - 0.57 to -3.825 (SD 0.10), 95% CI -3.82 - -3.43, $p < 0.001$. In the DBP model the beta value for sex increased from -0.23 (SD 0.41), 95% CI -1.01 - 0.57 to -1.968 (SD 0.07), 95% CI -2.10 - -1.84, $p < 0.001$. In the PP model, the beta effect of sex increased from -0.245 (0.37), 95% CI -0.97 - 0.48 to -1.657 (SD 0.01), 95% CI -1.81 - -1.51, $p < 0.001$.

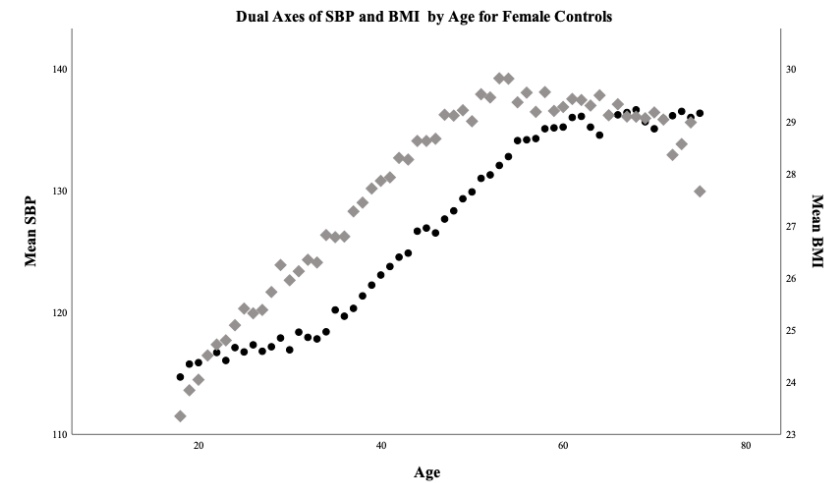
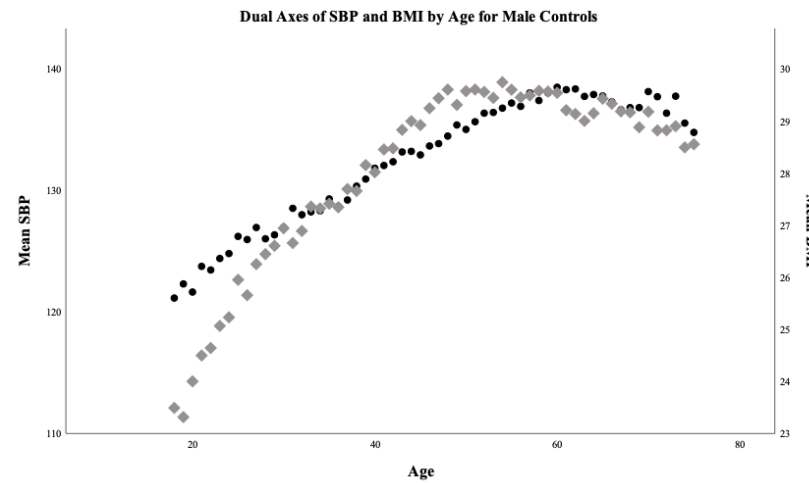
The mean SBP, DBP and PP values alongside BMI values were plotted using dual axes scatterplots to visualise the relationships by age, DS status and sex (figures 14-16). In controls there was a mirroring between blood pressure and BMI changes, particularly for SBP. On the

other hand, in people with DS the association between BMI and BP changes with age in both sexes were less well-defined. The differences in sex effects observed in blood pressure in people with DS therefore do not appear to be entirely accountable by BMI, and indeed there is an uncoupling of age-related associations between BMI and blood pressure in the DS cohort.

A)



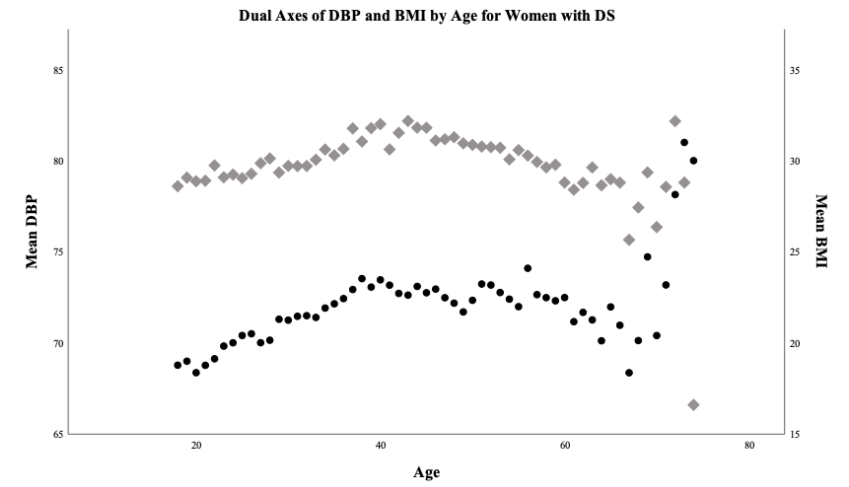
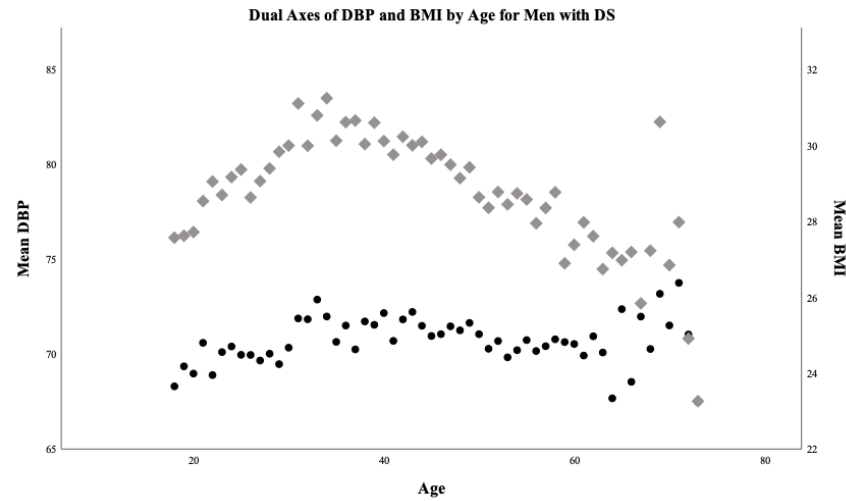
B)



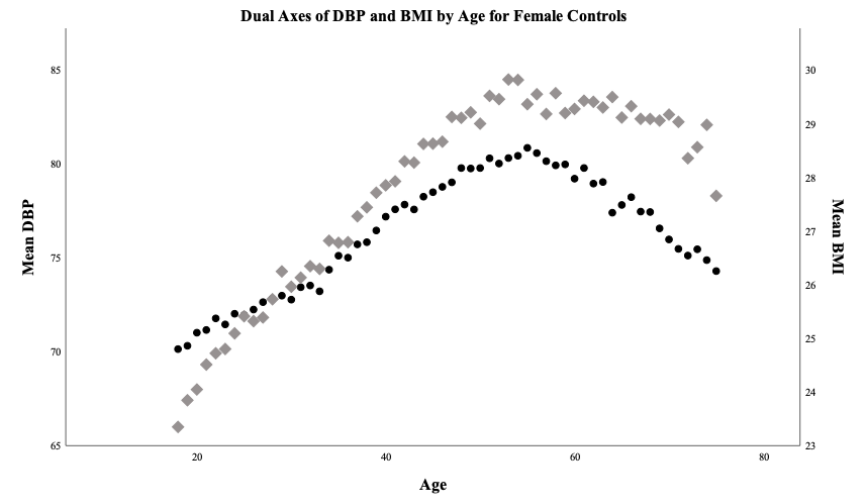
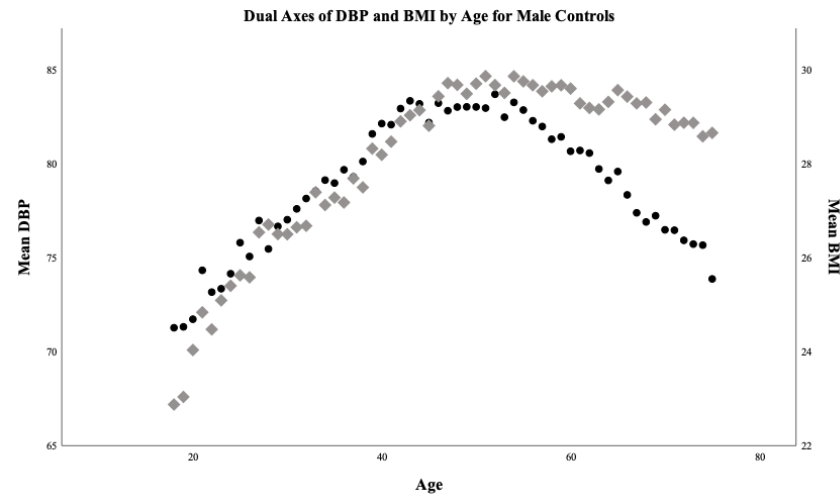
- ◆ BMI
- Blood Pressure

Figure 14. Dual axes scatterplots displaying mean values for SBP (mmHg) and BMI (kg/m^2) by age, with groupings by sex (males first column, females second column). A) Down syndrome cohort – top row. B). Control groups – bottom row. Error bars represent 95% confidence intervals.

A)



B)



◆ BMI
● Blood Pressure

Figure 15. Dual axes scatterplots displaying mean values for DBP (mmHg) and BMI (kg/m²) by age, with groupings by sex (males first column, females second column). A) Down syndrome cohort – top row. B). Control groups – bottom row. Error bars represent 95% confidence intervals.

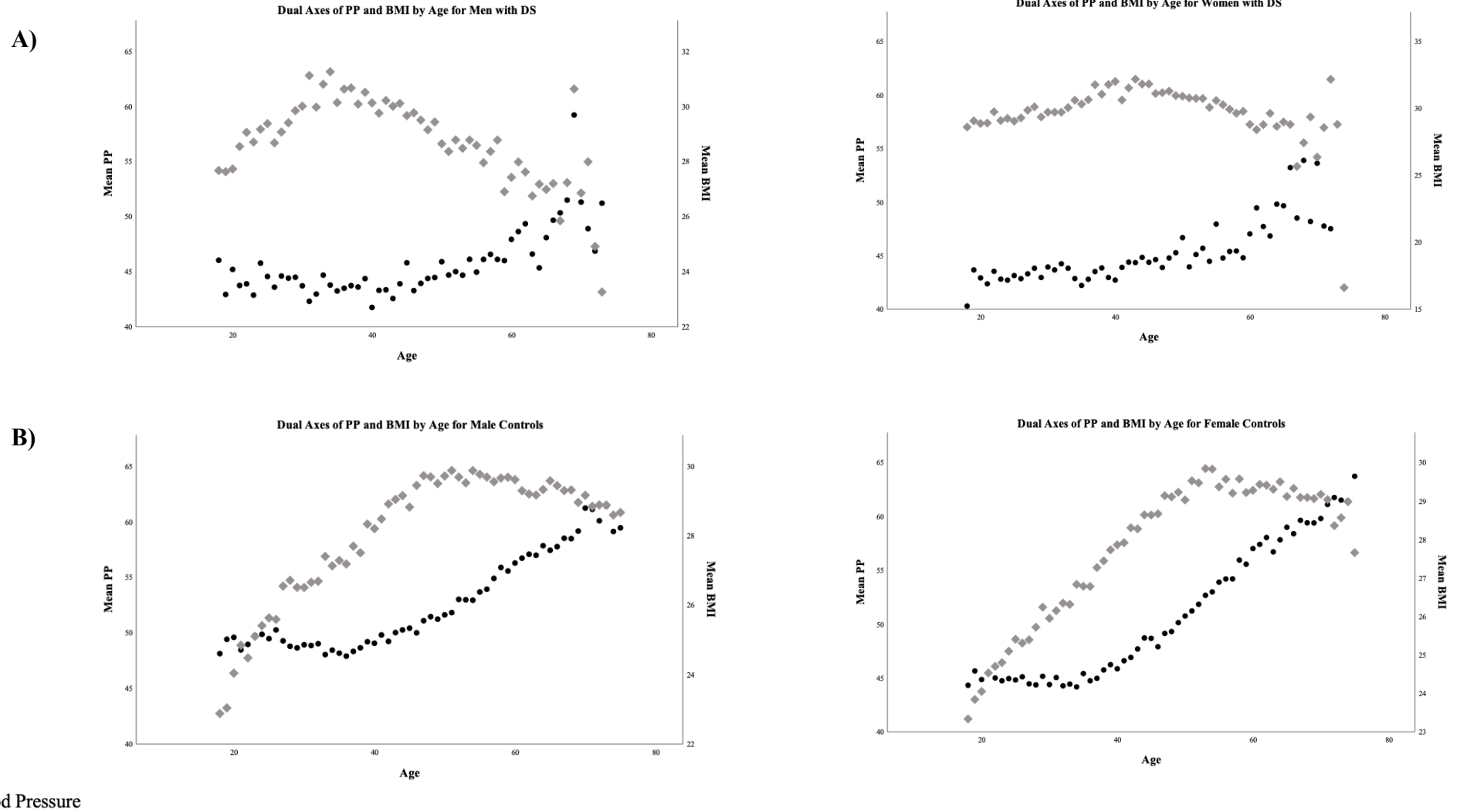


Figure 16. Dual axes scatterplots displaying mean values for PP (mmHg) and BMI (kg/m^2) by age, with groupings by sex (males first column, females second column). A) Down syndrome cohort – top row. B). Control groups – bottom row. Error bars represent 95% confidence intervals.

4.4. Chapter Summary

The results above demonstrate significant differences in the cardiovascular profiles of people with Down syndrome compared to controls. These differences were not universal, and were more pronounced in some cardiovascular health parameters than others. There were also differences in the way age and sex interacted with cardiovascular health parameters, and these interactions differed to that which was observed in controls (table 39).

There were significant differences between the DS group and general population controls in mean CVP values, patterns of CVP change with age, and the relationship between CVP and sex. Notably, blood pressure parameters and BMI had the most marked differences between the groups with both age and sex differences observed in people with DS compared to controls. Lipid profiles were affected by DS status, however the same sex effects were observed. DS appears to affect sex-patterns differentially depending on the CVP rather than exerting universal effects throughout the body.

In light of these significant differences in CVPs, it cannot be assumed that the dementia-related risks related to CVPs as observed in the general population will be the same for people with DS. The relationship between CVPs and dementia incidence in people with DS is quantified in the next section.

Variable	Magnitude difference between DS and Controls?	Age-related effects?	Difference in age effects between DS and controls?	Sex-related effects?	Difference in sex effects between DS and controls?
SBP	Yes (Lower in DS)	Yes	Yes	Yes	Yes (F>M in DS; M>F in controls)
DBP	Yes (Lower in DS)	Yes	Yes	Yes	Yes (F>M in DS; M>F in controls)
PP	Yes (Lower in DS)	Yes	Yes	Yes	Yes (F<M in DS; M=F in controls)
BMI	Yes (Higher in DS)	Yes	Yes	Yes	Yes (F>M in DS; M>F in controls)
TC	Yes (Lower in DS)	Yes	No	Yes	No
HDL	Yes (Lower in DS)	Yes	Yes	Yes	No
LDL	No	Yes	No	Yes	No
Tg	Yes (Lower in DS)	Yes	Yes	Yes	No
HbA1c (DM)	Yes (Higher in DS)	Yes	No	Yes	Yes (F=M in DS; M>F in controls)
HbA1c (no DM)	Yes (Lower in DS)	Yes	Yes	Yes	No

Table 39. Summary of group, age and sex effects on cardiovascular health parameters in the Down syndrome cohort compared to controls.

Chapter V

Analysis of Cardiovascular Health Markers in People with Down Syndrome and Their Relationship to Incident Dementia Risk Using Electronic Primary Care Records

Part B. Cardiovascular Risk Factors in Early-Mid Adulthood and Incident Dementia Risk in People with Down Syndrome

Contents

- 5.1. Summary of Methods and Aims
- 5.2. Power Calculations
- 5.3. Cohort Summary
 - 5.3.1. Overall Cohort
 - 5.3.2. Cohort for Survival Analyses
- 5.4. Survival Models
 - 5.4.1. Systolic Blood Pressure
 - 5.4.2. Diastolic Blood Pressure
 - 5.4.3. Pulse Pressure
 - 5.4.4. Body Mass Index
 - 5.4.5. Effects of Covariates
 - 5.4.6. Combined Risk Model
- 5.5. Exploratory Group Comparisons
- 5.6. Chapter Summary

5.1 Summary of Methods and Aims

In the literature from the general population it is reported that mid-life cardiovascular status can affect later life risks for cognitive decline and dementia^{199,200}. It is not known if the same is true for people with DS. I hypothesised that there are compounding effects on dementia risk from both genetic factors and cardiovascular health status in people with DS, and that the presence of certain cardiovascular factors such as obesity in younger adulthood may be associated with earlier onset of dementia, despite common genetic drivers behind the development of AD in DS.

To investigate this, data from the CPRD was used following the rationale and methods outlined in chapter 3. Briefly, measurements of cardiovascular health parameters (CVPs) taken between the ages 25-34 years (inclusive) were used as predictors for incident dementia over the age of 35 years, alongside relevant comorbidities. The primary CVPs considered were: systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), and body mass index (BMI) as these are measured routinely in annual health checks. The outcome of interest was first recorded diagnosis of new incident dementia. This was coded using the first record of a new dementia diagnosis, stroke, or epilepsy/seizures due to the common co-occurrence of these conditions. Total cholesterol (TC), high density and low density lipoproteins (HDL and LDL respectively), triglycerides (Tg) and glycated haemoglobin (HbA1c) were investigated as exploratory predictor variables.

Kaplan-Meier methods were used to assess mean survival time and calculate crude incident rates for each group. Cox regression models were then fitted to calculate hazard ratios for incident dementia based on CVP group, examining the effects of covariates. CVP groups were

entered individually and then models constructed with additional relevant covariates using the enter method: sex, age, and registration length. Relevant co-morbidities were included: cardiovascular disease (a combined group consisting of hypertension, IHD, hypercholesterolaemia and PVD), diabetes mellitus, hypothyroidism and CHD. Unless specified the middle value CVP group was used as the reference indicator, as was male sex. Epilepsy and stroke were not used as comorbidities as people with these pre-existing conditions were excluded from the analysis.

5.2. Power Calculations

SBP, BP, PP, and BMI measurements were assessed in the survival analyses. There were insufficient events in people with recorded TC, HDL, LDL, Tg or HbA1c values to allow for construction of meaningful regression models or adequately power the analyses. Exploratory group comparisons between people with dementia (DSdem) and without dementia (DScn) were completed using two-way independent t-tests for these five additional CVPs and are included at the end of the chapter.

Given the paucity of available research in this area for people with DS, estimated hazard ratios (HRs) based on existing literature in sporadic AD were used to estimate analytical power. For SBP, published HRs ranged between 1.57-1.77, with an average of 1.7^{336,337,338,339}. The ratio of people with DS and raised SBP taken from the results of the cross-sectional CPRD analysis was approximately 1:9. To ensure sensitivity and power were maintained at the standard values for α and β of 0.05 and 0.2, ideally there would be 310 events (incident dementia occurrences).

BMI HRs are estimated to be between 1.4-2.0 in the literature for incident dementia, with an average around 1.77,^{338,340}. The proportion of high BMI in the DS group was approximately 2:3. To ensure sensitivity and power are maintained at the standard values for α and β of 0.05 and 0.2, ideally there would be 116 events in the analysis.

For the SBP analysis there were actually 135 events, providing a $1-\beta$ value of 0.5 if alpha is retained at 0.05. This means the analysis may have been underpowered and there is a risk of type II errors when interpreting the results. For BMI there were 121 events which provided adequate power for the analysis.

Limited studies have reported HRs for mid-life DBP and dementia risk in sporadic AD. One study reported HRs of around 0.9 which suggests no or minimal impact of DBP on dementia risk. Similarly for PP there were no relevant HRs for PP group identified in the literature. The analysis here therefore proceeded as an exploratory investigation for these CVPs.

5.3. Cohort Summary

5.3.1. Overall Cohort

Inclusion and exclusion criteria were outlined in the methods section (chapter 3). The number of people who had CVP data available between the ages of 25-34 years and were eligible for inclusion in the survival analyses are described in table 40.

CVP	<i>n</i>	Events	CVP Value	Age at CVP Measurement
SBP	1622	135	115.64 (11.29)	31.38 (2.30)
DBP	1614	133	71.95 (7.71)	31.38 (2.30)
PP	1614	133	43.78 (8.18)	31.38 (2.30)
BMI	1375	121	28.73 (7.16)	31.44 (2.41)
TC	422	29	4.81 (0.96)	30.42 (3.05)
Tg	406	15	1.41 (0.95)	30.43 (3.09)
HDL	419	12	1.25 (0.36)	30.35 (3.07)
LDL	365	12	3.11 (2.19)	30.42 (3.03)
HbA1c	407	16	40.05 (16.16)	29.74 (2.91).

Table 40. Cohorts numbers for CVPs measured between the ages of 25-34 years. Results are presented as mean (SD). Given the low number of incident events in the TC, Tg, HDL, LDL and HbA1c groups only blood pressure and BMI were used in the survival analyses.

The cohort was divided into low, medium, and high groups for each CVP as follows. Firstly grouping was based on general population guidance for target CVP thresholds which were used to define the middle or reference group. Values lower than these thresholds were classified as “low” and those above as “high” for the purpose of the analysis. In a second grouping, to account for the different distributions of CVP values observed in DS, thresholds were derived from the DS population data presented in chapter 4. The CVP group limits were the 10th and 90th percentile values of the adults DS sample population. Table 41 summarises the CVP groups. The DS-population derived HbA1c cut-offs were based on percentiles for those without diabetes.

	<i>General Population Guidance</i>			<i>10th and 90th Percentile Values</i>		
	Low	Medium	High	Low	Medium	High
SBP (mmHg) ³⁴¹	<100	100-140	>140	<101	101 – 130	>130
<i>n</i>	71	1486	65	158	1266	198
Events (n)	11	113	11	11	99	25
DBP (mmHg) ³⁴¹	<70	70-90	>90	<62	62-80	>80
<i>n</i>	584	998	32	167	1132	315
Events (n)	45	83	5	25	90	18
PP (mmHg)	<40	40-60	>60	<34	34-54	>54
<i>n</i>	568	976	70	153	1305	156
Events (n)	63	61	9	12	103	18
BMI (kg/m²) ³⁴²	<18.5	18.5-25	>25	<24.3	24.3 – 33.7	>33.7
<i>n</i>	24	465	886	165	1097	113
Events (n)	3	28	90	10	103	8
TC (mmol/L)	<5	5-6.5	>6.5	<3.7	3.7-6.4	>6.4
<i>n</i>	224	176	22	36	358	9
Events (n)	12	13	4	2	23	4
Tg (mmol/L)	≤2.3	-	>2.3	0.72	0.72 – 2.5	>2.5
<i>n</i>	371	-	35	53	331	22
Events (n)	12	-	3	1	12	2
HDL (mmol/L)	<1.0	1.0 – 1.6	>1.6	<0.95	0.95 – 1.8	>1.8
<i>n</i>	68	294	57	349	52	18
Events (n)	3	8	1	3	9	0
LDL (mmol/L)	≤3	-	>3	<1.9	1.9-4.4	>4.4
<i>n</i>	198	-	167	31	315	19
Events (n)	3	-	9	0	10	2
HbA1c (mmol/mol)	<42	42-47	>47	<30.1	30.1 – 43.1	>43.1
<i>n</i>	326	12	69	56	275	76
Events (n)	10	1	5	3	8	5

Table 41. CVP groupings and number of available measurements taken between age 25-34 years. Groups based on guidance targeted towards general population and those derived from the DS sample based on 10th and 90th centiles.

5.3.2. Cohort Summary for Survival Analyses

BP data measured between 25-34 years were available for 2,919 individuals. After exclusion criteria were applied there were 1,622 cases with BP data available who were eligible for inclusion in the survival analyses. There were 527 males and 1095 females. There were 14,114 person years available. Mean registration length was 8.63 years (SD 5.45, range 1-25). Mean age at cohort entry was 36.58 (SD 3.50, range 35-58). One hundred and forty people (8.6%) had history of congenital heart disease (CHD), 465 (28.7%) had a recorded diagnosis of hypothyroidism and 120 had diabetes mellitus (7.4%). There were 135 incident events, with no left censored cases or missing values identified for SBP. There were 8 missing values for DBP. Cohort summaries are presented in table 42.

There were 7,491 BMI readings available that were measured in people aged 25-34 years, taken from 2,810 individuals. When exclusion criteria were applied there were 1,375 cases (481 men and 894 women) with 11,521 patient years available. Mean registration length was 8.38 years, (SD 5.70, range 1-25). Mean age at cohort entry was 36.39 years (SD 3.32, range 35 – 56). There were 121 incident events. There were no missing or left censored cases. One hundred and twenty-five people had CHD (9.1%), 428 had hypothyroidism (31.3%), and 117 had diabetes mellitus (8.5%).

The relatively low numbers of co-morbid CHD observed in the cohorts (which is estimated to occur in up to 50% of people with DS) are likely due to a combination of factors. One, as this analysis uses data extracted from GP records we are reliant on adequate histories being provided and entered. In the older population with DS, people with a history of CHD may no longer have this recorded, particularly if the condition did not require treatment or follow-up

in adulthood. It has been found that CHDs can be under-recognised and recorded in older adults with DS³⁴³. Secondly, the prevalence of CHD is generally calculated in populations including children. In these analyses people under 35 years old were excluded. There may be a healthy survivor effect in the older adults who may be less likely to have a significant CHD when younger as surgery and treatment may not have been readily available or as effective four decades ago.

CVP	<i>n</i>	Person years	Events	Male (%)	Registration Length	Age at Cohort Entry
Blood Pressure	1,622	14, 114	135	527 (32)	8.63 (5.45)	36.58 (3.5)
BMI	1,375	11,521	121	481 (35)	8.39 (5.70)	36.39 (3.3)

Table 42. Cohort Summary for BP and BMI Survival Analyses. Continuous variables presented as mean (SD). Sex presented as n (%).

Those with incident dementia (DSdem) were compared to those without (DScn) using two-way independent t-tests. The DSdem group were slightly older on average at the time of CVP measurement (32.46 years (SD 2.32) vs 31.28 (2.27), t 5.70, $p < 0.001$ for BP; 32.37 years (2.50) vs 31.35 years (2.39), t 4.296, $p < 0.001$ for BMI), and were slightly older at cohort entry (37.93 (3.97) vs 36.43 (3.35); t 4.25, $p < 0.001$ for BP; 37.57 (3.91) vs 36.27 (3.24), t 3.538, $p < 0.001$ for BMI).

When measured between 25-34 years old there was no difference between mean SBP, DBP or PP between those with incident dementia and those without (115.69 mmHg (SD 11.07) versus 115.19 mmHg (SD 13.48), t 0.41, $p = 0.68$ for SBP; 71.72 mmHg (9.05) versus 71.99 mmHg (7.58), t 0.31, $p = 0.76$ for DBP; 43.68 mmHg versus 43.79 mmHg (8.09), t 0.13, $p = 0.90$ for

PP). The DSdem group had a higher mean BMI but this was not significant (29.16 (6.37) vs 28.68 (7.24), t 0.781 $p=0.28$). There was a higher proportion of males in the DSdem group (11.6% vs 7.3%, chi square 7.447, $p=0.006$).

A combined cardiovascular comorbidity group (designated as “CVD”) which included history of ischaemic heart disease (IHD), hypertension, hypercholesterolaemia or peripheral vascular disease (PVD), was created as a covariate for inclusion in the survival models due to the low numbers of people with these comorbidities in the sample. There were 18 people with IHD, 64 with hypertension, 93 people with hypercholesterolemia, and 23 with PVD.

5.4. Survival Models

5.4.1. Systolic Blood Pressure Models

SBP Groups Based on Thresholds for the General Population

Applying the pre-defined low (<100mmHg), middle (100-140mmHg) and high (>140mmHg) SBP values based on general population guidance there were 113 events in the middle group, eleven in the low, and eleven in the high group. This provided person years of 12,824; 643; and 647 respectively. The median survival time was highest in the normotensive (middle) group. There were significant differences between the low and middle group, and between the high and middle group. The survival rates were not significantly different between the low and high SBP groups. Mean survival times were 259.52 months (SD 4.09) in the middle group compared to 208.49 months (SD 8.52) in the low group and 212.54 months (SD 12.97) in the high (Log rank chi square 2.925, $p=0.048$ and 5.009, $p=0.025$ for the low and high groups

compared against the normotensive group respectively). The crude incident rates were almost identical for the low and high groups, which were approximately twice as high as the normotensive group: 17.10 (95% CI 7.00 – 27.21) per 1000 person years, in the low group; 17.00 (95% CI 6.95 – 27.04) in the high group; 8.81 (95% CI 7.19 – 10.44) in the normotensive group.

Cox regression models were constructed with age, sex, registration period, hypothyroidism, CHD, diabetes, and CVD inputted as covariates. Significant associations between SBP group and incident dementia risk were maintained in the adjusted model (table 43). This was for both the high and low SBP groups, although the low group contribution was of borderline significance in the fully adjusted model. For those in the high SBP group (>140mmHg) compared to the reference group (100-140mmHg) the hazard ratio was 2.04 (95% CI 1.1 – 3.8, $p=0.026$). This suggests that people with high SBP values aged between 25-34 years are at double the risk of incident dementia at a specific time point compared to those who are normotensive. Those in the low SBP group (<100mmHg) also had an elevated hazard ratio of 1.84 (95% CI 0.99 – 3.43, $p=0.055$) compared to the normotensive group, although this was of borderline significance.

	-2LL	$\Delta\chi^2$	<i>p</i>
Block 0	1702.76		
Block 1	1695.99	6.76	0.034
Block 2	1641.13	54.86	<0.001
Block 3	1626.98	14.15	.007

	B	SE	Wald	<i>p</i>	Exp(B)	95% CI
Block 1						
SBP Group			7.859	0.020*		
Low SBP	0.614	0.317	3.758	0.053	1.847	0.99 – 3.44
High SBP	0.691	0.316	4.780	0.029*	1.996	1.07 – 3.71
Block 2						
SBP Group			8.328	0.016*		
Low SBP	0.648	0.318	4.152	0.042*	1.912	1.03 – 3.57
High SBP	0.707	0.318	4.954	0.026*	2.029	1.09 – 3.78
Sex (F)	-0.425	0.178	5.706	0.017*	0.654	0.46 - 0.93
Age	0.128	0.020	39.743	<0.001*	1.136	1.09 – 1.18
Time	-0.103	0.027	14.341	<0.001*	0.902	0.86 - 0.95
Block 3						
SBP Group			7.955	0.019*		
Low SBP	0.611	0.319	3.672	0.055	1.842	0.99 – 3.43
High SBP	0.712	0.319	4.981	0.026*	2.037	1.09 – 3.81
Sex (F)	-0.298	0.183	2.662	0.103	0.742	0.52 – 1.06
Age	0.131	0.020	41.49	<0.001*	1.140	1.10 – 1.19
Time	-0.107	0.027	15.111	<0.001*	0.899	0.85 - 0.95
CHD	0.530	0.266	3.966	0.046*	1.699	1.01 – 2.86
Hypothyroid	0.509	0.178	8.193	0.004*	1.664	1.17 – 2.36
Diabetes	0.228	0.267	1.161	0.281	1.334	0.79 - 2.25
CVD	0.027	0.229	0.014	0.907	1.027	0.66 – 1.61

Table 43. Cox regression model for SBP groups based on general population guidance. Indicator variables: normotensive SBP (100-140mmHg); male sex. CHD = Congenital Heart Disease. CVD = Cardiovascular Disease.

SBP Groups Derived from Down Syndrome Population Percentiles

The groups were defined using the 10th and 90th percentiles from the population data in chapter 4: Low SBP group <101mmHg; Middle SBP group 101 – 130mmHg; High SBP group >130mmHg. There were 99 events in the middle group, eleven in the low group and 25 in the high. There were 10,633 person years for the reference group, 1,496 for the low and 1,985 for the high. The mean survival times were not significantly different between the middle and high group (Log rank Chi Square 2.295, p=0.13), nor the low and high group (Log rank Chi square 0.578, p=0.45). There was a significant difference between the low and middle group. The mean survival time in the low group was 208.49 months (SD 8.52) compared to 259.85 months (SD 2.42) in the middle group (Log rank Chi square 4.094, p=0.04). Crude incident rates for the low group were the same as in the general population derived groups (17.10 per 1000 person years, 95% CI 7.00 – 27.21) as the limits applied for this group were almost identical to the previous analysis (<100mmHg in the general population cut offs and <101mmHg in the DS derived groups). In the middle SBP group the rate was 8.62 (95% CI 6.92 – 10.32) per 1000 person years. In the high SBP group the rate was 12.59 (95% CI 7.66 - 17.53) per 1000 person years. See figure 18 at the end of the chapter for survival curves.

In the fully adjusted model outlined in table 44, both the low and high SBP groups had an increased HR compared to the reference group. The significance was borderline for the low group (HR 1.84 (95% CI 0.98 – 3.45), p=0.06). The effect for the high group was not significant (HR 1.29 (95% CI 0.83 – 2.02), p=0.26).

	-2LL	$\Delta\chi^2$	p
Block 0	1702.76		
Block 1	1697.94	4.82	0.090
Block 2	1643.83	54.11	<0.001
Block 3	1629.92	13.90	0.008

	B	SE	Wald	p	Exp(B)	95% CI
Block 1						
SBP Group			5.337	0.069		
Low SBP	0.628	0.319	3.884	0.049*	1.874	1.01 – 3.50
High SBP	0.330	0.224	2.173	0.140	1.392	0.90 – 2.16
Block 2						
SBP Group			5.008	0.082		
Low SBP	0.651	0.320	4.139	0.042*	1.918	1.02 – 3.59
High SBP	0.277	0.226	1.500	0.221	1.319	0.85 – 2.06
Sex (F)	-0.425	0.179	5.667	0.017*	0.654	0.46 - 0.93
Age	0.125	0.020	38.116	<0.001*	1.133	1.09 – 1.18
Time	-.0104	0.027	14.546	<0.001*	0.902	0.86 - 0.95
Block 3						
SBP Group			4.363	0.113		
Low SBP	0.611	0.321	3.629	0.057	1.842	0.98 – 3.45
High SBP	0.256	0.229	1.256	0.262	1.292	0.83 – 2.02
Sex (F)	-0.298	0.183	2.650	0.104	0.742	0.52 – 1.06
Age	0.128	0.020	39.866	<0.001*	1.137	1.09 – 1.18
Time	-0.107	0.027	15.126	<0.001*	0.899	0.85 - 0.95
CHD	0.496	0.266	3.480	0.062	1.642	0.98 – 2.77
Hypothyroid	0.515	0.178	8.359	0.004*	1.674	1.18 – 2.37
Diabetes	0.297	0.268	1.226	0.268	1.345	0.80 – 2.28
CVD	0.017	0.231	0.006	0.941	1.017	0.65 – 1.60

Table 44. Cox regression model for SBP groups based on DS population 10th and 90th centiles. Indicator variables: middle SBP group (101-130mmHg); male sex. CHD = Congenital Heart Disease; CVD = Cardiovascular disease.

Systolic Blood Pressure Summary

There were significant effects of high SBP values on incident dementia risk. In those with SBP values >140mmHg the rate of incident dementia was approximately twice as high compared with people who were normotensive. This relationship held in adjusted models. It is notable however that a relatively small number of people had extreme SBP values ($n=65$) which is in agreement with the reduced heterogeneity in SBP values observed in people with DS. When SBP values derived from the DS population percentiles were used the effect of high blood pressure was reduced, suggesting that in people with DS the value of 140mmHg SBP to define at risk groups has clinical relevance. Having a low SBP (below 100mmHg) was of borderline significance, with HRs around 1.8 in both adjusted models and suggests a negative impact of low SBP on dementia onset.

Hazard ratios were comparable between the high and low SBP groups for both the general population and DS derived groups (HRs of 0.90 (95% CI 0.39 - 2.09) $p=0.81$ and 0.89 (95% CI 0.50 – 1.58), $p=0.69$ respectively). Having a low as well as a high SBP therefore appears to increase incident dementia risk. This may be indicative of a u-shaped risk relationship between SBP and dementia, with both high and low values being associated with increased incidence rates.

5.4.2. Diastolic Blood Pressure Models

DBP Groups Based on Thresholds for the General Population

After applying the criteria from the general population there were insufficient events in the high DBP group to conduct a survival analysis with only five events occurring in people with DBP values over 90mmHg (a minimum requirement of 10 events per group was pre-defined in the analysis design). A binary cut off at 70mmHg was therefore explored to allow for comparison between those with lower DBP values to a reference group with DBP values above 70mmHg (table 45). There was no significant difference between mean survival rates: 258.17 months (SD 6.36) in the low DBP group compared to 246.87 months (SD 4.31) in the reference group (Log Rank Chi Square 0.052, $p=0.82$). The effect of DBP group was not significant (HR 1.04 (95% CI 0.7 - 1.5), $p=0.92$). The model fit was improved by adding covariates, but DBP group remained a poor predictor with a fully adjusted HR of 0.94 (95% CI 0.65 - 1.35), $p=0.73$.

DBP Groups Derived from Down Syndrome Population Percentiles

The three DS derived groups were defined as: Low DBP <62mmHg; Middle DBP 62-80mmHg; High DBP >80mmHg. There were 90 events in the reference group, 25 in the low and 18 in the high group. There were 10,764 person years available for the reference group, 1,687 for the low group and 1,563 for the high. Average survival times between the groups were different between the low and reference group (237.51 months (SD 10.42) in the low compared to 253.45 months (SD 3.71) in the reference group, Log Rank Chi Square 4.84, $p=0.03$). The other group differences were not significant. The crude incident rate for the low

group was 14.82 (95% CI 9.01 - 20.63). For the reference group this was 8.36 (95% CI 6.63 – 10.09). For the high group the rate was 11.52 (95% CI 9.68 – 26.32).

In the adjusted model the overall contribution of DBP group was significant ($p=0.034$), see table 46. The low group compared to the reference group (with values of 62-80mmHg) showed a significantly increased hazard ratio (HR 1.77, 95% CI 1.1 – 2.8, $p=0.02$). Those in the high DBP group also appeared to be at increased risk compared to the reference group, but this lost significance in the adjusted model (HR 1.43, 95% CI 0.96 – 2.14, $p=0.08$). The difference between the low and high DBP groups was not significant (HR 0.81 (95% CI 0.48 - 0.135), $p=0.417$). See figure 18 at the end of the chapter for survival curves.

Diastolic Blood Pressure Summary

When general population BP targets were used to define DBP groups there were no significant effects on incident dementia ($p=0.73$). There were only a small number of people in the “high” DBP group ($n=32$) which prevented three-way group comparisons. On the other hand, there were effects of very low DBP values on incident dementia risk when using the DS population-derived limits. Those with low DBP ($<62\text{mmHg}$) had an approximately 1.7 times increased incident rate compared to the normotensive group. Those with high DBP had an approximately 1.4 times increased rate, although the effect lost significance in the fully adjusted model ($p=0.08$). Mirroring the trends observed in the SBP analysis, both high and low DBP may be a risk factor for earlier dementia onset in people with DS, although high SBP and DBP occurs less frequently in this population.

	-2LL	$\Delta\chi^2$	p
Block 0	1672.70		
Block 1	1671.75	0.05	0.821
Block 2	1621.18	51.47	<0.001
Block 3	1606.89	14.29	0.006

	B	SE	Wald	p	Exp(B)	95% CI
Block 1						
DBP Group	0.041	0.183	0.051	0.822	1.042	0.73 – 1.49
Block 2						
DBP Group	-0.027	0.184	0.022	0.883	0.973	0.68 – 1.40
Sex (F)	-0.475	0.179	7.049	0.008*	0.622	0.44 - 0.88
Age	0.124	0.020	36.801	<0.001*	1.132	1.09 – 1.18
Time	-0.10	0.027	13.290	<0.001*	0.905	0.86 - 0.96
Block 3						
DBP Group	-0.065	0.185	0.121	0.727	0.937	0.65 – 1.35
Sex (F)	-0.334	0.185	3.266	0.071	0.715	0.50 – 1.03
Age	0.128	0.021	38.557	<0.001*	1.136	1.09 – 1.18
Time	-0.102	0.028	13.855	<0.001*	0.903	0.86 - 0.95
CHD	0.448	0.273	2.698	0.100	1.565	0.92 – 2.67
Hypothyroid	0.554	0.180	9.475	0.002*	1.739	1.22 – 2.47
Diabetes	0.257	0.270	0.909	0.340	1.293	0.76 – 2.19
CVD	0.068	0.231	0.085	0.770	1.070	0.68 – 1.68

Table 45. Cox regression model for DBP groups based on general population guidance. Indicator variables: DBP (>70mmHg); male sex. CHD = Congenital Heart Disease; CVD = Cardiovascular Disease.

	-2LL	$\Delta\chi^2$	<i>p</i>
Block 0	1672.70		
Block 1	1664.13	8.57	0.014
Block 2	1614.05	50.08	<0.001
Block 3	1600.51	13.54	0.009

	B	SE	Wald	Sig	<i>p</i>	95% CI
Block 1						
DBP Group			8.785	0.012*		
Low DBP	0.563	0.237	5.636	0.018*	1.756	1.10 – 2.79
High DBP	0.483	0.201	5.771	0.016*	1.622	1.03 – 2.79
Block 2						
DBP Group			7.394	0.025*		
Low DBP	0.583	0.240	5.903	0.015*	1.791	1.12 - 2.87
High DBP	0.391	0.204	3.670	0.055	1.478	0.99 – 2.20
Sex (F)	-0.412	0.180	5.241	0.022*	0.662	0.47 - 0.94
Age	0.119	0.021	33.38	<0.001*	1.126	1.08 – 1.17
Time	-0.105	0.027	14.763	<0.001*	0.900	0.85 - 0.95
Block 3						
DBP Group			6.738	0.034*		
Low DBP	0.570	0.242	5.571	0.018*	1.769	1.10 – 2.84
High DBP	0.360	0.205	3.073	0.080	1.433	0.96 – 2.14
Sex (F)	-0.287	0.185	2.413	0.120	0.750	0.52 – 1.08
Age	0.122	0.021	34.734	<0.001*	1.130	1.09 – 1.18
Time	-0.108	0.028	15.429	<0.001*	0.897	0.85 - 0.95
CHD	0.490	0.274	3.214	0.073	1.633	0.96 – 2.80
Hypothyroid	0.507	0.180	7.881	0.005*	1.659	1.17 – 2.36
Diabetes	0.281	0.270	1.085	0.298	1.324	0.78 – 2.25
CVD	0.068	0.232	0.086	0.769	1.071	0.68 – 1.69

Table 46. Cox regression model for DBP groups based on DS population 10th and 90th centiles. Indicator variables: middle DBP group (62-80mmHg); male sex. CVD = history of cardiovascular disease.

5.5. Pulse Pressure Models

PP Groups Based on Thresholds for the General Population

When applying general population guidelines comparing a high PP group (with values >60mmHg), normotensive (PP 40-60mmHg) and low PP group (<40mmHg) there were only 70 people with nine events in the high group which was not sufficient to power the analysis. Therefore, as with DBP, a two-way comparison was performed between those with low PP values (<40mmHg) and those with values above 40mmHg (forming the reference group).

There were 568 people in the low group and 1,046 in the reference group. There were 63 events in the low group and 70 in the reference. There were 5,171 person years for the low PP group and 8,843 for the reference group. The mean survival time was significantly lower in the low PP group: 241.87 months (SD 6.89) compared to 264.99 (SD 4.51) in the reference group, Log rank Chi Square 5.59, $p=0.02$.

There was a significant effect of PP group, and this was retained in the fully adjusted model (table 47). Those with low PP showed an increased incidence of dementia (HR 1.45, (95% CI 1.03 – 2.05), $p=0.03$). The crude incident rate per 1000 person years for the low group was 12.18 (95% CI 9.17 – 15.19) compared to 7.92 (95% CI 6.06 – 9.77) per 1000 person years in the reference group. See figure 18 at the end of the chapter for survival curves.

	-2LL	$\Delta\chi^2$	<i>p</i>
Block 0	1672.703		
Block 1	1667.35	5.35	0.021
Block 2	1616.18	51.17	<0.001
Block 3	1602.56	13.62	0.009

	B	SE	Wald	<i>p</i>	Exp(B)	95% CI
Block 1						
PP Low	0.405	0.174	5.425	0.020*	1.500	1.07 – 2.11
Block 2						
PP Low	0.394	0.175	5.084	0.024*	1.484	1.05 – 2.09
Sex (F)	-0.446	0.179	6.184	0.013*	0.640	0.45 - 0.91
Age	0.123	0.020	36.145	<0.001*	1.131	1.09 – 1.18
Time	-0.102	0.027	14.038	<0.001*	0.903	0.86 - 0.95
Block 3						
PP Low	0.374	0.176	4.495	0.034*	1.453	1.03 – 2.05
Sex (F)	-0.326	0.185	3.115	0.078	0.722	0.50 – 1.04
Age	0.127	0.021	37.817	<0.001*	1.135	1.09 – 1.18
Time	-0.105	0.027	14.772	<0.001*	0.900	0.85 - 0.95
CHD	0.410	0.273	2.260	0.133	1.507	.88 – 2.57
Hypothyroid	0.532	0.180	8.754	0.003*	1.702	1.20 – 2.42
Diabetes	0.261	0.232	0.227	0.333	1.299	0.77 – 2.21
CVD	0.111	0.232	0.227	0.634	1.117	0.71 – 1.76

Table 47. Cox regression model for low PP group (<40mmHg) based on general population guidance. Indicator variables: normotensive PP (40-60mmHg); male sex. CHD = Congenital Heart Disease. CVD = Cardiovascular Disease.

PP Groups Derived from Down Syndrome Population Percentiles

Using the PP values defined from the DS population there were 103 events in the middle group, 12 in the low group (<34 mmHg) and 18 in the high (>54mmHg). The mean survival time was 256.92 months (SD 4.57) in the middle group, 224.53 (SD 9.02) in the low, and 242.53 (SD 9.84) in the high. There were no significant differences in the survival times between the groups (Log Rank Chi Square 0.05 – 1.40, $p>0.24$ for all). Both the high and low PP groups showed increased HRs compared to the reference group but these differences were not significant when entered initially or in the fully adjusted model (HR of 1.2 (95% CI 0.7 – 2.0), $p=0.51$ for the low PP group; HR 1.4 (95% CI 0.9 – 2.4), $p=0.16$ for the high PP group). See table 48.

Pulse Pressure Summary

There was a significant association between low PP (<40mmHg) and incident dementia risk with people in the low PP group having a HR 1.5 times higher than the reference group. There were few people in the high PP group which is in keeping with previous finding of lower BP values in people with DS compared to the general population. This prevented analysis of the effects of high PP (>60mmHg) on incident dementia risk. When using DS derived groups with lower cut offs (reference group 34-54mmHg) there were no group effects. This suggests that, at least for the lower PP values, the guidance for the general population appears to reflect a degree of risk in the DS population and that low PP is associated with earlier dementia onset. Given the challenges of differentiating blood pressure risks in people with DS who have reduced heterogeneity in their SBP and DBP values, PP may offer a more sensitive measure, particularly of low cardiac output and overall cardiovascular status in this population.

	-2LL	$\Delta\chi^2$	<i>p</i>
Block 0	1672.70		
Block 1	1670.97	1.73	0.421
Block 2	1619.27	51.70	<0.001
Block 3	1604.93	14.34	0.006

	B	SE	Wald	<i>p</i>	Exp(B)	95% CI
Block 1						
PP Group			1.817	0.403		
Low PP	0.224	0.270	0.692	0.405	1.251	0.74 – 2.12
High PP	0.300	0.257	1.369	0.242	1.350	0.82 – 2.23
Block 2						
PP Group			2.042	0.360		
Low PP	0.203	0.271	0.565	0.452	1.226	0.72 – 2.08
High PP	0.339	0.258	1.729	0.189	1.404	0.85 – 2.33
Sex (F)	-0.453	0.180	6.360	0.012*	0.636	0.45 - 0.90
Age	0.125	0.020	37.358	<0.001*	1.133	1.09 – 1.18
Time	-0.100	0.027	13.398	<0.001*	0.905	0.86 - 0.96
Block 3						
PP Group			2.217	0.330		
Low PP	0.180	0.272	0.438	0.508	1.198	0.70 – 2.04
High PP	0.367	0.259	2.012	0.156	1.444	0.87 – 2.40
Sex (F)	-0.314	0.185	2.880	0.090	0.730	0.51 – 1.05
Age	0.129	0.021	39.038	<0.001*	1.137	1.09 – 1.18
Time	-0.103	0.028	13.924	<0.001*	0.902	0.86 - 0.95
CHD	0.441	0.273	2.612	0.106	1.554	0.91 – 2.65
Hypothyroid	0.548	0.180	9.282	0.002*	1.731	1.22 – 2.46
Diabetes	0.294	0.270	1.184	0.277	1.342	0.79 – 2.28
CVD	0.044	0.231	0.036	0.850	1.045	0.66 – 1.64

Table 48. Cox regression model for PP groups based on DS population 10th and 90th centiles. Indicator variables: middle PP group (34-54mmHg); male sex. CHD = Congenital Heart Disease. CVD = Cardiovascular Disease.

