Investigating Cognitive Function in Clinical and Non-clinical Samples using Electroencephalography and Psychometric Assessment: A Comparative Study

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I. Declaration

I, George Kalatzis, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy (Science), in the School of Life Sciences at the University of Technology Sydney. This thesis is wholly my own work unless otherwise referenced or acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution.

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II. Acknowledgements

This thesis has been the most significant and exacting undertaking of my professional career, requiring sustained self-discipline, determination, and resilience. The individuals mentioned below merit appreciable praise; they contributed to maintaining not only my sanity, but also the final polished version of this thesis. Words alone are insufficient to reflect my appreciation of them.

Foremost, I must thank my principal supervisor, Associate Professor Sara Lal, for affording me with the opportunity to undertake this doctoral research. Your unshakeable support, wisdom, incisiveness, and scientific know-how empowered me to persevere during several stages of this zigzagging journey despite repeated setbacks. These same qualities also motivated me to continually advance myself intellectually and to always strive for high standards. In addition, your critical, constructive feedback during the preparation of this thesis, as well as seminar presentations, was also invaluable and helped develop a more robust piece of work. I must also thank my co-supervisors, Dr Najah Nassif and Associate Professor Chris Zaslawski. Your viewpoints, insights, and feedback on thesis chapters and seminar presentations helped strengthen the quality of this research. I also extend my thanks to the Faculty of Science for providing me with the necessary facilities and resources to undertake my doctoral candidature.

I am immeasurably grateful and indebted to Dr Elizabeth Louise May, who proofread the thesis and provided substantial editing input. Her uncompromising standards for writing emboldened me to become a stickler for proper English, using every word and comma with precision and economy, and to consult the acknowledged authority (Fowler's Modern English Usage) for all matters concerning English usage whenever uncertain. It is my hope that the examiners enjoy reading this dissertation half as much as they did reading hers.

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Considerable thanks are also due for my colleagues in the Neuroscience Research Unit (NRU). They inspired me to persevere in light of the highs and lows of academia, listened to countless seminar practice sessions, and helped maintained my marbles over numerous lunch outings. I also owe tremendous thanks to all study volunteers who participated in the study – whether immediate family, friends, or strangers. Without their involvement (and their enthusiasm and interest), this research would not have been possible or have advanced.

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III. Thesis Format

This thesis is formatted as a conventional thesis and hence is structured as a series of chapters.

IV. Publications and Presentations

Published Abstracts/Conference Presentations:

1. Lees, T., Maharaj, S., **Kalatzis, G.,** Nassif, N., Newton, P., & Lal, S. The neurocognitive relationship between stress and anxiety, memory and decision-making performance of Australian Nurses. Poster presentation: 58th Annual meeting of the Society for Psychophysiological Research 2018, Quebec City, Canada.
2. **Kalatzis, G.,** Lees, T., Nassif, N., Zaslawski, C., & Lal. S. Exploring cognitive function in diabetes and non-diabetes samples using electroencephalography (EEG) and psychometric assessment: A comparative study. Oral presentation: The 37th Annual Scientific Meeting of the Australasian Neuroscience Society 2017, Sydney, 3rd – 6th December.
3. **Kalatzis, G.,** Lees, T., Nassif, N., Zaslawski, C., & Lal, S. Investigating cognitive function in diabetes and healthy samples using electroencephalography (EEG) and psychometric assessment: a comparative study. Oral presentation: The Inter-University Neuroscience & Mental Health Conference 2016, Sydney, 20th – 21st September.
4. **Kalatzis, G.,** Lees, T., Nassif, N., Zaslawski, C., & Lal, S. Investigating cognitive function in diseased states: electroencephalography (EEG) and psychometric assessment. Poster presentation: The New Horizons Conference 2015, Sydney, 23rd – 25th November.
5. Lees, T., **Kalatzis, G.,** & Lal, S. (2015). Examining negative mental states and their association to psychometric and electroencephalographic measures of cognitive performance in Australian Nurses. Psychophysiology, 52 (S24), doi: 10.1111psyp.12495.
6. Lees, T., **Kalatzis, G.,** & Lal, S. Examining negative mental states and their association to psychometric and electroencephalographic measures of cognitive performance in Australian Nurses. Poster presentation: 55th Annual meeting of the Society for Psychophysiological Research 2015, Seattle, USA.

Other (manuscripts currently submitted or pending outcome):

1. Lees, T., Maharaj, S., **Kalatzis, G.,** Nassif, N., Newton, P., & Lal, S. (2020). Electroencephalographic prediction of global and domain-specific cognitive performance of clinically-active Australian Nurses. *Physiological Measurement* (Accepted – 20/08/2020).
2. **Kalatzis, G.,** Lees, T., Nassif, N., Zaslawski, C., & Lal, S. (2020). Changes in EEG activity as an indicator of early cognitive dysfunction in diabetes mellitus: a review. *Journal of Diabetes and Its Complications* (Due for resubmission).

V. Table of Contents

VI. List of Figures

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VIII. Abbreviations

α – Alpha

Aβ – Amyloid-beta

AChEI – Acetylcholinesterase Inhibitor

AD – Alzheimer's Disease

ADA – American Diabetes Association

AgCl – Silver Chloride

AGE – Advanced Glycation End Product

AIHW – Australian Institute of Health & Welfare

APOE – Apolipoprotein E gene

ARC – Arcuate Nucleus

β – Beta

BBB – Blood-Brain Barrier

BGL – Blood Glucose Level

BMI – Body Mass Index

BP – Blood Pressure

BPM – Beats per Minute

CBF – Cerebral Blood Flow

CGM – Continuous Glucose Monitoring

CKD – Chronic Kidney Disease

CNS – Central Nervous System

CPP – Cerebral Perfusion Pressure

CSF – Cerebrospinal Fluid

CVD – Cardiovascular Disease

DBP – Diastolic Blood Pressure

DC – Direct Current

DCCT – Diabetes Control and Complications Trial

DL – Dorsolateral

DM – Diabetes Mellitus

DPP4i – Dipeptidyl Peptidase-4 Inhibitor

DSM – Diagnostic & Statistical Manual of Mental Disorders

ECoG – Electro-Corticography

EEG – Electroencephalography

EOAD – Early Onset Alzheimer's Disease

EOG – Electro-oculogram

ERP – Event Related Potential

fAD – Familial Alzheimer's Disease

FFT – Fast Fourier Transform

FPG – Fasting Plasma Glucose

fMRI – Functional Magnetic Resonance Imaging

GLUT-1 – Glucose Transporter 1

$HbA1_C$ – Glycosylated Haemoglobin

HR – Heart Rate

HR – Hazard Ratio

HREC – Human Research Ethics Committee

HTN – Hypertension

Hz – Hertz

IDE – Insulin Degrading Enzyme

IDF – International Diabetes Federation

IR – Insulin Resistance

KΩ – Kilo-ohms

LAQ – Lifestyle Appraisal Questionnaire

LTP – Long-Term Potentiation

MANCOVA – Multiple Analysis of Covariance

MCI – Mild Cognitive Impairment

mm Hg – Millimetres of mercury

mmol/L – Millimoles per litre

MMSE – Mini-Mental State Examination

MoCA – Montreal Cognitive Assessment

MRI – Magnetic Resonance Imaging

Ms – Milliseconds

$\mu V/s^2$ – Microvolts per second squared

NFT – Neurofibrillary Tangles

NRU – Neuroscience Research Unit

NSW – New South Wales

PAD – Peripheral Arterial Disease

PNS – Peripheral Nervous System

PSEN1 – Presenilin 1

PSEN2 – Presenilin 2

PVN – Paraventricular Nucleus

RCT – Randomised Controlled Trial

ROS – Reactive Oxygen Species

RR – Relative Risk

r – rho value

sAD – Sporadic Alzheimer's Disease

SBP – Systolic Blood Pressure

SD – Standard Deviation

SGLT2i – Sodium Glucose Co-Transporter-2 Inhibitor

SNR – Signal-to-Noise Ratio

SVD – Small Vessel Disease

T1DM – Type 1 Diabetes Mellitus

T2DM – Type 2 Diabetes Mellitus

UKPDS – United Kingdom Prospective Diabetes Study

UTS – University of Technology Sydney

VaD – Vascular Dementia

VCI – Vascular Cognitive Impairment

VMPFC – Ventromedial Prefrontal Cortex

VMH – Ventromedial Hypothalamus

WAIS-R – Weschler Adult Intelligence Scale Revised

WHR – Waist-Hip Ratio

WMH – White Matter Hyperintensities

$>$ – greater than

$\geq$ – greater than or equal to

$<$ – less than

$\leq$ – less than or equal to

IX. Abstract

Diabetes mellitus (DM) (Type 1 (T1DM) and Type 2 (T2DM)) and hypertension (HTN) are associated with subtle cognitive dysfunction; however, few studies have explored the cognitive and electroencephalography (EEG) changes that occur in these conditions. The present cross-sectional study assessed cognitive performance (global and domain-specific) in clinical (T1DM, T2DM, and HTN) and non-clinical samples using established cognitive assessments and EEG, and investigated their associations with blood pressure (systolic (SBP) and diastolic (DBP)) and blood glucose level (BGL).

Results were obtained from 94 study participants divided into four groups: non-clinical (n = 49), T1DM (n = 13), T2DM (n = 17), and HTN (n = 15). The experimental protocol was commenced by obtaining pre-study BP measurements and a BGL measurement. Participant lifestyle factors and disease-specific variables (*e.g.* HbA1c, age of disease onset, *etc.*) were obtained using the Lifestyle Appraisal Questionnaire (LAQ) and disease-specific questionnaires, respectively. Brain activity was then measured using a 32-channel EEG over two five-minute study phases (baseline (quiet sitting) and active (Stroop Test)). Subsequently, two reliable and validated cognitive screening tools were administered, the Mini-Mental State Examination (MMSE) and the Cognistat. The study was concluded with post-study BP measurements and a BGL measurement.

No significant difference was found in global or domain-specific cognitive performance between the groups. In the non-clinical group, post-study BGL was inversely associated with total MMSE score ($p < 0.05$; $r = -0.32$). In the T1DM and T2DM groups, higher BGL was significantly associated ($p < 0.05$) with theta activity in anterior brain regions, while glycosylated haemoglobin ($HbA1_C$) and disease duration were found to be significantly associated ($p < 0.05$) with slow-wave oscillations. In the HTN group, higher SBP and DBP was significantly associated ($p < 0.05$) with slow-wave activities over central and parietal brain areas.

These findings provide novel insight into the associations between blood pressure (SBP and DBP) and BGL and EEG activity in non-clinical and clinical groups. The data obtained suggest that the EEG can consistently detect changes in oscillatory brain activity linked to small changes in BP and BGL, identifying the EEG as a potential neurophysiological instrument for early screening for the subtle changes in cognition linked to both DM and HTN. Future use of EEG as a screening tool could avert adverse cognitive outcomes linked to these chronic diseases, such as Alzheimer's disease (AD) and dementia, and help reduce the substantial socioeconomic and emotional burden associated with them.

1. Introduction

1.1 Ageing and Health

The health of our ageing population is a major and pressing socioeconomic issue. Significant advances in medicine, including but not limited to disease diagnosis and prevention, developments in medical technologies, improved accessibility to treatment, novel pharmacological interventions, and improved understanding of risk factors, have resulted in individuals worldwide, on average, living longer (Figure 1.1). In Australia, life expectancy at birth has increased considerably since the late 1800s, with Australians now living approximately 30 years longer (males: 33.2 years; females: 33.8 years) compared to a century ago (Australian Institute of Health & Welfare, 2018).

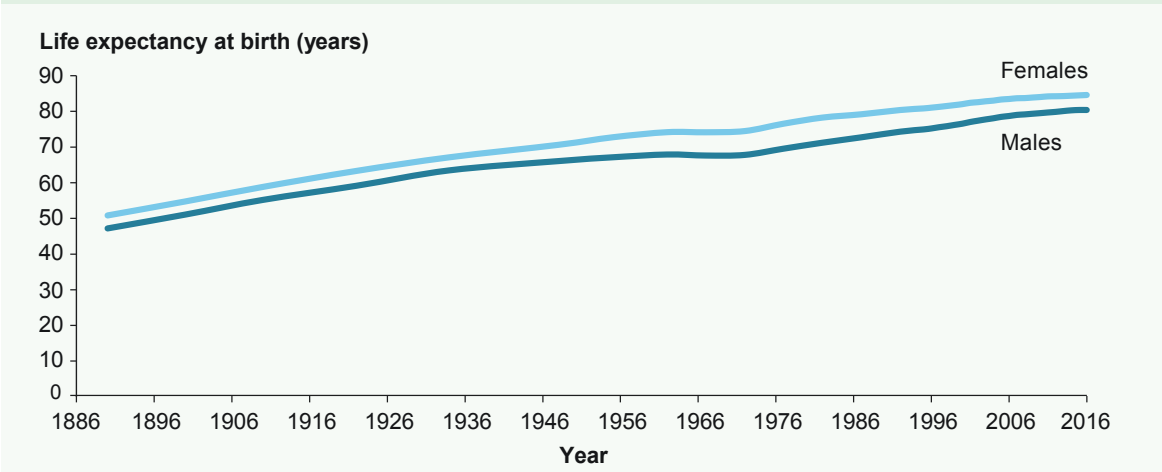


Figure 1.1. Life expectancy by birth year and sex from 1886 – 2016. Life expectancy for both sexes in Australia has increased gradually over the last century. Adapted and modified from Australian Institute of Health & Welfare (AIHW), (2018, p 10).

As longevity has increased worldwide, this has simultaneously increased demands and strain on health care systems. The natural human ageing process is accompanied by progressive, irreversible changes in physiological function (AIHW, 2018), including gradual hearing and visual loss, reduced mobility, and increased frailty (AIHW, 2018). One other irreversible physiological change associated with increasing age is age-related cognitive decline. This often manifests as Alzheimer's disease (AD), the most common form of cognitive impairment (defined as performance in neuropsychological assessment at 1.5 - 2 SDs (standard deviations) below the normative mean), although various other forms of age-related cognitive decline exist (Citron, 2010; Nordberg, 2015; Biessels & Despa, 2018). Currently, 3.9 million people are classified as older Australians (> 65 years old) (AIHW, 2018) (Figure 1.2) and, by 2056, projections suggest that Australia's ageing population will increase twofold, rising to approximately 8.7 million (AIHW, 2016). By 2096, estimates predict the number of older Australians will reach 12.8 million (Figure 1.3). As neurodegenerative diseases and pre-symptomatic stages of cognitive impairment typically begin manifesting in older Australians (~65 years old) (although they may manifest earlier), this trend in population ageing poses a challenging socioeconomic issue (Hampel & Lista, 2016; Elahi & Miller, 2017; Biessels & Despa, 2018).

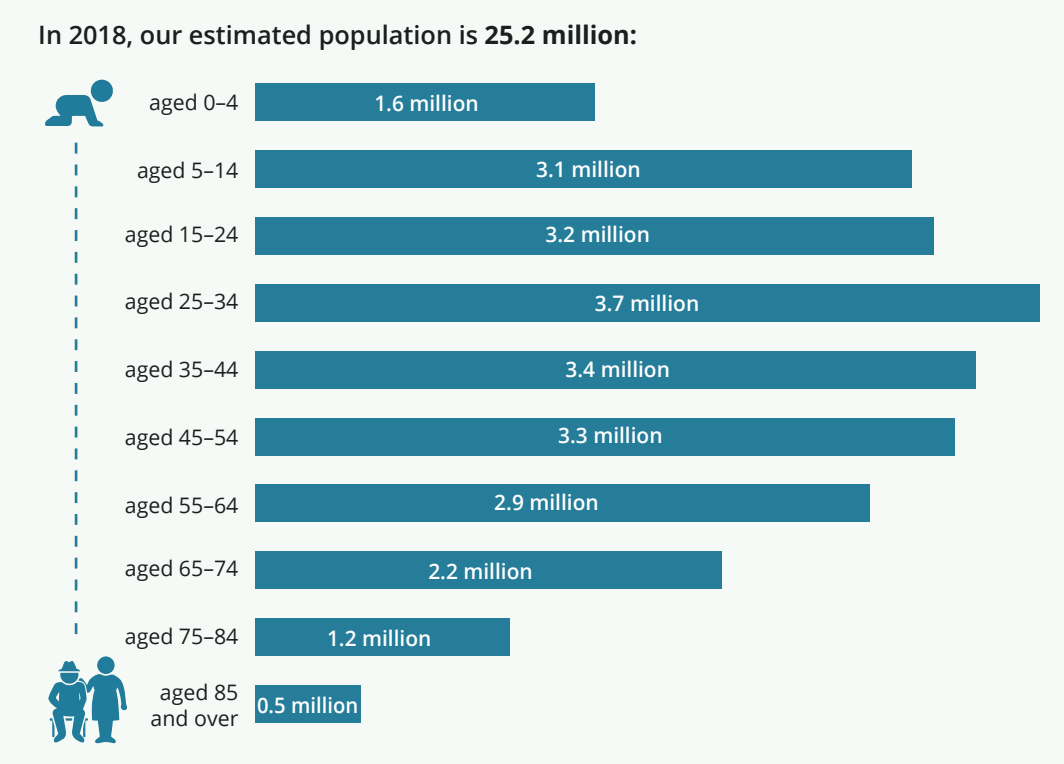


Figure 1.2. Estimated number of Australians, categorised by age group, in 2018. Adapted from AIHW, (2018, p 7).

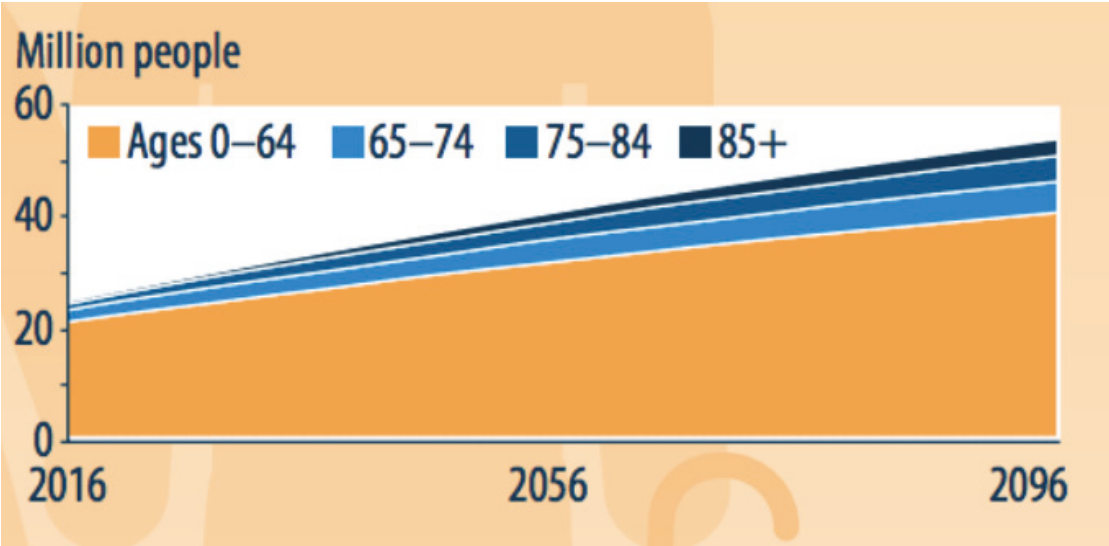


Figure 1.3. Predicted number of Australian individuals aged 65 years and over, categorised by age group, between the period 2016 - 2096. The number of individuals aged 65 years and over is expected to double by 2056. Adapted from Australian Institute of Health & Welfare (AIHW), (2016, p 17).

1.2 Cognition

The adult human brain – an integral component of the central nervous system (CNS) – is a complex organ comprised of billions of metabolically-active and nutrient-dependent brain cells (neurons) that exert precise control over all behavioural and physiological responses (Sweeney *et al.,* 2018). It is commonly referred to as the "control-centre", and plays crucial roles in the precise regulation of physiological parameters, including blood pressure (BP), blood glucose level (BGL), heart rate (HR), and body temperature (Herculano-Houzel, 2009; Tau & Peterson, 2010; Ando *et al.*, 2011; Grayson, Seeley, & Sandoval, 2013). Glucose- and oxygen-dependent neurons interspersed throughout the brain communicate with neighbouring cells to perform high-order functions, such as decision-making and reasoning. Collectively, these mental processes are referred to as cognitive function (Tau & Peterson, 2010). Interestingly, although the brain accounts for approximately 2% of total body weight, it is the most metabolically-demanding organ, consuming roughly 20% of the body's glucose needs (Kisler *et al.,* 2017; Sweeney *et al.,* 2018). It also relies on a continuous, uninterrupted supply of blood, consuming approximately one-fifth of total blood supply (Kisler *et al.,* 2017; Dementia Australia, 2020).

One broad, descriptive catch-all term commonly used to describe functions associated with the brain is cognition: mental processes of attention, perception, thinking, learning, and memory, which support various aspects of everyday behaviour (Holden, 2011; Spyridaki *et al.,* 2016; Biessels & Despa, 2018). Cognition is primarily controlled by prefrontal cortical regions (the dorsolateral (DL) and ventromedial prefrontal cortex (VMPFC)) and influences mental alertness, workplace productivity, conscious decision-making ability, and personal well-being (Wood & Grafman, 2003; Frederick, 2005; Ando *et al.*, 2011). It also plays an integral role in supporting disease self-management tasks, such as, for example, ongoing monitoring of blood glucose concentrations in patients with diabetes mellitus (Wood & Grafman, 2003; Frederick, 2005; Ando *et al.*, 2011; Biessels *et al.,* 2020). Therefore, preservation of optimal cognitive function for as long as possible, without pharmacotherapy or alternative cognitive strategies, is essential. Cognition may also be divided into several different domains, including attention (filtering of specific stimuli), memory (retention and recall of information), language (understanding, repeating, and vocalising individual words and sentences), visuospatial ability (processing visual information and reproducing drawings), and executive function (high-

order cognitive processes that orchestrate goal-directed behaviours) (Biessels & Despa, 2018; Viggiano *et al.,* 2020). Prior to describing the functions linked to specific brain areas, a basic understanding of gross brain anatomy is warranted.

The adult human brain comprises three major divisions: the cerebrum, cerebellum, and the brainstem. The cerebrum, which is highly folded, consists of two cerebral hemispheres – left and right – interconnected by the corpus callosum, a thick band of white matter connective tissue. It plays general roles in basic sensory and motor information processing and the regulation of skeletal muscle contractions (Martini *et al.,* 2011). An area central to understanding and controlling speech, the Broca's area, resides in the left temporal area (Martini *et al.,* 2011). Posterior to the cerebrum lies the cerebellum, colloquially referred to as the second brain, a structure crucial for motor command modulation and balance (Buckner, 2013). The cerebellum is divided into right and left hemispheres, termed left and right cerebellar hemispheres, respectively. Anchored to the cerebellum is the brain stem, which contains relay centres and nuclei critical for regulating autonomic functions, such as BP and HR, as well as tracts and nuclei that assist in the maintenance of consciousness (Martini *et al.,* 2011). Damage or lesions to this area of the brain can result in unconsciousness (Martini *et al.,* 2011).

Blanketing the cerebrum is a thin, superficial layer of grey matter tissue known as the cerebral cortex. The cerebral cortex consists of four major lobes: frontal, parietal, occipital, and temporal (Figure 1.4). Each lobe controls and performs general functions essential to everyday activities, although some functions are solely isolated to specific lobes (Martini *et al.,* 2011). For example, the temporal lobe contains auditory processing centres (Herschel's area) and brain structures involved in long-term memory storage, formation and retrieval, and stress modulation (*e.g.* the amygdala and the hippocampus) (Martini *et al.,* 2011). Visual processing and colour perception is processed predominantly by the occipital lobe. Increased activity in the parietal lobe has been linked to visuospatial functioning and somatosensory processing (*e.g.* touch and pain), whereas high-order cognitive functions, such as decision-making and executive function, are chiefly subserved by the frontal lobe (Martini *et al.,* 2011). Although specific functions are performed by discrete brain areas, it is important to acknowledge that cognitive function results from continuous communication between various brain regions.

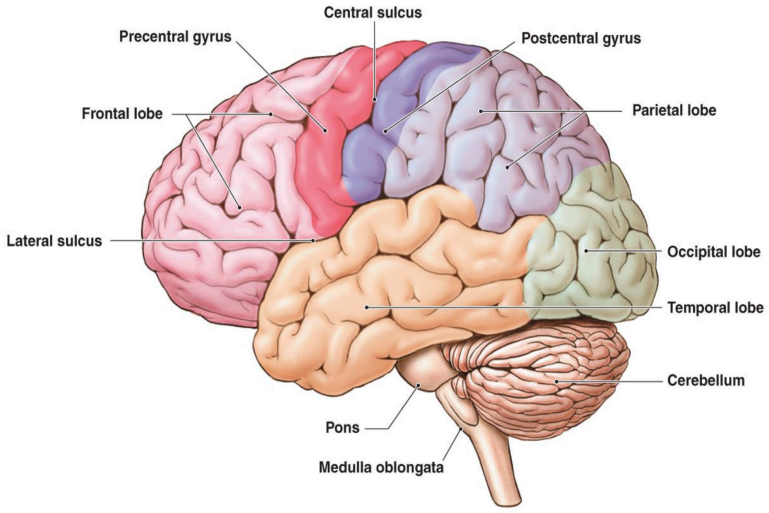


Figure 1.4. Lateral diagrammatic view of the cerebral cortex highlighting the different lobes of the brain as well as other landmark anatomical features. Each lobe (colour-coded) controls and performs specific functions essential for everyday activities. Adapted from Martini *et al.,* (2011, p 444).

Various pathologies and modifiable (*e.g.* lifestyle) and non-modifiable (*e.g.* age, genetics, race) risk factors, including but not limited to ageing, prolonged stress, head trauma, genetic predisposition, infection, diabetes mellitus, hypertension, chronic alcoholism, tobacco smoking, and high cholesterol, have been reported to directly or indirectly contribute to the onset and progression of cognitive dysfunction, leading to early development of cognitive impairment (Arnsten, 2009; Obisesan, 2009; Novak & Hajjar, 2010; Sims-Robinson *et al.*, 2013; Kivipelto *et al.,* 2018). Current estimates suggest cognitive impairment affects approximately 447, 115 individuals in Australia and, by 2025, is predicted to cost Australia upwards of $18.7 billion dollars (National Centre for Social and Economic Modelling, 2017). Alarmingly, the symptoms of cognitive impairment develop perniciously, often beginning decades prior to symptomatic presentation and, when overt, manifest as subtle disturbances in performing daily tasks (*e.g.* financial planning); consequently, accurate and timely diagnosis is challenging (Gauthier *et al.*, 2006; Hampel & Lista, 2016). Currently, early identification of mild cognitive impairment (MCI), the earliest detectable stage of cognitive decline, is the most reliable method for identifying populations at high-risk of converting to dementia (Hampel & Lista, 2016). In clinical practice, this is typically detected using formal neurocognitive assessments and analysing biomarkers in cerebrospinal fluid (CSF) (Hampel & Lista, 2016).

1.2.1 Mild Cognitive Impairment (MCI) (Mild Neurocognitive Disorder)

The concept of mild cognitive impairment (MCI) (mild neurocognitive disorder) was coined by Reisberg *et al.* (1982) to classify older individuals exhibiting impaired cognitive function, but not meeting criteria for dementia. It describes a transitional stage between normal cognition and cognitive decline that identifies individuals at high-risk of developing dementia (DeCarli, 2003; Reitz *et al.,* 2011; Hampel & Lista, 2016; Biessels & Despa, 2018; Viggiano *et al.,* 2020) and is defined as objective cognitive impairment (usually 1.5 SDs below the normative mean) in one or more cognitive domains. The main cognitive domain affected is memory (amnesic MCI), but others can also be impacted (Biessels & Despa, 2018; Viggiano *et al.,* 2020). While MCI typically does not interfere with performing complex daily tasks, which remain largely intact (Novak & Hajjar, 2010; Sachdev *et al.*, 2014; Hampel & Lista, 2016), engagement in everyday tasks and activities becomes increasingly effortful (Sachdev *et al.*, 2014; Hampel & Lista, 2016).

Although MCI is widely-viewed as a precursor to dementia, and investigators agree it is useful in identifying populations at an increased risk of developing AD (Reitz *et al.,* 2011), some researchers question the reliability of using MCI as a predictor of progression to dementia. This has been ascribed to the high number of individuals (~90–95%) who do not develop dementia (Viggiano *et al.,* 2020) and the mixed nature of MCI, which complicates accurate prediction of the likelihood of developing dementia (Richard & Brayne, 2014). In agreement with this, Roberts *et al.* (2014), in a recent large population-based study (n = 1939, age: 70-89 years at baseline), found reversion rates to normal cognition are relatively high. Following a 5.1 year median follow-up period, of the 534 cases of MCI identified at baseline, approximately 36% did not convert to dementia. Thirty-eight percent (38%) also reverted to normal cognition; however, after a later follow-up, it was reported that over half of these patients demonstrated symptoms fulfilling diagnostic criteria for MCI (Figure 1.5). In Australia, it is estimated that approximately 15% of patients with MCI convert to dementia per year (Dementia Australia, 2020). Current literature also indicates that patients with MCI have a three to five times increased risk of developing dementia compared to age-matched controls (Dementia Australia, 2020). Given no effective intervention strategies exist to delay the progression of MCI to AD or dementia, and the mechanisms linking MCI to these

cognitive diseases remain incomplete, early detection of MCI using non-invasive measures before irreversible cognitive deficits have manifested is critical.

Box 2 | Diagnostic criteria for mild neurocognitive disorder

A. Evidence of modest cognitive decline from a previous level of performance in one or more cognitive domains (complex attention, executive function, learning and memory, language, perceptual–motor, or social cognition) based on:
   1. Concern of the individual, a knowledgeable informant, or the clinician that there has been a mild decline in cognitive function; and
   2. A modest impairment in cognitive performance, preferably documented by standardized neuropsychological testing or, in its absence, another quantified clinical assessment.

B. The cognitive deficits do not interfere with capacity for independence in everyday activities (that is, complex instrumental activities of daily living such as paying bills or managing medications are preserved, but greater effort, compensatory strategies, or accommodation may be required).

C. The cognitive deficits do not occur exclusively in the context of a delirium.

D. The cognitive deficits are not better explained by another mental disorder (for example, major depressive disorder or schizophrenia).

Figure 1.5. Diagnostic criteria used widely by medical practitioners and clinicians to diagnose individuals with mild neurocognitive disorder, also known as mild cognitive impairment (MCI). Adapted from Sachdev *et al.,* (2014, p 637).

Accurate estimates of the prevalence of MCI, prognosis, and determination of individuals at increased risk of progression to dementia is also complex. This has been attributed to inconsistent definitions of MCI, varying prevalence rates of MCI reported in different populations, and difficulties in differentiating and defining boundaries between the various stages of cognitive impairment (*e.g.* mild cognitive impairment, AD, and dementia) (DeCarli, 2003; Hampel & Lista, 2016). Often, there is significant overlap. The published prevalence rates for MCI are also highly variable. A recent population-based epidemiological meta-analysis estimated the global prevalence of MCI in adults over 65 years to range between 3-37% (Sachdev *et al.*, 2015). In the same meta-analysis, which combined data from 11 longitudinal, population-based, cross-sectional studies incorporating the revised definition for MCI (Figure 1.5), the estimated global prevalence rate for MCI was considerably lower, ranging between 6-12% (Sachdev *et al.*, 2015). Therefore, it is imperative that future studies estimating the prevalence of MCI in populations comply with the revised criteria proposed recently for MCI to reduce misclassification of individuals.

Once diagnosed with MCI, it is hypothesised that individuals will follow one of three hypothetical trajectories: (i) reversion to normal healthy cognitive function, (ii) maintenance of stable MCI, or (iii) progression to dementia (impaired cognition) (Hampel & Lista, 2016) (Figure 1.6). Given the pathophysiological processes in MCI initiate decades prior to symptomatic presentation, and no effective disease-modifying pharmacological intervention strategies to delay progression to AD currently exist, early detection of cognitive decline using reliable and accurate screening tools is critical. Early identification of individuals at increased risk of developing dementia, particularly asymptomatic populations, would be conducive to reducing the substantial socioeconomic costs associated with AD. Addressing prevalent modifiable lifestyle risk factors known to exacerbate cognitive decline, such as mid-life obesity, dyslipidaemia, hypertension, and diabetes mellitus, would also be paramount to curbing the rising global MCI and dementia burden.

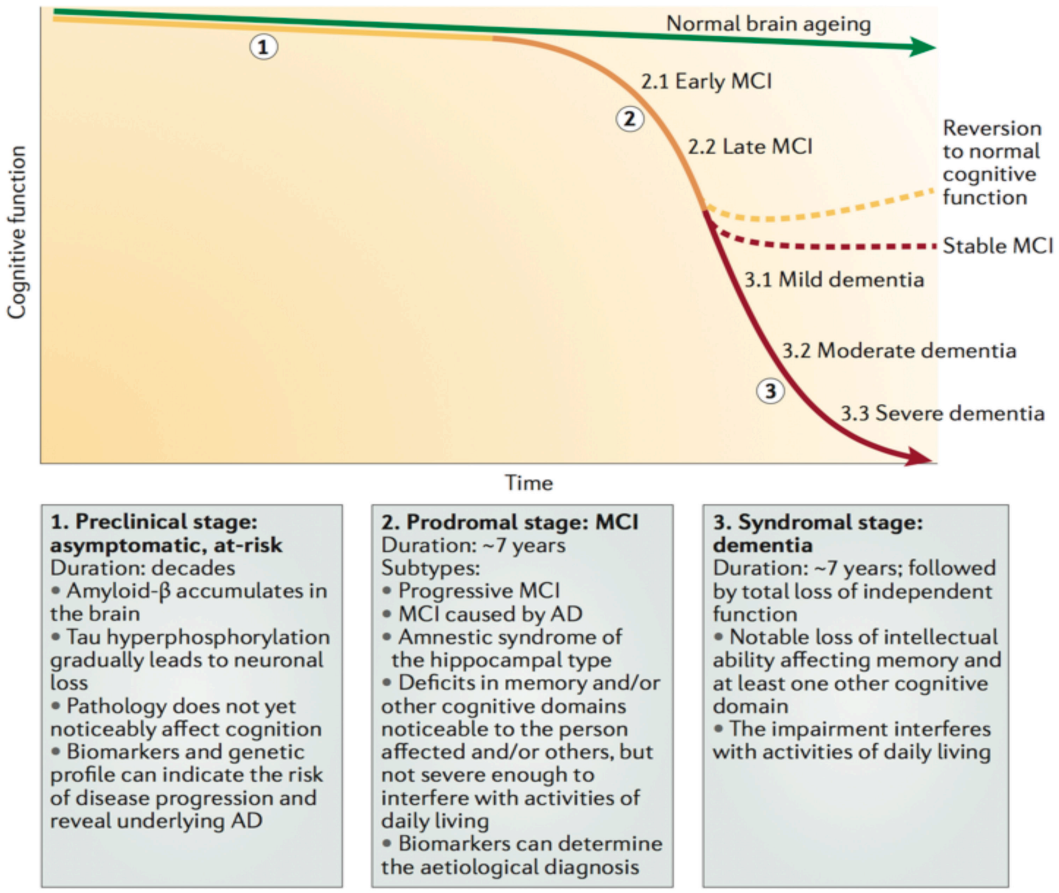


Figure 1.6. Proposed hypothetical trajectories in cognition that individuals follow during normal brain ageing and in the proposed major stages of cognitive impairment. Adapted from Hampel & Lista, (2016, p 2).

Key: **AD –** Alzheimer's Disease **MCI –** Mild Cognitive Impairment

β – beta

1.2.2 Major Neurocognitive Disorder (NCD) (Dementia)

Dementia, a crippling irreversible neurocognitive disorder commonly mistaken for Alzheimer's disease (AD), is an 'umbrella' term describing a syndrome characterised by pronounced deterioration in multiple cognitive domains (Reitz *et al.,* 2011; Ninomiya, 2014; Hampel & Lista, 2016). It is the most severe expression of cognitive impairment, causing a progressive loss of underlying cortical neurons, neuroinflammation and gliosis, profoundly disrupting vulnerable cortical networks responsible for normal social and occupational functioning (Sachdev *et al.*, 2014; Elahi & Miller, 2017; Kivipelto *et al.,* 2018). This complex neurodegenerative process results in irreversible impairments in cognition, including significant memory loss, disorientation, personality changes, and measurable decay in cognition from a previous grade of performance (AIHW, 2018). The strongest known risk factor for dementia is age, with approximately 90% of dementias manifesting after the age of 65 (Elahi & Miller, 2017;). The risk of dementia also nearly doubles every five years between the ages of 65 to 90 (Kivipelto *et al.,* 2018). Despite extensive research efforts dedicated to untangling the complex aetiology of the disease, the precise underlying pathophysiology of dementia remains elusive. Current literature suggests the pathophysiology is multi-factorial, involving complex interplay between lifestyle, vascular, inflammatory, and psychosocial processes (Kivipelto *et al.,* 2018; Sweeney *et al.,* 2018).

Similar to the growing unsettling trend in the prevalence of MCI, the prevalence of dementia is also rising: according to the World Alzheimer Report (2015), dementia affected approximately 46.8 million individuals worldwide. By 2050, primarily due to the ageing population and the increasing mean age of the population, estimates predict dementia will affect an estimated 131.5 million individuals globally (World Alzheimer Report, 2015; Elahi & Miller, 2017; Kivipelto *et al.,* 2018). Alarmingly, a similar pattern in prevalence has also been reported in Australia, with current estimates suggesting 459,000 Australians suffer from dementia (Dementia Australia, 2018) (Figure 1.7). This translates to approximately 250 individuals developing the syndrome every day (Dementia Australia, 2018). Unless effective pharmacotherapies are developed, or a significant medical breakthrough occurs, by 2028, the number of individuals affected by dementia will rise to 598,000 (Dementia Australia, 2018). Further estimates suggest this number could rise to 1,076,000 by 2058 (Dementia Australia, 2018). It is clear that dementia is a major, pressing socioeconomic burden requiring urgent scientific attention.

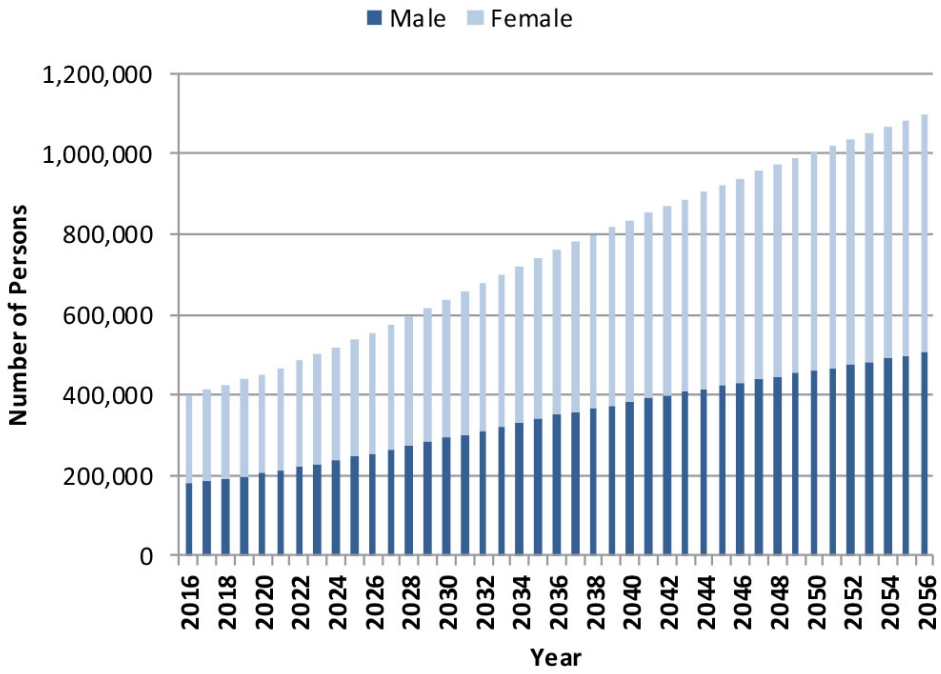


Figure 1.7. Current and projected number of Australians (male and female) estimated to be affected by dementia between 2016-2056. Adapted from Alzheimer's Australia, (2017, p 11).

The socioeconomic burden linked to treating and managing the various forms of dementia (AD, vascular dementia, dementia with Lewy bodies, and frontotemporal dementia), is also substantial. According to a report published in 2003, it was estimated that dementia cost Australia $6.6 billion (Access Economics, 2003). In 2016, costs associated with treating dementia exceeded $14 billion (direct costs: $8.8 billion; indirect costs: $5.5 billion), translating into an astounding average of $35,550 per individual (Alzheimer's Australia, 2017). By 2050, it is estimated that costs associated with treating dementia globally will exceed $1.1 trillion (Prince *et al.,* 2013). Given the rising global prevalence of dementia, and emerging data indicating diabetes mellitus and hypertension are associated with an increased risk of developing cognitive impairment, research aimed at identifying other modifiable risk factors associated with an increased risk of dementia and incipient signs and symptoms of early cognitive decline is urgently required. Exploration of alternative non-invasive detection methods that may facilitate reliable and early identification of cognitive deterioration, before irreversible cognitive deficits have occurred, would also be of equal clinical importance.

Dementia is hypothesised to result from a broad range of aetiologies, including modifiable lifestyle risk factors (*e.g.* diabetes mellitus, hypertension, substance abuse, and chronic alcohol intoxication), but is classified on the basis of underlying neuropathologies (Elahi & Miller, 2017). These include abnormal misfolded protein aggregates accumulating in vulnerable neurons and glia (Elahi & Miller, 2017). However, accurate and timely classification of dementia and differentiation of dementia from AD and other underlying mental disorders is difficult, requiring fulfilment of the following: (a) a comprehensive clinical examination of the patient's history by a medical professional supported by anecdotal observations from a dependable informant, (b) objective cognitive and neuropsychological assessment, and (c) fulfilment of specific diagnostic criteria published in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (5th edition) (DSM-5) (Figure 1.8) (Elahi & Miller, 2017). The DSM-5 manual, revised in 2013 to assist clinicians in diagnosing dementia, also identifies six key cognitive domains detrimentally affected by neurocognitive disorders (Figure 1.9).

A. Evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains (complex attention, executive function, learning and memory, language, perceptual–motor, or social cognition) based on:
   1. Concern of the individual, a knowledgeable informant, or the clinician that there has been a significant decline in cognitive function; and
   2. A substantial impairment in cognitive performance, preferably documented by standardized neuropsychological testing or, in its absence, another quantified clinical assessment.

B. The cognitive deficits interfere with independence in everyday activities (that is, at a minimum, requiring assistance with complex instrumental activities of daily living such as paying bills or managing medications).

C. The cognitive deficits do not occur exclusively in the context of a delirium.

D. The cognitive deficits are not better explained by another mental disorder.

Specify:

- Without behavioural disturbance: if the cognitive disturbance is not accompanied by any clinically significant behavioural disturbance
- With behavioural disturbance (specify disturbance): if the cognitive disturbance is accompanied by a clinically significant behavioural disturbance (for example, psychotic symptoms, mood disturbance, agitation, apathy, or other behavioural symptoms). For example, major depressive disorder or schizophrenia

Figure 1.8. Diagnostic criteria used widely by clinicians to diagnose patients with major neurocognitive disorder/dementia. Adapted from Sachdev *et al.,* (2014, p 638).

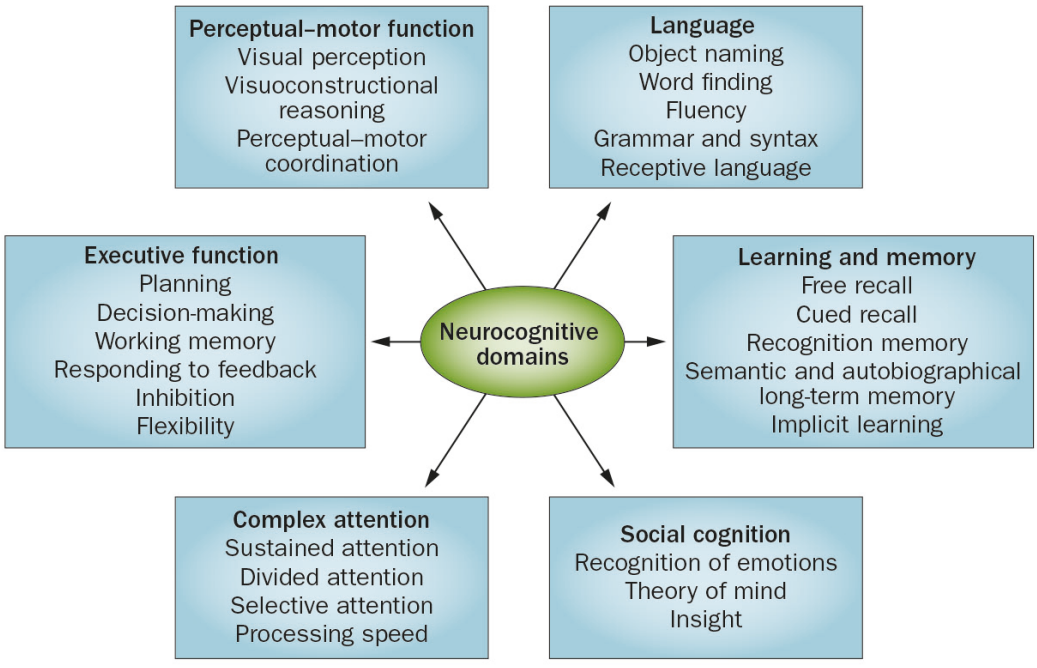


Figure 1.9. Key neurocognitive domains that are commonly affected by neurocognitive disorders, as recognised by the DSM-5 criteria. Adapted from Sachdev *et al.,* (2014, p 636).

1.2.3 Alzheimer's Disease (AD)

Alzheimer's disease (AD) is a serious and irreversible neurodegenerative disease characterised by notable memory loss, significant decay in cognitive function, and disturbances in personality and behaviour (Baugmart *et al.,* 2015; Elahi & Miller, 2017). It is the most common crippling form of dementia that primarily affects older populations (~65 years old), accounting for approximately 70-80% of dementia cases in Australia and an estimated 50-75% globally (Citron, 2010; Nordberg, 2015; AIHW, 2018; Dementia Australia, 2020). Advanced age is the most significant risk factor in AD development, with the risk of AD doubling every five years after age 65 (Citron, 2010; Nordberg, 2015; Dementia Australia, 2020). Other notable risk factors for AD include a family history of the condition, neuroinflammation, cerebrovascular disease, traumatic brain injury, vascular pathology, and low education (Baugmart *et al.,* 2015; Elahi & Miller, 2017). Worldwide, AD affects approximately 46.8 million individuals, and projections suggest this number will increase two-fold every twenty years, rising to 131.5 million by 2050 (World Alzheimer Report, 2015). Alarmingly, no effective disease-modifying treatment options or objective diagnostic tools to reliably and accurately detect the disease presently exist.