5.4.4. Body Mass Index Models

When split into three groups based on general population thresholds there were only three events in the low BMI group (people with BMI $<18.5 \text{ kg/m}^2$). Conversely, when using the DS derived groups there were only eight people in the high group ($>33.7 \text{ kg/m}^2$). It was therefore not possible to conduct survival analysis using either of these three groups.

To address the low event numbers in the groups, the cohort was instead classified based on obesity category. This was divided into those with BMI below 25 kg/m^2 , those with a BMI in the overweight range (BMI $25\text{-}30 \text{ kg/m}^2$) and those in the obese range (BMI $>30 \text{ kg/m}^2$). For this analysis the group with “normal” BMI of $<25 \text{ kg/m}^2$ was used as the reference group. There were 31 events and 4,389 person years in the reference group; 44 events and 3,408 person years in the overweight group; 46 events and 3,724 person years in the obese group. The mean survival time was highest in the reference group: 250.61 months (SD 4.38) compared to 246.67 months (SD 8.34) in the overweight group and 229.66 months (SD 6.57) in the obese group. The difference in survival time was significant between the reference and overweight group (Log Rank Chi Square 7.55, $p=0.01$), and between the reference and obese group (Log Rank Chi Square 8.70, $p=0.003$). The difference between the overweight and obese groups was not significant ($p=0.98$). The crude incident rate for the reference group was 7.06 (95% CI 4.58 – 9.55) per 1000 person years; for the overweight group this was 12.91 (95% CI 9.71 – 16.11) per 1000 person years; and for the high group 12.35 (95% CI 8.78 – 15.92) per 1000 person years.

Construction of Cox regression models indicated a meaningful effect of BMI category on incident risk for both the overweight and obese groups compared to the reference group (table

49). This remained significant when initial covariates were included, with HRs of 1.9 for both groups. In the fully adjusted model the overall group difference lost significance ($p=0.08$), but was retained for the overweight group (HR 1.66 (95% CI 1.03 – 2.67), $p=0.04$). The effect of the obese group was of borderline significance (HR 1.60 (95% CI 0.99 – 2.59), $p=0.05$). There was no difference between the obese and overweight group, HR 1.02 (95% CI 0.67 – 1.54), $p=0.94$). There were HRs of approximately 1.6 for both the overweight and obese groups.

Given the lack of difference observed between the overweight and obese group, the analysis was repeated with BMI recoded as a binary category split at 25kg/m^2 ($n=484$ in the normal weight group and $n=891$ in the overweight or obese group). There were 4,389 person years in the reference group and 7,132 in the high BMI group with 31 events in the reference group and 90 in the high. The crude incidence rate was 12.62 per 1000 person years (95% CI 10.01 – 15.23) in the overweight and obese group compared to 7.06 (95% CI 4.58 – 9.55) in the reference group.

In the adjusted model the high BMI group, which included both those who were overweight and those who were obese, was at significantly increased risk compared to the normal weight group. The hazard ratio was 1.6 (95% CI 1.1 – 2.5), $p=0.03$. Having a BMI above 25kg/m^2 in mid-life was therefore associated with a 60% increase in risk of incident dementia at a given time point over the age of 35 years compared to those with a BMI in the normal range (table 50). See figure 19 at the end of the chapter for survival curves.

	-2LL	$\Delta\chi^2$	<i>p</i>
Block 0	1501.29		
Block 1	1490.86	10.43	.005
Block 2	1445.19	45.67	<.001
Block 3	1432.83	12.36	.015

	B	SE	Wald	<i>p</i>	Exp(B)	95% CI
Block 1						
BMI Group			9.518	0.009*		
Overweight	0.639	0.235	7.418	0.006*	1.895	1.20 – 3.00
Obese	0.649	0.233	7.730	0.005*	1.913	1.21 – 3.02
Block 2						
BMI Group			7.326	0.026*		
Overweight	0.575	0.238	5.839	0.016*	1.777	1.12 – 2.83
Obese	0.573	0.236	5.884	0.015*	1.774	1.12 – 2.82
Sex (F)	-0.501	0.186	7.281	0.007*	0.606	0.42 - 0.87
Age	0.133	0.022	37.141	<0.001*	1.142	1.09 – 1.19
Time	-0.074	0.026	8.074	0.004*	0.928	0.88 - 0.98
Block 3						
BMI Group			5.005	0.082		
Overweight	0.505	0.243	4.323	0.038*	1.656	1.03 – 2.67
Obese	0.473	0.244	3.740	0.053	1.604	0.99 – 2.59
Sex (F)	-0.475	0.189	6.293	0.012*	0.622	0.43 - 0.90
Age	0.138	0.022	39.197	<0.001*	1.148	1.10 – 1.20
Time	-0.076	0.026	8.134	0.004	0.927	0.88 - 0.98
CHD	0.633	0.269	5.539	0.019*	1.883	1.11 – 3.19
Hypothyroid	0.440	0.189	5.421	0.020*	1.552	1.07 – 2.25
Diabetes	0.025	0.287	0.008	0.930	1.026	0.59 – 1.78
CVD	0.181	0.244	0.551	0.458	1.199	0.74 – 1.94

Table 49. Cox regression model for BMI groups based on obesity category. Indicator variables: normal weight (BMI <25kg/m²); male sex. CHD = Congenital Heart Disease; CVD = history of cardiovascular disease.

	-2LL	$\Delta\chi^2$	<i>p</i>
Block 0	1501.29		
Block 1	1490.86	10.43	0.001
Block 2	1445.19	45.67	<0.001
Block 3	1432.85	12.34	0.015

	B	SE	Wald	<i>p</i>	Exp(B)	95% CI
Block 1						
BMI Group	0.644	0.209	9.516	0.002	1.904	1.27 – 2.89
Block 2						
BMI Group	0.574	0.212	7.326	0.007	1.776	1.17 – 2.69
Sex (F)	-0.501	0.186	7.289	0.007	0.606	0.42 - 0.87
Age	0.133	0.022	37.141	<0.001	1.142	1.09 – 1.19
Time	-0.074	0.026	8.093	0.004	0.928	0.88 - 0.98
Block 3						
BMI Group	0.489	0.219	4.978	0.026*	1.630	1.06 – 2.50
Sex (F)	-0.477	0.189	6.364	0.012*	0.621	0.43 - 0.90
Age	0.138	0.022	39.271	<0.001*	1.148	1.10 – 1.20
Time	-0.075	0.026	8.110	0.004*	0.927	0.88 - 0.98
CHD	0.633	0.269	5.549	0.018*	1.884	1.11 – 3.19
Hypothyroid	0.438	0.188	5.400	0.020*	1.549	1.07 – 2.24
Diabetes	0.023	0.286	0.006	0.937	1.023	0.58 – 1.80
CVD	0.183	0.244	0.560	0.454	1.200	0.74 – 1.94

Table 50. Cox regression model for binary BMI groups. Indicator variables: BMI <25kg/m²; male sex. CHD = Congenital Heart Disease; CVD = Cardiovascular Disease.

BMI Summary

Being overweight or obese was associated with an increased HR for incident dementia compared to having a BMI below 25 kg/m². There was no clear difference between those who were overweight and those who were obese; having a BMI above 25 was the main driver of increased risk (crude incident rates of between 1.6 and 1.7 for the obese and overweight groups). When combining the overweight and obese groups the crude incident rate was 1.79 times that of the reference group. There were very few people with DS who had a BMI below 18.5kg/m² and the mean BMI of the whole sample would be classified as overweight (28.73 kg/m² (7.16)) reflecting the trend towards higher BMIs in this population. The guidance for the general population to maintain a BMI below 25kg/m² is relevant for reducing risk in people with DS despite the different trends observed in BMI patterns across the lifespan, at least in younger adults.

5.4.5. Effects of Covariates

As expected, age contributed significantly to all models with increasing age associated with increased risk of incident dementia. The HR was around 1.1 in all models, with an approximately 10% increased risk of incident dementia for each year of age at each time point.

A diagnosis of hypothyroidism contributed significantly to the models, whereas a history of diabetes mellitus did not. The association between hypothyroidism and increased hazard ratios appears robust as it was a significant contributor in all models with HRs estimated between 1.6-1.7. The effect of sex were significant in the BMI models, with a HR of 0.6 and a beta

coefficient of -0.5. This suggests that females compared to males are at a relatively reduced risk of incident dementia (up to a 40% reduced risk) at each time point in relationship to BMI. Female sex also approached significance in the blood pressure models with a similar HR around 0.7.

History of congenital heart disease was significant in the models alongside BMI as a predictor. CHD was associated with a HR of approximately 1.9 in the adjusted BMI models, which was significant (CHD HR 1.88, 95% CI 1.11 – 3.19, $p=0.02$). CHD was also a significant predictor in the SBP model based on general population targets (HR 1.7, 95% CI 1.01 – 2.86, $p=0.046$). CHD trended towards significance in the other blood pressure models with HRs between 1.5-1.6. A history of CHD appears to be a relevant interacting factor when considering the impact of cardiovascular health on incident dementia risk and this may be particularly important when understanding cumulative risks. The effect of prevalent cardiovascular disease (hypertension, hypercholesterolaemia, peripheral vascular disease or ischaemic heart disease) was not significant in either the blood pressure or BMI analyses. This may reflect the low prevalence of these conditions in people with DS.

In conclusion, there were associations between CHD, male sex, and a diagnosis of hypothyroidism with increased dementia incidence risk. In contrast to the general population there may be a disconnect between traditional cardiovascular risk factors and cardiovascular health in people with DS. This has implications for assessing and managing cardiovascular risk in this patient group.

5.4.6. Combined Risk Model

An exploratory combined risk model was constructed, with at risk groups defined by the significant parameters outlined above. These were PP<40mmHg; BMI>25kg/m²; hypothyroidism; and history of CHD. High SBP (>140mmHg) was not included given the low numbers of people in this group and the association with PP. Similarly low DBP (<62mmHg) was not included due to the correlation with PP to avoid potential over-fitting. Presence or absence of a risk factor was coded as binary absent (0) or present (1) and a combined risk group was calculated based on the sum of these risks. If data for one of the three risk factors were missing then this was dummy coded as absent. Risk was classified as low (zero factors), moderate (1-2 factors), and high (3-4 factors). There were 453 people in the low risk group (no risks present) with 3,802 patients years of data available; 1,009 people in the moderate risk group with 8,709 years available, and 178 in the high with 1,603 patient years. There were 13, 94 and 28 events in each outcome group respectively. Mean survival times were 272.53 months (SD 4.79); 251.02 months (SD 5.27); and 210.57 months (SD 8.00) for low, moderate and high risk groups with group significance (Log rank Chi Square 27.62, p<0.001).

Cox regression models were constructed, with combined risk group as the primary predictor (table 51). Age, sex, registration length, diabetes mellitus and CVD were included as relevant additional predictors. The low risk group (people with zero risk factors) was the indicator group, as was male sex and absence of each comorbidity. There was a significant effect of combined risk group on risk of incident dementia. The crude incident rates were 3.42 (95% CI 1.56 – 5.28) per 1000 person years for the low risk group; 10.79 (95% CI 8.61 – 12.98) in the moderate risk group; and 17.47 (95% CI 11.00 – 23.94) in the high risk group (see figure 17).

People with 1-2 or 3-4 risk factors had elevated hazard ratios compared to those with no risk factors, and the highest HR was observed in the group with the highest burden of risk factors (table 12). The relationship was sustained in the adjusted model: HR 3.15 (95% CI 1.8 – 5.7) for the moderate group; HR 5.69 (95% CI 2.9 – 11.1) for the high risk group, both $p < 0.001$. Compared to the moderate risk group, the high group had elevated risk (adjusted HR 1.81 (95% CI 1.18 - 2.76), $p = 0.006$).

The results indicate that people with one or two DS-related cardiovascular risk factors had incident rates approximately 2.5 times higher than those with no risk factors. People with three or four risk factors had incident rates approximately four times greater. Low PP, high BMI, CHD and hypothyroidism all appear to interact significantly with incident dementia risk, alongside sex and age effects. A cumulative effect of risk factors on incident dementia in people with DS is therefore implied and highlights a need for multi-domain risk reduction approaches. See figure 20 at the end of the chapter for survival curves.

	-2LL	$\Delta\chi^2$	<i>p</i>
Block 0	1702.76		
Block 1	1672.87	29.89	<0.001
Block 2	1616.91	55.96	<0.001
Block 3	1615.93	0.99	0.611

	B	SE	Wald	<i>p</i>	Exp(B)	95% CI for Exp(B)
Block 1						
Risk Group			23.987	<0.001*		
Moderate Risk	1.147	0.296	15.001	<0.001*	3.149	1.76 – 5.63
High Risk	1.646	0.336	23.971	<0.001*	5.187	2.68 – 10.02
Block 2						
Risk Group			26.360	<0.001*		
Moderate Risk	1.156	0.300	14.866	<0.001*	3.177	1.77 – 5.72
High Risk	1.748	0.341	26.309	<0.001*	5.75	2.95 – 11.21
Sex (F)	-0.294	0.179	2.701	1.00	0.746	0.53 – 1.06
Age	0.134	0.020	44.327	<0.001*	1.143	1.10 – 1.19
Time	-0.103	0.027	14.600	0.001*	0.902	0.86 - 0.95
Block 3						
Risk Group			26.012	<0.001*		
Moderate Risk	1.148	0.300	14.616	<0.001*	3.153	1.75 – 5.68
High Risk	1.739	0.341	25.955	<0.001*	5.694	2.92 – 11.12
Sex (F)	-0.284	0.181	2.461	0.117	0.753	0.53 – 1.07
Age	0.133	0.020	43.414	<0.001*	1.142	1.10 – 1.19
Time	-0.106	0.027	15.147	0.001*	0.900	0.85 - 0.95
Diabetes	0.187	0.268	0.488	0.485	1.206	0.71 – 2.04
CVD	0.118	0.228	0.269	0.604	1.126	0.72 – 1.76

Table 51. Cox regression model for combined risk groups. Indicator variables: Low risk group (0 risk factors); male sex. CVD = Cardiovascular Disease.

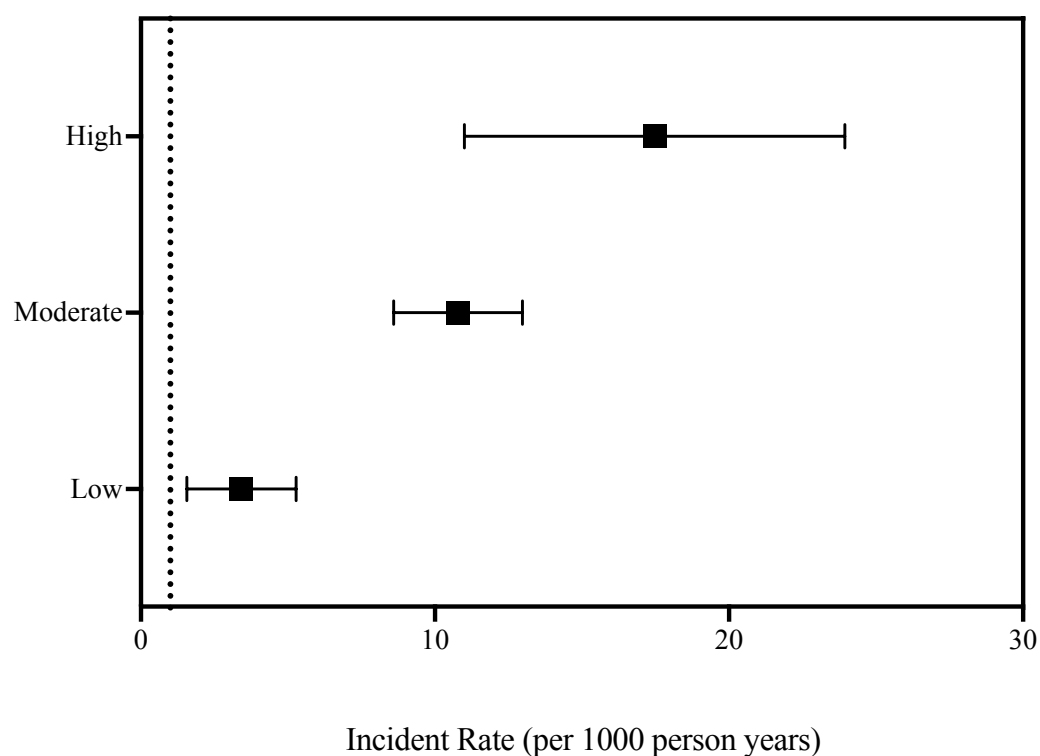


Figure 17. Crude incident rates per 1000 person years for each level of combined risk. Low risk = no risk factors; moderate risk = 1-2 factors; high risk = 3+ factors. Error bars represent 95% CI.

5.5. Exploratory Group Comparisons

For the CVPs with insufficient events to power survival analyses crude comparisons were performed between those with incident dementia (DSdem) and those without (DScn). These were total cholesterol (TC), high and low density lipoproteins (HDL and LDL), triglycerides (Tg) and glycated haemoglobin (HbA1c).

Total Cholesterol

There were 2,396 TC values measured in people with DS between the age of 25-34 years which were taken from 957 individuals. From this sample, 442 people also had data available over

the age of 35 years: 183 men and 239 women. There were a total of 29 incident events and 2,636 person years of data. Mean TC was 4.81 mmol/L (SD 0.96). Mean age at measurement was 30.42 years (SD 3.05).

There were no significant differences between the DSdem and DScn groups in terms of mean age at TC measurement (32.90 years (SD 2.16) in DSdem versus 32.10 (2.35) years in DScn, t 1.778, $p=0.08$). Mean registration period was also similar between the two groups (7.41 years (SD 4.69) in DSdem, versus 6.16 years (SD 4.32), t 1.40, $p=0.14$). There was a similar proportion of males and females in the groups (Pearson chi square 0.67, $p=0.347$). Mean TC was higher in those with incident dementia, but this was not significant: 5.44 mmol/L (SD 1.58) in DSdem compared to 4.91 mmol/L (SD 0.94) in DScn, t 1.797 $p=0.08$).

Triglycerides and Lipoproteins

There were 406 people with available Tg values between ages 25-34 years (207 males and 199 females) and a total of 15 outcome events. There were 4,999 person years available. Mean Tg was 1.41 mmol/L (SD 0.95). Those with DSdem had a higher mean Tg value but this did not reach significance (1.37 mmol/L (SD 0.71) in DScn compared to 2.33 mmol/L (SD 3.31) in DSdem, t 1.114, $p=0.28$).

There were 419 people with HDL values available, with 12 incident events. There were 365 people with available LDL data and 12 incident events. There were no differences between groups in terms of proportion of males and females (Pearson chi square 0.18, $p=0.67$; 0.30, $p=0.59$; and 0.67, $p=0.42$ for Tg, HDL and LDL respectively). Those in the DSdem group had lower mean HDL values but this was not significant (1.14 mmol/L, SD 0.28 in DSdem compared to 1.26 (0.36) in DScn, t 1.162, p 0.246). The DSdem group had higher mean LDL

values, however the difference was not significant (3.45 mmol/L (SD 0.67) in DSdem compared to 3.10 mmol/L (SD 2.22) in DScn, t 0.55, $p=0.58$).

Glycated Haemoglobin

There were 407 people with available HbA1c values between 25-34 years and health data over the age of 35 years. Mean HbA1c was 40.05mmol/mol (SD 16.16). Of these, 45 had recorded diagnoses of diabetes. The mean HbA1c was 77.71 mmol/mol (SD 17.96) in those with diabetes and 35.36 mmol/mol (SD 7.46) in those without. The mean age of measurement was similar (29.74 years (SD 2.90) in those without diabetes and 29.70 (SD 3.06) in those with). There were 16 incident events. Those in the DSdem group had higher HbA1c values but this did not reach two-sided significance due to large variation observed in the DSdem group (HbA1c 50.08 mmol/mol (SD 27.17) in DSdem compared to 39.63 mmol/mol (SD 15.47), t 1.527, $p=0.147$). There were no differences in the proportion of males and females in the two groups (Pearson chi square 0.074, $p=0.786$) and no difference in the proportion of people with comorbid diabetes (Pearson chi square 1.00, $p=0.317$).

Section Summary

There was a trend for people who develop incident dementia to have values of lipids and HbA1c that would be classed as at higher risk for dementia in the general population, however none of the group differences were significant. Those with incident dementia tended to have higher TC, Tg, LDL and HbA1c, but lower HDL values between the ages of 25-34 years compared to those without incident dementia. However, as the analyses were underpowered it is not possible to form firm conclusions from the results.

The reasons for the attrition of appropriate patients for the analyses is likely explained by the nature of the data used and the population under investigation. CPRD only contains data from 1990 onwards and is dependent on electronic health record usage. There has been an increase in its use as technology has advanced and become almost universally used in GP practices. For people with DS who are now 60, this means that there is less chance they would have recorded values in the system from 30 years ago. Data availability is also dependent on which GP practice a person is registered with. This means that people who move when they are older may not have linked follow up data. Similarly, older people with data may not have been with a linked practice when they were younger. In addition, as this is clinical data we are reliant on health practitioners having measured lipids and HbA1c. These are not currently essential for annual health checks which reduces how many records there are available. The regular measurements of BMI and blood pressure are routine and non-invasive, and therefore have more robust representation in CPRD.

5.6. Chapter Summary

Cardiovascular health factors in people with Down syndrome aged between 25-34 years are associated with an increased risk of incident dementia later in life. Having a raised SBP above 140mmHg was associated with double the risk of incident dementia (HR 2.04, 95% CI 1.09 – 3.81, $p=0.03$) with a 2:1 odds that someone in the high SBP group will receive a diagnosis before someone in normotensive group. Similarly a low SBP below 100mmHg was associated with increased risk although this was of borderline significance in the adjusted model (HR 1.84, 95% CI 0.99 – 3.43, $p=0.06$).

A low DBP below 62mmHg increased risk with a HR of 1.77, 95% CI 1.10 – 2.84, $p=0.02$. The association with low DBP was not observed when the cut-off of 70mmHg was applied which may be because this obscures risks associated with more extreme DBP values. There were a trend towards higher DBP values $>80\text{mmHg}$ and increased risk but this was not significant (HR 1.43, 95% CI 0.96 – 2.14, $p=0.08$). Having a low PP ($<40\text{mmHg}$) was associated with increased risk (HR 1.45, 95% CI 1.03 – 2.05, $p=0.03$).

A raised BMI above 25kg/m^2 was associated with a HR of 1.6, 95% CI 1.05 – 2.46, and being overweight or obese significantly increased the risk of incident dementia. This may be particularly important for people with DS given the increased rate of obesity observed in this population which tends to occur at younger ages than in the general population. There are also sex effects present, particularly in association with BMI, with females having lower HR compared to males. The interactions between sex, cardiovascular health and dementia risk warrant explicit investigation.

In people with Down syndrome, having abnormal blood pressure and BMI values between the age of 25-34 years is therefore associated with an increased chance of receiving a dementia diagnosis over the age of 35 before those with values within normal ranges. This means that poor cardiovascular health in mid-life is associated with earlier onset of dementia despite the common genetic drivers of the disease inherent to Down syndrome. A potentially cumulative effect of vascular and genetic risks is implied. This is supported by the results of the combined risk model which indicated a cumulative effect of risk factors. People with multiple risk factors (specifically $\text{PP}<40\text{mmHg}$, $\text{BMI}>25\text{ kg/m}^2$, hypothyroidism and CHD) were at greater risk of incident dementia. People with three or four factors had over five times the risk compared to

people with no factors (HR 5.69, 95% CI 2.92 – 11.12), $p < 0.001$). This suggests that multi-modal interventions may be essential in managing longer term risks.

The parameters advised in the general population for BMI and systolic blood pressure appear to be relevant for risk prediction in people with DS, but the values for DBP may need to be lowered to adequately capture risk. PP values below 40mmHg were more common in the DS population than very low or high SBP or DBP values and therefore PP may be a more useful marker of cardiovascular risk in people with DS, reflecting changes in BP that may be missed if using SBP or DBP thresholds. PP is less frequently used when assessing cardiovascular health status and its inclusion in annual health checks for people with DS may be merited.

A diagnosis of hypothyroidism was also associated with increased incident dementia risk alongside cardiovascular factors. Whilst hypothyroidism was included as a covariate and not a main predictor, the consistent effect suggests that it is an important contributor to risk. Similarly a history of congenital heart disease impacted risk, particularly in the BMI models.

Understanding the mechanisms underpinning this increased risk associated with CVPs will help establish pathways of AD progression in people with DS, and could lead to identification of risk modifying interventions to help delay disease onset. Knowledge of the unique cardiovascular profile in DS will also inform surveillance and disease monitoring, and specific indicators of risk may need to be created for people with DS.

The results presented in chapters 4 and 5 will be discussed in chapter 6. Following this, the results of the in-depth phenotyped cohort study are presented, where the association between CVPs, cognitive outcomes and plasma biomarkers are investigated (chapter 7). This

exploratory analysis was designed to consider possible mechanistic explanations for the associations found between CVP and incident dementia risk by starting to investigate the relationship between CVPs, blood-based biomarkers of neurodegeneration and inflammatory status.

A.

B.

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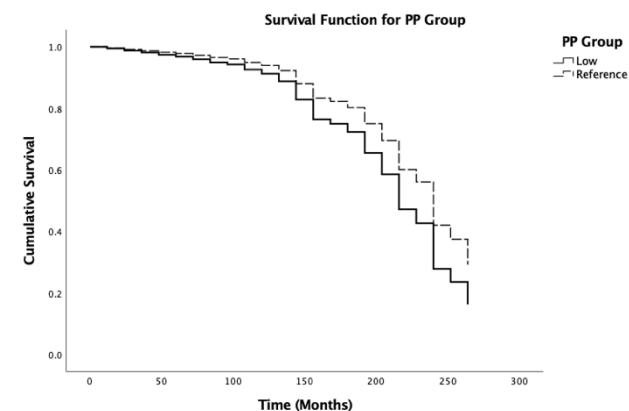
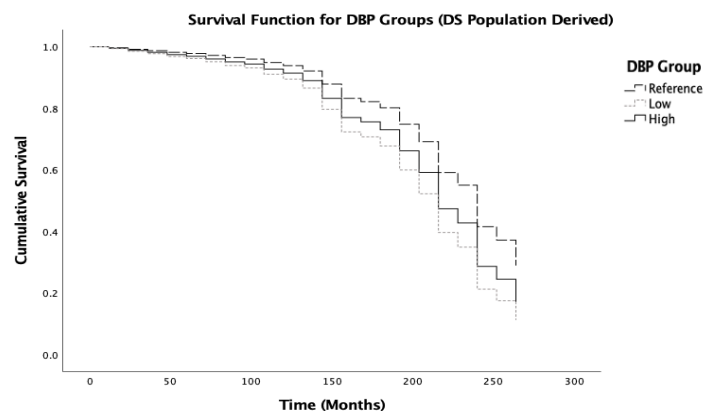
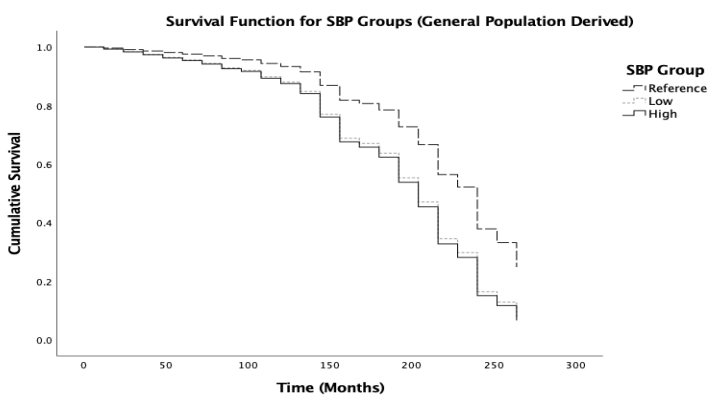


Figure 18. Adjusted survival curves constructed from cox regression for BP groups. A) Survival function for SBP Groups. Reference group SBP 100-140mmHg. B) Survival function for DBP groups. Reference group DBP 62-80mmHg. C) Survival Function for PP group. Reference group PP >40mmHg.

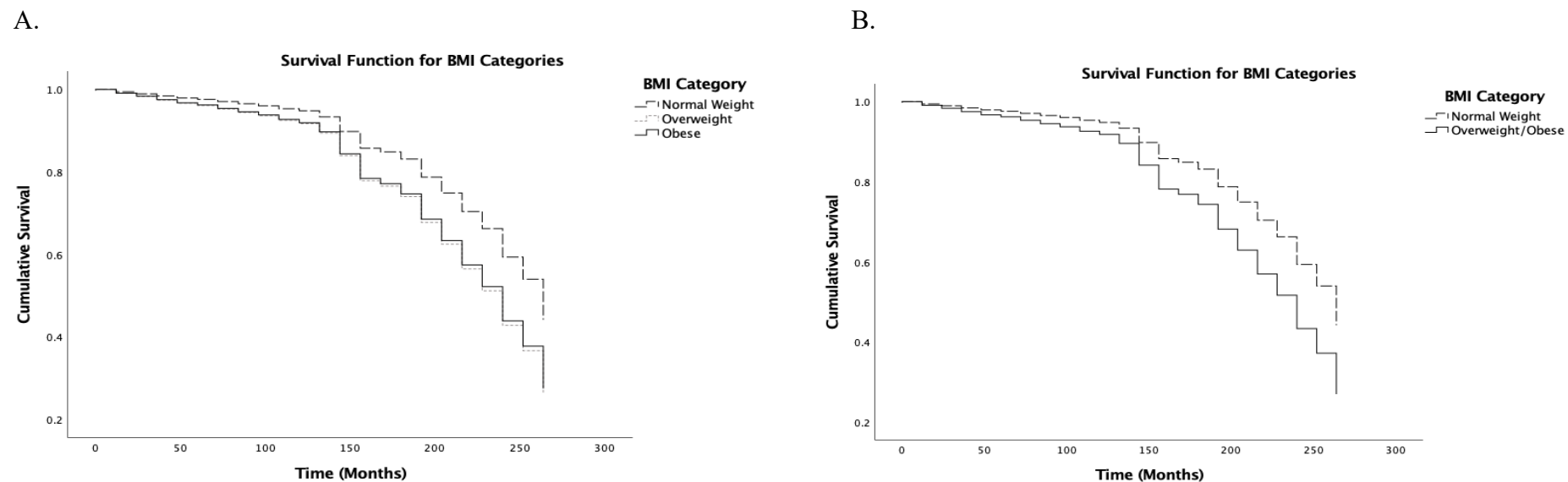


Figure 19. Adjusted survival curves constructed from cox regressions for BMI groups. Reference group normal weight (BMI 18.5-25kg/m²)
A) Survival function for three BMI Groups. B) Survival function for binary BMI categories.

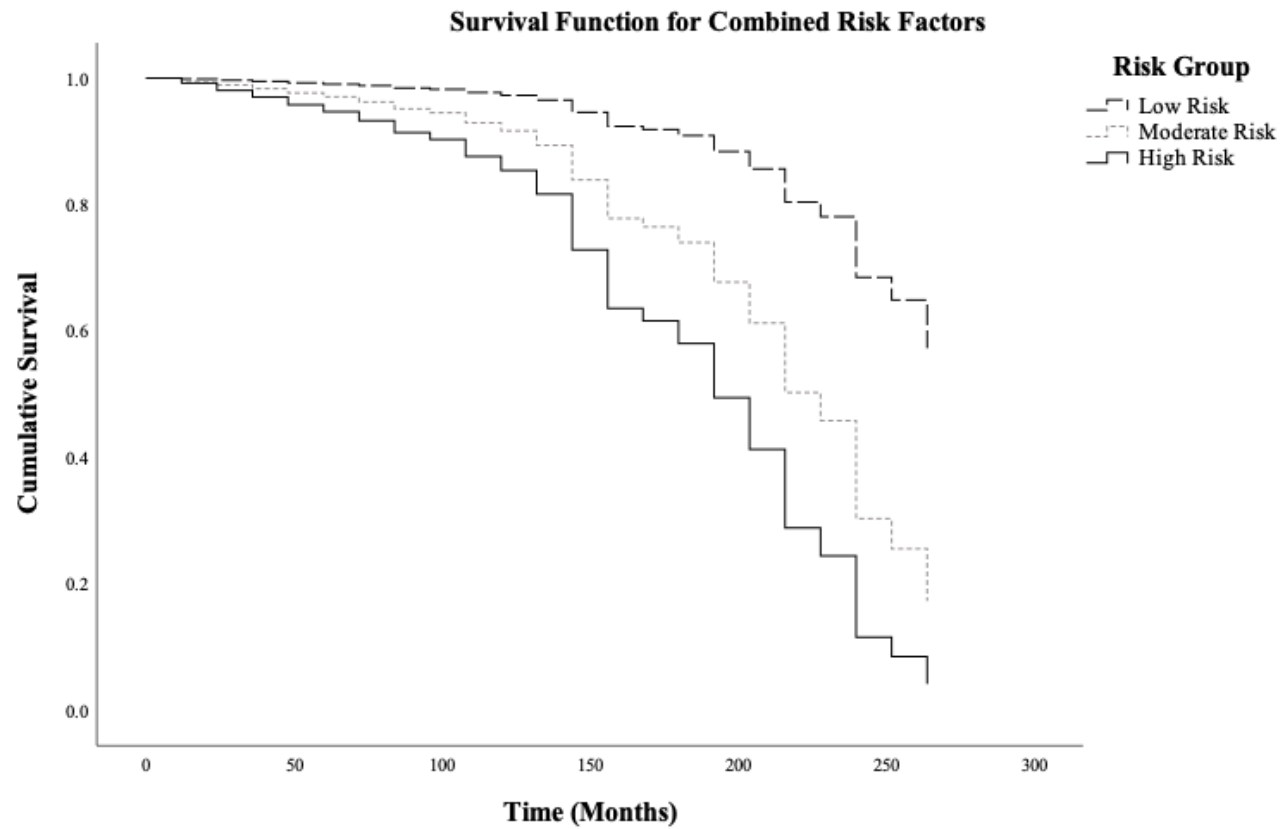


Figure 20. Adjusted survival curves constructed from cox regression for cumulative risk groups. Reference group, zero risk factors. Risk factors included: PP<40mmHg; BMI >25 kg/m²; Hypothyroidism; Congenital Heart Disease.

Chapter VI

Analysis of Cardiovascular Health Markers in People with Down Syndrome and Their Relationship to Incident Dementia Risk Using Electronic Primary Care Records

Part C. Discussion

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6.1. Summary of Results

6.1.1. *Cardiovascular Parameters Across Adulthood*

Using UK population-based data extracted from electronic health records in the UK a unique cardiovascular profile in people with DS has been clearly defined. A different pattern of comorbidities were confirmed in the DS group who had significantly lower incidence rate ratios (IRRs) for ischaemic heart disease (IRR 0.51, 95% CI 0.4 – 0.7), hypercholesterolaemia (IRR 0.55, 95% CI 0.5 – 0.6) and hypertension (IRR 0.24, 95% CI 0.2 – 0.3) compared to controls. There were higher rates of obesity (IRR 1.77, 95% CI 1.6. – 2.0) and peripheral vascular disease (IRR 1.40, 95% CI 1.1 – 1.8). In this comparison which only included adults, the incidence rates for diabetes mellitus were similar between the two groups (IRR 0.92, 95% CI 0.8 – 1.1). Hypothyroidism had a markedly increased incidence (IRR 6.07, 95% CI 5.5 – 6.7). People with DS were diagnosed with all of these conditions at a significantly earlier age than controls.

There were whole group differences for mean values of all nine analysed cardiovascular parameters (CVPs) between DS and control groups. SBP, DBP, PP, TC, HDL, Tg and HbA1c (in people without diabetes) were lower in people with DS whereas BMI, LDL and HbA1c (in people with diabetes) were higher. People with DS did not exhibit the same age-related changes in blood pressure throughout life with minimal increases observed. BMI increased earlier in life in people with DS, but then showed a decline with ageing around the age of 40 years. Lipid profiles were only partially dysregulated in the DS group. Although different absolute values were observed in this analysis, cholesterol and LDL patterns were not notably different between the DS and control populations, with similar sex-effects. Cholesterol peaked in middle-age with a classic parabolic distribution across the ages. LDL and HDL followed

similar trajectories. On the other hand triglycerides showed different patterns with age in people with DS compared to controls, with reduced age related increases in DS and lower overall values. Trisomy 21 appears to interact with glycaemic control pathways in people without diabetes, altering HbA1c patterns with age. HbA1c trends were similar in people with DS and controls who had diabetes, but plateaued with age in those with DS without diabetes. HbA1c continued to increase with age in controls. HbA1c levels were higher in people with DS and diabetes compared to controls, suggesting poorer glycaemic control.

Disconnects between CVPs were observed in people with DS, notably between BP and BMI. In the general population BP and BMI were strongly correlated (correlations 0.88, 0.82 and 0.67 for SBP, DBP and PP with BMI respectively) and the parameters tended to increase in tandem. In people with DS no correlation between SBP and BMI was identified (-0.06). Significant correlations between BMI and DBP and PP were present but were weaker than those observed in controls, with PP having a negative rather than a positive correlation with BMI (correlations 0.46 and -0.45 for DBP and PP).

There were differences in relationships between BMI and lipid profiles. In controls BMI positively correlated with TC, HDL, LDL and Tg. In the DS cohort only TC and LDL correlations were significant. BMI weakly correlated with HbA1c in DS (0.23), but not in controls (0.08). HbA1c was weakly positively correlated with LDL and Tg in people with DS (0.24 and 0.38). In controls HbA1c negatively correlated with HDL (-0.60) and positively with Tg (0.44).

Sex differences existed, most prominently in BP and BMI. These were in opposition to the trends reported in the general population. Women with Down syndrome had higher BMI and

higher SBP and DBP. The differences were not adequately explained by interactions between the two factors. Men with DS had higher PP than women whereas PP-related sex differences in controls were not significant. In regression models for blood pressure, age and DS status were significant contributing factors whereas sex was not, signifying that age and trisomy 21 exert the greatest effects on blood pressure differences between the groups, with reduced sex-related differences. For BMI there were greater sex differences in the DS group compared to controls. The DS population had similar sex differences in lipid profiles to controls. DS status did not appear to alter sex effects for TC and LDL, but for HDL and Tg the sex differences were of a greater magnitude in controls than DS. In controls men had significantly higher HbA1c levels than women, in DS the same pattern was observed but was only significant in people without diabetes.

6.1.2. Cardiovascular Health Factors and Incident Dementia Risk

Using survival analysis methods it was shown that people with Down syndrome with certain cardiovascular risks in younger adulthood (aged between 25 and 34 years old) were more vulnerable to earlier onset of dementia. People with high SBP ($>140\text{mmHg}$) appeared to be at increased risk (HR 2.04, 95% CI 1.1 – 3.8), and those with low SBP trended towards increased risk (HR 1.8, 95% CI 1.0 – 3.4). Similarly people with very low DBP ($<62\text{mmHg}$) were at increased risk (HR 1.8, 95% CI 1.1 – 2.8), as were people with low PP ($<40\text{mmHg}$) who had a HR of 1.5 (95% CI 1.0 – 2.1). There was a trend towards high DBP ($>80\text{mmHg}$) conferring an increased risk but this did not reach significance in the adjusted models (HR 1.4, 95% CI 0.96 – 2.14). The low numbers of people with DS who had high blood pressures make it difficult to quantify the potential risks of these conditions, but the data support a pattern that

both very low and very high blood pressures may increase dementia incidence risk in people with DS.

People with high BMI (above 25 kg/m²) had increased incident risk of dementia, with adjusted HRs of 1.6 (95% CI 1.1 – 2.5). Being obese or overweight were associated with similar HRs. Male sex, hypothyroidism and a history of congenital heart disease were associated with increased risk of dementia as incidental findings in the survival analysis. Despite having insufficient events to power survival analyses, group comparisons showed a trend for people with incident dementia to have poorer cardiovascular health profiles in relation to lipid and glycaemic control in young-mid adulthood. Those with incident dementia events had higher TC, lower HDL, higher LDL and higher HbA1c values, although the differences did not reach significance.

There was a cumulative effect of risk; people with more risk factors had increased risk of incidental dementia compared to those with no risk factors (HR of 3.2, 95% CI 1.8 – 5.7 for those with 1-2 risk factors; HR 5.7, 95% CI 2.9 – 11.1 for those with 3+ factors compared to people with no risk factors).

6.2. Results in the Context of Existing Literature

Blood Pressure

Low systolic and diastolic blood pressures have been well-documented in people with Down syndrome, but the prevalence and lifespan trends were not known^{330,344}. The patterns of pulse pressure have not been widely researched. PP is a measure of the difference between systolic

and diastolic blood pressures. In the general population pulse pressure increases after age fifty years as a result of arterial wall stiffening and accompanying increases in SBP and decreases in DBP. Pulse pressure has been suggested as a predictor of coronary heart disease and as a surrogate marker of central artery stiffness^{345,346}. In Down syndrome the causes of low or high PP are less clear given the relative stable DBP and SBP measurements with age, and the lower reported incidence of arterial stiffening secondary to atherosclerosis.

Body Mass Index

Increased prevalence of obesity in people with Down syndrome has been documented previously, however the lifespan trends were not clearly delineated. *Melville et al* examined obesity in adults (age 20-69 years) with DS using a case-control method matched to adults with ID on sex, age and accommodation type. A total of 247 pairs were included in the study. Women with DS had higher BMI, with an odds ratio 2.7 of being overweight and 1.43 of being obese compared to ID controls. Men showed no difference in BMI despite lower height and weight. They were more likely to be overweight (OR 1.6) but less likely to be obese (OR 0.85). Overall there was a relative risk of 1.26 for men and women to have a BMI greater than 25 compared to the matched ID controls³⁴⁷. These findings are consistent with those presented in the CPRD analysis in chapter four, particularly highlighting the increased risk in females of high BMI.

Echoing the disconnect between BP and BMI observed in my population analysis, the relationship between metabolic disorders in DS does not appear to match those observed in the general population. In a study of mortality in DS, the obesity-related standardised mortality odds ratio (SMOR) was increased in DS (SMOR 1.87) based on death certificate recording,

yet the SMOR for diabetes was reduced (0.63)²¹⁷. This supports the hypothesis that people with DS may have a unique relationship between cardiovascular and metabolic risk factors.

Lipid Profiles

In the large population sample performed in chapter four, adults with DS had lower mean TC, Tg and HDL values, with similar levels of LDL compared to the general population. Whilst the values differed slightly the patterns with age followed those of controls, apart from for Tg.

In the literature much of the focus has been on the lipid profiles of children or the effects of raised cholesterol on dementia risk in mice^{137,138}. Many of the studies were performed in very small samples, and interpretation of the results is therefore limited^{348,55,349}. In a literature review including 22 studies, the most commonly stated changes in people with DS were raised Tg and lower HDL levels, but with heterogeneity of findings and little literature about adult lipid profiles or their changes over the lifespan³⁵⁰. One study including 146 adults with DS (mean age 39.7 years for men and 36.9 for women) compared to a non-matched ID group had similar findings to the ones presented in this thesis, with lower TC values in people with DS (mean TC 4.98 mmol/L in DS compared to 5.30) although this was not statistically significant. Alpha cholesterol levels were also lower and this was significant (mean 0.86 compared to 1.15 in males and 1.15 compared to 1.53 in females). They found no difference in triglycerides³⁵¹. In a community study comparing 20 adults with DS to 22 age-matched controls there were no differences in TC or HDL levels, nor were there differences in levels of the metabolite 24S-OH-cholesterol, a marker of brain cholesterol turnover³⁵². A study of 51 adults with DS aged 18-62 and a comparison sample found no differences in lipid profiles, including Tg values, between groups³⁵³.

Therefore people with DS appear to have distinct lipid profiles, but not necessarily hyperlipidaemia. Sex differences may account for some of the discrepancies in the literature. In my analysis males had lower Tg levels compared to general population males which contributed to the overall lower Tg values. Such differences may introduce biases in smaller studies.

Glycated Haemoglobin and Diabetes

In chapter four, incidence rate ratios for diabetes were similar between the DS cohort and controls. Diabetes is common in Down syndrome, and increased rates have been reported. For example, higher incidences have been observed in children and younger adults, particularly for type 1 diabetes, with lower occurrences in older adults¹⁵. This shift may explain why in the overall group analyses there were similar rates of diabetes in adults with DS and controls.

HbA1c levels were lower in people with DS compared to controls in my population analysis (46.4 mmol/mol (SD 18.4) compared to 51.6 mmol/mol (SD 18.6), and this remained true for the subgroup without diabetes (36.1 mmol/mol (SD 5.8) compared to 38.5 mmol/mol (SD 6.1)). In a study of metabolic indices in 150 adolescents and young adults with DS aged 10-20years compared to 103 age-equivalent controls a similar pattern was observed, with lower HbA1c values in the DS group³⁵⁴.

On the other hand, in my analysis, people with DS and diabetes had higher mean HbA1c levels than controls with diabetes (68.3mmol/mol (SD 16.9) compared to 66.7mmol/mol (SD 16.3)) which indicates poorer glycaemic control. Poor glycaemic control has been reported in people

with intellectual disability including those with Down syndrome. A postal survey study found that 52% of respondents had HbA1c levels above recommended targets³⁵⁵. Poorer control was associated with younger people, obesity, and type 1 diabetes rather than type 2. All of these factors are common in people with DS. The differences in HbA1c levels could reflect intrinsic differences in insulin sensitivity and glucose processing in people with DS, and may reflect external or social factors in people with diabetes.

6.3. Potential Mechanisms Underlying Observed Patterns of Cardiovascular Parameters

A number of mechanisms may explain the observed CVP patterns in people with Down syndrome in the CPRD analysis. These can be broadly split into those related to autonomic dysfunction; atherosclerotic risks; immune dysregulation; direct genetic effects; metabolic and endocrine pathways; and social factors. Each of these will be considered in turn to consider the aetiology of the CVP profile presented in this research.

6.3.1. Autonomic Pathways

The autonomic system is part of the nervous system responsible for maintaining homeostasis and regulating essential bodily functions. It is not under conscious control, with responses mediated via sensory feedback loops that communicate with the CNS. The autonomic system is composed of three main pathways: the sympathetic, parasympathetic, and enteric systems. Broadly speaking the sympathetic pathway is involved in activating processes such as attention and arousal, leading to raised heart rates, higher blood pressure (caused by increasing

vasoconstriction) and glycogen breakdown. The parasympathetic system mediates resting and digestive pathways, diverting resources to the gastrointestinal system and reducing blood pressure and heart rate. The predominant carrier of parasympathetic signal is the vagus nerve which also delivers signals from baroreceptors in the vasculature to the CNS. Signals from the vagus nerve promote cardiac relaxation, reducing conduction speed through the heart and decreasing contractility of the chambers³⁵⁶. Dysfunction of the autonomic system can present as a wide-range of symptoms, but the most common finding is orthostatic hypotension³⁵⁷.

The autonomic system is implicated in immune regulation. The sympathetic nervous system innervates the thymus, spleen and lymph nodes and can affect activation of these immune organs. Parasympathetic nerves express cytokine receptors including IL-1 which are involved in the inflammatory response. When these receptors are bound downstream activation of the hypothalamic-pituitary-adrenal axis occurs, promoting further inflammatory responses³⁵⁶.

The autonomic system is reported to be intrinsically dysregulated in people with DS³⁵⁸. The aetiology of this dysfunction has not been well-defined, but is observed throughout the lifespan and appears to be a fundamental component of the DS phenotype^{59,61,359,360}. In addition, many of the conditions associated with autonomic dysfunction are common in Down syndrome including autoimmune conditions and the later stages of diabetes. Dysregulation of the enteric system in children can lead to gastroesophageal reflux, irritable bowel syndrome, constipation, and Hirschsprung disease. Autonomic dysfunction is common in amyloidopathies, and it may be driven by similar mechanisms in DS given the increased levels of amyloid-beta present throughout life. Alternatively, it has been proposed that excess expression of the *RCAN1* gene (which is also triplicated in DS) leads to deficits in neurotrophic support for neurones of the

autonomic system causing subsequent dysregulation. Correction of RCAN1 levels in mice has been associated with better neuronal survival in the peripheral nervous system³⁶¹.

Fernhall et al considered the effect of autonomic dysfunction on work capacity in people with Down syndrome. The authors proffered a model in which autonomic dysfunction diminishes catecholamine and sympathetic responses to stress, leading to reduced haemodynamic control, low cardiac output, poor peripheral blood flow and decreased magnitude of blood pressure changes. The effects could be exacerbated by a low intrinsic heart rate, and limited heart rate changes as a result of the autonomic dampening³⁶².

The low blood pressures observed across the lifespan may be attributable to autonomic dysregulation observed in DS. It has been suggested that low blood pressure related to autonomic dysfunction may work in tandem with other factors to further affect blood pressure. For example, low blood pressure in combination with reduced arterial stiffening may account for the increasing disparity of BP with age in the general population compared to DS. However, in a study using Ts65Dn mice models (an incomplete model of DS), whilst lower blood pressures (for SBP, DBP and PP) and heart rates were identified compared to wild type mice, similar pulse wave velocities were present³⁶³. This finding may implicate autonomic control differences rather than vascular resistance as the primary driver for low BP in DS, although the study tested the effects in relatively young mice (tested at 6 and 12 months) which may underestimate additional age-related changes.

Autonomic dysfunction has been linked to obesity, with greater impairment in those with obesity. In the general population a correlation between obesity and hypertension has been established. This has been particularly associated with central obesity (more associated with

men) than lower body obesity³⁶⁴. One of the mechanisms thought to drive this relationship is activation of the sympathetic nervous system in obesity, which then leads to increased arterial tone. Reduced baroreceptor sensitivity is also reported in obesity, which diminishes sympathoinhibitory effects in response to high blood pressure.