Two major clinical subtypes of AD are recognised: familial AD (fAD) (early onset) and sporadic AD (sAD) (late onset) (Baglietto-Vargas *et al.*, 2016; Elahi & Miller, 2017; Dementia Australia, 2020). Most AD cases are sporadic AD (~98%), but some patients develop familial AD (<1%), an uncommon form (Elahi & Miller, 2017). While both subtypes share similar neuropathological hallmarks (*e.g.* amyloid-beta (Aβ) plaques, neurofibrillary tangles (NFTs), and notable neuronal and synaptic loss), the determinants responsible for triggering neurodegeneration differ (Baglietto-Vargas *et al.*, 2016). Mutations in three specific genes – amyloid precursor protein (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2) – have been shown to promote Aβ protein deposition and have been implicated in fAD pathogenesis and early-onset AD (EOAD) (1-5% of cases); however, the pathogenesis of sAD remains largely unknown (Citron, 2010; Van Cauwenberghe *et al.,* 2016; Baglietto-Vargas *et al.*, 2016; Kandimalla *et al.,* 2017). Current literature suggests the aetiology is multifactorial, resulting from complex interactions between a combination of genetic risk alleles and lifestyle factors (both modifiable and non-modifiable) (Van Cauwenberghe *et al.,* 2016; Baglietto-Vargas *et al.*,

2016; Kandimila *et al.,* 2017). At the genetic level, the ε4 (epsilon) allele of the apolipoprotein E gene (APOE) is strongly associated with an increased risk of sAD (15-fold higher in homozygotes; three-fold higher in heterozygotes) (Farrer *et al.,* 1997; Van Cauwenberghe *et al.,* 2016).

Unlike MCI, which develops with no detectable changes in brain tissue, AD is characterised clinically by underlying neuropathological hallmarks (Figure 1.10). The two main pathologies include: (1) the accumulation of insoluble, neurotoxic amyloid-beta (Aβ) aggregates (neuritic plaques), and (2) intracellular neurofibrillary tangles (NFTs), caused by hyper-phosphorylated *tau* protein (Reitz *et al.,* 2011; Nordberg, 2015; Baglietto-Vargas *et al.*, 2016; Kandimila *et al.,* 2017). Substantial literature indicates these pathologies contribute synergistically to and underlie the cognitive deficits commonly reported in AD (Nordberg, 2015; Baglietto-Vargas *et al.*, 2016; Kandimila *et al.,* 2017). However, convincing data obtained by Braak *et al.* (2011), who found NFTs in the absence of amyloid in the brains of 2,332 patients with AD in the early stages of disease, have challenged the established view that Aβ predominantly underlies the progressive cognitive decline in AD. Such a finding has also sparked discussion between investigators to reconsider AD as a tauopathy, although AD is still considered by many as a dual proteinopathy (Elahi & Miller, 2017).

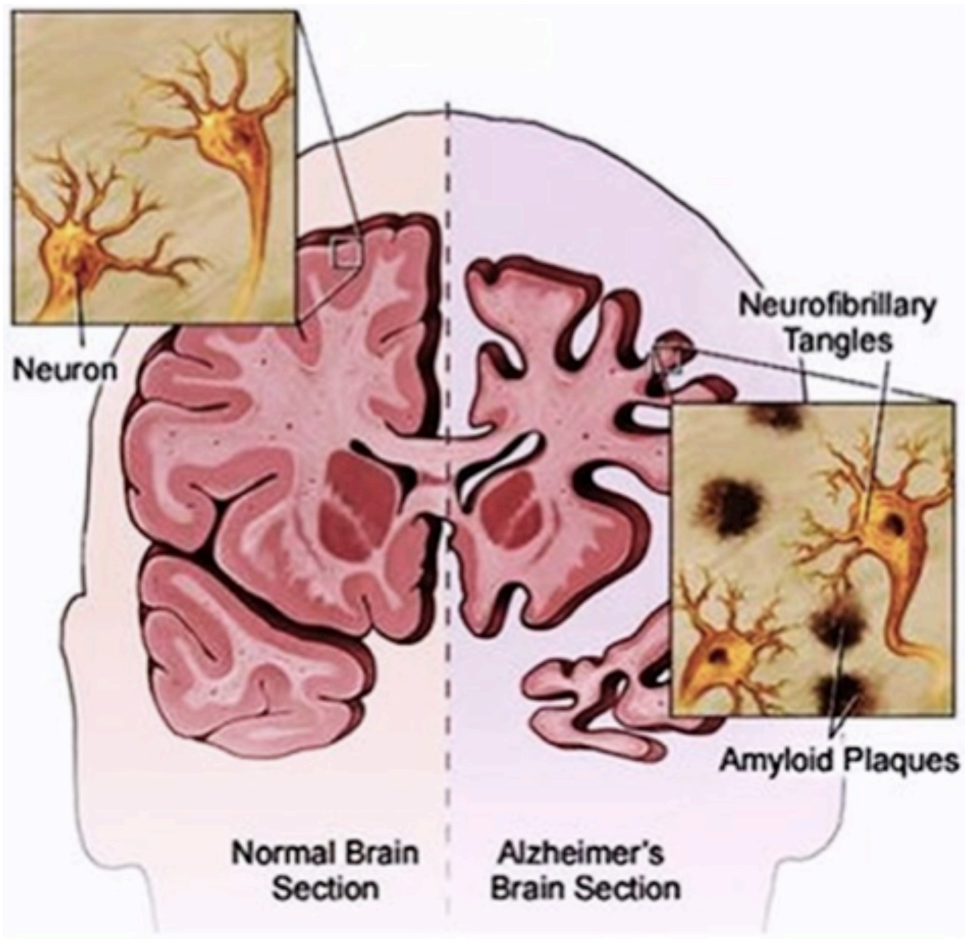


Figure 1.10. Cross-sectional representation comparing healthy brain tissue to Alzheimer's disease brain tissue. Adapted from U.S Department of Health & Human Services, (2008).

Alzheimer's disease is associated with global cortical shrinkage and perturbations in underlying cellular and molecular processes, including neuronal loss, oxidative stress, neuro-inflammation, disrupted cholinergic neurotransmission, gliosis, and synaptic degeneration (Baglietto-Vargas *et al.*, 2016; Kandimila *et al.,* 2017; Elahi & Miller, 2017). Such disturbances and cortical atrophy, purportedly triggered by the proteinopathies described above (Aβ and NFTs), cause neurodegeneration in underlying affected brain tissue, resulting in cognitive deficits such as memory loss and personality changes. These deficits, which often manifest perniciously and increase gradually in severity, correlate with the degree of neurodegeneration in underlying brain tissue (Mormino *et al.*, 2014).

Alzheimer's disease affects several brain regions and, once diagnosed, patients on average survive 9 years (Masters *et al.*, 2015; Elahi & Miller, 2017) (Figure 1.11). The neurodegenerative process is progressive: initially, the disease preferentially disrupts vulnerable neurons and glia in brain regions responsible for episodic memory, including the transentorhinal cortex of the medial temporal lobes, hippocampus, noradrenergic neurons of the locus coeruleus, and basal forebrain (Elahi & Miller, 2017). Degeneration in these brain areas causes deficits in attention and short-term episodic memory (Apostolova & Thompson, 2007; Elahi & Miller, 2017). As AD progresses, characterised by the gradual accumulation of abnormal neurotoxic protein aggregates (Aβ) and NFTs in the temporo-parietal cortices (Figure 1.12), it causes pronounced personality changes, visuospatial dysfunction, disturbances in attention, acalculia, and memory loss (Apostolova & Thompson, 2007; Elahi & Miller, 2017). By end-stage AD, patients demonstrate significant memory loss and lack the ability to recognise familiar faces (prosopagnosia) (Apostolova & Thompson, 2007; Elahi & Miller, 2017). They also struggle to perform simple daily tasks, developing complete dependence on caregivers and family members. This imposes appreciable strain on society, health care systems, and caregivers (Citron, 2010; Nordberg, 2015).

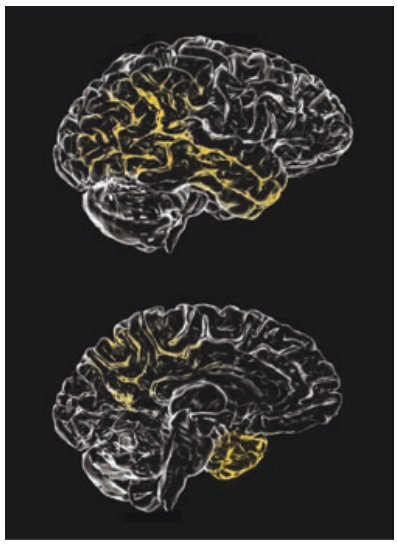


Figure 1.11. Neuroimaging scan highlighting brain areas affected by sporadic Alzheimer's disease (AD) in the early stages. Atrophy in the medial temporal lobes is a signature structural abnormality of early-stage AD. Affected regions are highlighted in yellow. Adapted from Elahi & Miller, (2017, p 464).

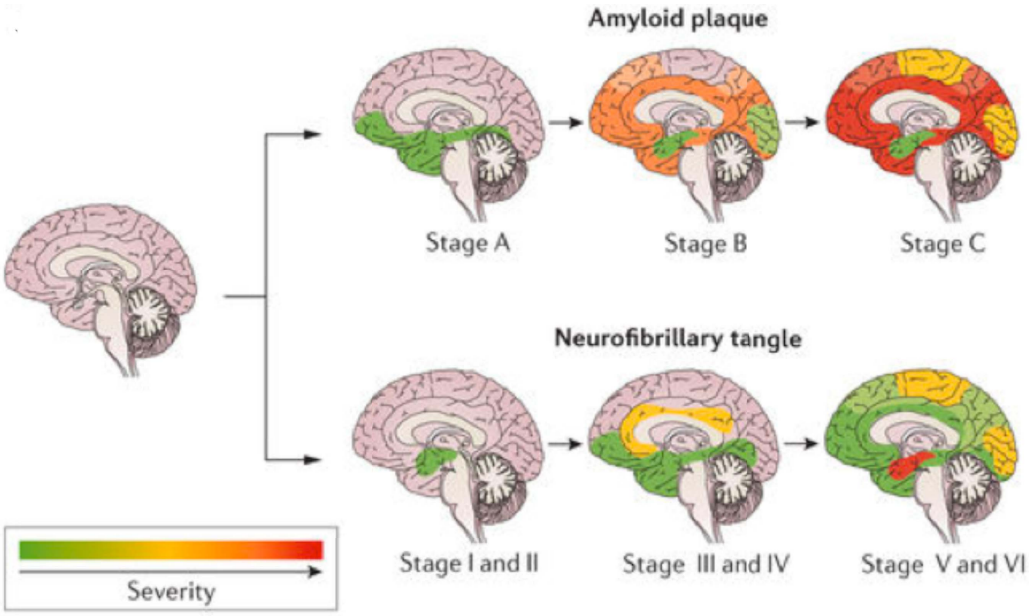


Figure 1.12. Patterns in the accumulation of neuropathologies (amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs)) in Alzheimer's disease and their relation to disease severity. Adapted from Masters *et al.,* (2015, p 2).

One prominent issue with current diagnostic criteria for cognitive impairment criticised repeatedly by clinicians, is that the aetiology is determined based on the nature of the symptoms; therefore, clinicians recommend using biomarkers for determining accurately the underlying aetiology. Although promising biomarkers of AD pathology and disease progression have been identified in cerebrospinal fluid (CSF) ($A\beta_{42}$: $A\beta_{40}$; highest diagnostic accuracy for AD), minimal progress has been made in recent years in developing effective disease-modifying therapies against AD (Citron, 2010). To date, no approved pharmacotherapy or disease-modifying interventions can impede the onset or progression of AD, so future cognitive-based research is urgently required. Current clinical treatment for AD includes acetylcholinesterase inhibitors (AChEI) together with psychosocial support; however, these treatment options do not confer neuroprotective benefits, providing only symptomatic improvement and delaying the progressive cognitive decline. In 2016, dementia and AD were the second leading cause of death in Australians, trailing closely behind coronary heart disease (AIHW, 2018) (Figure 1.13). Unless curative or effective pharmacotherapies are developed, the devastating socioeconomic burden of AD will continue to rise, impacting sufferers, caregivers, and society worldwide, costing Australia an estimated $18.7 billion dollars by 2025 (Dementia Australia, 2020).

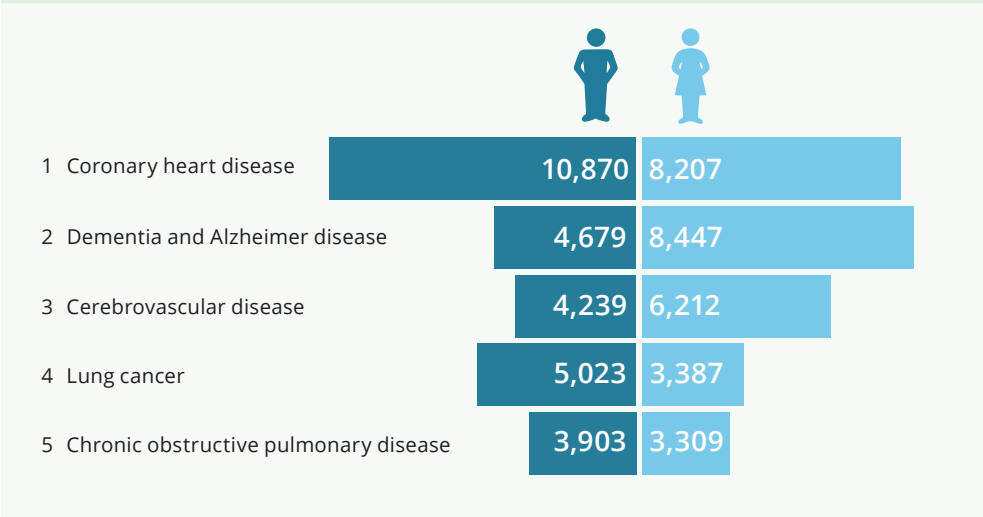


Figure 1.13. Leading causes of death in Australian males and females in 2016. Dementia and Alzheimer's disease were the second leading causes of death, accounting for an estimated 11% of deaths in females. Adapted from Australian Institute of Health & Welfare (AIHW), (2018, p 89).

In view of increased prevalence rates predicted for AD, future research must identify biomarkers of early cognitive impairment (MCI) or the various stages of cognitive impairment (pre-clinical, prodromal, and syndromal). This may enable early detection of incipient cognitive decline and identification of populations at increased risk of developing cognitive impairment long before irreversible cognitive deficits have manifested, thus allowing early therapeutic intervention. Elahi & Miller (2017) argue these biomarkers should reflect early-stage pathological processes, as their predictive utility deteriorates with age. Concurrent determination of prevalent modifiable lifestyle risk factors associated with accelerating cognitive decline, such as high cholesterol, mid-life obesity, sedentary lifestyle, diabetes mellitus, and hypertension, would also be advantageous. Such risk factors have been consistently linked to AD, dementia, and another increasingly-common form of dementia, vascular dementia.

1.2.4 Vascular Dementia (VaD)

Vascular dementia (VaD) (also vascular cognitive impairment (VCI)) is the second most common form of cognitive impairment, encompassing all vascular pathologies that contribute to cognitive decline (van der Flier, *et al.,* 2018; Iadecola *et al.,* 2019; Dementia Australia, 2020). It is caused by cerebrovascular events that disrupt cerebral blood flow or damage brain tissue directly (*e.g.* stroke) and accounts for ~15-20% and ~15-30% of total dementia cases in Australia and worldwide, respectively (van der Flier *et al.,* 2018). The resulting vascular pathologies – ischaemia-induced infarcts or white matter hypersensitivities (WMHs) – damage the highly-vascularised network of blood vessels of the brain, interrupting the supply of crucial oxygen, energy metabolites, and nutrients to glucose-dependent neurons (Sweeney *et al.,* 2018; Iadecola *et al.,* 2019; Dementia Australia, 2020). This causes noticeable impairment in cognition (van der Flier *et al.,* 2018).

Several risk factors (modifiable and non-modifiable) have been implicated in VaD development, notably advanced age and those outlined in section 1.2. Substantial epidemiological evidence has also revealed poorly-controlled diabetes mellitus (DM) and hypertension significantly elevate VaD risk, with recent estimates attributing 50% of VaD cases to hypertension (Dementia Australia, 2020). Most cases of VaD (>90%) are classified as mixed aetiology, as pure VaD (*i.e.* dementia directly attributable to cerebrovascular events) is uncommon (<10% dementia cases) (van der Flier *et al.,* 2018). Interestingly, emerging data indicate that patients with Type 2 diabetes mellitus (T2DM) who have dementia demonstrate abnormalities in vasculature similar to those observed in VaD. This finding has prompted investigators to refer to dementia in diabetes as "diabetic dementia", a unique form of dementia that differs from typical dementia (Morley, 2017).

VaD affects several brain regions (temporal and parietal cortices, thalamus, and basal ganglia), with the cognitive deficits typically correlating with the location of the underlying cerebrovascular pathology and degree of damage (van der Flier *et al.,* 2018). While memory and behaviour may be affected, VaD preferentially disrupts high-order cognitive processes controlled by the frontal lobe, such as executive functioning (planning, organising) and information processing (Garrett *et al.,* 2004; Iadecola *et al.,* 2016; van der Flier *et al.,* 2018). Similar to the neurocognitive diseases described earlier,

a diagnosis of VaD is also complex, requiring a comprehensive neurological examination using established, objective neuroimaging modalities (magnetic resonance imaging – MRI) and psychometric assessments to validate the observed cognitive disturbances (Elahi & Miller, 2017; van der Flier *et al.,* 2018). However, due to the limited time available in everyday clinical practice and the complexity in performing neuroimaging, VCI is commonly diagnosed by clinicians when evidence of vascular pathology is evident and other potential causes of cognitive impairment have been excluded (van der Flier *et al.,* 2018).

Unlike the progressive degenerative nature of AD, VaD causes abrupt deterioration in cognitive function that proceeds in a stepwise pattern following adverse cardiovascular events such as strokes. These are the most common cause of VaD. However, impairments in cognition may also result from subclinical vascular brain injury (Elahi & Miller, 2017). These sharp declines in cognition cause noticeable disruptions to day-to-day social and occupational functioning, resulting in reduced survival (approximately 3-5 years after diagnosis) (Iadecola *et al.,* 2016; van der Flier *et al.,* 2018; Dementia Australia, 2020). Although improvement in cognitive function may occur after cardiovascular events, it typically worsens following each successive deleterious cardiovascular event (Dementia Australia, 2020).

1.3 Risk factors for cognitive impairment

Several modifiable and non-modifiable lifestyle risk factors (Section 1.2) have been associated, to varying degrees, with directly and indirectly triggering or contributing to the development and progression of cognitive dysfunction. Such widely-recognised risk factors include advanced age, high cholesterol (hypercholesterolaemia), physical inactivity, poor diet, sedentary behaviour, mid-life obesity, low educational attainment, and tobacco smoking (Biessels & Reagan, 2015) (Figure 1.14); however, the mechanisms linking these specific lifestyle risk factors and cognitive dysfunction remain controversial and poorly elucidated. Prolonged wakefulness and disrupted sleep have recently also been suggested as a risk factor for cognitive impairment, although further evidence is required to confirm this relationship, which falls outside the scope of this thesis (Bubu *et al.,* 2017).

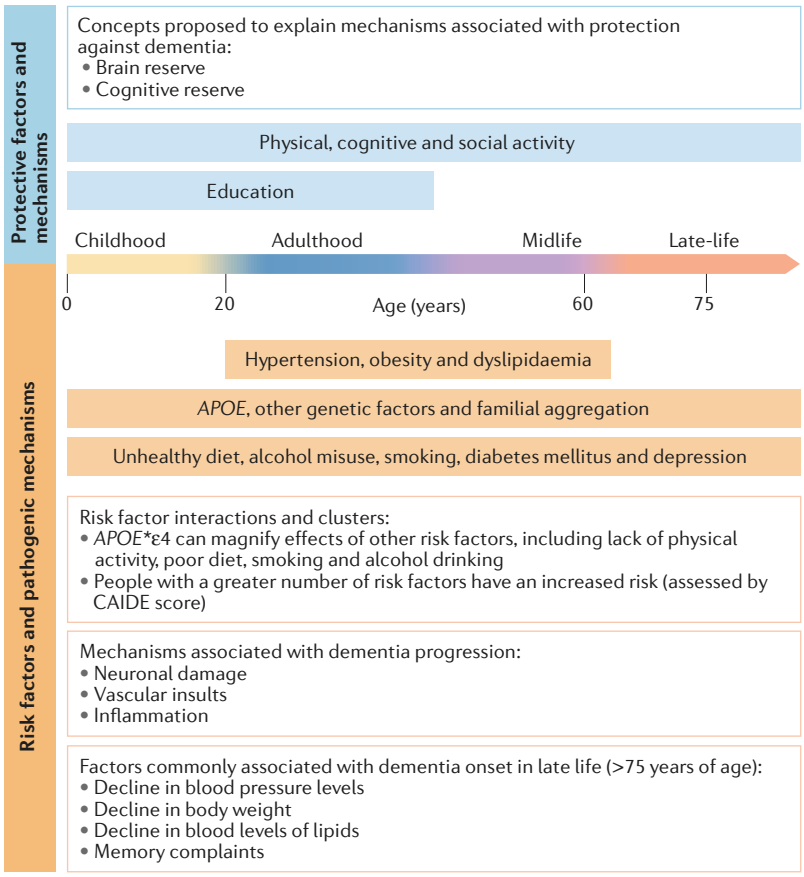


Figure 1.14. Modifiable and non-modifiable risk factors for cognitive impairment across the entire lifespan. Some lifestyle risk factors affect the risk of developing cognitive impairment between specific time periods (*e.g.* obesity, dyslipidaemia), whereas others affect dementia risk at any stage of life (*e.g.* diabetes mellitus, unhealthy diet, depression). Adapted from Kivipelto *et al.,* (2018, p 3).

Although increasing age is universally accepted as the strongest risk factor for the development of the various forms of cognitive impairment (Kivipelto *et al.,* 2018), substantial epidemiological evidence indicates that prevalent modifiable risk factors increase and accelerate the likelihood of developing cognitive impairment. Two prevalent modifiable lifestyle risk factors that have been consistently linked to exacerbating cognitive decline and are associated with an increased risk of developing neurodegenerative diseases, such as those described earlier, are diabetes mellitus and hypertension, and these conditions will comprise a major focus of this thesis.

1.3.1 Diabetes Mellitus

Diabetes mellitus (DM) is a modern-day global epidemic and its debilitating nature worldwide has been described as a "serious threat to global health that respects neither socioeconomic status nor national boundaries" (International Diabetes Federation Diabetes Atlas, 9th Edition, 2019, p2). It is a highly-complex, non-communicable metabolic disorder characterised by dysregulation of glucose homeostasis (Ashcroft & Rorsman, 2012; McCrimmon, Ryan, & Frier, 2012; Grayson, Reely, & Sandoval, 2013; Koekkoek *et al.*, 2015; International Diabetes Federation, 2019) (Figure 1.15). Various modifiable (*e.g.* physical inactivity, overweight/obesity, poor diet, high caloric intake, tobacco smoking, and alcoholism) and non-modifiable risk factors (*e.g.* age, sex, family history, and history of gestational diabetes) have been identified, but to date the precise underlying cause of diabetes remains elusive (Back & Kaufman, 2012; Rajagopalan, & Brook, 2012; Szendroedi *et al.,* 2012; Atkinson *et al.,* 2014). Diabetes mellitus causes transitory and sustained elevations in circulating blood glucose concentrations, a medical sequela known clinically as hyperglycaemia, which is associated with numerous deleterious and costly long-term complications (Section 1.4). Although pharmacotherapy (glucose-lowering therapies) and lifestyle modification (increased physical activity, nutritionally-balanced diet, cessation of smoking and alcohol consumption) may minimise the severity, progression, and onset of the disease, diabetes currently remains incurable (IDF, 2019).

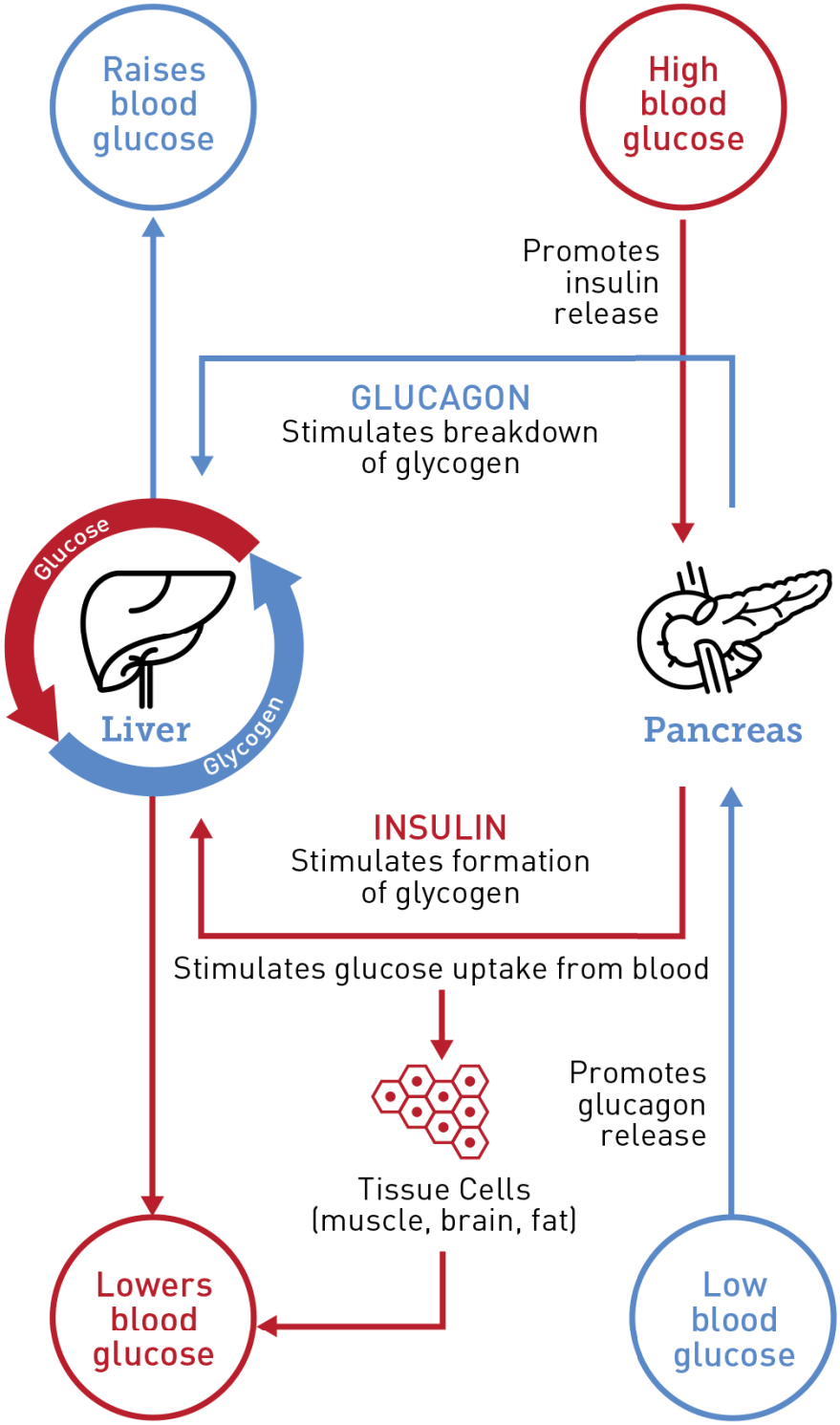


Figure 1.15. Diagrammatic representation of normal homeostatic regulation of blood glucose concentration. In both forms of diabetes mellitus, blood glucose homeostasis is dysregulated, leading to hyperglycaemia. Adapted from International Diabetes Federation (IDF), (2018, p 25).

Two major forms of DM are broadly recognised: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Both forms are characterised by raised blood glucose concentration (hyperglycaemia) (fasting plasma glucose (FPG) ≥ 7.0 mmol/L (millimole per litre); two-hour plasma glucose ≥ 11.1 mmol/L)), a strong risk factor for the development of microvascular complications (see section 1.4) (Sims-Robinson *et al.*, 2010; Baumgart *et al.*, 2015; Biessels & Reagan, 2015; Koekkoek *et al.*, 2015; DeFronzo *et al.,* 2017). While newer subtypes are being identified, this thesis will focus on the established forms, T1DM and T2DM.

1.3.2 Type 1 Diabetes Mellitus (T1DM)

Type 1 diabetes mellitus (formerly insulin-dependent diabetes mellitus) most commonly manifests in childhood (but may develop at any age) and accounts for ~5-10% of all diabetes cases (Koekkoek *et al.*, 2015; IDF, 2019). It is caused by the irreversible autoimmune-mediated destruction of insulin-secreting pancreatic beta (β) cells (Sims-Robinson *et al.*, 2010; Biessels & Reagan, 2015; Koekkoek *et al.*, 2015). This results in complete insulin deficiency, leading to hypergylcaemia (Sims-Robinson *et al.*, 2010; Biessels & Reagan, 2015; Koekkoek *et al.*, 2015). The irreversible destruction of pancreatic β-cells is posited to eventuate *via* complex interactions between genetic risk alleles and environmental factors; however, the precise underlying pathophysiology is currently unclear (Harcourt *et al.,* 2013; IDF, 2019). As insulin-secreting pancreatic β-cells are destroyed by an autoimmune reaction, patients with T1DM require lifelong dependency on exogenous insulin therapy and stringent ongoing monitoring of blood glucose concentrations to maintain acceptable glycaemic control (Harcourt *et al.,* 2013; IDF, 2019). This imposes a considerable burden for both the patient and the numerous allied health care professionals directly involved in the management of the disease. Symptoms of T1DM include excessive thirst, unexplained weight loss, frequent urination, lethargy, and constant hunger (IDF, 2019).

1.3.3 Type 2 Diabetes Mellitus (T2DM)

In contrast, T2DM is characterised by insulin resistance (IR), purportedly caused by a combination of progressive pancreatic β-cell dysfunction, diminished sensitivity to insulin, and impaired insulin secretion (Figure 1.16) (Harcourt *et al.,* 2013; Grayson *et al.,* 2013; Biessels & Reagan, 2015; Koekkoek *et al.*, 2015). Target cell (skeletal muscle, adipose tissue, and liver) unresponsiveness to insulin leads to reduced glucose uptake and stimulates pancreatic alpha (α) cells to secrete glucagon, stimulating hepatic glucose production (HPG), leading to hyperglycaemia (Zheng *et al.,* 2018). Similar to T1DM, the pathophysiology of T2DM is currently unknown, although several well-established lifestyle risk factors, including obesity, tobacco smoking, insufficient physical activity, genetic susceptibility, and poor diet have been implicated in IR and exacerbating pancreatic β-cell dysfunction (Sims-Robinson *et al.,* 2010; Koekkoek *et al.,* 2015). The strongest predictor of T2DM is overweight/obesity, with weight gain in early adulthood (25-40 years) being strongly associated with a higher risk and earlier onset of T2DM (Riboli *et al.,* 2002).

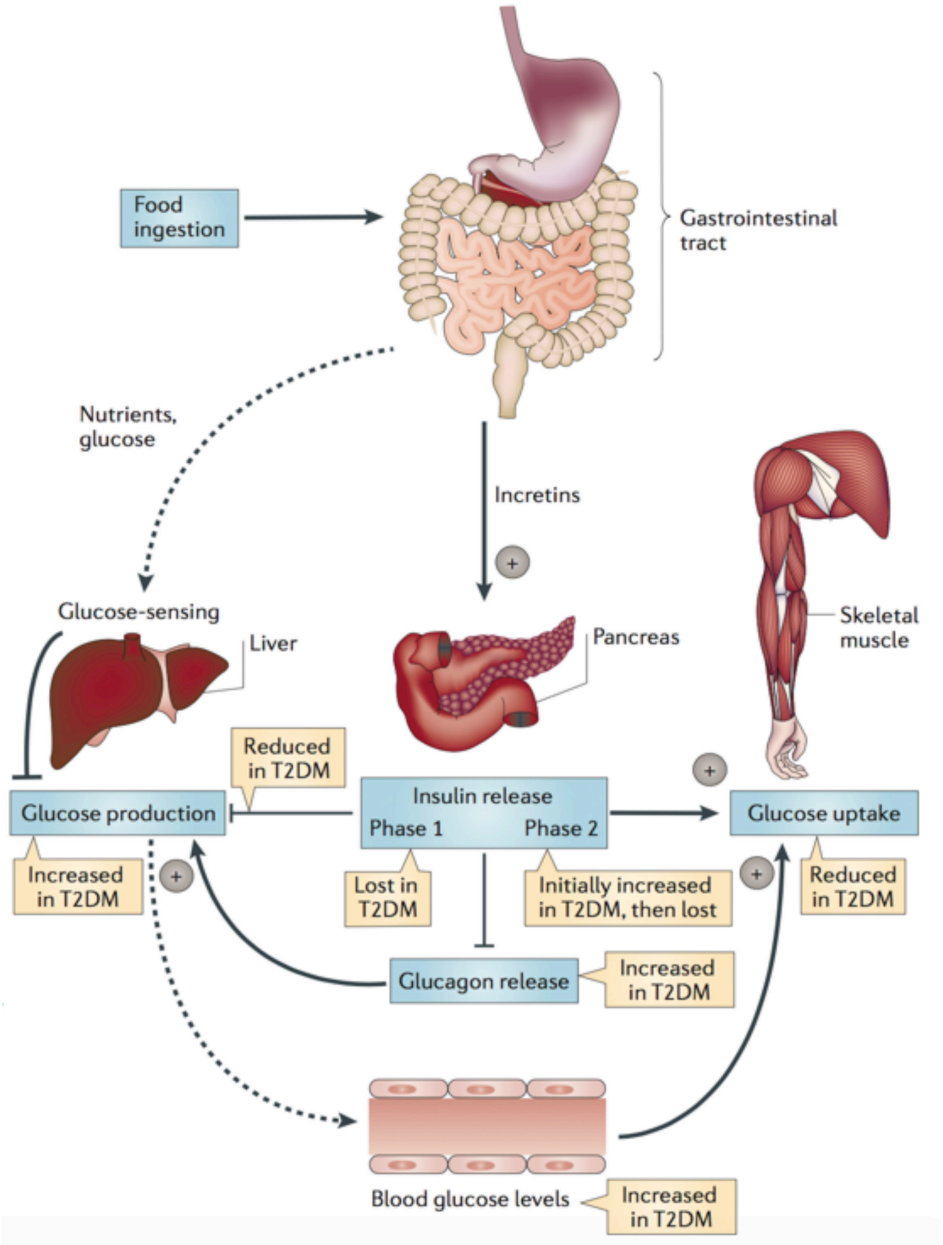


Figure 1.16. Dysregulation of blood glucose homeostasis in Type 2 diabetes mellitus (T2DM). In individuals with T2DM, the incretin response, which is responsible for ~70% of insulin secretion following an oral glucose load, is diminished. This results in impaired glucose-stimulated insulin secretion, leading to hyperglycaemia. Adapted from Grayson, Seeley, & Randoval, (2013, p 3).

Most risk factors for T2DM are modifiable; therefore, T2DM is considered a largely preventable and reversible disease (IDF, 2019). Unlike T1DM, T2DM chiefly manifests in adulthood and accounts for approximately 85-90% of all diabetes cases worldwide (IDF, 2019); however, due to sedentary behaviour, increased longevity, poor diet, and trends in the ageing of the population, T2DM is increasingly being observed in younger populations (Biessels & Despa, 2018; IDF, 2019). While T1DM and T2DM share similar core symptoms (*e.g.* hyperglycaemia), recurrent fungal infections, delayed wound healing, and numbness/tingling in extremities distinguish T2DM from T1DM (IDF, 2019).

Although T1DM and T2DM share similar pathophysiological hallmarks (impaired insulin secretion, pancreatic β-cell dysfunction, and hyperglycaemia), their respective epidemiology, accompanying comorbidities, and pathophysiology differ (McCrimmon *et al.,* 2012; Atkinson *et al.,* 2014). Key disease characteristics, including approximate prevalence rates and treatment options available for both forms, are summarised in Table 1.1.

Table 1.1. Key disease characteristics of type 1 and type 2 diabetes mellitus. Adapted and modified from Koekkoek *et al.*, (2015).

Characteristic	T1DM (insulin-dependent)	T2DM (non-insulin dependent)
Pathophysiology	Autoimmune disorder; irreversible destruction of insulin-secreting pancreatic beta (β) cells	Progressive pancreatic β-cell dysfunction, insulin resistance
Period of Onset	Incidence peaks during childhood (5 – 7 years) or early adulthood but can occur at any age	Peaks in adulthood; however, increasingly observed in younger populations due to sedentary behaviour
Prevalence (%)	~ 5-10%	~ 90-95%
Treatment	Constant glucose monitoring and lifelong exogenous insulin administration *via* injections or insulin pump therapy	Lifestyle modification (physical activity, nutritionally-balanced diet, patient education, cessation of alcohol and smoking, weight loss) in combination with pharmacotherapy (metformin, DPP-4is, and SGLT2is are the most commonly prescribed glucose-lowering therapies)

Key:

T1DM - Type 1 Diabetes Mellitus **T2DM -** Type 2 Diabetes Mellitus

DPP-4i - Dipeptidyl-peptidase 4 inhibitor

SGLT2i - Sodium Glucose Cotransporter 2 inhibitor

Over the last two decades, the global prevalence of diabetes mellitus has risen steadily. Current estimates suggest diabetes mellitus affects roughly 463 million individuals worldwide and, if current trends continue, attributable to increased life expectancy and sedentary lifestyles, the number of affected individuals is predicted to rise to 700 million (~20%) by 2045, with low- and middle-income populations primarily driving this trend (Zheng *et al.,* 2018; IDF, 2019) (Figure 1.17). An estimated 1.7 million Australians are affected by diabetes mellitus and a further 6.1% of adults self-report having the metabolic disorder (Diabetes Australia, 2017; Australia's Health, 2018). Similarly, projections indicate this number will also increase (Diabetes Australia, 2019). Alarmingly, estimates predict that by 2023 diabetes will supersede dementia as the fastest growing chronic disease in Australia (McCrimmon *et al.,* 2012; Atkinson *et al.,* 2014) (Figure 1.18).

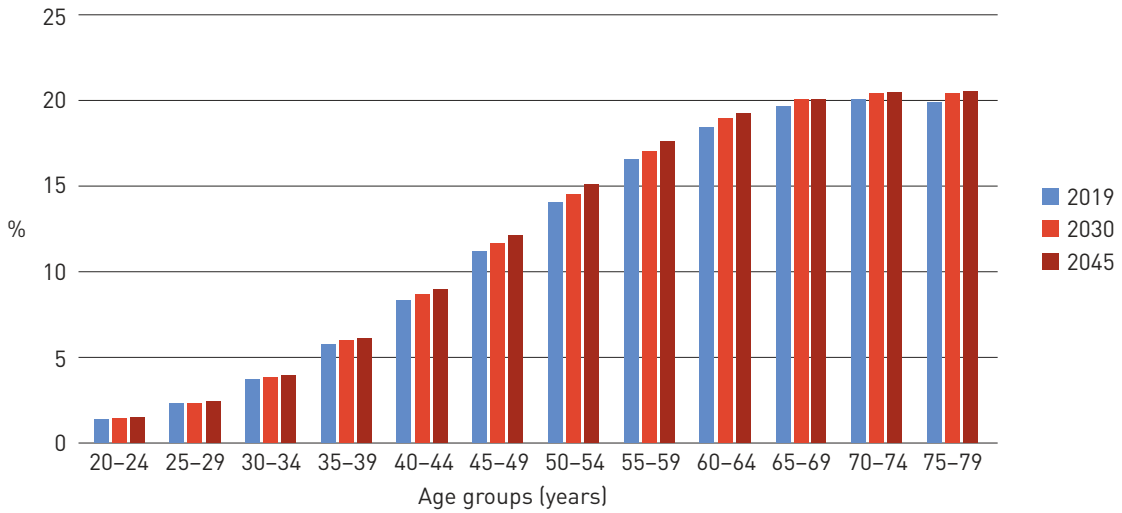


Figure 1.17. Predicted prevalence of diabetes mellitus, categorised by age group (20 - 79 years), in 2019, 2030, and 2045. The prevalence of diabetes mellitus is predicted to increase with increasing age. Adapted from IDF, (2019, p 37).

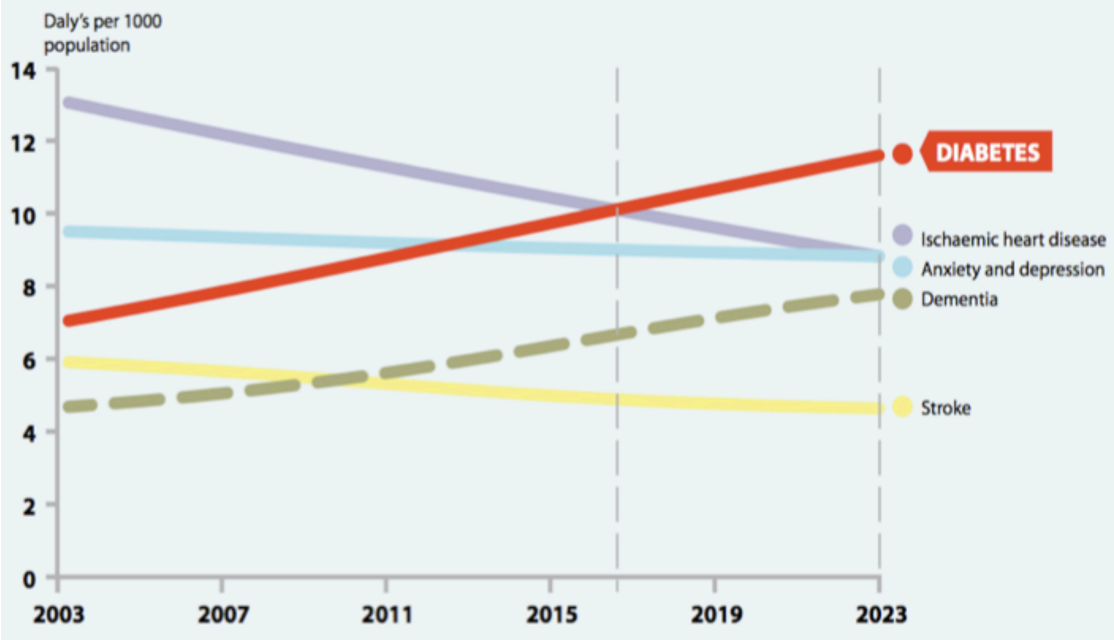


Figure 1.18. Trends in leading causes of disease and burden in Australia. Diabetes is predicted to supersede dementia as the fastest growing chronic disease in Australia by 2023. Adapted from AIHW, (2010, p 59).

Given the increasing prevalence rates predicted for DM globally and in Australia, research aimed at identifying the salient risk factors that increase the likelihood of developing diabetes is critical to reducing or preventing the deleterious complications associated with uncontrolled or untreated DM, which typically manifest insidiously.

1.4 Diabetes Mellitus: Complications

Diabetes mellitus is a multi-factorial systemic disease, which, when poorly-managed or uncontrolled, is associated with numerous life-threatening and debilitating complications (Harcourt *et al.,* 2013; IDF, 2019). The pathophysiology involves various organs (pancreas, gastrointestinal tract, heart, liver, kidney, and skeletal muscle) and the resulting complications – microvascular and macrovascular – have been consistently linked to hyperglycaemia, particularly the microvascular complications (Nathan, 1993; Vithian & Hurel, 2010; Forbes & Cooper, 2013). The microvascular complications (retinopathy, nephropathy, and neuropathy) chiefly affect highly-vascularised organs, whereas the macrovascular complications (myocardial infarction, stroke, peripheral arterial disease (PAD)) impair normal blood vessel functioning (Vithian & Hurel, 2010; Forbes & Cooper, 2013; Harcourt *et al.,* 2013; American Diabetes Association, 2020;

IDF, 2019). However, intensification of glycaemic control early in the disease has been shown to reduce and delay the development of these adverse long-term complications (The Diabetes Control and Complications Trial, 1993; The United Kingdom Prospective Diabetes Study, 1998), with a 1% reduction in glycosylated haemoglobin ($HbA1_C$) leading to a substantial decrease (37%) in all-cause mortality from diabetes-related complications (UKPDS, 1998). While the micro- and macrovascular complications of diabetes are well documented, it is important to recognise that diabetes is also associated with other complications, including sexual dysfunction, autonomic neuropathy, and depression (Mezuk *et al.,* 2008; Kuehl & Stevens, 2012).

The substantial socioeconomic burden of diabetes can also be illustrated by considering the rate of diabetes-related hospitalisations and deaths. Diabetes mellitus accounted for approximately 929,000 hospitalisations between 2013 and 2014, a disturbing 9% of total hospitalisations in Australia (Australian Institute of Health & Welfare, 2016). It was responsible for approximately 10% of all deaths in Australia (15,100) in 2013 and approximately 16,450 deaths in 2016, highlighting the devastating and wide-reaching burden of diabetes (Australia's Health, 2018). Diabetes is also the leading cause of kidney disease in Australia, with approximately 30% and 50% of patients with T1DM and T2DM developing kidney disease, respectively (Thomas *et al.,* 2015).

Although the peripheral complications of DM are well established, one other under-recognised complication of poorly-managed DM is cognitive dysfunction, manifesting as either diabetes-associated cognitive decrements, MCI, or mixed dementia (Koekkoek *et al.,* 2015; Biessels & Despa 2018; Biessels *et al.,* 2020). While documented for almost a century, this decline in cognition is frequently overlooked and under-recognised in standard diagnostic/medical practice. It is also commonly undetected by neuropsychological assessment, as it progresses insidiously. However, in light of emerging evidence suggesting diabetes exacerbates cognitive decline, the neurological complications of diabetes are being increasingly recognised as a significant co-morbidity (Biessels & Despa, 2018; Biessels *et al.,* 2020) and hence will comprise a major focus of this thesis.

1.5 Diabetes Mellitus and Cognitive Function

The relationship between diabetes mellitus and cognitive function has attracted significant debate, but to date remains highly controversial. Despite rigorous studies (cross-sectional and longitudinal) establishing a clear link between diabetes and CNS dysfunction, significant variability and contention exists in the literature concerning the precise cognitive modalities affected. The variability between studies has been ascribed to various factors: methodological limitations, the diverse cognitive assessments deployed, variation in cognitive assessment administration techniques, and investigators failing to account for the various diabetes-related metabolic factors (*e.g.* diabetes duration, glycosylated haemoglobin (HbA1c), fasting plasma glucose (FPG)). Unclear among investigators are also the underlying pathophysiological mechanisms linking diabetes to cognitive impairment, a rapidly-growing research area attracting considerable exploration with potentially broader societal implications for patient management, particularly in elderly age groups (>65 years of age) (Biessels & Despa, 2018; Biessels *et al.,* 2020; Srikanth *et al.,* 2020). Most forms of cognitive impairment begin manifesting in this age group, although it may occur earlier (*e.g.* early-onset Alzheimer's disease (EOAD)).

Several studies have assessed cognitive function in DM since Miles & Root (1922) first observed that patients with diabetes perform worse in assessments examining memory and attention compared to subjects without diabetes. However, the exact nature and pattern of cognitive dysfunction in DM (T1DM and T2DM) has, to date, confounded researchers, specifically in the case of T2DM. It is now increasingly recognised that cognitive dysfunction is an important complication of DM (both T1DM and T2DM) warranting urgent attention, due to the established association between diabetes and an increased risk of cognitive impairment and the increased co-occurrence of diabetes and cognitive impairment (Koekkoek *et al.*, 2015; Biessels & Despa, 2018; Biessels & Whitmer, 2019; ADA, 2020; Biessels *et al.,* 2020; Srikanth *et al.,* 2020). Although guidelines have been developed to assist general practitioners in clinical practice to address cognitive dysfunction in diabetes, some patients report that their healthcare professionals occasionally struggle to address diabetes-related cognitive dysfunction (Biessels & Whitmer, 2019; Srikanth *et al.,* 2020). This has been partly attributed to a lack of awareness of cognitive dysfunction in DM, which still reportedly lags behind that

of the other established peripheral complications (Biessels & Whitmer, 2019). Srikanth *et al.* (2020) suggest this results in delayed identification of cognitive dysfunction.

Various terms have been suggested to describe the subtle decline in cognition triggered by diabetes. Such terms include 'diabetic encephalopathy', 'diabetes-related cognitive dysfunction' and, more recently, 'diabetes-associated cognitive decline' (Koekkoek *et al.,* 2015; Biessels & Despa, 2018). Currently, the modest changes in cognition linked to diabetes are known as diabetes-associated cognitive decrements (Koekkoek *et al.,* 2015; Biessels & Despa, 2018; Biessels & Whitmer, 2019; Biessels *et al.,* 2020; Srikanth *et al.,* 2020). These are subtle decrements in cognitive functioning in one or more cognitive domains that often progress perniciously and affect all age groups (young adults to oldest age (>85 years of age) (Biessels & Despa, 2018; Biessels & Whitmer, 2019; Biessels *et al.,* 2020; Srikanth *et al.,* 2020). In T1DM, the decrements manifest early during the disease and remain relatively stable over time, whereas in T2DM they are hypothesised to develop during the pre-diabetes stage and progress insidiously as glycaemic control worsens (Biessels & Despa, 2018; Biessels & Whitmer, 2019). Alarmingly, it has been estimated that the decrements in cognition associated with T2DM develop approximately 50% faster than the normal ageing process (Biessels *et al.,* 2014; Biessels & Despa, 2018). This accelerated cognitive deterioration in T2DM is commonly referred to as "accelerated brain ageing" and is posited to be mediated by various pathophysiological pathways which, to date, also remain poorly-elucidated (section 1.6). While the cognitive decrements may cause cognitive complaints (often disclosed by the patient), they are, by definition, subtle; thus, they generally do not interfere with diabetes self-management or daily occupational and social functioning until advanced stages (Biessels & Despa, 2018; Biessels & Whitmer, 2019; Biessels *et al.,* 2020). They are also typically undetected by formal neuropsychological assessment, due to their slowly progressive nature (Koekkoek *et al.*, 2015; Biessels & Despa, 2018; Biessels *et al.,* 2020). Consequently, this complicates the ability of clinicians to establish whether individuals are affected by diabetes-associated cognitive dysfunction (Koekkoek *et al.*, 2015; Biessels & Despa, 2018).

Substantial epidemiological evidence indicates that DM is associated with an increased risk of all types of cognitive impairment (MCI, AD, VaD, dementia). The relative risk (RR) for all types of dementia has been documented as 1.5 to 2.5 times greater for T2DM (Biessels, 2006; Cheng *et al.,* 2014), whereas patients with T1DM have been estimated to have a 65% increased risk of dementia (Smolina *et al.,* 2015). Systematic reviews and meta-analyses of data from more than two million participants have estimated the relative risk (RR) for AD and VaD in diabetes as 1.53 and 2.27 greater than in individuals without diabetes, respectively (Gudala *et al.,* 2013; Zhang *et al.,* 2017). Alarmingly, data from a recent large cohort study in 2015 revealed that newly-diagnosed diabetes is associated with an elevated risk of dementia (hazard ratio - HR 1.16). Similarly, an increased risk of MCI (both amnesic and non-amnesic) and poorer prognosis of MCI has also been documented in subjects with DM compared to people without DM: one study reported a HR of 1.5 for amnesic MCI and 1.2 for non-amnesic MCI (Luchsinger *et al.,* 2007), whereas another documented a HR of 1.6 and 1.4 for amnesic and non-amnesic MCI, respectively (Robert *et al.,* 2014). In one meta-analysis examining the prognosis of MCI in patients with diabetes, the RR of conversion to dementia was determined to be 1.7 compared to subjects with MCI, but not diabetes. Despite both DM and cognitive impairment co-occurring frequently, and several studies having explored the association between diabetes and dementia, the precise relation between these conditions remains unclear.

Emerging data have recently also challenged the widely-accepted view that diabetes causes AD-like brain changes, suggesting that patients with T2DM demonstrate vascular abnormalities similar to those reported in VaD (Secnik *et al.,* 2017). This novel finding has prompted investigators to refer to dementia in diabetes as “diabetic dementia”, a unique form of dementia that differs from typical dementia and arises from different underlying mechanisms (Morley, 2017; Biessels & Despa, 2018) (Figure 1.19). Although most patients with T2DM develop dementia after the age of 65 years, evidence suggests that diabetes increases the risk of early-onset dementia (before the age of 65 years) (Biessels & Despa, 2018; Biessels *et al.,* 2020). However, compared to the persistent year-by-year decline in cognition reported in dementia, diabetes-associated cognitive decrements progress perniciously. Thus, due to dissimilar trajectories in cognitive decline, investigators recommend considering diabetes-associated cognitive dysfunction and dementia as distinct entities (Morley, 2017; Biessels & Despa, 2018; Biessels *et al.,*

2020). Interestingly, although both forms of diabetes share core similarities (hyperglycaemia), the affected cognitive domains and brain areas vulnerable to insult differ between both T1DM and T2DM. One derangement in cognition common to both forms is reduced information processing speed (Messier, 2005; Kodl & Seaquist, 2008; Koekkoek *et al.*, 2015).

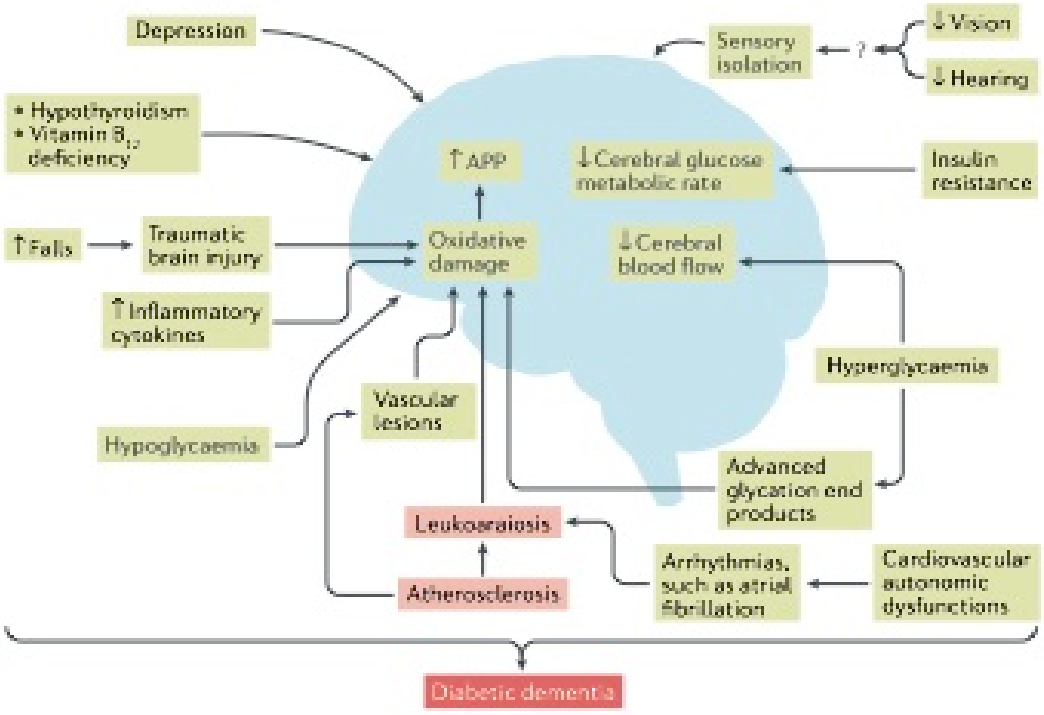


Figure 1.19. Possible pathophysiological pathways and contributors linking diabetes to diabetic dementia in Type 2 diabetes mellitus, a unique form of dementia similar to vascular dementia. Several key determinants are hypothesised to contribute directly to diabetes-associated cognitive dysfunction, including glycaemic events (hypo- and hyperglycaemia), advanced glycation end products (AGEs), and insulin resistance. Adapted from Morley, (2017, p 1).

1.5.1 Type 1 Diabetes Mellitus (T1DM) and Cognitive Function

Numerous cross-sectional and longitudinal studies have consistently shown that subjects with T1DM (children and adults) demonstrate modest yet detectable decrements (on average half a SD [0.3-0.7 SDs]) in cognitive function (compared to age-matched controls) across several cognitive domains, as measured by neuropsychological assessment (Brands *et al.*, 2005). The major cognitive modalities commonly reported to

be affected by T1DM include psychomotor speed, verbal fluency, general intelligence, cognitive flexibility, and attentional performance, with decrements in these domains having been ascribed primarily to poor glycaemic control, disease duration, and early disease onset (before 7 years of age) (Brands *et al.*, 2005; Brands *et al.*, 2006; Wessels *et al.*, 2007; Gaudieri *et al.*, 2008; Weinger *et al.*, 2008; Ryan *et al.,* 2016). While impairments in problem-solving, vocabulary, memory, and construction have also been documented, data supporting these observations are scarce. Occasionally, investigators have also found no differences in cognition between T1DM patients and those without diabetes (Lawson *et al.*, 1984). Thus, the relationship between T1DM and cognitive dysfunction, despite intensive exploration, remains unclear. It is also complicated by the administration of different cognitive assessments. Geijselaers *et al.* (2017) argue this results in inconsistent findings obtained between investigators and recommend deploying similar established neuro-psychometric batteries.

Ryan *et al.* (2003) investigated cognitive function in adults with T1DM and age- and education-matched healthy controls. The relationship between diabetes-related complications and cognitive dysfunction over a 7-year period was also examined (n = 160: 103 patients with diabetes [43 males and 60 females, mean age: 40.4 ± 6.2 years], and 57 healthy subjects [22 males and 35 females, mean age: 41.8 ± 7.1 years]). Cognitive function was divided into three major domains: (i) learning and memory, (ii) problem-solving and spatial ability, and (iii) psychomotor efficiency; and assessed using two established neuropsychological assessments: the revised Wechsler Adult Intelligence Scale (WAIS-R) (Wechsler, 1955) and Digit Vigilance Test (Lewis & Rennick, 1979). These tests assess psychomotor efficiency and sustained attention in children and adults, respectively. The investigators found that subjects with T1DM demonstrated significantly worse psychomotor speed compared to non-diabetes subjects (p-value < 0.001). No differences in performance were observed in other examined domains. The authors also reported that microvascular complications (retinopathy and autonomic neuropathy) were associated with exacerbating the decline in psychomotor function, a finding supported by recent studies (Brands *et al.*, 2005; Weinger *et al.*, 2008).

While the study of Ryan *et al.* (2003) was a well-designed longitudinal investigation, experimental limitations were evident. The study initially examined a large cohort, but several subjects with diabetes were not reassessed at the 7-year follow-up period (for undisclosed reasons), reducing the study's statistical power. The authors also did not account for other diabetes-related variables, such as disease duration and glycaemic control, and this may have moderated the relationship and contributed to the accelerated deterioration in psychomotor speed observed. The confounding effects of diabetes-related variables are well established and several investigators argue these should be accounted for in future studies if precise associations are to be determined (Munshi *et al.*, 2006; Roberts *et al.*, 2008; Roriz-Filho *et al.*, 2009).

Though often dismissed in cognitive investigations, the time at which cognitive function is assessed can influence experimental data, and the time of testing was not reported by Ryan *et al.* (2003). Circadian rhythm, controlled by hypothalamic suprachiasmatic nuclei, causes changes in alertness over time and alertness typically falls between 2-4pm (Valdez, 2019). Therefore, testing conducted during these hours poses the risk of obtaining inaccurate data as this may yield an imprecise representation of peak cognitive performance and a patient's cognitive profile. The reliability coefficients/psychometric properties for each assessment administered were also not reported. Future studies examining cognitive function in subjects with diabetes using standardised neuro-psychometric batteries should report the psychometric properties of all cognitive assessments administered. Investigators should also administer cognitive screening tools recommended by emerging clinical guidelines for screening diabetes-associated cognitive decrements (*e.g.* the Mini-Mental State Examination (MMSE) (Folstein, McHugh, & Folstein, 1975; ADA, 2020; Srikanth *et al.,* 2020). This will improve the likelihood of detecting potential subtle cognitive deficits and could potentially reduce the inconsistency in reports of the cognitive domains affected by diabetes.

One of the most seminal studies published exploring the relationship between T1DM and cognitive function is that of Brands *et al.* (2005), who conducted a large meta-analysis of the impact of T1DM on cognitive function. The meta-analysis included 33 studies and the sample population consisted of adults with diagnosed T1DM (aged >18, mean age not reported). The association between diabetes-related metabolic variables such as disease duration and glycaemic control, as well as the presence of complications, was also

assessed. Brands *et al.* (2005) concluded that subjects with T1DM demonstrate significantly worse performance compared to people without DM in several cognitive domains. Significantly worse performance was reported in seven cognitive domains: intelligence, speed of information processing, psychomotor efficiency, sustained attention, cognitive flexibility, and visual perception. Poor cognitive function was also found to be strongly linked to microvascular complications rather than severe hypoglycaemic episodes, disease duration, or poor metabolic control, a finding that both supports and contradicts current literature (Ryan *et al.,* 2016). Although the magnitude of deterioration in each affected cognitive domain was modest, as determined using effect sizes (Cohen's *d*), the authors suggested that the subtle cognitive decrements could potentially interfere with daily tasks central to diabetes self-management (*e.g.* monitoring of blood glucose concentrations).

1.5.2 Type 2 Diabetes Mellitus (T2DM) and Cognitive Function

Similar to the relationship observed in T1DM, neurocognitive assessments reveal patients with T2DM also exhibit subtle yet measurable decrements in cognition across various cognitive domains compared to non-diabetes samples (Cohen's *d* effect size [0.2 – 0.5]) (Kodl & Seaquist, 2008; Reijmer *et al.*, 2010; Palta *et al.*, 2014). However, unlike T1DM, which preferentially damages brain areas involved in cognitive flexibility and psychomotor speed, T2DM detrimentally affects brain centres associated with memory, learning, information processing speed, and executive functioning. These cognitive processes, excluding information processing, are typically preserved in T1DM. Modest decrements in visuoconstructional ability, language and perception have also been reported, although data supporting these findings are scarce (Brands *et al.*, 2007; Ruis, *et al.*, 2009). The stages and severity of cognitive dysfunction in adults with T2DM may also be divided into three approximate stages: (i) diabetes-associated cognitive decrements, (ii) MCI, and (iii) dementia (Biessels & Despa, 2018).

While vascular complications are common to both forms of poorly-managed DM, T2DM is frequently accompanied by various comorbidities, including obesity, depression, dyslipidaemia, and hypertension, which have been associated with exacerbating the cognitive decline in T2DM (Sims-Robinson *et al.*, 2010; Reijmer *et al.*, 2010; McCrimmon *et al.,* 2012). These comorbidities also often moderate strongly the relationship between T2DM and cognition, complicating determination of the exact

relation between T2DM and cognition (Messier, 2005; Ferrannini & Cushman, 2012; McCrimmon *et al.,* 2012). The confounding effects of these comorbidities have also been repeatedly argued to account for the diverse cognitive modalities commonly affected by T2DM. Current literature suggests obtaining information about the various diabetes-related moderating variables, such as glycosylated haemoglobin (HbA_{1C}) and any presenting comorbidities, as thoroughly as possible to address this limitation (Munshi *et al.*, 2006; Roberts *et al.*, 2008; Roriz-Filho *et al.*, 2009). In light of the increasing global prevalence of T2DM and the progressive nature of cognitive decrements in diabetes, preventive treatments for, and determination of the critical determinants contributing to the progression of the subtle cognitive dysfunction in DM, are urgently needed. Understanding the pathophysiological pathways that link DM to cognitive dysfunction would also be crucial.

1.6 Mechanistic Contributors to Cognitive Dysfunction in Diabetes

Several pathophysiological mechanisms have been proposed to account for the subtle cognitive dysfunction commonly observed in patients with diabetes (T1DM and T2DM); however, our understanding to date remains incomplete. The literature suggests the contribution of each risk factor to cognitive dysfunction is also small (Biessels & Despa, 2018). The key causative determinants suggested to contribute to the development and progression of diabetes-associated cognitive dysfunction are described below.

1.6.1 Hyperglycaemia

Neurons require a continuous, uninterrupted supply of glucose for optimum cognitive functioning (McNay & Cotero, 2010; Frier, 2014). Acute disturbances in BGL cause immediate and possibly permanent decrements in cognitive function (McNay & Cotero, 2010; Frier, 2014). Emerging evidence also suggests that fluctuations in blood glucose concentrations in T2DM may be linked to aggravating the cognitive decrements in diabetes and increasing the risk of dementia in late life (Rawlings *et al.,* 2017). When glucose concentrations remain persistently elevated (as in hyperglycaemia), glucose neurotoxicity may ensue (Tomlinson & Gardiner, 2008). This can lead to irreversible cellular damage and microvascular abnormalities, which accelerate the cognitive decline and result in cognitive dysfunction. (Biessels *et al.,* 2006). Therefore, hyperglycaemia is

widely considered a critical pathological determinant central to the aggravation of diabetes-associated cognitive dysfunction, particularly chronic hyperglycaemia.

The neurotoxic consequences of hyperglycaemia are well recognised and hypothesised to be mediated through various pathophysiological processes. Although hyperglycaemia has been associated with interrupting cerebral blood flow (CBF) and inducing brain hypoxia (Morley, 2017), depriving glucose and oxygen-dependent neurons of crucial nutrients, several lines of evidence suggest hyperglycaemia exacerbates cognitive decline in diabetes *via* three principal mechanisms: (i) increasing glucose flux *via* the polyol and hexosamine pathways, (ii) disrupting intracellular neuronal and second messenger pathways, and (iii) most commonly reported, triggering the formation of advanced glycation end products (AGEs) (Biessels *et al.,* 2006; Tomlinson & Gardner, 2008; Roriz-Filho *et al.*, 2009; Sims-Robinson *et al.*, 2010; Harcourt *et al.,* 2013; Baglietto-Vargas *et al.*, 2016; Morley, 2017). These complex pathophysiological processes are posited to damage underlying cerebral tissue directly and have been associated with inducing both microvascular and macrovascular abnormalities (Gispen & Biessels, 2000; Brownlee, 2001).

Advanced glycation end products (AGEs) are reactive substances formed by irreversible, non-enzymatic fusions of sugars with amino groups of proteins and lipids and have been associated with stimulating production of reactive oxygen species (ROS) (Sims-Robinson *et al.*, 2010; Morley, 2017; Biessels *et al.,* 2020). Elevated quantities of AGEs and ROS have been reported to trigger oxidative brain damage, damaging the integrity and function of critical biomolecules such as proteins and lipids (Brownlee, 2001; Cobb & Cole, 2015). Oxidative brain stress has been linked to activating inflammatory pathways and increasing inflammatory cytokine production, which has been associated with stimulating amyloid precursor protein and accelerating deposition of amyloid beta in neurodegenerative diseases such as AD and dementia. Taken together, these processes could potentially trigger early AD pathology or accelerate conversion to AD (Sims-Robinson *et al.*, 2010; Morley, 2017).

Further to adversely affecting the described processes, hyperglycaemia has also been linked to perturbations in neuronal function at the cellular level. Such disturbances include altered axonal transport, demyelination, and impaired neurotrophic support (Tomlinson & Gardiner, 2008) (Figure 1.20). It is through these putative molecular mechanisms that hyperglycaemia is hypothesised to contribute to the accelerated cognitive decline and progression of cognitive dysfunction observed in diabetes patients.

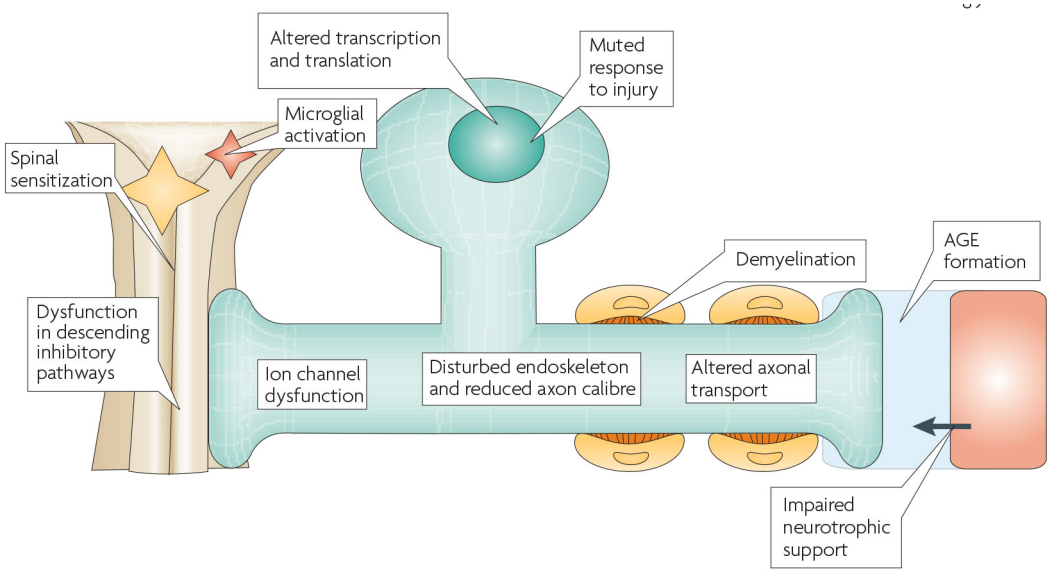


Figure 1.20. Adverse molecular effects associated with glucose neurotoxicity in neurons. At the molecular level, glucose neurotoxicity can disrupt normal neuronal function, leading to altered axonal transport, ion channel dysfunction, and demyelination. Adapted from Tomlinson & Gardiner, (2008, p 42).

1.6.2 Recurrent Hypoglycaemia (RH)

Abnormally low blood glucose (plasma glucose concentration < 3.0 mmol/l) is a common, reversible adverse effect of intensive insulin therapy commonly reported in both T1DM and T2DM patients (Frier, 2014). It is classified clinically according to whether an individual can self-treat (mild) or not self-treat (severe) and has also been associated with deficits in cognitive function (Frier, 2014). It is estimated that patients with T1DM experience one to two episodes of mild hypoglycaemia per week, whereas those with T2DM experience 0.3-0.7 episodes per week (Ostenson *et al.*, 2014). However, accurate retrospective recall of hypoglycaemic episodes is poor; severe episodes can be accurately remembered up to one year, whereas mild episodes can only be reliably recalled for up to one week (Frier, 2014). This complicates ascertainment of the precise

relationship between hypoglycaemia and cognitive function, but it is clear that hypoglycaemia is an important risk factor for diabetes-associated cognitive dysfunction.

The cognitive domains affected by moderate and severe hypoglycaemic episodes are predominantly those associated with frontal lobe function, such as short-term memory and reaction time (Frier, 2014). Alarmingly, it has been reported that complete cognitive recovery in these domains following the return to normoglycaemia may not occur for approximately 60 minutes (Zammitt *et al.*, 2008). Although the short-term neurological sequelae of acute hypoglycaemia are well understood, the literature is unclear as to whether recurrent hypoglycaemia causes long-term, irreversible cognitive deterioration. Mixed findings have been obtained: some studies have reported permanent cognitive disturbances, substantiated using neuroimaging modalities (Chalmers *et al.* 1991), whereas others have observed no association (Bruce *et al.,* 2009). These conflicting results have been ascribed to difficulty in determining and controlling for patient glycaemic history as well as the many diabetes-specific variables such as disease duration, hypoglycaemic episodes, underlying micro- or macrovascular complications, and pre-existing comorbidities (McNay & Cotero, 2010). Frier (2014) argues the relationship is age-dependent.

The literature is not particularly helpful in elucidating the mechanisms through which hypoglycaemia, and particularly recurrent hypoglycaemia, may induce cognitive dysfunction in diabetes. While disturbances in cognition caused by acute hypoglycaemia are understood to result directly from glucose deprivation, impairing glucose-sensitive hippocampal and cortical brain areas, the pathophysiological processes mediated by recurrent hypoglycaemia remain elusive (Languren *et al.*, 2013). Emerging data from animal studies indicate recurrent hypoglycaemia exacerbates brain oxidative damage, causing irreversible neuronal death and leading to cognitive dysfunction (Languren *et al.*, 2017). Support for this view is provided by Languren *et al.,* (2017), who observed that moderate recurrent hypoglycaemia, after an episode of severe hypoglycaemia, over seven days, aggravated brain oxidative damage in three-month-old male Wistar rats (280-300g). Similarly, Won *et al.* (2012b) also observed brain oxidative damage, indicated by lipoperoxidation of 4-hydroxynonenal, in the rat hippocampal CA1 dendritic layer. Taken together, these data suggest that recurrent hypoglycaemia may contribute to cognitive dysfunction by inducing brain oxidative damage, specifically in the hippocampus, instead of directly causing neuronal death.

Counterregulatory failure, a maladaptive homeostatic response that prevents timely detection of falling blood glucose concentrations and coordination of appropriate counter-regulatory responses, has also been proposed (Sprague & Arbelaez, 2011). The brain contains specialised neuronal populations (glucose-excitatory and glucose-inhibitory) that play crucial roles in maintaining glucose homeostasis and coordinating the counterregulatory response (Roh e*t al.,* 2016). Evidence from animal studies indicates that sustained hypoglycaemia diminishes the sensitivity of these specialised glucose-sensing and glucose-inhibiting neurons of the ventromedial hypothalamus (VMH), arcuate nucleus (ARC), and paraventricular nucleus (PVN) responsible for initiating the counterregulatory response (Song & Routh, 2006). This finding has led researchers to suggest that recurrent hypoglycaemia may cause defects in the counterregulatory response, such as alteration of the threshold for the onset of the counterregulatory response and blunting of the counterregulatory response to subsequent hypoglycaemic episodes (Cryer, 2006; Beall *et al.,* 2012; Languren *et al.,* 2017). Conversely, other researchers argue that moderate recurrent hypoglycaemia, paradoxically, provides a beneficial adaptive response, shielding the brain against the detrimental effects induced by severe hypoglycaemia (Puente *et al.*, 2010). Therefore, it can be seen that the mechanisms underlying hypoglycaemia-associated cognitive dysfunction in diabetes remain controversial and warrant further exploration to clarify the exact pathophysiological mechanisms induced by recurrent hypoglycaemia.

1.6.3 Altered Insulin Signalling

Accumulating evidence indicates that defective insulin signalling may also contribute to the development and progression of diabetes-associated cognitive dysfunction and may accelerate AD pathology (Sims-Robinson *et al.*, 2010; Cholerton *et al.,* 2013; Baglietto-Vargas *et al.*, 2016). In fact, AD has been referred to as Type 3 diabetes or an insulin-resistant brain state (de la Monte & Wands, 2008; Sims-Robinson *et al.*, 2010), suggesting that both diabetes and AD share similar pathophysiological pathways.

The brain contains numerous insulin receptors interspersed throughout several key CNS areas, notably hippocampal and cortical regions (Biessels *et al.,* 2006; Cholerton *et al.,* 2013; Biessels & Reagan, 2015). Although the brain was once considered an insulin-independent organ, it is now understood that insulin in the brain plays crucial roles in influencing memory and learning (Cholerton *et al.,* 2013; Biessels & Despa, 2018). Insulin plays central roles in the maintenance of synaptogenesis and long-term potentiation (LTP), the latter an important process for memory formation. Insulin in the brain has also been linked to influencing the activity of major excitatory neurotransmitters implicated in cognition, specifically acetylcholine and norepinephrine (Kopf & Baratti, 1999).

Impaired insulin signalling in both forms of diabetes has been associated with disturbances in cognition (Sims-Robinson *et al.,* 2010). In T1DM, chronic brain insulin deficiency blunts long-term potentiation (LTP), disrupting hippocampal and spatial functioning (Sims-Robinson *et al.,* 2010). In contrast, insulin resistance (IR) – a pathophysiological hallmark of T2DM – has been associated with compensatory hyperinsulinaemia, particularly in early stage T2DM (Biessels *et al.,* 2006) (Figure 1.21). Evidence exists in the literature that hyperinsulinaemia is a modifiable risk factor for cognitive decline, attributable to the vasoactive effects of insulin (Kalmijn *et al.,* 1995). Support for this view is provided by Kalmijn *et al.,* (1995), who assessed global cognitive function using the MMSE and found that subjects without DM, but with hyperinsulinaemia, performed worse than those with DM. Prolonged compensatory hyperinsulinaemia has also been hypothesised to promote hyperphosphorylation of *tau* and Aβ deposition, causing irreversible neuronal death (Biessels *et al.,* 2006; Sims-Robinson *et al.,* 2010). Interestingly, investigators have also reported hyperinsulinaemia in patients with sAD. This raises the possibility that (i) diabetes may contribute to AD pathophysiology, and (ii) that both diabetes and AD share similar pathophysiological pathways.

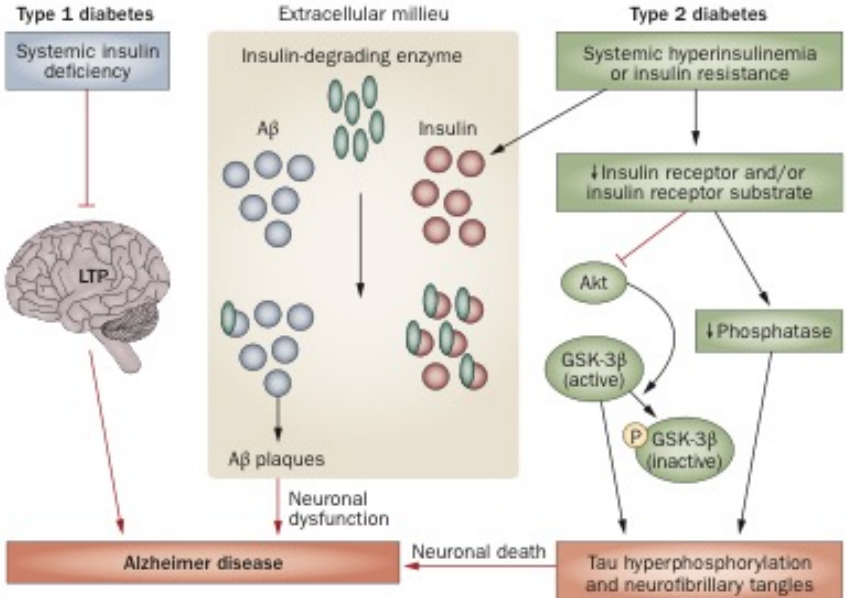


Figure 1.21. Proposed mechanisms linking impaired insulin signalling in diabetes (Type 1 and Type 2) to Alzheimer's disease. In Type 1 diabetes mellitus, chronic insulin deficiency disrupts long-term potentiation (LTP), impairing hippocampal function, leading to AD. In Type 2 diabetes mellitus, insulin resistance and corresponding compensatory hyperinsulinaemia causes hyperphosphorylation of *tau* and neurofibrillary tangles, leading to irreversible neuronal death. Adapted from Sims-Robinson *et al.,* (2010, p 553).

Disrupted cerebral insulin signalling has also been implicated in influencing the activity of insulin-degrading enzyme (IDE) (Sims-Robinson *et al.,* 2010), which is primarily responsible for the degradation of insulin in neurons and microglia. However, evidence indicates that IDE also plays important roles in the intracellular degradation and clearance of amyloidogenic proteins involved in the pathogenesis of AD pathology, notably amyloid beta (Kurauti *et al.,* 2017). Excessive insulin has been linked to stimulating amyloid beta secretion and obstructing the extracellular proteolytic degradation of amyloid beta by directly competing with IDE. This results in decreased clearance of amyloid beta. Together, elevated insulin levels and reduced clearance of amyloid beta due to altered insulin signalling are hypothesised to contribute synergistically to amyloid beta aggregation and plaque formation. Such a pathophysiological synergistic interaction could potentially account for and contribute to diabetes-associated cognitive dysfunction (Sims-Robinson *et al.,* 2010).

1.6.4 Blood-Brain Barrier (BBB) Dysfunction

The blood-brain barrier (BBB) is a highly-selective and protective physiological barrier that isolates the central nervous system (CNS) from all non-neural tissue (Tomlinson & Gardiner, 2008; Sweeney *et al.,* 2018) (Figure 1.22). Its integrity is maintained by sealed tight junctions, formed by continuous capillary brain endothelial cells and perivascular astrocytic end-feet that line cerebral microvessels (Tomlinson & Gardiner, 2008; Sweeney *et al.,* 2018). This specialised barrier performs various critical functions, including the stringent maintenance of the highly-regulated internal milieu of the CNS (ionic composition, neurotransmitters, water), and the shielding of vulnerable neuronal populations and glia from insult from potentially neurotoxic substances circulating in the blood (Abbott *et al.,* 2006; Tomlinson & Gardiner, 2008; Sweeney *et al.,* 2018). Growing evidence from clinical and experimental studies indicates that uncontrolled DM is associated with BBB breakdown (Horani & Mooradian, 2003; Prasad *et al.,* 2014; Takechi *et al.,* 2017). Breakdown of the BBB putatively permits the entry of potentially neurotoxic substances, disturbing the highly-regulated microenvironment of the CNS. The unrestricted influx of toxic blood-derived substances into the brain has been linked to activating inflammatory chemicals and triggering the onset and progression of the neurodegenerative processes (Prasad *et al.,* 2014; Takechi *et al.,* 2017; Sweeney *et al.,* 2018).

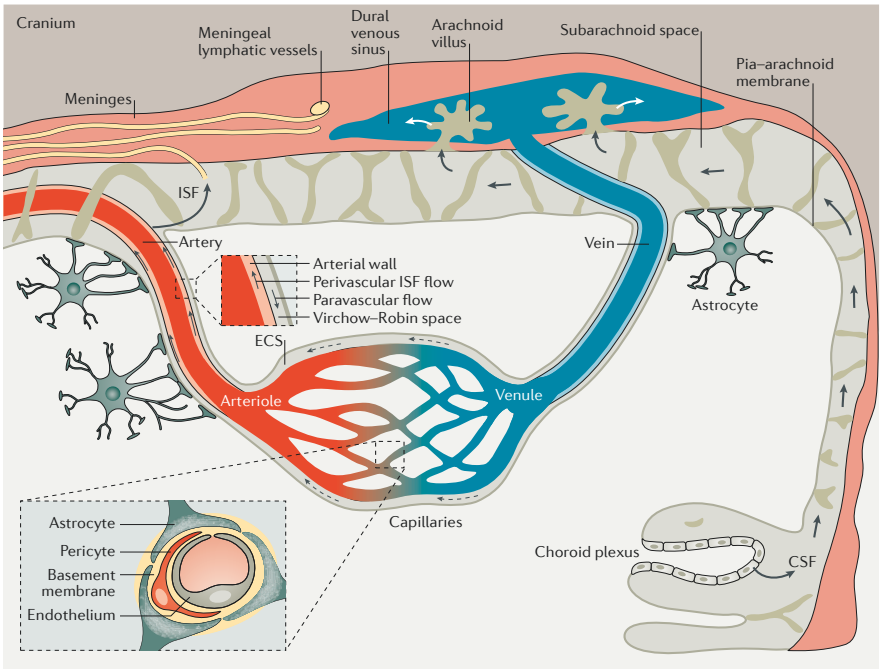


Figure 1.22. The blood-brain barrier (BBB). It is comprised of endothelium, pericytes, a basement membrane, and astrocytes. Breakdown of the BBB putatively permits the unrestricted entry of plasma-derived substances into the sensitive micro-environment of the CNS, disrupting normal neuronal functioning. Adapted from Sweeney *et al.,* (2018, p 135).

Disruption of the BBB in diabetes has been hypothesised to result from several possible pathophysiological mechanisms, but the literature generally suggests that poor glycaemic control and glycaemic events (hypo- and hyperglycaemia) are the key causative determinants (Takechi *et al.,* 2017; Sweeney *et al.,* 2018). Glucose, which rapidly traverses the BBB *via* the insulin-dependent, facilitated glucose transport member 1 (GLUT1), is a key energy substrate for the brain; however, in dangerously low (hypoglycaemia) and abnormally high concentrations (hyperglycaemia) it becomes especially neurotoxic (Tomlinson & Gardner, 2008). In animal models of diabetes, studies have reported that hyperglycaemia (acute and chronic) is associated with a down-regulation of essential BBB glucose transporters, notably GLUT-1, decreasing glucose uptake into the brain (Lorenzi *et al.,* 1986). Some, however, have not observed this effect. In human studies, which are few (likely attributable to the confounding effects of common diabetes-related metabolic variables), researchers have observed increased BBB permeability using magnetic resonance imaging (MRI) in subjects with well-controlled T2DM (Starr *et al.,* 2003).

While the molecular mechanisms underpinning how glycaemic events (hypo- and hyperglycaemia) disrupt BBB integrity are currently unclear, recent literature suggests they cause BBB leakiness by disrupting the continuous, end-to-end sealed tight junction complex (Tomlinson & Gardner, 2008; Sweeney *et al.,* 2018). This perforation of the BBB putatively permits the entry of neurotoxic chemicals and excessive glucose, leading to glucose neurotoxicity and neuroinflammation (Tomlinson & Gardner, 2008; Sweeney *et al.,* 2018). This downstream process triggers a cascade of pathophysiological processes, including the production of ROS and oxidative stress, which have been associated with directly inducing both microvascular and macrovascular abnormalities and activating inflammatory molecules linked to triggering the neurodegenerative process (Tomlinson & Gardner, 2008; Sweeney *et al.,* 2018).

Given the pathophysiological mechanisms underlying diabetes-associated cognitive dysfunction and pathways linking diabetes to various forms of cognitive impairment remain unclear, there is an urgent need for objective neurophysiological measures that can reliably and accurately detect the subtle cognitive decrements associated with diabetes.

1.7 High Blood Pressure

Hypertension (HTN) (also high blood pressure, raised blood pressure, chronic arterial hypertension, or systemic arterial hypertension)) is a highly-prevalent chronic condition characterised by abnormally elevated blood pressure (BP) in the systemic arteries (systolic blood pressure ≥ 140mm/Hg and diastolic blood pressure ≥ 90mm/Hg) following repeated examination (Sörös *et al.,* 2013; Iadecola *et al.,* 2016; Oparil *et al.,* 2018; Unger *et al.,* 2020). Current estimates suggest hypertension affects 1.3 billion individuals worldwide (~31.1%) and projections indicate this number will increase in a similar way to the prevalence patterns predicted for diabetes (Iadecola *et al.,* 2016; Drummond *et al.,* 2019; Mills *et al.,* 2020; Unger *et al.,* 2020). It is also estimated that 3.5 billion adults globally have sub-optimal systolic BP (≥ 110 – 115mm/Hg), driven largely by non-adherence to anti-hypertensive therapy, with hypertension preferentially affecting individuals in undeveloped geographical areas with weak healthcare systems (Oparil *et al.,* 2018; Unger *et al.,* 2020). This translates to an unsettling almost one in four adults suffering from hypertension globally (Forouzanfar *et al.,* 2017). While raised blood pressure (BP) predominantly affects older populations (> 65 years of age), it may also affect young and middle-aged populations and is increasingly being reported in these groups (Lyngdoh *et al.,* 2013; Unger *et al.,* 2020). Investigators ascribe this latter pattern to sedentary behaviour, physical inactivity, and poor diet (Mills *et al.,* 2020).

Several prevalent modifiable (*e.g.* tobacco smoking, alcohol intake, obesity, poor diet, physical inactivity, and sedentary behaviour) and non-modifiable (*e.g.* age, ethnicity, and gender) lifestyle risk factors have been implicated in the development of hypertension (Figure 1.22); however, as with diabetes, the precise underlying cause of hypertension currently remains elusive (Iadecola *et al.,* 2016; Oparil *et al.,* 2018; Unger *et al.,* 2020). The aetiology appears to be multi-factorial, resulting from a complex interplay between genetic and environmental factors (Oparil *et al.,* 2018; Unger *et al.,* 2020) (Figure 1.23). Interestingly, other researchers have suggested that hypertension results from a disruption in normally tightly-regulated physiological processes, chiefly cardiovascular, renal, and vascular function (Drummond *et al.,* 2019). While various forms of hypertension have been described (monogenic forms, treatment-resistant, Liddle syndrome), this thesis will concentrate on the well-documented heterogeneous form, commonly referred to as

'essential' or primary hypertension. This form is generally asymptomatic and affects up to 90% of individuals with hypertension (Bartoloni *et al.,* 2018; Oparil *et al.,* 2018).

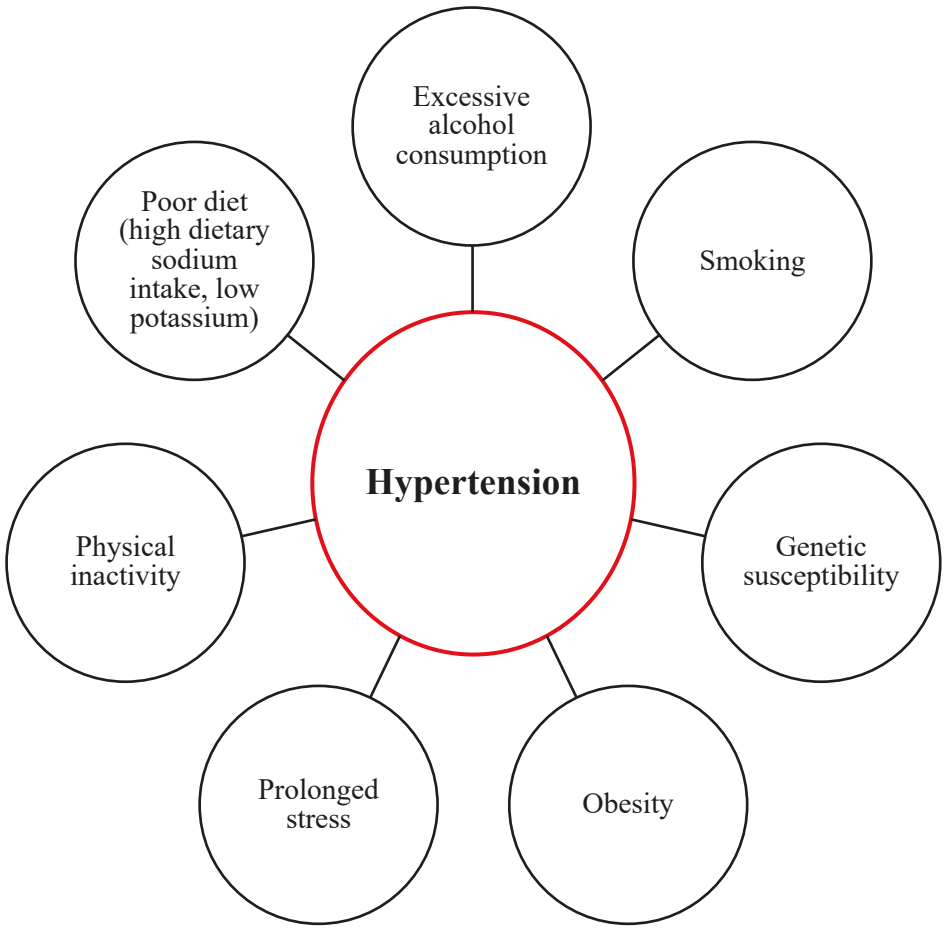


Figure 1.23. Common modifiable lifestyle risk factors associated with triggering and contributing to the development of hypertension. Adapted and modified from Oparil *et al.,* (2018).

Hypertension is commonly referred to as the "silent killer" and is associated with significant premature morbidity worldwide (Mills *et al.,* 2020). The prevalence of HTN (both in Australia and worldwide) has also increased steadily: according to the World Health Organisation (2018), in 2008, hypertension affected approximately 40% of individuals aged 25 years and more worldwide. In Australia, during the period 2012 - 2013, approximately six million individuals (34%) aged 18 years and over had hypertension and, in the same period, it was recognised as the most common chronic disorder treated and managed by Australian general practitioners (Australian Institute of

Health & Welfare, 2014). Alarmingly, recent published data revealed an estimated 68% (4.1 million) of Australians did not control or treat their hypertension (AIHW, 2016). Although the global prevalence of hypertension decreased marginally between 1980 - 2008, estimates suggest the prevalence of hypertension will continue to increase, largely due to changes in population trends in ageing, increased life expectancy, and modifiable lifestyle risk factors characteristic of sedentary lifestyles described above (Weber *et al.,* 2014).