People with DS have a high incidence of obesity and are also suspected to have reduced baroreflex receptivity, therefore they may be expected to have higher blood pressures³⁶⁵. This is not the case. Results from a study using health data from 291 adults with DS and unmatched controls found that people with DS were three times more likely to be overweight, but only 0.7% reported a history of hypertension³⁶⁶. This supports the hypothesis that intrinsic autonomic dysregulation affects blood pressure values in DS, rather than blood pressure being secondary to obesity-related autonomic changes. This also provides a rationale as to why the relationship between BP and BMI is disrupted in people with DS.

6.3.2. Metabolic and Endocrine Pathways

Metabolic and endocrine pathways are complex and inter-related mechanisms broadly involved in the regulation of hormones, glucose, lipids, and proteins, with wide-ranging effects on the body and roles in maintaining bodily homeostasis. Down syndrome has been presented as a metabolic disease and dysregulation is observed in many of these pathways³⁶⁷. Notably there are high rates of obesity and diabetes, especially in younger people, and high rates of hypothyroidism. Lipid imbalances, insulin resistance, and alterations in hormonal profiles are observed^{15,217,366-370}. Dysregulation of these pathways may explain the increased prevalence of obesity observed in the CPRD cohort, and contribute to the HDL, Tg and HbA1c alterations.

It has been suggested that metabolic dysfunction not only promotes obesity, but that obesity further affects metabolic processes. Whilst metabolic and endocrine imbalances in DS are well-documented, the aetiology and consequences of these imbalances are less clear. Much of the work to date has been performed in children with DS, often in small and potentially unrepresentative samples which may not translate to adult profiles.

In male mice models of DS, Ts65Dn mice were found to have increased fat mass and reduced lean mass compared to controls, despite similar body weights. Ts65Dn mice also had increased energy intake but similar levels of activity. Dysregulation of hormones that regulate appetite were implicated as the mechanism driving this difference. Ghrelin, a marker of hunger and an anti-inflammatory adipokine, was lower in the DS mice during fasting. Increased or equivalent leptin levels, a marker of satiety and a pro-inflammatory adipokine, were observed after feeding. When exposed to a high-fat diet the Ts65Dn mice had increased body weight gain compared to controls, with increased percentage of body fat³⁷¹. There is the suggestion of a leptin-resistant phenotype in DS, with reduced sensitivity to satiety markers and reduced responsiveness of appetite-regulation pathways^{371,372}. Hence obesity in DS may be related to inherent dysfunctions of hormone pathways rather than simply external factors such as exercise levels.

Metabolic syndrome is generally associated with obesity and is thought to be related to insulin resistance, pro-inflammatory states and increased levels of fatty acids. It is linked to increased morbidity and mortality in the general population, particularly related to cardiovascular disease and diabetes. Metabolic syndrome involves the grouping of four main conditions: glucose intolerance/insulin resistance, central obesity, hypertension and dyslipidaemia. Insulin resistance is typically stated as the driver of metabolic syndrome. Insulin resistance is

associated with reduced inhibition of lipolysis in adipose tissue and a subsequent increase in fatty acids³⁷³.

People with DS appear more susceptible to obesity and insulin resistance than the general population, but the other components of metabolic syndrome appear rarer, despite there being common drivers for all four. In the population analysis presented in chapter four, triglyceride levels were lower in people with DS, as were HDL levels and cholesterol, with LDL levels being similar. This suggests differences in lipid processing in people with DS, but does not necessarily mean they are at increased risk of lipid-associated disorders. The lower incidence of hypercholesterolaemia attests to this. One proposed reason is the presence of protective factors in people with DS that counteract some of the metabolic risks. For example, adiponectin is a peptide secreted by adipocytes which interacts with endocrine pathways with anti-inflammatory and anti-atherogenic properties. It may also enhance insulin sensitivity³⁷⁴. Adiponectin levels are abnormal in people with obesity in the general population³⁷⁵. In contrast, increases in adiponectin levels have been observed with age in people with DS and may exert protective effects against the development of type-two diabetes, atherosclerosis and hypertension despite other risks being present^{331,376,377}. This could also partly explain the declines in BMI observed with age in people with DS.

People with DS are at increased risk of diabetes, particularly at a younger age, with a high incidence of T1DM. The risk of T2DM is also raised at a younger age, but in older ages the general population have higher incidence¹⁵. It is possible that the increase in T2DM incidence at younger ages is related to the higher rates of obesity observed, although as discussed the interplay between obesity and other metabolic pathways is not straightforward. Similar mechanisms related to the presence of protective molecules such as adiponectin could explain

the plateauing of HbA1c levels and reduction in T2DM levels observed in older adults with DS (without diabetes) in the CPRD cohort. If there are higher levels of peptides that promote insulin sensitivity with age, then glucose control and hence HbA1c levels would be expected to be maintained within normal ranges in people with DS, which matches the observed patterns.

Interactions Between Sex and Metabolic Pathways

Sex hormones are important contributors to metabolic and endocrine pathways and may explain some of the sex effects observed in the analysis. In the general population blood pressure is higher in men than women prior to menopause³⁷⁸. Blood pressure increases in women after menopause and reaches the same or higher values than in men. It may be that the renin-angiotensin system of the kidneys and actions of androgens are the main driver of these sex differences in BP as males typically have higher androgen levels than females. This theory is supported by the fact that during periods of increased androgens, such as during puberty, males show a greater increase in BP than females. Similarly, in polycystic ovary syndrome there are elevated levels of the androgen testosterone and a higher prevalence of hypertension³⁷⁹. It is suggested that oestrogen confers a protective effect against cardiovascular complications of obesity, including on blood pressure, in women prior to menopause. Sex hormones have been directly implicated into regulation of pathways of lipid metabolism, as well as hormone-independent effects generated by the X and Y chromosomes (for a review see *Palmisano et al, 2018*³⁸⁰). In DS the effects of sex are a current area of research interest and have not been clearly defined to date²³.

In the population analysis presented in chapter four, reduced variation between the sexes was observed in blood pressure and the majority of the lipids measured in people with DS compared

to controls. One potential explanation could be related to the dysregulation of sex hormones, particularly in men with DS. Primary hypogonadism has been described in male children and young adults with DS³⁸¹. Hypogonadism has been associated with changes in fat distribution and an association between hypogonadism and increased deposition of adipose tissue in femoral areas has been reported³⁸². This distribution is more commonly associated with females rather than males (who tend to have greater fat deposition in the intra-abdominal space). Conversely, a reduction in oestrogen after menopause has been associated with increased visceral adipose-tissue deposition, a more “male” pattern³⁸³. Women with DS tend to experience menopause earlier than the general population, therefore may have a prolonged period with lower oestrogen levels³⁸⁴. In both of these situations (men with hypogonadism, women with early menopause) the reduction in sex hormones could partly explain the reduced heterogeneity between the sexes, with some convergence of their relative hormone profiles. Similar mechanisms could also explain the reduced sex-differences in HbA1c in people with DS without diabetes. The presence of oestrogen is known to promote insulin sensitivity which should lower HbA1c values. In the general population women did indeed have lower HbA1c values than men. In people with DS there was no significant difference between HbA1c values which would be in keeping with reduced effects of oestrogen and its protective effects against diabetes.

It is apparent that metabolic and hormonal pathways are affected in people with DS. There are conflicting effects on CVPs, with effects stemming from trisomy of chromosome 21 altering the relationships between CVPs in both pathogenic and potentially protective ways,

6.3.3. Immune Dysregulation and Inflammation

Immune dysregulation is commonly observed in people with Down syndrome, affecting both the innate and adaptive immune systems³⁸⁵. One of the consequences is the increased prevalence of autoimmune conditions including type 1 diabetes mellitus and hypothyroidism, but also coeliac disease, alopecia areata, psoriasis and arthritis^{386,387}. Increased prevalence of autoimmunity has been related to dysregulation of the *AIRE* gene on chromosome 21³⁸⁸. *AIRE* is a transcription factor involved in the regulation of gene expression. Counterintuitively, despite triplication of the *AIRE* gene, lower expression has been consistently discovered in the thymus of people with DS^{389,390}. *AIRE* is vital for the negative selection of autoreactive T-cells and dysregulation can lead to increased autoimmunity³⁹¹. The higher incidence of type 1 diabetes may explain the higher levels of HbA1c observed in the DS cohort with diabetes, however external factors including medical supervision and diet cannot be discounted.

Another highly prevalent morbidity in DS is hypothyroidism. This can be the result of different aetiologies but is frequently due to the presence of anti-thyroid antibodies. Thyroid hormones are involved in multiple processes and affect CVPs including blood pressure, lipid levels and body weight. Hypothyroidism is associated with a number of related diseases including obesity, diabetes and heart disease in the general population. Low concentrations of thyroid hormones have been associated with decreased myocardial contractility and reduced cardiac output, alongside ventricular dysfunction. These effects have not been consistently observed in DS and it is still debated to what extent thyroid function affects cardiac function³⁹². However, hypothyroidism, especially if uncorrected, could potentially exacerbate low cardiovascular perfusion and vascular-related risks in DS. More research is needed to understand this association. The incident dementia risk linked to hypothyroidism could in part be mediated via

potentiation of other vascular risk factors such as low blood pressure. This could explain why there cumulative effects of risks (including hypothyroidism).

As well as an increased preponderance to autoimmune conditions, the innate immune system is dysregulated in DS, with alterations in complement components observed across all pathways compared to controls³⁰⁶. Immune function is closely related to other health factors and obesity has been implicated in immune dysregulation and development of a pro-inflammatory state. In the cross-sectional Up & Down study, adiposity was evaluated in 95 people with DS (aged between 11-18 years) using BMI, waist circumference, skinfold thickness, and body fat percentage. People with DS had higher measurements in all variables compared to controls. Waist to height ratio was positively associated with complement components C3 and C4 in the whole sample, with higher levels in those with DS (C3 119.8mg/dl in DS compared to 91.9mg/dl in controls; C4 30.1 mg/dl in DS compared to 20.1mg/dl). CRP was also raised in the DS group (4.5mg/L compared to 0.9mg/L)¹⁷. Therefore DS was associated with increased complement components (in adolescents) and this was further increased by higher body fat percentage. Obesity may be both a pro-inflammatory state and the consequence of a pro-inflammatory state further driving obesity.

DS is also associated with a reduction in regulator complement factors. Factor H (FH) works to dampen the complement system response and reduce the chance of spontaneous activation. Deficiencies in FH have been associated with chronic inflammation and autoimmunity. In DS, over-expression of genes including *miRNA-155* are related to down-regulation of FH, which may promote increased inflammation and autoimmunity⁴⁷. Conversely, over-expression of *miRNA-155* has been associated with down-regulation of genes associated with hypertension (e.g. *AGTR1*) which could enhance the hypotensive profile³⁹³. This demonstrates the potential

for competing pathways to function synergistically in DS, leading to unpredicted pairings of symptoms (such as low blood pressure and high inflammation, or high prevalence of obesity and low prevalence of metabolic syndrome) and may explain some of the disparities in CVP profiles observed in DS.

6.3.4. Genetic Effects

In Down syndrome the triplication of chromosome 21 leads to specific gene dosage effects, but also global genome expression alterations^{23,394,395}. *APP* is perhaps the most important gene that is overexpressed in DS when considering dementia risk. It also potentially exerts effects on body mass and obesity. A positive correlation between APP levels and body weight has been observed, and high APP was found to disturb mitochondrial function in adipocytes, promoting further weight gain^{396,397}. Male transgenic *APP* mice were more susceptible to diet-induced weight gain than wild-type controls³⁹⁸. As people with DS have increased expression of *APP*, this may compound the risks of obesity in this population.

RCAN1 (also known as the down syndrome critical region gene (DSCR)) is over-expressed in people with DS and, as discussed above, has been implicated as a potential contributor to autonomic dysfunction. RCAN1 is involved in pathways of angiogenesis, and has an inverse relationship with VEGF signalling, a protein that promotes angiogenesis³⁹⁹. RCAN1 has been associated with CHD development in DS, and is therefore a potentially powerful modifier of both the nervous and cardiovascular system⁴⁰⁰. Loss of *RCAN1* in combination with *APOE* knock-out led to increased levels of serum cholesterol in mice models, suggestive of a role for RCAN1 in the metabolism of cholesterol⁴⁰¹. This would be in keeping with the finding of lower

average cholesterol in people with DS who have over-expression of *RCANI* which could modulate plasma cholesterol.

DRYK1A is similarly over-expressed in DS and can disturb angiogenesis. These pathways have been speculated to contribute to inhibited pulmonary angiogenesis and reduced vascularity of lung tissue in DS with increased risk of developing pulmonary hypertension³⁹⁹. *DRYK1A* is also associated with cardiac disease, with mouse models overexpressing *DRYK1A* developing dilated cardiomyopathy and heart failure⁴⁰². Thus, whilst exact mechanisms are not yet understood, it is possible that genetic changes related to trisomy 21 disrupt vascular system development which could contribute to the CVP phenotype observed in DS, particularly in blood pressure differences and congenital heart defects.

Increased inflammation has been linked to increased risk of obesity. There are a number of pro-inflammatory genes on chromosome 21 that may contribute to increased levels of inflammation, and trisomy 21 has been associated with chronic activation of inflammatory pathways including interferon responses³⁸⁵. These genes include superoxide dismutase (*SOD1*), *S100B* and carbonyl reductase, all of which has been linked to increased oxidative stress and inflammation⁴⁰³.

In addition to the effect of trisomy 21 directly, there are complex genetic interactions between trisomy 21 and other genetic risks. For example, people with Down syndrome are at increased risk of congenital heart disease, with estimated prevalence of approximately 50%. They occur more commonly in women and the most frequently reported is AVSD⁴⁰⁴. As half of people with DS do not develop CHD, other contributing factors are being investigated. Trisomy 21 may prime the system, making people more sensitive to other genetic changes that are

associated with CHD. In mice models the presence of mutations in *CRELD1* and *HEY2*, located on chromosomes 3 and 6 respectively, in conjunction with trisomy 21 increased the incidence of CHD. Additive risks were observed, with the presence of both mutations further increasing risk⁴⁰⁵. Hence, despite DS being considered a well-documented genotype, there are complex genetic interactions between trisomy 21 and other genetic risks that affect the cardiovascular system. Similar interactions could affect other parts of the cardiovascular system and contribute to the observed CVP patterns. Defining the interactions between genes on the sex chromosomes and trisomy 21 may also shed light on the sex differences observed.

Sex differences are observed across all aspects of biology. It is recognised that these differences go beyond direct chromosomal and hormone related effects, and manifest across a wide-range of systems. In DS there is evidence of interactions between sex and aneuploidy which can alter the pattern of gene expression. *Block et al* (2015) studied the effects of trisomy on expression of approximately 100 proteins in the hippocampus, cortex and cerebellum of Dp(10)1Yey mice (Dp10, a mouse model of DS) compared to controls. They found sex differences in protein expression which were genotype dependent⁴⁰⁶. In some cases the effects on protein expression exacerbated or inverted the sex effects in Dp10 mice compared to controls, in other cases the differences led to increased similarity between Dp10 males and females. For example, PRMT2 expression, a protein which interacts with oestrogen receptors, was reduced in the cerebellum of female control mice compared to males whereas in the Dp10 mice this pattern was reversed. Other genes such as *ADAR2* were affected differentially by the presence of trisomy in males and females; *ADAR2* was increased in Dp10 males compared to controls but there were no significant differences in the levels for females. The authors also noted brain-region specific differences. Female Dp10 mice showed greater differences in cerebellar protein levels compared to controls whereas male Dp10 mice had greater differences in the hippocampus.

These findings demonstrate complex interactions between sex and trisomy 21, with differential effects in the expression of certain genes depending on sex. However, the differences are not uniform and further work is needed to understand the mechanisms driving these differences and their consequences.

6.3.5. *Atherosclerotic Risk*

Atherosclerosis results from the deposition of lipids in the walls of arterial blood vessels which form plaques⁴⁰⁷. It causes the stiffening of vessels and in the general population atherosclerotic risk is associated with increased mortality with age. Plaques can expand over time causing narrowing of arteries and blocking blood flow. Plaques act as sites that promote thrombus formation increasing the risk of acute thrombotic episodes and ischaemic events including strokes. Atherosclerosis is associated with increased blood lipids, particularly low density lipoprotein and triglycerides. It is more common in certain conditions including obesity, diabetes and hypertension. People with genetic conditions such as familial hyperlipidaemia are at increased risk. Lifestyle factors including smoking, low levels of physical activity, pollution and poor sleep have also been implicated as potential drivers of atherosclerosis⁴⁰⁸.

People with Down syndrome do not appear to experience the same atherosclerotic consequences due with age-related risks as the general population. This is despite the high prevalence of obesity, especially in younger people with DS. Intima-media thickness (IMT), a marker of atherosclerosis, was measured in 52 adults with DS (age between 35–60 years) and the relationship to other cardiovascular risk factors compared to age and sex matched controls. People with DS had thinner intima-media as well as lower SBP and DBP measurements

compared to controls. On the other hand the DS cohort had higher triglycerides, total body fat, and markers of general inflammation (CRP). Fasting Cholesterol, HDL, LDL and glucose levels were similar between the groups, as was waist circumference. Those with DS did less moderate to vigorous physical activity and were less likely to be smokers. Physical activity and male sex were the only predictors of IMT in people with DS⁴⁰⁹. Therefore, despite higher body fat, systemic inflammation and triglycerides which are generally associated with increased atherosclerosis, people with DS had lower IMT measurements.

Higher levels of atherosclerosis are associated with increased blood pressures with age in the general population. Lifestyle factors (e.g. low levels of cigarette smoking) may reduce some of the atherosclerotic risk in DS, but reduced atherosclerosis alone does not appear to explain the different BP profiles adequately. Inflammation has also been linked to the onset of atherosclerosis by altering the function of cells in the arterial walls. People with DS have a pro-inflammatory profile, and yet lower risks of atherosclerosis. In a review of reported causes of mortality from death certificates in the US a reduced standardised mortality rate (SMOR) from ischaemic heart disease in DS (0.42) was reported despite a high obesity SMOR (1.87)²¹⁷. This supports the lower incidence of atherosclerosis in this population and its uncoupling from risk factors such as obesity.

It appears that people with DS are not immune to atherosclerosis, but that it is less common, and non-traditional risk factors may be important in determining atherosclerotic risk. The association with male sex could indicate a potential cumulative risk factor that may put men at increased risk of incident dementia than women when considered alongside other cardiometabolic factors such as obesity. Similarly, even mild atherosclerosis in combination

with a high baseline dementia risk could be speculated to advance onset of dementia in this population through cerebrovascular disease pathways.

6.3.6. Social and Lifestyle Factors

Dietary intake can promote obesity, and people with DS and other intellectual disabilities may be more likely to be exposed to high fat diets. In a Nordic study, people with DS living in supported accommodation were found to eat a higher frequency of pre-cooked convenience meals which often had high salt and fat content⁴¹⁰. Only approximately 30% of the DS group consumed fruit or vegetables daily. People with DS who were of normal weight or overweight were more likely to consume fruit and vegetable compared to obese participants. BMI was inversely associated with plasma carotenoid concentration (which has been used as a proxy marker of fruit and vegetable intake). Therefore obesity in people with DS may be compounded by dietary and social factors such as where a person lives. This is supported by the fact that people with ID not associated with DS also show increased levels of obesity compared to the general population, with common social and environmental factors contributing to raised BMI in DS. In a review, women with ID were found to have higher BMI than men which could account for some of the sex differences in BMI observed in the population analysis⁴¹¹.

People with DS have been reported to engage in reduced levels of physical activity and there are recognised barriers for people with DS engaging in certain physical health activities. These include cost and transport concerns, lack of availability and accessibility, reliance on support from others to attend, and limited health knowledge, among others^{104,105,412}. Low exercise

levels in the general population are associated with increased BMI and could be a contributing factor for the higher BMIs observed in people with DS. However in a systematic review no effect from fitness programmes were found on body weight, BMI or body fat percentage in people with DS. Only two trials provided data in this area and the exercise programmes were predominantly of low impact activities such as jogging, cycling or brisk walking⁴¹³. In a separate study the relationship between physical activity and BMI in DS was low ($r=-0.2$), and associations with percentage body fat were only moderate ($r=-0.4$)⁴¹⁴. In the Up&Down cohort, 111 adolescents with DS aged 11-20 years were sex-matched 2:1 with a control group to assess the relationship between body fat and physical fitness. No association between BMI or waist circumference with fitness markers were identified in people with DS, but in controls there was a significant correlation between these measures and handgrip strength, motor skills and cardiorespiratory fitness. Therefore obesity does not appear to be caused only by low physical fitness in people with DS⁴¹⁵.

Exercise levels could interact with cholesterol processing, with lower lipid levels found in people who engage in more exercise in conjunction with a low fat diet in the general population⁴¹⁶. Therefore low activity levels in people with DS may in part explain the raised HDL levels seen but do not fully explain the observations including lower TC and Tg. There may also be confounding effects such as medical surveillance and lifestyle factors that could be relevant factors, particularly when considering the increased HbA1c values observed in people with DS and diabetes.

The effects of external factors on blood pressure do not follow expected patterns from a review which summarised the effects of exercise in BP in the general population. The authors conclude that there is strong evidence for exercise, particularly aerobic exercise, having a positive effect

on blood pressure (both SBP and BP) in a dose-dependent manner, with lower BPs associated with more exercise⁴¹⁷. If people with DS engage in lower levels of physical activity, it would be expected that they may have higher blood pressures. It has been proposed that part of the beneficial effect of exercise on BP is mediated via effects that relax the smooth muscle surrounding distributing arteries, counteracting negative effects related to aortic stiffening. People with DS have relatively reduced levels of atherosclerotic disease and do not appear to experience age-related arterial stiffening⁴¹⁸. Hence the effects of exercise on arterial smooth muscle may not necessarily have the same effect on the vascular system. The impact of social factors such as physical activity might therefore be different for people with DS.

The low blood pressures observed in people with DS do not solely appear to be related to lower levels of atherosclerosis. People with DS are less likely to engage in certain high risk behaviours such as smoking which could contribute to reduced atherosclerosis. However this would not fully explain the reduced BP and limited vascular stiffening observed in studies with DS participants. Blood pressure trends in other populations with low lifestyle risks still show age-related BP increases that are absent in DS. For example, in a study of forager-farmers, representing a population with relatively low modern cardiovascular lifestyle risks, BP trajectories with age were plotted. Blunted BP increases with age were observed in the forager population compared to national population averages, but followed similar patterns including age related increases⁴¹⁹. This suggests that some BP changes are related to intrinsic age-related changes in the general population, distinct from lifestyle factors, but may not be present in DS.

Social and lifestyle factors may therefore interact and exacerbate cardiovascular differences in the DS population, particularly related to lipid dysregulation and obesity, but do not explain the cardiovascular profile observed.

6.4. Relationships Between Cardiovascular Health and Dementia

6.4.1. Blood Pressure

In survival analyses presented in chapter five, high SBP ($>140\text{mmHg}$), low DBP ($<62\text{mmHg}$) and low PP ($<40\text{mmHg}$) were associated with significantly increased hazard ratios for incident dementia. Low SBP ($<100\text{mmHg}$) and high DBP ($>80\text{mmHg}$) trended towards significance. A u-shaped pattern may be present, with both high and low BP values increasing risk. In DS this is in the context of generally low blood pressures; there were more people in the extreme-low blood pressure groups than in the extreme-high blood pressure groups in young-mid adulthood. In the general population more people appear to have high BP in young-to-mid adulthood. In a National Health Statistics Report in the USA, 23.4% of people between 18-39 years old had hypertension⁴²⁰. The relative risk of low blood pressure on dementia incident in DS may therefore be higher than the relative risk of high blood pressures due to the population distribution of these factors.

The association between high systolic blood pressure in mid-life and later dementia risk has been well-documented in the general population⁴²¹. A HR of 1.57 (95% CI 1.05 – 2.35) in a longitudinal study of 1,440 participants was estimated^{200,422}. An association with raised DBP and dementia has been reported, but less consistently. An increased risk of mortality was associated with younger, fitter people with raised DBP, whereas low DBP ($<70\text{mmHg}$) was associated with an increased risk of mortality (HR 1.36) which was more marked in people over the age of 80 years⁴²³. In a meta-analysis, trends towards an association between mid-life diastolic hypertension with incident AD risk were reported, but the authors acknowledge significant discrepancies in the literature⁴²⁴.

The effects of low blood pressures on dementia risk have been less well-researched and is usually reported as a risk in older rather than younger people. A cross-sectional study observed an inverse relationship between both systolic and diastolic blood pressure and dementia, however this was felt to be secondary to the dementing process as the association was only significant in those already diagnosed with moderate or severe dementia⁴²⁵. In a study of 488 adults over the age of 75 years, HRs of 1.2 for each 10mmHg decrease in DBP were reported, with the effects related to AD rather than vascular dementia. People with low DBP (<70mmHg) were found to have a HR of 1.9 for AD compared to normotensive people. Persistent low BP over a period of two years was associated with a HR of 2.19 of any dementia⁴²⁶. A separate study reported similar HRs of 1.7 for incident AD in people over the age of 75 years with DBP <65mmHg, compared to normotensive individuals.

The associations between mid-life systolic and diastolic blood pressures are therefore complex with limited current research about the risk of low blood pressures in this period. The risks associated with high blood systolic blood pressure in mid-life are usually attributed to associated increased atherosclerotic risks and cerebral small vessel disease alongside direct organ and vessel damage from longstanding exposure to high pressures. However, both AD and vascular dementia are increased with high blood pressures. This association between hypertension and AD may be more specifically related to reduced clearance of A β and increased cerebral amyloid angiopathy which have been related to high blood pressures⁴²⁷.

The available literature studying the effects of PP on incident dementia risk is more limited and variable. A prospective cohort study with 15 year follow-up made a comprehensive analysis of BP changes and mortality. In younger people (below 65 years) there was an association between decreasing PP (caused by increasing diastolic pressure above 80mmHg)

and increased risk of mortality. Similarly there was a J-shaped pattern between PP and mortality with a change point estimated at 41.8mmHg (CI 34.4 – 48.5mmHg) in people over 65 years. This suggests a non-linear association between PP and mortality. The authors point to the complexities of using PP alone as a measure as it can result from either changes in DBP, SBP, or both⁴²⁸. A cohort study of an elderly Korean population used data from health insurance claims to investigate effects of PP in people over the age of 60 on incident dementia, with PP values stratified in groups of 10 mmHg between 50-90mmHg. The study reported higher hazard ratios for dementia in women with PP above 50, but no difference in men⁴²⁹. In a perspective paper, PP was cited an emerging target for dementia treatments. Associations between increased PP, BBB dysfunction and neuronal damage were suggested which could promote oxidative stress and inflammation, driving AD pathology. This was supported by the observation that high PP was associated with cerebrovascular damage and white matter changes in older people⁴³⁰. The consequences of low PP were not discussed.

6.4.2. Cerebral Blood Flow

Reduced cerebral blood flow (CBF) has been identified as an early event in AD progression in sporadic AD^{431,432}. This has been less well documented in DS, but a recent study reported similar findings. In a preliminary study of 35 adults with DS, those with probable AD had lower cerebral blood flow measured using pulsed arterial spin labelling. Cerebral blood flow was associated with cognitive measures, with worse performance in people with lower cerebral blood flow⁴³³. An interaction between CBF and amyloid-beta positivity has been observed⁴³⁴. Amyloid-beta deposition is increased in areas of reduced cerebral blood flow, and promotes inflammation and capillary constriction which further reduces CBF. In people with DS and

chronic hypotension, reduced cerebral blood flow could potentiate amyloid-beta deposition, decrease clearance and promote early progression of neuropathology⁴³⁵.

Poor cerebral perfusion has been associated with hypertension⁴³⁶. The association between hypotension and cerebral blood flow is less established. In a study of 40 people (without DS) with chronic hypotension, reduced middle cerebral artery blood flow at rest was observed, with latency in flow response times following cognitive stimulation^{437,438}. People with DS may be more vulnerable to the effects of low blood pressure on cerebral perfusion. Increased APP expression has been associated with vasoconstriction in the brain prior to frank amyloid-beta deposition, and *APP* mouse models have demonstrated impaired vascular reactivity and autoregulation with increased susceptibility to ischaemic brain injury before amyloid-beta plaque formation⁴³⁹. Deficits in cerebrovascular autoregulation in mice overexpressing APP meant that they were less able to maintain cerebral vascular homeostasis in the face of changing mean arterial pressures, and cerebral blood flow was increasingly dependent on arterial pressures as A β concentrations increased⁴⁴⁰. People with DS may exhibit a similar relationship: low blood pressures could therefore have direct consequences on cerebral perfusion and exacerbate amyloid-beta deposition and neurodegeneration.

Work in people with atherosclerotic disease found that low blood pressures were insufficient to cause brain atrophy in isolation, but in combination with low CBF there was an increased risk of cortical atrophy⁴⁴¹. Whilst people with DS are at low risk of atherosclerosis, the compounding risks of low blood pressure and cerebral hypoperfusion could increase cerebral tissue loss. Further support for the role of hypoperfusion and vascular factors in increasing AD progression in DS comes from neuropathological studies of autopsied brain tissue. Cerebral amyloid angiopathy (CAA) is common in DS-AD but has a different distribution to CAA

observed in other forms of AD. Prominent CAA was found in more superficial leptomeningeal vessels in brains from people with DS rather than more deeply penetrating vessels. The accumulation of more amyloid-beta in superficial vessels could be a product of poorer cerebral blood flow leading to increased vascular stasis and earlier deposition of amyloid-beta⁴⁴².

A relationship between cardiovascular risk in mid-life, cerebral hypoperfusion and incident dementia is in accordance with a longitudinal study of 116 adults over 20 years. Cerebral hypoperfusion in later life was associated with higher cardiovascular risk in mid-life⁴⁴³. The Framingham risk score was used to quantify cardiovascular risk. This score considers total cholesterol, smoking status, HDL and SBP values. Higher cumulative risk scores were associated with reduced grey matter perfusion at the end of the study, with the relationship being strongest with the earliest risk measurement.

6.4.3. *Body Mass Index*

Prolonged obesity, particularly starting in earlier life, has been associated with neurodegeneration and AD. A meta-analysis provided a review of mid-life BMI as a risk factor for dementia using data extracted from 15 prospective studies. Based on three studies which looked specifically at mid-life BMI and AD they calculated a pooled relative risk (RR) of 1.35 for AD in those who were overweight in midlife. For obese individuals this was 2.04. An association between being underweight in midlife and AD was also observed with a RR of 1.96⁷. An American longitudinal ageing study with a median follow up period of 23.4 years and data from 2,322 participants investigated the relationship between BMI and incident AD⁴⁴⁴. In men they found an association between being underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$) at age 30, 40

and 45 years and increased likelihood of incident AD (HR 5.76, 95% CI 2.1 – 16.0). In women having a BMI >30 kg/m² at age 30, 40 or 45 and central obesity was associated with increased risk (HR 6.57, 95% CI 2.0 – 22.0). Women who lost weight between 30-45 years were at increased risk (HR 2.02, 95% CI 1.1 – 3.9) whereas weight gain in men increased risk (HR 3.7, 95% CI 1.4 – 9.6).

Obesity has been associated with increased atherosclerotic deposition and organ damage, and this could explain some of the increased risk for incident dementia observed in DS in the survival analyses presented in chapter five. Whilst atherosclerotic disease occurs less commonly, appears milder, and is not directly associated with obesity in people with DS in the same way as in the general population, atherosclerosis can still occur in people with DS and could theoretically contribute to incident dementia risk^{409,445}. This is relevant when we consider the stacking of cumulative risk factors which increased the risk of incident dementia in DS, even though atherosclerosis does not appear to be the primary mechanism through which mid-life obesity increases incident dementia risk.

Obesity may increase dementia risk via other pathways. The lack of difference between the risks in overweight and obese people with DS may be an indicator that other inflammatory and endocrine changes (which may be similar in both overweight and obese people) are driving the pathology. Obesity has been associated with upregulation of APP. Microarray studies of adipocytes from people without DS found approximately 2.5 times increased levels of APP mRNA expression in subcutaneous abdominal adipocytes from obese adults compared to non-obese⁴⁴⁶. APP expression was correlated with measures of insulin resistance and proinflammatory gene expression (including IL-6), independent of BMI. To date a similar study has not been carried out in people with DS, but it could be hypothesised that increased

adiposity (related to increased BMI) may serve to augment the elevated APP expression observed in DS and promote inflammation, both of which can increase amyloid-beta deposition to further aggravate AD pathology and subsequent neurodegeneration.

Raised BMI has been associated with a pro-inflammatory state which has in turn been associated with cognitive decline and accelerated dementia onset. It has been suggested that obesity induces systemic inflammation resulting in inflammation of brain tissue and neurodegeneration, possibly with preferential effects in the hypothalamus⁴⁴⁷. In DS inflammation may mediate amyloid-beta deposition and drive neuropathology. Therefore, having a raised BMI and hence more inflammation earlier in life may result in earlier progression to AD. The increased risk associated with hypothyroidism and dementia incidence would be in agreement with an immune modulatory process, with a pro-inflammatory state being a risk for accelerated dementia pathology.

Cerebral amyloid angiopathy (CAA) is a common finding in the brains of people with DS. An association between increased CAA severity and faster progression of AD has been posited^{448,449}. In CAA, amyloid-beta is deposited into the cerebral vessels, weakening the walls and increasing the risk of intracerebral microbleeds and haemorrhages. CAA is associated with immune activation in the cerebral cortex, with neuroinflammation and reactive gliosis observed. The relationship between obesity and CAA has been studied using CAA mouse models (Tg-SwDI mice). Obesity was induced through dietary measures. High fat diet decreased soluble A β 40 in both CAA and control mice. There were limited alterations in neuropathology, however localised changes in microglial reactivity were observed, with regional cerebral increases of IL-6 in the CAA mice who were obese⁴⁵⁰. Obesity may exacerbate inflammation in the cortex of people with increased CAA. Obesity-induced

inflammation may therefore be a key process in which mid-life obesity increases incident dementia risk in people with DS, with a cumulative effect in combination with other risk factors such as amyloid-beta pathology.

Diabetes is another significant risk associated with DS which is linked with the development of AD in the general population. In the analysis presented in chapter five, diabetes was not significant in the models as a predictor of incidence dementia risk, however this is in the context of other CVP variables and diabetes was not examined as a primary factor. People with DS have been found to have increased insulin resistance associated with obesity, but the other components of metabolic syndrome are not present. The risks associated with diabetes and dementia in the general population could be linked to metabolic syndrome rather than diabetes in isolation. Perhaps in DS obesity is the primary metabolic factor driving dementia risk. This would be in keeping with the fact that both overweight and obese people with DS were found to have similar hazard ratios for incident dementia in the CPRD analysis. In the general population there are increasing risks in obese compared to overweight individuals and metabolic syndrome risk is increased in obesity compared to being overweight. The similar or lower levels of cholesterol related proteins and HbA1c in people with DS compared to controls would be in support of obesity mediated inflammatory pathways rather than lipid and atherosclerotic pathways driving risk.

6.4.4. Dementia Risk Associated with Covariates

Congenital Heart Disease

CHD is common in DS and affected by sex. Data from 230 live births of people with DS were used to compare relative risks of congenital heart defects in males and females. A reduced risk was calculated for males compared to females, with relative risks between 0.4 and 0.6. In contrast there were similar risks in males and females without DS (relative risks between 0.9 and 1.0) apart from in severe CHDs where there was an increased risk in males (RR 1.54 in controls). *Takano et al* (2019) similarly found higher prevalence of CHD in females with DS using data from medical records in Japan, as did *Freeman et al* (2008) with nearly double the odds for CHD in females compared to males (OR 1.93, 95% CI 1.4 – 2.7). Differences in cardiovascular development between males and females with DS may therefore be present which could interact with other risks and dementia onset.

Associations between CHD and dementia have not been established. In a small retrospective exploratory pilot study including 29 unmatched adults with DS (12 of whom had heart disease), the combined score from a dementia test battery was used to examine the relationship between non-specific heart disease (including CHD), thyroid status, and cognition¹⁴¹. Heart disease was associated with lower DTB scores, particularly in those with hypothyroidism. There was a negative correlation between DTB score and age, but only in those with heart disease. The very small sample size and non-specific heart disease grouping make the significance of the results difficult to determine

Until relatively recently the focus of congenital heart disease research was on repair and childhood longevity. Due to advances in medical care there is now an ageing population of people who were born with CHD and the long-term sequelae of these conditions are gaining importance. This is true in people with DS, and there were lower numbers of people with a history of CHD in the over 35 cohort than in children. There is evidence that CHD may interact with other acquired comorbidities through life, and may exacerbate other risks in people with Down syndrome⁴⁵¹. The exact effects are likely to depend on the type and severity of CHD and the treatment received. Further research is needed into the long-term consequences of CHD in people with DS.

Hypothyroidism

In studies in the general population there are conflicting results regarding the relationship between thyroid status and risk of incident dementia. Increased hazard ratios of 2.03 (95% CI 1.62 – 2.53) have been suggested in younger people with hypothyroidism (under 56 years old) for combined AD and vascular dementia in a Danish study⁴⁵². For AD specifically there was an increased HR but this lost significance in an adjusted model (HR 1.34, 95% CI .78 – 2.28). An association between raised TSH levels and dementia incidence was also reported, with HR of 1.12 (95% CI 1.07 – 1.16) for each 6 month period of elevated TSH. An adjusted odds ratio of 1.81 (95% CI 1.14 – 2.87) for dementia in people over the age of 65 years with a history of hypothyroidism compared to those without was estimated in another study⁴⁵³.

On the other hand, a meta-analysis conducted using data from five prospective cohort studies of subclinical hypothyroidism found a non-significant pooled adjusted risk ratio of 1.14 (95% CI .84 – 1.55) for dementia which suggests subclinical hypothyroidism is not associated with

elevated dementia risk. In the same analysis a pooled risk ratio of 1.67 (95% CI 1.04 – 2.69) was estimated for subclinical hyperthyroidism⁴⁵⁴. A later study found no association between autoimmune thyroid disease or overt hypothyroidism and incident dementia while hyperthyroidism was associated with increased risk, with a HR 1.40, 95% CI 1.02 – 1.92⁴⁵⁵.

Similar conflicts are apparent in Down syndrome-specific studies. A study of 232 adults stratified into three AD-onset groups (before age 47, between 48-59, and over 59 years) identified a positive association between age of dementia onset and age of hypothyroidism diagnosis. Being diagnosed with hypothyroidism at an earlier age and having *APOE* ε4 alleles were independently associated with an earlier age of AD onset⁴⁵⁶. In an older study no difference in prevalence of hypothyroidism was found between people with and without dementia⁴⁵⁷. Hypothyroidism was not associated with age of AD diagnosis in survival analyses using UK data extracted from intellectual disability health service records⁴⁵⁸.

Thyroid hormones have wide-ranging effects, but a number of interactions may be relevant in the development of AD pathology. These include associations with oxidative stress pathways, regulation of *APP* gene expression and tau phosphorylation, and modulating acetylcholine synthesis⁴⁵⁹. Both thyroid dysfunction and dementia incidence increase with age, and it may be that the relationships between hypothyroidism and AD reported could be the result of common pathophysiological mechanisms rather than one being causative of the other. In DS, hypothyroidism is common throughout the lifespan which may make this less likely.

Sex Effects

For the majority of the CVPs studied the magnitude of sex effects were reduced in Down syndrome compared to controls, yet in survival models male sex was a significant contributor to dementia risk suggesting that sex may modulate cardiovascular risk for dementia.

The current literature is inconsistent as to whether men or women with DS are more likely to be diagnosed with AD earlier. Based on a cross-sectional community sample of 111 adults with DS, it was estimated that men with DS were three times more likely to develop AD by the age of 65 years than women⁴⁶⁰. No sex effects on incidence dementia were identified in a study of people in their fifth decade, although there were issues with power in the analyses⁴⁶¹. Using survival analyses adjusted for *APOE* status, ethnicity and level of ID, age-dependent sex differences were identified for incident AD. Over the age of 60 years men were six times more likely to develop AD than women, but not at younger ages (RR 6.32, 95% CI 2.11 – 18.96, $p < 0.001$)⁴⁶². No interactions between sex and *APOE* status were acknowledged.

In preliminary work presented in a conference abstract, sex differences in NfL and total-tau levels were measured in a cross-sectional analysis of 275 adults with DS. When adjusted for age, NfL concentrations did not differ by sex, but t-tau concentrations did⁴⁶³. As expected, NfL levels were higher in people with prodromal and confirmed AD. T-tau levels were also higher, but only in females. T-tau only showed classification utility for women (AUC 0.7 – 0.9 for women; 0.5 - 0.7 for men).

A review published in August 2022 summarised the literature around sex differences in DS and their relationship to AD⁴⁶⁴. The authors highlight inconsistencies in the DS literature

regarding sex effects. Some papers report higher incidences of AD in women compared to men, with earlier onset. It has been suggested that post-menopause, women may be at increased risk due to lower levels of bioavailable oestradiol. In an Irish study tracking the age of menopause in 143 women with DS the age of menopause positively correlated with the age of dementia onset ($r=0.572$), with later onset of menopause associated with later dementia onset. The sample in this study was small ($n=12$)⁴⁶⁵. Other studies reported the opposite effect or a lack of sex effect. One report described increased white matter hyperintensities in the brains of men with DS and *APOE* $\epsilon 4$, and it is possible that men may be more vulnerable to cerebrovascular pathology with age compared to women which could increase their incidence dementia risk in combination with other cardiovascular risk factors⁴⁶⁴.

6.5. Chapter Summary

People with DS have unique cardiovascular profiles. They have low blood pressures and limited age-related changes, higher rates of obesity when younger, lipid dysregulation, and differences in glucose metabolism which are more prominent with ageing. Sex differences were also observed, with more homogeneity between the sexes in most parameters. These differences are likely related to a number of intrinsic and external factors including autoimmune dysfunction, genetic effects, metabolic and endocrine dysregulation, and social factors.

Cardiovascular risk factors, particularly low blood pressure and raised BMI, affect incident dementia risk in people with DS. Blood pressure risk may be mediated in part through hypoperfusion pathway. BMI may impart risk through a combination of pro-inflammatory

effects and interactions with lipid pathways and atherosclerosis. Additional variables including sex, history of congenital heart disease and hypothyroidism also increase risk and may interact with cardiovascular parameters and genetic factors to modulate AD pathology and dementia onset in people with Down syndrome.

In the next chapter plasma biomarkers related to AD (NfL, GFAP, p-tau181, A β 40 and A β 42) alongside markers of inflammation (complement components) are analysed to explore their association with cardiovascular parameters and cognitive outcomes. The aim is to consider which potential mechanisms discussed here may be most likely to drive the interactions between cardiovascular status and incident dementia risk in people with DS.

Chapter VII

Cardiovascular Parameters and their Relationship to Cognitive and Blood-based Markers of Dementia Using Data from the LonDowns Prospective Cohort Study

Part A. Results

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7.1. Background

7.1.1. *Summary of Methods and Aims*

In the preceding chapters it was established that adults with Down syndrome have a distinct cardiovascular profile. They have lower blood pressures and do not exhibit the same age-related changes in blood pressure observed in the general population. In addition, people with DS have, on average, a higher BMI at a younger age, with a decline in BMI in later years. The risk factors assessed in this chapter were identified as significant contributors to incident dementia risk in the preceding chapters: systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) and body mass index (BMI). People with CHD, men, and those with hypothyroidism also appear to be at increased risk, although it is not clear the mechanism through which these factors interact with CVP status and AD pathways. In the literature, exercise level is also suggested as a predictor of dementia risk in the general population. Robust population-level data recording exercise habits is not available to date in GP records, hence it was not included as a factor in the CPRD analysis. Instead, preliminary work outlined in chapter 3 (and included in appendix 1) aimed to define the relationship between level of physical activity and dementia symptoms in people with DS. The study identified level of regular physical activity as an important risk factor for dementia in the DS group.

In this section, data from the LonDowns cohort - a deeply phenotyped prospective study of AD in adults with DS - were used to explore the relationships between CVPs, cognitive health, dementia-related symptoms, and plasma biomarkers. Cross-sectional and longitudinal analyses were performed using Pearson two-way correlations, regression models, and repeated measures ANCOVA. Dementia-related profiles were determined using principal factor analysis with logistic regression techniques.

Relationships between these CVPs with cognition and blood based biomarker of neurodegeneration (NfL), dementia pathology (p-tau181), amyloid-beta (A β 40 and A β 42) and neuroinflammation (GFAP), were ascertained in exploratory analyses. Components of the complement system were measured in an additional analysis as a marker of general inflammatory processes. The aim was to identify possible mechanistic pathways to explain the increased incident dementia rates associated with cardiovascular health status.

7.1.2. Hypotheses to be Tested

Based on the results and discussion presented in chapters 4-6 of this thesis, it was speculated that raised BMI and hypothyroidism increase risk of incident dementia by promoting inflammatory processes which accelerate neuropathology. Low blood pressures may potentiate amyloid-beta deposition via effects on cerebral perfusion, reducing amyloid-beta clearance and leading to ischaemic injury. Hence different patterns of amyloid-beta markers may be identifiable. The presence of CHD could further exacerbate these effects. Regular physical exercise boosts cognitive performance but the associations between exercise and biomarkers of AD have been inconsistent in the literature, with limited direct relationships identified.

The following hypotheses were therefore tested in the subsequent analyses:

- Raised BMI will be associated with increased markers of amyloid-beta burden in the form of disrupted A β protein profiles, and inflammation in the form of complement activation.

- Low blood pressures will be correlated with increased markers of neurodegeneration and neuronal injury, particularly NfL and GFAP, as a result of chronic hypoperfusion and resulting ischaemic insult to cortical tissues. Low blood pressures may also be related to higher A β levels and different ratios of A β 40 and A β 42 as a result of impaired amyloid-beta clearance.
- Higher levels of NfL and p-tau181 will be observed in people with multiple risk factors compared to people without established risk factors.
- People with a history of CHD will have lower blood pressures and may have increased levels of NfL and GFAP at younger ages than people without.
- People with hypothyroidism will have disrupted inflammatory pathways, with dysregulation of complement markers.
- Engaging in higher levels of regular exercise will be related to reduce dementia symptom burden, and may be accompanied by reduced markers of neurodegeneration and inflammation.

7.2. Sample Description

Demographics

One hundred and ten assessments were completed, following the protocol outlined in the methods section (chapter 3), including plasma sample collection for biomarkers analysis. Seventeen of these assessments were follow-ups of the same participants, leaving a cohort of 92 individuals for cross-sectional analysis. Additional longitudinal CVP measurements were available for 70 people. The mean time between the two assessment was 5.06 years (SD 1.61). The characteristics of the cohort are described in table 52.

Fifty-one participants were male (55.4%) and 41 female (44.6%). Forty-two had mild intellectual disability, and 43 moderate (45.7% and 46.7%). Seven had severe or profound ID (7.6%). Ethnicity was categorised based on the 2021 UK census groups: 75 (81.5%) were white British. Seventy-seven participants had *APOE* data available with 55 (59.8%) possessing no $\epsilon 4$ allele and 22 (23.9%) having at least one $\epsilon 4$ allele. Thirty-six participants were under 35 years of age and fifty-six were 35 years or older.

The majority of the sample (95.7%) had never smoked, two were ex-smokers and two were current smokers (both 2.2%). Nearly two-thirds of the participants (62.6%) did not drink alcohol, 26 (28.6%) drank between 0.5-3 units a week and seven people drank four or more units a week (7.7%).

The most common type of exercise performed was walking with 54.6% of people participating in this activity each week. Swimming was the next most common ($n=30$, 21.2%), followed by dance ($n=25$, 22.7%) and going to the gym ($n=24$, 21.8%). Other exercise activities included cycling, football, badminton, basketball, cricket, gymnastics, Ju jitsu, Pilates, yoga, skiing, tennis, trampolining, tai chi, and electronic games such as Wii Fit.

Co-Morbidities

There were high incidences of congenital heart disease (31.5%), hypothyroidism (47.8%) and other autoimmune conditions (45.7%) in the sample cohort. Diabetes mellitus (either type 1 or type 2) was present in 9.8%. There were low rates of hypertension, with only one recorded diagnosis, as well as low occurrences of clinically diagnosed hypercholesterolaemia (5.4%).

Thirteen people had a diagnosis of dementia at the time of assessment and a further eight had possible dementia (i.e. they were in the process of being assessed for dementia at the time of assessment, presenting with symptoms consistent with a dementia diagnosis but which had not been confirmed clinically). For the purpose of this analysis those with either a confirmed or query dementia diagnosis were grouped into a single “dementia” group ($n=21$) unless otherwise specified.

		<i>n</i>	%
Total		92	100
Sex	Male	51	55.4
	Female	41	44.6
Ethnicity	Asian or Asian British	2	2.2
	Black, Black British, Caribbean or African	5	5.4
	Mixed or Multiple Ethnic Groups	3	3.3
	White	81	88.0
	Not Recorded	1	1.1
Level of ID	Mild	42	45.7
	Moderate	43	46.7
	Severe/Profound	7	7.6
Smoking Status	Never Smoked	88	95.7
	Ex-Smoker	2	2.2
	Current Smoker	2	2.2
Alcohol Use	None	57	62.6
	Up to 3 units a week	26	28.6
	4 or more units a week	7	7.7
Dementia Status	None	71	77.2
	Query	8	8.7
	Confirmed	13	14.1
Exercise Level	Low	19	20.7
	Moderate	46	50.0
	High	26	28.3
APOE ε4 Status	Present	22	23.9
	Absent	55	59.8
	Unknown	15	16.3

Table 52. Sample demographics for the LonDowns cohort.

7.3. Data Quality Checks

Plasma Biomarker Quality Controls

All participants had NfL levels measured at the time of assessment. A subgroup had additional plasma biomarkers A β 40, A β 42, GFAP, p-tau181, and complement components measured. There were 92 people with NfL results, 76 with additional A β 40, A β 42, p-tau181 and GFAP measurements, and 65 participants with complement measurements. The mean time between sample acquisition and initial processing and storage time was 59 minutes (SD 46).