The significant socioeconomic burden attributable to hypertension can also be illustrated by considering the substantial contribution of hypertension to the burden of cardiovascular disease (CVD) and death both globally and in Australia. Worldwide, hypertension is the leading preventable risk factor for CVD and all-cause mortality, accounting for approximately 10.4 million deaths per year (Global Burden of Disease Study, 2018; Mills *et al.,* 2020). It also accounts for a staggering 12.8% of all cardiovascular-related deaths, with vascular events directly linked to HTN responsible for approximately 9.4 million deaths (Oparil *et al.,* 2018). In Australia, during the period 2007 - 2008, CVD was the highest-costing disease group, costing the Australian healthcare system approximately $7.7 billion (Oparil *et al.,* 2018). Unless a significant medical breakthrough occurs, or patients begin controlling their blood pressure using effective blood pressure-lowering medications or alternative therapies, the substantial CVD burden associated with hypertension will continue to rise, imposing considerable strain on healthcare systems worldwide. Therefore, given hypertension is a well-established risk factor for both CVD and chronic kidney disease (CKD), research aimed at identifying the major determinants responsible for accelerating hypertension development is urgently needed. The concurrent development and implementation of appropriate risk-reduction countermeasures for known risk factors (*e.g.* dietary salt reduction, *etc.*) to delay or prevent the development of hypertension would also be of clinical significance.

1.7.1 Hypertension: Complications

The adverse peripheral vascular complications associated with uncontrolled hypertension have been extensively reported (Oparil *et al.,* 2018). Such complications include myocardial infarction, atrial fibrillation, and stroke, with a study estimating 54% of strokes being attributable to HTN (Lawes *et al.,* 2008). Poorly-controlled or untreated

hypertension may also be both a direct cause or consequence of CKD, with HTN strongly aggravating progression of renal failure (Bartoloni *et al.,* 2018). One other deleterious complication of raised BP is cognitive dysfunction, manifesting as deficits in cognitive domains (Iadecola *et al.,* 2016). This received little attention until observational studies in 1960, when diminished cognitive function was observed in air traffic controllers and pilots with high blood pressure. Despite the deleterious relationship between high blood pressure and cognition having been documented for over half a century, as is also the case with DM, the relation between hypertension and cognition remains highly controversial, evidenced by conflicting data obtained from numerous studies (cross-sectional and longitudinal) (section 1.7.2).

Similar to the decrements in cognitive function reported in diabetes, the decline in cognition triggered by hypertension progresses insidiously, complicating timely detection. They also often remain undetected by neurocognitive assessment (Iadecola *et al.,* 2016). Iadecola *et al.* (2016) emphasise that hypertension-associated cognitive decline represents a significant public health challenge and argue that research elucidating the link between BP and cognition is urgently required. Given the predicted increases in hypertension prevalence rates, and the substantial epidemiological evidence revealing that hypertension exacerbates cognitive decline and contributes to dementia, research geared towards understanding the mechanisms linking hypertension to cognitive impairment is critical. The identification of non-invasive neurological measures that can reliably and accurately detect early hypertension-associated cognitive dysfunction would also be of great advantage.

1.7.2 Hypertension and Cognitive Function

The relationship between blood pressure and cognitive function has been extensively examined since first documented by Elias (1969), who observed reduced psychomotor speed performance in hypertensive air traffic controllers and pilots compared to normotensive subjects. However, despite numerous investigations (cross-sectional and longitudinal) exploring the relationship between BP and cognition, the precise association remains highly controversial (Birns & Kalra, 2009). The pathophysiological mechanisms underlying hypertension-associated cognitive dysfunction (Section 1.8) and the electroencephalography (EEG) changes that occur in hypertension also remain unclear and largely uninvestigated; hence, they will comprise a significant focus of this thesis (Section 1.11).

One prominent issue raised commonly by investigators exploring the relationship between blood pressure and cognition, is that the relation is often complicated by several factors. This has led to inconsistent findings being obtained between studies. The mixed results between studies have been ascribed to many possible factors, including:

- the highly dynamic and variable nature of blood pressure (Schulze *et al.* 2000*;* Franklin *et al.,* 2013);
- potential interference from white-coat hypertension (Franklin *et al.,* 2013);
- differences in blood pressure measurement technique (Schulze *et al.,* 2000);
- the number of blood pressure measurements recorded (Goldstein *et al.,* 2013);
- use and duration of anti-hypertensive treatments (Obesisan, 2009);
- different classifications of hypertension (Birns & Kalra, 2009);
- irreversible organ damage resulting directly from uncontrolled or poorly-managed hypertension (Harrington *et al.,* 2000);
- co-morbidity with pre-existing chronic diseases, *e.g.* T2DM (Ferrannini & Cushman, 2012);
- consideration of important covariates associated with hypertension (Obesisan, 2009);
- variable participant exclusion criteria between studies (Birns & Kalra, 2009,);
- dissimilar age groups examined;
- duration of follow-up in longitudinal studies (Iadecola *et al.,* 2016); and

- the significant variability in cognitive instruments administered to assess cognitive performance (Birns & Kalra, 2009; Iadecola *et al.,* 2016).

Although researchers recommend administering neuropsychological assessments sensitive to cognitive domains detrimentally affected by hypertension, Birns and Kalra (2009) argue that randomised controlled trials (RCTs) are required for robust assessment of the association between high blood pressure and cognitive function. Sörös *et al.* (2013) also argue that different participant inclusion/exclusion criteria create a significant methodological limitation, emphasising this complicates comparability between studies. Therefore, future research exploring the relationship between high blood pressure and cognitive function is urgently warranted and should attempt to address the limitations listed above as much as possible for meaningful comparisons to be made between studies.

1.7.3 High Blood Pressure and Cognition: Evidence from Cross-Sectional Studies

Mixed findings concerning raised blood pressure and cognitive function have been obtained in cross-sectional investigations across all age groups: mid-life (age 40 - 64 years), late-life (age 65 - 84 years) and oldest age (≥ 85 years). While most studies have reported negative associations between raised blood pressure and cognition (Starr *et al*., 1993; Kilander *et al.,* 1998; Obisesan *et al.,* 2008), others have observed U-shaped and J-shaped associations (Waldstein *et al.,* 2005). Some studies have reported no association at all (Farmer *et al.,* 1987). Interestingly, some investigators have reported an inverse relationship: that is, elevated blood pressure confers improvements in cognitive performance (Launer *et al.,* 1995). Support for this view was provided from a study exploring BP links to cognition in centenarian Australians, which found higher systolic BP was associated with stronger global cognitive performance (Richmond *et al.,* 2011).

1.7.4 High Blood Pressure and Cognition: Evidence from Longitudinal Studies

Although various investigators suggest longitudinal studies provide the best assessment of the temporal relation between BP and cognition, inconsistent findings have also been obtained across all age groups (Birns & Kalra, 2009). Most longitudinal studies have consistently observed strong negative associations between high blood pressure and cognitive decline (Yaffe *et al.,* 2014); however, some have observed J- and U-shaped associations (as reported for cross-sectional studies) (Waldstein *et al.,* 2005). Interestingly, some studies examining BP and cognitive function have failed to replicate the well-documented negative association, reporting improved cognitive function with elevated BP in the oldest age groups. This is the same unexpected outcome observed in cross-sectional studies described above (Guo *et al.,* 1997; Kähönen-Väre *et al.,* 2004). On the opposite end of the age continuum, no association between raised BP and cognitive function has been found in younger populations (adolescents) (Lyngdoh *et al.,* 2013).

The most well-documented association reported in longitudinal investigations has been between mid-life BP and cognition. Several studies have consistently demonstrated a strong negative association between mid-life hypertension (especially high systolic BP) and late-life cognitive impairment and dementia (Elias *et al.,* 1993; Kilander *et al.,* 2000; Elias *et al.,* 2004), but some have observed no relationship (Kesse-Guyot *et al.,* 2015). One study found that 10 mmHg (millimetres of mercury) increases in systolic blood pressure (SBP) and diastolic blood pressure (DSP) in stroke-free subjects during mid-life were linked to reduced overall global cognitive performance and poor performance in attention and memory domains (Elias *et al.,* 1993). Similarly, using an adjusted cognitive model, another large-scale study found subjects with high SBP (≥160 mmHg, now classified as Grade 2 Hypertension) in mid-life had a two-fold heightened risk of performing poorly in global cognitive measures after 25 years (Launer *et al.,* 1995). Another study found that pre-hypertension in both middle-aged and older women was associated with impaired information processing and verbal memory ten years later (Chen *et al.,* 2015). Therefore, the relation between mid-life raised blood pressure and cognitive function is well established.

In addition to the controversial relationships reported above, numerous studies (cross-sectional and longitudinal) assessing global and domain-specific cognitive performance using validated neuro-psychometric batteries have also demonstrated that hypertensive subjects perform worse in global cognition and specific domains of cognition compared to non-hypertensive subjects (Harrington *et al.,* 2000; Lande *et al.,* 2003; Waldstein *et al.,* 2005). This has been shown predominantly in commonly-administered cognitive screening tools of global cognitive performance, such as the Mini-Mental State Examination (MMSE) (Folstein, McHugh, & Folstein, 1975), but subtle decrements in specific cognitive domains (executive function) have also been reported. Interestingly, diminished cognitive function has also been observed in hypertensive subjects at the time of cognitive assessment, irrespective of a prior diagnosis of hypertension, suggesting short-term changes in BP detrimentally influence cognitive function (Waldstein *et al.,* 2005). In patients presenting with comorbidity (*e.g.* T2DM), worse cognitive function has been reported, potentially indicating that both conditions contribute synergistically to deteriorating cognitive function (Ferrannini & Cushman, 2012).

Unlike DM, which affects a diverse range of cognitive domains, the cognitive modalities detrimentally impacted by hypertension are those controlled by frontal lobe functioning, such as information processing and executive functioning. The latter is a complex cognitive domain vital for adequate everyday functioning and ongoing disease self-management (Harrington *et al.,* 2000; Lande *et al.,* 2003; Waldstein *et al.,* 2005; Novak & Hajjar, 2010; Iadecola *et al.,* 2016). Reduced memory performance has also been documented, although deterioration in this domain is modest and data supporting these findings are limited. Function in other cognitive domains non-dependent on frontal lobe functioning, such as visuospatial function and calculation, typically remain preserved. While it is known that the cognitive decrements in DM progress perniciously and are irreversible, it is unknown whether the cognitive decline in hypertension is reversible. Given hypertension is widely recognised as an established modifiable risk factor for cognitive impairment, and no effective disease-modifying treatments to delay the onset of cognitive decline exist, research aimed at understanding the mechanistic pathways linking hypertension to cognitive impairment is crucial for the development of future therapies.

1.8 Mechanisms Underlying Cognitive Dysfunction in Hypertension

Several pathophysiological mechanisms have been suggested to underlie hypertension-associated cognitive decline; however, similar to findings related to diabetes-associated cognitive dysfunction, the exact pathophysiological mechanisms that link hypertension to cognitive impairment still remain unclear. Current literature suggests the pathophysiology is multi-factorial, resulting from the interplay between several pathways. The major mechanisms proposed to contribute to the development and progression of hypertension-associated cognitive dysfunction and link hypertension to cognitive impairment are described briefly below.

1.8.1 Blood-Brain Barrier (BBB) dysfunction

Accumulating evidence from experimental and clinical studies indicates that persistently-elevated blood pressure disrupts the integrity of the BBB, leading to BBB breakdown and increased permeability (Iadecola *et al.,* 2016) (Figure 1.24). Disruption of this critical physiological barrier disturbs the highly-regulated internal CNS milieu, resulting in impaired neuronal connectivity, synaptic function, and information processing (Sweeney *et al.,* 2018). (Figure 1.24). Although the precise mechanisms underlying hypertension-associated BBB dysfunction are unclear and probably multi-factorial, current literature suggests that hypertension causes BBB breakdown by inducing degeneration of pericytes and the underlying endothelium. These are both critical structural components of the BBB (Sweeney *et al.,* 2018). Endothelial and pericyte degeneration promotes the destabilisation of essential tight junction proteins and adherens junctions in the vessel wall, leading to BBB leakiness (Sweeney *et al.,* 2018). As the BBB is compromised, neurotoxic blood-derived factors circulating in the blood (plasminogen, thrombin, pathogens, and Fe^{2+} from the breakdown of iron-containing proteins) flow unregulated into the sensitive brain parenchyma. The uncontrolled influx of neurotoxic chemicals into the CNS has been linked to activating inflammatory mediators, such as microglia and astrocytes, and inducing oxidative stress, releasing harmful reactive oxygen species that impair normal brain function. These processes have been associated with triggering and contributing to the complex neurodegenerative cascade that eventually leads to AD and dementia (Sweeney *et al.,* 2018).

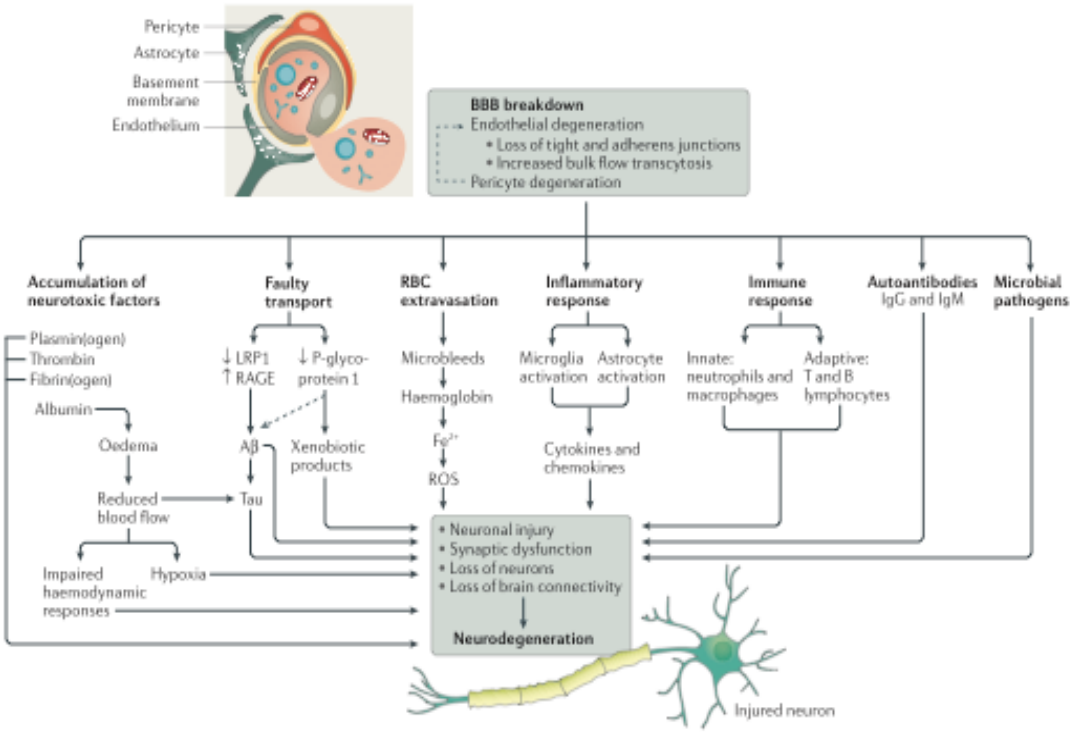


Figure 1.24. Blood-brain barrier (BBB) breakdown and its accompanying adverse molecular effects. Disruption of the BBB results in pericyte and endothelium degeneration, triggering a cascade of physiological responses. Such responses, which include impaired transport, erythrocyte extravasation, and inflammatory responses, lead to impaired CNS function (synaptic dysfunction, irreversible neuronal injury, and loss of neurons), causing neurodegeneration. Adapted from Sweeney *et al.,* (2018, p 144).

1.8.2 Impaired Neurovascular Coupling

The adult human brain is dependent on a continuous, uninterrupted supply of blood, consuming approximately one-fifth of total blood supply (Iadecola, 2004; Novak & Hajjar, 2010; Iadecola *et al.,* 2016; Kisler *et al.,* 2017; Dementia Australia, 2020). Acute and sustained interruptions in cerebral blood flow (CBF) can severely impair underlying vulnerable nutrient-dependent brain cells responsible for cognitive function, with irreversible neuronal damage occurring within minutes (Iadecola, 2004; Novak & Hajjar, 2010; Iadecola *et al.,* 2016). Thus, an appropriately regulated CBF is essential for optimal brain homeostasis (Kisler *et al.,* 2017).

Neurovascular coupling (also known as functional hyperaemia) refers to ongoing cell-cell interactions between brain cells (astrocytes and other neuroglia) and adjacent smooth muscle and endothelial cells that function together as a single functional unit. This is known as a neurovascular unit (Figure 1.25) (Iadecola, 2004; Novak & Hajjar, 2010; Iadecola *et al.,* 2016). It is a pivotal cerebrovascular mechanism that enables selective redistribution of cerebral blood flow (CBF) to metabolically-demanding brain areas and the simultaneous elimination of toxic metabolic by-products (Iadecola, 2004; Novak & Hajjar, 2010; Iadecola *et al.,* 2016). Such a dynamic and adaptive physiological process enables the brain to continuously receive adequate quantities of crucial nutrients (O_2 and glucose) during intensive neural activation, safeguarding the highly-regulated internal milieu of the CNS and maintaining normal cognitive functioning (Iadecola, 2004; Novak & Hajjar, 2010; Iadecola *et al.,* 2016).

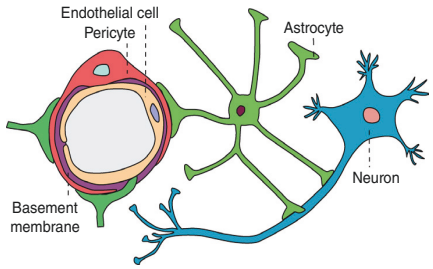


Figure 1.25. A simplified representation of a neurovascular unit (NVU), which consists of brain cells (astrocytes and other neuroglia) and adjacent smooth muscle and endothelial cells that function together as a single unit. Adapted from Sweeney *et al.,* (2016, p 772).

Changes in blood pressure (hypo- and hypertension) have been associated with interruptions in CBF, resulting in disturbances in cerebral perfusion, oxygenation, and vascular reserve capacity (Novak & Hajjar, 2010). Convincing evidence from experimental studies suggest that hypertension detrimentally affects the dynamic neurovascular coupling process, disrupting cerebral blood flow to metabolically-active cortical areas and leading to reduced cerebral perfusion, oxygenation, and vascular reserve capacity (Novak & Hajjar, 2010). As neurons in metabolically-active regions are deprived of crucial nutrients (O_2 and glucose), brain ischaemia, neuronal dysfunction and irreversible cellular damage will occur, which manifests as cognitive decline. Sustained deprivation of nutrients and blood to brain cells in activated brain regions due to impaired cerebral blood flow is hypothesised to contribute to the progression of hypertension-associated cognitive dysfunction and link hypertension to cognitive impairment.

1.8.3 Small-Vessel Disease (SVD)

The adult human brain contains an estimated 644 kilometres (km) of small and large cerebral vessels, including arteries, arterioles, capillaries, and venules that serve dual functions: (i) delivering oxygen and nutrient-rich blood (energy metabolites) to brain cells to maintain optimal perfusion, and (ii) eliminating neurotoxic metabolic by-products (CO_2) from the brain parenchyma to the systemic circulation (Sörös *et al.,* 2013; Iadecola *et al.,* 2016; Sweeney *et al.,* 2018). It is also the most metabolically-demanding organ, consuming approximately 20% of the body's oxygen; therefore, healthy blood vessels play a central role in maintaining optimal brain homeostasis and perfusion (Iadecola *et al.,* 2016; Kisler *et al.,* 2017; Sweeney *et al.,* 2018).

Findings from brain imaging studies (MRI) suggest that exposure to persistently-elevated BP progressively disrupts the vasculature of vulnerable cerebral blood vessels (Sörös *et al.,* 2013). The major arteries susceptible to early damage from chronic arterial HTN primarily include the middle cerebral artery and the lenticulostriate arteries (Figure 1.26). This has been ascribed to their short extension from the base of the brain (Sörös *et al.,* 2013). These arteries play crucial roles in supplying oxygen, energy metabolites, and nutrients to key brain centres, including the brainstem, basal ganglia, and thalamus (Sörös *et al.,* 2013). Damage to these vital blood vessels causes vascular remodelling and fibrinoid degeneration, resulting in arterial stiffness and reduced lumen diameter. Micro-aneurysms in the vessel wall lead to a gradual narrowing (lacunar infarct) and rupturing (intracerebral haemorrhage) of arteries (Sörös *et al.,* 2012; Iadecola *et al.,* 2016). This is known as small-vessel disease (SVD) and commonly manifests clinically as arteriosclerosis which, in advanced stages, is associated with microbleeds and thickened vessel walls. Taken together, alterations in blood vessel integrity and diameter result in impaired cerebral blood flow (CBF), causing hypoperfusion and insult to white matter brain areas. This increases the risk of stroke (ischaemic and haemorrhagic). Such pathophysiology could contribute to cognitive dysfunction and link hypertension to the early cognitive dysfunction commonly reported in patients with hypertension.

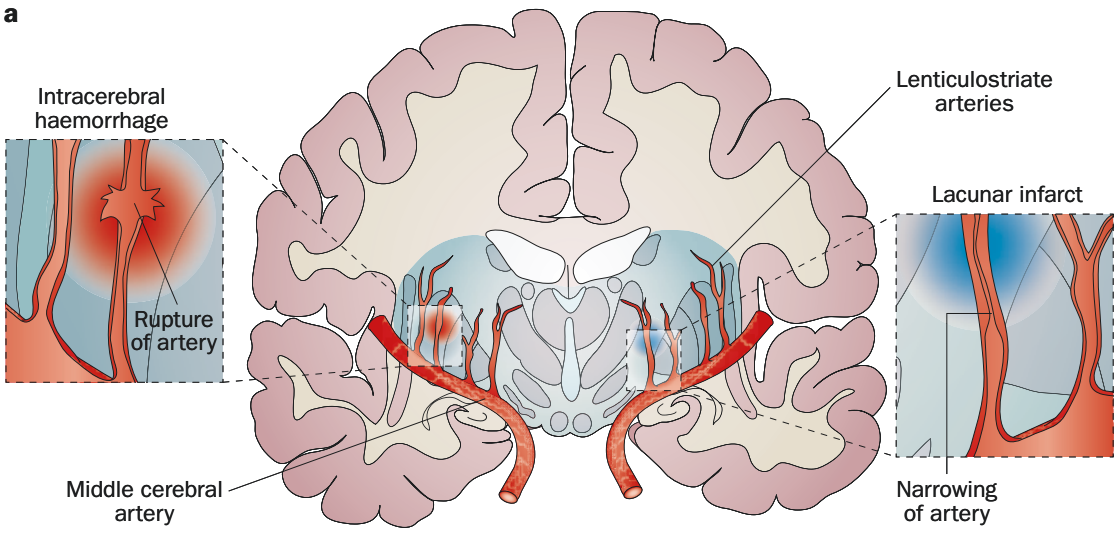


Figure 1.26. The major cerebral arteries hypothesised to be disrupted by untreated hypertension. Adapted from Sörös *et al.,* (2013, p 2).

Given prevalence rates for both diabetes and hypertension are predicted to increase, and both conditions have been consistently linked to exacerbating cognitive dysfunction *via* mechanisms currently unknown, it is clear an objective indicator and cognitive screening tools that can accurately and consistently detect the subtle changes in cognition associated with these conditions is urgently required. Such cognitive measures could have broader societal implications, including the early and accurate detection of incipient cognitive decline. They could also alert clinicians of individuals at high risk of progressing to these cognitive diseases, enabling the instigation of robust risk-reduction measures currently recommended in emerging guidelines to avert adverse cognitive outcomes (*e.g.* adequate cardiovascular risk factor management). One objective, non-invasive neurophysiological measure that has shown promising potential in monitoring changes and trajectories in cognition in progressive neurodegenerative diseases and early stages of cognitive impairment (MCI), is electroencephalography (EEG).

1.9 Electroencephalography (EEG)

First recorded from the scalp of humans in 1929 by German neurophysiologist, Hans Berger, electroencephalography (EEG) is a sensitive neurophysiological technique that records ongoing electrical brain activity generated by underlying cortical pyramidal neurons (Da Silva, 1991; Smith, 2005; Jia & Kohn, 2011; Kaiboriboon *et al.,* 2012; Michel & Murray, 2012; Huster *et al.,* 2013; Khanna *et al.,* 2015). This electrical activity is recorded using electrodes attached non-invasively to the scalp according to the standard *international 10-20 system* of electrode placement (Jasper, 1958), a universally-adopted and standardised system that ensures uniform scalp coverage (Figure 1.27). While the electroencephalogram is widely considered the 'gold-standard' in clinical practice for diagnosing, screening, classifying, and monitoring changes in brain activity in neurological disorders (*e.g.* epilepsy, sleep disorders), it has also shown promising potential in monitoring trajectories in cognition in progressive, neurodegenerative diseases, such as AD and dementia (Smith, 2005; Kaiboriboon *et al.,* 2012; Babiloni *et al.,* 2013; Straaten *et al.,* 2014, McBride *et al.,* 2014; Khanna *et al.,* 2015).

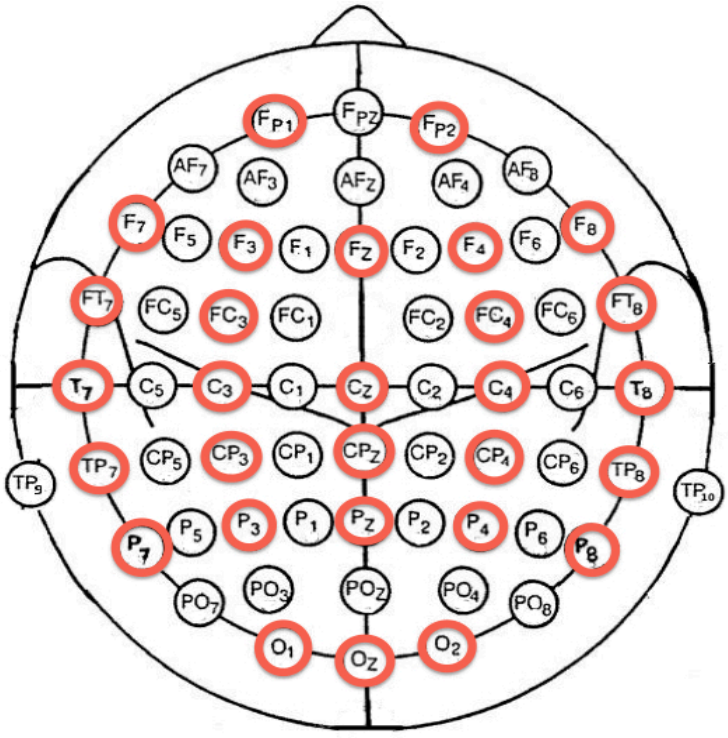


Figure 1.27. The standard international 10-20 system of electrode placement. Letters correspond to underlying brain areas. Odd numbers represent left-hemispheric postions; even numbers, right hemispheric positions. The electrodes circled in red are the most common sites of the 10-20 system used in clinical practice. Adapted and modified from Mert & Akan, (2018, p 5).

Although the low spatial resolution of the EEG is often criticised (Fazli *et al.,* 2012; Michel & Murray, 2012; Burle *et al.,* 2015), many researchers argue the EEG demonstrates several advantageous properties (Srinivasan, 2007; Michel & Murray, 2012; Khanna *et al.,* 2015, Modi & Sahin, 2017). First, the EEG complements existing cognitive assessments and neuroimaging technology, validating abnormalities observed. The EEG is non-invasive, cost-effective, and its application is straightforward (Srinivasan, 2007; Michel & Murray, 2012; Khanna *et al.,* 2015; Houmani *et al.,* 2018; Lord *et al.,* 2020). It is readily available, unaffected by habituation or repetitive effects, portable and, unlike other neuroimaging modalities, does not expose patients to radiation (Lord *et al.,* 2020). It also provides researchers robust temporal resolution (Srinivasan, 2007; Sauseng & Klimesch, 2008; Michel & Murray, 2012; Giacino *et al.,* 2014; Khanna *et al.,* 2015; Modi & Sahin, 2017). This high temporal resolution affords novel insight into cerebral function on a millisecond scale, allowing investigators to explore brain electrical activity non-invasively in different brain areas during cognitive tasks in real-time (Srinivasan, 2007; Sauseng & Klimesch, 2008; Khanna *et al.,* 2015). For these reasons, electroencephalography was selected to assess cognitive function in the present study.

The electroencephalogram reflects brain activity from summated postsynaptic potentials generated by millions of cortical neurons distributed in the cerebral cortex (Sauseng & Klimesch, 2008; Modi & Sahin, 2017). Excitation of these cortical neurons results in the generation of distinct electrical signatures, referred to as brain waves (Da Silva, 1991; Sauseng & Klimesch, 2008; Martini *et al.,* 2011; Campisi & La Rocca, 2014). Berger (1929) first observed high-amplitude alpha oscillations while examining brain activity in resting healthy subjects and since then, several other brain waves of varying frequencies have been described (Sauseng & Klimesch, 2008; Campisi & La Rocca, 2014; Khanna *et al.,* 2015). Five major brain waves can be derived from EEG tracings (Sauseng & Klimesch, 2008; Campisi & La Rocca, 2014; Khanna *et al.,* 2015). These are classified as delta, theta, alpha, beta or gamma, and are associated with various psychophysiological states (Sauseng & Klimesch, 2008; Campisi & La Rocca, 2014; Straaten *et al.,* 2014; Khanna *et al.,* 2015) (Table 1.2).

1.9.1 Delta Waves

Delta waves are low-frequency (< 4 Hz), large-amplitude (75-200 μV) brain waves generated by thalamo-cortical circuits (Sauseng & Klimesch, 2008; Campisi & La Rocca, 2014). Delta waves predominate the electroencephalogram recording in deep sleep (Modi & Sahin, 2017). In cognitive studies, they have been implicated in attention processess (Schroeder & Lakatos, 2009).

1.9.2 Theta Waves

Hypothesised to originate from thalamic and hippocampal nuclei, theta rhythms are large-amplitude waves with a frequency range of 4-8 Hz (Sauseng & Klimesch, 2008; Massar *et al.,* 2014). Together with delta waves, they are collectively referred to as 'slow-wave activity'. Theta waves are primarily associated with drowsiness and reduced mental alertness, but have been linked with learning and memory as well as rapid eye movement (REM), sleep, and hypnagogic imagery (Sauseng & Klimesch, 2008; Da Rosa, & Rodrigues, 2011; Massar *et al.,* 2014; Modi & Sahin, 2017). Increased theta activity has also been observed in subjects with cognitive impairment (Jelic *et al.,* 1996; Jelic *et al.,* 2000).

1.9.3 Alpha Waves

First observed by Berger (1929) in resting healthy subjects, alpha rhythms are high-amplitude, high-frequency (8-13 Hz) oscillations evident in EEG recordings during relaxed wakefulness (Sauseng & Klimesch, 2008; Bazanova & Vernon, 2013; Campisi & La Rocca, 2014). Alpha waves are abundant over parieto-occipital and cortical brain areas and vanish from EEG recordings during cognitively-demanding activities, such as attention and executive functioning (Martini *et al.,* 2011; Campisi & La Rocca, 2014).

1.9.4 Beta Waves

Known as "fast-wave oscillations", beta oscillations are high-frequency (14-30 Hz) brain waves associated with mental alertness, motor activity, and an activated cortex (Sauseng & Klimesch, 2008; Campisi & La Rocca, 2014). They are pronounced over all cortical areas, including frontal, parietal, somatosensory and motor regions of the brain (Sauseng & Klimesch, 2008; Campisi & La Rocca, 2014; Modi & Sahin, 2017).

1.9.5 Gamma Waves

Gamma rhythms are cortically-generated high-frequency (30-80 Hz) brain waves. They are posited to underlie high-order cognitive processes, including attention, perception, consciousness, and sensory and memory processing (Fries, 2009; Sauseng & Klimesch, 2008; Campisi & La Rocca, 2014; Ray & Maunsell, 2015; Modi & Sahin, 2017). Reduced gamma activity has been associated with abnormalities in cognitive function, such as cognitive impairment.

Table 1.2. The main brain waves commonly observed in an electroencephalogram and their corresponding psychophysiological state(s). Adapted and modified from Modi & Sahin, (2017).

Brain Wave	Frequency (Hz)	Psychophysiological State(s)
Delta	1 – 4	Deep sleep, attentional processes,
Theta	4 - 8	Drowsiness, fatigue, rapid eye movement, learning and memory processing,
Alpha	8 - 12	Relaxed wakefulness, attention, selective processing
Beta	12 - 30	Mental alertness, sustained concentration, motor activity
Gamma	30 - 100	Attention, short-term memory processing, consciousness, selective inhibition

Key:

Hz - Hertz

1.10 Electroencephalography Changes in Diabetes Mellitus

Research investigating electroencephalographic changes associated with diabetes mellitus (T1DM and T2DM) is sparse. There is a lack of recent data reporting and validating EEG changes that occur in diabetes and most studies have examined EEG activity from limited electrode sites; consequently, the precise underlying neurophysiological changes associated with diabetes remain unclear. Research in this area is also predominantly exploratory, with few longitudinal investigations having been conducted. This scarcity limits understanding of the electroencephalography changes that occur during disease progression and the clinical utility of the electreoncephalogram. Early published reports classify abnormal EEG activity into broad arbitrary groups (*e.g.* abnormal/normal activity), limiting understanding of the precise electrophysiological abnormalities. Many studies also fail to report and account for the various diabetes-related mediating factors in detail, which are known to moderate the relationship between diabetes and cognition (Munshi *et al.*, 2006; Roberts *et al.*, 2008; Roriz-Filho *et al.*, 2009). This results in conflicting data being obtained.

Evidence from early EEG studies indicate that both children and adults with DM (T1DM or T2DM) demonstrate electrophysiological abnormalities compared to subjects without diabetes (Greenblatt, Murray, & Root, 1946; Izzo *et al.,* 1953; Eeg-Olofsson & Petersen 1971; Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005). The main changes in EEG activity commonly reported in subjects with DM include: (i) sharp increases in slow-wave brain activities (theta and delta) and (ii) declines in fast-wave brain activities (alpha, beta, and gamma) (Eeg-Olofsson & Petersen 1971; Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005). These EEG abnormalities have been primarily observed over temporo-occipital and frontal brain areas; however, global and focal changes in alpha, beta, and theta frequency bands have also been reported (Izzo *et al.,* 1953; Eeg-Olofsson, 1971; Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005; Cooray *et al.,* 2011a). Less reported have been modest reductions in fast-frequency gamma activity. Several investigators suggest the electrophysiological abnormalities observed in subjects with diabetes result from abnormal blood glucose concentrations (Soltèz & Acsádi, 1989). This view is supported by recent studies, showing that abnormal blood glucose concentrations influence the electrical activity of the brain (Graveling *et al.,* 2013; Rachmiel *et al.,* 2016). Therefore, ascertainment of blood glucose concentration at the time of cognitive assessment is pivotal to mitigate potential influence from hypo- or hyperglycaemic states.

1.10.1 Changes in slow-wave EEG activity in diabetes mellitus

Several early EEG studies investigating neurophysiological changes have found both children and adults with diabetes demonstrate global and focal increases in slow-wave activity compared to healthy subjects (Greenblatt, Murray, & Root, 1946; Izzo, Schuster, & Engel, 1953; Herrlin *et al.,* 1962; Eeg-Olofsson & Petersen 1977). Izzo *et al.* (1953) found young adults and elderly subjects with diabetes (n = 81: mean age: 50.1 years, age range: 15-76 years) exhibited increased slow-wave activity (delta and theta) and reduced alpha activity in frontal brain regions using a Grass, six-channel, model III C electroencephalogram (electrode positions not reported). Herrlin *et al.* (1962) recorded EEG activity from twenty-one electrode positions in young children and adults (n = 80, age range: 16-38 years) with long duration DM (diabetes duration: 15 years) over a two-year period and reported pronounced increases in slow-wave activity in the left parietal region. Interestingly, Herrlin *et al.* (1962) found no association between BGL and abnormal EEG activity or common diabetes-mediating factors, including diabetes duration, onset of diabetes, and degree of metabolic control. The precise EEG electrode positions explored by Herrlin *et al.* (1962) were also not reported. Similarly, Eeg-Olofsson and Petersen (1966) reported increased theta activity in children with diabetes; however, unlike Herrlin *et al.* (1962), this pattern was strongly linked to hypoglycaemia. Supporting Eeg-Olofsson and Petersen (1966), Tsalikian *et al.* (1981) also observed similar abnormalities in low-frequency EEG activity in newly- and previously-diagnosed children with uncontrolled T1DM (n = 39; 24 boys, 15 girls, age range: 11.5 months – 16.5 years) with ketosis, suggesting poor glycaemic control causes the observed electrophysiological abnormalities. However, Hung *et al.* (2010) suggest that more reliable associations may be observed bewteen $HbA1_C$ and EEG activity than with finger prick blood glucose tests, as blood glucose concentrations vary continuously.

The available evidence generally suggests there is a generalised slowing of EEG activity in patients with DM (T1DM and T2DM), particularly in temporal and occipital brain regions (Mooradian *et al.,* 1988; Pramming *et al.,* 1988; Tallroth *et al.,* 1990). Mooradian *et al.* (1988) reported increased slow-wave activity over the central cortex (electrode locations: F_Z, C_Z, and P_Z) and reductions in alpha activity in the parietal region in elderly subjects (n = 43, mean age: 66.3 ± 0.3 years, diabetes duration: 13.3 ± 1.8 years) with T2DM compared to age-matched controls. No relationship between blood glucose

concentration and EEG activity was documented. In contrast, Pramming *et al.* (1988) found marked increases in theta oscillations over temporal and parieto-occipital areas in patients with T1DM (n = 13, mean age: 28 years, diabetes duration: 8 years) during induced hypoglycaemia. Similar to Pramming *et al.* (1988), Tallroth *et al.* (1990) observed sharp increases in slow-wave frequency activity in T1DM subjects (n = 8, mean age: 28.0 ± 7.4 years, duration of diabetes: 15.5 ± 5.1 years) during insulin-induced hypoglycaemia over anterior brain regions. Interestingly, the authors reported that EEG activity normalised after blood glucose concentrations stabilised, reinforcing that hypoglycaemia causes transitory alterations in brain electrical activity (Deary, & Frier, 2013; Rachmiel, *et al.,* 2015).

Other researchers have reported similar findings in subjects with DM. Brismar *et al.* (2002) investigated EEG activity in young adults with well-controlled T1DM without a history of recurrent hypoglycaemia (n = 100: 49 patients with diabetes, 51 healthy controls, age: 21-41 years) and found pronounced increases in slow-wave brain activity: increased delta activity in frontal and temporo-parietal areas, and elevated theta activity in frontal and left central brain regions. During controlled hypoglycaemia, Bjorgaas *et al.* (1998) similarly showed that children with T1DM (n = 19, diabetes duration: >1.5 years) exhibit global increases in theta activity in cortical areas compared to healthy subjects using quantitative EEG.

Conversely, a recent study by Cooray *et al.* (2011) reported diminished global slow-wave power in patients with T1DM (n = 119, age range: 22-56 years, diabetes duration: > 5 years). The dissimilar outcome obtained could be due to several factors: differences in the degree of metabolic control of diabetes participants recruited, the shorter duration of diabetes in participants recruited by Bjorgaas *et al.* (1998), and potential influence from the mediating effects of diabetes-related factors. Bjorgaas *et al.* (1998) also predicted the degree of metabolic control solely from glycosylated haemoglobin ($HbA1_C$) data provided. Although $HbA1_C$ is widely considered the 'gold-standard' marker of long-term glycaemic control, it does not reflect minute-to-minute fluctuations in BGL (Kovatchev, 2017; ADA, 2020). It is also insensitive to hypoglycaemic episodes (Kovatchev, 2017; ADA, 2020). Hence, this may have accounted for the different outcome observed. Importantly, the presence of electrophysiological abnormalities in children suggests the developing human brain is vulnerable to the early neurotoxic effects of diabetes (Ryan,

2006; Biessels, Deary, & Ryan, 2008). However, the ability of the EEG to consistently detect changes in cortical activity non-invasively supports the use of EEG as a potential suitable cognitive measure for rapidly monitoring ongoing changes in brain activity in diabetes.

1.10.2 Changes in fast-wave EEG activity in diabetes mellitus

Neurophysiological studies have also revealed that subjects with diabetes demonstrate measurable reductions in fast-wave brain activities compared to subjects without diabetes. One consistently-reported finding in recent studies has been diminished beta power. This has been primarily observed over temporal brain areas (Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005; Cooray, Hyllienmark, & Brismar, 2011). Howorka *et al.* (2000) found T1DM patients (n = 13, mean age: 36.1 ± 10.2, mean diabetes duration: 16.7 ± 7.4 years) with a recurrent history of severe hypoglycaemia demonstrated global widespread reductions in beta power. Slowing of central beta activity was also documented. In three more recent studies, all of which investigated EEG activity in young adults with T1DM, the investigators reported declines in beta power compared to age-matched healthy controls (Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005, Cooray *et al.,* 2011). Interestingly, no study found an association between beta power and a history of hypoglycaemia. Diminished beta power was also reported over different brain regions: Brismar *et al.* (2002) observed this diminution over posterior temporal and occipital areas, Hyllienmark *et al.* (2005) over the posterior temporal region, while Cooray *et al.* (2011) reported this pronounced decline in beta power in temporal regions.

Marked reductions in upper alpha activity have also been reported, although mixed findings have been obtained (Eeg-Olofsson and Petersen, 1966). In an early study investigating oscillatory activity in children and young adults with DM (n = 80, mean age: 10 years, age range: 2-16 years, diabetes duration: 4.6 years), Eeg-Olofsson and Petersen (1966) found these patients demonstrated reduced alpha activity. However, the location of this diminished alpha activity was not reported. No relationship was found also between age, metabolic control, age of diabetes onset, or diabetes duration, and the observed abnormalities in alpha activity were determined to be significantly correlated to the frequency of hypoglycaemic comas. Three decades later, Tribl *et al.* (1996) observed similar abnormalities in alpha activity during induced hypoglycaemia in adults with T1DM (n = 14; 8 males, 6 females, mean age: 33.1 ± 8.9 years, diabetes duration: 12.8 ±

6.0 years, mean $HbA1_C$: 7.2 ± 1.1 %), suggesting that glycaemic events are associated with detectable changes in cerebral electrical activity. In a study conducted two-years later in children during controlled hypoglycaemia using quantitative EEG, Bjorgaas *et al.* (1998) obtained contradictory results, observing modest increases in alpha activity over fronto-central and temporal regions.

One EEG frequency band largely unexplored in neurophysiological studies in diabetes is the gamma wave. Gamma waves are high-frequency brain waves associated with high-order cognitive processes, including short-term information processing and attention (Engel, Fries, & Singer, 2001; Sauseng & Klimesch, 2008; Blokland *et al.,* 2015; Ray & Maunsell, 2015). Available evidence indicates that subjects with diabetes exhibit reduced gamma activity (Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005, Cooray *et al.,* 2011). Brismar *et al.* (2002) and Hyllienmark *et al.* (2005) found young adults with T1DM exhibited reduced gamma power in posterior temporal brain regions. This pattern of reduced gamma activity was similarly reproduced more recently by Cooray *et al.* (2011), who found pronounced reductions in gamma power over the mid-parietal region. However, to date, only few studies have reported such patterns. No recent investigations have replicated this pattern in neuronal gamma oscillations in patients with DM (T1DM and T2DM). Therefore, it is critical that future studies continue investigating high-frequency gamma band oscillations in subjects with diabetes (Engel, Fries, & Singer, 2002). Exploration of activity in this frequency band may reveal possible disturbances in cortical regions responsible for generating these brain waves, indicating possible early cognitive deterioration.

Whether the duration of diabetes correlates with the severity of EEG abnormalities observed also remains unclear. While the duration of AD is understood to correlate strongly with the severity of EEG abnormalities, the literature is not helpful in clarifying whether such a relationship also exists in diabetes mellitus. Mixed results have been obtained: some early studies have reported an association (Izzo *et al.,* 1953), whereas others have not (Haumont *et al.,* 1979; Soltèz & Acsádi, 1989). Soltèz & Acsádi (1989) acknowledge the lack of an association between duration of diabetes and EEG abnormalities could have been ascribed to the short duration of diabetes (mean duration of diabetes: 5 years) in participants recruited in their study. Other researchers attribute conflicting findings to arbitrary classifications of metabolic control and limited

information obtained by early studies regarding the various diabetes-related moderating factors. The complex and multi-factorial nature of diabetes also complicates this relation. Thus, it is clear from the mixed findings obtained that this area of research requires further exploration. Table 1.3 summarises the main changes in EEG activity reported in patients with DM (T1DM and T2DM) from the studies described above.

Table 1.3. Summary of main findings from studies investigating electroencephalography activity in diabetes mellitus.

Study	Sample Group	Main EEG Findings
Izzo *et al.* (1953)	Young adults and elderly patients with DM (n = 81: mean age: 50.1 years, age range: 15-76 years, type of diabetes: not specified)	↑ delta, ↑ theta (global), ↓ alpha in frontal regions
Herrlin *et al.* (1962)	Young children and adults (n = 80, age range: 16-38) with long duration T1DM (diabetes duration: 15 years)	↑ delta, ↑ theta in left parietal regions
Eeg-Olofsson and Petersen (1966)	Young children with T1DM (n = 80, mean age: 10 years, age range: 2-16 years, diabetes duration: 4.6 years) with a history of hypoglycaemia	↑ theta, ↓ alpha
Tsalikian *et al.* (1981)	Newly- and previously-diagnosed children with uncontrolled T1DM (n = 39; 24 boys, 15 girls, age range: 11.5 months – 16.5 years) and ketosis	↑ delta, ↑ theta
Mooradian *et al.* (1988)	Elderly subjects with T2DM (n = 43, mean age: 66.3 ± 0.3 years, diabetes duration: 13.3 ± 1.8 years) and controls (n = 41, mean age: 65.3 ± 0.6 years)	↑ delta, ↑ theta over central regions, ↓ alpha in parietal regions

Pramming *et al.* (1988)	Patients with T1DM (n = 13, mean age: 28 years, diabetes duration: 8 years) during induced hypoglycaemia	↑ theta over temporal and parieto-occipital areas
Tallroth *et al.* (1990)	Patients with T1DM (n = 8, mean age: 28.0 ± 7.4 years) with long duration diabetes (15.5 ± 5.1 years) during insulin-induced hypoglycaemia and control (n = 12, 26.4 ± 4.2 years)	↑ delta, ↑ theta over anterior brain regions
Tribl *et al.* (1996)	Patients with T1DM (n = 14; 8 males, 6 females, mean age: 33.1 ± 8.9 years, diabetes duration: 12.8 ± 6.0 years, mean $HbA1_C$: 7.2 ± 1.1 %)	*Slight hypoglycaemia*: ↑ delta, ↑ theta over lateral frontal regions *Hypoglycaemia:* ↑ delta ↑ theta, ↓ alpha *Severe hypoglycaemia:* ↑ delta ↑ theta in centro-temporal and parieto-occipital regions
Bjorgaas *et al.* (1998)	Children with T1DM (n = 19, diabetes duration: >1.5 years)	↑ theta over cortical areas, ↑ alpha over fronto-central and temporal regions.
Howorka *et al.* (2000)	T1DM patients (n = 13, mean age: 36.1 ± 10.2, diabetes duration: 16.7 ± 7.4 years) with a recurrent history of severe hypoglycaemia	↓ beta (global)

Brismar *et al.* (2002)	Young adults with T1DM (n = 49, 21 – 41 years of age, diabetes duration: 9.4 ± 3.5 years, mean $HbA1_C$: 6.9 ± 1.2 %) and controls (n = 51)	↓ alpha, beta, gamma in posterior and temporal regions, ↓ beta in occipital regions
Hyllienmark *et al.* (2005)	Young adults with T1DM (n = 35, mean age:17.1 ± 1.7 years, diabetes duration: 7.6 ± 4.6 years, age of diabetes onset: 9.6 ± 4.6 years, glycaemic control: not reported) and controls (n = 45, mean age:16.8 ± 1.6 years)	↑ delta, theta in frontal regions, ↓ alpha, beta, gamma in posterior temporal regions
Cooray *et al.* (2011)	Patients with T1DM (n = 119, mean age: 43.3 ± 7.6 years, mean $HbA1_C$: 7.3 ± 1.2 %, diabetes duration: 27.1 ± 11.6 years)	↓ beta over temporal regions, ↓ gamma over mid-parietal region

Key:

T1DM – Type 1 Diabetes Mellitus
T2DM – Type 2 Diabetes Mellitus
n – sample size
$HbA1_C$ – Glycosylated haemoglobin
↑ **–** increase
↓ **–** decrease

1.11 Electroencephalography Changes in Hypertension

Studies exploring neurophysiological changes in hypertensive subjects are lacking. There are no recent studies that have investigated EEG activity in subjects with hypertension. The longstanding neglection of this area of research may be partly due to the dilemma that hypertensive subjects are often medicated or reliant upon medication to maintain acceptable blood pressure. Consequently, this complicates recruiting non-medicated patients for EEG testing. It is understood that changes in cerebral blood flow (CBF) are associated with changes in oscillatory activity (Hossmann, 1994; Jordan, 2004). Clinical studies have also reported that blood pressure-lowering medication can enhance cerebral perfusion, potentially preserving cognitive function (Obisesan, 2009). Current evidence suggests that anti-hypertensive therapy can lower the risk of dementia and AD by 12 and 16%, respectively (Ding *et al.,* 2020), but the optimum age to initiate therapy and the duration of antihypertensive medication required to observe such a benefit remains unclear (Iadecola & Gottesman, 2019). No convincing data also exist to indicate the most effective blood-pressure lowering therapy for favourable cognitive outcomes (Iadecola & Gottesman, 2019; Ding *et al.,* 2020).

The only available study exploring EEG activity in hypertension was that conducted by Mani and Townsend (1964), who investigated EEG activity in subjects with clinically-diagnosed benign intracranial hypertension (BIH) (n = 14; 7 males and 7 females, mean age not reported) and obstructive hydrocephalus (n = 31). No study participants had a prior history of epilepsy or cerebrovascular disease. EEG activity was recorded from eight electrode positions (not disclosed) and categorised into five arbitrary groups (A: well-defined alpha rhythm, little other activity, B: good alpha rhythm, slight excess of other frequencies, C: little alpha rhythm, excess of other activity, D: dominant fast activity, E: dominant slow activity). Burst activity was also categorised into three different gradations (Grade 1: minimal, Grade 2: definite, Grade 3: marked). The authors found that subjects with BIH demonstrated mostly normal EEG activity compared to subjects with obstructive hydrocephalus, with BIH subjects exhibiting mostly EEG activity fulfilling category B criteria. Frequent bursts in EEG activity were also observed mostly in the BIH group, which the investigators ascribed to rising intracranial pressure.

While the study of Mani and Townsend (1964) demonstrated that BIH is associated with changes in brain oscillatory activity, experimental limitations weakened the study. Predominantly normal EEG activity was observed in BIH sufferers; however, this could have been linked to the small sample size examined (n = 14), which reduced the study's statistical power and the number of adjustments performed in the final analysis. Limited electrode positions were also assessed (8-channel Ediswan EEG system). Such a limited assessment of brain activity overlooks potential changes in brain activity occurring across the entire cortex. Lal & Craig (2001) suggest research validity may be improved by assessing more scalp locations.

Additionally, although the electrodes were distributed evenly over the cortex, they were not attached in accordance with the standard international *10-20* system of EEG. Future studies exploring changes in EEG activity in hypertension should utilise the universally-accepted standardised international *10-20 system* using a montage that ensures uniform scalp coverage (*e.g.* the 19-channel 10-20 montage). This will improve the comparability of the findings between subsequent studies. Future investigations should also report the grade/classification of hypertension at the time of electrophysiological assessment. Various grades of hypertension have been described (*e.g.* Grade 1, Grade 2, *etc.*) and these could influence electroencephalography activity. Taken together, these factors may account for the unusual findings obtained by Mani and Townsend (1964). However, it is clear that there is a paucity of data examining neurophysiological changes in hypertension.

1.12 Effect of glucose lowering and anti-hypertensive medication on EEG

Whether anti-hypertensive and glucose-lowering medication influence EEG activity and can reverse the electrophysiological abnormalities observed in these conditions remains unclear. It has been suggested that blood pressure-lowering therapy enhances cerebral perfusion (Obisesan, 2009). Current evidence also indicates that anti-hypertensive medication lowers the risk of dementia and AD by 12% and 16%, respectively (Ding *et al.,* 2020); however, no convincing data exist to suggest the most effective class for optimum cognitive outcomes (Iadecola & Gottesman, 2019; Ding *et al.,* 2020). Interestingly, no studies have examined the impact of anti-hyperglycaemic medication on brain oscillatory activity in patients with T2DM. Observational studies suggest that some anti-diabetic agents (*e.g.* sodium glucose cotransporter 2 inhibitors (SGLT2is) and glucagon-like peptide 1 receptor agonists (GLP-1RAs)) improve cognition by influencing critical brain processes (*e.g.* metabolism, inflammation, regeneration, synaptogenesis), but no conclusive evidence indicates that these pharmacotherapies modify the risk of cognitive dysfunction in T2DM (De Galan *et al.,* 2009; Areosa Sastre *et al.,* 2017). No data also exist to suggest that glucose-lowering therapy can reverse aberrant oscillatory activity. Investigators recommend that future randomised controlled trials (RCTs) should explore cognitive outcomes as a secondary endpoint (Biessels & Despa, 2018; Biessels & Whitmer, 2019). This may identify associations between specific anti-hyperglycaemic agents and cognitive outcomes. It is clear this area of research requires further exploration and that future studies exploring EEG activity in patients with DM (T1DM and T2DM) and HTN should report medications taken to establish possible associations.

1.13 Basis and Study Significance

1.13.1 Implications of the Present Study

Diabetes mellitus (T1DM and T2DM) and hypertension (HTN) are highly-prevalent chronic diseases that are increasing in incidence and prevalence globally. Both are also associated with numerous debilitating and life-threatening complications. One previously under-recognised complication common to both conditions, now being increasingly recognised as an important comorbidity in clinical practice, is cognitive dysfunction. This often manifests as subtle, irreversible cognitive decrements in DM, and cognitive decline in HTN. Although diabetes-associated cognitive decrements are known to progress perniciously, it is unknown whether hypertension-associated cognitive dysfunction is reversible or can be attenuated by pharmacotherapy. The mechanisms underlying the accelerated cognitive decline in both conditions remain unclear, so further research is urgently warranted to understand better the relationship between these conditions and cognition.

Numerous studies (cross-sectional and longitudinal) have assessed cognitive functioning in subjects with DM (T1DM and T2DM) and HTN using objective neuroimaging modalities and psychometric assessments; however, the precise cognitive domains affected remain unclear, as does the relationship between BGL and BP (SBP and DBP) and EEG activity and performance in individual domains of cognition. Given the trends in prevalence predicted for each condition, it is clear that future cognitive research is urgently warranted. Administration of reliable and validated cognitive screening tools, such as the Mini-Mental State Examination (MMSE) (Folstein, McHugh, & Folstein, 1975) and the Cognistat (Kiernan *et al.,* 1987), could reveal the cognitive domains detrimentally affected by these conditions and determine the suitability of these assessments for screening for the subtle cognitive decrements linked to these conditions. They could also identify individuals at high-risk of progressing to MCI, the earliest detectable stage of cognitive impairment, before irreversible deterioration in cognition has occurred. This would avert the adverse cognitive outcomes and the substantial socioeconomic and emotional costs associated with both conditions.

Substantial epidemiological evidence has revealed an association between both DM (T1DM and T2DM) and HTN and an increased risk of cognitive impairment (MCI, AD, VaD, and dementia) (Sierra *et al.,* 2012; Koekkoek *et al.,* 2015). The relative risk for dementia has been documented as a 1.5 to 2.5 times increased risk in patients with T2DM (Strachan *et al.,* 2011) compared to healthy subjects, whereas those with T1DM have a 60% increased risk of dementia (Smolina *et al.,* 2015). In contrast, the relative risk for dementia in hypertension remains unknown. It has also been estimated that one in three AD cases worldwide indirectly arises from these conditions (Norton *et al.,* 2014). Given evidence consistently indicates that both conditions are associated with an increased risk of cognitive impairment, it is paramount that further research is undertaken to understand better the pathophysiological pathways that link these conditions to cognitive impairment, particularly in the early stages. Such research could ultimately have broader societal implications, including the identification of the prominent causative determinants responsible for aggravating the cognitive decline commonly reported in these conditions. This could enable early therapeutic intervention and delay the onset and progression to neurodegerenative diseases such as AD and dementia. It could also contribute to reducing the substantial socioeconomic and emotional costs linked to these cognitive diseases.

Several cognitive measures are available to assess cognition in DM and HTN; however, there is currently no consensus among investigators concerning the most suitable cognitive measures for screening the cognitive decrements associated with these conditions. No objective neurological instruments or cognitive measures can also reliably and accurately detect the subtle cognitive decrements triggered by these conditions, as they manifest and progress insidiously (Biessels & Despa, 2018; Biessels & Whitmer, 2019). The pernicious nature of these decrements complicates timely and accurate detection of incipient signs and symptoms, leading to delays in identification and appropriate intervention (Srikanth *et al.,* 2020). The EEG is an established neurophysiological measure that has shown promising potential in monitoring changes in brain activity in MCI and in both DM and HTN and trajectories in cognition. Previous research indicates the EEG can consistently and reliably detect changes in oscillatory activity associated with these conditions and early stages of cognitive impairment (MCI) (Jelic *et al.,* 2000; Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005; Cooray, Hyllienmark, & Brismar, 2011). The EEG is also cost-effective, non-invasive, does not emit radiation, and demonstrates high temporal resolution. Such robust temporal resolution allows for

rapid and accurate detection of abnormalities in brain activity, which compares favourably with costly neuroimaging technologies. The latter also often require formal training for proper usage and repetition, due to head movements during scans (Lord *et al.,* 2020). Given the rising global tide of neurodegenerative diseases, there is an urgent need for a non-invasive biomarker that can accurately and consistently detect incipient cognitive decline. The present study could yield evidence to support the widespread deployment of EEG in clinical practice to facilitate reliable and accurate identification of the subtle and slowly-progressing cognitive dysfunction triggered by both DM and hypertension. This is often undetected by formal neuropsychological screening assessments.

There is a lack of research examining the neurophysiological changes associated with DM and HTN, specifically in the case of the latter and T2DM. The research in this area is also predominantly exploratory, limiting an in-depth understanding of the precise electroencephalography changes associated with these disorders. Consequently, the neurophysiological changes remain poorly understood. While studies have investigated EEG activity in subjects with DM, no study has explored the electroencephalography changes linked to hypertension. The deleterious relationship between raised blood pressure and cognition has been documented since the 1960s yet the mechanisms underlying hypertension-associated cognitive dysfunction remain elusive. Given the prevalence of hypertension is increasing, exploring the EEG changes that occur in hypertension is critical and relevant. Such research could reveal the brain areas susceptible to early deterioration from hypertension and possible signature EEG-based biomarkers of raised blood pressure. It could also determine the suitability of the EEG for detecting the subtle cognitive dysfunction linked to hypertension.

The comparative nature of the study comparing cognitive functioning between DM and HTN using objective neurophysiological measures and subjective psychometric tools is also novel. Previous investigations have compared cognitive function between clinical and non-clinical samples, but none have compared cognitive functioning (global and domain-specific) in subjects with DM (T1DM and T2DM) and HTN using established cognitive screening tools (the MMSE and the Cognistat). Both are reliable and validated neurocognitive assessments widely administered in clinical contexts to screen for early cognitive impairment (Folstein, McHugh, & Folstein, 1975; Tombaugh & McHugh,

1992; Pangman *et al.,* 2000; Lancu & Olmer, 2006). Notably, the MMSE is currently recommended in emerging guidelines for screening for subtle cognitive decrements and cognitive impairment in elderly patients with DM (ADA, 2020; Srikanth *et al.,* 2020). Therefore, the present research could provide preliminary insight into the suitability of these cognitive assessments for identifying the slowly-progressing and subtle changes in cogntion associated with these conditions.

The present exploratory study, and subject of this thesis, is a novel, cross-sectional investigation that aims to address the limitations described herein by exploring cognitive function in four sample groups (n = 49, non-clinical; n = 30 diabetes subjects (n = 13, T1DM; and n = 17, T2DM) and n = 15 HTN patients) using electroencephalography and non-invasive cognitive measures (the Mini-Mental State Examination (Folstein, Folstein, & McHugh, 1975) and the Cognistat (Kiernan *et al.,* 1987)).

1.14 Hypotheses

In clinical (T1DM, T2DM, and HTN) and non-clinical samples using cognitive measures (EEG and psychometric assessment), it is hypothesised that:

1. There will be differences in cognitive performance between clinical and non-clinical samples, and
2. There will be correlations between cognitive measures and blood pressure (BP) and blood glucose level (BGL)

1.15 General Aims

1. Investigate differences in cognitive performance between clinical and non-clinical samples
2. Investigate associations between cognitive measures (MMSE, the Cognistat, and EEG) and blood pressure (BP) and blood glucose level (BGL)

1.16 Specific Aims (Aim 1)

1. Investigate differences in global cognitive performance (Mini-Mental State Examination) between clinical (T1DM, T2DM, and HTN) and non-clinical samples
2. Investigate differences in domain-specific cognitive performance (the Cognistat) between clinical (T1DM, T2DM, and HTN) and non-clinical samples
3. Investigate differences in electroencephalography (EEG) activity between clinical (T1DM, T2DM, and HTN) and non-clinical samples

1.17 Specific Aims (Aim 2)

1. Investigate associations between cognitive measures (MMSE, the Cognistat, and EEG) and pre-study and post-study systolic and diastolic blood pressure (BP)
2. Investigate associations between cognitive measures (MMSE, the Cognistat, and EEG) and pre-study and post-study blood glucose level (BGL)

2. Methodology

2.1 Methodology Summary

The methodology described in this chapter was developed to address the aims and hypotheses introduced in Chapter 1. The cross-sectional study was conducted in the Neuroscience Research Unit (NRU) at the University of Technology Sydney (UTS), in a temperature- and lighting-controlled and sound-attenuated neurophysiology laboratory, with minimal ambient interference. Testing involved one session, taking approximately two hours for each participant. All instrumentation (objective and subjective measures) used in this investigation was reliable and validated at the time of assessment. Given circadian rhythms have been shown to influence cognitive performance (Valdez, Ramírez, & García, 2012; Wright, Lowry, & LeBourgeois, 2012), testing was conducted during peak wakefulness periods (9 am – 2 pm and 4 pm – 8 pm).

2.2 Ethics Approval and Consent

The present study was conducted under ethics approval (HREC: 201400010) obtained from the UTS Human Research Ethics Committee (HREC). Prior to commencing experimental testing, all test subjects were provided with a concise overview of the study, methodology involved, and the study inclusion/exclusion criteria. Study volunteers were additionally informed that participation was voluntary and that they could discontinue involvement in the research at any time, without providing reasons for withdrawal. If the inclusion criteria were fulfilled (Section 2.4), written informed consent was then obtained from all test participants agreeing to participate in the study, prior to data collection. Both the participant and the researcher then read and signed the consent form and, in accordance with UTS ethics requirements, retained a copy of the consent form (Appendix 8.1).

2.3 Recruitment of Study Participants

49 healthy (hereafter referred to as non-clinical) volunteers, 30 participants with DM (T1DM: n = 13; T2DM: n = 17) and 15 with HTN (systolic and/or diastolic blood pressure ≥ 140mmHg/90mmHg) aged between 18-80 years were recruited from the local Sydney community for the present study. Study volunteers (both non-clinical and clinical) were recruited using various strategies: advertisement *via* posters (electronic and physical) (Appendix 8.7) in populated urban spaces and relevant medical clinics throughout Sydney; advertisement *via* relevant and professional organisations, including Diabetes Australia, Diabetes NSW, and Alzheimer's Australia; presentations at relevant community events; and by word-of-mouth.

2.4 Study Inclusion/Exclusion Criteria

Present study inclusion criteria required participants from the non-clinical cohort to be between 18-80 years of age with no underlying chronic disease (such as diabetes, hypertension, asthma, *etc*.), intellectual impairment, or psychosis (depression, substance abuse) that could potentially limit compliance in the study or influence the data. Participants reporting clinically-diagnosed DM (T1DM or T2DM) or HTN were eligible for inclusion in the clinical group and this was determined by ascertaining the participant's response to Question 18 of the Lifestyle Appraisal Questionnaire (LAQ) (Craig, Hancock, & Craig, 1996). Participants with DM (T1DM or T2DM) or HTN who reported taking medications to control their condition or complications linked to their respective chronic disease (*e.g.* microvascular or macrovascular complications), were also eligible for inclusion.

If participants (from either the non-clinical or clinical sample) indicated one or more of the following, as solicited by the Lifestyle Appraisal Questionnaire (LAQ) (Craig, Hancock, & Craig, 1996), they were immediately excluded from further study participation: illicit substance use/dependence, psychotropic medication, alcoholism (>16 standard alcoholic drinks per day), smoking (>10 cigarettes daily), severe intellectual disorder or psychosis. The literature suggests these lifestyle risk factors can cause irreversible changes in underlying brain structures, affecting normal cognitive function (Le Berre *et al.,* 2014; Karama *et al.,* 2015); hence, this would have influenced the data obtained in the present study.

2.5 Blood Pressure (BP) Measurement

In accordance with established and recommended blood pressure (BP) measurement guidelines (Pickering *et al.,* 2005; Unger *et al.,* 2020), after a five-minute rest period, participant brachial blood pressure was recorded three times, both before and after cognitive testing. Blood pressure was measured using a reliable and validated automatic non-invasive digital BP monitor (OMRON Healthcare Co., Ltd, IA1B (HEM-7000-C1L), Kyoto, Japan)) (Figure 2.1) and recorded from the participant's right upper arm (positioned at the level of the heart) in the upright sitting position, with feet flat on the floor and no talking before, between, or during measurements (Figure 2.2). Three measurements were recorded, since the literature indicates this improves accuracy and attenuates potential influence of the effects of 'white-coat hypertension' (office systolic blood pressure ≥ 140/90 mm Hg at least three times), a syndrome affecting approximately 10-30% of individuals worldwide (Pickering *et al.,* 2005; Unger *et al.,* 2020). This technique also negates auscultation-induced errors (Unger *et al.,* 2020). To minimise potential carry-over from previous measurements and permit the restoration of elastic properties of blood vessels, participants were given a rest period (1-2 minutes) between each BP measurement (Unger *et al.,* 2020). Subsequently, each of the three BP readings were averaged to determine mean BP before and after the study, for each participant.

Figure 2.1. The non-invasive automatic blood pressure device used to record participant brachial blood pressure.

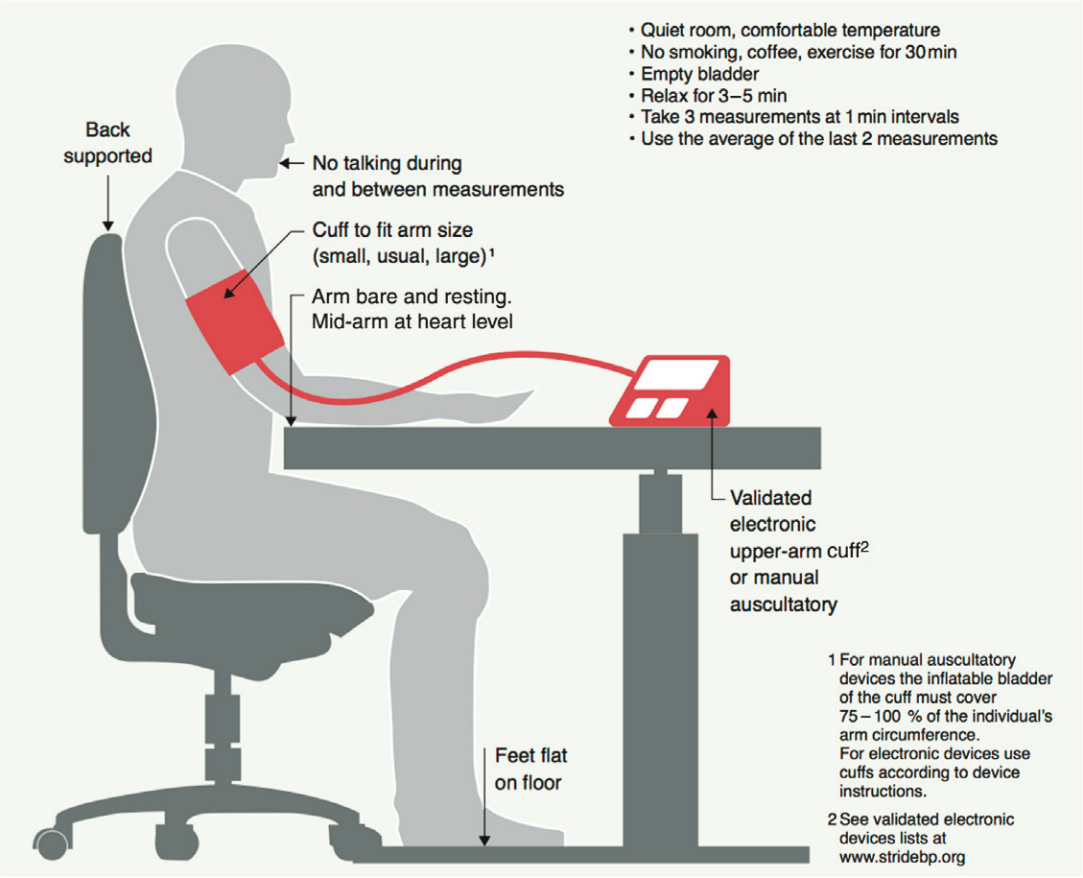


Figure 2.2. Appropriate posture and seating position for recording blood pressure using a non-invasive automatic blood pressure monitor. Adapted from Unger *et al.,* (2020, p 4).

2.5.1 BP Inclusion/Exclusion Criteria

Blood pressure of subjects from the non-clinical sample determined their inclusion or exclusion from the study. Subjects from the non-clinical group with average BP (for either systolic BP or diastolic BP, or both) meeting Grade 2 HTN criteria (≥ 160/100 mm Hg) were immediately excluded from further study involvement and, in accordance with the UTS HREC approved emergency protocol, were offered to be escorted to a nearby medical centre (Appendix 8.2). Blood pressure meeting this threshold is associated with premature cardiovascular mortality and long-term adverse cardiovascular outcomes (Weber *et al.,* 2014; Unger *et al.,* 2020).

If BP measurements (for systolic or diastolic alone, or both) for the non-clinical group met Grade 1 HTN criteria (≥ 140/90 mm Hg but ≤ 160/100 mm Hg), they were also excluded from the study. In this instance, the participant was notified of their elevated BP and advised to consult their medical professional for further clinical evaluation. No exclusion threshold for BP applied for participants with clinically-diagnosed T1DM or T2DM or HTN, as BP is typically elevated in these chronic diseases (Deshpande, Harris-Hayes, & Schootman, 2008; DeFronzo *et al.,* 2017). However, as outlined in the UTS HREC approved emergency protocol, participants from the clinical cohort (T1DM or T2DM or HTN) were still advised to consult their medical practitioner (BP ≥ 140/90 mm Hg) and were offered to be escorted to the nearest medical centre if their BP met Grade 1 or 2 hypertension criteria (Table 2.1).

Table 2.1. Blood pressure inclusion and exclusion limit thresholds.

Category	SBP (mm Hg)	DBP (mm Hg)	Non-clinical Sample	Clinical Sample (T1DM, T2DM, and HTN)
Normal BP	< 130	< 85	Included	**Excluded**
High – normal	130 – 139	85 – 89	Included	**Excluded**
Grade 1 hypertension	140 – 159	90 – 99	**Excluded** and offered an escort to accessible medical centre	Included and advised to consult physician
Grade 2 hypertension	≥ 160	≥ 100	**Excluded** and offered an escort to accessible medical centre	Included and advised to consult physician

Key:

BP – Blood Pressure

mm Hg – millimetres of mercury

HTN – Hypertension

HREC – Human Research Ethics Committee

≥ – greater than or equal to

T1DM –Type 1 Diabetes Mellitus

SBP –systolic blood pressure

< – less than

T2DM – Type 2 Diabetes Mellitus

DBP – diastolic blood pressure

If the respective BP inclusion criteria were fulfilled for each group, blood glucose concentrations for each study participant were then determined.

2.6 Blood Glucose Level (BGL) Determination

Following pre-study (baseline) BP measurement, the blood glucose level (BGL) of all test participants was determined. Two-hour (2-hr) fasting blood glucose concentrations were determined before cognitive testing using a sterile, single-use lancing device, which included three depth settings (Accu-Chek Safe-T-Pro Plus, Roche Diabetes Care Australia Pty Ltd), and measured using a reliable and validated blood glucometer device (Accu-Chek Performa, Roche Diagnostics Australia Pty Ltd) (Figure 2.3). All blood glucose concentration values were reported in millimoles per litre (mmol/L). This spot-test was performed for all subjects as glycaemic events (hypo- and hyperglycaemia) are associated with detectable changes in electroencephalography activity (Sommerfield, Deary, & Frier, 2004; Cox *et al.,* 2005; Graveling, Deary, & Frier, 2013; An *et al.,* 2015). Therefore, the impact of hypo- or hyperglycaemia could be negated and the relationship between other moderating variables (*e.g.* glycaemic control and disease duration) could be established. If blood glucose concentrations fell outside the normal recommended blood glucose concentration range (Figure 2.4), participants were still included for further study participation.

Figure 2.3. Blood glucometer device (left) and sterile, single-use lancing device (right) used to determine blood glucose concentrations for all study participants

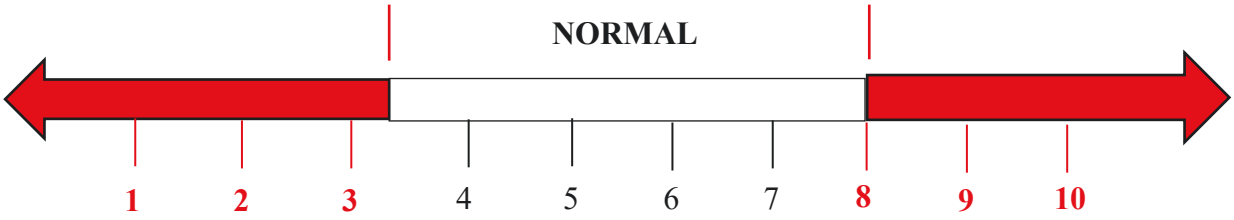


Figure 2.4. Normal 2-hour postprandial blood glucose concentration range. After 2-hours of fasting, blood glucose concentrations falling between 3.5-8mmol/L are typically considered normal, whereas concentrations outside this range are considered abnormal (Diabetes Australia, 2020).

After the BGL was determined for each participant, they then completed the Lifestyle Appraisal Questionnaire (LAQ) (Craig, Hancock, & Craig, 1996). Participants reporting diagnosed T1DM or T2DM or HTN completed an additional questionnaire developed in-house by the researcher for each disease type. These disease-specific questionnaires solicited additional characteristics relevant to their respective chronic disease not already obtained from the Lifestyle Appraisal Questionnaire (*e.g.* disease duration, glycosylated haemoglobin ($HbA1_C$), *etc.*) (Appendix 8.3 and 8.4).

2.7 Demographic Data Acquisition

2.7.1 Lifestyle Appraisal Questionnaire (LAQ) (Craig, Hancock, & Craig, 1996)

Lifestyle factors, perceived stress levels, and demographic characteristics of all participants (non-clinical and clinical) were obtained using a reliable and validated Lifestyle Appraisal Questionnaire (LAQ) (Craig, Hancock, & Craig, 1996). The LAQ is a sensitive two-part questionnaire frequently administered in clinical and research contexts that assesses lifestyle risk factors and perceived stress over an 8-week period (Craig, Hancock, & Craig, 1996). It is also widely considered a reliable measure of long-term health outcomes (Craig, Hancock, & Craig, 1996).

The LAQ comprises two parts: Part I consists of 22 questions that solicit lifestyle risk factors associated with an increased risk of developing lifestyle-associated chronic diseases (*e.g.* alcoholic beverage consumption, smoking patterns, sleep quality, lifestyle disease history, drug intake). Additional lifestyle data, including body mass index (BMI), diet, exercise patterns, and alternative relaxation techniques undertaken, are also obtained. The maximum obtainable score is 73, with higher scores indicating an increased likelihood of developing long-term lifestyle-related diseases, such as coronary heart disease and diabetes mellitus (Craig, Hancock, & Craig, 1996). In contrast, Part II assesses participant lifestyle pressures and perceived stress levels, evaluated by 25 Likert scale type questions scored based on severity (0 – 3: 0 – almost never, 1 – sometimes, 2 – often, 3 – almost always). The maximum attainable score for Part II is 75, with higher scores suggesting greater perceived stress levels (Craig, Hancock, & Craig, 1996).

Participants reporting T1DM or T2DM or HTN completed additional questionnaires developed in-house. The purpose of these questionnaires was to solicit additional disease-specific characteristics relevant to each condition not already obtained by the LAQ (*e.g.* duration, age of disease onset, glycosylated haemoglobin ($HbA1_C$), frequency of blood glucose/blood pressure monitoring, alternative therapies used, medications taken and frequency, and self-scored disease management) (Appendix 8.3 and 8.4). Current literature suggests these factors moderate the relationship between each chronic disease and cognition (Ryan, Geckle, & Orchard 2003; Roberts *et al.,* 2008; Wessels *et al.,* 2008). Participants indicating no chronic condition (non-clinical cohort) were exempt from completing these questionnaires and only completed the LAQ.

Following completion of the LAQ, participant responses to questions 1, 2, 7, 8 and 18 were reviewed to determine if the participant fulfilled the study inclusion criteria (Section 2.4). Question 18 of the LAQ, "Do you at present suffer from a chronic condition", did not apply for participants from the clinical sample. If participants fulfilled the study inclusion criteria, a non-invasive, elastic 32-channel EEG cap with pre-determined electrode positions complying with the standard International *10-20 system* (Jasper, 1958) for EEG electrode placement was attached to the participant's scalp.

2.8 Electroencephalography Data Acquisition

Thirty channels of electroencephalography (EEG) recording were obtained using a NeuroScan Synamps amplifier and Scan 4.3 recording software (Compumedics NeuroScan, Charlotte, NC, USA). Brain electrical activity was recorded using a non-invasive 32-channel elastic cap embedded with Ag/AgCl electrodes (Quik-Cap, Compumedics, sampling rate: 1000 Hz) (Figure 2.5) in predetermined positions conforming to the standard international *10-20 system* of EEG electrode placement (Jasper, 1958) (Figure 2.6). The scalp electrode positions examined were: (Fp_1 (Fronto polar 1, Fp_2), (F_7 (Frontal 7, F_3, F_z, F_4, F_8), (FT_7 (Fronto-temporal 7, FT_8), (FC_3 (Fronto-central 3, FC_Z, FC_4), (T_7 (Temporal 7, T_8), (TP_7 (Temporo-parietal 7, TP_8), (C_3 (Central 3, C_Z, C_4), (CP_3 (Centro-parietal 3, CP_Z, CP_4), (P_7 (Parietal 7, P_3, P_Z, P_4, P_8), and (O_1 (Occipital 1, O_Z, O_2) (Figure 2.6).

Figure 2.5. The 32-channel non-invasive, elastic EEG cap used to record brain electrical activity from study participants.

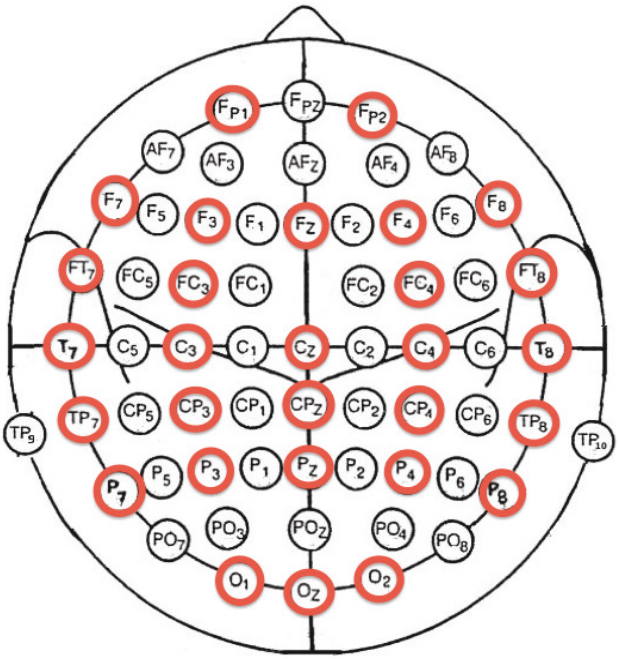


Figure 2.6. Topographic representation of the standard international *10-20* system of electroencephalography (EEG). Areas circled in red indicate electrode positions investigated in the present study. Odd numbers represent left hemispheric positions; even numbers, right hemispheric positions. The 'Z' notation indicates positions occurring along the midline of the cranium. Adapted and modified from Mert & Akan, (2018, p 5).

Key:

Fp – Fronto-polar **F** – Frontal **FT** – Fronto-temporal **FC** – Fronto-central
T – Temporal **C** – Central **TP** – Temporo-parietal **CP** – Centro-parietal
O – Occipital **PO** – Parieto-occipital **Z** – midline

Electrodes were referenced against a ground electrode and electrode impedance was maintained below five kilo-ohms (KΩ) (Keil *et al.,* 2014). Electroencephalography data acquisition occurred while the participant was seated in a comfortable upright position in a sound-attenuated, temperature- and lighting-controlled laboratory with minimal interference. An additional electrode pair positioned above and below the orbit participant's left eye (VEOU and VEOL) recorded electro-oculogram (EOG) activity to attenuate eye movement artifacts from the EEG signal (refer to section 2.10.1). Participants were also instructed to minimise movement during EEG recordings to reduce potential contamination of the electroencephalography signal from movement artifacts. Following accurate EEG cap placement and filling of relevant EEG electrodes on the cap with highly-conductive gel (Signa Gel, Parker Laboratories Inc, USA) (Figure 2.7), the

EEG signal was examined visually on the computer to determine whether adequate signals from each electrode were being generated (Figure 2.8), as well as to remove any potential artifacts evident (refer to section 2.10.1). If a poor tracing was generated, the following adjustments were performed until a reasonable signal was observed: nearby electrical equipment potentially interfering with the EEG signal was switched off; adjustments to electrical leads and gel quantities in electrode positions yielding poor signals were made; and the EEG cap was re-positioned. Once electrode impedance for each electrode was below the specified threshold value indicated above, and signals from each electrode position were considered acceptable, a baseline electroencephalography recording was obtained.

Figure 2.7. Electrode gel used to fill electrodes in the EEG cap, sterile syringe and blunted needle.

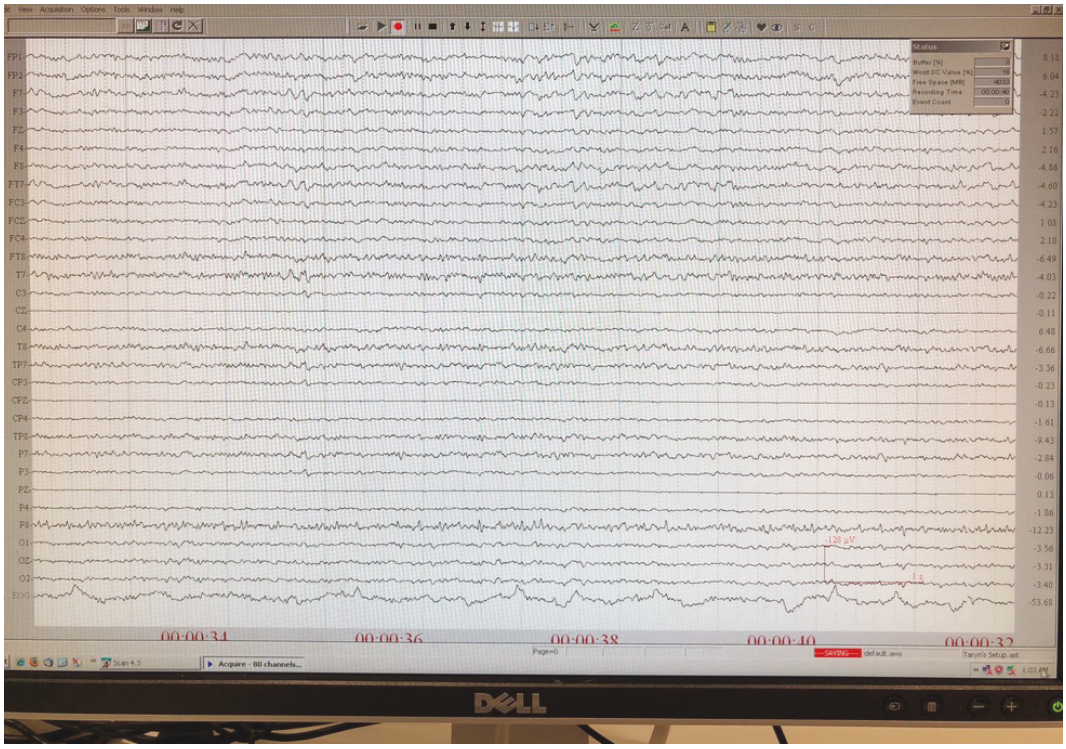


Figure 2.8. Example of an unprocessed EEG recording considered acceptable. The 'X' axis indicates time, whereas the 'Y' axis represents microvolts for the electrode positions examined.

Electrophysiological data were obtained from each of the 30 electrode positions (see section 2.8 for electrode locations) according to the standard international *10-20* system (Jasper, 1958) over two study phases, baseline and active, each 5-minutes in duration. The baseline phase involved the participant sitting quietly observing a blank computer screen, whereas the active phase involved engagement with a cognitively-stimulating task *via* a computer screen using pre-loaded software developed in-house, the Stroop Colour Word Test (Stroop, 1935) (refer to section 2.8.1) (Figure 2.9). Prior to recording the active Stroop test-linked EEG, a short practice run was conducted to determine whether the participant understood the instructions provided about the Stroop test and responded appropriately.

Figure 2.9. Screenshot depicting the Stroop Colour Word Test Program. This assessment prompts participants to process matched and mismatched stimuli (*i.e.* correctly identify the colour of the text shown on the screen as fast as possible). For example, in the screenshot above, the correct answer would be blue.

2.8.1 Stroop Colour Word Test (Stroop, 1935)

The Stroop Colour-Word Test was developed in 1935 and is a commonly administered and reliable neuropsychological measure that assesses several cognitive modalities, including cognitive flexibility, selective attention, psychomotor efficiency, and executive cognitive control (Adleman *et al.,* 2002; Homack & Riccio, 2004; Van der Elst *et al.,* 2006; Pilli *et al.,* 2013). It is widely considered a sensitive indicator of executive cognitive functioning, a broad cognitive domain concerned with multiple high-order cognitive processes mediated by the frontal lobe. The instrument has been deployed extensively in cognitive investigations in several areas of research, including frontal lobe function, developmental changes in the frontal lobe, disruptions in cognitive function triggered by neuropsychiatric disorders and, of relevance to this thesis, progressive changes in psychomotor efficiency and executive control, two cognitive domains that are consistently detrimentally affected by both DM and hypertension (Adleman *et al.,* 2002; Alvarez & Emory, 2006; Beratis *et al.,* 2010).

A clinically-valuable characteristic of the tool is the generation of an observable "Stroop interference effect", elicited when participants must correctly identify text colour when text is displayed in mismatched colours (*i.e.* the word GREEN printed in black ink) (Liotti *et al.,* 2000; Adleman *et al.,* 2002; Van der Elst *et al.,* 2006). This perceptible Stroop interference effect, which is characterised by an elongation in response time and caused by the simultaneous activation of two converging cortical pathways controlling attention (MacLeod & MacDonald, 2000), is considered a sensitive indicator of selective attention and executive cognitive functioning (Van der Elst *et al.,* 2006). Both DM and HTN have been consistently shown to affect cognitive flexibility and executive cognitive function (Adleman *et al.,* 2002; Alvarez & Emory, 2006; Beratis *et al.,* 2010); therefore, the Stroop test can be considered a suitable assessment for exploring disturbances in cognitive function triggered by either of these chronic diseases. While the assessment is available in several versions, the computerised version of the tool was utilised in the present investigation. Higher accuracy in stimulus presentation has been reported using the computer version (Pilli *et al.,* 2013).

Following acquisition of EEG data (baseline and active), two reliable and validated neurocognitive assessments, the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) and the Cognistat (Kiernan *et al.,* 1987), were then administered.

2.9 Cognitive Assessment

Cognitive function in several domains of cognition was assessed subjectively using two reliable and validated neuro-psychometric batteries; the Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) and the Cognistat (Kiernan *et al.,* 1987). Both are frequently deployed cognitive screening tools, but key differences exist between the two (discussed below). To negate any potential bias due to order effect, the cognitive assessments were administered in a randomised order.

2.9.1 Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975)

A reliable and validated neuro-psychometric tool available in several languages, the Mini-Mental State Examination (MMSE) is a brief clinical measure frequently administered in clinical and research environments that screens for early cognitive impairment (Folstein, McHugh, & Folstein, 1975; Tombaugh & McHugh, 1992; Pangman *et al.,* 2000; Lancu & Olmer, 2006) (Figure 2.10). Administered in a command-response based manner, and taking approximately 5-10 minutes, the assessment consists of 11 questions that assess essential cognitive functions. The MMSE comprises two parts: Part 1, which requires vocal responses, assesses cognitive domains of orientation to time and place (10 points), registration (3 points), attention/calculation (5 points) and recall (3 points), whereas Part 2, examined *via* verbal and written responses, assesses language (naming, repetition, reading, comprehension, visuoconstruction) (9 points) (Figure 2.11) (Folstein, McHugh, & Folstein, 1975; Tombaugh & McHugh, 1992; Pangman, Sloan, & Guse, 2000). The maximum obtainable score is 30, which represents the summed score from each cognitive domain (Folstein, McHugh, & Folstein, 1975). Scores ≤ 23 typically indicate probable cognitive dysfunction and have been linked with a subsequent dementia diagnosis in approximately 79% of cases (Folstein, McHugh, & Folstein, 1975; Lancu & Olmer, 2006; Marioni *et al.,* 2011).

Subject.....

Date.....

"MINI MENTAL STATE"

Max Score	*Score*	
		Orientation
5		What is the (year) (date) (day) (month) (season)?
5		Where are we: (state) (country) (town) (building) (level)?
3		**Registration**
		Name 3 objects: BALL, FLAG, TREE
		1 second to say each. Then ask the subject all 3 after you have said them. Give 1 point for each correct answer. Then repeat until subject has learnt all 3. Count trials and record.
5		**Attention and Calculation**
		Serial 7's
		( )93 ( )86 ( )79 ( ) 72 ( ) 65 ( ) 58
		1 point for each correct. Stop after 5 answers. Alternatively spell world backwards: DLROW
3		**Recall**
		Ask for the 3 objects repeated above. Give 1 point for each correct.
9		**Language**
		Name a pencil, and watch (2 points)
		Repeat the following "No ifs, ands, or buts". (1 point)
		Follow a 3-stage command: "Take a piece of paper in your right hand, fold it in half and put it on the floor" (3 points)
		Read and obey the following: CLOSE YOUR EYES
		Write a sentence (1 point); (subject, object and verb)
		Copy design (1 point)
		TOTAL SCORE

Assess level of consciousness along a continuum

Alert Drowsy Stupor Coma

Figure 2.10. The Mini-Mental State Examination (MMSE). Adapted from Folstein, McHugh, & Folstein (1975).

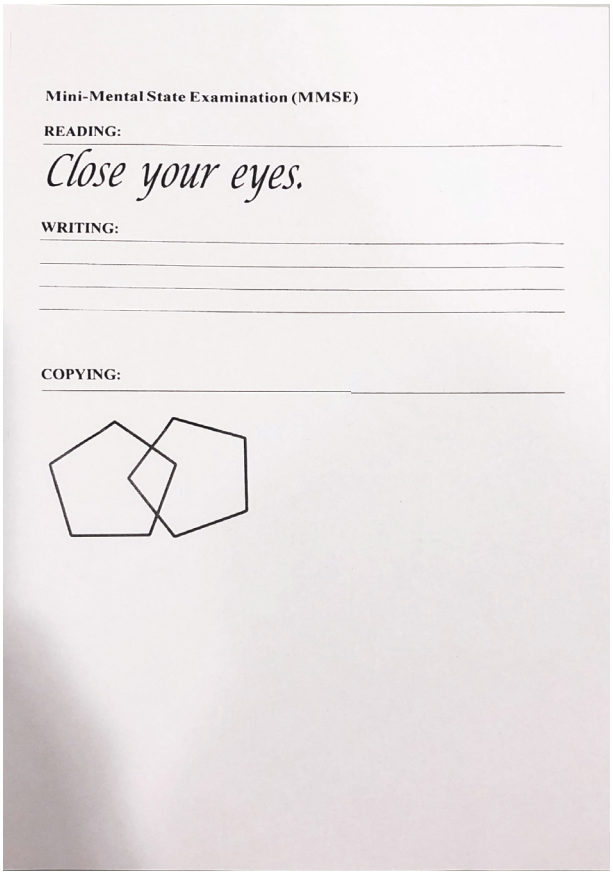


Figure 2.11. Stimulus sheet for assessing language in Part 2 of the MMSE.