The differences in sample number between the biomarkers were related to sample volumes and logistical issues during the pandemic. Recruitment was extended to compensate for research pauses and procedural amendments during the Covid-19 pandemic and associated restrictions. Samples for NfL analysis were prioritised for this project. Other biomarkers were processed according to sample and laboratory availability.

Repeatability and intermediate precision of the SIMOA assays were all high with minimal variation. Repeatability for NfL was 4.5%; GFAP 3.9%; p-tau181 5.2%; A β 40 1.1%; A β 42 4.1%. Precision was 6% for NfL; 4.1% for GFAP; 9.6% for p-tau181; 1.2% for A β 40 and 4.1% for A β 42. Variation of less than 10% was considered acceptable.

There was a strong correlation between NfL values run between the two sites (R^2 0.978). Samples analysed at KCL consistently had values approximately 1.15 greater than those processed in Sweden and a correction was applied to the NfL values to harmonise the results. This procedure has been validated in large multi-centre studies of NfL, including the two sites where the samples were processed in this project¹⁵³.

Data Distributions

Normality of distribution for variables was checked visually using P-P plots and confirmed based on skewness and kurtosis. One SBP and one DBP value were identified as outliers and removed (alongside the corresponding PP value). Both values were significantly higher than the accepted mean ± 3 SDs, were likely to represent measurement or recording errors, and were causing significant skew in the data (SBP 177mmHg, DBP 112 mmHg). When these were removed, SBP and DBP values met normality requirements.

Values for BMI were positively skewed, but no outliers were identified. Positive skew is common in health data, and is widely recognised when using BMI data. Ln transformations were applied with resulting normality within acceptable limits. CAMDEX values were also positively skewed, again with no clear outliers. To account for presence of zero values (occurring in people without dementia symptoms) a $Ln(x+1)$ transformation was applied. The resulting data were within normality limits.

One A β 42 measurement was excluded as it was lower than the calibrator and may represent an error (A β 42 0.81pg/ml). The corresponding A β 40 value was also removed. No other significant outliers were identified in the biomarker panel. NfL and p-tau181 were significantly positively skewed (NfL skewness 2.68, Shapiro-Wilk 0.701; p-tau181 skewness 1.68, Shapiro-Wilk 0.785, both $p < 0.001$). A β 40 and GFAP were also positively skewed (skewness 2.90, Shapiro-Wilk 0.927; and skewness 1.576, Shapiro-Wilk 0.978 respectively, $p < 0.001$ for both). A β 42 values were within acceptable levels (skewness -0.44, Shapiro-Wilk 0.214). In light of the above, Ln transformations were applied to NfL, p-tau181, A β 40, A β 42 and GFAP values to allow for application of parametric methods and ensure internal consistency.

All other data was within acceptable limits for normality. SABS values were in the borderline range but accepted due to the robustness of the statistical tests being applied.

7.4. Distributions of Outcome Measures in the Cohort

Cardiovascular Parameters

Blood pressure, BMI and abdominal circumference (AC) data were available for 86 participants. The mean (SD) age at assessment was 41.46 years (13.35). The mean SBP was 112.45mmHg (12.32), DBP was 68.88mmHg (9.69) and PP was 43.37mmHg (11.70). Mean BMI was 29.57 kg/m² (6.69) and AC was 95.40cm (13.22). As in the CPRD population analysis, only BMI showed a significant relationship with age, increasing until mid-life and then decreasing (results from multivariate analysis F 1.782, partial eta squared 0.593, p=0.034). For all other CVPs, p>0.268 for age comparisons (see figures 21 and 22 for age-related patterns of blood pressure and BMI measurements).

The only significant difference between men and women was observed in BMI, with higher BMI values observed in women (32.54 kg/m² (SD 7.36) compared to 27.34 kg/m² (SD 5.42), p<0.001). There were no differences in the prevalence of comorbidities including dementia between the sexes (Pearson Chi square all p>0.283). See table 53 for comparisons.

Cognitive Outcomes

Variations in the complexity of the direct cognitive tasks led to inconsistencies in completion rates with floor effects evident. The paired associate learning – first attempt memory score (PALFAMS) task had the lowest rates of completion, with data available for 64 people (69.6%

of the sample). Verbal fluency was completed by 77 people (83.7%). The informant questionnaires had higher completion rates with 88 (95.7%) participants having complete SABS data and 91 (98.9%) with complete CAMDEX-DS data.

The mean score for PALFAMS was 8.78 (SD 5.42), and for adjusted verbal fluency (VF) was 2.65 (SD 0.93). The mean SABS score was 76.10 (SD 21.80) and calculated CAMDEX score was 8.76 (SD 16.52).

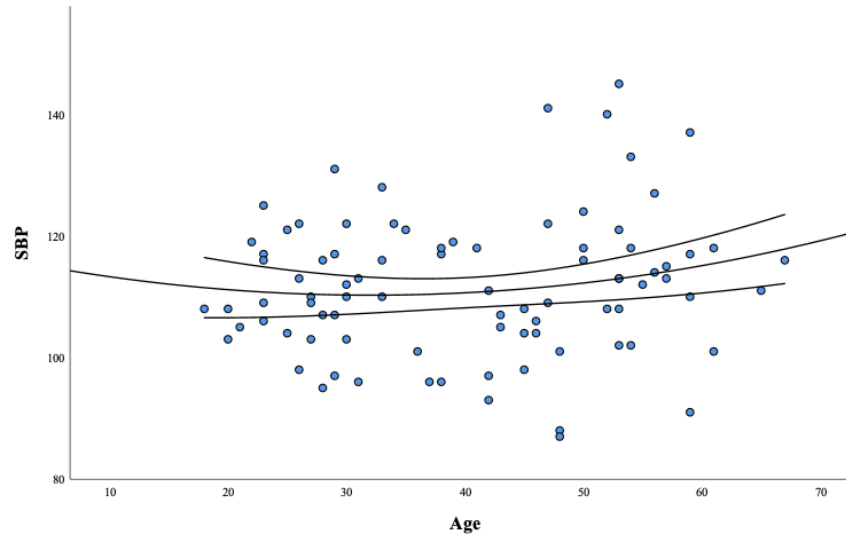
Plasma Biomarkers

Values for plasma biomarkers NfL, GFAP, p-tau181, A β 40, A β 42 and A β 42/40 ratios were visualised graphically using scatterplots. NfL, GFAP and p-tau181 showed associations with age and dementia status; people with dementia showed steeper increases with age (see figure 23 for scatterplots of NfL, GFAP and p-tau181 with age). A β 42/40 was only weakly negatively correlated with age, and values were not different between people with and without dementia. There was no meaningful correlation between A β 40 or A β 42 with age or dementia status. There were no significant sex differences. Mean values for men and women are presented in table 53.

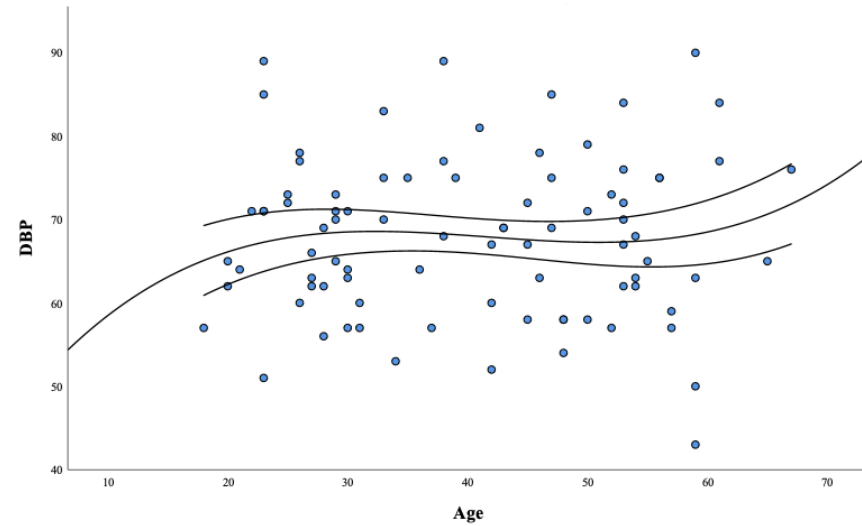
	Male	Female	t	Mean difference	95% CI	p
Age	42.00 (13.83)	40.17 (13.12)	0.645	1.829	-3.80 – 7.46	0.520
SBP	113.62 (10.57)	109.08 (12.44)	1.823	4.537	-0.41 – 9.49	0.072
DBP	68.80 (9.52)	66.58 (9.77)	1.054	2.217	-1.97 – 6.40	0.295
PP	44.82 (12.08)	42.50 (10.92)	0.914	2.320	-2.73 – 7.37	0.363
BMI	27.34 (5.42)	32.54 (7.36)	-3.598	-0.161*	-0.25 - -0.07	<0.001
AC	94.18 (12.32)	96.90 (14.79)	-0.745	-2.168	-7.96 – 3.62	0.458
PALFAMS	8.19 (4.94)	9.42 (5.88)	-0.917	-1.237	-3.93 – 1.56	0.363
VF	2.70 (0.91)	2.57 (0.92)	0.606	0.126	-0.29 – 0.54	0.546
SABS	73.94 (23.00)	79.29 (19.73)	-1.148	-5.349	-14.61 – 3.92	0.254
CAMDEX	11.73 (18.85)	4.43 (10.95)	1.799	0.528	-0.06 – 1.11	0.075
NfL	20.70 (20.35)	18.32 (16.22)	0.336	0.053	-0.26 – 0.36	0.737
GFAP	123.60 (94.58)	120.81 (87.90)	0.081	0.003	-0.33 – 0.32	0.985
Aβ40	149.45 (34.08)	149.42 (38.70)	0.609	0.063	-0.27 – 0.14	0.544
Aβ42	8.43 (2.63)	8.23 (2.51)	0.212	0.019	-0.16 – 0.19	0.833
Aβ42/40	0.056 (0.013)	0.055 (0.013)	0.262	0.016	-0.11 – 0.14	0.794
p-tau181	8.24 (6.30)	9.24 (8.52)	-0.586	-0.999	-4.38 – 2.40	0.560

Table 53. Parameters for male and female participants in the LonDowns cohort. Values presented as mean (standard deviation). Comparisons made using two-tailed independent t-tests, with significance set at $p < 0.05$.

A.



B.



C.

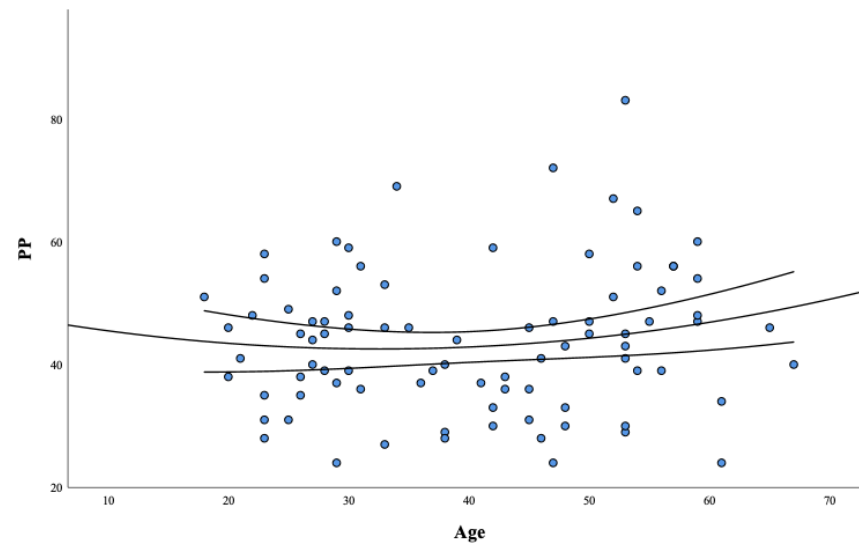


Figure 21. Scatter plots of blood pressure parameters (in mmHg) by age (years) in the LonDowns cohort. A) SBP by age. Quadratic R^2 0.021. B) DBP by age. Quadratic R^2 0.008. C) PP by age. Cubic R^2 0.017. Error bars represent 95% CI.

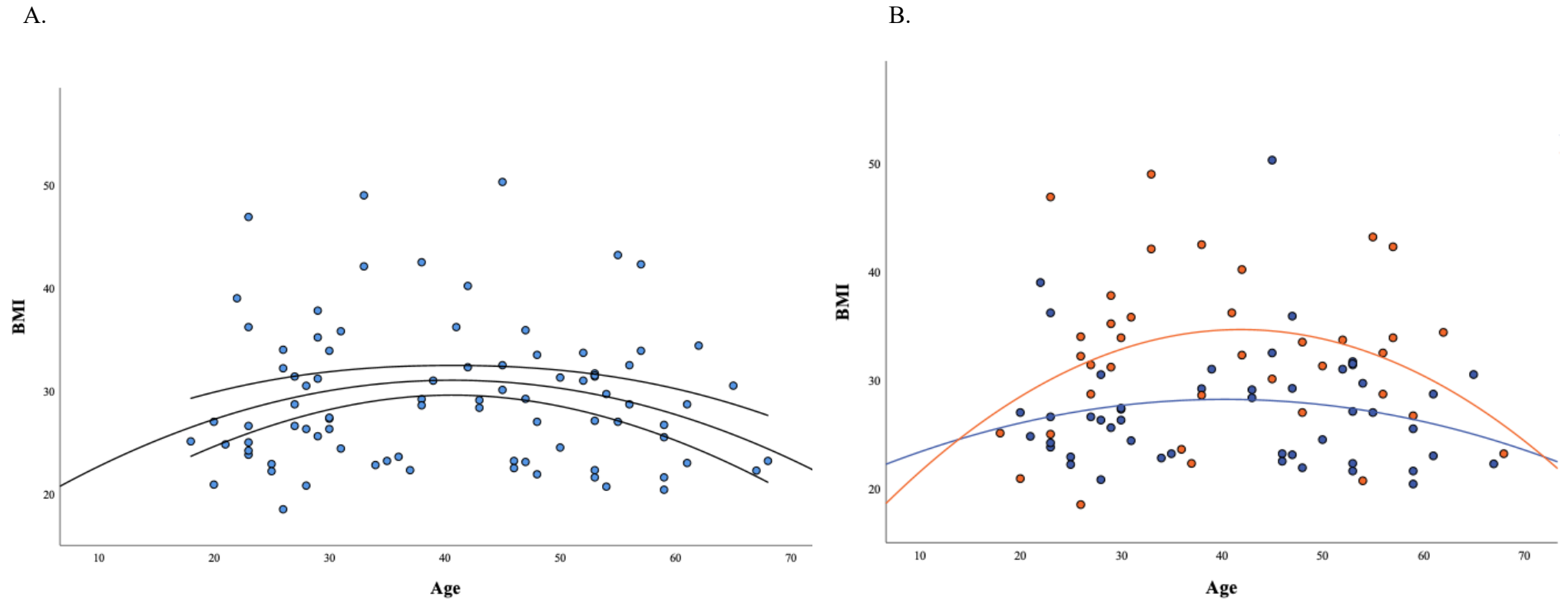


Figure 22. Scatter plots of BMI (in kg/m²) by age (years) in the LonDowns cohort. A) BMI by age. Quadratic R^2 -0.044. B) BMI by age and sex. Females (orange), males (blue). Error bars represent 95% CI.

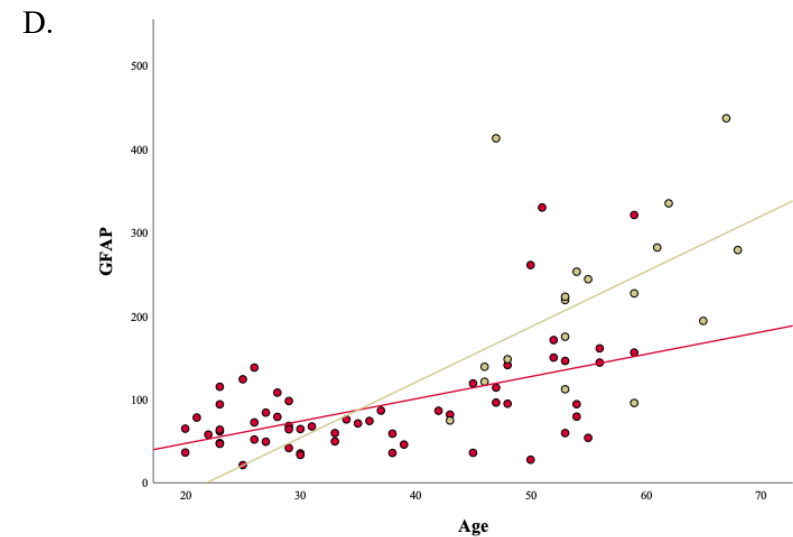
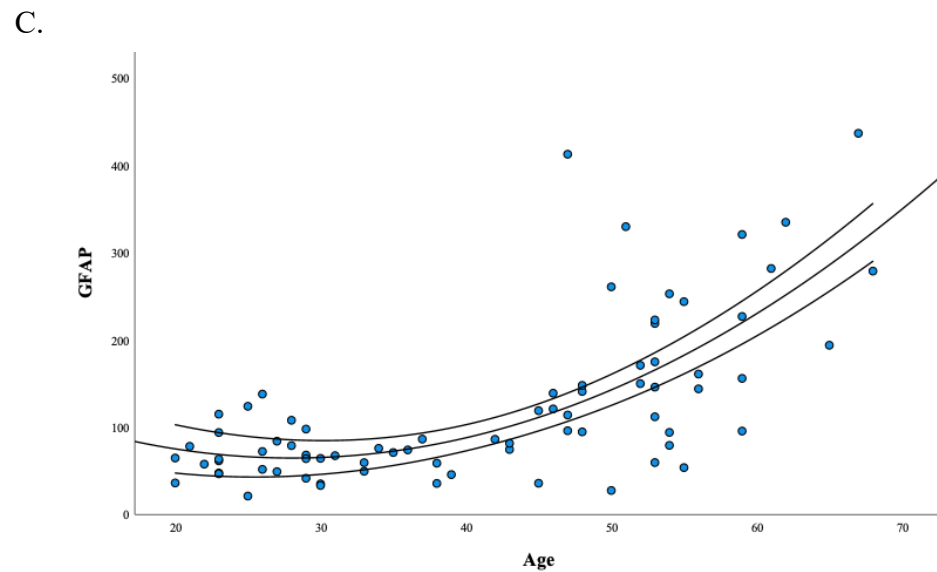
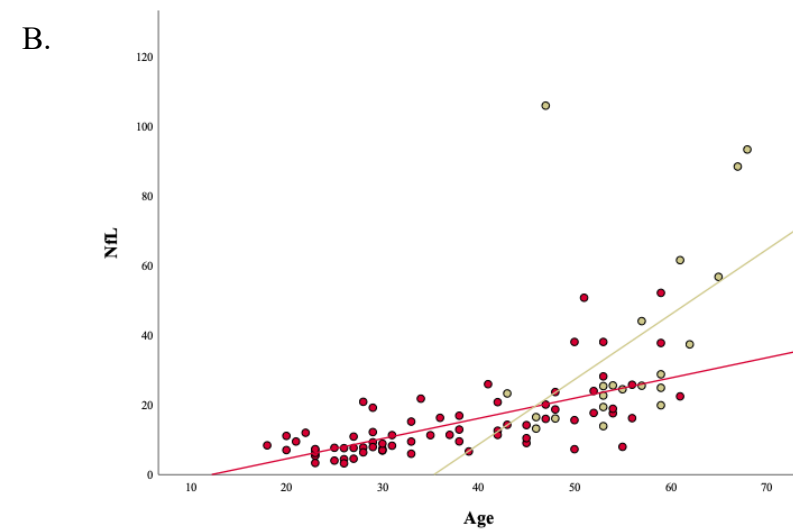
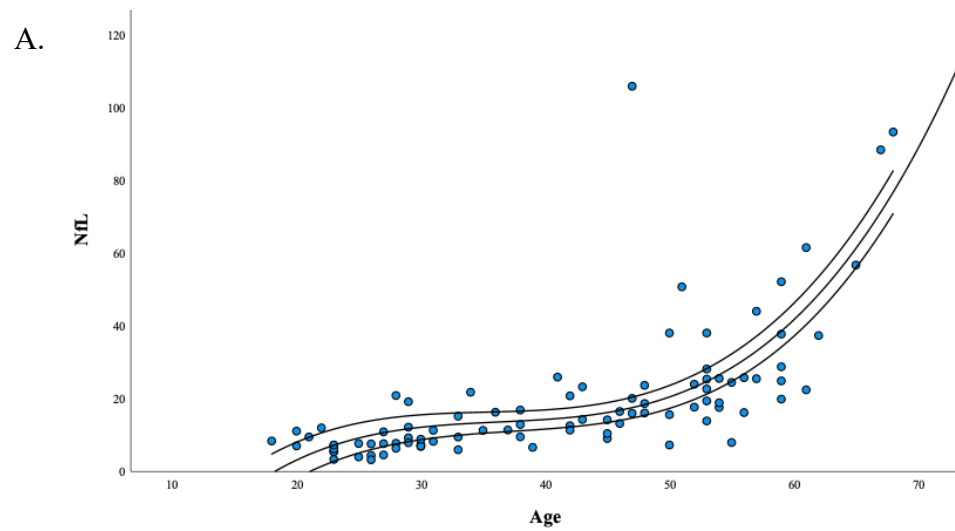


Figure 23a. Scatter plots of NfL and GFAP (in pg/ml) by age (years) in the LonDowns cohort. A) NfL by age. Cubic R^2 0.547. B) NfL split on dementia status (yellow indicates people with dementia) with lines of best fit. C). GFAP by age. Quadratic R^2 0.503. D). GFAP split on dementia status. Error bars represent 95% CI.

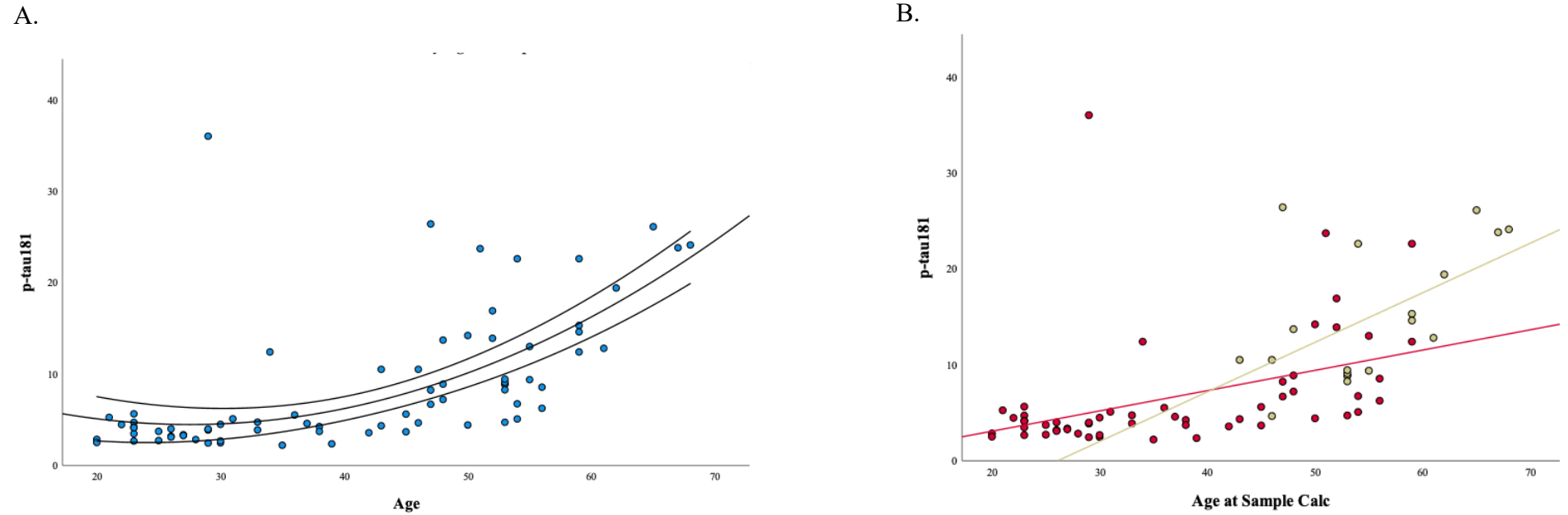


Figure 23b. Scatter plots of p-tau181 (in pg/ml) by age (years) in the LonDowns cohort. A) p-tau181 by age. Quadratic R^2 0.409. B) p-tau181 split on dementia status (yellow indicates people with dementia) with lines of best fit. Error bars represent 95% CI.

7.5. Relationships between Cardiovascular Parameters, Cognitive Outcomes and Plasma Biomarkers

In the initial analysis the whole cohort was analysed, including both those with dementia and without. Unadjusted two-tailed Pearson correlations were calculated between age, CVPs, cognitive outcomes and plasma proteins. Two-tailed independent t-tests were performed for sex and exercise-group comparisons.

7.5.1. Whole Sample Comparisons

Pearson Correlations

Table 54 summarises the significant correlations. As expected, blood pressure parameters were closely correlated (SBP:DBP correlation 0.41, 95% CI 0.27 - 0.61; SBP:PP correlation 0.65, 95% CI 0.53 - 0.77; DBP:PP correlation -0.42, 95% CI -0.53 - -0.15, all $p < 0.001$). BMI and AC were also closely correlated (Pearson correlation 0.82, 95% CI 0.72 - 0.87, $p < 0.001$).

The cognitive and functional measures were correlated with each other (PALFAMS:VF correlation 0.51, 95% CI 0.29 - 0.67, $p < 0.001$; PALFAMS:SABS correlation 0.30, 95% CI 0.06 - 0.51, $p = 0.02$; PALFAMS:CAMDEX correlation -0.27, 95% CI -0.49 - -0.03, $p = 0.03$; VF:SABS correlation 0.51, 95% CI 0.31 - 0.66, $p < 0.001$; VF:CAMDEX correlation -0.30, -0.49 - -0.07, $p = 0.01$; SABS:CAMDEX correlation -0.61, -0.73 - -0.46, $p < 0.001$). Age was negatively correlated with cognitive measures (PALFAMS, VF) and functional measures (SABS), but positively correlated with CAMDEX score where a higher score suggests greater decline (age:PALFAMS correlation -0.50, 95% CI -0.66 - -0.28, $p < 0.001$; age:VF -0.38, 95%

CI 0.16 – 0.54, $p=0.01$; age:SABS correlation -0.33, 95% CI -0.51 - -0.13, $p=0.002$; age:CAMDEX correlation 0.50, 95% CI 0.32 - 0.64, $p<0.001$). Age was not correlated with blood pressure parameters (see figure 21).

NfL, GFAP and p-tau181 were positively correlated with age (Pearson correlation for NfL 0.79, 95% CI 0.69 – 0.85; for GFAP 0.66, 95% CI 0.52 – 0.77; for p-tau181 0.72, 95% CI 0.59 – 0.82, all $p<0.001$). NfL, GFAP and p-tau181 were all positively correlated with each other (NfL:GFAP 0.78, 95% CI 0.68 – 0.86; NfL:p-tau181 0.731, 95% CI 0.60 – 0.82; GFAP:p-tau181 0.728, 95% CI 0.60 – 0.82, all $p<0.001$). NfL and p-tau181 were not associated with amyloid-beta measures whereas GFAP was positively correlated with A β 40 (0.231), but not A β 42 (see figure 24).

NfL was negatively correlated with the cognitive outcomes and positively correlated with CAMDEX score (NfL: PALFAMS correlation -0.444, 95% CI -0.62 - -0.22; NfL:VF correlation -0.386, 95% CI -0.56 - -0.18; NfL:SABS correlation -0.373, 95% CI -0.54 - -0.18; NfL:CAMDEX correlation 0.538, 95% CI 0.37 – 0.67, all $p<0.001$). GFAP and p-tau181 also correlated with these measures (GFAP:PALFAMS correlation -0.415, 95% CI -0.62 - -0.16, $p=0.02$; GFAP:VF correlation -0.395, 95% CI -0.58 - -0.17, $p=0.001$; GFAP:SABS correlation -0.429, 95% CI -0.60 - -0.22, $p<0.001$; GFAP:CAMDEX correlation 0.546, 95% CI 0.36 – 0.69, $p<0.001$; p-tau181:PALFAMS -0.465, 95% CI -0.66 - -0.22, $p<0.001$; p-tau181:VF - 0.293, 95% CI -0.50 - -0.05, $p=0.02$; p-tau181:SABS -0.391, 95% CI -0.57 - -0.17, $p<0.001$; p-tau181:CAMDEX 0.579, 95% CI 0.40 – 0.71, $p<0.001$).

Neither NfL nor p-tau181 were correlated with any of the CVPs. GFAP was negatively correlated with BMI but not AC (GFAP:BMI correlation -0.310, 95% CI -0.51 - -0.08,

p=0.008). BMI was negatively correlated with CAMDEX score, but was not correlated with amyloid-beta measures nor other cognitive outcomes (BMI:CAMDEX correlation -0.240, 95% CI -0.43 - -0.03, p=0.03).

PP was negatively correlated with both amyloid-beta measures but not their ratio (PP:A β 40 correlation -0.338, 95% CI -0.53 - -0.11, p=0.004; PP:A β 42 correlation -0.292, 95% CI -0.49 - -0.06, p=0.01). PP was not correlated with NfL, p-tau181 nor GFAP. SBP and DBP were not significantly correlated with any biomarkers. None of the BP measurements were correlated with BMI or cognitive outcomes. A β 42/40 ratios only showed significant correlations with A β 40 and A β 42 (A β 40 correlation -0.264, 95% CI -0.46 - -0.04, p=0.02; A β 42 ratio 0.345, 95% CI 0.13 – 0.53, p=0.002).

	Age	SBP	DBP	PP	BMI	AC	NfL	A β 40	A β 42	A β 42/40	GFAP	pTau181	PALFAMS	VF	SABS	CAMDEX
Age		0.124	0.019	0.108	-0.031	0.142	0.785**	0.140	0.005	-0.219	0.664**	0.723**	-0.502**	-0.377**	-0.330**	0.495**
SBP	0.124		0.410**	0.654**	0.159	0.199	-0.007	-0.189	-0.155	0.061	-0.046	0.168	-0.134	0.058	-0.064	0.089
DBP	0.019	0.410**		-0.422**	0.088	0.166	-0.011	0.199	0.184	-0.028	0.067	-0.063	-0.203	-0.119	-0.110	0.125
PP	0.108	0.654**	-0.422**		0.089	0.067	0.002	-0.338**	-0.292*	0.081	-0.097	0.210	0.017	0.149	0.027	-0.016
BMI	-0.031	0.159	0.088	0.089		0.817**	-0.150	0.029	-0.047	-0.104	-0.310**	-0.078	0.074	0.004	0.082	-0.240*
AC	0.142	0.199	0.166	0.067	0.817**		-0.024	0.086	0.029	-0.091	-0.199	-0.010	0.078	-0.016	-0.114	-0.039
NfL	0.785**	-0.007	-0.011	0.002	-0.150	-0.024		0.124	0.047	-0.122	0.782**	0.731**	-0.444**	-0.386**	-0.373**	0.538**
A β 40	0.140	-0.189	0.199	-0.338**	0.029	0.086	0.124		0.815**	-0.264*	0.231*	-0.017	0.118	-0.033	-0.057	0.020
A β 42	0.005	-0.155	0.184	-0.292*	-0.047	0.029	0.047	0.815**		0.345**	0.127	-0.120	0.099	0.061	0.012	-0.102
A β 42/40	-0.219	0.061	-0.028	0.081	-0.104	-0.091	-0.122	-0.264*	0.345**		-0.162	-0.172	0.019	0.152	0.076	-0.197
GFAP	0.664**	-0.046	0.067	-0.097	-0.310*	-0.199	0.782**	0.231*	0.127	-0.162		0.728**	-0.415**	-0.395**	-0.429**	0.546**
pTau181	0.723**	0.168	-0.063	0.210	-0.078	-0.010	0.731**	-0.017	-0.120	-0.172	0.728**		-0.465**	-0.293*	-0.391**	0.579**

Table 54. Pearson correlations between cardiovascular parameters, cognitive outcomes and plasma biomarkers.

**Group difference is significant at the 0.01 level

*Group difference is significant at the 0.05 level

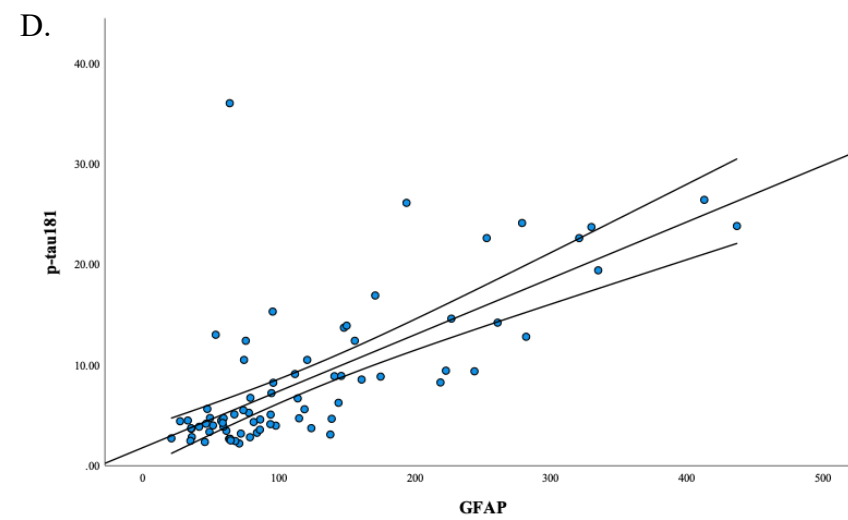
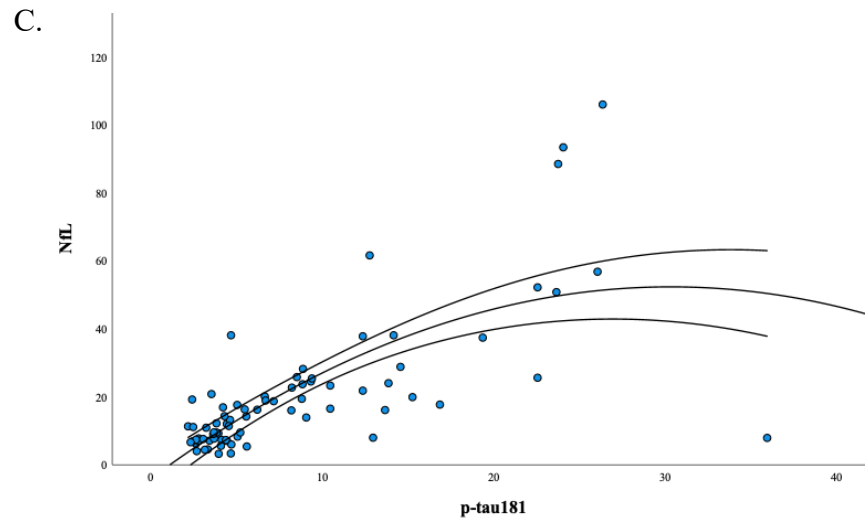
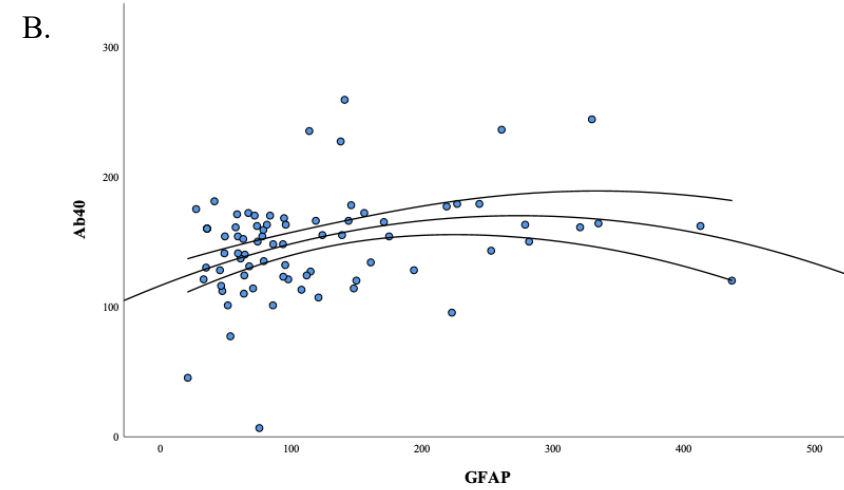
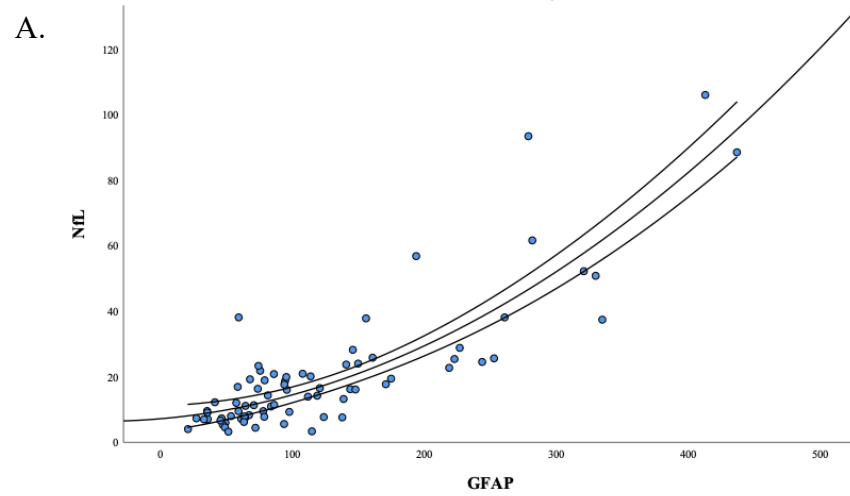


Figure 24. Scatter plots of plasma biomarker correlations in the LonDowns cohort (all in pg/ml). A) NfL by GFAP correlation. Quadratic R^2 0.743. B) A β 40 by GFAP. Quadratic R^2 -0.111. C) NfL by p-tau181. Quadratic R^2 0.532. D) P-tau181 by GFAP. Linear R^2 0.510. Error bars represent 95% CI.

Exercise Group Comparisons

Two-tailed independent t-tests were used to compare parameters between exercise groups. Low and moderate exercise levels were grouped together to allow for examination of the effects of high exercise compared to other groups (see table 55). There were significant differences in age between the groups, with the high exercise group being significantly younger (29.64 years (SD 8.2) compared to 43.47 years (SD 12.6), $p < 0.001$).

These age differences likely contributed to the observed differences in the cognitive outcomes, NfL and GFAP measurements between the groups as these outcomes were all correlated with age. Both NfL and GFAP were higher in the low exercise group whilst the cognitive outcomes were poorer. These relationships will be explored further in the next section. The difference in p-tau181 between exercise groups was not significant, despite this biomarker also having a positive correlation with age.

Other differences were not obviously age-related. Abdominal circumference was lowest in the high exercise group (AC 89.53cm (SD 12.7) in the high exercise group compared to 95.75 cm (SD 13.9) in the low, $p = 0.04$). BMI was lower in the high exercise group although the difference did not reach significance (mean BMI 27.06 kg/m² (SD 4.97) in the high exercise group compared to 29.95 kg/m² (SD 7.2) in the low, $p = 0.09$). Blood pressure measurements, particularly SBP, trended towards lower values in the high exercise group (SBP 108.64 mmHg (SD 7.9) in the high exercise group and 113.31 mmHg (SD 11.1) in the low exercise group, $p = 0.07$).

	Low/Moderate	High	t	Mean difference	95% CI	p
Age	43.47 (12.61)	29.64 (8.18)	5.618	13.828	8.92 – 18.74	<0.001*
SBP	113.31 (11.06)	108.64 (7.93)	1.860	4.671	-0.34 – 9.68	0.067
DBP	69.02 (9.07)	66.12 (9.18)	1.278	2.902	-1.63 – 7.43	0.206
PP	44.29 (11.53)	42.52 (7.68)	0.686	1.769	-3.37 – 6.91	0.495
BMI	29.95 (7.20)	27.06 (4.97)	1.741	0.0918	-0.01 – 0.20	0.086
AC	96.75 (13.85)	89.53 (12.66)	2.146	7.224	0.51 – 13.94	0.035*
PALFAMS	7.91 (6.07)	10.67 (3.43)	-2.112	-2.760	-5.39 - -0.14	0.040*
VF	2.65 (0.86)	3.00 (0.85)	-1.555	-0.350	-0.80 – 0.10	0.125
SABS	74.70 (19.79)	84.36 (20.12)	-1.945	-9.664	-19.53 – 0.20	0.055
CAMDEX	8.51 (15.97)	2.68 (8.75)	3.753	0.964	0.45 – 1.48	<0.001*
NfL	22.39 (19.92)	9.05 (4.20)	4.661	0.739	0.42 – 1.06	<0.001*
GFAP	131.46 (98.36)	72.38 (30.49)	3.124	0.500	0.16 – 0.74	0.003*
p-tau181	7.69 (6.90)	6.40 (4.24)	0.340	0.057	-0.28 – 0.39	0.735
Aβ40	150.21 (45.66)	148.26 (29.27)	-0.430	-0.057	-0.32 – 0.21	0.669
Aβ42	8.45 (2.47)	9.13 (2.74)	-0.806	-0.082	0.10 - -0.28	0.423
Aβ42/40	0.055 (0.012)	0.061 (0.014)	-1.398	-0.098	-0.24 – 0.04	0.167

Table 55. Two-way comparisons between exercise groups. Significance set at $p < 0.05$.

Age Group Comparisons

The analysis was split into a younger cohort aged below 35 years, and an older cohort aged 35 years and older. Cardiovascular parameters did not significantly differ between the age groups (differences for SBP, DBP, PP, BMI, and AC all $p>0.378$). The younger cohort performed better on all cognitive outcomes and had lower CAMDEX scores (mean PALFAMS 11.59 (SD 3.87) in the younger group compared to 6.58 (SD 5.52) in the older, t 4.286, $p<0.001$; verbal fluency 2.94 (SD 0.84) compared to 2.40 (SD 0.90), t 2.749, $p=0.007$; SABS 82.76 (SD 18.32) compared to 72.15 (SD 22.79), t 2.289, $p=0.03$; mean CAMDEX 2.14 (SD 7.42) compared to 12.69 (SD 18.90), t 3.726, $p<0.001$).

There were significant biomarker differences between the younger and older cohort (figure 25). The older cohort had higher mean NfL, GFAP and p-tau181 levels (mean NfL 8.85 pg/ml (SD 4.45) in the younger group compared to 26.58 pg/ml (SD 20.79); mean GFAP 68.26 pg/ml (SD 27.73) compared to 157.93 pg/ml (SD 101.07); mean p-tau181 3.69 pg/ml (SD 0.93) in the younger group compared to 9.07 pg/ml (SD 5.84), all $p<0.001$). The difference in A β 40 was of borderline significance with higher levels in the older age group (mean A β 40 136.43 pg/ml (SD 40.05) in the younger cohort compared to 154.82 pg/ml (SD 37.02), $p=0.051$). There were no differences in A β 42 or A β 42/40 values at a group level.

A greater proportion of participants in the younger group engaged in high levels of exercise compared to the older cohort (55.6% compared to 9.1%). A greater proportion of the older group engaged in moderate exercise (61.8% compared to 36.1% in the younger group). Only 8.3% of the younger cohort were classified as engaging in low exercise compared to 29.1% of the older group. Group differences were significant (all $p<0.001$ Pearson Chi Square tests).

	Younger	Older	t	Mean difference	95% CI	p
Age	26.75 (4.10)	50.46 (8.09)	-18.525	-23.714	-26.26 - -21.17	<0.001
SBP	111.37 (9.03)	111.96 (13.07)	-0.231	-0.589	-5.65 – 4.48	0.818
DBP	67.31 (8.83)	68.25 (10.21)	-0.443	-0.941	-5.16 – 3.28	0.659
PP	44.06 (10.20)	43.71 (12.58)	0.137	0.351	-4.74 – 5.44	0.891
BMI	29.50 (7.28)	29.34 (6.53)	0.107	0.160	-2.83 – 3.15	0.915
AC	93.59 (13.82)	96.20 (13.09)	-0.887	-2.607	-8.45 – 3.24	0.378
PALFAMS	11.59 (3.87)	6.58 (5.52)	4.286	5.003	2.58 – 7.42	<0.001
VF	2.94 (0.84)	2.40 (0.90)	2.749	0.548	0.15 – 0.94	0.007
SABS	82.76 (18.32)	72.15 (22.79)	2.289	10.617	1.40 – 19.84	0.025
CAMDEX	2.14 (7.42)	12.69 (18.90)	-5.697	-1.346	-1.82 - -0.88	<0.001
NfL	8.85 (4.45)	26.58 (20.79)	-8.277	-0.993	-0.75 – -1.23	<0.001
GFAP	68.26 (27.73)	157.93 (101.07)	-5.699	-0.714	-0.46 – -0.96	<0.001
p-tau181	3.69 (0.93)	9.07 (5.84)	-6.686	-0.748	-0.97 – -0.52	<0.001
Aβ40	136.43 (40.05)	154.82 (37.02)	-1.982	-0.199	0.01 – 0.40	0.051
Aβ42	8.39 (2.89)	8.33 (2.382)	0.235	0.021	-0.19 – 0.15	0.814
Aβ42/40	0.059 (0.02)	0.054 (0.01)	1.291	0.078	-0.04 – 0.20	0.201

Table 56. Parameters for younger (<35 years) and older participants. Values presented as mean (standard deviation). Comparisons made using two-tailed independent t-tests, with significance set at $p<0.05$.

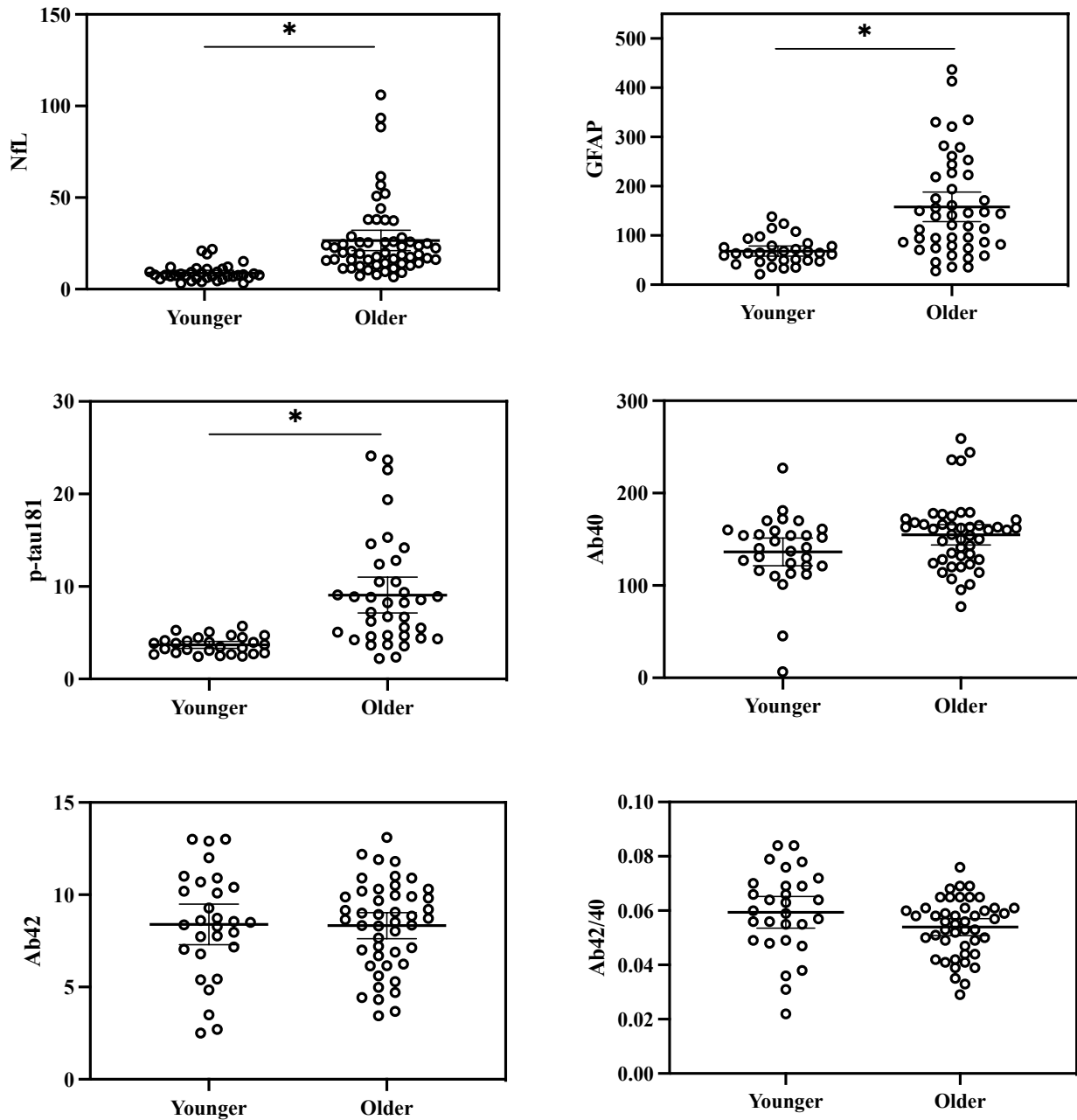


Figure 25. Grouped scatter plots comparing plasma biomarkers in the younger (below 35 years) and older (35 years and above) cohorts. All values presented in pg/ml. Error bars represent mean and 95% CI. Stars indicate significant group differences ($p < 0.05$).

7.5.2. Older Adult Cohort

7.5.2.1. Cross-sectional Analysis

There were 56 participants aged 35 years and older, 32 men (57.1%) and 24 women (42.9%). Twenty-seven had mild ID (48.2%), 23 had moderate (41.1%) and six had severe ID (10.7%). Sixteen people (28.6%) engaged in low exercise, 34 people (60.7%) in moderate and five (8.9%) in high. Fifty-two never smoked (92.9%). Thirty-two people (57.1%) never drank alcohol; only four people drank more than 4 units a week (5.5%). Ten people (17.9%) had a history of CHD, and 28 had hypothyroidism (50%). Thirteen people had dementia (23.2%) and eight had suspected dementia (14.3%). All 56 had NfL measurements and 46 had A β 40, A β 42, p-tau181 and GFAP. See table 56 for parameter values. There were sex differences observed for BMI, with lower measurements in men (27.64 kg/m² in men compared to 31.76 in women, t -2.328, mean difference -0.137, 95% CI -0.25 - -0.02, p =0.02). There were no significant sex differences for the other CVPs.

There were significant differences based on exercise level in this older cohort. Verbal fluency scores were higher in the high exercise group (mean VF 2.61 (SD 0.88) in the high compared to 1.82 (SD 0.75) in the low, t -2.660, mean difference -0.80, 95% CI -1.40 - -0.19, p =0.01). There was a trend towards higher SABS scores and lower CAMDEX scores in the high exercise group (mean SABS 75.12 (SD 21.53) in the high compared to 63.07 (SD 25.26) in the low exercise group, t -1.717, mean difference -12.06, 95% CI -26.15 – 2.04, p =0.09; mean CAMDEX 10.03 (SD 17.12) in the high compared to 20.47 (SD 22.03), t 1.840, mean difference 0.808, 95% CI -0.08 – 1.69, p =0.07). There was a trend towards higher A β 42 and A β 40 levels in the high exercise group (mean A β 42 8.81 pg/ml (SD 2.19) in the high compared to 7.17 pg/ml (SD 2.60) in the low exercise group, t -1.938, mean difference -0.24, 95% CI -

0.50 – 0.02, $p=0.07$; mean A β 40 160.85 pg/ml (SD 37.89) in the high compared to 141.13 (SD 31.76) in the low exercise group, t -1.695, mean difference -0.133, 95% CI -0.29 – 0.03, $p=0.09$). The A β 42/40 ratio was higher in the high exercise group, but the difference was not significant (mean A β 42/40 0.055 (SD 0.01) in the high exercise group compared to 0.050 (SD 0.01) in the low, t -1.267, $p=0.22$).

NfL, GFAP and p-tau181 concentrations were lower in the high exercise group, but only the difference for p-tau181 was significant (mean NFL 25.43 pg/ml (SD 20.47) in the high compared to 29.55 pg/ml (SD 22.60) in the low exercise group, t 0.668, mean difference 0.125, 95% CI -0.25 – 0.50, $p=0.51$; mean GFAP 149.74 pg/ml (SD 103.30) in the high compared to 181.13 pg/ml (SD 99.78) in the low exercise group, t 0.931, mean difference 0.214, 95% CI -0.25 – 0.67, $p=0.36$; mean p-tau181 13.68 pg/ml (SD 7.72) in the low exercise group compared to 7.80 pg/ml (SD 4.60), t 2.343, mean difference 0.54, 95% CI 0.07 – 1.01, $p=0.03$). Blood pressure values and BMI did not vary between the exercise groups.

Controlling for the Effects of Covariates

Individual regression models were constructed with each CVP as the primary predictor variable. Cognitive outcomes or plasma biomarkers were the dependents. Age, sex, and level of ID were inputted as covariates. Alcohol and smoking status was not included in the analysis due to low prevalence. Moderate and high exercise were grouped together due to sample size limitations, as were moderate and severe ID. Values for SBP, DBP, PP and BMI were added as continuous variables in linear regression models. Group comparisons were performed using logistic regression models, with comparisons made between low and moderate/high exercise; presence or absence of hypothyroidism; presence or absence of congenital heart disease.

There was a relationship between raised DBP and poorer performance on PALFAMS (B -0.22, 95% CI -0.41 - -0.04, $p=0.02$ for DBP), see table 58. The opposite relationship was observed between BMI and PALFAMS (B 7.36, 95% CI 0.10 – 14.62, $p=0.047$). A negative relationship between BMI and GFAP was identified (B -1.06, 95% CI -1.81 - -0.31, $p=0.01$) whilst the same relationship with NfL trended towards significance (B -0.63, 95% CI -1.32 – 0.05, $p=0.07$), see table 60. A negative association between PP and A β 40 was suggested (B -0.01, 95% CI -0.01 - 0.001, $p=0.08$), see table 59. SBP was not related to any cognitive or biomarker outcomes (table 57).

A significant difference in verbal fluency was retained between exercise groups, with higher scores in the high exercise group (B 1.68, SE 0.71, Exp(B) 5.37, 95% CI 1.3 – 21.8, $p=0.02$) A β 42 trended towards increased values in the high exercise group (B 2.07, SE 1.17, Exp(B) 7.93, 95% CI 0.8 – 77.9, $p=0.08$). There was a pattern towards lower CAMDEX scores (indicating fewer dementia symptoms) and higher SABS scores in the high exercise group, but the differences did not reach significance (see table 61). There were no other significant differences between exercise groups.

There were no significant associations between history of congenital heart disease or hypothyroidism and cognitive or biomarkers outcomes (see tables 62 and 63). People with a diagnosis of hypothyroidism tended to have higher CAMDEX scores compared to those without (B 0.422, 95% CI 0.94 – 2.49, $p=0.09$). There were no differences observed in blood pressure or BMI parameters between the CHD or the hypothyroid groups.

	B	SE	Beta	t	p	95%CI
PALFAMS	0.001	0.065	0.004	0.023	0.982	-0.13 – 0.13
VF	0.008	0.009	0.118	0.847	0.403	-0.01 – 0.03
SABS	0.097	0.210	0.058	0.460	0.648	-0.33 – 0.52
CAMDEX	-0.002	0.015	-0.018	-0.140	0.889	-0.03 – 0.03
NfL	-0.003	0.005	-0.069	-0.572	0.570	-0.01 – 0.01
GFAP	-0.005	0.006	-0.109	-0.812	0.422	-0.02 – 0.01
p-tau181	-0.061	0.062	-0.135	-1.001	0.324	-0.19 – 0.06
Aβ40	-0.003	0.003	-0.172	-1.013	0.318	-0.01 – 0.003
Aβ42	-0.001	0.004	-0.048	-0.292	0.772	-0.01 – 0.01
Aβ42/40	0.002	0.003	0.097	0.619	0.540	-0.004 – 0.007

Table 57. Results from SBP regression analyses for the older adult cohort. Systolic blood pressure inputted as the main predictor for each cognitive outcome and plasma biomarker. Analyses were adjusted for age, sex, and level of ID.

	B	SE	Beta	t	p	95%CI
PALFAMS	-0.223	0.090	-0.374	-2.476	0.019*	-0.41 - -0.04
VF	-0.008	0.013	-0.080	-0.569	0.573	-0.03 – 0.02
SABS	0.116	0.263	0.055	0.440	0.662	-0.41 – 0.65
CAMDEX	0.004	0.019	0.029	0.231	0.819	-0.03 – 1.25
NfL	0.008	0.007	0.140	1.186	0.242	-0.01 – 0.02
GFAP	0.005	0.009	0.073	0.561	0.578	-0.01 – 0.02
p-tau181	0.023	0.088	0.034	0.255	0.800	-0.16 – 0.20
Aβ40	0.003	0.004	0.149	0.894	0.377	-0.004 – 0.01
Aβ42	0.005	0.006	0.142	0.888	0.380	-0.01 – 0.02
Aβ42/40	0.002	0.004	0.067	0.437	0.664	-0.01 – 0.01

Table 58. Results from DBP regression analyses for the older adult cohort. Diastolic blood pressure inputted as the main predictor for each cognitive outcome and plasma biomarker. Analyses were adjusted for age, sex, and level of ID.

	B	SE	Beta	t	p	95%CI
PALFAMS	0.104	0.064	0.260	1.622	0.115	-0.03 – 0.24
VF	0.011	0.009	0.165	1.233	0.225	-0.01 – 0.03
SABS	0.023	0.212	0.014	0.109	0.914	-0.40 – 0.45
CAMDEX	-0.005	0.015	-0.042	-0.326	0.746	-0.04 – 0.03
NfL	-0.008	0.005	-0.182	-1.569	0.123	-0.02 – 0.002
GFAP	-0.008	0.007	-0.170	-1.292	0.205	-0.02 – 0.01
p-tau181	-0.081	0.064	-0.168	-1.258	0.217	-0.21 – 0.05
Aβ40	-0.005	0.003	-0.294	-1.783	0.083	-0.01 – 0.001
Aβ42	-0.004	0.004	-0.158	-0.969	0.339	-0.01 – 0.004
Aβ42/40	0.001	0.003	0.052	0.331	0.742	-0.01 – 0.01

Table 59. Results from PP regression analyses for the older adult cohort. Pulse pressure inputted as the main predictor for each cognitive outcome and plasma biomarker. Analyses were adjusted for age, sex, and level of ID.

	B	SE	Beta	t	p	95%CI
PALFAMS	7.357	3.554	0.299	2.070	0.047*	0.10 – 14.62
VF	0.029	0.630	0.007	0.046	0.964	-1.25 – 1.31
SABS	20.758	13.617	0.198	1.524	0.135	-6.69 – 48.20
CAMDEX	-0.933	0.904	-0.132	-1.032	0.308	-2.76 – 0.89
NfL	-0.632	0.339	-0.216	-1.864	0.069	-1.32 – 0.05
GFAP	-1.059	0.370	-0.332	-2.687	0.007*	-1.81 - -0.31
p-tau181	-0.414	0.346	-0.137	-1.194	0.240	-1.12 – 0.29
Aβ40	-0.169	0.176	-0.159	-0.958	0.344	-0.53 – 0.19
Aβ42	-0.232	0.252	-0.151	-0.922	0.362	-0.74 – 0.28
Aβ42/40	-0.063	0.160	-0.062	-0.397	0.694	-0.39 – 0.26

Table 60. Results from BMI regression analyses for the older adult cohort. BMI inputted as the main predictor for each cognitive outcome and plasma biomarker. Analyses were adjusted for age, sex, and level of ID.

	B	SE	Exp (B)	p	95%CI
PALFAMS	-0.037	0.093	0.964	0.695	-0.80 -1.16
VF	1.680	0.714	5.365	0.019*	1.32 – 21.75
SABS	0.029	0.019	1.029	0.136	0.99 – 1.07
CAMDEX	-0.030	0.021	0.971	0.162	-0.93 – 1.01
NfL	0.640	0.758	1.897	0.399	0.43 – 8.84
GFAP	0.548	0.711	1.729	0.441	0.43 – 6.97
p-tau181	-0.411	0.860	0.663	0.633	0.12 – 3.58
Aβ40	2.663	1.771	14.335	0.133	0.45 – 460.73
Aβ42	2.071	1.166	7.929	0.076	0.81 – 77.93
Aβ42/40	2.031	1.796	7.621	0.258	0.23 – 257.75

Table 61. Binary logistic regression results for exercise groups in the older adult cohort. Low exercise is the indicator group. Sex, age and level of ID included as covariates.

	B	SE	Exp (B)	p	95%CI
PALFAMS	-0.140	0.115	0.869	0.222	0.70 – 1.09
VF	-0.040	0.520	0.961	0.938	0.35 – 2.66
SABS	-0.021	0.018	0.979	0.254	0.94 – 1.02
CAMDEX	0.350	0.298	1.418	0.241	0.79 – 2.54
NfL	0.348	0.728	1.417	0.632	0.34 – 0.59
GFAP	0.416	0.720	1.517	0.563	0.37 – 6.22
p-tau181	-0.649	0.923	0.524	0.484	0.09 – 3.20
Aβ40	-2.049	1.711	0.129	0.231	0.01 – 3.68
Aβ42	-0.435	1.106	0.647	0.694	0.07 – 5.65
Aβ42/40	1.131	1.764	3.098	0.521	0.10 – 98.23

Table 62. Binary logistic regression results for CHD groups in the older adult cohort. Absence of CHD is the indicator group. Sex, age and level of ID included as covariates.

	B	SE	Exp (B)	p	95%CI
PALFAMS	0.022	0.080	1.023	0.781	0.87 – 1.20
VF	0.571	0.471	1.770	0.226	0.70 – 4.46
SABS	-0.001	0.015	0.999	0.969	0.97 – 1.03
CAMDEX	0.422	0.249	1.526	0.090	0.94 – 2.49
NfL	0.009	0.587	1.009	0.988	0.32 – 3.19
GFAP	-0.341	0.596	0.711	0.567	0.22 – 2.29
p-tau181	0.728	0.727	2.070	0.317	0.50 – 8.61
Aβ40	-0.081	1.290	0.922	0.950	0.07 – 11.55
Aβ42	-0.044	0.958	0.957	0.964	0.15 – 6.26
Aβ42/40	0.003	1.501	1.003	0.998	0.05 – 19.03

Table 63. Binary logistic regression results for hypothyroid status in the older adult cohort. Absence of thyroid disorder is the indicator group. Sex, age and level of ID included as covariates.

Comorbid Risk Group

A low and a high risk group were created based on the presence of variables related to increased risk in the population analysis in chapter 5 and in pilot work discussed in the methods section (chapter 3). The risks groups were PP<40mmHg; low exercise; presence of CHD; presence of hypothyroidism. Low DBP was not included due to collinearity with PP. Raised BMI ($>25\text{mg}/\text{m}^2$) was excluded from the analysis in this older cohort due to the cross-sectional associations between low BMI and poorer outcomes. Reductions in BMI are observed as part of the dementia process in people with DS²⁰¹. Due to this interaction, the associations between BMI, cognition and biomarkers are more likely to be related to dementia-processes in this older cohort rather than representing a pure cardiovascular risk factor.

There were 11 people with no risk factors, 19 with one factor, 23 with two, and three with three risk factors. These were collapsed into a low risk (0-1 risk factors, $n=30$) and a high risk group (2+ factors, $n=26$). Higher dementia symptom load (as measured by higher CAMDEX scores) was observed in people with a greater number of risk factors (B 0.94, SE 0.32, Exp(B) 2.56, 95% CI 1.4 – 4.8, $p=0.003$). There were trends towards worse performance on SABS, VF and PALFAMS in the higher risk group but these were not significant (see table 64). There were higher NfL, p-tau181 and GFAP values associated with the high risk group in conjunction with lower A β levels, however none of the biomarker associations reached significance. No differences in SBP, DBP, heart rate or BMI measures were identified between the risk groups.

The results imply that the impact of cardiovascular health factors may be cumulative at the cognitive and dementia symptom level, but are not necessarily reflected in biomarker changes in older adults with Down syndrome. It may be that these risk factors negatively affect

cognition via indirect processes, rather than having direct neurodegenerative effects. Alternatively the cross-sectional nature of the analysis may mask longer-term changes.

	B	SE	Exp (B)	p	95%CI
PALFAMS	-0.123	0.082	0.884	0.135	0.75 – 1.04
VF	-0.782	0.471	0.457	0.097	0.18 – 1.15
SABS	-0.029	0.016	0.972	0.079	0.94 – 1.00
CAMDEX	0.940	0.318	2.561	0.003*	1.37 – 4.78
NfL	0.466	0.586	1.593	0.427	0.51 – 5.03
GFAP	0.329	0.593	1.390	0.579	0.44 – 4.44
p-tau181	0.631	0.936	1.880	0.500	0.30 – 11.76
Aβ40	-1.414	1.386	0.243	0.307	0.02 – 3.67
Aβ42	-0.920	0.984	0.399	0.350	0.06 – 2.75
Aβ42/40	-0.436	1.517	0.647	0.774	0.03 – 12.65

Table 64. Results from binary logistic regression models comparing risk groups (without BMI). People with low risk (0-1 comorbidities) formed the indicator group. Sex, age and level of ID were included as covariates.

When high BMI was added as a risk factor all associations lost significance (see table 65). When low BMI was instead added as a risk factor, the relationships between risk group and CAMDEX were strengthened, as were the negative associations between SABS which gained significance, and with PALFAMS which became of borderline significance. The relationships with biomarkers remained non-significant (see table 66). In older people with DS, low BMI may have an additive effect with other comorbidities which negatively impact cognition. When

comparing blood pressure parameters in the combined risk groups which include low BMI, there was a significant difference in SBP levels which were not observed in group comparisons without low BMI included as a risk factor. There were lower values of SBP observed in the high combination risk group (B -0.057, Exp(B) 0.945, 95% CI 0.90 – 0.99, p=0.04). DBP values did not differ between the groups. Hence BMI and SBP may have a synergistic association with risk.

	B	SE	Exp (B)	p	95%CI
PALFAMS	0.024	0.092	1.024	0.796	0.86 – 1.23
VF	0.028	0.471	1.029	0.952	0.41 – 2.59
SABS	0.000	0.016	1.000	0.984	0.97 – 1.03
CAMDEX	0.265	0.247	1.303	0.283	0.80 – 2.11
NfL	-0.242	0.632	0.785	0.701	0.23 – 2.71
GFAP	-0.464	0.624	0.629	0.457	0.19 – 2.14
p-tau181	-1.247	1.007	0.287	0.215	0.04 – 2.07
Aβ40	-2.828	1.586	0.059	0.075	0.01 – 1.32
Aβ42	-1.732	1.127	0.177	0.124	0.02 – 1.61
Aβ42/40	-0.549	1.542	0.578	0.722	0.03 – 11.85

Table 65. Results from binary logistic regression models comparing comorbid risk groups (with BMI >25kg/m² included as a risk factor). People with low risk (0-1 comorbidities) formed the indicator group. Sex, age and level of ID were included as covariates.

	B	SE	Exp (B)	p	95%CI
PALFAMS	-0.174	0.092	0.841	0.060	0.70 – 1.01
VF	-0.435	0.438	0.647	0.320	0.27 – 1.53
SABS	-0.047	0.021	0.954	0.026*	0.92 – 0.99
CAMDEX	1.068	0.347	2.908	0.002*	1.47 – 5.74
NfL	0.554	0.631	1.739	0.381	0.51 – 5.99
GFAP	0.474	0.659	1.606	0.472	0.44 – 5.84
p-tau181	0.298	1.397	1.347	0.831	0.09 – 20.82
Aβ40	0.592	1.520	1.808	0.697	0.09 – 35.56
Aβ42	-0.225	1.067	0.799	0.833	0.10 – 6.47
Aβ42/40	-1.267	1.699	0.282	0.456	0.01 – 7.87

Table 66. Results from binary logistic regression models comparing comorbid risk groups (with BMI <25kg/m² included as a risk factor). People with low risk (0-1 comorbidities) formed the indicator group. Sex, age and level of ID were included as covariates.

Section Summary

Overall there were poorer cognitive outcomes associated with higher DBP and lower BMI (relating to PALFAMS measures), with better cognitive performance in the high exercise group (relating to verbal fluency measures). Low BMI was also associated with increased GFAP which is a marker of neuroinflammation and neurodegeneration. Neither a history of hypothyroidism nor CHD were related directly to any of the examined outcomes, although hypothyroidism trended towards an association with increased dementia symptom load. There was evidence of a cumulative negative effect on cognitive outcomes in people with increased

numbers of risk factors, but no significant associations were identified with biomarker levels. Low BMI was a significant risk predictor rather than high BMI in this cross-sectional analysis.

5.2.2. Longitudinal Analysis Using Baseline Data

A subset of the sample had been assessed previously with baseline cognitive and health data available. The historical assessment is referred to as the baseline assessment. The most recent assessment (which was used in the cross sectional analysis above) is referred to as T1. The CVP values at baseline were used to evaluate the longer-term impact of cardiovascular health on cognitive and biomarker outcomes at T1.

There were 39 people aged 35 and over with baseline data available. The mean time between assessments was 5.23 years (SD 1.54), mean age at T1 was 44.31 years (SD 7.34). Nine (23.1%) had at least one *APOE* ϵ 4 allele; 24 were male (61.5%); 18 (46.2%) had mild LD; 12 (30.8%) had dementia; seven (17.9%) had a history of CHD; 23 (59%) had hypothyroidism. Fourteen (35.9%) had PP values below 40 mmHg baseline; 25 (64.1%) had BMI values $>25\text{kg/m}^2$; 13 engaged in low exercise (33.3%); 21 (53.8%) in moderate and only five (12.8%) in high levels of exercise. Those who engaged in moderate and high physical activity at baseline were collapsed into one group.

The effect of baseline CVP values and risk groups on biomarker levels at T1 (approximately 5 years later) were analysed by constructing linear regression models. Age, sex, *APOE* status (presence or absence of at least one ϵ 4 allele) and time between baseline assessment and sample acquisition were included as covariates.

BMI at baseline was significantly positively associated with PALFAMS at T1 indicating that higher BMI at baseline was associated with better performance at T1 (B 7.84, SE 3.43, t 2.282, 95% CI 0.81 – 14.87), $p=0.03$). There was a trend towards similar patterns with SABS score (B 18.63, SE 13.06, t 1.427, 95% CI -7.65 – 44.91, $p=0.16$). PP at baseline trended towards a positive association with PALFAMS at T1 (B 0.125, SE 0.066, t 1.893, 95% CI -0.01 – 0.26, $p=0.07$), and VF (B 0.019, SE 0.011, t 1.731, 95% CI 0.003 – 0.041, $p=0.09$), with lower PP associated with poorer performance. High exercise at baseline trended towards an association with lower CAMDEX scores at T1 indicative of lower dementia symptom burden (B -0.58, SE 0.36, t -1.61, 95% CI -1.30 – 0.15, $p=0.12$). There were no other associations between baseline CVP measures and cognitive outcomes at T1.

Lower SBP measures at baseline were significantly associated with higher NfL at T1 (B -0.14, SE 0.007, t -0.262, 95% CI 0.03 – 0.01, $p=0.05$). There was a trend towards lower SBP at baseline and higher p-tau181 at T1 (B -0.011, SE 0.006, t -1.669, 95% CI -0.02 – 0.002, $p=0.10$). The associations between SBP at baseline and other biomarkers at T1 were not significant. PP at baseline had a borderline significant negative relationship with NfL at T1 (B -0.014, SE 0.010, t -1.999, 95% CI -0.02 – 0.001, $p=0.052$) with a trend towards A β 40 (B -0.006, SE 0.04, t -1.454, 95% -CI 0.01 – 0.01, $p=0.155$). No associations with GFAP, p-tau181, A β 42, or A β 42/40 ratio were observed. Associations between DBP at baseline and biomarkers at T1 were not significant.

Lower BMI at baseline was significantly associated with higher GFAP at T1 (-1.147, SE 0.428, t -2.681, 95% CI -2.02 - -0.28, $p=0.011$). There was a trend towards higher NfL (B -0.623, SE 0.356, t -1.751, 95% CI -1.34 – 0.10, $p=0.08$). No associations with p-tau181 or A β proteins

were identified. Exercise group and hypothyroid status at baseline were not significantly associated with any biomarkers at T1.

Combined Risk Group

A combined risk group at baseline was created using the same variables as in the cross-sectional analysis (PP<40 mmHg, history of CHD, hypothyroidism, and low exercise identified as the risk groups). BMI was excluded due to complex interactions with dementia and cognition that may affect the analysis. Repeated measures ANCOVA were performed to compare change in cognitive outcomes between baseline and T1 in the combined risk groups. Regression models were created to determine the relationship between baseline combined risk group and biomarker outcomes at T1.

There were five people with zero risk factors at baseline, 17 with one factor, 12 people with two factors, four people with three factors, and one person with all four. These were combined into a crude low and higher risk group ($n=22$ in low risk group with 0-1 risk factors, $n=17$ in higher risk group with 2+ factors). The association with PALFAMS in the adjusted model was significant ($F\ 16.572$, partial eta squared 0.768, $p=0.01$). PALFAMS score was significantly higher at baseline in the low risk group (11.0 (SD 4.12) compared to 6.75 (SD 5.63), mean difference 4.681 (SE 1.44), $p=0.022$). At T1 the same relationship was observed (9.36 (SD 4.43) vs 4.92 (SD 4.85), mean difference 6.127 (SE 1.70), $p=0.015$). There was a deterioration in score over time in both groups, but the differences were not significant (for low risk group, mean difference 0.566 (SE 1.40), $p=0.70$; for high risk, mean difference 2.013 (SE 0.90), $p=0.08$). Therefore PALFAMS score was higher in the low risk group at both time points, but with similar trends overtime. See figure 26.

In the linear regression model, risk group was a significant predictor of CAMDEX score with a positive association suggesting higher CAMDEX scores were associated with being in the higher risk group (B 0.765, t 2.384, 95% CI 0.11 – 1.42, p=0.02). Two-way group differences were significant (t 2.223, p=0.03). VF score was higher in the low risk group at baseline and T1, but the differences were not significant (mean VF 3.13 (SD 0.89) compared to 2.55 (SD 1.29), mean difference 0.512 (SE 0.47), p=0.29 at baseline; mean VF 2.63 (SD 1.03) compared to 2.55 (SD 0.93), mean difference 0.291 (SE 0.41), p=0.49 at T1). The association between risk group and VF score was not significant in the adjusted model (F 2.318, partial eta squared 0.174, p=0.16). The differences in scores between baseline and T1 were also not significant for either risk group (mean difference 0.297 (SE 0.20), p=0.17 in the low risk group; mean difference 0.076 (SE 0.22), p=0.73 in the high risk group). There were no significant differences between risk groups for SABS score at baseline or T1 (mean SABS 75.77 (SD 21.57) compared to 77.79 (SD 19.92), mean difference 5.703 (SE 6.70), p=0.41 at baseline; mean SABS 72.36 (SD 23.21) compared to 72.00 (SD 22.97), mean difference 3.121 (SE 7.70), p=0.69 at T1). In the adjusted model risk group was not significant (F 0.007, partial eta 0.001, p=0.93).

Risk group at baseline was positively associated with NfL at T1 (B 0.309, SE 0.152, t 2.028, 95% CI 0.001 – 0.62, p=0.05), and had a borderline positive association with GFAP (B 0.378, SE 0.189, t 2.007, 95% CI -0.01 – 0.76, p=0.053). There was a trend towards negative associations with A β 42/40 ratio (B -0.104, SE 0.070, t -1.485, 95% CI -0.25 – 0.04, p=0.147). Associations with p-tau181, A β 40 and A β 42 proteins individually were not significant (see figure 27).

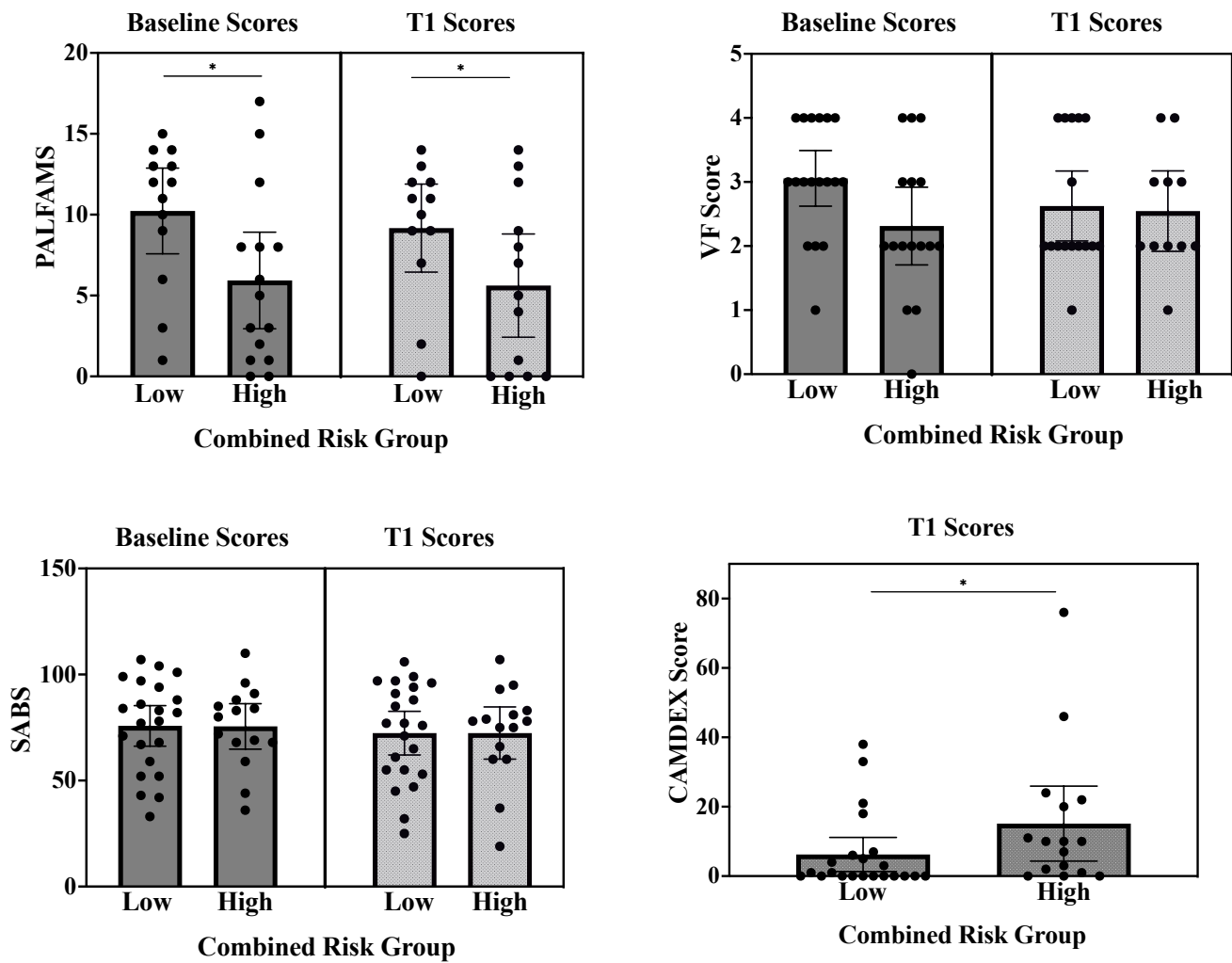


Figure 26. Cognitive outcomes at baseline and T1, grouped by baseline combined risk group in the older adult cohort. Error bars represent mean and 95% CI. Baseline risk measurements were collected approximately 5 years before T1 cognitive outcomes.

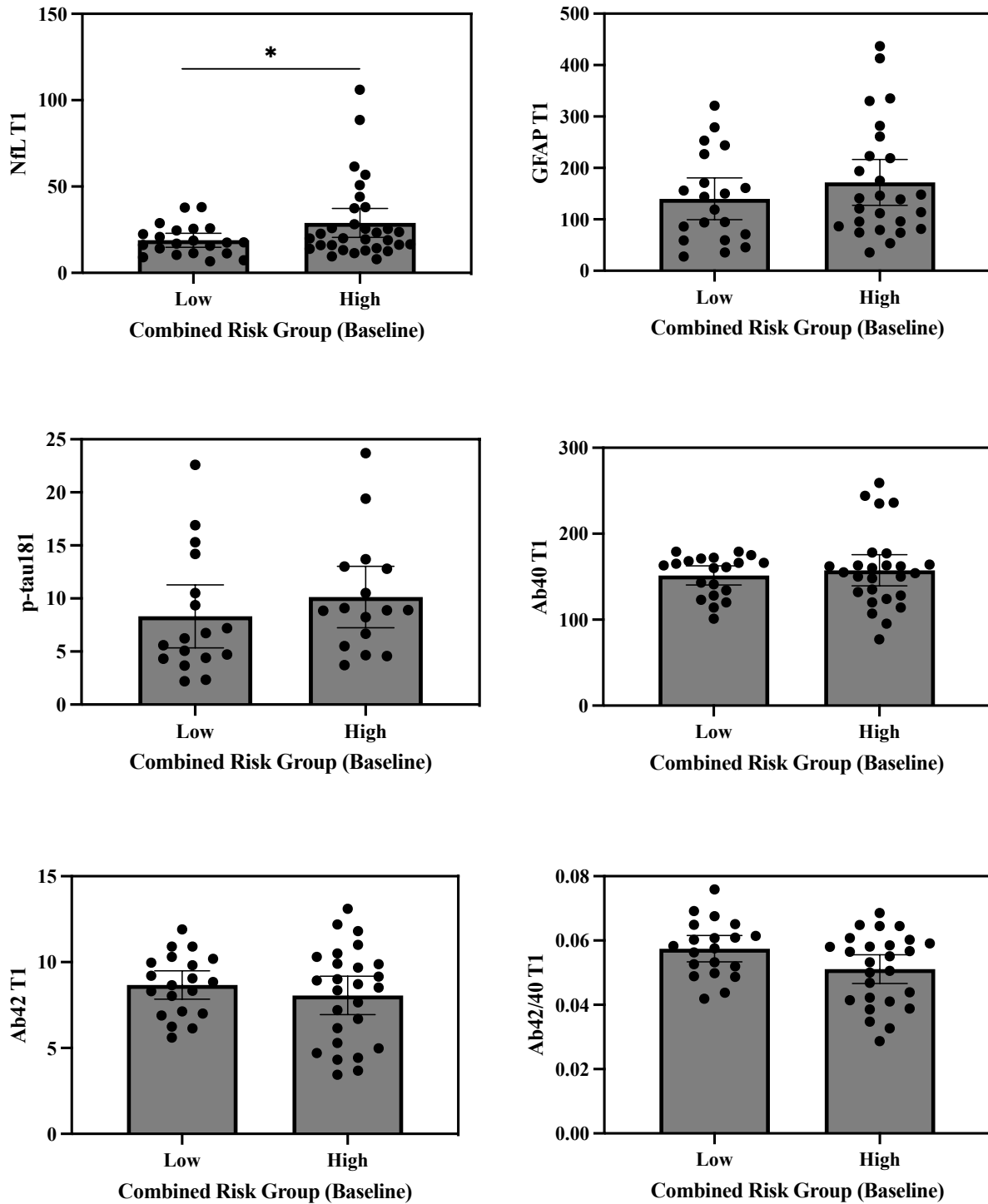


Figure 27. Biomarker concentrations (pg/ml) at T1 grouped by baseline risk group in the older adult cohort. Error bars represent mean and 95% CI. Baseline risk measurements were collected on average 5 years before biomarker samples. For NfL, adjusted comparisons were significant at $p=0.05$.

Section Summary

Patterns suggest that CVP factors affect cognition and dementia neuropathology in the older adult cohort. BMI was the most prominently associated factor. Cross-sectionally, lower BMI ($<25\text{kg/m}^2$) was associated with worse performance on PALFAMS, with higher GFAP and NfL levels. Longitudinally there were negative associations between baseline BMI with NfL and GFAP levels at T1. This is in keeping with a lowering of BMI being associated with dementia onset. It suggests that in older adults a lower BMI is related to increased dementia-burden and neurodegenerative changes, although the directionality of this relationship is unclear.

DBP was negatively associated with PALFAMS score cross-sectionally but not longitudinally. Conversely, SBP at baseline was negatively associated with NfL at T1, with trends towards negative associations with p-tau181 at T1, but did not have cross-sectional associations. PP at baseline trended towards positive associations with PALFAMS cross-sectionally and longitudinally, with trends towards negative associations with NfL and A β 40. Hence lower PP was associated with poorer cognitive outcomes and higher markers of neurodegeneration. Cross-sectionally exercise was positively associated with verbal fluency scores, and there was a trend towards higher A β 42 in the high exercise group. Longitudinally there were no biomarker associations at T1 with baseline exercise, but there was a trend towards lower CAMDEX scores (representing lower dementia symptom burden) at T1 in people engaging in high exercise at baseline.

There is evidence for a cumulative effect of cardiovascular risk on cognition and dementia symptoms. Cross-sectionally those with higher risks had higher CAMDEX scores, with lower

scores in PALFAMS, VF and SABS measures. Longitudinally, higher burden of cardiovascular risk at baseline was associated both with poorer cognitive outcomes (lower PALFAMS scores and higher CAMDEX scores) and increased concentrations of NfL and GFAP. This demonstrates that CVP factors may have detrimental effects over time.

7.5.3. Younger Adult Cohort

The younger adult cohort were used to explore the association between CVPs and biomarkers before the onset of major AD-related neuropathology.

5.3.1. Cross-sectional Analysis

There were 36 participants under the age of 35 years: 19 males (52.8%) and 17 females (47.2%). Fifteen people had mild ID (41.7%), 20 had moderate (55.6%) and one had severe (2.8%). Three people engaged in low exercise (8.3%), 12 in moderate (33.3%) and 21 in high (58.3%). No one smoked or had previously smoked. Twenty-five (69.4%) did not drink alcohol and only two people (5.6%) drank more than four units a week. Nineteen people (52.8%) had CHD, and 16 (44.4%) had hypothyroidism. All participants had NfL measurements (mean 8.85 pg/ml (SD 4.45)); 30 had A β 40, A β 42, p-tau181 and GFAP levels (mean A β 40 136.43 pg/ml (SD 40.05); mean p-tau181 3.69 pg/ml (SD 0.93); mean GFAP 68.26 pg/ml (SD 27.73); mean A β 42 8.39 pg/ml (SD 2.89) and mean A β 42/40 0.06 (SD 0.02)). Values for all parameters are presented in table 56.

Given the low prevalence of regular alcohol intake and lack of smokers, these factors were not included in further analyses. Low exercise was grouped with moderate exercise, and severe ID was grouped with moderate ID to create dichotomous groups due to the low numbers in these groups. The only significant sex difference was identified for BMI with men having lower average BMI (26.56 (SD 4.51) compared to 32.98 (SD 8.48), t 2.721, mean difference 6.42, 95% CI 1.53 – 11.31, $p=0.01$). BMI was significantly lower in the high exercise group (27.11 (SD 5.15) compared to 32.68, t 2.298, mean difference 5.57, 95% CI 0.83 – 10.31, $p=0.03$).

There was a trend towards lower SBP in those engaging in higher levels of exercise, but this did not reach significance (mean SBP 109.1mmHg in the high exercise group compared to 114.5mmHg, t 1.814, mean difference 5.417, 95% CI -0.66 – 11.49, $p=0.08$). There was no difference in DBP or PP between exercise groups, and exercise group was not associated with differences in biomarkers measures in these younger participants.

Regression models were constructed as in the older cohort using each continuous CVP measurement as the primary predictor with plasma biomarkers (NfL, GFAP, p-tau181, A β 40, A β 42 and A β 42/40 ratio) as the dependent variables. Age, sex, and level of ID were included as covariates. Risk group comparisons were made by constructing logistic regression models for each binary outcome (exercise level, history of CHD, diagnosis of hypothyroidism).

Limited associations were observed in the regression analyses using continuous variables (tables 67-69). PP had a positive relationship with p-tau181 (B 0.01, 95% CI -0.002 – 0.03, $p=0.03$). SBP had a borderline significant negative relationship with both NfL and GFAP levels (B -0.02, 95% CI -0.03 – 0.001, $p=0.06$ for NfL; B -0.02, 95% CI -0.04 – 0.001, $p=0.050$ for GFAP). There was a trend towards a negative association between PP and A β 40 levels, but this did not reach significance (B -0.02, $p=0.08$). Similarly there was a trend towards a negative association between DBP and NfL (B -0.02, $p=0.07$). BMI was not associated with any of the plasma biomarkers, including amyloid-beta (table 70).

	B	SE	Beta	t	p	95%CI
NfL	-0.016	0.008	-0.321	-1.986	0.056	-0.03 – 0.001
GFAP	-0.019	0.009	-0.394	-2.065	0.050*	-0.04 – 0.001
p-tau181	0.010	0.009	0.217	1.074	0.293	-0.01 – 0.03
Aβ40	-0.021	0.013	-0.296	-1.683	0.105	-0.05 – 0.005
Aβ42	-0.008	0.009	-0.183	-0.895	0.380	-0.03 – 0.01
Aβ42/40	0.003	0.006	0.106	0.522	0.606	-0.01 – 0.02

Table 67. Results from SBP regression analyses for the under 35 year old cohort. Systolic blood pressure inputted as the main predictor for each cognitive outcome and plasma biomarker. Analyses were adjusted for age, sex, and level of ID.

	B	SE	Beta	t	p	95%CI
NfL	-0.016	0.009	-0.316	-1.872	0.071	-0.03 – 0.002
GFAP	-0.001	0.011	-0.022	-0.096	0.924	-0.02 – 0.02
p-tau181	-0.012	0.008	-0.297	-1.436	0.164	-0.03 – 0.01
Aβ40	0.006	0.014	0.081	0.397	0.695	-0.02 – 0.04
Aβ42	-0.005	0.010	-0.116	-0.527	0.603	-0.03 – 0.02
Aβ42/40	0.001	0.006	0.025	0.114	0.910	-0.01 – 0.01

Table 68. Results from DBP regression analyses for the under 35 year old cohort. Diastolic blood pressure inputted as the main predictor for each cognitive outcome and plasma biomarker. Analyses were adjusted for age, sex, and level of ID.

	B	SE	Beta	t	p	95%CI
NfL	-0.001	0.008	-0.032	-0.180	0.858	-0.02 – 0.02
GFAP	-0.014	0.008	-0.362	-1.690	0.104	-0.03 – 0.003
p-tau181	0.016	0.007	0.456	2.305	0.030*	0.002 – 0.03
Aβ40	-0.020	0.011	-0.352	-1.849	0.077	-0.04 – 0.002
Aβ42	-0.003	0.008	-0.077	-0.356	0.725	-0.02 – 0.01
Aβ42/40	0.002	0.005	0.080	0.377	0.709	-0.01 – 0.01

Table 69. Results from PP regression analyses for the under 35 year old cohort. Pulse pressure inputted as the main predictor for each cognitive outcome and plasma biomarker. Analyses were adjusted for age, sex, and level of ID.

	B	SE	Beta	t	p	95%CI
NfL	-0.149	0.395	-0.076	-0.378	0.708	-0.96 – 0.66
GFAP	-0.509	0.411	-0.287	-1.238	0.228	-1.36 – 0.34
p-tau181	0.334	0.332	0.223	1.007	0.324	-0.35 – 1.02
Aβ40	0.227	0.556	0.087	0.407	0.687	-0.92 – 1.38
Aβ42	-0.295	0.436	-0.163	-0.677	0.505	-1.20 – 0.61
Aβ42/40	-0.240	0.305	-0.185	-0.784	0.441	-0.87 – 0.39

Table 70. Results from BMI regression analyses for the under 35 year old cohort. BMI inputted as the main predictor for each cognitive outcome and plasma biomarker. Analyses were adjusted for age, sex, and level of ID.

Risk Group Comparisons

Logistic regression models were constructed for group comparisons. These were exercise group, history of CHD and hypothyroid status. There was a trend towards a positive association between exercise and GFAP in this younger group but this was not significant (table 71). The high exercise group also had lower systolic blood pressures, so an interaction between SBP and exercise could be driving this association with GFAP. CHD trended towards a positive association with p-tau181 (B 2.78, 95% CI 0.77 – 34.60, p=0.07). Hypothyroid status had no significant associations in the models (tables 72 and 73).

	B	SE	Exp (B)	p	95%CI
NfL	-0.383	0.889	0.682	0.667	0.12 – 3.90
GFAP	2.331	1.377	10.285	0.090	0.69 – 152.80
p-tau181	-0.022	1.309	0.978	0.986	0.08 – 12.71
Aβ40	2.168	1.546	8.744	0.161	0.42 – 180.88
Aβ42	-0.069	1.069	4.113	0.186	0.51 – 33.45
Aβ42/40	0.906	1.436	2.475	0.528	0.15 – 41.29

Table 71. Binary logistic regression results for exercise group status in the younger adult cohort. Low exercise is the indicator group. Sex, age and level of ID included as covariates.

	B	SE	Exp (B)	p	95%CI
NfL	1.448	0.958	4.256	0.131	0.65 – 27.84
GFAP	-0.151	0.934	0.860	0.872	0.14 – 5.37
p-tau181	1.612	1.394	5.014	0.247	0.33 – 77.04
Aβ40	-1.442	1.496	0.236	0.335	0.01 – 4.44
Aβ42	-0.162	0.922	0.850	0.860	0.14 – 5.18
Aβ42/40	0.959	1.458	2.608	0.511	0.15 – 45.42

Table 72. Binary logistic regression results for hypothyroid group status in the younger adult cohort. Absence of hypothyroidism is the indicator group. Sex, age and level of ID included as covariates.

	B	SE	Exp (B)	p	95%CI
NfL	0.006	0.839	1.006	0.994	0.19 – 5.21
GFAP	-0.063	0.910	0.939	0.945	0.16 – 5.59
p-tau181	2.779	1.548	3.221	0.073	0.77 – 34.60
Aβ40	-1.097	0.942	0.334	0.244	0.05 – 2.12
Aβ42	-0.757	0.964	0.469	0.432	0.07 – 3.10
Aβ42/40	-1.063	1.419	0.345	0.454	0.02 – 5.58

Table 73. Binary logistic regression results for CHD group status in the younger adult cohort. Absence of CHD is the indicator group. Sex, age and level of ID included as covariates.

Combined Risk Group

Comorbidity risk groups were created as in the earlier analyses by combining risks: CHD, hypothyroid, PP<40mmHg, and low exercise levels. Age, sex and level of ID were included as covariates. One-way ANCOVA was performed using comorbidity group as the main predictor and the biomarker outcomes as the dependents. The combined group was run with and without BMI (BMI>25 kg/m²) given the conflicting interactions between BMI and dementia risk observed in older adults.

There were five people with zero risks, eight with one risk factor, 15 with two, six with three, and two with four factors. Those with three and four risk factors were combined into one group. Combined risk group without BMI had a significant association with NfL (F 3.626, partial eta squared 0.341, p=0.03), with increasing NfL observed as the risk groups increased (figure 28). Post hoc tests were borderline significant between the low and high risk group (mean difference 0.545 (SE 0.22), p=0.058). The difference between the low and moderate risk group trended towards a difference (mean difference 0.399 (SE 0.18), p=0.117). There was no difference between the moderate and high risk groups (mean difference 0.145 (SE 0.21), p=1.0).

A β 40 was associated with risk group (F 6.605, partial eta squared 0.569, p=0.005). Post hoc tests confirmed significantly lower A β 40 values in the highest risk group (mean difference 0.409 (SE 0.13), p=0.031 and 0.402 (SE 0.10), p=0.008 between those with one or two risks compared to those with three). The other group differences were not significant. There were no significant associations between risk group and A β 42 levels (F 1.914, partial eta squared 0.264, p=0.168); A β 42/40 ratio (F 1.730, partial eta squared 0.245, p=0.201); p-tau181 (F

1.432, partial eta squared 0.059, $p=0.244$); or GFAP (F 0.539, partial eta squared 0.092, $p=0.662$).

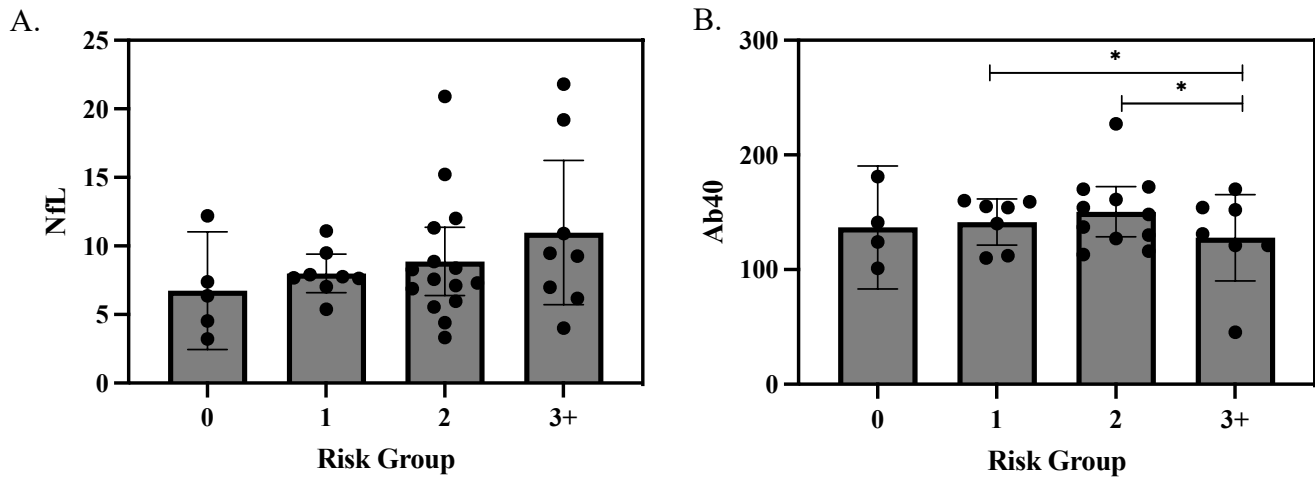


Figure 28. Biomarker values(in pg/ml) per combined risk group in adults under 35 years old. Error bars represent mean and 95% CI. A. Mean NfL per risk group. B. Mean Aβ40 per risk group.

BMI was then added as an additional risk factor. The trend for increasing NfL across the risk groups was maintained, but this did not reach significance (F 1.555, partial eta squared 0.182, $p=0.230$). The association with Aβ40 was not significant (F 0.065, partial eta squared 0.012, $p=0.977$). On the other hand, when BMI was controlled as a covariate in the earlier analysis the relationship between risk group and amyloid-beta was retained (F 5.876, partial eta squared 0.579, $p=0.009$). This is consistent with BMI having complex interactions with Aβ and dementia risk in people with DS. When BMI was included there was still no associations with Aβ42 (F 0.861, partial eta squared 0.139, $p=0.482$), p-tau181 (F 0.236, partial eta squared 0.01, $p=0.632$); GFAP (F 0.341, partial eta squared 0.060, $p=0.796$), or Aβ42/40 ratio (F 1.201, partial eta squared 0.184, $p=0.341$) between the risk groups.