Although the rapid administration of the assessment is widely praised, and broad consensus exists among researchers that the psychometric properties of the assessment are considered reasonable despite its low sensitivity (test-retest reliability values: 0.56-0.99, Cronbach's alpha: 0.54-0.96) (Tombaugh & McHugh, 1992), the cognitive tool has been criticised for being susceptible to various demographic and cognitive variables. Such variables include age, education level/attainment, and cultural background, potentially resulting in misclassification of actual cognitive status (Tombaugh & McHugh, 1992). Unlike sex, which has not been shown to influence MMSE performance, age and education have both been consistently reported as powerful moderators affecting MMSE scores (Crum *et al.,* 1993). In a large population-based study conducted by Crum *et al.* (1993), MMSE performance was strongly associated with both age and education level, with higher education levels correlating with higher median test scores and stronger cognitive performance in younger test populations. To address this limitation, authors have suggested adjusting cut-off scores relative to the age and education level of the population examined. This ensures that an unbiased outcome is achieved (Crum *et al.,* 1993; Galasko *et al.,* 1996).

The widely-recommended 23-point cut-off score suggestive of potential cognitive impairment has also attracted considerable debate, as some investigators argue this recommended cut-off threshold may not yield optimal classification accuracy (van Gorp *et al.,* 1999; O'Bryant *et al.,* 2008). Measurable improvements in both test accuracy and sensitivity were observed by Van Gorp *et al.* (1999) when the cut-off score was increased to ≤ 26/30. In agreement with this, O'Bryant *et al.* (2008) also reported improved diagnostic accuracy in identifying dementia in highly-educated individuals when the cut-off score was increased to ≤ 27/30. Therefore, on account of the improved diagnostic accuracy and sensitivity reported, Van Gorp *et al.* (1999) and O'Bryant *et al.* (2008) advise dismissing the recommended 23-point cut-off threshold to maximise diagnostic accuracy, especially when assessing highly-educated populations.

2.9.2 Cognistat (Kiernan *et al.*, 1987)

The Cognistat (formerly known as the Neurobehavioural Cognitive Status Examination (NCSE) is another reliable, validated and widely-deployed brief cognitive examination that quantitatively screens for cognitive dysfunction in several key cognitive domains (Kiernan *et al.,* 1987; Whiteside *et al.,* 1996; Drane *et al.,* 2003; Macaulay *et al.,* 2003). It is available in several languages and is routinely administered conjointly with or instead of the MMSE, taking approximately 15-20 minutes to administer (Kiernan *et al.,* 1987; Logue *et al.,* 1993; Engelhart, Eisenstein, & Meininger, 1994; Eisenstein *et al.,* 2002).

The Cognistat consists of 10 subtests that examine a diverse range of cognitive domains: orientation and attention, construction ability, memory, language, calculation, and reasoning, the latter subdivided into two further modalities, similarities and judgment (Schwamm *et al.,* 1997; Engelhart, Eisenstein, & Meininger, 1994; Oehlert *et al.,* 1997; Eisenstein *et al.,* 2002) (Figure 2.12). However, unlike the MMSE and other cognitive screening tools that only yield a summed global score, the Cognistat measures domain-specific cognitive performance (*i.e.* performance in individual cognitive domains), yielding a graphical representation of cognitive performance (Figure 2.13). This provides researchers with a quick snapshot and differentiated profile of overall patient cognitive status and enables prompt recognition of potential early cognitive dysfunction (Logue *et al.,* 1993; Macaulay *et al.,* 2003).

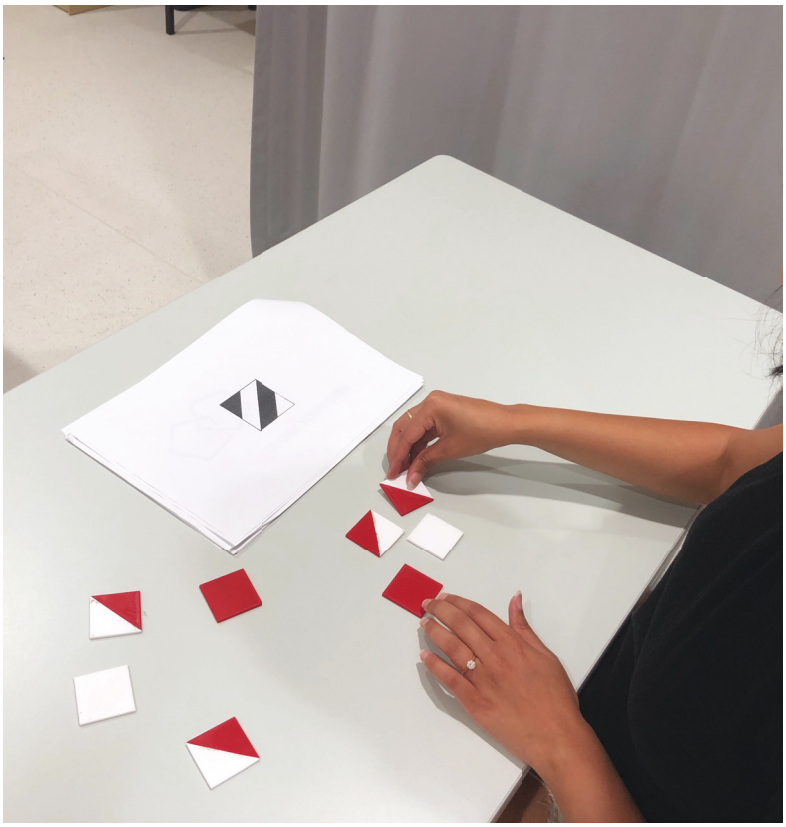


Figure 2.12. Participant completing the Construction domain subtest of the Cognistat. This subtest requires participants to create the images shown in the stimulus manual using the coloured square tiles provided. Permission to reproduce the image has been obtained from the participant.

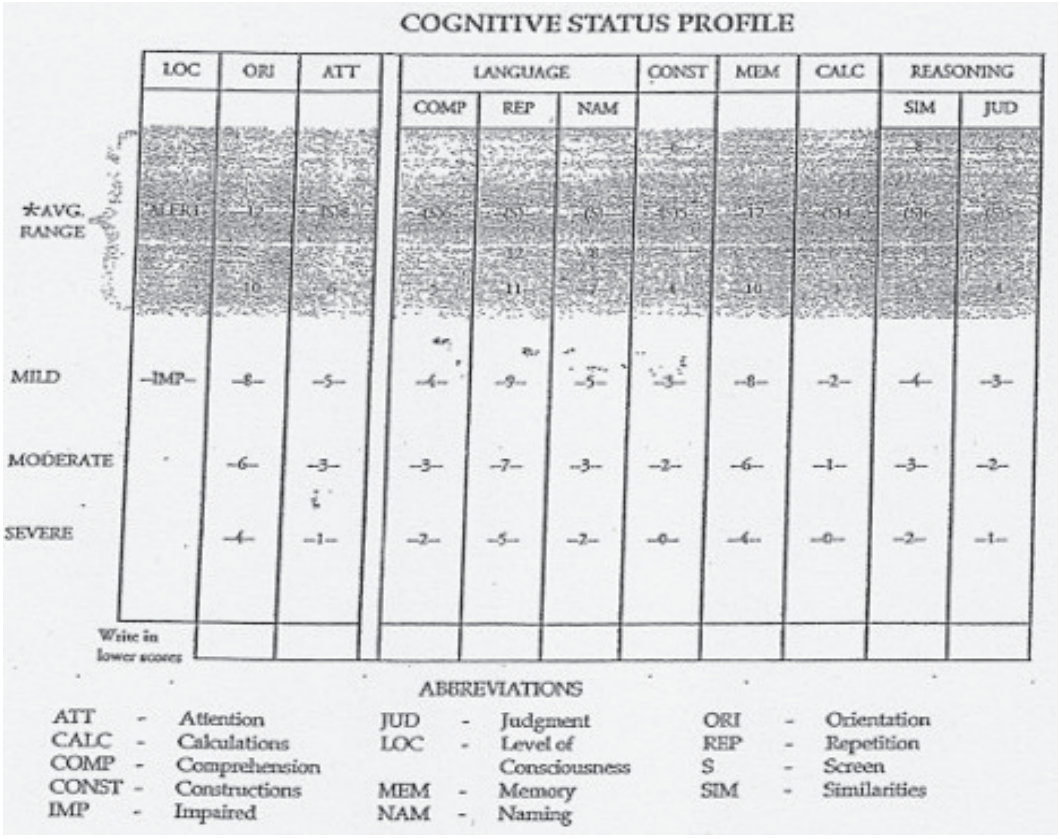


Figure 2.13. The Cognistat cognitive status profile. Scores beneath the average range for each cognitive domain are graded (mild, moderate, severe) and indicate the degree of cognitive impairment. Adapted from the Cognistat manual (2007).

One characteristic that differentiates the Cognistat from other cognitive assessments is its unique "screen-metric" construct. This identifies whether impairment is evident in a particular cognitive domain, or if further cognitive evaluation is required (Oehlert *et al.,* 1997; Gupta & Kumar, 2009; Rice *et al.,* 2015). First, the participant is asked a 'screen' question, representative of the cognitive domain being assessed. This question is typically more difficult than questions presented subsequently in the 'metric' section. If the participant answers the 'screen' question correctly, cognitive function for that particular domain is considered intact, the maximum subtest score is awarded, and the examiner advances to the next cognitive domain. Conversely, if the participant fails the 'screen' question the remaining 'metric' questions, which progressively increase in difficulty and determine whether function is intact, are then administered (Oehlert *et al.,* 1997; Nøkleby *et al.,* 2008; Rice *et al.,* 2015).

Although the Cognistat demonstrates reasonable psychometric properties (high internal consistency (Cronbach's alpha: 0.94)) (Kiernan *et al.,* 1987), limitations have been identified with the popular clinical measure. While the Cognistat possesses reasonably high sensitivity (sensitivity: 93%), Drane and Ossato (1997) found the assessment lacked sound specificity. This effect was pronounced in memory and construction domains, where it was found to yield an unacceptably high number of false positive results in healthy elderly patients. Consequently, Drane and Ossato (1997) recommend extending the average range score in each cognitive domain to address this limitation. Similar to the MMSE, the Cognistat has also been shown to be susceptible to the effects of cognitive variables (age and education level) (Drane *et al.,* 2003). Drane *et al.* (2003) found that age and education level strongly influenced Cognistat test performance. While age was shown to primarily affect performance in memory and attention domains, both age and education level influenced construction ability performance. Drane *et al.* (2003) recommend considering age and education level when evaluating cognitive performance to reduce the influence of these demographic variables on Cognistat performance. Drane *et al.* (2003) also suggest disregarding the recommended normative cut-off point scores for classifying impairment for non-cognitively healthy populations, arguing this may potentially result in misclassification of a patient's cognitive status.

The appropriateness, feasibility, and accuracy of the "screen-metric" approach of the Cognistat in identifying cognitive deficits has also been scrutinised in recent decades. Although the 'screen' question is typically of greater difficulty than subsequent 'metric' items, Oehlert *et al.* (1997) argue the recommended "screen-metric" format has the potential to overlook possible cognitive deficits, particularly when screen questions are only administered. In agreement with this view, Rice *et al.* (2015) found the "screen-metric" approach failed to adequately detect cognitive deficits in a large sample of stroke rehabilitation patients when screen questions were only administered.

It has also been reported that approximately 20% of normal cognitively-intact adults fail screen items when the routine screen-metric format is deployed (Kiernan *et al.,* 1987). While administering all screen and metric items extends the average administration time to roughly 30 minutes, Oehlert *et al.* (1997), Van Gorp *et al.* (1997) and Rice *et al.* (2015) argue administrating the Cognistat in its entirety (screen and metric items) minimises the likelihood of overlooking potential cognitive deficits, improves test reliability, and enhances the overall predictability of the battery. Therefore, to reduce the possibility of missing potential cognitive dysfunction, particularly the subtle cognitive dysfunction associated with diabetes and hypertension, all questions of the Cognistat (screen and metric) were administered in the present investigation.

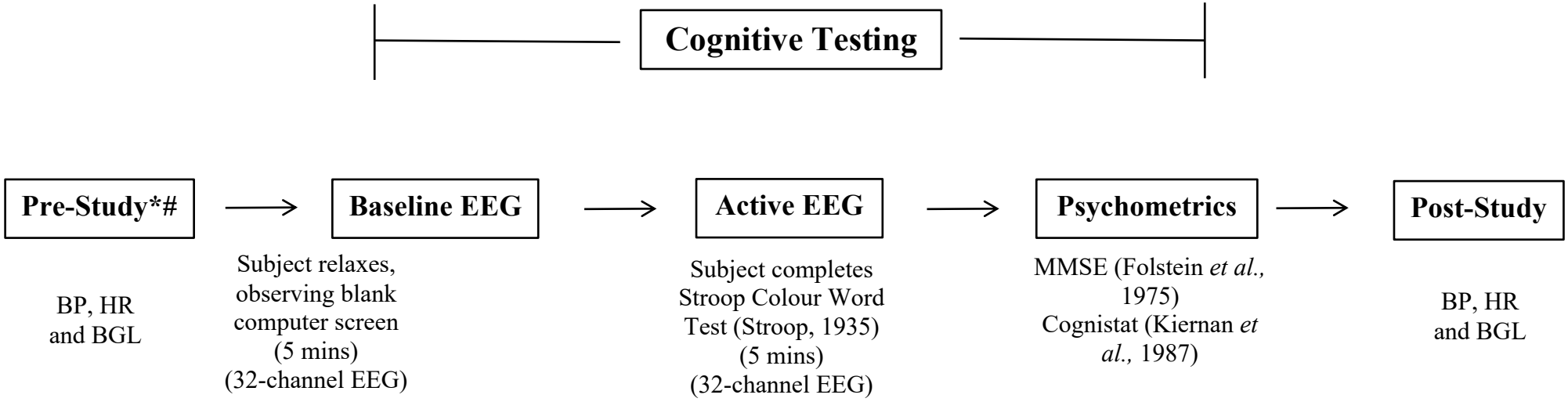
Although some researchers question the diagnostic utility of the Cognistat as a screening tool for cognitive impairment, most researchers laud the neuropsychological battery for its conciseness, broad range of cognitive domains assessed, brief administration time, low cost, and its multidimensional construct (Engelhart *et al.,* 1994; Whiteside *et al.,* 1996; Eisenstein *et al.,* 2002; Brown, Mapleston, & Nairn, 2011). As mentioned earlier, dependent on the score obtained in each subtest, scores in the Cognistat are plotted on a cognitive status profile. Performance is then subsequently graded on a scale: average, mild, moderate or severe impairment (Figure 2.12) (Kiernan *et al.,* 1987). This allows for rapid identification of cognitively-intact areas and alerts clinicians to evidence of any potential cognitive deficits present (Kiernan *et al.,* 1987). Unlike the MMSE, each cognitive domain in the Cognistat has a domain-specific impairment threshold score. Scores in proximity to or beneath these thresholds indicate a degree/gradation (mild, moderate, or severe) of cognitive impairment. Maximum obtainable scores, as well as domain-specific cut-off scores indicative of impairment for each respective cognitive domain, are presented below (Table 2.2). As previous research suggests Cognistat test accuracy and sensitivity improves when administered in concert with other cognitive screening tools (Schwamm *et al.,* 1987; Macaulay *et al.,* 2003), both the MMSE and Cognistat were administered conjointly in the present study.

Table 2.2. Maximum achievable scores and domain-specific impairment threshold scores for each cognitive domain of the Cognistat. Adapted and modified from (Kiernan *et al.,* 1987).

			Grade of Cognitive Impairment			
Cognitive Domain		**Maximum Score**	**Impairment Threshold**	**Mild**	**Moderate**	**Severe**
Orientation		12	< 10	8	6	4
Attention		8	< 6	5	3	1
Language	*Comprehension*	6	< 5	4	3	2
	Repetition	12	< 11	9	7	5
	Naming	8	< 7	5	3	2
Construction		6	< 4	3	2	0
Memory		12	< 10	8	6	4
Calculation		4	< 3	2	1	0
Reasoning	*Similarities*	8	< 5	4	3	2
	Judgement	6	< 4	3	2	1
Total		82	< 65	51	36	21

After completion of the cognitive assessment, three post-study BP measurements as well as one post-study BGL measurement were recorded (as outlined in the experimental protocol detailed in section 2.4) and this concluded the experimental testing (Figure 2.14). Participants were then acknowledged for their participation in the study with a cognitive profile (Appendix 8.5) and remuneration (clinical cohorts) (Appendix 8.8) and were supplied with a copy of the signed consent form signed at the beginning of the study (Appendix 8.1), with a copy also retained by the investigator. Finally, in accordance with the UTS HREC requirements, the researcher completed a study summary sheet, reporting on how the study was conducted and any unusual events that occurred throughout the investigation (Appendix 8.6).

Figure 2.14. Present study experimental protocol



Key:

***** – Lifestyle Appraisal Questionnaire
# – In-house Chronic Disease Questionnaire
BP – Blood Pressure
BGL – Blood Glucose Level
EEG – Electroencephalography
HR – Heart Rate
MMSE – Mini Mental State Examination

2.10 Data Processing and Analysis

Data analysed in the present cross-sectional investigation included:

- Demographic data (age, BMI, years of education, LAQ Part 1, LAQ Part 2) (Craig, Hancock, & Craig, 1996)
- Clinical data (solicited by questionnaires developed in-house as part of this research)
- Cardiovascular variables (pre-study and post-study SBP and DBP)
- Blood glucose concentrations (determined pre- and post-study)
- Electroencephalography data (baseline, active, (Stroop Test)) (Stroop, 1935)
- Cognitive function, assessed by the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) and the Cognistat (Kiernan *et al.,* 1987)

2.10.1 Electroencephalography Data Pre-Processing

Several artefacts can contaminate the electroencephalography signal (*e.g.* direct current, movement, non-electrically shielded room, *etc.*), resulting in unreliable data (Kaiboriboon *et al.,* 2012). Therefore, all raw EEG data obtained (during baseline and active recordings) was subjected to pre-processing (noise-reduction) prior to statistical analysis (refer to Section 2.11) according to the steps below. Investigators suggest this improves the signal-to-noise ratio (SNR) (Kaiboriboon *et al.,* 2012).

1. Direct current (DC) interference or sources of high-frequency movement artefacts (*e.g.* muscular, fast-paced movements) were eliminated using a Butterworth IIR bandpass filter set at 1.5 and 50 Hz.
2. Ocular artefacts, such as blinking, were attenuated by applying an electro-oculography algorithm (aligned-artefact average procedure).
3. 5-minute baseline and active EEG recordings were subsequently divided into 300 one-second epochs.
4. The individual epoch values were then examined for outliers, which were removed using the modified Z-score statistic (epoch values $\geq$10 excluded) (Maharaj, Lees, & Lal, 2019). Previous studies in our research unit have used this method (Maharaj, Lees, & Lal, 2019). The modified Z-score statistic was calculated using the following equation:

$$z = \frac{X - \tilde{x}}{MAD}$$

X = epoch value $\tilde{x}$ *= median* *MAD = Median Absolute Deviation*

The equation (below) can be used to derive the median absolute deviation:

$$MAD = \tilde{x}_i \left(|X_i - \tilde{x}_j(X_j)| \right)$$

X = epoch value $\tilde{x}$ *= median*

5. A fast-Fourier transform application (FFT) then enabled the derivation of average EEG activity for each individual frequency band (delta: 1 – 4 Hz; theta: 4 – 8 Hz; alpha: 8 –12 Hz; beta: 12 – 30 Hz; and gamma: 35 – 100 Hz) (Modi & Sahin, 2017).

All EEG values were recorded in microvolts per second squared ($\mu V/s^2$).

2.11 Statistical Analysis

2.11.1 Power Analysis

Statistical power refers to the probability of rejecting correctly the null hypothesis when it is false (*i.e.* not making a false negative) (Button *et al.,* 2013). High statistical power reduces Type II errors and increases the likelihood of identifying true effects and/or relationships (Biau *et al.,* 2008; Button *et al.,* 2013). Cohen (1992) determined that the minimum sample size required for the analyses in the present research, with adequate power (0.8) and moderate to large effect size (0.5 – 0.8), is ~30. In groups where n ≤30 (T1DM, T2DM, and HTN), appropriate non-parametric analyses were performed. Previous studies conducted in our research unit (Rothberg *et al.,* 2016; Maharaj, Lees, & Lal, 2019; Chalmers *et al.,* 2020) and the literature (Pramming *et al.,* 1988; Tallroth *et al.,* 1990; Bjorgaas *et al.,* 1998; Howorka *et al.,* 2000) have utilised smaller or similar sample sizes to that reported in the present thesis.

2.11.2 Dependent Sample T-test

T-tests detect significant differences in means of paired (dependent) and unpaired (independent) samples (Lund Research Ltd, 2019). Dependent sample t-tests (paired) were conducted in the non-clinical group to determine significant differences between the following variables: pre-study and post-study blood pressure (SBP and DBP); and pre-study and post-study blood glucose level (BGL).

2.11.3 Wilcoxon Signed Rank Test

The Wilcoxon Signed Rank Test is a robust non-parametric equivalent of a dependent sample t-test (Lund Research Ltd, 2019). It determines significant differences in the medians of paired non-normally distributed data (skewed or ranked). This test was performed to identify significant differences between pre-study and post-study physiological variables (SBP, DBP, and BGL) in the clinical samples (T1DM, T2DM, and HTN), with n ≤30.

2.11.4 Mann Whitney U Test

The Mann Whitney U Test is a non-parametric equivalent of an independent sample t-test that identifies significant differences in the ranks of non-normally distributed data in unpaired samples (Lund Research Ltd, 2019). This test was conducted to identify significant differences in pre-study and post-study physiological variables (SBP, DBP, and BGL) between the clinical samples (T1DM, T2DM, and HTN).

2.11.5 Partial Pearson's Correlation

A partial Pearson's correlation examines the strength and direction of an association between two continuous dependent variables in a sample while controlling for confounding variables (Lund Research Ltd, 2019). A partial Pearson's correlation controlling for age and BMI was applied to determine associations of BP and BGL with cognitive function (EEG and cognitive measures, MMSE and the Cognistat) in the non-clinical population. The literature indicates these lifestyle risk factors influence cognitive outcomes (Kivipelto *et al.,* 2018).

The Pearson's correlation generates an *r* (rho) value (the Pearson's correlation coefficient). This value ranges between $r = 1$ and $r = -1$ and indicates the strength and direction of the association. Positive *r* values indicate positive relationships (*i.e.* as one variable increases, the other variable also increases), while negative *r* values indicate negative associations (*i.e.* as one variable decreases, the other increases). No consensus exists for specific cut-off values for different strengths of association, but the following values are generally accepted: negligible (± 0.0 – 0.3), low (± 0.3 – 0.5), moderate (± 0.5 – 0.7), strong (± 0.7 – 0.9) (Hinkle *et al.,* 2003).

2.11.6 Spearman's Rank-Order Correlation

Spearman's rank-order correlation is a non-parametric equivalent of Pearson's correlation (Lund Research Ltd, 2019). Similarly, it determines the strength and direction of an association/relationship between two continuous variables in a sample. However, unlike Pearson's correlation, which uses individual data values, Spearman's rank-order correlation uses the ranks of data. This statistical test was applied to investigate associations in study samples with $n \leq 30$ participants (the clinical cohorts –T1DM, T2DM, and HTN).

2.11.7 Multiple Analysis of Covariance (MANCOVA)

The multiple analysis of covariance (MANCOVA) is considered an extension of the ANOVA and compares differences in means of multiple independent samples while controlling for confounding variables (Lund Research Ltd, 2019). This test was applied to determine differences in global and domain-specific cognitive variables between the non-clinical and clinical cohorts while controlling for covariates (*e.g.* age and BMI). As the MANCOVA is an omnibus test, *post hoc* Tukey tests were performed subsequently to ascertain where significant differences, if any, occurred between the groups.

3. Results: Demographic characteristics (Non-clinical and Clinical)

This chapter reports descriptive statistics of demographic data (age, BMI, LAQ Part 1, LAQ Part 2, and years of education) for both the non-clinical and clinical populations (T1DM, T2DM, and HTN). Physiological variables obtained before and after cognitive assessment (SBP, DBP, HR, and BGL) for all sample groups are also reported. All data are presented as mean ± SD. Figure 3.1 displays a breakdown of the sample populations (non-clinical and clinical) reported in the current study.

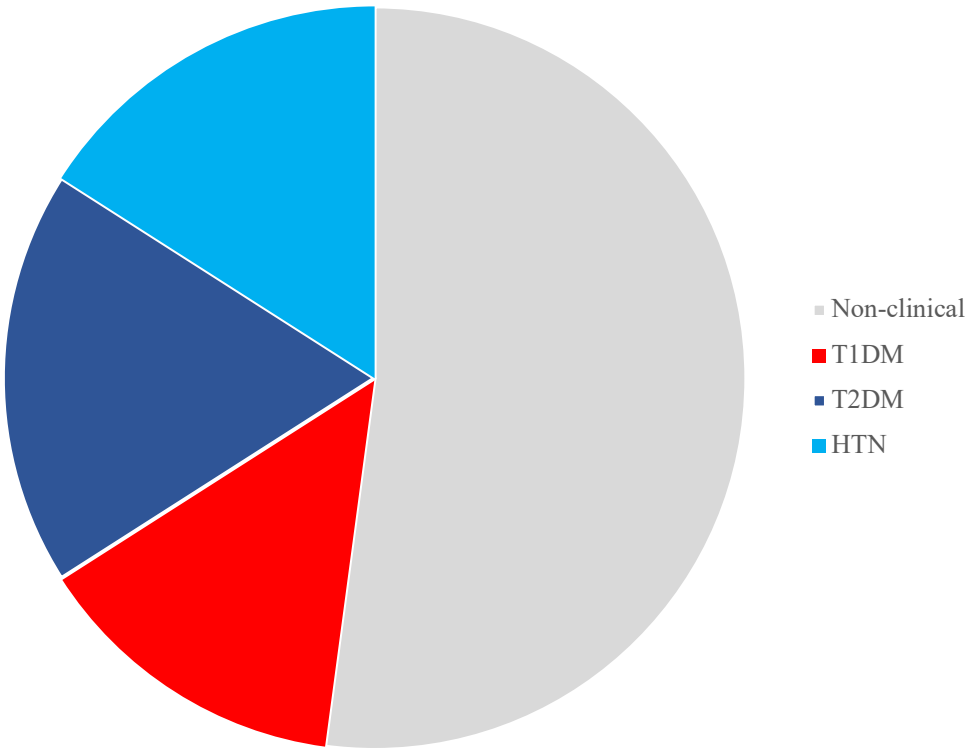


Figure 3.1. Diagrammatic breakdown of sample groups (non-clinical and clinical (T1DM, T2DM, and HTN)) comprising the total study cohort (n = 94)

3.1 Participant Summary

A total of ninety-four (n = 94) study participants, aged between 18-80 years, were recruited from the local Sydney community for involvement in the present cross-sectional investigation. The study cohort was comprised of the following four groups: (1) non-clinical (n = 49, mean age: 31.3 ± 15.9 years), (2) Type 1 diabetes mellitus (T1DM) (n = 13, mean age: 35.1 ± 16.1 years), (3) Type 2 diabetes mellitus (T2DM) (n = 17, mean age: 54.7 ± 12.1 years), and (4) hypertension (HTN) (n = 15, mean age: 61.0 ± 16.9 years). All participants from the clinical populations (T1DM, T2DM, HTN) reported medication use for their respective conditions (glucose-lowering or anti-hypertensive medication) (see Section 8.9). Age and BMI differed significantly between the groups and were therefore used as covariates in the final analyses.

3.2 Demographic Variables

Demographic characteristics (age, BMI, LAQ Part 1, LAQ Part 2, and years of education) for all participants were obtained using a reliable and validated questionnaire (the Lifestyle Appraisal Questionnaire (LAQ) (Craig, Hancock, & Craig, 1996). Age, BMI, and LAQ Part 1 were determined to be significantly different between the groups (Table 3.1). The age of the populations ranged between 30-61 years, with the non-clinical group being the youngest (31.3 ± 15.8 years) and the hypertension group (61.0 ± 16.9 years) as the older cohort. The age of the T1DM (35.1 ± 16.1 years) and T2DM (54.7 ± 11.8 years) cohorts fell between the non-clinical and HTN sample groups. Analysis revealed age differed significantly between the following groups: non-clinical and T2DM ($p<0.001$); non-clinical and hypertension ($p <0.001$); T1DM and T2DM ($p = 0.01$) and T1DM and hypertension ($p <0.001$). The BMI of participants from the non-clinical (24.6 ± 5.1 kg/m^2) and T1DM (23.8 ± 1.60 kg/m^2) groups resided within the healthy range (BMI: 18.5 – 24.9) (Australian Government Department of Health, 2020). In contrast, it fell within the unhealthy range (BMI: > 25) (Australian Government Department of Health, 2020) for both the T2DM (31.7 ± 1.4 kg/m^2) and HTN groups (28.1 ± 1.50 kg/m^2), with the T2DM group demonstrating the highest BMI. A significant difference in BMI was also found between the groups ($p <0.001$). *Post hoc* analysis showed this difference occurred between the following groups: non-clinical and T2DM ($p <0.001$); non-clinical and hypertension ($p = 0.04$) and T1DM and T2DM ($p <0.001$).

In relation to participant lifestyle risk factors (measured using the Lifestyle Appraisal Questionnaire (LAQ Part 1)), scores ranged between 11-20 points, with the non-clinical group scoring the lowest (11.7 ± 6.6) and the HTN population scoring the highest (19.7 ± 4.4). A significant difference was observed in LAQ Part 1 score between the groups ($p<0.001$). *Post hoc* analysis revealed this difference was between the non-clinical and T2DM ($p<0.001$) and non-clinical and HTN ($p <0.001$) groups. While LAQ Part 2 scores and years of education varied slightly between the groups, the differences were not statistically significant.

Table 3.1. Key demographic characteristics (age, BMI, LAQ Part 1, LAQ Part 2, and years of education) for all groups. P values are parametric multivariate.

Demographic Variable	Sample Group	Mean ± SD	p
Age (Yrs)	Non-clinical	31.3 ± 15.8	<0.001*
	T1DM	35.1 ± 16.1	
	T2DM	54.7 ± 11.8	
	HTN	61.0 ± 16.9	
BMI (kg/m^2)	Non-clinical	24.6 ± 5.1	<0.001*
	T1DM	23.8 ± 8.5	
	T2DM	31.7 ± 5.6	
	HTN	28.1 ± 5.2	
LAQ Part 1	Non-clinical	11.7 ± 6.6	<0.001*
	T1DM	15.3 ± 6.9	
	T2DM	19.4 ± 6.3	
	HTN	19.7 ± 4.4	
LAQ Part 2	Non-clinical	15.9 ± 11.7	0.22
	T1DM	22.4 ± 10.9	
	T2DM	19.9 ± 9.3	
	HTN	15.5 ± 12.5	
Years of Education (Yrs)	Non-clinical	18.0 ± 5.6	0.27
	T1DM	21.0 ± 13.1	
	T2DM	21.7 ± 13.7	
	HTN	16.6 ± 4.9	

Key:

Yrs – Years

LAQ – Lifestyle Appraisal Questionnaire

T2DM – Type 2 diabetes mellitus

* – statistical significance

kg/m^2 – kilograms per metre squared

T1DM – Type 1 diabetes mellitus

HTN – Hypertension

p – p-value

3.2.1 Cardiovascular Variables (SBP, DBP, and HR)

Blood pressure (BP) and heart rate (HR) were recorded both before and after cognitive testing (see section 2.5 under methods for BP protocol). The change (Δ) that occurred in each variable (BP and HR) was calculated by subtracting the pre-study value from the post-study value. Generally, blood pressure (SBP and DBP) increased after the study in all groups, whereas heart rate (HR) decreased. Blood pressure (SBP and DBP) was found to be highest in the HTN population and lowest in the non-clinical group (Table 3.2).

With respect to systolic blood pressure (SBP), a significant difference was found between groups for both pre-study ($p < 0.001$) and post-study SBP ($p < 0.001$). For pre-study SBP, *post hoc* analysis revealed differences between the following groups: non-clinical and T1DM ($p = 0.02$), non-clinical and HTN ($p < 0.001$), and T2DM and HTN ($p = 0.02$). For post-study SBP, significant differences were found between the following groups: non-clinical and T1DM ($p < 0.05$), non-clinical and T2DM ($p = 0.01$), non-clinical and HTN ($p < 0.001$); T1DM and HTN ($p = 0.02$); and T2DM and HTN ($p < 0.001$). Interestingly, no significant difference was found within each of the groups between pre-study and post-study SBP for all groups.

Similarly, significant differences between groups were observed for pre-study ($p < 0.05$) and post-study DBP ($p < 0.001$). For pre-study DBP, *post hoc* analysis indicated significant difference between the following groups: non-clinical and T1DM ($p = 0.02$), non-clinical and T2DM ($p = 0.03$), and non-clinical and HTN ($p < 0.05$). For post-study DBP, it was found between the non-clinical and T1DM group ($p < 0.05$) and non-clinical and HTN ($p < 0.001$) groups. Similar to SBP, no significant differences were observed between pre-study and post-study DBP within the four groups.

Although pre-study HR varied slightly between the groups, significance was not reached; however, an overall significant difference was found between the groups in post-study HR ($p < 0.05$). *Post hoc* analysis revealed this significance was between the following groups: non-clinical and T1DM ($p < 0.05$), non-clinical and T2DM ($p = 0.01$), T1DM and HTN ($p = 0.03$), and T2DM and HTN ($p = 0.04$). Significant within-group differences between pre-study and post-study HR were found in the following groups: non-clinical ($p < 0.001$), T2DM ($p = 0.01$), and HTN ($p = 0.03$).

Table 3.2. Pre-study and post-study cardiovascular variables (SBP, DBP and HR), as well as the change that occurred, for each group. P-values are parametric multivariate.

Cardiovascular Variable	Sample Group	Pre-study	p	Post-study	p	Δ (post-study – pre-study)	p
SBP (mm Hg)	Non-clinical	112.5 ± 12.1	<0.001*	111.9 ± 13.4	<0.001*	- 0.55 ± 7.3	0.60
	T1DM	125.9 ± 18.5		126.5 ± 17.2		0.62 ± 12.3	0.97
	T2DM	120.2 ± 12.9		123.1 ± 11.5		2.7 ± 6.4	0.10
	HTN	135.7 ± 21.0		140.1 ±19.8		4.4 ± 8.1	0.07
DBP (mm Hg)	Non-clinical	73.7 ± 9.7	<0.05*	75.0 ± 9.4	<0.001*	1.3 ± 5.4	0.09
	T1DM	81.2 ± 10.0		82.9 ± 10.1		1.7 ± 6.2	0.35
	T2DM	79.9 ± 6.3		80.3 ± 7.1		0.30 ± 4.5	0.73
	HTN	82.9 ± 12.2		85.5 ± 10.0		2.5 ± 5.3	0.11
HR (bpm)	Non-clinical	70.0 ± 9.0	0.16	64.9 ± 7.4	<0.05*	- 5.1 ± 6.9	<0.001*
	T1DM	74.2 ± 9.0		72.9 ± 8.7		- 1.3 ± 4.0	0.20
	T2DM	75.2 ± 10.6		71.8 ± 9.5		- 3.4 ± 4.5	0.01*
	HTN	70.5 ± 10.5		65.9 ± 8.7		- 4.6 ± 7.4	0.03*

Key:

SBP – systolic blood pressure **DBP –** diastolic blood pressure **HR –** heart rate **mm Hg –** millimetres of mercury

bpm – beats per minute **Δ –** change **T1DM –** Type 1 diabetes mellitus

T2DM – Type 2 diabetes mellitus * - statistical significance **HTN –** hypertension **p** – p-value

3.2.2 Blood Glucose Level (BGL)

Two-hour (2-hr) fasting blood glucose concentrations were determined for each participant before cognitive testing (refer to section 2.6 under methodology for BGL measurement protocol). The change (Δ) that occurred in BGL was calculated by subtracting the pre-study value from the post-study value. All BGL values were reported in millimoles per litre (mmol/L) (Table 3.3). Excluding the HTN population where BGL increased marginally, BGL generally decreased after the study in all groups. It was within the normal range (3.5 – 8 mmol/L) (ADA, 2020) for both the non-clinical and HTN groups but was slightly elevated in the T1DM and T2DM groups, confirming diabetes status.

Significant differences were found between pre-study ($p < 0.001$) and post-study ($p < 0.001$) BGL in all the groups. For pre-study BGL, *post hoc* analysis revealed the differences occurred between the following groups: non-clinical and T1DM ($p < 0.001$), non-clinical and T2DM ($p < 0.001$), T1DM and HTN ($p < 0.001$), and T2DM and HTN ($p < 0.001$). Conversely, for post-study BGL, the differences were found between the non-clinical and T1DM ($p < 0.05$), non-clinical and T2DM ($p < 0.001$), and T2DM and HTN ($p < 0.001$). Analysis also revealed significant within-group differences between pre-study and post-study BGL in non-clinical ($p = 0.01$) and T2DM groups ($p = 0.01$).

Table 3.3. Pre-study and post-study BGL, as well as the change in BGL that occurred, in all groups.

Physiological Variable	Sample Group	Pre-Study	p	Post-Study	p	Δ (post-study – pre-study)	p
BGL (mmol/L)	Non-clinical	5.2 ± 0.72	<0.001*	4.9 ± 0.58	<0.001*	- 0.26 ± 0.65	0.01*
	T1DM	8.0 ± 2.9		6.9 ± 2.6		- 1.07 ± 4.1	0.40
	T2DM	9.0 ± 4.1		7.7 ± 3.2		- 1.36 ± 1.43	0.01*
	HTN	5.2 ± 0.66		5.3 ± 0.58		0.1 ± 0.64	0.66

Key:

BGL – blood glucose level
mmol/L – millimoles per litre
Δ – change
T1DM – Type 1 diabetes mellitus
T2DM – Type 2 diabetes mellitus
HTN – Hypertension
* – statistical significance
p – p-value

3.2.3 Disease-specific variables (disease duration, glycosylated haemoglobin)

Disease-specific variables, such as disease duration (chronicity) and age of disease onset, were reported for the clinical populations (T1DM, T2DM, and HTN) in the present study *via* a questionnaire developed in-house (see Appendix 9.3 and 9.4). Glycosylated haemoglobin ($HbA1_C$) was also provided. These variables were obtained as they have been shown to moderate the relationship between these chronic diseases and cognition (Biessels *et al.,* 2014; Feinkohl *et al.,* 2015; Biessels & Despa, 2018). Although frequency and severity of hypoglycaemia (mild or severe) (for T1DM and T2DM) and duration of disease (for HTN) were solicited, it could not be recalled reliably by study participants and was therefore not reported. Table 3.4 summarises key disease-specific variables sought from participants in the clinical samples.

Table 3.4. Disease-specific variables solicited from the clinical groups.

Variable	Sample Group	Value	p
$HbA1_C$	T1DM	6.9 %	0.27
	T2DM	8.1 %	
Disease Duration (Yrs)	T1DM	17.8 ± 9.2	0.02*
	T2DM	11.3 ± 5.9	
Disease Onset (Yrs)	T1DM	16.8 ± 14.9	<0.001*
	T2DM	44.9 ± 14.7	

Key:

$HbA1_C$ – glycosylated haemoglobin
Yrs – years
T1DM – Type 1 diabetes mellitus
T2DM – Type 2 diabetes mellitus
p – p-value
***** – statistical significance

The glycaemic control of patients with DM was slightly elevated ($HbA1_{C:}$ 7 – 8 %) (ADA, 2020), with $HbA1_C$ ranging between 7-8% (T1DM: 6.9%; T2DM: 8.0%) (Table 3.4). A Wilcoxon signed rank test revealed no significant difference in glycosylated haemoglobin between the T1DM and T2DM groups. On average, patients with T1DM generally had diabetes for longer (mean duration: 17.8 ± 9.2 years) than those with T2DM (mean duration: 11.3 ± 5.9 years) or HTN (7.5 ± 2.9 years). Most patients with T1DM developed their condition after 7 years of age or earlier (disease onset: 16.8 ± 14.9 years) than those with T2DM (disease onset: 44.9 ± 14.4 years), with analysis revealing a significant difference in disease onset between T1DM and T2DM ($p < 0.001$).

4. Associations between blood pressure, blood glucose level and cognitive performance (Non-clinical and Clinical)

This chapter reports the cognitive performance data (global and domain-specific) for all four samples (non-clinical, T1DM, T2DM, and HTN), assessed using the reliable and validated cognitive screening tools, the Mini-Mental State Examination (Folstein, Folstein, & McHugh, 1975) and the Cognistat (Kiernan *et al.,* 1987). It also reports the performance of all groups for the Stroop Colour Word Test (Stroop, 1935), which was used to simulate cognitive activity (measured using EEG) (see section 2.8 under methodology for details). Associations between BP (SBP and DBP), BGL, and disease-specific variables (disease duration and HbA1c level for the clinical groups) and cognitive function are also reported for each group. All data are presented as mean ± SD.

4.1 Cognitive Performance

4.1.1 Global Cognitive Performance (Mini-Mental State Examination) (Folstein, Folstein, & McHugh, 1975)

With respect to global cognitive performance, as assessed using the Mini-Mental State Examination (MMSE), all groups performed above the impairment threshold for the assessment (scores ≤ 23 indicative of cognitive dysfunction) (Table 4.1). Interestingly, the T1DM group performed the strongest (28.5 ± 1.1), whereas the T2DM group performed the worst (27.5 ± 2.9). The global cognitive performance scores of the non-clinical and HTN groups resided between that of the T1DM and T2DM groups, scoring 28.3 ± 1.6 and 28.0 ± 1.5, respectively. Although global cognitive performance varied slightly between the groups, it was not statistically significant.

Table 4.1. Mean scores obtained in the Mini-Mental State Examination by each of the study sample groups.

Variable	Sample Group	Score	p
MMSE	Non-clinical	28.3 ± 1.6	0.89
	T1DM	28.5 ± 1.1	
	T2DM	27.5 ± 2.9	
	HTN	28.0 ± 1.5	

Key:

MMSE – Mini-Mental State Examination **T1DM** – Type 1 diabetes mellitus

T2DM – Type 2 diabetes mellitus **HTN** – Hypertension

p – p-value

4.1.2 Domain-specific Cognitive Performance (Cognistat) (Kiernan *et al.*, 1987)

In relation to domain-specific cognitive performance, as assessed using the Cognistat, all groups performed above the impairment threshold (> 65) for the Cognistat, as well as for each domain-specific impairment threshold (refer to section 2.9.2), confirming intact cognitive function within the study cohort. Performance in most cognitive domains was similar between the groups, with the non-clinical and T1DM groups demonstrating consistently strong cognitive performance. Interestingly, performance differed considerably in the memory domain, with the T1DM group performing the best (11.3 ± 0.95) and the HTN group performing the worst (9.2 ± 2.9). However, no significant difference in domain-specific cognitive performance was observed between the groups (Table 4.2).

Table 4.2. Mean scores obtained by each sample group (non-clinical, T1DM, T2DM, and HTN) for individual domains of the Cognistat, as well as the total Cognistat score.

Variable	Sample Group	Score	p
Orientation	Non-clinical	11.5 ± 0.71	0.58
	T1DM	11.6 ± 0.77	
	T2DM	11.3 ± 0.69	
	HTN	11.5 ± 1.5	
Attention	Non-clinical	7.5 ± 0.77	0.92
	T1DM	7.5 ± 0.66	
	T2DM	6.9 ± 1.1	
	HTN	7.3 ± 1.0	
Comprehension	Non-clinical	5.8 ± 0.39	0.40
	T1DM	5.8 ± 0.38	
	T2DM	5.8 ± 0.56	
	HTN	5.5 ± 0.64	
Repetition	Non-clinical	11.8 ± 0.49	0.07
	T1DM	11.9 ± 0.28	
	T2DM	11.8 ± 0.56	
	HTN	11.1 ± 1.36	
Naming	Non-clinical	7.7 ± 0.75	0.73
	T1DM	7.9 ± 0.28	
	T2DM	7.6 ± 0.87	
	HTN	7.5 ± 1.13	
Construction	Non-clinical	5.6 ± 0.74	0.42
	T1DM	5.7 ± 0.63	
	T2DM	5.4 ± 0.94	
	HTN	5.1 ± 0.88	
Memory	Non-clinical	10.7 ± 1.9	0.49
	T1DM	11.3 ± 0.95	
	T2DM	9.4 ± 3.5	

	HTN	9.2 ± 2.9	
Calculation	Non-clinical	3.7 ± 0.49	0.84
	T1DM	3.6 ± 0.65	
	T2DM	3.6 ± 0.86	
	HTN	3.8 ± 0.41	
Similarities	Non-clinical	7.2 ± 1.2	0.79
	T1DM	6.9 ± 1.3	
	T2DM	6.6 ± 2.1	
	HTN	7.1 ± 1.1	
Judgement	Non-clinical	3.9 ± 0.92	0.98
	T1DM	3.9 ± 0.76	
	T2DM	4.1 ± 0.78	
	HTN	4.1 ± 1.2	
Total Cognistat	Non-clinical	75.4 ± 3.49	0.76
	T1DM	76.3 ± 2.90	
	T2DM	72.4 ± 6.7	
	HTN	72.7 ± 4.64	

Key:

T1DM – Type 1 diabetes mellitus

T2DM – Type 2 diabetes mellitus

HTN – Hypertension

p – p-value

4.1.3 Stroop Colour Word Test (Stroop, 1935)

A significant difference was found in average response time (measured in milliseconds) between the sample groups ($p = 0.01$) for matched stimuli in the Stroop Test (*e.g.* the word BLUE printed in blue ink) (refer to Table 4.3). The non-clinical group displayed the fastest average response time (1213.67 ± 240.9 ms), whereas the T2DM group demonstrated the slowest response time (1779.9 ± 631.0 ms). The average response times for the T1DM (1246.00 ± 171.27 ms) and HTN (1523.33 ± 364.96 ms) groups fell between those of the non-clinical and the T2DM groups. *Post hoc* analysis also indicated significant differences in response time for matched stimuli in the Stroop test between the following groups: non-clinical and T2DM ($p<0.001$), T1DM and T2DM ($p = 0.01$), and T2DM and HTN ($p = 0.04$).