7.2.2.1. Longitudinal Analysis Using Baseline Data

There were 31 people below 35 years old who also had baseline data available from a previous assessment. Eleven (35.5%) had at least one *APOE* $\epsilon 4$ allele, and 17 (54.8%) did not. Three people had missing *APOE* data. Twelve (38.7%) had mild ID, 18 (58.1%) had moderate ID and one (3.2%) had severe ID. There were 17 males (54.8%) and 14 females (45.2%). The mean time between assessments was 4.85 years (SD 1.70). Mean age at baseline was 22.26 years (SD 3.59) and at follow up assessment was 27.19 years (SD 3.99). Ten people (32.3%) had PP values below 40mmHg at baseline; eight people (25.8%) had BMI <25kg/m², eleven had BMI 25-30 (35.5%) and eleven had a BMI >30 (35.5%); 13 had a diagnosis of hypothyroid (41.9%) and 15 (48.4%) had a history of CHD. Only one person (3.2%) engaged in low exercise. Eleven (35.5%) engaged in moderate, and 19 (61.3%) engaged in high. Low and moderate exercise were combined into one group.

Regression models were constructed with each plasma biomarker as a dependent factor and continuous CVP variables as predictors. Logistic regression models were used to compare risk groups. Covariates were age, sex, level of LD, *APOE* $\epsilon 4$ status, and time between assessments. There were no significant associations between CVPs at baseline and biomarkers at T1. There was a trend towards a positive association between PP at baseline and p-tau181 at T1 (B 0.012, SE 0.006, t 1.851, 95% CI -0.002 – 0.03, p=0.08).

Combined Risk Group

At baseline there were two people (6.5%) with zero risk factors, six (19.4%) with one, four people (12.9%) with two factors, 17 (54.8%) with three, and two people (6.5%) with four

factors. No participant had all five risk factors. There were no significant associations between baseline risk group and biomarkers at T1 whether or not BMI was included in the model.

Summary of Section

Overall there were fewer associations between CVP factors and biomarker outcomes in people under the age of 35 years. The lack of associations may be in relation to the relative stability of neuropathology within the younger cohort, with less magnitude of change expected in the time-frame used. There is a suggestion of increased neuronal injury associated with low systolic blood pressures demonstrated in higher NfL and GFAP levels. PP was positively associated with p-tau181 levels, with trends towards negative associations with GFAP. BMI was not significantly associated with the biomarkers analysed here. This may be because BMI exerts detrimental effects via other mechanisms, such as via inflammatory pathways.

PP was negatively associated with A β 40 in the whole sample correlation analysis. Adjusted regression models in this younger cohort also suggested a trend towards higher A β 40 levels with lower PP. A β 42 was related to PP at a whole group level but not in this younger group. This could suggest that changes in A β 42 are of a smaller magnitude than A β 40 and require larger sample sizes to detect. Hence younger people with lower PP tended towards increased GFAP and A β 40 levels but lower p-tau181. In this younger cohort p-tau181 and GFAP may be reflecting factors other than direct dementia-processes in people with DS and this will be considered further in the discussion.

When the risk factors were combined there were trends towards cumulative risks of low PP, low exercise, CHD and hypothyroidism being related to higher NfL levels, indicative of

increased neurodegeneration even in these young adults. The same trend was observed when high BMI was added to the model, but with modulated relationships. High BMI may therefore be exerting some risk effects, but the relationship is not linear.

7.5.4. Longitudinal NfL Analysis

A small subset of people ($n=17$) from both the older and younger cohorts had repeat NfL measures from an additional assessment (T2) conducted two years after the current T1 assessment. There were 11 men and six women. Eight people had mild ID and nine had moderate or severe ID. Mean age was 38.46 years (SD 10.99), range 23-61 years. The mean time between assessments was 2.17 years (SD 0.66). Mean NfL at the first assessment was 11.46 pg/ml (SD 6.70) and at the second was 14.13 pg/ml (SD 8.97)

Repeated measures ANCOVA were performed for NfL measurements between T1 and T2, controlling for age, sex, ID and time between assessments. NfL increased between the time points and age was a significant factor ($F\ 13.613$, partial eta squared 0.660, $p=0.008$). Exercise group was significant ($F\ 10.939$, partial eta squared 0.814, $p=0.02$). Post hoc tests showed that in people with moderate exercise there was less change between the time points but this was not significant (mean difference 1.249 (SE 1.10), $p=0.31$). BMI also showed a relationship with NfL change and BMI was a significant predictor in the model ($F\ 11.577$, partial eta squared 0.591, $p=0.009$). Those with BMI under 25 kg/m² at T1 had greater NfL increases between time points (mean difference 11.474 (SE 2.04), $p<0.001$ in those with low BMI compared to 1.615 (SE 1.38), $p=0.28$).

There was a significant effect of CHD in this small sample ($F\ 7.907$, partial eta squared 0.530 , $p=0.026$). Post hoc corrections confirmed a greater increase in NfL in people with CHD (mean difference 6.920 (SE 1.87), $p=0.01$). The difference in those without CHD was not significant (mean difference 0.742 (SE 1.84), $p=0.70$). There were no significant effects of blood pressure on change in NfL between time-points. *APOE* status approached significance with greater change observed in those with an $\epsilon 4$ allele, however there were only two people in the sample with an $\epsilon 4$ allele ($F\ 4.774$, partial eta squared 0.374 , $p=0.06$) and therefore *APOE* status was not included in the wider analysis. Only three people in the sample had dementia. They showed greater NfL change (mean difference 10.992 (SE 4.70), $p=0.044$ compared to 2.932 (SE 1.65), $p=0.11$ in DScon) but the difference was not significant ($F\ 2.586$, partial eta squared 0.223 , $p=0.142$).

A combined risk group was created as before. The risks included were CHD, hypothyroidism, $PP<40\text{mmHg}$ and low exercise. There were five people with zero risks, three with one risk, six with two, and two with three risk factors. Risk group in the overall model was not significant ($F\ 3.880$, partial eta squared 0.700 , $p=0.089$). Post hoc tests confirmed that an increase in NfL was observed between the time points in all risk groups, but only the change in the group with three risks was significant (mean difference 8.776 (SE 2.52), $p=0.02$). The difference between those with two risks trended towards significance (mean difference 3.385 (SE 1.53), $p=0.07$). The differences in NfL over time in the 0 and 1 risk groups were not significant ($p>0.15$ for both). See figure 29.

The small numbers in the sample make it difficult to draw firm conclusions, but the observed trends support the hypothesis that there is a cumulative effect of cardiovascular risk on neuropathology, and reveal NfL to be a potentially useful marker of risk in people with DS.

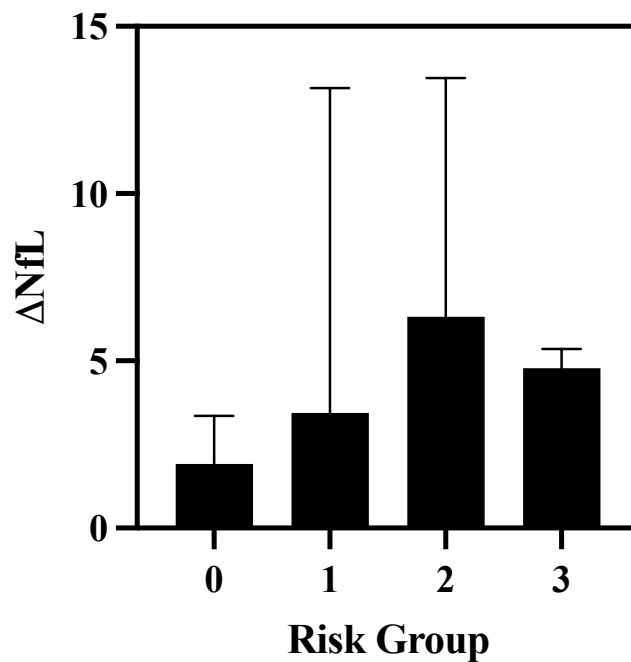


Figure 29. Mean change in NfL value between T1 and T2 for small subgroup ($n=17$), classified by combined CVP risk group at T1. Bar represent mean NfL (pg/ml) with error bars representing 95% CIs.

7.6. Cardiovascular Parameters and Plasma Biomarkers in People with Dementia

7.6.1. Two-way Group Comparisons

Thirteen people had a diagnosis of dementia at the time of assessment and a further eight had possible dementia. For the purpose of this analysis those with either a confirmed or query dementia diagnosis were grouped into a single “dementia” group ($n=21$) referred to as DSdem. Excluding people with query dementia did not affect the whole group comparisons and the combined group was used for all analyses. Group comparisons were made between those with and without dementia (classified as DScn). Two-way independent t-tests were performed for continuous variables and Pearson’s chi square tests for categorical values (see tables 74 and 75 for group comparisons).

Those with dementia were significantly older than those without (55.71 vs 37.24 years). There was a greater proportion of people with severe intellectual disability in the dementia group, although the numbers in this group were small. Only seven participants in the whole cohort had severe or profound ID. There were no significant differences in mean cardiovascular parameters, although there was a trend towards those with dementia having a lower BMI than those without (27.47 vs 30.25 kg/m², $p=0.07$). Those with dementia showed poorer performance on all direct cognitive and informant measures ($p<0.001$ for all).

Biomarker comparisons between groups are displayed in figure 30. People with dementia had significantly higher NfL levels (37.50 pg/ml compared to 14.36 pg/ml, $t\ 6.142$, $p<0.001$), GFAP levels (220.62 pg/ml compared to 92.10 pg/ml, $t\ 6.269$, $p<0.001$) and p-tau181 levels (14.97 pg/ml compared to 6.13 pg/ml, $t\ 6.46$, $p<0.001$). A β 40 and A β 42 were both slightly lower in people with dementia but differences were not significant (A β 40 144.24 pg/ml in people with dementia compared to 148.59 pg/ml in those without, $t\ 0.218$, $p=0.83$; A β 42 7.38 pg/ml compared to 8.53 pg/ml, $t\ 1.102$, $p=0.14$). A β 42/40 ratio was lower in people with dementia (0.050 compared to 0.059, $t\ 2.242$, $p=0.03$).

In terms of health co-morbidities the prevalence of congenital heart disease, hypothyroidism and other autoimmune conditions did not differ between the groups. Those with dementia had a significantly higher prevalence of late onset epilepsy (19.0% vs 2.8%, $p=0.01$) and stroke (9.5% vs 0, $p=0.01$). A significantly smaller proportion of those in the DSdem group engaged in moderate or high exercise (52.4% vs 87.1%), but there were similar rates of alcohol use and smoking between the groups.

		Whole Sample	Dementia Absent	Dementia Present	t-test	p value
	<i>n</i>	92	71	21		
	<i>n</i>	<i>Mean (SD)</i>	<i>Mean (SD)</i>	<i>Mean (SD)</i>		
Age (years)	92	41.46 (13.35)	37.24 (11.80)	55.71 (6.91)	-8.98*	<0.001
<i>Cardiovascular Parameters</i>						
SBP (mmHg)	86	112.45 (12.32)	112.30 (11.33)	113.00 (15.67)	-0.22	0.414
DBP (mmHg)	86	68.88 (9.69)	68.58 (9.02)	69.95 (11.97)	-0.54	0.591
PP (mmHg)	86	43.73 (11.70)	43.93 (10.78)	43.05 (14.84)	0.29	0.388
HR (bpm)	87	64.24 (12.00)	64.36 (11.35)	63.85 (14.3)	0.17	0.869
BMI (kg/m²)	86	29.57 (6.69)	30.25 (6.90)	27.47 (5.68)	1.85	0.072
AC (cm)	86	95.40 (13.22)	95.56 (13.66)	94.87 (11.94)	0.21	0.419
<i>Cognitive Measures</i>						
PALFAMS	64	8.78 (5.42)	9.89 (4.87)	2.80 (4.39)	4.29*	<0.001
Verbal Fluency	77	2.65 (.93)	2.84 (0.87)	1.87 (0.74)	3.98*	<0.001
Short SABS	88	76.10 (21.80)	80.86 (19.25)	58.84 (22.28)	4.27*	<0.001
CAMDEX	91	8.76 (16.52)	3.03 (6.87)	29.10 (23.69)	-4.87*	<0.001
<i>Plasma Biomarkers</i>						
NfL (pg/ml)	92	19.64 (18.56)	14.36 (10.23)	37.50 (27.71)	-6.14*	<0.001
GFAP (pg/ml)	76	122.54 (91.51)	92.10 (62.40)	220.62 (102.66)	-6.27*	<0.001
p-tau181 (pg/ml)	76	8.66 (7.23)	6.13 (4.67)	14.97 (6.97)	-6.46*	<0.001
Aβ40 (pg/ml)	75	147.56 (39.04)	148.59 (42.54)	144.24 (25.35)	0.22	0.830
Aβ42 (pg/ml)	75	8.25 (2.70)	8.52 (2.71)	7.38 (2.53)	1.10	0.140
Aβ42/40 ratio	75	0.06 (0.01)	0.06 (0.02)	0.05 (0.01)	2.24*	0.030

Table 74. Mean values for cardiovascular parameters, cognitive outcomes, and plasma biomarkers at time of blood sample based on dementia status.. Two-way independent t-tests compared values for those with and without dementia. Significance set at $p < 0.05$.

	Whole Sample <i>n (%)</i>	Dementia Absent <i>n (% of group)</i>	Dementia Present <i>n (% of group)</i>	Chi Square	p value
Sex: Female	41 (44.6)	34 (47.9)	7 (33.3)	1.390	0.238
LD: Mild	42 (45.7)	32 (45.1)	10 (47.6)	0.042	0.837
LD: Severe	7 (7.6)	3 (4.2)	4 (19.0)	5.065*	0.024
APOE ε4	22 (28.6)	18 (23.4)	4 (23.5)	0.272	0.602
Alcohol Use	34 (37.4)	26 (36.6)	8 (40.0)	0.076	0.783
Smoker	4 (4.3)	2 (2.8)	2 (9.5)	1.753	0.186
Exercise Mod/High	72 (79.1)	61 (87.1)	11 (52.4)	11.816*	<0.001
Diabetes	9 (9.8)	8 (11.3)	1 (4.8)	0.777	0.378
CHD	29 (31.5)	23 (32.4)	6 (28.6)	0.110	0.740
Hypertension	1 (1.1)	1 (1.4)	0 (0)	0.299	0.584
High Cholesterol	5 (5.4)	4 (4.3)	1 (4.8)	0.024	0.877
Hypothyroid	44 (47.8)	33 (46.5)	11 (52.4)	0.226	0.634
Autoimmune Disease	42 (46.2)	33 (47.1)	9 (42.9)	0.119	0.730
Epilepsy <30	8 (8.7)	5 (7.0)	3 (14.3)	1.071	0.301
Epilepsy >30	6 (6.5)	2 (2.8)	4 (19.0)	7.003*	0.008
Stroke	2 (2.2)	0 (0)	2 (9.5)	6.721*	0.010

Table 75. Characteristics and comorbidities present in the LonDowns cohort based on dementia status. Group comparisons made using Pearson's Chi square statistic. Significance set at $p < 0.05$.

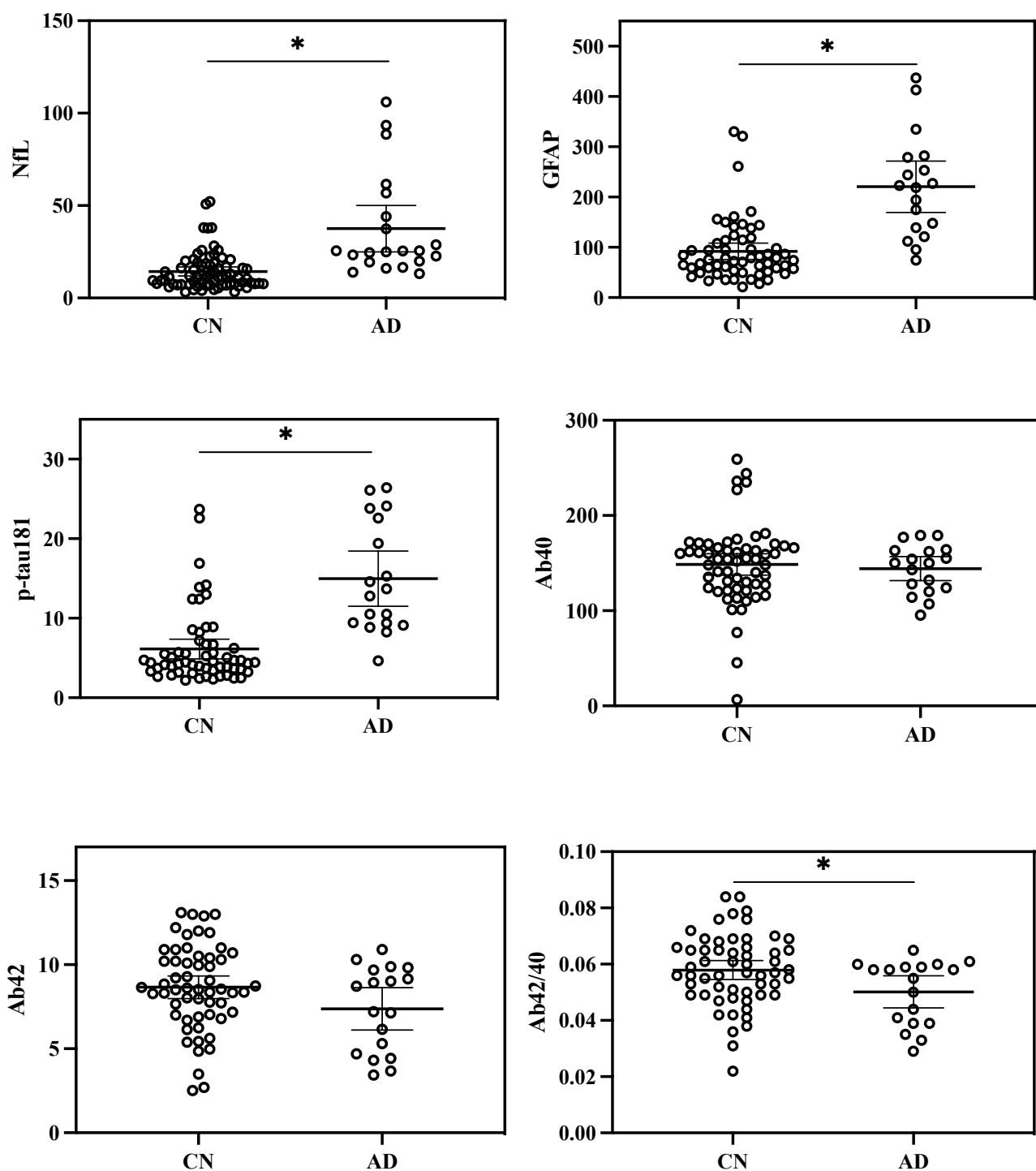


Figure 30. Grouped scatter plots comparing plasma biomarkers in those with dementia (AD) and those without (CN). All values presented in pg/ml. Error bars represent mean and 95% CI. Starred differences were significant at $p < 0.05$.

7.6.2. *Principal Component Analysis*

Principal component analyses were performed to determine relationships across the multiple variables. The data included were the CVPs (SBP, DBP, PP, BMI), cognitive outcomes (PALFAMS, VF, SABS, CAMDEX) and the plasma biomarkers (NfL, GFAP, p-tau181, A β 40, A β 42, A β 42/40).

There were five principal components (PCs) which met minimum requirements for inclusion and contributed 76.04% of the total variance. A loading table (table 76) is presented for each PC. Variables with loadings >0.5 were considered significant contributors to the PC. The first PC (PC1) contributed 26.4% of the variance; PC2 contributed 18.2%; PC3 13.1%; PC4 10.4% and PC5 8.0%. The Eigenvalue of PC5 was 1.19 (figure 31).

The first identified component (PC1) was associated with age, NfL, GFAP, p-tau181, CAMDEX (all positively associated with each other) and PALFAMS (negatively associated with the other variables). PC2 was associated with SBP and PP, which were positively associated with each other, as well as A β 40 and A β 42 which the blood pressure variables were negatively associated with. PC3 has contributions from DBP which had positive associations with A β 42 and A β 42/40 ratios and a negative association with SABS score. PC4 was only significantly related to PP value. The final included PC (PC5) was the only component to have contributions from BMI. In this factor, BMI was negatively associated with A β 42/40 ratio.

	PC1	PC2	PC3	PC4	PC5
Age	-0.811	0.052	-0.159	0.161	-0.215
SBP	-0.201	-0.799	0.096	0.451	-0.007
DBP	-0.09	-0.339	0.640	-0.206	-0.424
PP	-0.125	-0.565	-0.365	0.606	0.325
BMI	0.090	-0.328	0.324	0.352	-0.600
NfL	-0.795	0.341	-0.151	0.117	-0.035
GFAP	-0.801	0.315	-0.045	-0.061	0.096
p-tau181	-0.774	0.122	-0.194	0.219	-0.044
Aβ40	-0.113	0.747	0.287	0.263	-0.235
Aβ42	0.053	0.674	0.551	0.453	0.142
Aβ42/40	0.225	0.143	0.523	0.393	0.518
PALFAMS	0.685	0.208	-0.189	0.308	-0.182
VF	0.400	0.177	-0.463	0.411	-0.307
SABS	0.323	0.442	-0.547	0.042	-0.150
CAMDEX	-0.747	-0.111	0.059	0.027	-0.047

Table 76. Loading table for the five principle components identified. Variables with a loading +/- 0.5 were considered significant contributors to the component.

PC1 is consistent with a dementia-related grouping. Increased CAMDEX score and poorer performance on cognitive tasks such as PALFAMS are recognised indicators of dementia. In this PC they were related to increased NfL, p-tau181 and GFAP suggesting that clinical markers of dementia are reflective of underlying neurodegenerative changes. A combination of age, NfL, p-tau181 and GFAP could be explored for its diagnostic potential.

PC2 was not associated with cognitive outcomes, but low SBP and low PP were related to increased values of A β 40 and A β 42. This component can be considered a low blood pressure and non-specific amyloid-beta promoting group. Similarly PC3 represents interactions between raised DBP and A β 42 promoting effects. PC4 may reflect a unique association for PP,

and helps us to consider how PP interacts with incident dementia risk. PP can be associated with low SBP (as in PC2), but can also be a product of increased DBP which has its own associations (as in PC3). PC4 is therefore a PP factor. The disconnect between BMI and other factors represented by PC5 signals that the risks associated with BMI and the risks associated with BP measures may be related to different mechanisms. BMI was inversely related to A β 42/40 ratio and no other factors. PC5 is a BMI-related pathology group.

As the greatest contributors of variance (explaining 45% of the variance), the first two PCs (PC1 and PC2) were plotted on a score chart and coded according to dementia status (figure 32). There was a clear group division between those with and without dementia, highlighting that these groups may be differentiated from each other based on the factors in the analysis.

One participant (* on the score chart, figure 32) appeared to be a relative outlier from the no-dementia group, co-locating more closely with the dementia group. On review of the participant details they were an older person (greater than 50 years old), without dementia but with changes in multiple CAMDEX domains (in everyday skills, memory, other cognitive, personality and behavioural domains). This participant could represent a prodromal dementia case. There is evidence that the PC grouping could therefore be predictive for someone likely to develop dementia, with plasma biomarkers enhancing the predictive value of cognitive assessments at an individual level. It also supports the use of biomarkers as proxy indicators of dementia-related changes in people with DS, as used in this project.

Blood pressure values were associated with PC2, PC3 and PC4, rather than with the dementia-related PC1. Blood pressure parameters were related to amyloid-beta levels. Low SBP, raised DBP and low PP were all associated with increased plasma amyloid-beta levels. Low SBP and

PP were associated with increased A β 40 and A β 42, whereas high DBP was associated with raised A β 42 only. BMI was not related to cognitive outcomes nor biomarkers of neurodegeneration, and had only a relatively weak association with A β 42/40 ratio. The incident dementia risk associated with raised BMI in earlier adulthood may be mediated by additional processes not represented by the biomarkers used in the current analysis.

Principal component logistic regression was performed, with dementia status as the binary dependent variable (see table 77). Age, NfL, GFAP, p-tau181, PALFAMS and CAMDEX were all significant contributors to the model. Hence, age, NfL, p-tau181 and GFAP alongside direct cognitive and informant measures are strong predictors of dementia status in people with DS.

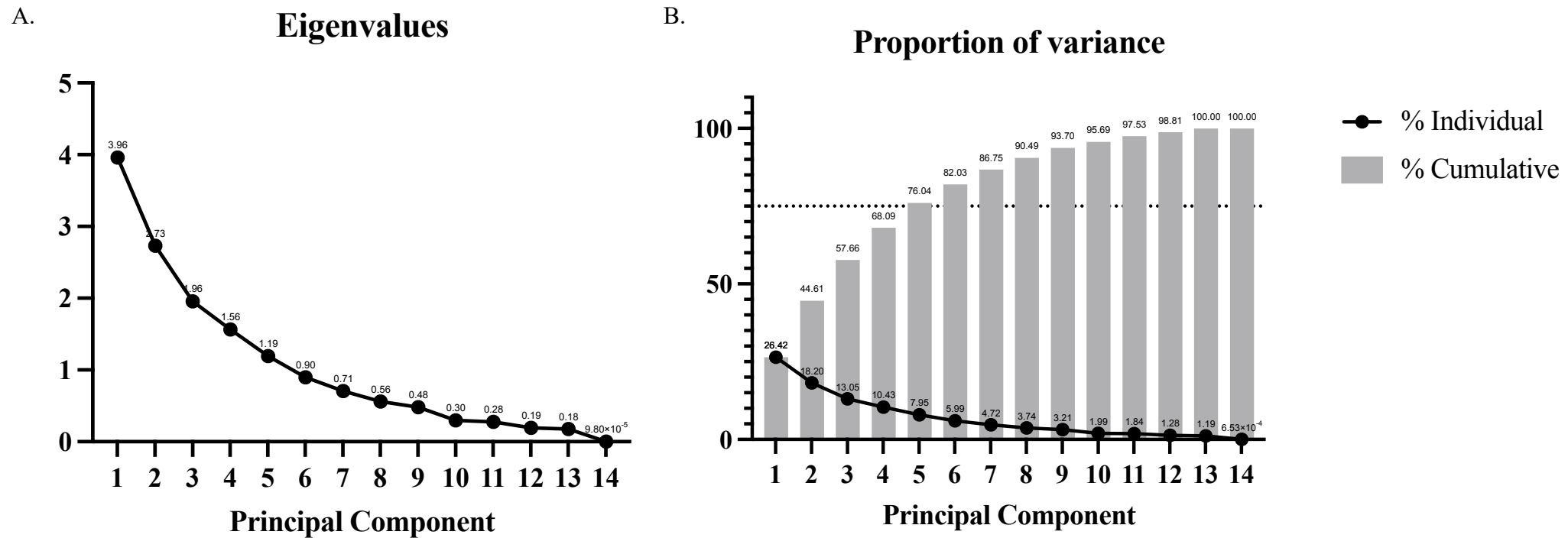
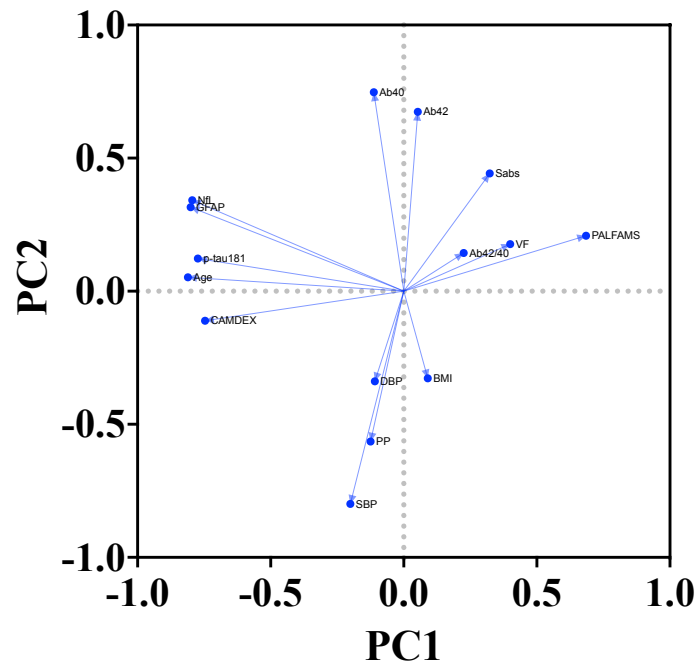


Figure 31. Plots representing Eigenvalues and proportion of explained variance in the principal component analysis.

A. Plot of relative Eigenvalues for each principal component. B. Proportion of Variance plot displaying individual and cumulative variance explained by each principal component.

A.



B.

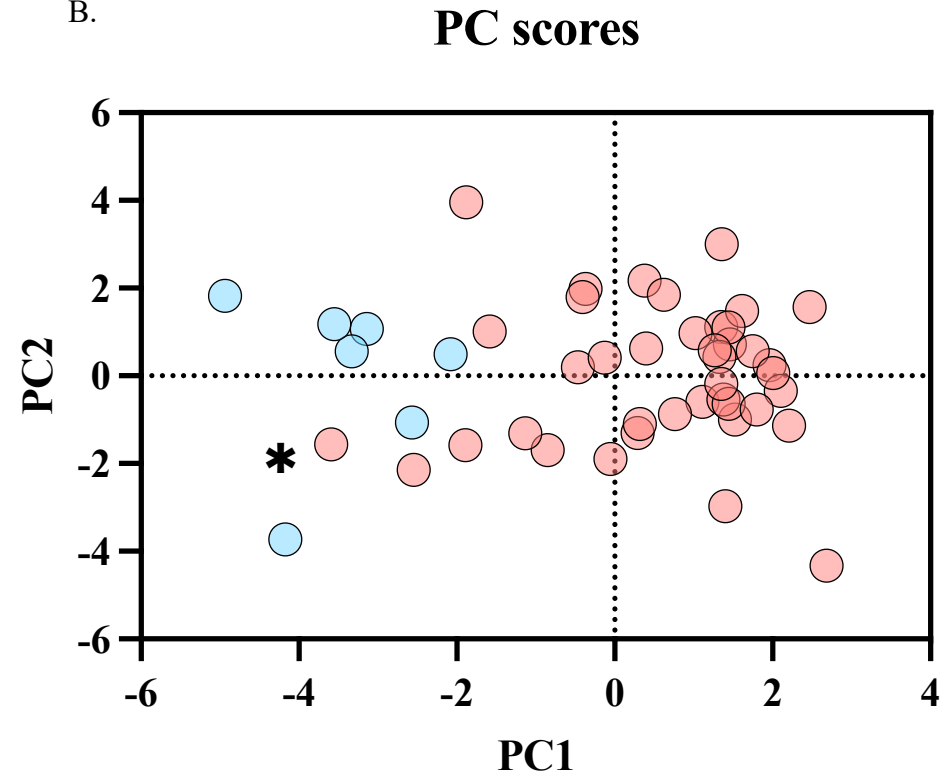


Figure 32. A) Loading plot for principal components 1 and 2, displaying the relative factor contributions for each PC. B) Principal Component score chart for PC1 and PC2, colour coded based on dementia status (DSdem = blue; DScn = pink). The participant labelled * is discussed above.

	B	SE	t	p	95% CI
Age	0.005	0.001	3.869	<0.001	0.002 – 0.006
SBP	-0.001	0.001	0.114	0.919	-0.002 – 0.003
DBP	0.001	0.002	0.603	0.550	-0.003 – 0.005
PP	-0.001	0.002	0.493	0.624	-0.004 – 0.003
BMI	-0.117	0.101	1.153	0.255	-0.32 – 0.09
NfL	0.083	0.018	4.695	<0.001	0.05 – 0.12
GFAP	0.103	0.017	5.966	<0.001	0.07 – 0.14
p-tau181	0.067	0.017	4.049	<0.001	0.03 – 0.10
A β 40	0.007	0.060	0.108	0.914	-0.12 – 0.13
A β 42	-0.037	0.056	0.671	0.506	-0.15 – 0.07
A β 42/40	-0.104	0.104	1.003	0.322	-0.32 – 0.11
PALFAMS	-0.011	0.002	5.019	<0.001	-0.02 - -0.007
VF	-0.056	0.020	2.808	0.008	-0.10 - -0.02
SABS	-0.002	0.001	2.050	0.040	-0.004 - -0.001
CAMDEX	0.047	0.008	6.003	<0.001	0.03 – 0.06

*Table 77. Principle component regression table.
Outcome variable = dementia status. R^2 0.548.*

Section Summary

NfL, p-tau181 and GFAP appear to be robust markers of dementia-related changes in people with DS, and in combination with age or cognitive outcome measures may be useful in differentiating people at increased risk of dementia at an individual level. Blood pressure parameters were associated with A β levels, suggesting an interaction between blood pressure and A β burden in people with DS.

BMI was not associated with markers of neurodegeneration, and only weakly with A β 42/40 ratios. The lack of associations leads us to consider whether BMI interacts with other pathways to affect risk. In the next section that relationship with complement will be explored to assess interactions with inflammatory processes, a proposed mechanism in which BMI and other cardiovascular risks may exert their effects.

7.7. Relationship between Cardiovascular Parameters, Plasma Biomarkers and Complement Components

65 people (70.7% of the original cohort) had additional complement data that was analysed in an exploratory manner to consider inflammatory mechanisms related to CVPs. Eight complement components were measured for each person: complement component 3 (C3), complement component 4 (C4), clusterin, factor H (FH), factor I (FI), complement receptor 1 (CR1), complement component 1q (C1q), and C1 esterase inhibitor (C1inh).

The mean age at sample was 39.69 years (SD 13.67). Fifty-six people had available *APOE* data, and 16 of these (24.6%) had a least one *APOE* ϵ 4 allele. There were 27 people (41.5%) with mild intellectual disability, 33 (50.8%) with moderate, and five with severe or profound. The majority of the sample were white British ($n=58$, 89.2%). There were 40 men (61.5%) and 25 women. Seven people (10.8%) had query dementia and five had confirmed dementia, giving a combined dementia group $n=12$. There were 21 people with CHD (32.3%), seven with diabetes, 31 people (47.7%) with hypothyroidism, and 32 (49.2%) with other autoimmune conditions. Ten people had epilepsy, six (9.2%) of which had onset before age 30 and four in

later life. Only two people had a diagnosis of hypercholesterolaemia (3.1%), one person had experienced a stroke, nobody had a diagnosis of hypertension.

All eight complement factors were measured for each person. Table 78 describes the mean complement values for the whole cohort.

	Minimum	Maximum	Mean	Std. Deviation
Clusterin	130.88	661.36	293.01	105.85
C3	885.19	2924.45	1535.09	416.08
C4	133.95	480.36	262.06	65.31
CR1	6.56	24.12	12.61	4.11
C1q	106.02	329.00	193.20	47.09
C1inh	83.14	245.93	150.82	32.54
FH	205.16	1398.53	512.21	222.04
FI	16.29	69.54	32.60	8.51

Table 78. Mean (SD) values for the eight complement components analysed. Complement levels presented in ug/ml.

Complement Correlations

Initially whole-sample two-sided Pearson correlations were performed between complement factors, CVPs, cognitive outcomes and biomarker measures (see table 79 for results). Correlations are presented as: Pearson Correlation (95% CI) 2-tailed significance.

Clusterin was significantly positively correlated with C3 (0.66, 0.49 – 0.78, $p<0.001$) and FH (0.43, 0.21 – 0.61, $p<0.001$). Clusterin was negatively correlated with CR1 (-0.35, -0.55 - -0.12, $p=0.004$). C3 was also positively correlated with FH and FI (0.72, 0.57 – 0.82, $p<0.001$ and 0.26, 0.02 – 0.47, $p=0.04$). C4 was positively correlated with C1q and FH (0.25, 0.01 – 0.47, $p=0.04$ and 0.26, 0.02 – 0.47, $p=0.03$). C1inh was positively correlated with C1q (0.57, 0.38 – 0.71, $p<0.001$) and F1 (0.27, 0.03 – 0.48, $p=0.03$). FH and FI were positively correlated (0.33, 0.09 – 0.53, $p=0.01$).

C3, FH and FI were positively correlated with BMI and AC (C3: 0.29, 0.04 – 0.51, $p=0.03$ and 0.31, 0.06 – 0.52, $p=0.02$; FH: 0.29, 0.04 – 0.51, $p=0.02$ and 0.28, 0.02 – 0.50 $p=0.03$; FI: 0.29, 0.04 – 0.51, $p=0.02$ and 0.34, 0.09 – 0.55, $p=0.01$). C4 was positively correlated with age (0.262, 0.02 – 0.48, $p=0.035$).

SABS had a negative correlation with C1q (-0.30, -0.51 - -0.06, $p=0.02$), whilst CAMDEX score was positively correlated with C1q (0.30, 0.06 – 0.50, $p=0.02$). C4 was positively correlated with NfL (0.26, 0.01 – 0.47, $p=0.04$). There was a borderline correlation between C4 and A β 42 (0.24, -0.01 – 0.46, $p=0.05$). CR1 approached a significant negative correlation with NfL (-0.23, -0.45 – 0.02, $p=0.068$). C1q approached a significant positive correlation with NfL (0.21, -0.03 – 0.44, $p=0.087$), GFAP (0.24, -0.01 – 0.46, $p=0.054$), and A β 42/40 (0.22, -0.03 – 0.44, $p=0.080$). There were positive correlations between p-tau181 with C4 (0.254, 95% CI 0.01 – 0.47, $p=0.043$) and borderline with C1q (0.246, 95% CI 0.001 – 0.46, $p=0.050$).

	Age	SBP	DBP	PP	BMI	AC	NfL	GFAP	Ptau181	A β 40	A β 42	A β 42/40	PALFAMS	VF	SABS	CAMDEX
Clusterin	-0.032	-0.091	-0.044	-0.059	-0.003	-0.029	-0.067	-0.033	-0.082	0.146	0.109	0.006	-0.028	-0.175	-0.016	-0.187
C3	-0.120	0.010	-0.017	0.024	0.289*	0.309*	-0.199	-0.080	-0.145	-0.025	0.104	0.172	0.084	-0.135	-0.067	-0.181
C4	0.262*	0.113	0.049	0.078	0.128	0.117	0.257*	0.190	0.254*	0.177	0.238	0.155	-0.123	-0.055	-0.133	0.035
CR1	-0.117	0.010	0.217	-0.170	0.130	0.121	-0.228	-0.097	-0.179	-0.144	-0.068	0.050	0.027	-0.005	0.001	-0.037
C1q	0.197	0.018	-0.073	0.079	-0.121	0.025	0.214	0.240	0.246*	-0.010	0.149	0.219	-0.039	0.067	-0.303*	0.296*
C1in	0.151	-0.177	-0.215	-0.008	-0.054	0.033	0.190	0.128	0.161	0.013	0.027	0.025	0.100	0.067	-0.119	0.054
FH	-0.050	0.097	-0.015	0.114	0.290*	0.277*	-0.108	-0.115	-0.099	-0.137	0.003	0.142	0.078	0.058	-0.035	-0.066
FI	0.034	0.111	0.003	0.114	0.290*	0.340*	0.025	-0.111	-0.051	0.004	-0.088	-0.127	0.133	0.143	0.022	-0.157

Table 79. Pearson correlations for complement components, cardiovascular parameters, and cognitive outcomes.

*correlation is significant at the 0.05 level (two-tailed)

** correlation is significant at the 0.01 level (two-tailed).

Sex differences in Complement Profiles

There was a general trend for complement values to be higher in women, and this was consistent for all factors apart from FI. Using two-way independent t-tests there were no significant sex differences, although clusterin and C3 trended towards being higher in women (mean clusterin 274.26 ug/ml (SD 86.98) in men and 323.02 ug/ml (126.72) in women, $t = 1.840$, $p=0.07$; mean C3 1456.11 ug/ml (SD 371.42) in men and 1661.46 ug/ml (SD 458.75) in women, $t = -1.980$, $p=0.052$).

Effects of APOE $\epsilon 4$ status on Complement

There were no significant differences in mean complement values between the two groups. Clusterin was higher in those with an $\epsilon 4$ allele but this was not significant (331.48 ug/ml (SD 145.61) compared to 281.80 ug/ml (94.62), $t = -1.262$, $p=0.22$).

Effects of Exercise Groups on Complement

Two-sided independent t-tests were used to compare the high exercise group to those engaging in low or moderate exercise. Only C4 was significantly different between the groups. There were lower C4 levels in the high exercise group (225.97 ug/ml (SD 41.06) compared to 275.89 ug/ml (SD 67.87) in the low group, $t = -3.606$, mean difference -49.92, 95% CI -77.72 – 22.12, $p<0.001$).

Complement Factors in Dementia

Two-tailed independent t-tests were performed comparing the dementia group (DSdem) to people without dementia (DScn). There were significant differences between the groups for C1q, which was higher people with dementia, and FI which was lower (mean C1q 219.6 ug/ml (SD 53.8) in DSdem compared to 187.2 ug/ml (SD 53.8) in DScn, $p=0.03$; mean FI 27.9 ug/ml (SD 9.0) in DSdem and 33.7 ug/ml (SD 8.1) in DScn, $p=0.03$). There were trends towards clusterin and C3 being lower in the dementia group (see table 80 and figure 33).

	DScn	DSdem	t	p	Mean diff	95% CI
n	53	12				
Clusterin	303.34 (110.57)	247.42 (67.89)	1.675	0.099	55.91	-10.77 – 122.60
C3	1576.99 (423.05)	1350.00 (339.65)	1.733	0.088	226.99	-34.76 – 488.73
C4	259.46 (67.74)	273.57 (54.29)	-0.673	0.504	-14.11	-56.01 – 27.79
CR1	12.69 (3.98)	12.25 (4.85)	0.332	0.741	0.44	-2.21 – 3.09
C1q	187.22 (43.81)	219.58 (53.80)	-2.214	0.030*	-32.36	-61.57 - -3.15
C1inh	150.15 (31.10)	153.77 (39.71)	-0.345	0.731	-3.62	-24.55 – 17.32
FH	526.36 (237.90)	449.69 (118.65)	1.082	0.284	76.67	-64.99 – 218.34
FI	33.68 (8.10)	27.85 (8.99)	2.204	0.031*	5.82	0.54 – 11.10

Table 80. Complement levels (ug/ml) in people with and without dementia. Data presented as mean (SD). Comparisons made using two-sided independent t-tests. DScn = Participants without dementia. DSdem = Participants with dementia.

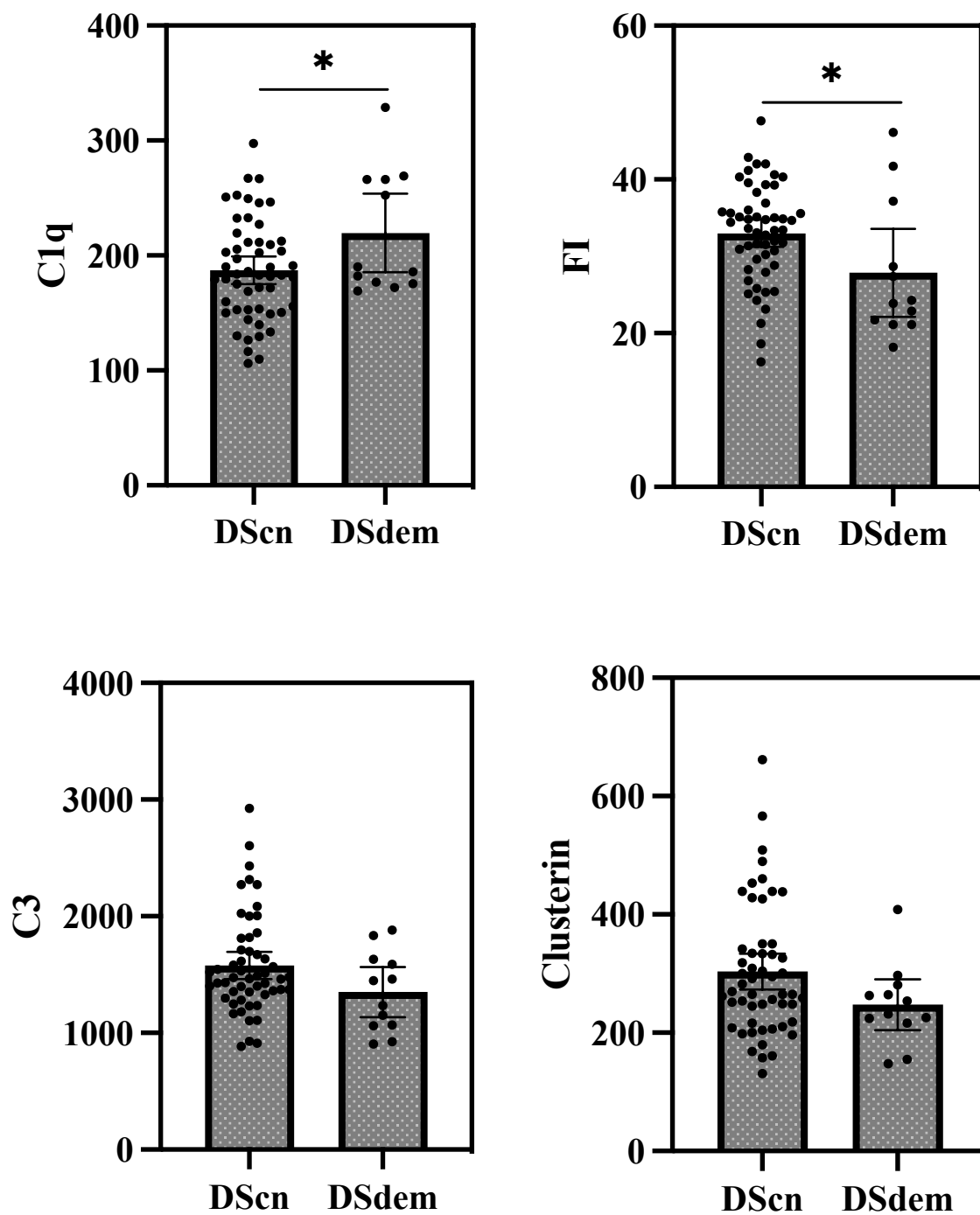


Figure 33. Mean complement levels (ug/ml) in people with and without dementia. Error bars represent 95% CI. DScn = Participants without dementia. DSdem = Participants with dementia. * indicates group difference significant at $p < 0.05$.

Cardiovascular Parameters and Complement Factors

Linear regressions were performed to analyse the relationships between complement factors and CVPs, adjusting for age, sex, level of ID and *APOE* status. Logistic regression models were constructed to make comparisons between binary risk groups. There was a significant positive association between BMI and FI (B 20.62, 95% CI 9.15 – 32.10, $p < 0.001$), and a trend towards a positive association with FH (B 263.92, 95% CI -70.86 – 55.23, $p = 0.12$). There were no other significant associations between complement and BMI (see table 81). There were no significant relationships between SBP, DBP or PP and complement components. Sex was significant in the models for clusterin and C3, with higher levels in women. Sex was a predictor for FH in blood pressure models, but not in the BMI model. *APOE* status was not a significant predictor in any of the models.

Clusterin had a significant relationship with exercise group, with higher clusterin levels in the high exercise group (mean clusterin 306.29 ug/ml (SD 113.99) in the high exercise group and 265.19 ug/ml in the low exercise group (SD 81.93), B 0.01, 95% CI 1.01 – 1.02, $p = 0.03$). There was a trend towards a negative association between CR1 and exercise group (B -0.12, 95% CI 0.76 – 1.04), $p = 0.15$). The association with C4 lost significance in the adjusted model. There were no other associations between complement and exercise group (see table 82). No significant associations were observed between CHD or hypothyroidism and complement component measurements (all $p > 0.185$).

	B	SE	Beta	t	p	95% CI
Clusterin	-81.536	80.553	-0.154	-1.012	0.317	-243.59 – 80.52
C3	308.436	309.455	0.152	0.997	0.324	-314.12 – 930.98
C4	58.607	47.289	0.192	1.239	0.221	-36.53 – 153.74
CR1	1.582	3.069	0.082	0.515	0.609	-4.59 – 7.76
C1q	6.558	30.238	0.034	0.217	0.829	-54.27 – 67.39
C1inh	-12.229	21.775	-0.091	-0.562	0.577	-56.03 – 31.58
FH	263.92	166.410	0.244	1.586	0.119	-70.86 – 55.23
FI	20.622	5.705	0.521	3.615	<0.001*	9.15 – 32.10

Table 81. Results from complement and BMI regression analyses. BMI inputted as the main predictor for each complement component. Age, sex, APOE ε4 status, and level of ID were covariates. .

	B	SE	Exp (B)	p	95%CI
Clusterin	0.008	0.004	1.009	0.028*	1.01 – 1.02
C3	0.001	0.001	1.001	0.309	0.99 – 1.01
C4	-0.005	0.005	0.995	0.268	0.98 – 1.01
CR1	-0.117	0.081	0.889	0.146	0.76 – 1.04
C1q	0.002	0.008	1.002	0.755	0.99 – 1.02
C1inh	0.007	0.011	1.007	0.557	0.99 - 1.03
FH	0.000	0.001	1.000	0.830	1.00 – 1.00
FI	0.032	0.044	1.032	0.467	0.95 – 1.12

Table 82. Results from binary logistic models for exercise group and complement associations. Age, sex, APOE ε4 status, and level of ID were covariates.

Combined Risk Group

As in previous analyses, the CVP risks were combined to form a high and a low comorbidity risk group which was modelled with and without the inclusion of raised BMI as a risk factor. There were no significant differences between the low (0-1 risk factors) and high risk group in either the model without BMI or with high BMI included (tables 83 and 84). When raised BMI was included as a risk factor all the associations between risk group and complement were weakened apart from CR1 and C4. The relationships between CR1, C4 and risk group were strengthened by the inclusion of raised BMI in the combined risk model, and there was a trend towards higher CR1 levels and C4 in the higher risk group. This could imply that CR1 and C4 are integral to BMI associated inflammatory changes. There was a trend for CR1 and C4 levels to be lower in the high exercise group, which could be a reflection of reduced BMI occurring in people with higher exercise levels.

	B	SE	Exp (B)	p	95%CI
Clusterin	-0.005	0.003	0.995	0.089	0.99 – 1.00
C3	-0.001	0.001	0.999	0.116	1.00 – 1.01
C4	-0.002	0.005	0.998	0.629	0.99 – 1.01
CR1	0.116	0.078	1.123	0.137	0.96 – 1.31
C1q	-0.013	0.008	0.987	0.093	0.97 – 1.00
C1inh	-0.002	0.010	0.998	0.863	0.98 – 1.02
FH	-0.002	0.002	0.998	0.110	0.99 – 1.00
FI	-0.054	0.040	0.947	0.179	0.88 – 1.03

Table 83. Results from binary logistic models for combined risk group (without BMI included) and complement. Age, sex, APOE ε4 status, and level of ID were covariates.

	B	SE	Exp (B)	p	95%CI
Clusterin	-0.003	0.003	0.997	0.314	0.99 – 1.00
C3	-0.001	0.001	0.999	0.357	1.00 – 1.00
C4	0.008	0.006	1.001	0.158	1.00 – 1.02
CR1	0.136	0.086	1.145	0.115	1.00 – 1.46
C1q	0.000	0.000	1.000	0.998	0.99 – 1.02
C1inh	0.011	0.011	1.011	0.297	0.99 – 1.04
FH	0.000	0.001	1.000	0.937	1.00 – 1.00
FI	0.015	0.034	1.015	0.664	0.95 – 1.09

Table 84. Results from binary logistic models for combined risk group (raised BMI included) and complement. Age, sex, APOE ε4 status, and level of ID were covariates.

Principal Component Analysis

PCA was performed to analyse the relationship between complement components and CVPs following the same methods as outlined previously. There were six PCs identified as significant with cumulative explanation of 77.6% of the variance (see table 85). PC1 explained 20.95%, PC2 15.61%, PC3 12.55%, PC4 11.07%, PC5 9.72% and PC6 7.69%. PC6 did not have any contributing factors that reached limits for significance (loading >0.5), and PC5 only had significant contributions from age. Therefore the main PCs considered were PC1-4 which explained 60.2% of the variance. The Eigenvalue of PC4 was 1.44 and PC6 was 1.00 (figure 34).

	PC1	PC2	PC3	PC4	PC5	PC6
Age	-0.095	0.263	-0.040	0.287	-0.676	0.096
SBP	0.236	0.872	0.150	0.127	0.046	-0.354
DBP	0.041	0.479	-0.181	-0.503	-0.477	-0.456
PP	0.227	0.575	0.312	0.548	0.437	-0.022
BMI	0.544	0.372	-0.042	-0.265	0.012	0.391
Clusterin	0.599	-0.461	0.407	0.014	-0.245	-0.303
C3	0.847	-0.247	0.232	-0.191	0.018	-0.063
C4	0.318	0.127	-0.121	0.375	-0.495	0.481
CR1	0.011	0.254	-0.611	-0.471	0.238	0.183
C1q	0.178	-0.055	-0.669	0.452	0.028	-0.202
C1inh	0.251	-0.362	-0.573	0.315	0.016	-0.291
FH	0.863	-0.049	-0.059	-0.113	0.044	0.130
FI	0.541	0.097	-0.298	0.026	0.145	-0.037

Table 85. Loading scores for the identified principal components. Factors with a loading score +/-0.5 were considered significant contributors to the PC.

PC1 had contributions from BMI, clusterin, C3, FH and FI, all of which were positively correlated. PC1 can be considered a BMI associated complement group. PC2 was similar to PC2 in the earlier PCA, with contributions from SBP and PP that were positively correlated with each other. There were no contributions with complement components. PC3 had contributions from the three complement factors: CR1, C1q and C1inh which were all positively correlated. Increases in C1q were found in the dementia group. These factors could represent a dementia-related complement group. PC4 has contributions from PP and DBP which were negatively correlated with each other and is similar to PC3 and PC4 observed earlier.

A score plot was generated based on PC1 and PC2, with colour coding by dementia status (figure 35). Differentiation of the dementia subgroup was less well defined than in the earlier PCA. Despite this, the complement system does appear to contribute to predictive models of dementia in people with DS. The complement profile in dementia may be less clearly demarcated, with overlap between people with and without dementia.

Principal component logistic regression was performed with dementia as the binary outcome measure (see table 86). There were significant effects of age, DBP, C3, C4, FH and FI. Age, DBP and C4 had positive coefficients. C3, FH and FI had negative coefficients. PP approached significance with a negative coefficient ($p=0.06$). BMI was not a significant predictor ($p=0.18$).

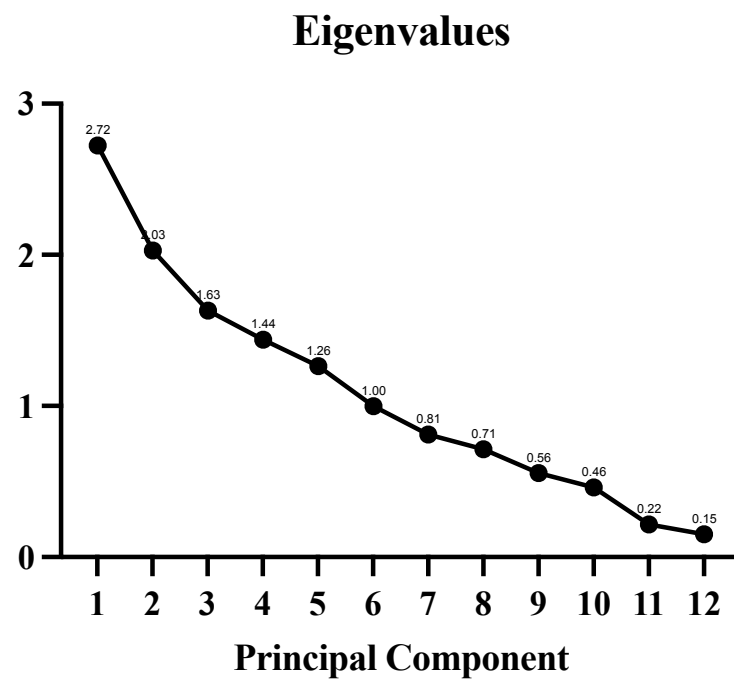
BMI was therefore related to C3, FH and FI, but not related to most of the other plasma biomarkers (NfL, GFAP, A β 40 or A β 42). The risk effects exerted by BMI may be mediated through inflammatory pathways such as complement rather than direct effects on other cardiovascular risks, amyloid-beta or neuronal damage. There were associations between C3, C4, FH and FI with dementia status. BMI was associated with clusterin as well as C3, FH, FI. Despite these associations, in this analysis BMI was not a significant predictor of dementia status. The results suggest interactions between BMI and complement, and complement and dementia, but the exact nature of these relationships warrant further investigation.

Blood pressure parameters did not co-locate with complement factors which suggests poor associations between blood pressure parameters and complement pathways. On the other hand, they did correspond to amyloid-beta levels in the previous analysis.

	B	SE	t	p	95% CI
Age	0.010	0.002	4.560	<0.001*	0.005 – 0.01
SBP	0.001	0.002	0.3564	0.723	-0.004 – 0.005
DBP	0.009	0.004	2.376	0.021*	0.001 – 0.02
PP	-0.005	0.003	1.905	0.062	-0.01 – 0.0002
BMI	-0.141	0.105	1.351	0.183	-0.35 – 0.07
Clusterin	-0.0001	0.0002	0.367	0.716	-0.0001 – 0.001
C3	-0.0001	0.00001	3.409	0.001*	-0.0002 – -0.0003
C4	0.001	0.0004	2.288	0.027*	0.0001 – 0.002
CR1	-0.007	0.006	1.181	0.244	-0.02 – 0.004
C1q	0.0005	0.0005	0.876	0.385	-0.0007 – 0.002
C1inh	0.0002	0.0008	0.291	0.772	-0.001 – 0.002
FH	-0.0002	0.0001	3.258	0.002*	-0.0003 - -0.0004
FI	-0.004	0.002	2.607	0.012*	-0.007 - -0.0009

Table 86. Principle component regression table. Outcome variable = dementia status. R^2 0.376.

A.



B.

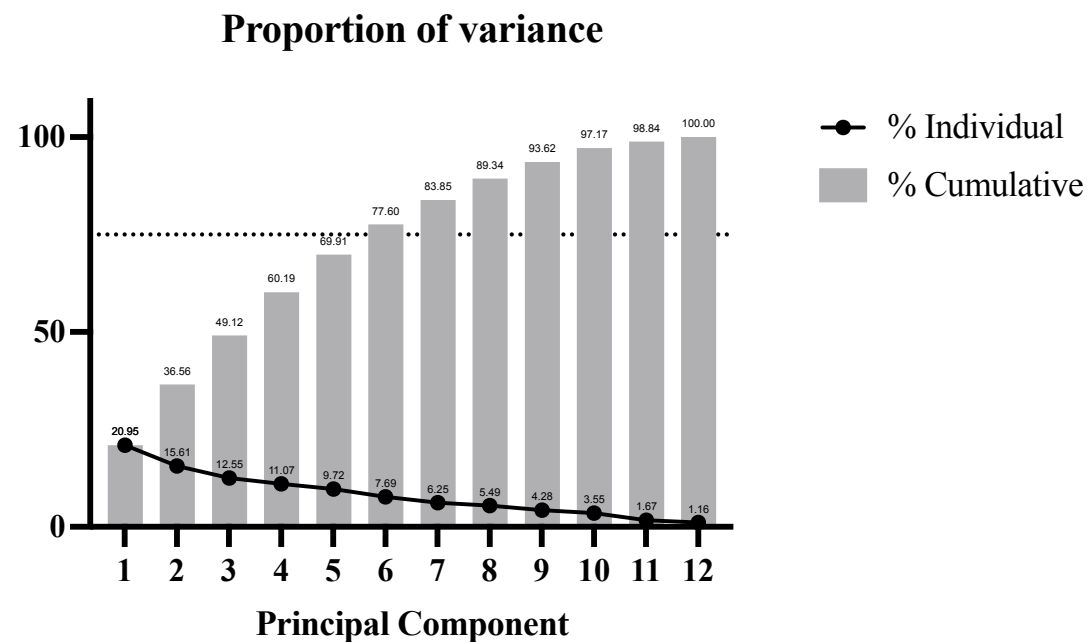


Figure 34. Plots representing Eigenvalues and proportion of explained variance in the principal component analysis. A) Plot of relative Eigenvalues for each principal component. B) Proportion of variance plot displaying individual and cumulative variance explained by each principal component.

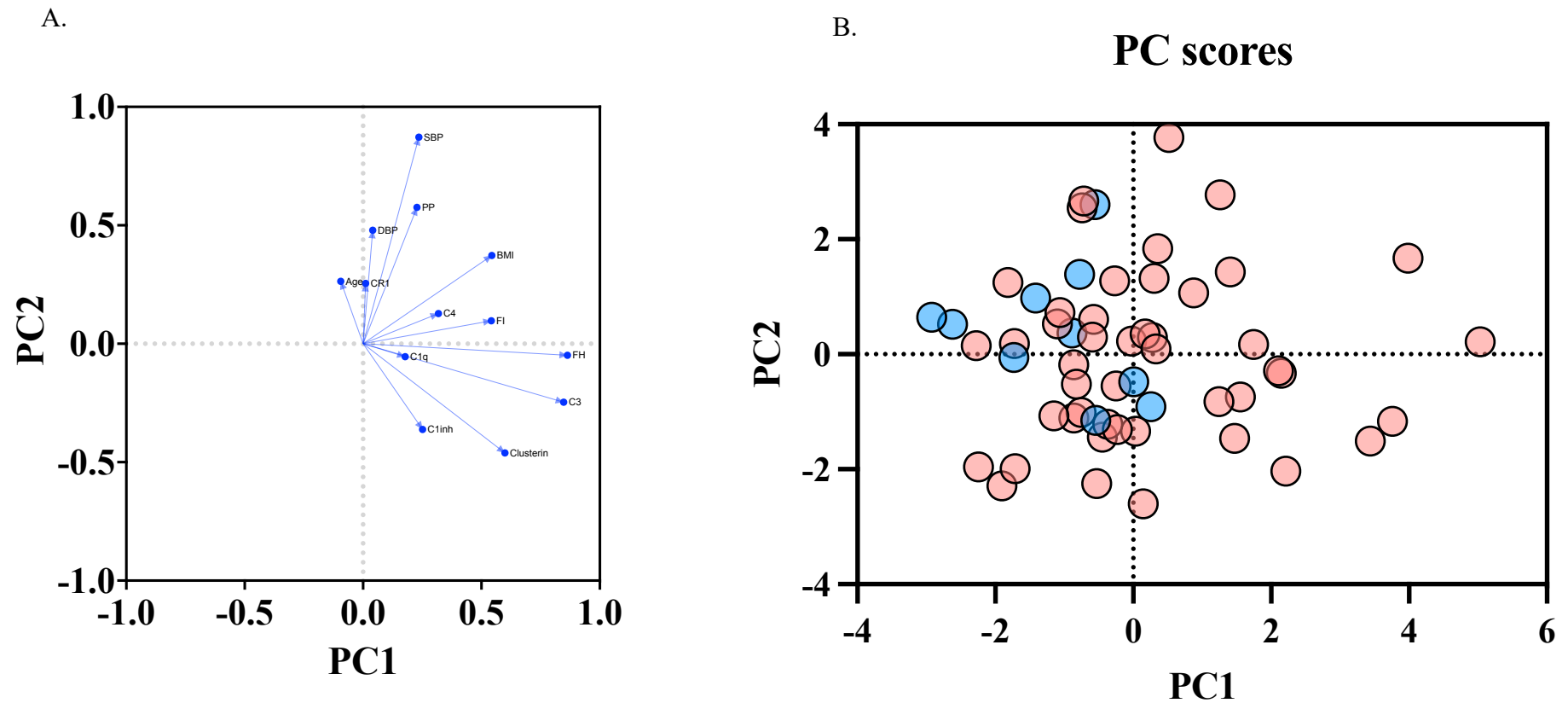


Figure 35. A) Loading plot for principal components 1 and 2, displaying the relative factor contributions for each PC. B) Principal Component score chart for PC1 and PC2, colour coded based on dementia status (DSdem = blue; DScn = pink).

Section Summary

The results present evidence of complex interactions between lifestyle, dementia status, and complement markers. Despite only small effects there are differences in complement factors based on CVP status. Overall the results are suggestive that exercise may have anti-inflammatory properties whereas AD appears to be pro-inflammatory. Complement activation occurs in those with raised BMI, and compounding risks of lifestyle, dementia status and sex may alter the inflammatory profile of people with DS.

There were associations between complement factors and NfL although these were weak correlations. CR1 had a negative association with NfL which trended towards significance. CR1 levels were increased in the high combined risk group, and this relationship was strengthened with the addition of raised BMI as a risk factor. On the other hand, C4 and C1q were positively associated with NfL and p-tau181. C4 is a non-specific complement protein that is activated by C1q. Both C4 and C1q can be co-located around amyloid-beta plaques and these findings support the hypothesis that increased activation of certain complement factors is involved in the pathogenesis of AD in DS. GFAP has been associated with both neurodegeneration and neuroinflammation and the trend towards a relationship between C1q and GFAP adds further weight to this. There were differences between the group with dementia and those without. In particular people with dementia had lower FI levels and higher C1q. There was a trend towards C3 and clusterin being lower in dementia.

The link between CVPs and complement factors were relatively modest, and the analysis may be underpowered given the size of the sample. Despite this there were patterns related to CVPs and complement profiles. Higher levels of exercise were associated with higher clusterin and

lower CR1 levels which may represent anti-inflammatory mechanisms related to exercise, or enhanced regulation of complement in people engaging in greater amounts exercise.

High BMI was associated with higher FI, whereas low FI was associated with dementia. Low BMI is now well recognised as a change observed in many people with DS and dementia. It is not clear whether the low FI levels observed in the dementia group are secondary to low BMI or related directly to AD-related pathology. Strikingly BMI co-located with complement components in the principal component analyses, but not with other cardiovascular parameters, or plasma biomarkers apart from A β 42/40 ratio.

7.8. Chapter Summary

There is evidence that cardiovascular risk factors interact with cognition and dementia-related pathology in people with Down syndrome. The patterns appear to be particularly pronounced in people over the age of 35 which may reflect the long-term, cumulative nature of these effects. This is consistent with literature in sporadic AD where mid-life risk factors affect later risks of cognitive decline and dementia, rather than affecting cognition or neuropathology at that time point.

Blood pressure parameters were associated with amyloid-beta proteins, whilst BMI was related to complement factors. Low SBP was associated with higher levels of GFAP and NfL, and low PP trended towards the same association with GFAP. On the other hand, PP had a positive association with p-tau181 in younger people. There was a trend for increased NfL to be present in people with multiple risk factors, and these effects were present in both cross-sectional and

longitudinal analyses. The results provide tangible hints towards the mechanisms through which CVP status may affect incident dementia risk in people with DS.

Reviewing the hypotheses presented at the beginning of this chapter, raised BMI was associated with increased markers of inflammation, but was not clearly associated with increased plasma amyloid-beta levels. Low blood pressures were associated with increased markers of neuronal injury, particularly longitudinally in the older cohort. On the other hand, low PP was associated with low p-tau181 in the younger cohort. There were cumulative effects of risk factors, and this was associated with higher levels of NfL but not GFAP. There was not a link between CHD and low blood pressure, nor increased measures of neurodegeneration. CHD did affect cumulative risk with an additive effect. People with hypothyroidism did not have noticeable inflammatory dysregulation compared to those without. Higher levels of exercise were associated with better cognitive performance, but there were limited associations at the biomarker level.

Potential pathways and mechanisms explaining these results and how the link to dementia pathogenesis will be considered in the following discussion sections.

Chapter VIII

Cardiovascular Parameters and their Relationship to Cognitive and Blood-based Markers of Dementia Using Data from the LonDowns Prospective Cohort Study

Part B. Discussion

Chapter Contents

- 8.1. Results Summary
 - 8.1.1. Relationships Between Cardiovascular Health and Cognitive Outcomes
 - 8.1.2. Patterns of Plasma Biomarkers
 - 8.1.3. Relationships between Cardiovascular Health and Plasma Biomarkers
- 8.2. Plasma Biomarkers in Context
 - 8.2.1. Amyloid-beta
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- 8.3. Potential Mechanisms Underlying the Observed Associations
 - 8.3.1. Blood Pressure
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- 8.4. Plasma Biomarkers and Dementia in People with Down Syndrome
- 8.5. Chapter Summary

8.1. Results Summary

8.1.1. *Relationships between Cardiovascular Parameters, and with Cognitive Outcomes*

Results from the LonDowns cohort study corroborate findings from the population analysis presented in chapter 4. Males and females in the cohort had minimal inter-participant sex and age differences in cardiovascular parameters (CVPs). Only BMI showed a significant increase in females compared to males (mean BMI 32.54 kg/m² (SD 7.36) in women compared to 27.34 kg/m² (SD 5.42), in men). BMI was also the only CVP that varied significantly with age, increasing in both sexes until a peak occurred at approximately age 40 years.

Blood pressure parameters (SBP, DBP and PP) did not correlate with BMI or age. There was a positive correlation between SBP and PP (0.65), and a negative correlation between DBP and PP (-0.42). Blood pressure measurements and BMI were modulated by exercise level. BMI was lowest in the high exercise group, and SBP trended towards being lower in the high exercise group. There was a negative correlation between BMI and CAMDEX (-2.40). This is in contrast to results in the population analysis where high BMI in younger adulthood was associated with increased risk of incident dementia. The difference is likely due to the cross-sectional nature of the cohort analysis, and the older population group.

8.1.2. *Patterns of Plasma Biomarkers*

At a whole group level NfL, p-tau181 and GFAP showed a positive correlation with age (0.79, 0.72, and 0.66 respectively). NfL and GFAP were positively correlated with each other (0.78), as were NfL and p-tau181 (0.73), and p-tau181 and GFAP (0.73). Neither NfL nor p-tau181

were associated with other biomarkers, whereas GFAP had a modest positive correlation with A β 40 (0.23).

Whilst NfL, p-tau181 and GFAP all increased with age there were slight differences in the pattern with evidence of non-linear relationships. The rate of increase was higher after the age of 40 years (cubic R^2 0.55 for NfL; quadratic R^2 0.41 for p-tau181; quadratic R^2 0.50 for GFAP). NfL showed the greatest increase with age. GFAP and p-tau181 values showed greater variation, and the increase at older ages was less steep than for NfL. This suggests that GFAP and p-tau181 may be more affected by non-ageing related processes than NfL in people with DS.

NfL, p-tau181 and GFAP increased in people with dementia at greater magnitudes than in people without dementia. The weak association between GFAP and A β 40 may therefore reflect the decreases in A β 40 observed in people with dementia, creating a non-linear relationship in later years. It was surprising that p-tau181 did not correlate with A β markers. This may be because amyloid processing can be dysregulated in people with DS throughout life, whereas p-tau181 appears to be a clearer marker of later AD pathology. It may also be related to the relatively lower sensitivity that SIMOA has for A β measurements, the small sample size leading to underpowered analyses, or the differences between plasma and brain levels of these biomarkers.

NfL, p-tau181 and GFAP were positively correlated with CAMDEX score which is a marker of dementia-symptom burden (0.54, 0.58, and 0.55 respectively). NfL, p-tau181 and GFAP were negatively correlated with cognitive outcomes measured using PALFAMS, VF and SABS (NfL correlations -0.44, -0.39 and -0.37; p-tau181 correlations -0.47, -0.29, -0.39; GFAP

correlations -0.42, -0.40, -0.43 respectively). In contrast the amyloid-beta protein markers (A β 40, A β 42 and A β 42/40 ratios) were not correlated with any of the cognitive outcomes analysed. NfL, GFAP and p-tau181 reflect neurodegeneration and neuronal injury, and the extent of damage corresponds with clinical signs of dementia and cognitive deficits. Plasma amyloid-beta concentrations do not have the same direct relationship with clinical outcomes. Changes in A β may represent earlier stages in dementia pathology rather than later-stage neurodegeneration, or relate more directly to general amyloid-beta burden in people with DS, instead of AD pathology.

Out of the complement components examined only C4 was correlated with age (0.26). C4 correlated with NfL (0.26) and p-tau181 (0.25) which were also correlated with age. NfL and p-tau181 were positively correlated with C1q which was found to be increased in people with dementia. GFAP did not show the same association with complement and was not significantly correlated with C4 or C1q as may be expected. GFAP levels may be affected by processes in addition to AD neuropathology, modulating its relationship with other biomarkers such as C4, C1q and A β .

8.1.3. Relationships between Cardiovascular Parameters and Plasma Biomarkers

The results indicate that blood pressure, BMI and exercise are related to outcomes measured at a cognitive and biomarker level. Low systolic and pulse pressures are related to raised amyloid-beta markers, whereas BMI was associated with raised inflammatory markers. NfL, p-tau181 and GFAP were most strongly associated with other markers of dementia rather than CVPs, but in the older sample low SBP and PP at baseline were related to increased NfL and GFAP

five years later. Exercise on the other hand appeared to promote better cognitive functioning, most strikingly observed in higher scores in a verbal fluency task in people engaging in more exercise. There were limited associations at a biomarker level.

Presented trends imply that BMI and blood pressure risks are associated with different mechanisms. Blood pressure parameters were associated with A β changes, whereas BMI was more closely correlated with GFAP and complement, which are measures of inflammation. The lack of correlation between blood pressure parameters and cognitive outcomes indicates that the increased risk of incident dementia associated with low PP and extremes of SBP/DBP may be mediated via promoting AD neuropathology in the long-term rather than causing a direct negative effect on cognition or causing neuropathology. BMI correlated with dementia symptomology most strongly (CAMDEX-DS) with weaker associations between less specific cognitive outcomes (PALFAMS, VF, SABS). BMI may therefore promote dementia neuropathology as a primary mechanism. The lack of association between BMI and A β proteins is unexpected. Obesity has been linked to increased A β levels⁴⁶⁶. The lack of association may be related to the generally higher levels of A β present in the DS population, sample size, or represent a difference in A β processing in people with DS.

In both younger and older adults there were trends towards increased markers of neurodegeneration associated with higher number of cumulative risks, but the relationship was more pronounced in the older adult cohort. The different biomarker outcomes and CVPs will be considered in turn to discuss potential pathways explaining the observed results.

8.2. Plasma Biomarkers in Context

8.2.1. *Amyloid-beta Proteins*

A β is formed by the proteolytic cleavage of amyloid precursor protein (APP) and is continuously generated throughout life. A β is primarily produced in the brain and transported into the CSF and plasma where it is broken down. Dysregulation of the APP processing pathway occurs in AD, leading to increased concentrations of APP in brain tissue, with a shift towards beta and gamma proteolytic pathways^{77,467,468}. This enhances production of A β 42 which is more prone to aggregation and deposition compared to other A β fragments.

Whilst it has been well-established that A β levels are increased in DS and are related to AD development, there is a lack of consensus about the patterns of plasma A β in association with dementia onset. AD has been described as a consequence of chronic imbalance between A β production and clearance⁷⁷. Levels of A β , particularly A β 40, decrease around the time of AD onset in the general population, and low A β levels at this time point are hypothesised to represent increased deposition in cerebral plaques. In DS raised levels of plasma A β 40 and A β 42 have also been associated with dementia²⁸⁸. These differences likely reflect samples being taken at different disease stages and from different populations, and highlight the difficulties in interpreting plasma amyloid-beta levels in isolation.

The relationship between neuropathology and plasma A β prior to dementia onset is not entirely understood. Soluble oligomeric forms of A β have been identified as playing key roles in synaptic disruption, activation of inflammatory pathways and resulting cognitive dysfunction prior to plaque formation⁴⁶⁸. Higher plasma A β could represent increased production in the brain, indicate poor peripheral breakdown of A β , or be a result of both conditions. Higher

plasma A β levels prior to dementia onset may therefore represent amplified dysregulation of amyloid processing which itself is associated with increased neuronal injury and synaptic loss. It is recognised that soluble A β can cause cognitive and neuronal dysfunction before more overt neuropathology is present⁴⁶⁹. Therefore higher levels of A β 40 and A β 42 could be an AD-promoting state in people with DS.

8.2.2. *NfL and GFAP*

NfL increases with age in people with DS and the general population. Higher levels have been well documented in AD both in the general population and in people with DS¹⁵³. Increased NfL is a marker of increased neurodegeneration and indicates greater neuronal damage. It is a non-specific marker but, due to the high prevalence of AD in people with DS, is also viewed as a representative marker of AD-pathology in people with DS.

GFAP increases with ageing due to increased transcription in astrocytes. The association between increased GFAP transcription with advancing age has been postulated to be the result of increased oxidative damage which also occurs with ageing⁴⁷⁰. Hence increases in GFAP are an indicator of reactive gliosis. Increased expression of GFAP has been observed as a compensatory response to cerebral ischaemia, and GFAP-nul mice were more vulnerable to cerebral ischaemia⁴⁷¹. Higher levels of GFAP may therefore be interpreted as a marker of increased oxidative stress and of cerebral ischaemia, as well as being associated with age-related neurodegeneration.

8.2.3. *P-tau 181*

Hyperphosphorylation of tau proteins is observed in AD, and leads to the formation of characteristic neurofibrillary tangles. Raised plasma p-tau181 levels are associated with clinical symptoms of dementia, but the increases are observed later in the disease progression than other markers^{300,301}. Plasma p-tau181 has been identified as a robust marker of AD in the general population²⁹⁸. In people with DS p-tau181 has been found to correlate with other dementia indicators including cerebral atrophy and hypoperfusion²⁹⁹. The relationship between p-tau and DS prior to dementia onset has not been established. Total tau appears to decrease from childhood into young adulthood before increasing again in later years, but patterns of p-tau were not reported²⁹⁰. P-tau 181 can therefore be considered a marker of dementia severity, with higher levels in older adults reflecting greater neuropathological burden of disease.

However, p-tau181 may also be useful in identifying other pathological processes. In a study using mice models of AD (3xTg-AD mice), transient hypoperfusion was induced to create a state of oligoemia (rather than frank ischaemia). The researchers measured tau and amyloid-beta markers following this⁴⁷². Increased levels of A β proteins were found in hypo-perfused mice, which was speculated to represent enhanced β -secretase activity. In contrast, decreased levels of tau were present, with lower levels of both total-tau and phosphorylated tau, and there was evidence of dysregulated phosphorylation. This suggests that tau could be a marker of chronic hypoperfusion in people with genetic risk of AD, such as people with DS.

8.3. Potential Mechanisms Underlying the Observed Associations

8.3.1. Blood Pressure

At a whole group level lower systolic and pulse pressures were associated with higher A β 40 and A β 42. Higher DBP was associated with lower A β 42 and A β 42/40 ratios. In older adults there was an association between low SBP and PP with higher NfL longitudinally; higher DBP was associated with poorer PALFAMS performance. In younger adults lower SBP was associated with higher GFAP. There was a positive association between PP and p-tau181. Low blood pressure was therefore associated with increased levels of plasma A β and NfL and GFAP, but reduced p-tau181, with differences in the relationships between younger and older adults.

In the Honolulu ageing study mid-life BP measures (assessed at a mean age of 58.9 years) were compared to A β measures collected approximately eight years later (at a mean age of 67.2). A β 40 and A β 42 levels increased as BP levels increased⁴⁷³. An interaction between DBP and amyloid-beta proteins was identified. Higher DBP in mid-life was associated with greater A β -related risk for AD at later follow up. People with higher DBP who displayed later decreases in A β 40 (an early indicator of AD) were at increased risk of developing AD. The associations with systolic and pulse pressures were not significant. It was hypothesised that elevated blood pressures compromise vascular integrity with reduced A β clearance and increased levels of CAA leading to increased risk of AD.

In middle-aged cognitively normal participants aged between 47-68 years, both systolic and pulse pressures were positively correlated with cerebral amyloid-beta burden as measured via Pittsburgh Compound-B (PiB) in Positron Emission Tomography (PET) scans⁴⁷⁴. DBP was

inversely correlated with A β so that lower DBP appeared to be associated with higher load. There was no effect of *APOE* status on the relationship. The authors identified PP as a potentially more sensitive measure of blood pressure risk than SBP in younger adults, prior to onset of significant arterial stiffness with age.

In a study of 51 people with AD, 36 people with MCI and 53 controls, a negative correlation between DBP and plasma A β 40 was reported and this was consistent in the dementia, MCI and control groups⁴⁷⁵. In contrast, in the LonDowns cohort DBP was positively associated with A β 40 and A β 42 levels at a whole group level and higher DBP was associated with worse cognitive performance in the older adults.

In the SPRINT study (systolic blood pressure intervention trial), the consequences of hypertensive management were determined⁴⁷⁶. People who received more intensive blood pressure management which sustained SBP below 120mmHg experienced larger increases in NfL compared to those with standard treatments. The difference was attributed to corresponding changes in the haemodynamics of renal perfusion as there were a higher frequency of eGFR reductions observed in the intensive treatment group, but without increases in markers of acute kidney injury. The results suggest a possible explanation for the link between low SBP and raised NfL at follow up which was observed in the older adult LonDowns cohort. If low SBP and reduced renal perfusion lead to increased NfL, then the mechanism could be chronic hypoperfusion leading to neuronal injury and neurodegeneration. Reduced perfusion could also contribute to reduced A β clearance.

Pulse pressure has been identified as a predictor of plasma A β 40 levels. Higher PP values (>50mmHg) were associated with greater concentrations of A β 40 in a general population

study⁴⁷⁷. It was suggested that this was secondary to impaired A β clearance and greater burden of A β in brain tissue as there was a reduction in the expression of A β transporter proteins in people with higher PP. In people with DS there are associations between PP and A β , but in the opposite direction to those reported in the general population; low PP was associated with raised plasma A β protein concentrations. Low PP rather than high PP may interfere with peripheral A β clearance and promote increasing A β in people with DS.

Existing research supports the hypothesis that low PP is an indicator of chronic hypoperfusion. In the study inducing hypoperfusion in 3xTg-AD mice discussed above, low levels of tau and raised levels of A β were observed following hypoperfusion⁴⁷². Despite this being a transient event, the effects on the biomarkers were evident for a few weeks. It can be speculated that chronic hypoperfusion may therefore result in similar effects on cerebral tissue. The results presented in this thesis mirror those reported in the mice study with low PP being associated both with raised A β and reduced p-tau181.

High blood pressures are associated with cerebral hypoperfusion secondary to cerebral autoregulation failures. These also leave the system more vulnerable to damage in the event of sudden blood pressure changes⁴⁷⁸. An inverse association could be present in DS due to the different blood pressure profiles and the low occurrence of high blood pressures. Low systemic blood pressures in combination with compromised cerebrovascular autoregulation could exert a similar pathological effect as high blood pressures, promoting cerebral hypoperfusion, further A β deposition and earlier incidence of dementia symptoms. Low blood pressures may also compromise the integrity of the blood brain barrier. This is supported by the fact that low SBP and PP were associated with increased levels of GFAP. GFAP is associated with astrocytes which form an integral part of the blood brain barrier and are essential in maintaining its

function⁴⁷⁹. Higher levels of GFAP have been found in people with cerebrovascular compromise and following ischaemic strokes⁴⁸⁰⁻⁴⁸².

The relationship between raised DBP and amyloid-beta markers ($A\beta_{42}$ and $A\beta_{42/40}$ ratios) provides insight into how risks may accumulate. Low PP results from SBP and DBP pressures being closer in value. This can be driven primarily through lower SBP values or higher DBP values. In the LonDowns cohort PP and SBP were positively correlated, whereas PP and DBP were negatively correlated. Low PP in people with DS therefore appears to be a product of reduced SBP and higher DBP. Higher DBP can occur in the presence of increased atherosclerosis. Hence people with DS may have low SBP which increases the risk of hypoperfusion and neurodegeneration in the long-term, evidenced by the association with raised GFAP and NfL longitudinally. Those with high DBP alongside low SBP (and hence low PP) may have additional atherosclerotic risks which could further affect cerebrovascular integrity. The relative risk of atherosclerotic disease in those with high DBP could increase incidence dementia risk compared to other people with DS, despite low prevalence of atherosclerosis overall. The relative homogeneity of blood pressures in DS may explain why PP was more sensitive in the population analyses than SBP and DBP measures, but does not mean that both SBP and DBP are not important in the pathogenesis of AD.

8.3.2. *Body Mass Index*

BMI was positively correlated with increased FH and FI. There was a positive relationship between C3 and BMI but this was moderated when sex was included in the model. BMI was negatively correlated with GFAP. The GFAP relationship was retained in the older cohort when

the sample was stratified on age group, but was not present in the younger cohort. The relationship between BMI and complement, and BMI and GFAP will be discussed separately.

The complement system is part of the innate immune system which promotes and regulates inflammation. Obesity is associated with chronic activation of the innate immune system within adipocytes, with accompanying increased production of pro-inflammatory molecules, including complement components⁴⁸³. Upregulation of complement factors have been observed in adipose tissue, particularly those early in the complement pathways.

C3, FH and C1q can be synthesised by adipose tissue and exaggerated activation of the classical component pathway is observed in obesity⁴⁸⁴. Raised complement factors C3 and C4 have been reported to be indicators of metabolic syndrome and reveal an association between obesity and increased complement activation⁴⁸⁵. In the LonDowns cohort raised BMI was associated with raised FH and FI. There was a relationship between C3 and BMI, but this was modulated by the effects of sex, with women having higher C3 values than men. The lack of adjusted association between C3 and BMI is likely due to the fact that in DS women have higher BMI rather than suggesting that there is no association between BMI and C3.

The consequences of obesity in relation to inflammation have been categorised into three main phases: an initial stressor related to positive energy balances in adipocytes; an acute inflammatory response; and a longer-term pathological phase⁴⁸⁶. The positive correlation between BMI and complement components in people with DS identified in the LonDowns cohort supports the hypothesis that obesity promotes additional inflammation in this population. Activation of the complement system causes increased synaptic labelling and clearance thereby increasing neuronal loss and worsening neurodegeneration. Knock-out mice

models of AD (generated without specific complement components) demonstrated similar A β deposition patterns to non-knock out mice but maintained better cognitive function⁴⁸⁷. This suggest that neuroinflammation may be a driver for neuronal destruction which is activated by A β .

Factor H and I are regulators of the complement system. Increased levels of these factors associated with obesity could indicate increased activation of the complement system and subsequent increased expression of regulators in people with higher BMI. On the other hand, the association could be viewed as a reduction in FH and FI in people with lower BMI. Knock out mice without FH have uncontrollable activation of the alternative pathway, with excess consumption of C3 and extremely low levels of serum C3. In the LonDowns cohort, people with dementia had lower levels of FI, FH and C3 than people without dementia which could represent a similar dysregulation process occurring.

Mirroring this is the negative association observed between BMI and GFAP. This is likely secondary to confounding effects of dementia processes wherein weight loss is associated with dementia onset, rather than raised BMI having direct neuroimmune dampening effects. This is supported by the results of the combined risk group. People in the higher combined risk group had raised CR1 levels (another complement component) and this association was strengthened when raised BMI was added as an additional risk factor. CR1 is involved in the removal of immune complexes opsonised by C3 and C4 components and regulates the classical and alternative pathways, preventing onward activation cascades⁴⁸⁸. CR1 is implicated in A β clearance. Reduced CR1-amyloid complex binding has been found in people with AD compared to controls, suggesting dysfunctional A β clearance in AD. The presence of obesity

may accelerate inflammatory processes earlier in life, leading to increased dysregulation and burn out in later years, amplifying cycles of degeneration.

Chronic inflammation has long-term sequelae and can negatively impact cognition and promote neurodegeneration. Hence obesity in middle-life in people with DS may promote a pro-inflammatory environment which can accelerate neuropathological changes as a person ages. There is evidence that obesity promotes inflammation in people with DS and may enhance the negative effects of other cardiovascular risk factors. However the relationships are obscured by confounding effects of AD neuropathology and subsequent weight loss making it difficult to untangle the pro-inflammatory effects of obesity from the amyloidogenic processes associated with weight loss in a small sample of people with mixed ages. There is likely a balance of benefits and negatives from inflammatory processes depending on age, stage of development and pathology. Further work is needed to understand how inflammatory processes evolve and influence pathology in people with DS^{93,175}.

8.3.3. Exercise

Higher levels of exercise were associated with better cognitive performance, particularly on a verbal fluency task, and trended towards lower CAMDEX scores which are indicative of lower levels of dementia symptomatology. This is in keeping with initial work performed for this thesis and wider literature reporting enhanced cognition after acute and longer term exercise programmes in both the general population and people with DS (see appendix 1).

On the other hand there were no significant associations between exercise and AD-related biomarkers in adjusted models. Other studies comparing the effects of exercise at a biomarker in people with DS have not found a relationship between physical activity and biomarker changes, despite reproducing cognitive benefits^{133,489,490}. The results from the LonDowns cohort would be in keeping with these findings. Exercise may stimulate cognitive benefits via other pathways rather than modulating amyloid- β -related neuropathology. For example, exercise may instead enhance cognitive reserve via increasing Brain-Derived Neurotrophic Factor (BDNF) levels or by promoting angiogenesis³⁰⁵. People with increased cognitive reserve may be able to sustain cognitive abilities for longer in the presence of AD-related neuropathology. The results in the older adult cohort support this theory. People who engaged in higher levels of exercise had higher PALFAM scores at both baseline and T1 than people with engaged in low levels of exercise, but a similar deterioration was observed in both groups between baseline and T1.

The high exercise group was associated with increased levels of clusterin. In a recent study, higher plasma levels of clusterin were observed in people with cognitive impairment following six months of structured exercise, and clusterin was associated with reduced expression of neuroinflammatory genes in complementary experiments⁴⁹¹. The role of clusterin is controversial and other studies have linked higher clusterin levels to increased risk of AD⁴⁹². These inconsistencies reflect the multifunctional nature of clusterin, and interpreting its levels is likely to be context dependent⁴⁹³. Relationships between other complement components with exercise have not been closely studied. In a study of 15 endurance athletes, suppression of CR1 was reported following acute exercise, with greater effects after longer durations of exercise⁴⁹⁴. Similar results were found in male rats⁴⁹⁵. This supports the hypothesis that exercise may exert positive effects partly via reduction of inflammation.

8.3.4. Congenital Heart Disease and Hypothyroidism

There were no clear associations between CHD with cognitive outcomes or plasma biomarkers. The presence of CHD has been associated with increased dementia risk in other studies although there is limited information at present⁴⁹⁶. The lack of associations here may be for a number of reasons. CHD may not directly affect cognition and therefore not relate to the cognitive outcomes assessed in this analysis. Alternatively the effects of CHD may occur over a longer period, not captured here. The numbers of older adults with CHD in this sample is lower than would be expected which may lead the analysis to be underpowered, but may also be an indicator of missed diagnoses which mean the CHD group is not representing all of those people with CHD. There may also be survival effects, with fewer older adults with CHD living into older age.

Hypothyroidism has not been consistently associated with dementia risk in the literature. As explored in chapter 6, hyperthyroidism has also been implicated. The trend towards higher NfL in people with hypothyroidism as part of a combined risk may suggest that hypothyroidism potentiates neurodegeneration and could therefore contribute to risk of decline in a cumulative fashion not demonstrated in the time frame examined. This suggests that the effects may be relatively modest or develop over a longer time period.

Hypothyroidism was not associated with changes in the complement components assessed, despite being implicated in autoimmunity. This may be because the complement system is a closer marker of inflammation rather than specific autoimmune status, or a larger sample size may be required to observe any differences. It is also of note that in this analysis, people with DS and hypothyroidism were compared to people with DS without hypothyroidism, rather than

controls. People with Down syndrome have immune and inflammatory dysregulation regardless of thyroid status and therefore the differences in complement components may be attenuated⁴⁷.

8.3.5. Cumulative Risk

People with a greater number of risk factors had higher levels of NfL which is suggestive of greater neurodegeneration. The relevant risk factors were low pulse pressure, low exercise levels, history of CHD and a diagnosis of hypothyroidism. Raised BMI was not a clear risk indicator in the cohort analysis, and results were confounded by the association between low BMI and dementia onset which was particularly prominent in the older adult cohort. There are a number of possible reasons why higher numbers of risk factors may increase neurodegeneration.

Interactions between A β , blood lipids and blood pressure have been reported. In a cross-sectional study of people aged between 40-85 (mean age 55.7 years (SD 10.2)), blood lipids were not correlated with plasma A β levels. However, when the sample was stratified by blood pressure, associations were identified. In people who were normotensive higher levels of total cholesterol and low density lipoproteins were associated with higher levels of plasma A β 42. In hypertensive people (with SBP >140mmHg or DBP>90mmHg) low LDL was associated with elevated plasma A β 40⁴⁹⁷.

Similarly an interaction between blood pressure and obesity may occur. It has been hypothesised that inflammation related to obesity may be triggered by relative hypoxia of

adipocytes as they expand and consume additional oxygen⁴⁹⁸. People with DS often have low blood pressures and relative hypoperfusion. This could contribute to further hypoxia of adipocytes and exacerbate inflammation. The presence of inflammation and reactive oxygen species (ROS) can modify APP leading to increased A β production⁴⁶⁹. A perpetuating feedback loop potentiating risks could therefore be established.

The presence of CHD in the general population has been associated with increased dementia risk, and it may be that CHD interacts with other processes in a cumulative manner. For example, CHD could exacerbate vascular perfusion difficulties, amplifying the risks associated with low blood pressures. There is evidence that alternations in brain structure related to congenital heart disease may affect brain development with adverse effects on executive functions and other cognitive skills⁴⁹⁹. Hence these people could be more vulnerable to early dementia related decline, especially in the context of other risk factors. Thyroid dysfunction has been associated with increased dementia risk, although the exact mechanisms are debated. Further research into the neuropathological associations between CHD and thyroid dysfunction is required to better understand the nature of these risks.

8.4. Plasma Biomarkers and Dementia in People with Down Syndrome

Plasma biomarkers were used in this study in an exploratory capacity as a means to understand the mechanisms through which CVPs may affect dementia onset and underlying neuropathology. Despite not being the primary focus, the results support the use of plasma biomarkers to augment clinical dementia diagnosis in people with DS. In particular, NFL, GFAP, and p-tau181 demonstrated clear associations with dementia symptomatology,

measured through both cognitive outcomes and clinical dementia diagnoses, with a high level of discrimination. In principal component analyses, one person with features of dementia but without a clinical diagnosis co-located with the dementia group. It is easy to see how the use of biomarkers alongside clinical assessment may improve confidence and accuracy of diagnoses in such individuals. The relationship between plasma complement components and dementia diagnosis has not been established. In this study, C1q and C3 showed promise as potential markers of AD, and suggest that inflammatory processes contribute to neuropathology.

In addition, this study suggests that plasma biomarkers may be useful in quantifying and predicting dementia risk in younger people with DS, as well as identifying potential targets for intervention. The presence of multiple risk factors was associated with raised NfL and GFAP, suggesting that people with poorer global health may have more rapid accumulation of neuropathology in intervening years. By monitoring NfL and GFAP in response to medical management of these conditions the effectiveness of the treatments on dementia risk could be assessed. Similarly, younger people with low blood pressures, low tau and raised A β levels could be experiencing chronic hypoperfusion leading to long-term cerebral compromise and earlier dementia onset. By measuring these factors together it may be possible to quantify risks associated with CVPs and identify people who may need further intervention. For example, if someone presents with low blood pressure but normal plasma biomarkers this may provide reassurance that cerebral homeostasis is being maintained. On the other hand, a patient with low blood pressure and altered biomarkers may be a candidate for intervention trials to improve cerebrovascular health. Due to the complex interplay between blood pressure and other homeostatic processes, mice models could be integral to pre-clinical research designs before interventions targeting vascular perfusion can be evaluated.

8.5. Chapter Summary

The results from the cohort study provide significant insight into how cardiovascular risk factors may influence cognition and pathways associated with AD neuropathology in people with Down syndrome. Most notably, risk factors appear to interact to negatively impact cognition and dementia pathology in a cumulative fashion, promoting increased disease burden and earlier onset of clinical symptoms. At a biomarker level, obesity was related to increased markers of inflammation, whereas low blood pressured appeared to promote dysregulation of A β and tau processing. Over the longer-term, the results of my analyses suggested that low blood pressures exacerbate neuronal injury, measurable using NfL.

In the following chapter the results from the population survival analyses and the cohort study will be synthesised to formulate a cohesive theory as to how cardiovascular risk affects dementia onset in people with Down syndrome.

Chapter IX

Combined Discussion and Conclusions

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- 9.1. Introduction
- 9.2. Summary of Research Questions
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- 9.6. Recommendations for Implementation and Future Research
- 9.7. Conclusions

9.1. Introduction

The preceding discussion chapters have considered analyses at population (results and discussion presented in chapters 4-6) and cohort study levels (results and discussion presented in chapters 7-8). The current chapter is focused on synthesising the data to consider overarching mechanisms and interconnecting pathways linking cardiovascular health status in people with DS and incident dementia risk. The benefits and limitations of the research methods are discussed, as well as clinical implications and areas requiring further research.

9.2. Summary of Research Questions

Given the strong associations between cardiovascular health and incident risk of Alzheimer's disease observed in the general population, it was hypothesised that cardiovascular health status would also affect dementia onset in people with DS. However, it was suspected that unique patterns of cardiovascular health parameters occur in people with Down syndrome compared to the general population. Therefore the cardiovascular profile in adults with DS was first defined using population data from electronic health records. This ensured that cardiovascular risk could be accurately understood in the context of trisomy 21. Following this, incidence dementia risk was calculated for key cardiovascular parameters in early adulthood. I then replicated the analyses in a cohort of individuals with DS who underwent physical health and cognitive assessments using a mixture of direct and indirect measurements. Blood samples were taken for exploratory plasma biomarker analysis; NfL, GFAP, p-tau181, A β 40, A β 42 and complement components were measured. These results were used to explore the relationships between cardiovascular health markers with clinical and biomarker changes in people with DS.

It was theorised that cardiovascular health status would be related to differences measurable at a biomarker level in younger people with DS, and predict changes at both a cognitive and biomarker level in older adults. These analyses could shed light on potential mechanisms that indicate why cardiovascular health status influences incident dementia risk in people with DS.

9.3. Key Findings

The results confirmed that people with DS have a distinct cardiovascular profile. Despite this there appear to be similar associations between the presence of hypertension and obesity in young adulthood and earlier dementia onset in people with DS and the general population. On the other hand, low systolic blood pressure (SBP) and pulse pressure (PP) were associated with increased risk of incident dementia in DS. This may represent a unique association in people with DS, or may reflect a common risk that is more prevalent in DS and therefore more readily detectable. Risks were additive, with higher incidence dementia risk in people with multiple risk factors.

Differences related to CVPs were observed at a biomarker level. Both SBP and PP were inversely related to A β 40 and A β 42 measures, with high levels of A β proteins in people with low SBP and PP. DBP showed a positive relationship with A β markers, and PP was positively associated with p-tau181. Longitudinally, low SBP and PP were associated with increased markers of neurodegeneration (GFAP and NfL) in older people with DS. Both low SBP and PP were therefore associated with increased plasma A β and dysregulation of processing pathways. There was evidence of age-specific associations between CVPs. Low DBP was

associated with increased NfL in younger people, whereas high DBP was associated with poorer cognitive performance in older adults.