A similar pattern in average response time was observed for mismatched stimuli in the Stroop Test (*e.g.* the word BLUE printed in yellow ink). The non-clinical group showed the fastest average response time (1440.25 ± 281.17 ms), whereas the T2DM group demonstrated the slowest response time (2202.62 ± 765.84 ms). Similarly, response times for the T1DM (1445.66 ± 227.94 ms) and HTN (1849.16 ± 387.09 ms) groups fell between those of the non-clinical and T2DM groups. A significant difference in average response time for mismatched stimuli in the Stroop test was found between the groups ($p < 0.001$). *Post hoc* analysis revealed significant differences between the following groups: non-clinical and T2DM ($p <0.001$), T1DM and T2DM ($p <0.001$), and T2DM and HTN ($p = 0.01$).

Interestingly, no significant associations were found between the pre-study and post-study physiological variables (BP and BGL) and the matched and mismatched aspects of the Stroop Colour Word Test for the non-clinical and clinical groups. While no significant associations were identified between disease-specific variables ($HbA1_C$, age of disease onset, disease duration) and the matched and mismatched aspects of the Stroop Colour Word Test for the T1DM and HTN groups, a significant association was found between age of disease onset and average response time for matched stimuli ($p = 0.01$) for the T2DM group (Table 4.4) (Figure 4.1).

Table 4.3. Mean average response times obtained for each group (non-clinical, T1DM, T2DM, and HTN) for matched and mismatched stimuli of the Stroop Colour Word Test (Stroop, 1935).

Variable	Sample Group	Average Response Time (ms)	p
Matched Stimuli	Non-clinical	1213.67 ± 240.9	0.01*
	T1DM	1246.00 ± 171.27	
	T2DM	1779.9 ± 631.0	
	HTN	1523.33 ± 364.96	
Mismatched Stimuli	Non-clinical	1440.25 ± 281.17	<0.001*
	T1DM	1445.66 ± 227.94	
	T2DM	2202.62 ± 765.84	
	HTN	1849.16 ± 387.09	

Key:

T1DM – Type 1 Diabetes Mellitus

T2DM – Type 2 Diabetes Mellitus

HTN – Hypertension

ms – milliseconds

***** – statistical significance

p – p-value

Table 4.4. Associations between disease-specific variables ($HbA1_C$, age of disease onset, and disease duration) and matched aspects of the Stroop Colour Word Test.

Dependent Variable	Independent Variable	Group	p	r
Matched	$HbA1_C$	T1DM	0.76	- 0.10
		T2DM	0.14	- 0.53
	Disease Duration	T1DM	0.76	- 0.09
		T2DM	0.52	- 0.17
	Disease Onset	T1DM	0.09	0.49
		T2DM	0.01*	0.65

Key:

T1DM – Type 1 Diabetes Mellitus

T2DM – Type 2 Diabetes Mellitus

$HbA1_C$ – glycosylated haemoglobin

***** – statistical significance

p – p-value

r – rho value

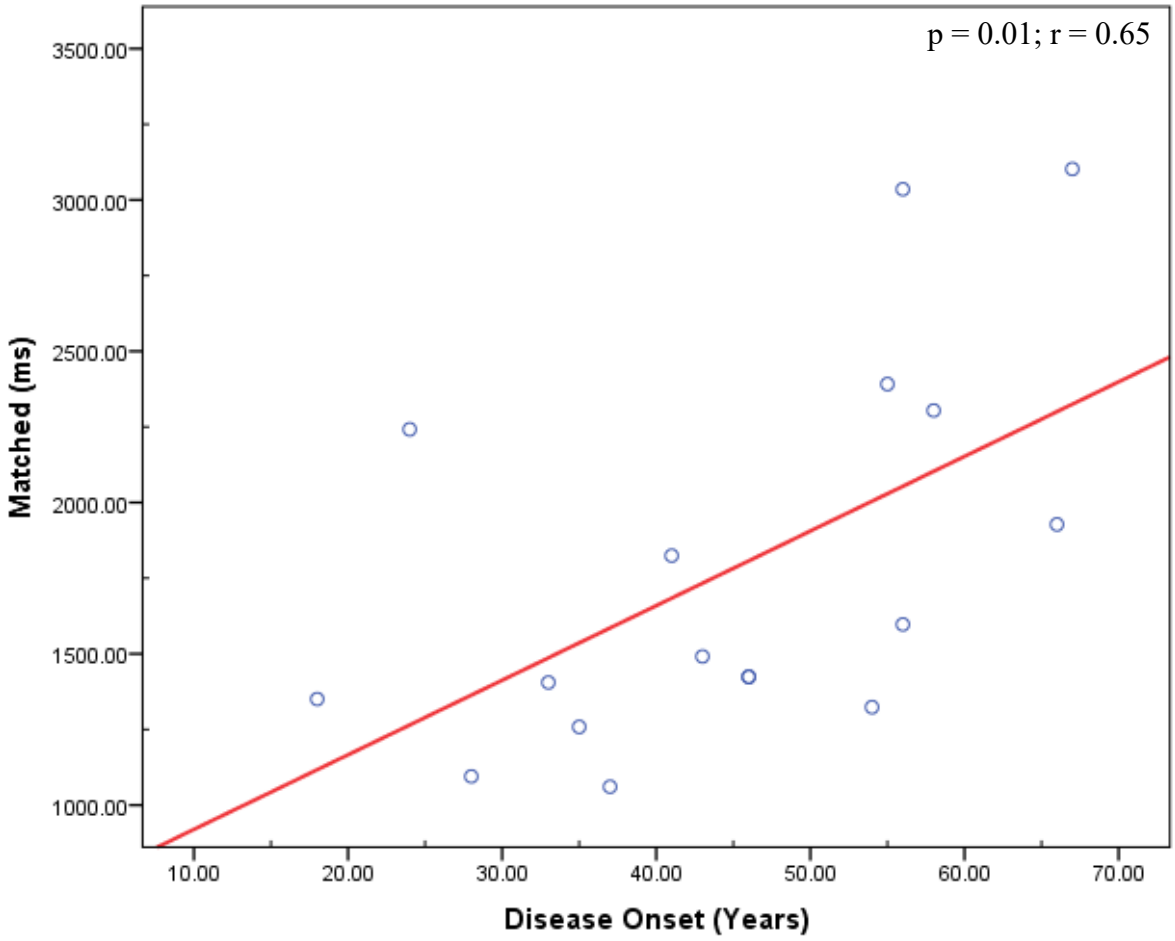


Figure 4.1. Positive correlation between age of disease onset and average response time for matched stimuli in the Stroop Colour Word Test for the Type 2 diabetes mellitus group.

Key:

ms – milliseconds **p** – p-value **r** – rho value

4.2 Associations between BP and cognition for the non-clinical sample group

A partial Pearson's correlation was conducted to identify associations between pre-study and post-study BP (SBP and DBP) and cognition (MMSE and Cognistat) in the non-clinical group. No significant associations were found between SBP and DBP (pre-study or post-study) and total MMSE score. There were also no significant associations between pre-study and post-study BP variables (SBP and DBP) with individual cognitive domain scores or the total Cognistat score.

4.3 Associations between BGL and cognition for the non-clinical sample group

No significant associations were found between pre-study BGL and total MMSE score in the non-clinical group; however, post-study BGL was inversely associated with total MMSE score ($p = 0.03$; $r = - 0.32$) (Figure 4.2). No significant associations were observed between BGL (pre-study and post-study) and individual cognitive domain scores or the total Cognistat score.

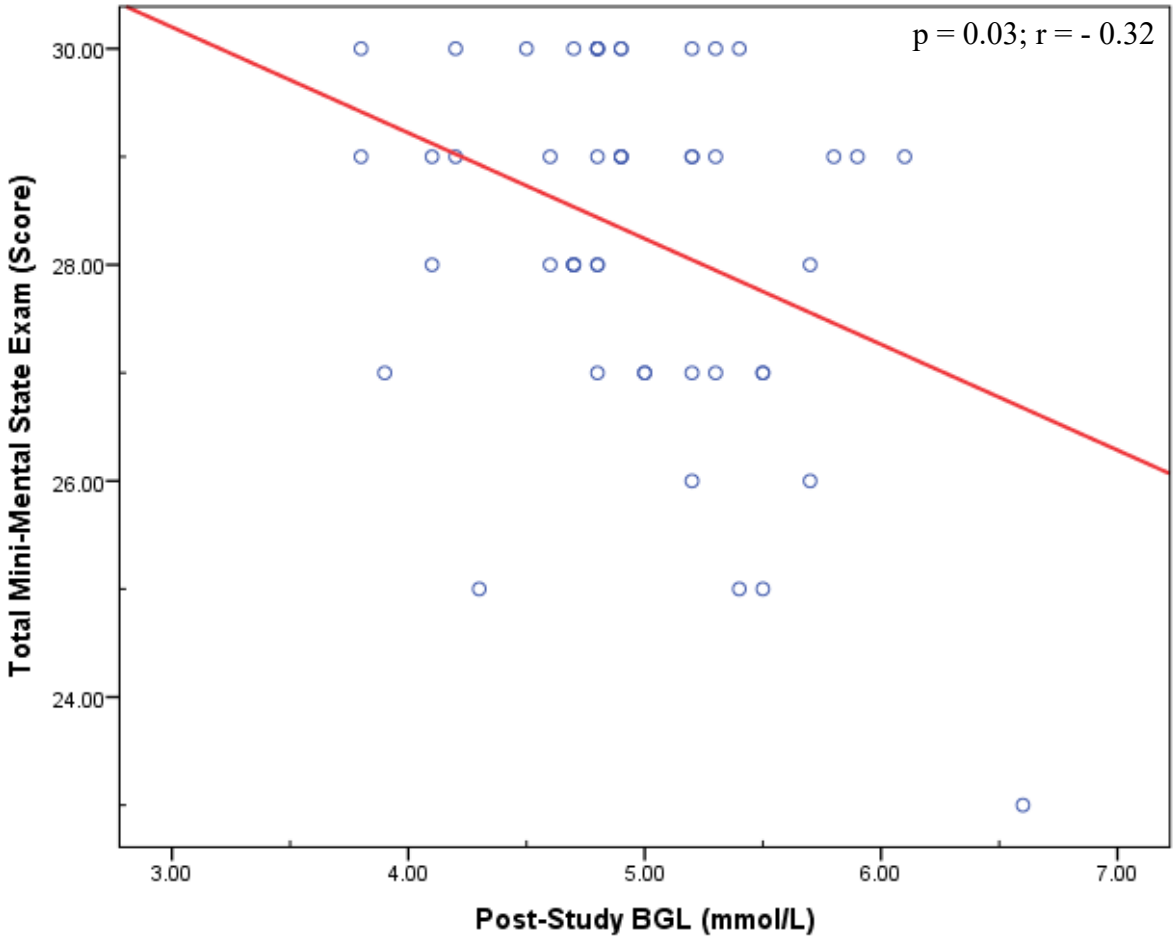


Figure 4.2. Inverse correlation between post-study blood glucose level (BGL) and total Mini-Mental State Examination score for the non-clinical group.

Key:

BGL – blood glucose level

mmol/L – millimoles per litre

p – p-value

r – rho value

4.4 Associations between BP, BGL and cognition for the T1DM sample group

A Spearman's Rank-Order correlation was performed to identify associations between pre-study and post-study physiological variables (SBP, DBP, and BGL) and cognitive function (MMSE and the Cognistat) in the T1DM group. No significant associations were observed between SBP (pre-study and post-study) and cognitive performance; however, pre-study DBP was significantly associated with performance in the similarities domain of the Cognistat ($p = 0.02$; $r = 0.62$), while post-study DBP was significantly associated with judgement performance in the Cognistat ($p = 0.04$; $r = 0.56$) (Figure 4.3). There were no significant associations between BGL (pre-study and post-study) and individual cognitive domain scores and the total Cognistat score.

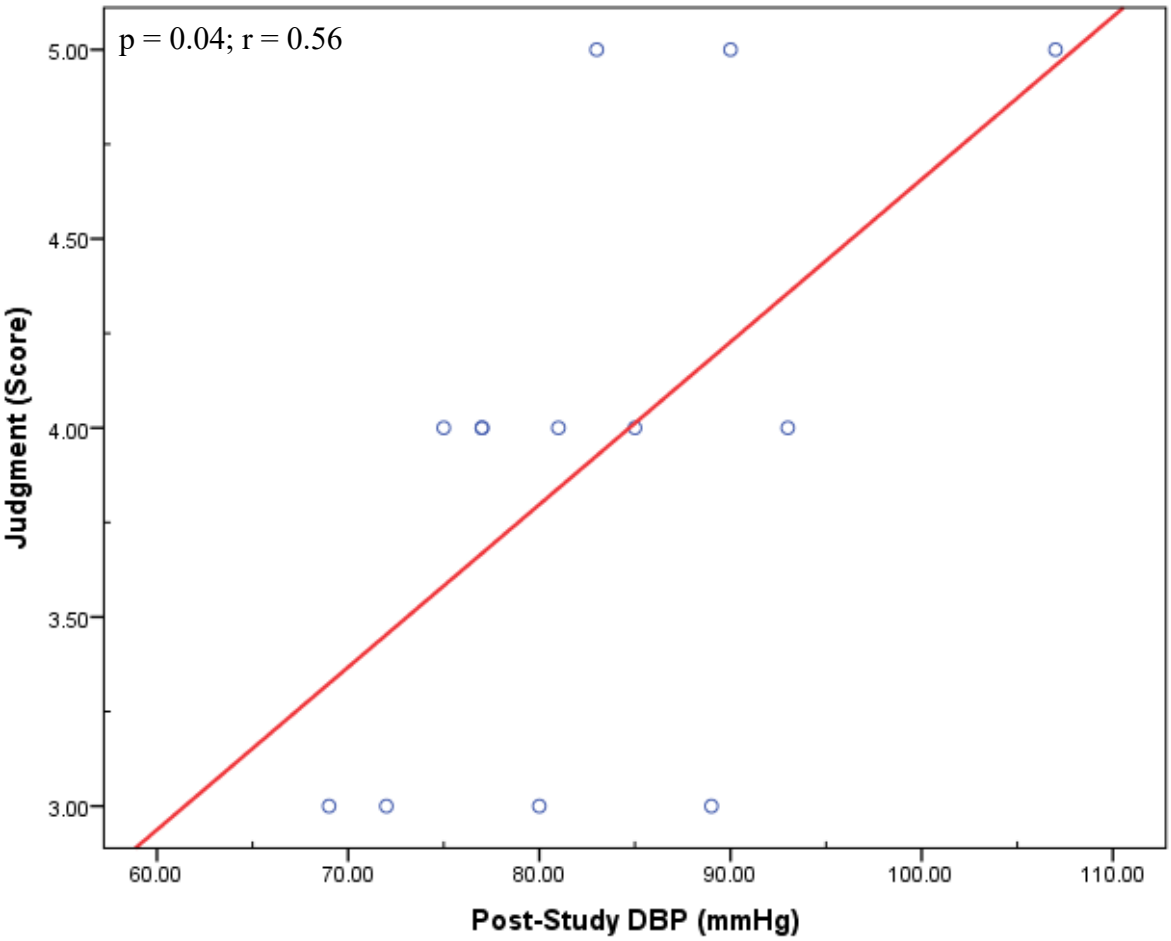


Figure 4.3. Positive correlation between post-study diastolic blood pressure and judgement performance in the Cognistat for the Type 1 diabetes mellitus sample group.

Key:

DBP – diastolic blood pressure

mm Hg – millimetres of mercury

p – p-value

r – rho value

With respect to disease-specific variables in the T1DM group, $HbA1_C$ level was significantly associated with the comprehension domain of the Cognistat ($p = 0.04$; $r = 0.58$), but no other domains. The age of disease onset was also found to be significantly associated with the similarities domain of the Cognistat ($p = 0.02$; $r = 0.65$). However, no association was found between disease duration and global cognitive performance (MMSE) or domain-specific cognitive performance.

4.5 Associations between BP, BGL and cognition for the T2DM sample group

A Spearman's Rank-Order correlation was similarly applied to identify associations between pre-study and post-study BP (SBP and DBP) and BGL and cognitive performance (MMSE and the Cognistat) in the T2DM group. No significant associations were found between pre-study SBP and cognitive measures, but post-study SBP was significantly associated with construction performance in the Cognistat ($p = 0.04$; $r = 0.52$) (Figure 4.4). Similarly, no significant associations were observed between pre-study DBP and cognitive function (global and domain-specific); however, post-study DBP was significantly associated with the attention ($p = 0.04$; $r = 0.52$) and judgement ($p = 0.01$; $r = 0.65$) (Figure 4.5) domains of the Cognistat, as well as the total Cognistat score ($p = 0.02$; $r = 0.61$) (Figure 4.6).

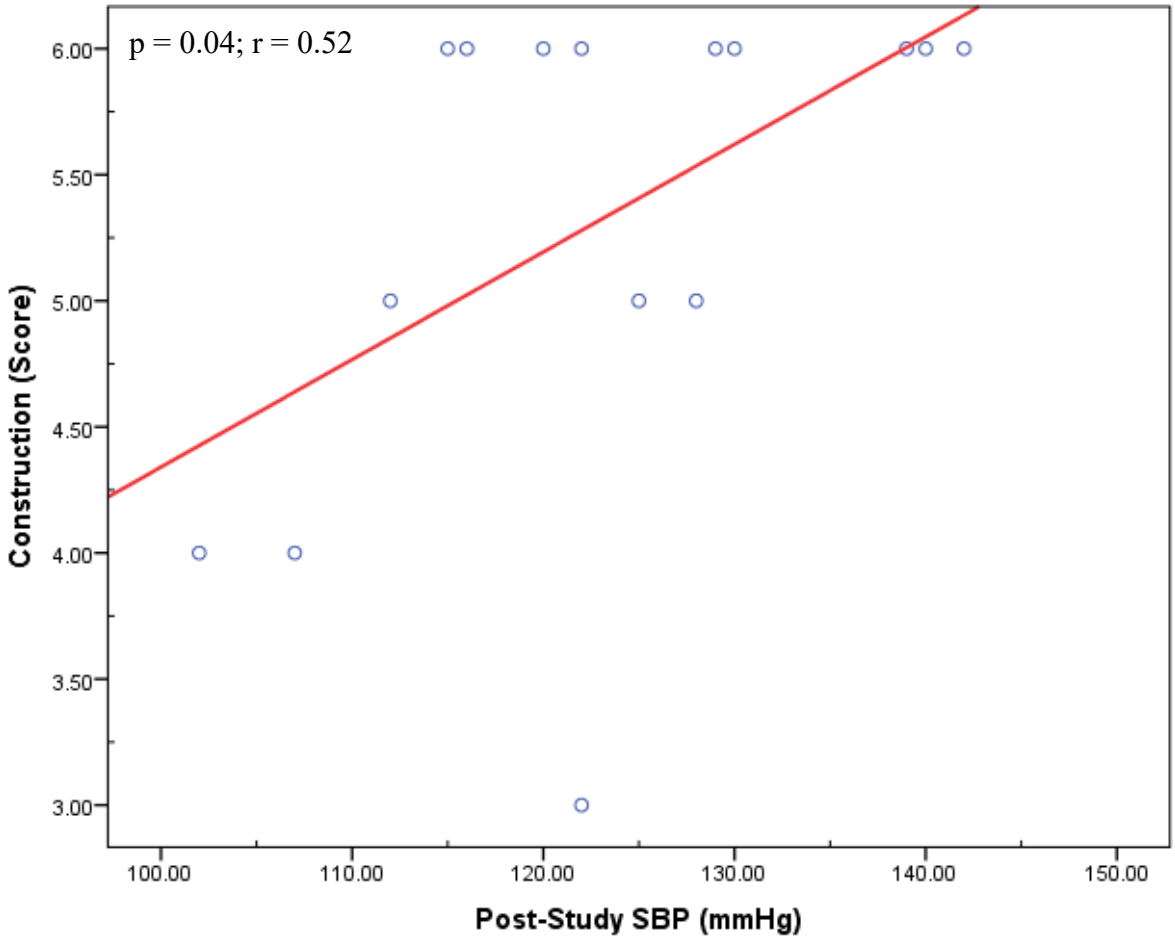


Figure 4.4. Positive correlation between post-study systolic blood pressure and construction performance in the Cognistat for the Type 2 diabetes mellitus sample group.

Key:

SBP – systolic blood pressure

mm Hg – millimetres of mercury

p – p-value

r – rho value

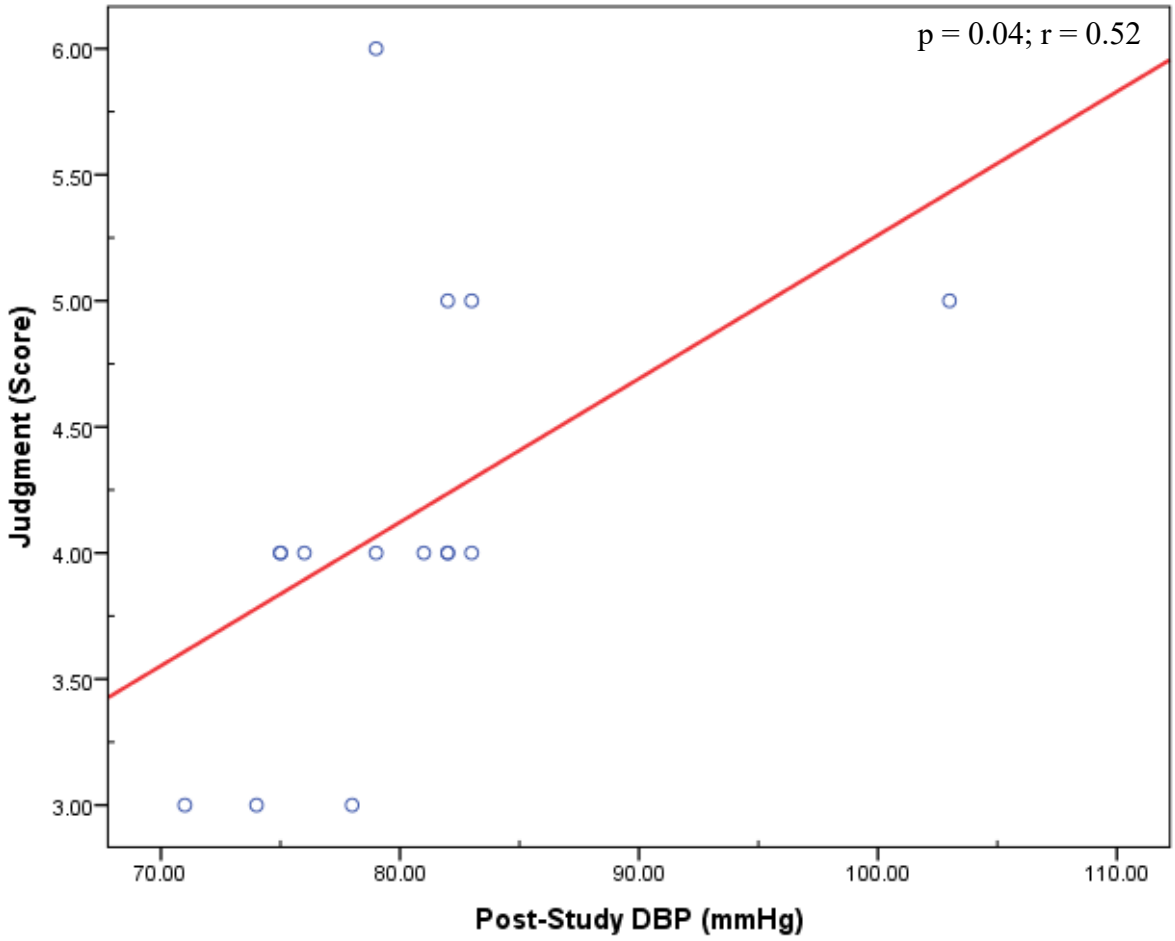


Figure 4.5. Positive correlation between post-study diastolic blood pressure and judgement performance in the Cognistat for the Type 2 diabetes mellitus sample group.

Key:

DBP – diastolic blood pressure

mm Hg – millimetres of mercury

p – p-value

r – rho value

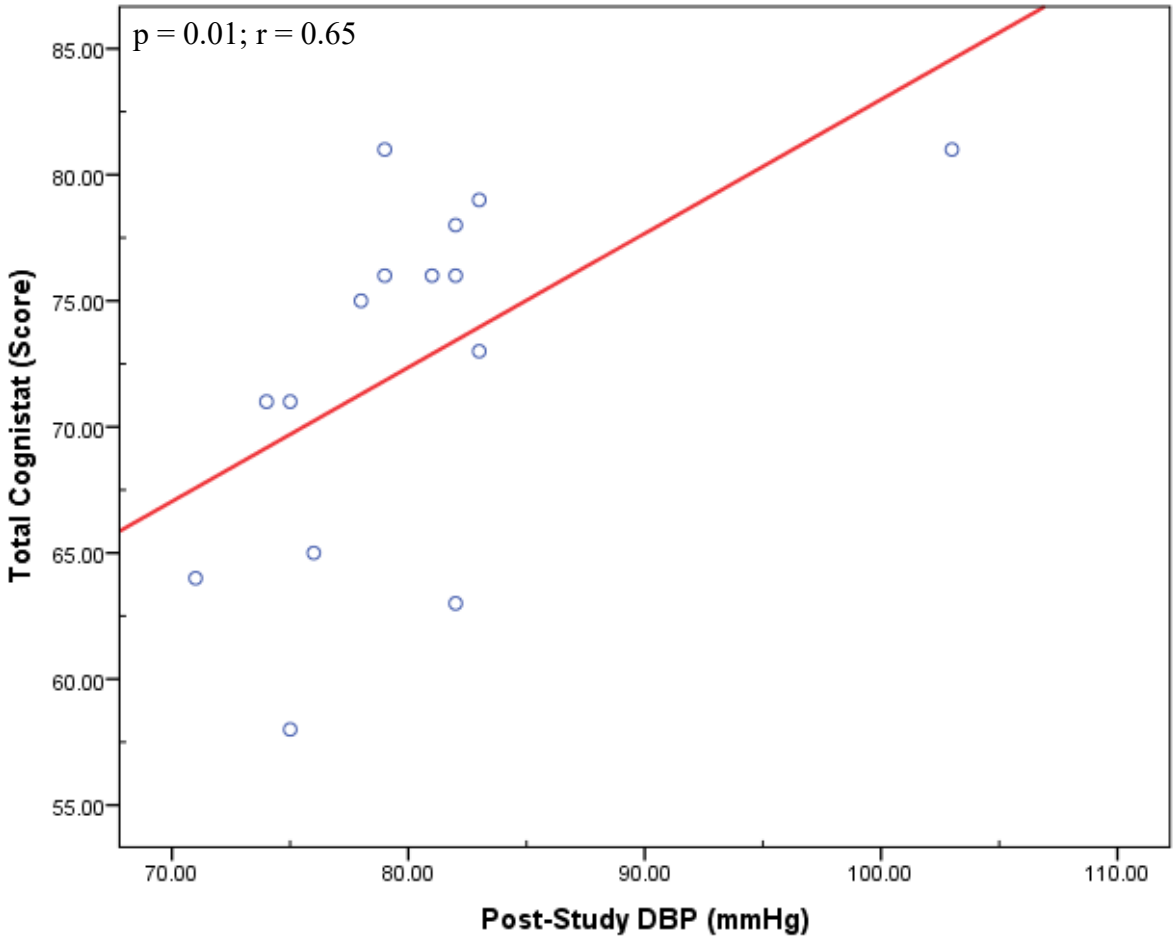


Figure 4.6. Positive correlation between post-study diastolic blood pressure and total Cognistat score for the Type 2 diabetes mellitus sample group.

Key:

DBP – diastolic blood pressure

mm Hg – millimetres of mercury

p – p-value

r – rho value

Regarding BGL, pre-study BGL was significantly associated with the attention ($p = 0.04$; $r = -0.52$) (Figure 4.7) and memory domains ($p = 0.04$; $r = 0.52$) of the Cognistat. In contrast, post-study BGL was significantly associated with memory performance in the Cognistat ($p < 0.05$; $r = 0.70$). No other significant associations were found between post-study BGL and global and domain-specific cognitive performance. There were also no significant associations observed between disease-specific variables ($HbA1_C$, disease duration, and age of disease onset) and global and domain-specific cognitive performance for the T2DM group.

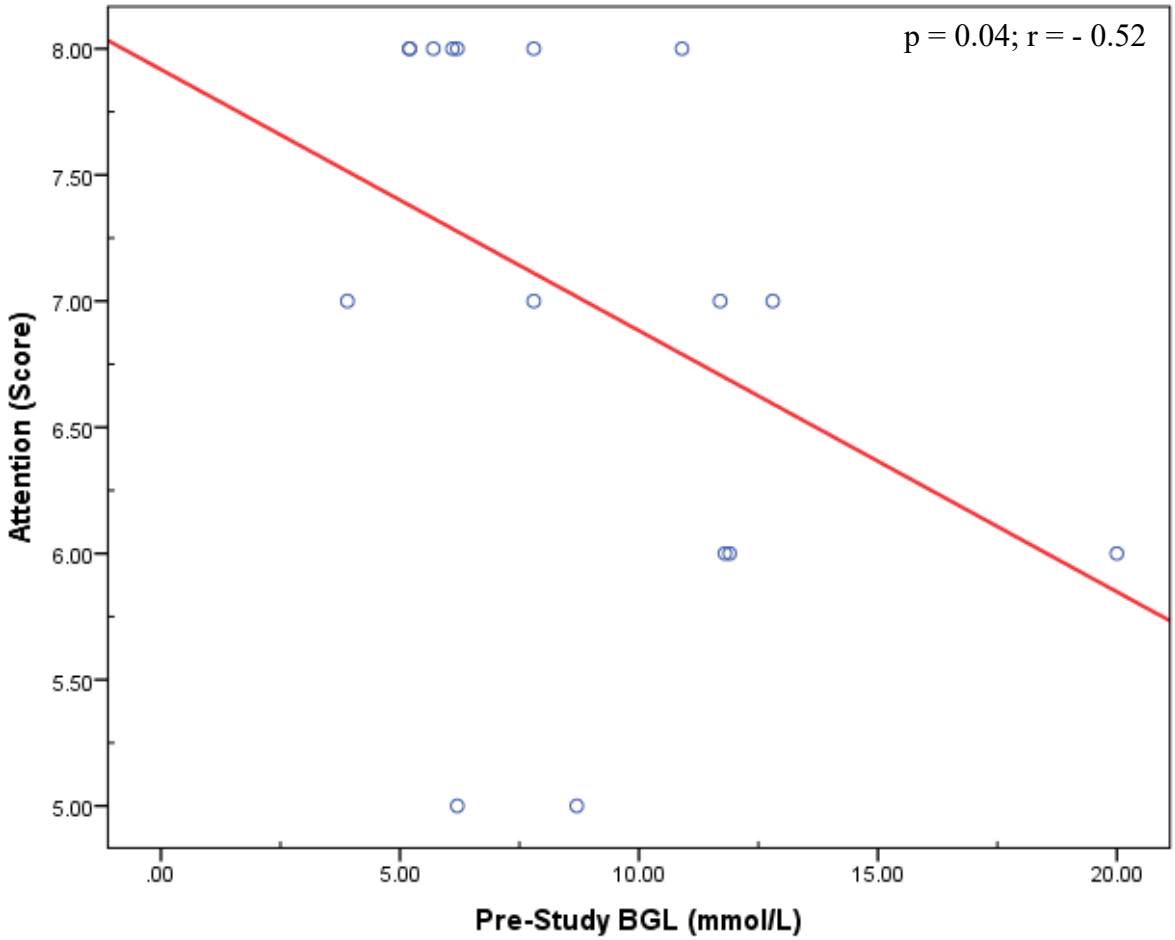


Figure 4.7. Inverse correlation between pre-study blood glucose level and the attention domain of the Cognistat for the Type 2 diabetes mellitus sample group.

Key:

BGL – blood glucose level

mmol/L – millimoles per litre

p – p-value

r – rho value

4.6 Associations between BP, BGL and cognition for the HTN sample group

A Spearman's Rank Order correlation was also conducted to identify associations between pre-study and post-study physiological variables (SBP, DBP, and BGL) and global and domain-specific cognitive performance in the HTN group. No significant associations were found between pre-study and post-study SBP and total MMSE score and total score of the Cognsitat. Similarly, no significant associations were found between pre-study and post-study DBP and the cognitive measures.

With respect to BGL, pre-study BGL was inversely associated with performance in the construction (p = 0.01; r = - 0.67) and similarities (p= 0.03; r = - 0.56) (Figure 4.8) domains of the Cognistat. Conversely, post-study BGL was significantly associated with memory performance (p= 0.01; r = 0.69) in the Cognistat.

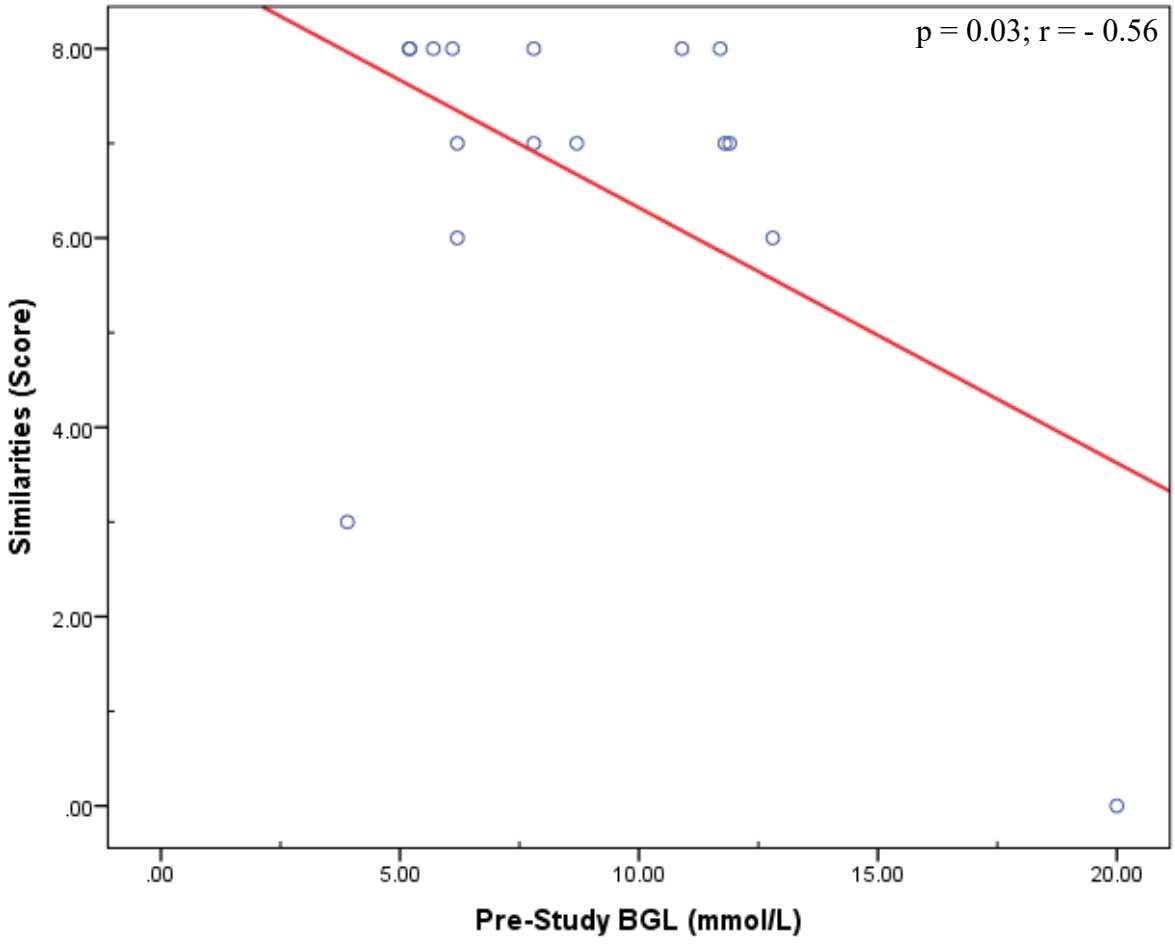


Figure 4.8. Inverse correlation between pre-study blood glucose level and the similarities domain of the Cognistat for the hypertension sample group.

Key:

BGL – blood glucose level

mmol/L – millimoles per litre

p – p-value

r – rho value

4.7 Discussion: Cognitive Performance of Non-Clinical and Clinical Populations

This chapter discusses findings concerning the cognitive performance (global and domain-specific) of both the non-clinical and clinical sample groups, as assessed using the reliable and validated cognitive screening tools, the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) and the Cognistat (Kiernan *et al.,* 1987). Associations identified between pre-study and post-study physiological variables (SBP, DBP, and BGL) and global and domain-specific cognitive performance, as well as the different Stroop Colour Word Test variables (matched and mismatched stimuli), are also discussed.

4.7.1 Cognitive Performance: Clinical Groups (T1DM, T2DM, HTN)

This study was the first to assess and compare global and domain-specific cognitive performance between non-clinical and clinical groups (T1DM, T2DM, and HTN) using the Mini-Mental State Examination (MMSE) and the Cognistat. The main findings from this investigation are: (1) global and domain-specific cognitive performance does not differ between the non-clinical and clinical cohorts; (2) higher blood glucose level (BGL) in the non-clinical group is inversely associated with global cognitive performance; and (3) blood pressure (SBP and DBP) is significantly correlated with global cognitive performance and individual domains of cognition in clinical groups (T1DM, T2DM, HTN).

Substantial evidence shows that patients with diabetes mellitus (T1DM or T2DM) perform slightly worse in cognitive assessments compared to age-matched healthy controls and demonstrate modest decrements in multiple cognitive domains (Kalmijn *et al.,* 1995; Brands *et al.,* 2005, Kilander *et al.,* 1998; Yaffe *et al.,* 2014). In their seminal meta-analysis, Brands *et al.* (2005) investigated the effect of T1DM on cognitive function and the magnitude of deterioration in cognitive domains and found that patients with T1DM exhibit subtle cognitive dysfunction in psychomotor speed and information processing (Cohen's *d* effect size: 0.3 – 0.7). Interestingly, the investigators reported that memory and learning domains were preserved, suggesting T1DM does not affect brain areas involved in long-term memory storage and retrieval, such as the hippocampus. This finding is substantiated by neuroimaging studies, which reveal that patients with T1DM display subtle abnormalities in cortical structure, such as reduced cortical grey matter

density and lower total grey volume, compared to age-matched healthy controls (Musen *et al.,* 2006; Wessels *et al.,* 2006), but not brain regions responsible for memory. Although statistical significance was not reached in the present study, memory performance for the T1DM group in the Cognistat was largely preserved, supporting existing literature that patients with T1DM demonstrate intact memory function (Brands *et al.,* 2005). The precise mechanisms accounting for this remain poorly elucidated, but exogenous insulin has been implicated. Conversely, impairments in memory are commonly documented in patients with T2DM (Monette *et al.,* 2014; Biessels & Despa, 2018). This has been ascribed to several abnormal molecular processes triggered by insulin resistance, which current literature suggests induces cerebral insulin resistance and disrupts insulin signalling (Sims-Robinson *et al.,* 2010; Biessels & Despa, 2018).

No difference was observed in global or domain-specific cognitive performance between the non-clinical group and the diabetes groups (T1DM and T2DM). Not all studies have found differences in cognitive function between patients with diabetes and healthy controls. Lawson *et al.* (1984) found no difference in cognitive function between patients with T1DM (n = 48) with some degree of peripheral and autonomic neuropathy and age-matched healthy controls (n = 40) despite assessing cognition using several reputable neurocognitive batteries (Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955), Wechsler Memory Scale, Kimura Repeated Figures Test, and Repeated Words Test). The investigators attributed this incongruity to methodological issues, such as the significant heterogeneity in characteristics of the diabetes patients recruited (*e.g.* disease duration, glycaemic control, frequency of hypoglycaemia). This, together with the numerous neuro-psychometric batteries available for administration and inconsistency in cognitive assessments administered, has frequently been raised by other researchers as a longstanding limitation in this area of research that complicates comparability of data between studies (Munshi *et al.,* 2006; Roberts *et al.,* 2008; Roriz-Filho *et al.,* 2009; Geijselaers *et al.,* 2017). Geijselaers *et al.* (2017) recommend administering similar neurocognitive batteries to assess the subtle changes in cognition linked to these conditions in future investigations to address this limitation.

The absence of a difference in global and domain-specific cognitive performance between the study groups in the present study conflicts with available evidence and may be explained by several factors. The main confounding factors suggested in the literature for diabetes include glycaemic control, glycaemic variability, age of disease onset, disease duration, recurrent hypoglycaemia, and glucose-lowering agents, but the contribution of each to cognitive dysfunction in diabetes is reportedly modest (Biessels *et al.,* 2014; Feinkohl *et al.,* 2015; Biessels & Despa, 2018). First, the glycaemic control of patients with diabetes recruited for the present study was generally fair (T1DM: 7.5%; T2DM: 8.0%) and fell within the standard, less-rigid range for glycaemic control ($HbA1_C$: 7.5 – 8.0%). While the $HbA1_C$ level was slightly elevated in both groups, it was found not to be associated with global cognitive performance, suggesting that deterioration in cognitive function occurs at higher $HbA1_C$ values. Support for this view is provided by Jacobson *et al.* (2007), who reported accelerated deterioration in psychomotor speed at $HbA1_C$ values > 8.8%. Similarly, Cukierman-Yaffe and colleagues (2009) found that a 1% increase in $HbA1_C$ in patients with T2DM was associated with a 0.20-point lower score in global cognitive performance (MMSE). Alarmingly, at high concentrations ($HbA1_C$ > 10%) it has been linked to an increased risk of dementia based on risk score models (Exalto *et al.,* 2013) and also substantial morbidity and mortality (Ricks *et al.,* 2012).

Although most authors have reported negative associations between $HbA1_C$ level and cognitive function, there are other investigators who have not. For example, in a large sample of elderly Japanese patients with T2DM (n = 1173; mean age: 71.8 ± 4.6 years), Akisaki *et al.* (2006) observed no association between glycosylated haemoglobin (mean $HbA1_C$: 7.9%) and global cognitive performance and information processing speed using the MMSE and Stroop B test, respectively. Similarly, Christman *et al.* (2011) found no association between glycosylated haemoglobin (mean $HbA1_C$: 8.5%) and cognitive function, assessed using three reputable neuropsychological assessments (Digit Symbol Substitution Test (DSST), the Delayed Word Recall Test (DWRT), and the Wechsler Adult Intelligence Scale-Revised (WAIS-R)) (Wechsler, (1955), in a large sample of patients with T2DM (n = 516). These data collectively substantiate prior evidence that cognitive function is impacted at higher $HbA1_C$ values (Jacobson *et al.,* 2007; Cukierman-Yaffe *et al.,* 2009; Exalto *et al.,* 2013). The contribution of other glycaemic

indices, such as fasting plasma glucose (FPG) and postprandial glucose (PPG), to cognitive dysfunction should also be investigated in future studies.

Although poor glycaemic control ($HbA1_C \geq 8\%$) is a well-established risk factor for cognitive dysfunction and the subtle diabetes-associated cognitive decrements, the strength of the relationship is reportedly weak (Geijselaers *et al.,* 2017). Geijselaers *et al.* (2017) reviewed numerous studies (cross-sectional and longitudinal) examining associations between $HbA1_C$ and cognitive function and found that the association, although significant, was relatively weak. Interestingly, Crane *et al.* (2013) observed that higher than normal $HbA1_C$, but not in the range diagnostic of diabetes, was implicated with an increased risk of dementia in people without diabetes, suggesting elevated blood glucose concentrations are a modifiable risk factor for cognitive impairment. This finding is also supported by emerging literature, which argues that glycaemic variability, instead of chronic hyperglycaemia alone, could be linked to dementia and possibly diabetes-associated cognitive decrements (Rawlings *et al.,* 2017). Given the glycaemic control of patients with diabetes (T1DM and T2DM) in the present study was fair, this may have accounted for the relatively intact global and domain-specific cognitive performance observed. It could have also explained the positive association observed between $HbA1_C$ and comprehension performance in the Cognistat for the T1DM group.

Early intensive glycaemic control is known to reduce the development and progression of long-term adverse microvascular complications (The Diabetes Control and Complications Trial Research Group, 1993; The UK Prospective Diabetes Study (UKPDS) Group, 1998); however, it confers little benefit on macrovascular complications (Holman *et al.,* 2008; Hayward *et al.,* 2015) and cognitive outcomes, with any meaningful benefit of glycaemic control on macrovascular complications taking approximately 10 years to manifest (UKPDS, 1998, DeFronzo *et al.,* 2017). Conversely, it can also precipitate unwanted hypoglycaemic episodes, potentially offsetting meaningful vascular benefits mediated by aggressive glucose control (Herzog & Sherwin, 2012). The landmark Diabetes Control and Complications Trial (DCCT), which assessed cognition as an endpoint and monitored cognitive functioning closely in two groups (intensive glycaemic control vs conventional control), found intensive glycaemic control did not improve cognitive outcomes despite $HbA1_C$ being 20 mmol/L lower in the intensive group compared to the conventional control group after an 18.5 year follow-up

period. Therefore, it is clear that several risk factors beyond chronic hyperglycaemia mediate the relationship and contribute to the development and progression of cognitive dysfunction in patients with diabetes (T1DM and T2DM).

Another risk factor known to moderate the relationship between diabetes and cognition is age of disease onset. Substantial literature indicates that age of disease onset of diabetes is a strong predictor of long-term cognitive outcomes, especially in T1DM (Biessels, Deary, & Ryan, 2008; Ryan *et al.,* 2016). Neuroimaging and neuropsychological data consistently reveal that early diabetes onset (< 7 years of age) is associated with subtle changes in cognitive development and brain structure (cortical atrophy) that persists into adulthood (Ferguson *et al.,* 2005; Musen *et al.,* 2006; Wessels *et al.,* 2006). This early-onset effect, known as the diathesis hypothesis, has been ascribed to the susceptibility of the developing brain to diabetes-induced metabolic disturbances, notably glycaemic events (hypo- and hyperglycaemia) (Ryan, 2006). These glycaemic fluctuations disrupt blood-brain-barrier (BBB) permeability and integrity and the highly regulated internal milieu of the CNS, disturbing several vital brain processes known to be labile during the first four to five years of life, such as synaptogenesis and neuronal signalling (Ryan, 2006; Sweeney *et al.,* 2016). This results in the unregulated entry of plasma-derived substances into the CNS, which is thought to interfere with brain development, leading to an increased likelihood of cognitive dysfunction (Ryan, 2006; Sweeney *et al.,* 2016).