Raised BMI in mid-life was associated with increased incidence dementia risk in the population analysis. In the cohort sample there were conflicting results. Low BMI, particularly in older adults, was associated with poorer outcomes on cognitive assessments and increased markers of neurodegeneration. This supports previous observations that declines in BMI are associated with AD pathology in people with DS. The direction of the relationship remains unclear, i.e. whether low BMI drives increased neuropathology or whether neuropathology and associated functional impairment lead to reductions in BMI. Despite the conflicting results, there were clues as to the how risks associated with raised BMI may manifest. A key observation was the disconnect between BMI and blood pressure in people with DS, and this was also evident at the biomarker level. Blood pressure and A β 40 and A β 42 levels corresponded with each other in principal component analyses, but not with BMI. Instead BMI only corresponded with complement components and the A β 42/40 ratio. This indicates that raised BMI is related to inflammatory pathways and associated with dysregulation of the complement system.

Unexpectedly, whilst exercise appeared to have neuroprotective effects demonstrated via enhanced cognitive performance, there was limited evidence of a positive impact at a biomarker level. The research presented here is supportive of exercise promoting cognitive reserve rather than directly influencing neuropathology. Other studies have similarly reported negative or limited biomarker changes in relation to exercise levels in people with DS, and the pathways through which exercise exerts cognitive effects remain unconfirmed.

Overall the results provide five key messages:

1. People with Down syndrome have a unique cardiovascular profile. Most noticeably they have lower blood pressures throughout adulthood, and there is a disassociation between blood pressure and body mass index measurements.
2. Low systolic blood pressure and pulse pressure are associated with increased incident dementia risk in people with DS. This appears to be via promotion of increased amyloid-beta processing dysregulation which may be the result of chronic hypoperfusion and failures of cerebral autoregulation.
3. Being overweight or obese in earlier adulthood increases risk of incidence dementia in people with Down syndrome, despite the confounding effects of BMI reductions which are associated with dementia onset in later life. The effects of obesity are at least partly mediated via inflammatory processes.
4. Exercise has a positive effect on cognition. The positive effects were not captured by biomarker changes. The mechanism in which exercise exerts cognitive benefits remains unclear in people with DS, and may involve unexamined pathways such as promoting angiogenesis, reducing oxidative stress, or increasing BDNF levels.
5. There is a cumulative effect of risk. People with higher numbers of cardiovascular risk factors experienced earlier decline and higher levels of neuropathology compared to people with fewer risk factors. The effects appear to be primarily mediated via increasing neurodegeneration and may be quantifiable by using NfL as a marker of risk burden.

These results suggest targets for therapeutic interventions to help optimise and maintain brain health in people with DS. This has implications for guidance on health care monitoring and

risk prediction for people with Down syndrome and suggests potential therapeutic targets to help maintain brain health and quality of life in the absence of definitive preventative or curative treatments.

9.4. Interpretation of Findings

9.4.1. *Blood Pressure*

To understand the results related to blood pressure and AD risk in people with DS, two separate but complementary processes present themselves which may act in synergy. Low blood pressure (driven primarily by low SBP) can be a sign of chronic systemic hypoperfusion. People with DS have disrupted vascular homeostasis as a result of autoimmune dysfunction. Therefore the effects of systemic hypoperfusion may translate more readily into deficits of cerebral perfusion because the autoregulatory mechanisms of the cerebrovasculature are compromised. Cerebral hypoperfusion leads to ischaemia of cerebral tissues indicated by increased expression of GFAP. Direct neuronal injury and release of NfL occurs, alongside activation of inflammatory systems.

Increases in DBP result in lower pulse pressures, a reflection of persistent low SBP in the presence of relatively raised DBP. Increased DBP is generally a marker of increased arterial stiffness often subsequent to atherosclerotic disease. Hence, people with DS and relatively raised DBP may be the people with higher levels of atherosclerotic disease, despite generally low prevalence. Alternatively, increased arterial stiffness in people with DS may be related to other, non-atherosclerotic pathways. Regardless of the causes of increased vascular rigidity, A β deposition has been associated with measures of peripheral vascular stiffness in the general

population. Increased A β burden has been related to higher degrees of arterial stiffness, with increased deposition over time in people with increased central arterial stiffness⁵⁰⁰. A similar pattern of increased A β was observed in people with high DBP and low PPs in the analyses presented here.

These two processes (chronic hypoperfusion and increased arterial stiffness) could explain the biomarker patterns observed and the increased risks associated with blood pressure changes in people with DS. Sustained low blood pressure may create a hypoxic cerebral environment resulting in disturbance of A β and tau processing: increased A β and reduced p-tau181 were associated with low PP. Over-time this could lead to neuronal injury and inflammation which further potentiates damage and causes NfL and GFAP to be released: increased levels of NfL and GFAP were associated with low SBP. Arterial stiffening, potentially as a result of atherosclerotic disease, may increase A β levels by reducing A β clearance and degradation: high DBP and low PP were associated with increased levels of A β 42 and A β 42/40 ratios. Toxic amyloid-beta oligomers are known to cause neuronal damage and inflammation and lead to plaque formation, with negative effects on cognition. Persistent hypoperfusion may exacerbate deficits in amyloid-beta clearing, promoting further A β deposition and neuropathology: in my analyses low SBP was associated with increased plasma A β , and DBP was inversely associated with PALFAMS score in the older cohort.

We can understand low PP measures in the context of DS to represent people with both a low SBP and relatively high DBP, and therefore may identify people who have two increased risks for the development of AD pathology. This could partly explain why low PP was a significant risk indicator in the survival models. The greater number of people with DS who have low PP rather than extremes of SBP or DBP may suggest PP as a more sensitive indicator of risk in

this group. The hypothesis above also accounts for the u-shaped pattern of risk associated with SBP and DBP observed, despite low numbers of people with high blood pressures. Both very low and very high values appearing to increase incident dementia risk. Low SBP and DBP could promote hypoperfusion; high values may represent people with more atherosclerotic changes and arterial stiffening. Both of these conditions appear to have an accelerating effect on AD-related neuropathology.

9.4.2. Body Mass Index

I found evidence that raised BMI induces complement activation and inflammatory processes in people with DS. Increased inflammation is associated with neuronal injury and neurodegeneration and could explain the relationship between raised BMI in early adulthood and increased risk of dementia incidence later in life. Complement dysregulation can drive inflammation and exacerbate disease. It is hypothesised that activation of the complement system causes increased synaptic labelling and clearance thereby increasing neuronal loss and worsening neurodegeneration. Knock-out mice models of AD without specific complement components showed similar patterns of A β deposition but better cognitive function than those with intact complement, suggesting that neuroinflammation may be the driver for neuronal destruction rather than direct effects of amyloid-beta⁴⁸⁷. However, intact complement pathways have also been shown to be integral for cognitive development and appropriate synaptic pruning and plasticity. The awareness of the role of complement in brain development has complicated our understanding of its significance in the development of neuropathology and AD, and suggests a multi-faceted contribution to disease prevention and acceleration¹⁷⁵.

Understanding pathology related to complement dysregulation may be affected by age, stage of cognitive development and other comorbidities⁹³.

Another potential pathway may be related to the link between obesity, diabetes and metabolic syndrome. Obesity is commonly associated with type 2 diabetes. Diabetes is another significant comorbidity associated with DS, and has been linked to the development of AD in the general population^{15,501}. In the analysis presented here, diabetes was not significant in the models as a predictor of incident dementia risk. People with DS have been shown to have increased insulin resistance associated with obesity, yet the other components of metabolic syndrome are not as commonly present³⁷⁷. The risks associated with diabetes and dementia in the general population could be linked to metabolic syndrome rather than diabetes in isolation. Perhaps in DS, obesity could be the primary metabolic factor driving dementia risk rather than secondary to other metabolic risks such as diabetes. In the general population there are increasing risks in obese compared to overweight individuals and the risk of developing metabolic syndrome is increased in obesity compared to being overweight⁵⁰². In this analyses both overweight and obese people with DS were found to have similar hazard ratios for incident dementia. The similar or lower levels of cholesterol related proteins and HbA1c in people with DS compared to controls is suggestive that in DS obesity mediated inflammatory pathways may be driving risk, rather than lipid pathways.

9.4.3. Exercise

It was not possible to examine the impact of exercise at a population level given the limitations of available, reliable data about exercise habits captured in CPRD. In the cohort study, exercise

did not prevent age-related decline between time-points, but people in the high exercise group performed better than those in the low exercise groups at both baseline and follow up. This pattern suggests that higher levels of exercise may increase cognitive reserve wherein individuals can tolerate higher levels of neuropathology before significant cognitive declines occur⁵⁰³. There were no strong associations between exercise groups and the AD-related biomarkers examined.

Various mechanisms have been proposed that may explain the link between exercise and improved cognitive reserve including upregulation of BDNF, enhancing neurotransmitter secretion, improving brain metabolism, promoting angiogenesis, and reducing co-morbid depression⁵⁰⁴. Studies involving people with Down syndrome have reported mixed results on the effect of exercise on cognition. Whilst a positive relationship between exercise and cognitive performance has been observed, the relationship with neuropathology and blood biomarkers is less established. In a recent study, higher levels of exercise were associated with higher composite memory scores, but were not directly associated with hippocampal volume. This is despite a relationship between greater memory scores and larger hippocampal volumes¹³³. Similarly, a positive relationships between higher activity levels and performance on cognitive tasks, with lower DLD scores (Dementia Questionnaire for People with Learning Disabilities) were observed. In the same study no associations between exercise and A β or tau measures using PET imaging techniques were identified. The only significant biomarker associations were between sedentary behaviour and poorer white matter integrity in specific cortical tracts⁴⁹⁰.

The results presented in this thesis are in keeping with the literature where exercise levels have not been strongly associated with AD biomarkers. There were suggestions in the data that

exercise exerts effects on inflammatory processes, with possible dampening effects. These anti-inflammatory effects could explain some of the positive cognitive benefits observed in the high exercise group. As discussed above, mice with lower levels of complement maintained better cognitive functioning in the face of similar A β loads compared to mice with intact inflammatory systems. Engaging in more exercise may therefore work as a disrupter to inflammatory feedback loops, reducing inflammatory responses to A β deposition and the subsequent neuronal injury.

9.4.4. Cumulative Risks

Alzheimer's disease has been conceptualised as a self-amplifying cycle of neurotoxicity, neuroinflammation and amyloid-beta production⁵⁰⁵. There is reason to believe this model is true in DS-AD as well as sporadic AD. In the population study (chapters 4 and 5), people with higher numbers of risk factors were at increased risk of incident dementia compared to people without any risk factors. This was mirrored in the cohort study (chapter 7) where higher NfL levels were present in people with greater numbers of risk factors. As NfL is a marker of neurodegeneration, this indicates that cardiovascular risk factors may interact to promote neuropathology in people with DS, enhancing dementia progression.

The unique cardiovascular, immune and metabolic environment in DS may modulate these processes and implicate different key risk factors, but the narrative that multiple complementary pathways increase risks appears to be as relevant for people with DS as the general population. By acknowledging the importance of additional pathways beyond A β overproduction as fundamental in the progression of AD in DS, a broader range of therapeutic

interventions can be identified to help delay onset. Better understanding of these processes also helps gives autonomy to people with DS to make informed lifestyle decisions to help mitigate risk in later life. A theoretical model of how key cardiovascular risk factors may interact with dementia neuropathology is presented in figure 36.

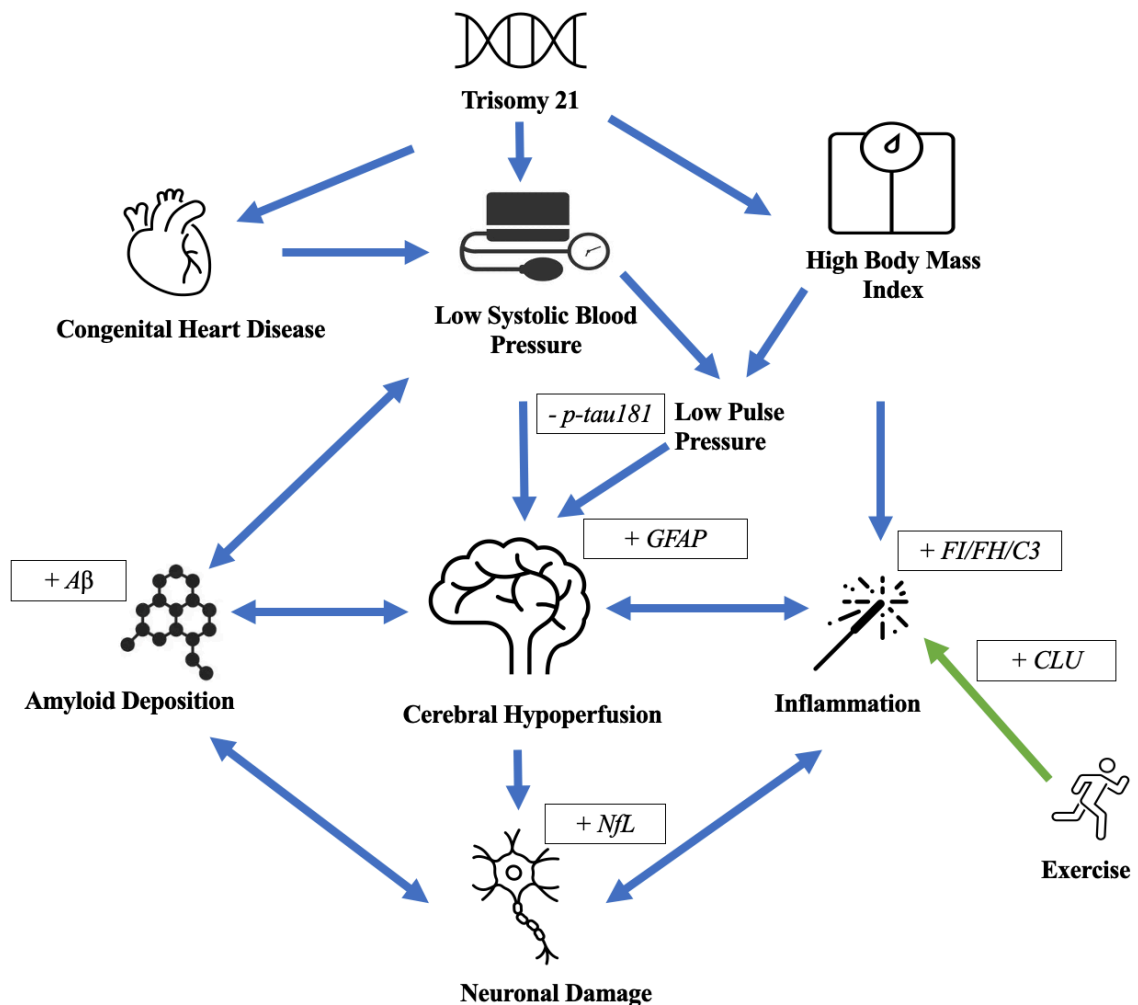


Figure 36. Potential interactions between cardiovascular risk factors and AD neuropathology. Blue arrows represent promoting effects. Green arrows representing diminishing effects. CLU = clusterin; $A\beta$ = Amyloid beta proteins 40 and 42; NfL = neurofilament light; GFAP = glial fibrillary acidic protein; FI = Factor I; FH = Factor H; C3 = complement component C3.

9.5. Strengths and Limitations

The ability to synthesise population data with data from individuals give this project both broad scope and depth which would not be achievable if either approach were used in isolation. Use of electronic health records allowed data to be collected from a large number of people with Down syndrome, with identification of longitudinal effects and comparisons with general population controls which would not be feasible to capture within a smaller cohort study. This also meant that representation from people at each year of age was included, something logistically challenging in smaller studies. Using clinical data means that real-world rather than theoretical circumstances were represented, increasing the clinical applicability of results. On the other hand, prospective cohort studies allow for the collection of in-depth individual data (including biomarkers) with scope to understand population trends at a person-level. This is essential to translate risks observed at a population level to processes that affect individual people and consider potential interventions. The approach of directly synthesising population and biomarker measures is relatively unique, especially in people with Down syndrome, and bolsters existing research by generating potential mechanistic explanations for observed risk trends combined in one study.

These approaches both have limitations. The CPRD uses data extracted from electronic health records which provide clinical quality data. Certain aspects are not well-recorded in this data, such as exercise and lifestyle factors. Similarly, data were not collected in a research environment meaning there are differences in when and why certain tests were performed for individuals. This can introduce biases, for instance if tests are only ordered in people at high risk of dysfunction then more abnormal results may be identified than are representative of the population. The methodological design of the study ensured steps were taken to reduce the

implicit biases present when using clinical health data. The CPRD was selected due to the high quality of the available data which has been analysed successfully in numerous previous studies including those which have been used to influence health care guidelines⁵⁰⁶. Commonly measured health parameters were used, including those which are measured in routine health checks. People DS were compared to age, sex and GP practice-matched controls to allow equivalent comparisons between parameters. Only data within “up to standard” periods within CPRD were included in the analyses, and multiple read codes were entered to ensure the data capture was robust.

An additional limitation related to the population analysis were the relatively low numbers of people with both CVP measurements available between the ages of 25-34 years and sufficient data after the age of 35 years to power survival analyses. This can be attributed to the ageing nature of the Down syndrome population as life expectancy increases, and the relatively recent introduction of reliable electronic health records which has been increasing since the 1990s. I anticipate that if the analyses were repeated in 5-10 years it is likely that a greater number of people would have the necessary data recorded which may improve the power of the analyses to allow for the additional CVPs (lipid profiles and HbA1c) to also be included. It was not possible to make comparative survival analyses using the matched general population sample given the differences in age trajectories for dementia in people with DS. The samples were censored at age 75, and this would lead to an under-estimate of the dementia risk in the general population and biased comparisons.

In the in-depth phenotyped LonDowns study there were potential power issues. Whilst a sample of 92 people was calculated as providing adequate power for regression analyses for NfL, smaller changes may not reach significance in a sample of this size. In addition, due to

limitations in biomarker processing availability related to the covid-19 pandemic there were fewer people who had GFAP, p-tau181, A β and complement data available. Repeating the analyses on a larger sample would increase the strength of the results.

The sample populations may also not be representative of all communities. The CPRD dataset is broadly representative of the UK population, but this differs from the populations of other countries. In the cohort analysis the sample was predominantly White British and the results may not translate for other ethnicities.

Finally, the methods presented here are based on observational studies rather than interventional. The results provide intriguing hints towards the relationship between cardiovascular health parameters and dementia processes, and demonstrate the potential utility of using plasma biomarkers alongside cognitive outcomes to quantify these effects. Directly investigating the consequences of exercise, blood pressure and BMI changes on cognitive and biomarker changes employing experimental designs would enhance the research presented here and develop the mechanistic theories.

9.6. Recommendations for Implementation and Future Research

Results outlined in this thesis can inform guidance designed to promote cognitive health in people with Down syndrome. Certain cardiovascular health parameters should be measured routinely as part of the annual health check. In particular, low blood pressures as well as high blood pressures should trigger further investigations. It is hard to determine exact cut-offs from the data analysed in this study, but systolic blood pressures consistently below 100mmHg, and

diastolic blood pressures consistently lower than 62mmHg may be indicators of chronic hypoperfusion. Pulse pressure should be recorded for people with DS and may provide a more sensitive measure of vascular health than SBP and DBP in isolation. People with low pulse pressures <40mmHg may be at increased risk of incident dementia. High blood pressures should also prompt treatment but these occur much less commonly in people with DS.

Support should be provided to help people with DS maintain a healthy weight, with target BMI <25kg/m² for younger people. Exercise interventions may be beneficial both in helping to maintain weight and to afford direct cognitive protection. People with Down syndrome should therefore be counselled on the positive effects of exercise. Local exercise facilities should be accessible for people with Down syndrome, and efforts should be made to identify physical activities that a person enjoys. People with congenital heart disease should be closely monitored and comorbidities adequately treated to reduce the cumulative effects of risk. Hypothyroidism appears to be a particularly pertinent risk factor which increases overall incident dementia risk in combination with cardiovascular risk. Thyroid function should therefore be carefully managed and monitored in people with DS throughout adulthood.

There are also significant implications for the use of plasma biomarkers as clinical diagnostic and risk prediction tools in people with DS. Longitudinal measurements of NfL and GFAP provide information about the rate of neurodegeneration and could be useful in monitoring cognitive health in response to interventions. Cross-sectionally, combinations of biomarkers and CVPs may enhance predictive risk models. For example, someone with low SBP and high A β or low p-tau181 may be at higher risk of incident dementia. This can inform patient counselling and planning. Such measures could also identify people most likely to benefit from intervention trials.

The association between GFAP, p-tau181 and NfL with cognitive measures of dementia including CAMDEX-DS scores demonstrates how clinical assessments could be enriched by the complementary use of biomarkers. This is especially pertinent in cases where the diagnosis is unclear when based on clinical symptoms alone. The use of biomarkers could therefore help ensure these cases are diagnosed earlier on in the disease progression to allow for timely interventions to be implemented.

The results also provide avenues for potential treatments to reduce incident dementia risk in people with Down syndrome. As well as promoting weight management in younger adults, treatment targeting inflammatory pathways may be beneficial for long-term risk. Any such interventions will need to be highly specific as there are risks involved in the whole scale modulating of inflammatory pathways which serve protective functions as well as contributing to AD pathology. Boosting clusterin could potentially be beneficial, or blocking down-stream complement activation in early dementia stages. More research is needed into the potential effects of such interventions. Increasing blood pressure has similar conflicting issues. Vasoconstriction may help maintain perfusion, but it could also increase the pressure in the system resulting in compromise of vascular integrity. Whether central autonomic dysfunction could be targeted remains a question and is a possible target for foetal medicines to enhance neuronal development in people with DS. This could improve perfusion and provide cognitive protection in the longer term. Similarly, the association between CHD and increased dementia risk highlights the importance of these conditions being identified and treated early. Lifelong monitoring may be beneficial for some people, with reactive and timely treatment of any associated comorbidities that arise.

It will be important to link cardiovascular health to direct observation of brain pathology. The vascular hypoperfusion theory could be tested using fMRI techniques to measure blood flow in people with low blood pressures. Signs of cerebral ischemia and inflammation would also support this theory. Longitudinal studies will be vital to understand the effects of mid-life risk factors on incident dementia risk, with comprehensive health, cognitive, plasma biomarker and imaging protocols such as the LonDowns and Alzheimer's Biomarker Consortium – Down Syndrome (ABC-DS) projects⁵⁰⁷.

9.7. Conclusions

Cardiovascular health status in people with Down syndrome can modulate the risk of dementia incidence. The risks associated with mid-life cardiovascular health on dementia pathology may have been overlooked in DS for a number of reasons. Firstly, in sporadic AD research there has been a focus on atherosclerotic-mediated risks, and it is now widely accepted that people with DS have relatively low prevalence of atherosclerotic disease and generally only experience mild changes. Secondly, the presence of low BP may not activate risk checking (for instance for other factors of CVD) which could lead to an assumption that cardiovascular disease risks in general are low in DS. Thirdly, the association between reduced BMI, progressive dementia and poorer cognitive performance in older adults confounds results, especially in cross-sectional and studies of short duration, with a presumption that obesity is either neutral or protective for cognition in DS. Fourthly, there has been a tendency to attribute obesity to low physical activity rather than a primary pathological process in people with DS, and therefore risks were attributed to low exercise rates rather than obesity itself. By using BMI in younger adults with DS to inform later risk, this study was able to uniquely identify the

additional risks posed by having a raised BMI on later dementia incidence. As for the general population, obesity does appear to be a significant factor driving pathology despite not being associated with additional classical cardiovascular risk factors.

By analysing population results alongside individual measures it was possible to identify why some of the disparities in the literature arise. Cross-sectionally there were few associations between CVPs and outcomes, particularly in younger people. In older people associations were confounded by the presence of dementia pathology, especially when considering the effects of BMI, with lower BMI being associated with worse outcomes. The cohort study was not able to replicate the longer duration of the population data, nor consider wider incident risks. On the other hand the cohort study provided additional mechanistic insights, allowing the linkage of CVP, cognitive, lifestyle data and blood plasma analysis.

Overall this thesis highlights the importance of recognising and managing cardiovascular risk in people with Down syndrome. In particular the potential negative sequelae of low as well as high blood pressure and raised BMI in mid-life are key factors to consider. The feasibility of using plasma biomarkers to monitor the effects of health interventions, enhance risk predictions, and strengthen clinical diagnoses in people with Down syndrome has also been demonstrated. The research presented here lays groundwork for future observational and interventional trials to further elicit the relationships between cardiovascular health and cerebral functioning, and to consider therapeutic interventions to delay dementia onset.

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Appendix 1.

The Association Between Physical Activity and CAMDEX-DS Changes Prior to the Onset of Alzheimer's Disease in Down Syndrome

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Abstract

Background: People with Down syndrome are at ultra-high risk of developing Alzheimer's dementia. At present, there are no preventative or curative treatments. Evidence from sporadic Alzheimer's disease literature suggests that lifestyle factors including physical activity may help maintain cognitive and functional skills and reduce dementia risk. Our study aimed to explore the association between regular exercise undertaken by participants with Down syndrome and changes in dementia-related domains of cognition and function. This was to consider whether physical activity may be a protective measure to delay cognitive decline and dementia in Down syndrome. Methods: Demographic, lifestyle, and health information was collected at baseline and at a two year follow up from 214 adults with Down syndrome without dementia, who also underwent assessment using the Cambridge Examination for Mental Disorders of Older People with Down Syndrome and Others with Intellectual Disabilities (CAMDEX-DS) and genetic analysis. Logistic regression models were used to examine the potential associations between decline in CAMDEX-DS domains and exercise whilst controlling for key variables. Results: At baseline, engaging in moderate intensity exercise was associated with a 47% reduced risk of everyday skills decline and engaging in high intensity exercise was associated with a 62% reduced risk of decline in personality and behaviour. At follow-up, high levels of exercise were associated with an 87% reduced risk of decline in

personality and behaviour. Moderate intensity exercise at baseline was associated with a 62% reduction in risk of decline during the follow-up period in memory and orientation. Discussion: Based on our data it appears that regular moderate and high intensity exercise could reduce the risk of clinically detectable decline in a Down syndrome population with possible long-term benefits. People with Down syndrome may engage in less physical activity than their peers, and barriers remain which can prevent people with Down syndrome engaging in exercise. Our work highlights how important it is that people with Down syndrome are supported to be physically active, and to promote exercise as part of a healthy ageing plan. Clinical trials in this area would be justified to determine if engaging in exercise can lead to realistic improvements in maintaining functioning and delaying dementia onset in Down syndrome and to help develop guidance in this area.

Keywords: dementia; Down syndrome; exercise; modifiable risk factors; cognitive decline; alzheimer's disease; physical activity

Introduction

Down syndrome (DS) is a chromosomal disorder most commonly caused by triplication of chromosome 21. It affects around 1 in 800 to 1 in 1000 live births globally and is the most common genetic cause of intellectual disability (ID) [1–3]. As life expectancy of adults with DS has dramatically improved, it has become evident that they are also at ultra-high risk of developing AD at an earlier age than the general population [4–6]. Characteristic neuropathologic changes are observed with amyloid-beta ($A\beta$) plaques and neurofibrillary tangles present in brain tissue [7–9].

The primary cause of the high AD risk is triplication of the amyloid precursor protein (*APP*) gene located on chromosome 21 [10]. However, despite this common genetic driver there is considerable variation in the age of symptom onset, speed of progression, and pattern of decline [11]. Some of these variations have been attributed to other genetic factors such as the presence of APOε4 alleles, which are associated with increased risk of AD in the general population [12] and an earlier age of AD onset in DS [13].

Modifiable or “lifestyle” factors have also been suggested as possible modulators of dementia risk. Studies in sporadic onset AD (SOAD) have suggested associations between factors such as obesity, smoking, poor diet, high blood pressure, cardiovascular disease (CVD), fewer years in education, low levels of socialisation, reduced exercise, raised cholesterol and alcohol consumption with worse cognitive health and increased risk of dementia [14,15]. A population-based analysis concluded that relative reductions of 10% per decade in the prevalence of seven such risk factors could reduce the worldwide prevalence of AD by 8.3% by the year 2050, with approximately one third of SOAD cases worldwide potentially attributable to modifiable risk factors [16]. These factors also appear to be relevant to those with genetic drivers of AD [17]. Reports in the literature indicate potential neuroprotective effects of physical activity (PA) and an inverse relationship between PA and dementia risk in the general population [18,19]. The association between levels of PA and cognitive decline in Down syndrome is argued to be of particular relevance for a number of reasons. There are disparities in the amount of PA people with Down syndrome engage in, with many not having equal opportunity to participate in regular activity [20–23]; methods to increase PA are cheap, adaptable, and easily attainable; PA can positively affect other areas of life including fitness, balance and social participation [24–26]; and higher levels of PA could be implemented from early life with limited ethical concerns as a form of pre-symptomatic disease modification. In addition, people with Down

syndrome in the UK generally do not smoke or drink excess amounts of alcohol and have low rates of hypertension and cholesterol-related illnesses [27–31]. They are therefore an important population in which to study the effects of PA that are not associated with lowering general cardiovascular risk.

Compared to the literature in SOAD, there have been relatively few studies into the effects of PA on cognition and dementia in Down syndrome. Experiments using Ts65Dn mice, a model for Down syndrome, provide initial evidence that PA could play a role in enhancing cognition. For example, voluntary wheel running in mice has been associated with better performance in cognitive tasks compared to sedentary controls, particularly when considering hippocampal mediated processes which are integral to AD progression [32–34]. Alongside cognitive changes, mice studies have also demonstrated effects of PA on gene and protein expression, neurogenesis, and morphology in the brain [32,33,35].

In human studies involving people with Down syndrome, episodic memory improvement has been associated with participation in a 12-week exercise programme [36]. Improvements have also been reported in post-intervention measures of executive function including inhibition [37,38], attention shifting [39], reaction time [40,41] and semantic fluency [42,43], although effect size and results vary between studies. This may be related to variations in the intensity, type and consistency of PA involved, as well as the study design. One study compared lifestyle factors including physical activity in a group of individuals with Down syndrome and AD to a sample of adults with Down syndrome but no clinical signs of AD [44]. No significant difference was observed between the groups in levels of PA. However, there was missing data in the sample, and it is noted that across the groups the levels of PA were low.

Overall, the current literature is suggestive of a positive relationship between PA and cognitive functioning in Down syndrome, with PA having possible neuromodulating and neuroprotective effects. Further research is needed in larger samples with consistent measures to better understand the link between PA, cognition, and dementia in Down syndrome. Our study therefore aimed to explore the association between the amount of regular exercise undertaken by participants and longitudinal changes in dementia-related domains of cognition and function prior to AD onset. This was to consider whether physical activity could be a protective measure to delay cognitive decline and dementia in Down syndrome.

Materials and Methods

Participants

Participants were taken from the LonDowns cohort, an observational and longitudinal cohort study of individuals with Down syndrome. The cohort has been described previously [45] and consists of people over the age of 16 years with DS living in England and Wales. All adults with a clinical diagnosis of DS were eligible for inclusion. Those with acute physical or mental health conditions were excluded until recovered.

Ethical approval for the LonDowns study was granted from the North West Wales Research Ethics Committee (13/WA/0194). Written informed consent was obtained from individuals with capacity to consent after a full explanation of the study. In the event that individuals lacked capacity to provide informed consent, a consultee signed a form on their behalf to indicate their decision regarding the individual's inclusion based on their knowledge of the individual and his/her wishes. This is in accordance with the UK Mental Capacity Act 2005.

Participants with a clinical diagnosis of dementia at baseline (T1) or at follow up two years later (T2) were removed from the dataset. This is because we were interested in the changes in CAMDEX-DS domains prior to a dementia diagnosis and to avoid the potential confounding effect of dementia on exercise levels. Eighty-six participants had dementia at T1 (mean age 54.59, SD 6.75) and were removed from the analysis. Eleven had converted to dementia at T2 (mean age 56.8, S.D. 4.81). Our sample therefore consisted of data from 214 participants with data at T1 (122 males) and 91 participants at T2 (54 males). The smaller group at T2 is due to the loss of participants who converted to dementia, those lost to follow up, and those with incomplete data at T2.

Measures

The CAMDEX-DS (Cambridge Examination for Mental Disorders of Older People with Down Syndrome and Others with Intellectual Disabilities) is an informant-based interview used to identify symptoms of decline in cognition, adaptive functioning, or behaviour, which are suggestive of early dementia-related change [46,47]. It assesses the following domains: everyday skills, memory and orientation, other cognitive skills (covering general mental functioning, language, perception, praxis, and executive functions), personality and behaviour, and self-care. Example questions include: “Does he/she have difficulty with shopping?” (everyday skills); “Does he/she have difficulty in remembering short lists of items” (memory and orientation); “Does he/she have difficulty in following instructions (other cognitive skills); “Is he/she often irritable/angry” (personality and behaviour); “Does he/she have difficulty with bathing?” (self-care). For each domain a number of questions prompt the informant to state whether there has been no deterioration, a slight deterioration, or a great deterioration in that particular area. For this study a symptom-level definition of decline was used based on results from the CAMDEX-DS interview. The presence of dementia-related symptoms was classified

using a binary ‘no decline’ or ‘decline’ categorisation based on informant responses for each participant. For each domain, participants were assigned to one of two groups based on whether they exhibited deterioration in their abilities compared to their best ever functioning. If one ‘great’ deterioration or two ‘slight’ deteriorations were recorded on individual CAMDEX-DS items, then the participant was regarded as showing a decline in this domain. Participants were considered to show no decline in a domain if no items were recorded as having a great deterioration, or there were less than two slight deteriorations reported.

Participants’ current average exercise levels were split into three categories: low, moderate, and high intensity. Coding for this variable was based on informant data relating to participants’ weekly activity levels collected during a semi-structured interview. Open questions about weekly activity and exercise levels were followed-up with focused inquiries to establish the type, duration, and frequency of PA during an average week for the participant. This approach was adapted from the methods used in Kenshole et al. (2017) and Laurin et al. (2001) [44,48]. The following criteria were used for group classification:

- i. High intensity. Exercise of an intensity greater than walking at least three times a week, or for at least three hours a week.
- ii. Moderate intensity. Exercise at an intensity equal to that of walking at least three times a week, or exercise less than three hours a week/three times a week of an intensity greater than walking, or at least two hours a week of exercise where intensity or type was not specified, or a combination of walking and other exercise when duration or intensity not specified.
- iii. Low intensity. All exercise not meeting the above thresholds, including individuals who do not engage in any exercise.

Using medical history and current medication data, participants were coded as having CVD or high cholesterol. *APOE* genotype was determined from saliva or blood samples. As outlined in previous studies, this was performed using a Thermo Fisher Scientific Taqman assay for rs7412 and rs429358 SNPs [49,50]. Participants were classified as $\epsilon 4$ carriers if they had $\epsilon 3:\epsilon 4$ or $\epsilon 4:\epsilon 4$ genotype. The non- $\epsilon 4$ group were those without an $\epsilon 4$ allele ($\epsilon 2:\epsilon 2$, $\epsilon 2:\epsilon 3$ or $\epsilon 3:\epsilon 3$). We excluded any participants with a $\epsilon 2:\epsilon 4$ genotype from the dataset due to the possible counteracting effects of the $\epsilon 2$ and $\epsilon 4$ alleles [51].

Statistical Analysis

Logistic regression models were used to examine the associations between decline in CAMDEX-DS domains and PA while adjusting for the effect of age, sex (male and female), intellectual disability level (ID; mild, moderate, and severe), *APOE* genotype (non- $\epsilon 4$ carriers and $\epsilon 4$ carriers), CVD and high cholesterol. For T2 models we additionally included exercise levels at T1 to examine if PA at baseline was associated with potential decline at follow-up. Participants required data for at least one outcome domain on the CAMDEX-DS at T1 or T2 for inclusion. The reference groups for categorical variables were female sex, ID level: mild, non- $\epsilon 4$ carriers, no CVD, no history of high cholesterol, low intensity of exercise. Hence, we compared those who engage in moderate or high intensity PA to those who perform very little or no PA.

Results

Participants ages ranged between 17 and 74 years. As shown in Table 1, the mean age at T1 was 46.11 (S.D 10.47) and 49.04 (S.D. 6.32) at T2. The average follow-up time in months between visits was 24.12 (S.D = 0.81) ranging from 23 to 28 months. The age difference between participants at T1 and T2 was 2.93 years and there were approximately the same

proportion of males and females in our cohort. Over 85% of our participants were white, the majority had either mild or moderate ID and were not $\epsilon 4$ carriers. Nearly a third of participants had CVD at T1 and this increased to 35% at T2, however very few participants had high cholesterol, were smokers, or drank alcohol at either timepoints. The majority of individuals participated in low or moderate intensity exercise.

Declines in cognition, adaptive functioning and personality and behaviour were common in our sample at both timepoints (Table 2). The most common domains of change at T1 were personality and behaviour followed by memory and orientation and other cognitive skills and then everyday skills and self-care abilities. At T2, the domains that showed the highest prevalence of decline were personality and behaviour and other cognitive skills. Approximately the same percentage of participants exhibited declines in everyday skills and memory and orientation abilities, and again decline in self-care abilities was reported in the fewest participants.

To examine the associations between CAMDEX-DS domains and PA at T1, we fitted regression models that included the domains of the CAMDEX-DS in separate models while adjusting age, sex, ID, *APOE* genotype and CVD. At T2, the same models were fitted with T2 data, but we also included PA at T1 to examine the longitudinal effects of exercise on potential decline at follow-up. Due to the low prevalence of high cholesterol in our sample (1.9% at T1 and 2.2% at T2) this variable was not included in the regression models.

		Frequencies (%)	
		T1	T2
<i>n</i>		214	91
Follow-up time in months (mean, S.D)			24.12 (0.81)
Age (mean, S.D)		46.11 (10.47)	49.04 (6.32)
Gender	Female	92 (43.0)	37 (40.7)
	Male	122 (57.0)	54 (59.3)
Ethnicity	Bangladeshi	1 (0.5)	0 (0.0)
	Black African	4 (1.9)	2 (2.2)
	Black Caribbean	7 (3.3)	5 (5.5)
	Black other	1 (0.5)	1 (1.1)
	Chinese	2 (0.9)	1 (1.1)
	Indian	3 (1.4)	1 (1.1)
	Mixed black African/white	1 (0.5)	1 (1.1)
	Mixed other	1 (0.5)	0 (0.0)
	Other/not available	2 (0.9)	2 (2.2)
	White British	185 (86.4)	74 (81.3)
	White Irish	3 (1.4)	2 (2.2)
	White other	4 (1.9)	2 (2.2)
ID level	Mild	75 (35.7)	33 (36.3)
	Moderate	96 (45.7)	37 (40.7)
	Severe/profound	39 (18.6)	21 (23.1)
<i>APOE</i> genotype	Non-ε4 carriers	149 (77.2)	68 (80.0)
	ε4 carriers	44 (22.8)	17 (20.0)
CVD	No	147 (72.4)	59 (64.8)
	Yes	56 (27.6)	32 (35.2)
High cholesterol	No	203 (98.1)	89 (97.8)
	Yes	4 (1.9)	2 (2.2)
Smoking	Ex-smoker	3 (1.4)	2 (2.3)
	No	209 (98.1)	84 (96.6)
	Yes	1 (0.5)	1 (1.1)
Alcohol	Any alcohol consumption	68 (31.9)	31 (34.8)
	No alcohol consumption	145 (68.1)	58 (65.2)
Exercise levels	Low intensity	78 (37.7)	31 (36.0)
	Moderate intensity	100 (48.3)	47 (54.7)
	High intensity	29 (14.0)	8 (9.3)

Table 1. Demographics of participants at T1 and T2.

CAMDEX-DS Domain		T1	T2
<i>n</i>		214	91
Everyday skills	No	147 (70.3)	62 (68.1)
	Yes	62 (29.7)	29 (31.9)
Memory and orientation	No	132 (62.6)	63 (69.2)
	Yes	79 (37.4)	28 (30.8)
Other cognitive skills	No	132 (63.5)	57 (62.6)
	Yes	76 (36.5)	34 (37.4)
Personality and behaviour	No	129 (61.4)	51 (56.0)
	Yes	81 (38.6)	40 (44.0)
Self-care	No	152 (73.8)	66 (72.5)
	Yes	54 (26.2)	25 (27.5)

Table 2. Changes in CAMDEX-DS domain at T1 and T2.

At T1 we found that people who engaged in moderate intensity PA were significantly less likely to exhibit decline in everyday skills (RR = 0.53 (95% CI 0.26–0.95), $p = 0.03$). Specifically, for these individuals, moderate levels of PA were associated with a 47% reduced risk of experiencing changes in everyday skills compared to people who participated in low levels of PA. High intensity PA was associated with decreased risk of changes in personality and behaviour (RR = 0.38 (0.11–0.92), $p = 0.042$). Here, engaging in high intensity PA was associated with a 62% reduction in risk of decline compared to those who engaged in low levels of PA. No other CAMDEX-DS domain was associated with PA (all $p > 0.05$).

Age at T1 was associated with an increased risk of decline in memory and orientation (RR = 1.03 (1.00–1.05), $p = 0.03$) and self-care abilities (RR = 1.03, (1.01–1.06), $p = 0.02$), while severe/profound ID was associated with increased risk of decline in other cognitive skills (RR= 1.73 (1.06–2.35), $p = 0.03$). Additionally, having an *APOE* $\epsilon 4$ allele was significantly associated with increased risk of decline in memory and orientation (RR = 1.77 (1.17–2.31), $p = 0.01$). We found that moderate intensity PA at T1 was associated with a 62% reduced risk of decline at T2 in memory and orientation compared to low intensity PA (RR = 0.38 (0.10–0.97),

$p = 0.047$). High levels of PA at T2 were associated with reduced risk of decline in personality and behaviour (RR = 0.13 (0.005–0.83), $p = 0.043$), here high levels of PA resulted in an 87% reduction in risk of decline compared to low intensity PA. Again, age at T2 was associated with increased risk of decline in self-care abilities (RR = 1.12 (1.04–1.21), $p = 0.005$) and we found that people with moderate ID had a 156% increase in risk of decline in personality and behaviour (RR = 2.56 (1.38–3.58), $p = 0.008$). A history of cardiovascular disease was not associated with decline at T1 or T2.

In summary, moderate, and high levels of exercise may be protective factors against cognitive and functional decline in adults with DS. A reduced risk of decline was observed in dementia-related domains of everyday skills, memory and orientation, and personality and behaviour while adjusting for the effect of age, sex, ID level, *APOE* genotype, and CVD.

Discussion

People with DS are at an ultra-high risk of developing AD as they grow older. At present, there are no effective interventions to significantly delay or prevent the onset of this disease. As such, it is vital to identify any potential modifiable factors to try and reduce the burden of AD. In studies of SOAD, low levels of PA have been suggested as a risk factor associated with cognitive decline and dementia, although the results in the literature are mixed. Similarly, studies involving mice models and participants with Down syndrome have demonstrated that both regular exercise and acute bouts of activity can have a positive impact on cognitive function but there is limited information as to how PA may impact dementia related symptoms. Our study aimed to address this knowledge gap by using the CAMDEX-DS questionnaire to evaluate longitudinal associations between day-to-day levels of PA and decline in five AD related domains whilst controlling for key covariables. We found a positive association

between engaging in moderate and high levels of exercise and maintenance of memory, personality, and behaviour, and everyday skills in our Down syndrome cohort.

Cross-sectionally we found that moderate levels of PA were associated with a significantly lower risk of reported decline in everyday skills. High levels of PA were associated with a reduced risk of personality and behaviour decline. Whilst the association with moderate exercise was only observed at T1, the association between high levels of PA and reduced risk of personality and behaviour decline was robust across both T1 and T2. Longitudinally, moderate levels of PA were associated with a reduced risk of decline in memory and orientation two years later. These results suggest that moderate and high levels of PA may confer protective effects against dementia-related decline and that these effects could have an enduring impact on risk.

Based on our data, it therefore appears that regular moderate and high intensity exercise could reduce the risk of clinically detectable decline in a DS population with possible long-term benefits. This is the first study to consider the longitudinal association of regular exercise and AD-related decline in a relatively large cohort of people with Down syndrome. Our results show promise that lifestyle factors related to the risk of SOAD may also be relevant for delaying symptom onset in Down syndrome, despite the genetic driver behind AD in this population.

These findings are consistent with the proposed neuromodulatory effects of PA. It has been suggested that engaging in regular exercise promotes a number of changes in the brain. For example, exercise has been positively associated with hippocampal volume, an area that is implicated in learning and memory decline in AD [52–54]. Other proposed mechanisms

include alterations in cerebral blood flow [55–57], increasing neurotrophic factors such as BDNF in the brain [58,59], protecting neural tissue and promoting neurogenesis [60], strengthening neural connections and networks, and reducing inflammation and related tissue degeneration [61]. It has also been suggested that cardiovascular risks may be related to the progression to AD and increased amyloidosis in brain tissue, although the picture remains unclear [62,63]. We could not examine these factors in the present study due to a lack of neuroimaging data. Consideration also needs to be given to potential psychological, social, and environmental factors associated with PA, which may modulate some of the positive cognitive impact and warrant further study [64,65].

In our study, we found no association between the presence of cardiovascular disease with decline. This may be due to the specific cardiovascular profile in Down syndrome where individuals often have lower blood pressure and less atherosclerotic disease when compared to the general population [27]. It may also suggest that the associations between PA and risk of cognitive decline are not mediated simply by reducing cardiovascular risk factors. This highlights the potential for PA to exert its effects directly by modulating pathways in the brain. It also demonstrates the benefits of working with people with Down syndrome when studying AD. They represent a specific model of AD that could help disentangle the indirect impact of exercise on cardiovascular disease from direct neuroprotective effects.

Our study has a number of strengths. Cognitive assessments used in other studies have often shown only small magnitude changes with floor effects making it difficult to accurately assess response following an intervention. In our design we chose to use the CAMDEX-DS questionnaire which can detect longitudinal changes over a period of years, using the individual's own best ever level of functioning as a baseline. Our sample size was relatively

large, and included people with all levels of ID. Our information about PA is naturalistic and representative of what individuals perform on a day-to-day basis, giving our results real-world relevance. This means that the “high intensity” level of PA we studied is achievable by people with DS in the community. Exercise studies often employ highly structured and specific routines that are not feasible for individuals to incorporate into their daily lives.

We acknowledge certain limitations. The exercise data was taken from semi-structured interviews and not collected using a standardised tool. On one hand, this prevented us from being able to analyse whether certain types of exercise may confer more or less benefit to individuals. On the other hand, it means that our data is a realistic representation of the level of PA that adults with DS actually engage in. We also used informant data rather than direct cognitive assessment. This was a deliberate choice to avoid possible floor effects and allow for the inclusion of a representative sample of people with Down syndrome, including those with severe/profound ID who often score at floor or are unable to engage in direct assessment. Finally, it is difficult to ascertain the impact of social contact and other environmental factors during PA, particularly when exercise is done in a group environment. It is possible that this social aspect of PA could be contributing to the observed effects.

We excluded participants with a diagnosis of dementia. Primarily this was to allow focus on the effects of PA on early cognitive changes prior to diagnosis. This approach also helped to avoid confounding effects. People with dementia generally engage in less PA due to the physical and cognitive changes and increased frailty associated with the disease [66,67]. It would therefore be difficult to interpret the associations between levels of PA and cognitive decline in people with dementia. However, given the potential neuroprotective effects of PA on maintaining cognition it may be that our results are also applicable to those already

diagnosed with AD and could help maintain their daily functioning and quality of life for longer. Further work is needed in this area.

Our findings have significant implications for the Down syndrome community and highlight the importance of promoting healthy lifestyle and exercise alongside the search for pharmacological treatments of AD. People with Down syndrome can engage in less PA than their peers, and in our sample only 14% engaged in high levels of exercise, reducing to 9% at T2. There are concerns that barriers remain, which prevent people with Down syndrome engaging in exercise, including stigma, lack of reasonable adjustments, assumptions about the physical capabilities of people with Down syndrome, and a lack of opportunities and access [68,69]. It is vital that people with Down syndrome are supported to engage in regular exercise, and that structures are in place to encourage and support this. It can be tempting to try and find the “optimum” type, timing, and duration of exercise to provide the most benefit for individuals, but our results suggest that any substantial PA of moderate or high intensity may be of benefit. Our priority should therefore be on supporting people with Down syndrome to be healthy and active in whichever way they enjoy.

Future work should focus on the effect of PA on direct measures of cognition, the associations with neuropathological changes using blood and imaging biomarkers, and the effect of exercise at different life stages. In addition, more in-depth analysis of the type and duration of PA would help draw conclusions as to the potential role that social contact and other factors may play in maintaining cognition, and whether certain types of activity provide greater cognitive protection than others. In this exploratory work we examined the association between longitudinal change in cognition and exercise within the LonDowns cognitive test battery [45]. Due to a limited sample size and missing data, our analyses were underpowered; however,

there was preliminary evidence to suggest cognition may be affected by exercise in Down syndrome. While the overall model was not significant, participants who engaged in moderate intensity exercise showed less change in their raw KBIT-2 verbal scores ($p = 0.046$) between the two time-points compared to those who engaged in low levels of exercise, adjusting for the effect of age, sex, and ID level and *APOE* status.

We have presented preliminary data regarding the potential positive impact of PA on AD-related cognitive decline, which is achievable for people with Down syndrome within everyday routines. Clinical trials in this area would therefore be justified to determine if engaging in exercise can lead to realistic improvements in maintaining functioning and delaying dementia onset in Down syndrome and to help develop consensus guidance in this area.

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