Brain susceptibility to diabetes-induced metabolic derangements has been hypothesised to occur at two critical life periods: (1) during childhood when the brain is developing, and (2) during neurodegenerative processes linked to ageing (> 65 years of age) (Biessels, Deary, & Ryan, 2008; Koekkoek *et al.*, 2015). Conversely, the brain appears resistant to metabolic disturbances/neuroglycopaenia outside these two key periods, with clinically relevant diabetes-associated cognitive decrements mainly affecting specific sub-groups of patients, such as those with advanced micro- and macrovascular complications (Biessels, Deary, & Ryan, 2008). Most patients with T1DM and T2DM recruited for the present study developed their condition after 7 years of age and did not report complications (retinopathy, nephropathy, neuropathy). Taken collectively, these diabetes-related characteristics may have accounted for the largely preserved and slightly better global cognitive performance observed for the T1DM group in the Mini-Mental State Examination (MMSE) and most domains of the Cognistat compared to the non-

clinical group and other clinical groups, supporting evidence that the brain is resistant to metabolic insult outside the critical periods outlined above (youth to middle age). The lack of clinically-relevant complications (micro- or macrovascular) in the T2DM group may have also explained the absence of a difference in global and domain-specific cognitive performance between the T2DM group and the other groups.

Whether recurrent hypoglycaemia contributes to cognitive dysfunction in patients with DM remains contentious. The literature is mixed: some studies have found associations between recurrent hypoglycaemia and cognitive decline (Exalto *et al.,* 2013), whereas others have not (Bruce *et al.,* 2009). Some investigators have also suggested the relationship is possibly bidirectional (Yaffe *et al.,* 2013; Feinkohl *et al.,* 2014). Frier (2014) argues the relationship is age-dependent. Convincing evidence supporting this observation is provided by Asvold *et al.* (2010), who reported that young children with T1DM who experienced severe hypoglycaemic episodes and developed their condition at < five years of age demonstrated poorer cognitive function than those with T1DM not exposed to severe hypoglycaemia when retested as adults. These data suggest recurrent hypoglycaemia elicits lasting effects on cognitive function and corroborate the diathesis hypothesis discussed previously (Ryan, 2006). Hypoglycaemic episodes – mild or severe – are the most common reversible and feared adverse effects of diabetes management; however, accurate retrospective recall of prior hypoglycaemic episodes is poor (Frier, 2014). While severe hypoglycaemic episodes can be reliably recalled for up to one year, mild episodes can only be accurately recalled for no longer than one week (Frier, 2014). This complicates ascertainment of the precise contribution of hypoglycaemia to diabetes-associated cognitive dysfunction and may account for the conflicting relationship currently reported. Although frequency of hypoglycaemia was solicited in the present investigation, it could not be reliably recalled by study participants; therefore, the relationship between hypoglycaemia and cognition could not be established. Other investigators have encountered this issue and suggest monitoring blood glucose using continuous glucose monitoring (CGM) devices to address this limitation (Geijselaers *et al.,* 2017).

Although no differences in global and domain-specific cognitive performance were found between the groups in the present study, these findings neither support nor refute the clinical utility of the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) or the Cognistat (Kiernan *et al.,* 1987) for the detection of early cognitive impairment or early cognitive dysfunction associated with both diabetes and hypertension. Both are reliable, validated and established cognitive screening instruments frequently deployed in clinical contexts for the rapid identification of incipient and/or early cognitive impairment (Folstein, McHugh, & Folstein, 1975; Tombaugh & McHugh, 1992; Pangman *et al.,* 2000; Lancu & Olmer, 2006). Notably, the Mini-Mental State Examination demonstrates reasonable psychometric properties (test-retest reliability values: 0.56-0.99, Cronbach's alpha: 0.54-0.96) (Tombaugh & McHugh, 1992) and is currently recommended in emerging clinical guidelines for screening elderly patients with diabetes for subtle cognitive decrements (American Diabetes Association (ADA, 2020)). Implementation of its recommended cut-off score (scores ≤ 23) has also been linked to a subsequent dementia diagnosis in approximately 79% of cases, and it has also been the most frequently used cognitive assessment for screening cognitive dysfunction in subjects with hypertension (Iadecola *et al.,* 2016). Therefore, it is likely the small sample size of the clinical populations in the present study, paired with the variables described earlier, accounted for the lack of a difference in global and domain-specific cognitive performance observed between the study groups.

No significant differences in global and domain-specific cognitive performance were found between the non-clinical group and HTN group in the present study. This finding conflicts with available literature and could be attributable to several factors outlined earlier (see Chapter 1, Section 1.7.2), although it is plausible to suggest that the well-controlled BP of the participants with hypertension, due to anti-hypertensive therapy, primarily explains the discrepancy. Other studies have reported similar findings; Nilsson *et al.* (1998) assessed cognitive function using the MMSE in treated hypertensive patients (n = 123) and subjects without hypertension (n = 76) and found performance did not differ between the groups, suggesting blood pressure-lowering therapy may confer favourable cognitive benefits. Whether anti-hypertensive therapy delays or prevents cognitive decline remains inconclusive and is beyond the scope of this thesis, but data from a recently published meta-analysis suggest it lowers the risk of dementia and AD by 16 and 12%, respectively (Ding *et al.,* 2020). The authors also reported similar effects between

different classes of anti-hypertensive therapy (Ding *et al.,* 2020). Most patients with hypertension recruited for the study also did not present with hypertension-related complications (*e.g.* stroke, myocardial infarction). These are strong risk factors for vascular cognitive impairment (VCI), which causes abrupt, stepwise declines in cognition (van der Flier *et al.,* 2018). Taken together, this may have accounted for the lack of a difference in global and domain-specific cognitive performance between the non-clinical and HTN group.

A significant difference in average response time was observed between the non-clinical and clinical groups using a computerised version of the Stroop Colour Word Test, with the clinical groups, on average, taking longer than the non-clinical group to process matched and mismatched stimuli. This finding is in accordance with existing literature (Ryan *et al.,* 2003; Brands *et al.,* 2006; Wessels *et al.,* 2007), which consistently indicates that information processing and executive cognitive functioning are common cognitive modalities detrimentally impacted by both DM and HTN (Ryan *et al.,* 2003; Brands *et al.,* 2006; Wessels *et al.,* 2007; Graveling, Deary, & Frier, 2013). Ample evidence indicates that abnormalities in white matter correlate with poorer performance in tasks of information processing tasks, executive function, and memory (Gunning-Dixon *et al.,* 2000; Hedden & Gabrieli, 2004). Consistent with this observation, Wessels *et al.* (2007) reported an association between white matter atrophy and decreased performance in information processing, executive function, and attention performance in a small sample of patients with T1DM (n = 10) with proliferative retinopathy (PN, Grade 5), measured using 13 neuropsychological tasks (see Wessels *et al.,* 2007 for all cognitive assessments administered). Imaging studies also consistently show that DM (T1DM and T2DM) and HTN are associated with modest changes in cerebral integrity, with hypertension recognised as the most significant risk factor for small vessel disease (SVD) (Iadecola *et al.,* 2016). Therefore, the slower average response time observed in the clinical groups, particularly the T2DM and hypertension groups, could potentially indicate some degree of damage to underlying white matter, such as, at the molecular level, demyelination or axonal loss induced by chronic ischaemia. This usually results from SVD, which causes irreversible cerebrovascular damage (Prins & Scheltens *et al.,* 2015; Van de Flier *et al.,* 2018).

The finding that patients with T2DM demonstrate longer average response times compared to both patients with T1DM and HTN is novel and suggests other risk factors common to T2DM may have contributed to the reduced speed of information processing. First, the glycaemic control of the T2DM group was slightly elevated, potentially contributing to the slowing of information processing. Another emerging risk factor attracting considerable attention that may have contributed is cerebral insulin resistance (Biessels & Despa, 2018). In the brain, insulin plays important roles in influencing crucial cognitive functions such as learning and memory (Cholerton *et al.,* 2013; Biessels & Reagan, 2015); however, disrupted insulin signalling has been reported in the brains of individuals with AD, but without T2DM (Arnold *et al.,* 2018). Evidence from experimental models has also shown that impaired insulin signalling promotes amyloid beta aggregation and hyperphosphorylation of *tau* protein (Sims-Robinson *et al.,* 2010). Taken together, these findings raise the possibility that defective insulin signalling – a core pathophysiological hallmark of T2DM – could contribute to diabetes-associated cognitive dysfunction and possibly permit crosstalk and accelerate progression to AD, accounting for the reduced information processing observed.

4.8 Associations: Non-clinical Group

In the non-clinical group, increasing BGL was found to be inversely associated with global cognitive performance (MMSE); however, the strength of the association was weak. This finding is in accordance with prior research. For example, disruptions in BGL, such as hypo- and hyperglycaemia, are known to compromise brain glucose homeostasis, leading to impairments in cognition (McNay & Cotero, 2010; Frier, 2014). The cognitive sequelae resulting from hyperglycaemia typically include reduced information processing, altered cognitive flexibility, and global cognitive dysfunction (Brands *et al.,* 2005). Fluctuations in BGL have also been linked to aggravating diabetes-associated cognitive decrements and increasing the risk of dementia. Interestingly, Geijselaers *et al.* (2017) hypothesise that dysglycaemia contributes to the development, but not progression, of cognitive dysfunction in patients with diabetes, a hypothesis supported by longitudinal investigations that found cognitive decline at baseline but not after considerable follow-up (Jacobson *et al.,* 2007).

Several putative mechanisms have been proposed to explain how elevated glucose concentrations may impair cognitive function. In addition to the pathophysiological mechanisms described earlier, elevated blood glucose induces hypoxia and interrupts cerebral blood flow, depriving neurons of crucial glucose and oxygen, particularly in frontal brain regions (Baglietto-Vargas *et al.*, 2016; Morley, 2017). At the molecular level, experimental evidence indicates hyperglycaemia also disturbs critical neurological processes, such as axonal transport and myelination (Sims-Robinson *et al.*, 2010; Morley, 2017). Thus, the weak inverse association reported between global cognition and attention performance in the Cognistat potentially implies that neurons in frontal brain regions may become vulnerable to the neurotoxic effects of elevated blood glucose concentrations nearing hyperglycaemia, even for short periods of time, resulting in poorer performance in tasks subserved by frontal lobe function.

No associations were found between blood pressure (SBP or DBP) and global and domain-specific cognitive performance in the non-clinical group. Other studies assessing cognitive function in subjects with and without hypertension have replicated this finding (Harrington *et al.,* 2000), suggesting the brain becomes vulnerable to damage at higher BP. Although to date the relation between blood pressure and cognition remains largely controversial, convincing evidence from cross-sectional and longitudinal studies consistently suggests that high blood pressure (> 140mmHg/90mmHg) is associated with poor cognitive performance (global) across all age groups (mid-life and late-life), with the strongest association found for mid-life BP (Kilander *et al.,* 1998; Yaffe *et al.,* 2014). Interestingly, an inverse relationship between BP and cognition has been documented in the older populations (>85 years of age) (Richmond *et al.,* 2011). As the blood pressure (both SBP and DBP) of the non-clinical population was normotensive, this likely explained the lack of associations identified between blood pressure and global and domain-specific cognitive performance. The cross-sectional study design of the present study also limits inferences about causality and cannot capture the minute-to-minute variability of BP. Iaedecola *et al.,* (2016) recommend longitudinal studies can establish better temporal associations between blood pressure and cognition.

4.9 Associations: Clinical Group

The literature provides strong, consistent evidence that acute and chronic glycaemic events (hypo- and hyperglycaemia) detrimentally affect performance in several cognitive domains (McNay & Cotero, 2010; Brands *et al.,* 2005; Frier, 2014). Hypoglycaemia (BGL < 3mmol/L) causes neuroglycopenia, resulting in marked impairment in attention-dependent activities, such as driving and executive function (Warren & Frier, 2005; Inkster & Frier, 2012; Graveling, Deary, & Frier, 2013), whereas hyperglycaemia (BGL > 11mmol/L) causes slowing in psychomotor efficiency and cognitive flexibility (Brands *et al.,* 2005; Cox *et al.,* 2005). In the T1DM group, no associations were found between BGL (pre-study and post-study) and global and domain-specific cognitive performance. This could have been due to participant blood glucose concentrations, which, although marginally elevated (8 mmol/L), resided within the normoglycaemic range, suggesting that brain vulnerability to hyperglycaemia potentially occurs at higher BGL values (Cox *et al.,* 2005). It is noteworthy that in the non-clinical group, increasing BGL (>7 mmol/L) was inversely associated with global cognitive performance, but not in the T1DM group. This finding implies a degree of cerebral adaptation, suggesting the brain develops a protective and adaptive mechanism to reduce further damage/insult (such as oxidative stress and interruptions in cerebral blood flow) to vulnerable neuronal populations from recurrent hyperglycaemia (Wyke, 1959; Hwang *et al.,* 2019). Such an adaptive mechanism purportedly attenuates the magnitude of impairment in cognition and could explain the lack of an association identified (Zammitt *et al.,* 2008; Graveling, Deary, & Frier, 2013).

In the T2DM population assessed, BGL was found to be inversely associated with attention performance in the Cognistat. This result is supported by the literature suggesting that elevated glucose concentrations cause modest cognitive dysfunction in frontal brain regions (Brands *et al.,* 2005; Cox *et al.,* 2005). However, it was also found to be significantly correlated with memory performance in the Cognistat in the HTN group, indicating that brain areas involved in memory, such as the hippocampus, potentially require slightly elevated glucose for optimum function during cognitive demand. In accordance with this observation, McNay *et al.* (2000) and McNay *et al.* (2001) showed hippocampal function is impaired substantially by hypoglycaemia. Although hippocampal glucocorticoid receptors are sensitive to the neurotoxic effects of chronic hyperglycaemia, the current findings suggest that short periods of elevated

glucose appear beneficial for optimum functioning during cognitive demand, especially memory performance.

Several associations were found between BP (SBP and DBP) and global and domain-specific cognitive performance in the clinical groups. It is estimated that approximately 60-70% of patients with T2DM develop hypertension, primarily due to increased reabsorption of glucose and sodium (DeFronzo *et al.,* 2017), but the BP (SBP and DBP) of participants with DM (T1DM and T2DM) and HTN in the present study resided within the normotensive range and was well controlled (HTN group). Blood pressure in the normal range supports optimum cognitive functioning, likely attributable to adequate, uninterrupted cerebral perfusion and neurovascular coupling (Iadecola *et al.,* 2016). Taken together, this may have potentially accounted for the various significant associations reported and lack of cognitive dysfunction demonstrated by these clinical groups (T1DM, T2DM, and HTN). The present study also found that increasing DBP in the HTN group was inversely associated with recall performance in the MMSE. The literature also reports that higher BP (SBP and DBP) is associated with poor cognitive function in global and domain-specific performance (Starr *et al.,* 1993; Kilander *et al.,* 1998; Obisesan *et al.,* 2008), although not all studies have reported such associations (Farmer *et al.,* 1987). Investigators link these mixed findings to the various methodological limitations and confounding variables, which frequently complicate determination of the precise relationship between BP and cognition (Obisesan *et al.,* 2009; Iadecola *et al.,* 2016).

4.10 Conclusions: Cognitive Performance

Using established cognitive screening tools, the present study has yielded evidence to suggest that cognitive performance (global and domain-specific), as assessed using the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) and the Cognistat (Kiernan *et al.,* 1987), does not vary between non-clinical and clinical populations (reject aims 1.1 and 1.2). This result contrasts with the available literature but could be attributable to the methodological limitations outlined in Chapter 1 and disease-specific variables, as discussed in this chapter, which have been shown consistently to moderate the relationship between these chronic diseases and cognition (Munshi *et al.,* 2006; Geijselaers *et al.,* 2017; Biessels & Despa, 2018). It may also be ascribed to the small sample sizes of each group, particularly the clinical groups. However, significant differences in average response time when processing congruent and incongruent stimuli using the Stroop Colour Word Test were found. This finding is novel and both supports and contributes to the established body of evidence indicating that both DM and HTN are associated with slowing of information processing and impaired executive functioning. It is also relevant clinically, as impairment in these cognitive domains can interfere with crucial disease self-management tasks (*e.g.* blood glucose monitoring, medication dosing, blood pressure monitoring *etc.*), leading to diminished self-management of DM and HTN. This may subsequently result in more diabetes and hypertension-related hospitalisations and increased frequency of complications, including diabetes and hypertension-associated cognitive dysfunction (Srikanth *et al.,* 2020).

It is clear that larger, adequately powered, and controlled investigations (cross-sectional and longitudinal) using similar neurocognitive batteries are required to further understand the pattern of the subtle cognitive dysfunction in these chronic diseases. Consistency in cognitive assessments administered in future studies will improve the comparability of data between studies (Geijselaers *et al.,* 2017). It will also help researchers understand the appropriateness of each cognitive battery for the screening of the subtle and slowly progressing diabetes-associated cognitive decrements and hypertension-induced cognitive dysfunction, a currently debated and still unanswered question. Given both DM and HTN frequently affect cognitive domains of attention, information processing, memory, and executive function, investigators recommend choosing cognitive screening tools that assess these domains (Geijselaers *et al.,* 2017) and have a high negative predictive value (*i.e.* accurately exclude cognitive dysfunction) (Srikanth *et al.,* 2020)).

5. Associations between Blood Pressure, Blood Glucose Level and Electroencephalography (Non-Clinical)

This chapter reports associations found between BP (SBP and DBP) and BGL and EEG variables (delta, theta, alpha, beta, and gamma) during baseline and active recordings (see section 2.8 in methodology) in the non-clinical cohort. The findings are presented commencing with links to pre-study variables followed by post-study variables. As age and BMI were found to be significantly correlated to dependent variables, a partial Pearson's correlation (controlling for age and BMI as covariates) was applied.

5.1 Associations between pre-study SBP and EEG activity (non-clinical)

Pre-study SBP was found to be significantly associated with alpha, beta, and gamma activity during the baseline phase in the non-clinical group (Table 5.1). For alpha activity, these associations with pre-study SBP were found at the following locations: T_7 ($p = 0.04$; $r = 0.45$) (Figure 5.1), C_3 ($p = 0.01$; $r = 0.60$), C_Z ($p = 0.01$; $r = 0.63$), TP_7 ($p = 0.03$; $r = 0.48$), CP_3 ($p = 0.04$; $r = 0.47$), CP_Z ($p = 0.02$, $r = 0.51$), and CP_4 ($p = 0.02$; $r = 0.53$). For beta activity, they were found at F_3 ($p = 0.03$; $r = 0.51$), FT_8 ($p = 0.02$; $r = 0.55$) (Figure 5.2), and P_8 ($p = 0.02$; $r = 0.54$). In contrast, links with pre-study SBP for gamma activity were at TP_8 ($p = 0.03$; $r = 0.42$), P_7 ($p = 0.04$; $r = 0.39$), and P_8 ($p = 0.04$; $r = 0.39$). No significant associations were identified between pre-study SBP and theta or delta activity during the baseline phase.

Table 5.1. Associations between pre-study SBP and EEG activity during the baseline phase for the non-clinical group.

Independent variable	Dependent variable (Baseline)	Brain Area	p	r
Pre-Study SBP	Alpha (α)	T_7	0.04*	0.45
		C_3	0.01*	0.60
		C_z	0.01*	0.63
		TP_7	0.03*	0.48
		CP_3	0.04*	0.47
		CP_Z	0.02*	0.51
		CP_4	0.02*	0.53
	Beta (β)	F_3	0.03*	0.51
		FT_8	0.02*	0.55
		P_8	0.02*	0.54
	Gamma (γ)	TP_8	0.03*	0.42
		P_7	0.04*	0.39
		P_8	0.04*	0.39

Key:

SBP – systolic blood pressure
F – Frontal
T – Temporal
C – Central
P – Parietal
* – statistical significance
p – p-value
r – rho value

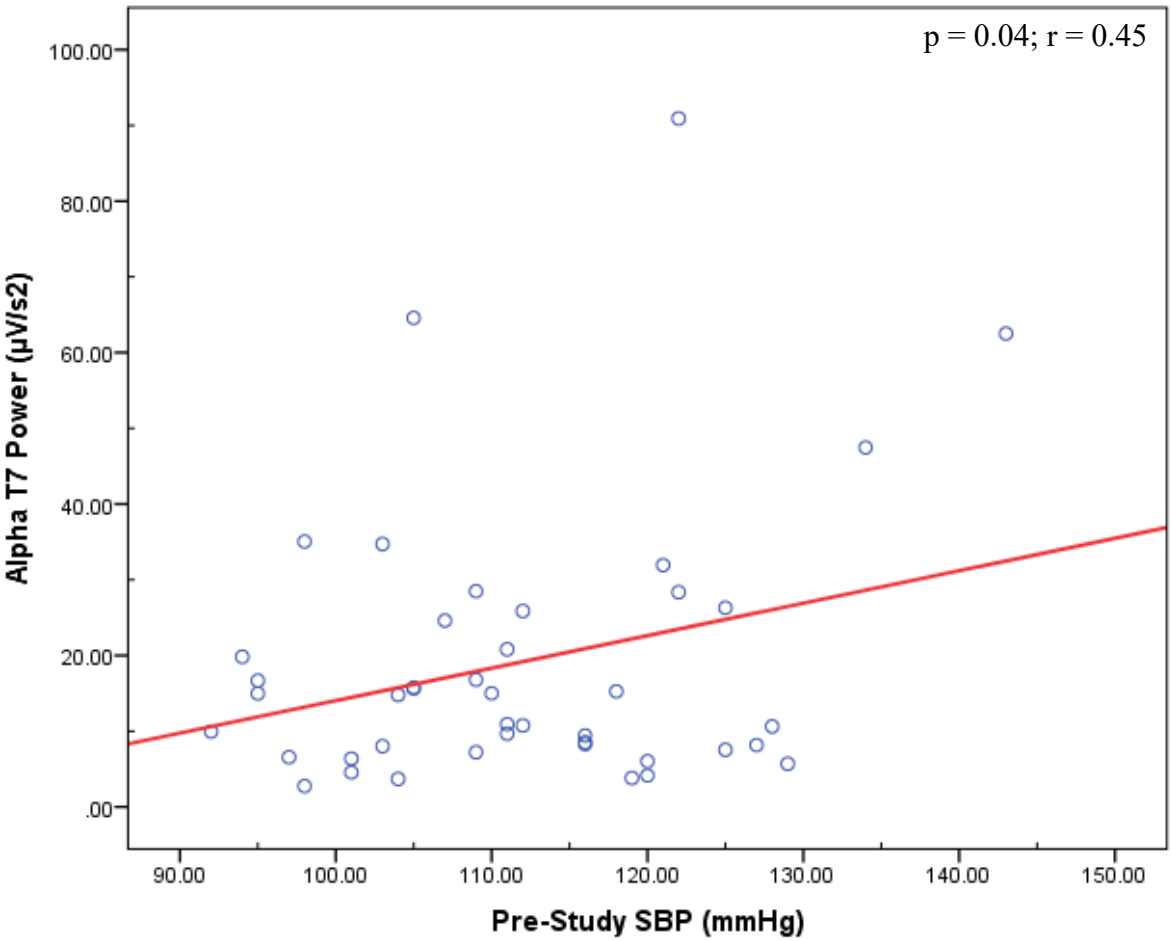


Figure 5.1. Positive correlation between pre-study systolic blood pressure and alpha power at T_7 during the baseline phase for the non-clinical group.

Key:

SBP – systolic blood pressure

T – Temporal

p – p-value

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

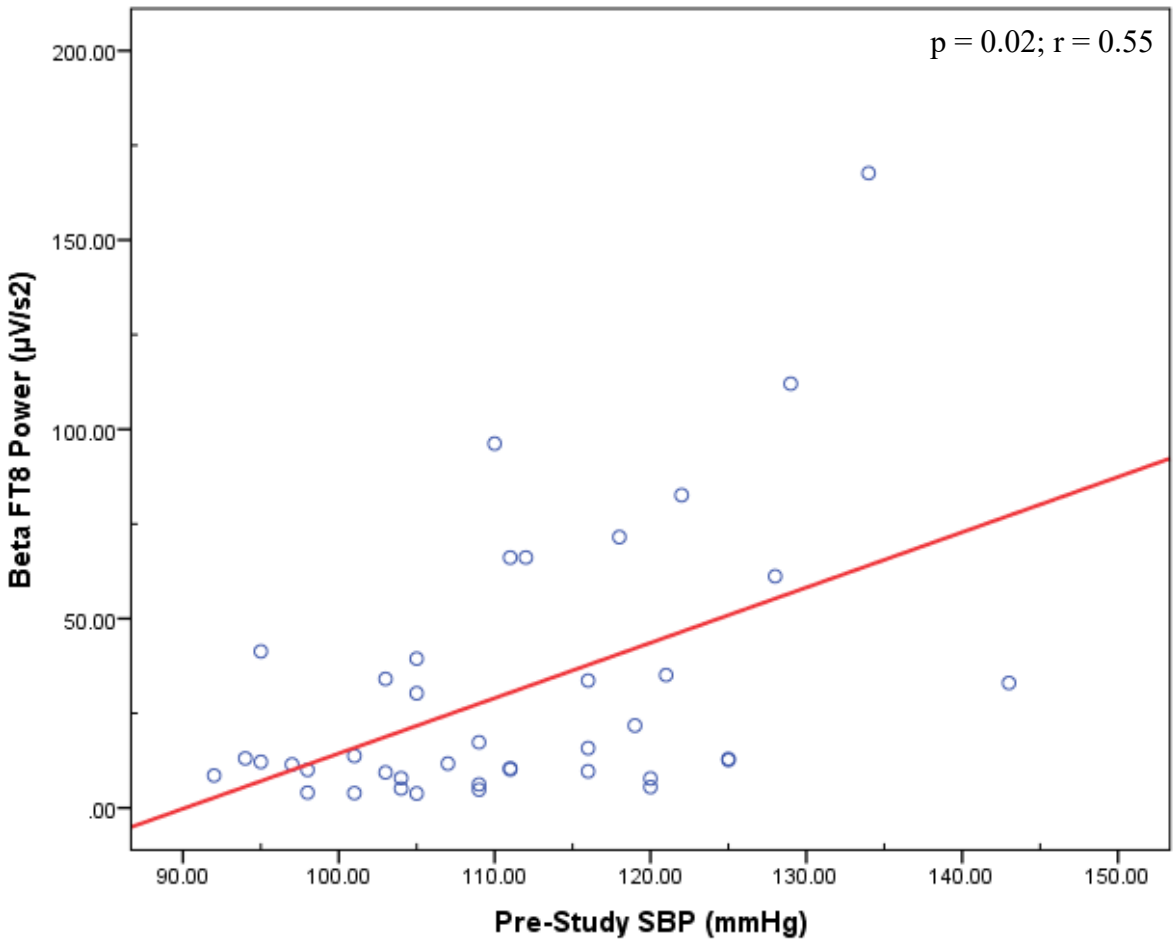


Figure 5.2. Positive correlation between pre-study systolic blood pressure and beta power at FT_8 during the baseline phase for the non-clinical group.

Key:

SBP – systolic blood pressure

T – Temporal

p – p-value

F – Frontal

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

Several significant associations were found between pre-study SBP and EEG variables during the active phase (Table 5.2). These links with pre-study SBP were found in fast-wave activities (alpha, beta, and gamma). For alpha activity, these associations were found at: FC_3 ($p < 0.001$; $r = 0.55$) (Figure 5.3); T_7 ($p = 0.01$; $r = 0.41$); C_3 ($p = 0.01$; $r = 0.39$); CP_3 ($p < 0.05$; $r = 0.47$); P_Z ($p = 0.01$; $r = 0.38$); P_8 ($p = 0.01$; $r = 0.40$), and O_1 ($p = 0.02$; $r = 0.36$). For beta activity, they were found at FP_1 ($p = 0.01$; $r = 0.42$), FP_2 ($p = 0.01$; 0.44), F_7 ($p = 0.04$; $r = 0.32$); F_3 ($p = 0.01$; $r = 0.42$), F_Z ($p = 0.04$; $r = 0.37$), FC_3 ($p < 0.001$; $r = 0.52$), FC_Z ($p = 0.01$; $r = 0.40$), TP_7 ($p = 0.01$; $r = 0.42$), CP_3 ($p < 0.05$; $r = 0.50$), CP_4 ($p < 0.05$; $r = 0.46$); P_Z ($p = 0.04$; $r = 0.32$), P_8 ($p = 0.001$; $r = 0.52$), O_1 ($p = 0.04$; $r = 0.34$), and O_2 ($p = 0.04$; $r = 0.33$). In contrast, pre-study SBP was associated with gamma activity at the following locations: FP_2 ($p < 0.001$; $r = 0.71$); FC_3 ($p = < 0.001$; $r = 0.54$) (Figure 5.4); T_7 ($p = < 0.001$; $r = 0.55$); C_3 ($p < 0.001$; $r = 0.62$); C_Z ($p = 0.001$; $r = 0.44$); TP_7 ($p = 0.01$; $r = 0.51$); P_Z ($p = < 0.001$; $r = 0.64$). There were no significant associations between pre-study SBP and slow-wave brain activities (theta and delta) during the active phase.

Table 5.2. Associations between pre-study SBP and EEG activity during the active phase for the non-clinical group.

Independent variable	Dependent variable (Active)	Brain Area	p	r
Pre-Study SBP	Alpha (α)	FC_3	<0.001*	0.55
		T_7	0.01*	0.41
		C_3	0.01*	0.39
		CP_3	<0.05*	0.47
		P_Z	0.01*	0.38
		P_8	0.01*	0.40
		O_1	0.02*	0.36
	Beta (β)	FP_1	0.01*	0.42
		FP_2	0.01*	0.44
		F_7	0.04*	0.32
		F_3	0.01*	0.42
		F_Z	0.04*	0.37
		FC_3	<0.001*	0.52
		FC_z	0.01*	0.40
		TP_7	0.01*	0.42
		CP_3	<0.05*	0.50
		CP_4	<0.05*	0.46
		P_Z	0.04*	0.32
		P_8	0.001*	0.52
		O_1	0.04*	0.34
		O_2	0.04*	0.33
	Gamma (γ)	FP_2	<0.001*	0.71
		FC_3	<0.001*	0.54
		T_7	<0.001*	0.55
		C_3	<0.001*	0.62
		C_Z	0.001*	0.44
		TP_7	0.01*	0.51
		P_Z	<0.001*	0.64

Key:

SBP – systolic blood pressure
F – Frontal
T – Temporal
C – Central
P – Parietal
p – p-value
***** – statistical significance
r – rho value

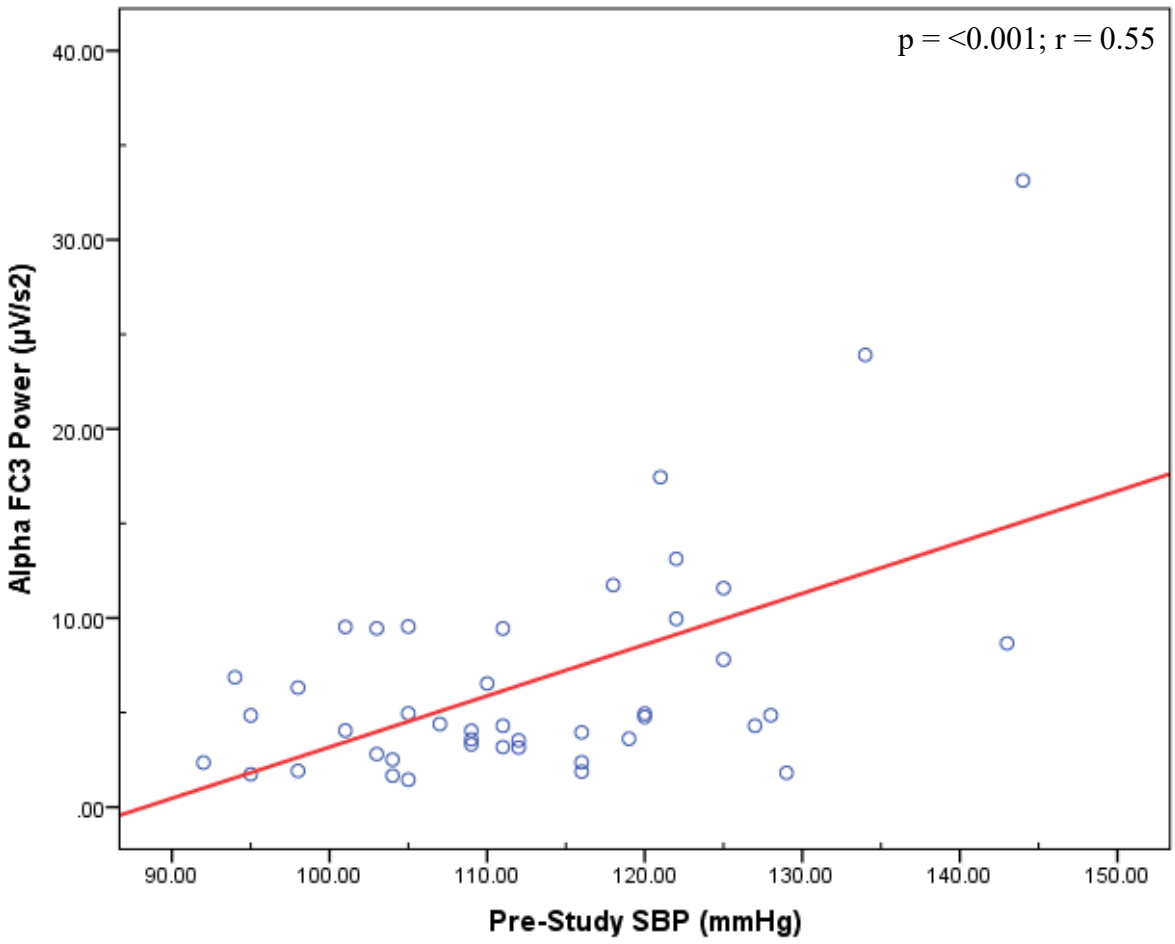


Figure 5.3. Positive correlation between pre-study systolic blood pressure and alpha power at FC_3 during the active phase for the non-clinical group.

Key:

SBP – systolic blood pressure

F – Frontal

T – Temporal

µV/s² – microvolts per second squared

p – p-value

r – rho value

mm Hg – millimetres of mercury

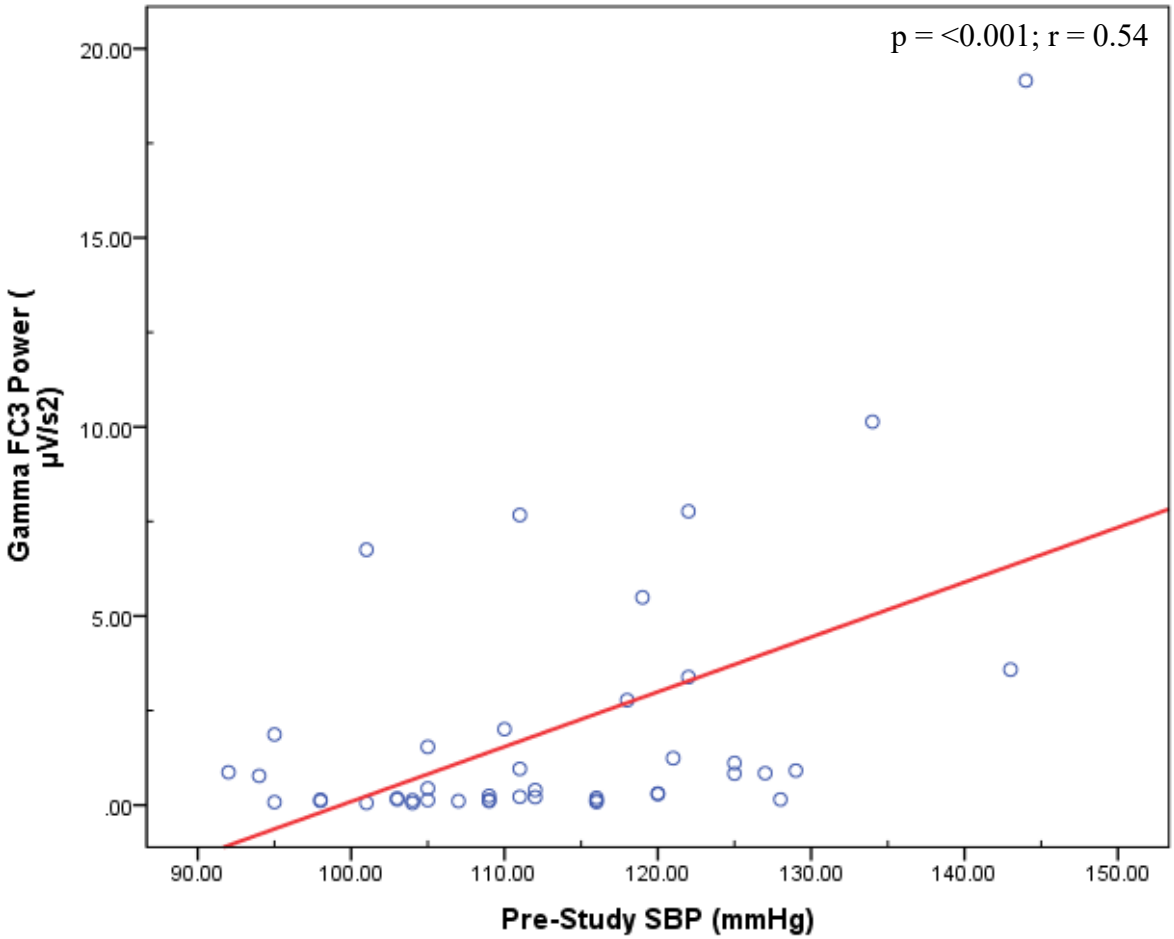


Figure 5.4. Positive correlation between pre-study systolic blood pressure and gamma power at FC_3 during the active phase for the non-clinical group.

Key:

SBP – systolic blood pressure

C – Central

p – p-value

F – Frontal

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

5.2 Associations between post-study SBP and EEG activity (non-clinical)

No significant associations were found between post-study SBP and the EEG variables during the baseline phase. However, multiple links were identified between post-study SBP and EEG activity during the active phase, mainly in the fast-wave activities (alpha, beta, and gamma) (Table 5.3). Post-study SBP was linked with alpha activity at FC_3 ($p = 0.01$; $r = 0.42$) and T_7 ($p = 0.01$; $r = 0.51$) during the active phase, while for beta activity the associations were observed at several locations as follows: FP_1 ($p = 0.02$; $r = 0.37$), FP_2 ($p = 0.01$; $r = 0.38$), F_3 ($p = 0.01$; $r = 0.41$), FC_3 ($p = 0.01$; $r = 0.42$), FC_Z ($p<0.05$; $r = 0.46$), TP_7 ($p = 0.01$; $r = 0.39$), CP_3 ($p<0.001$; $r = 0.40$), CP_4 ($p =0.01$; $r = 0.40$), and P_8 ($p = 0.01$; $r = 0.40$). In contrast, associations between post-study SBP and gamma activity were at FP_1 ($p = 0.02$; $r = 0.37$), FP_2 ($p = 0.01$; $r = 0.41$) (Figure 5.5), FC_3 ($p = 0.02$; $r = 0.37$), C_3 ($p = 0.01$; $r = 0.38$), TP_7 ($p = 0.02$; $r = 0.37$), TP_8 ($p = 0.01$; $r = 0.37$), P_7 ($p =0.01$; $r = 0.41$), and P_Z ($p = 0.01$; $r = 0.42$). Similar to pre-study SBP, no significant associations were found between post-study SBP and slow-wave brain frequencies (theta and delta) during the active phase.

Table 5.3. Associations between post-study SBP and EEG activity during the active phase for the non-clinical group.

Independent variable	Dependent variable (Active)	Brain Area	p	r
Post-Study SBP	Alpha (α)	FC_3	0.01*	0.42
		T_7	0.01*	0.51
	Beta (β)	FP_1	0.02*	0.37
		FP_2	0.01*	0.38
		F_3	0.01*	0.41
		FC_3	0.01*	0.42
		FC_Z	<0.05*	0.46
		TP_7	0.01*	0.39
		CP_3	<0.001*	0.40
	Gamma (γ)	FP_1	0.02*	0.37
		FP_2	0.01*	0.41
		FC_3	0.02*	0.37
		C_3	0.01*	0.38
		TP_7	0.02*	0.37
		TP_8	0.01*	0.37
		P_7	0.01*	0.41
		P_Z	0.01*	0.42

Key:

SBP – systolic blood pressure
F – Frontal
T – Temporal
C – Central
P – Parietal
p – p-value
***** – statistical significance
r – rho value

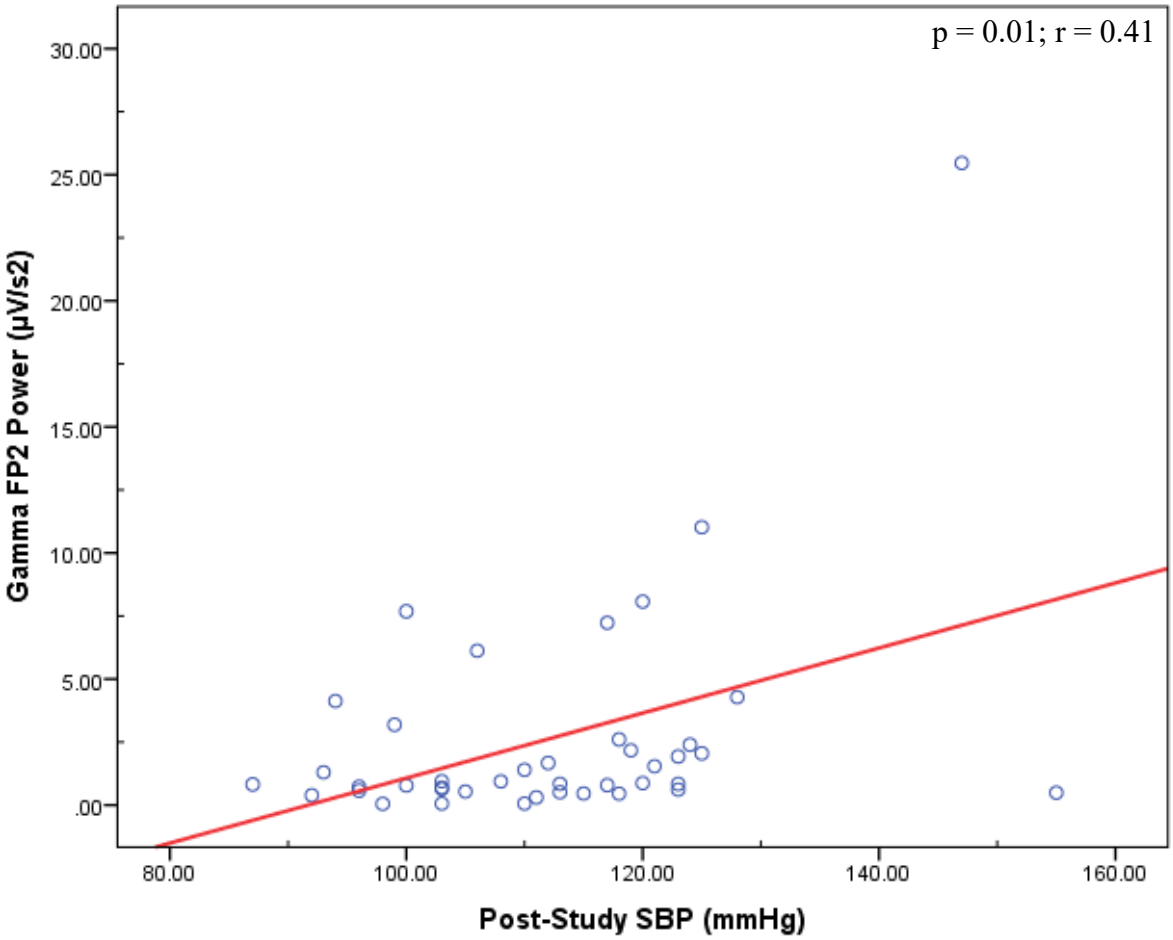


Figure 5.5. Positive correlation between post-study systolic blood pressure and gamma power at FP_2 during the active phase for the non-clinical group.

Key:

SBP – systolic blood pressure

P – Parietal

p – p-value

F – Frontal

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

5.3 Associations between pre-study DBP and EEG activity (non-clinical)

There were several significant associations between pre-study DBP and EEG variables during the baseline phase for alpha and gamma activity (Table 5.4). Pre-study DBP was associated with alpha activity at F_Z ($p = 0.02$; $r = 0.51$), FC_4 ($p = 0.02$; $r = 0.54$), T_7 ($p = 0.02$; $r = 0.51$), C_4 ($p = 0.04$; $r = 0.45$), TP_7 ($p = 0.01$; $r = 0.54$), CP_4 ($p = 0.02$; $r = 0.52$), and P_Z ($p < 0.05$; $r = 0.64$) (Figure 5.6). In contrast, pre-study DBP was associated with gamma activity at TP_7 ($p = 0.04$; $r = 0.40$), TP_8 ($p = 0.04$; $r = 0.40$), and P_7 ($p = 0.01$; $r = 0.44$). There were no significant associations between pre-study DBP and other EEG frequency bands (beta, theta, and delta) during the baseline phase.

Table 5.4. Associations between pre-study DBP and EEG activity during the baseline phase for the non-clinical group.

Independent variable	Dependent variable (Baseline)	Brain Area	p	r
Pre-Study DBP	Alpha (α)	F_Z	0.02*	0.51
		FC_4	0.02*	0.54
		T_7	0.02*	0.51
		C_4	0.04*	0.45
		TP_7	0.01*	0.54
		CP_4	0.02*	0.52
		P_Z	<0.05*	0.64
	Gamma (γ)	TP_7	0.04*	0.40
		TP_8	0.04*	0.40
		P_7	0.01*	0.44

Key:

DBP – diastolic blood pressure
F – Frontal
T – Temporal
C – Central
P – Parietal
* – statistical significance
p – p-value
r – rho value

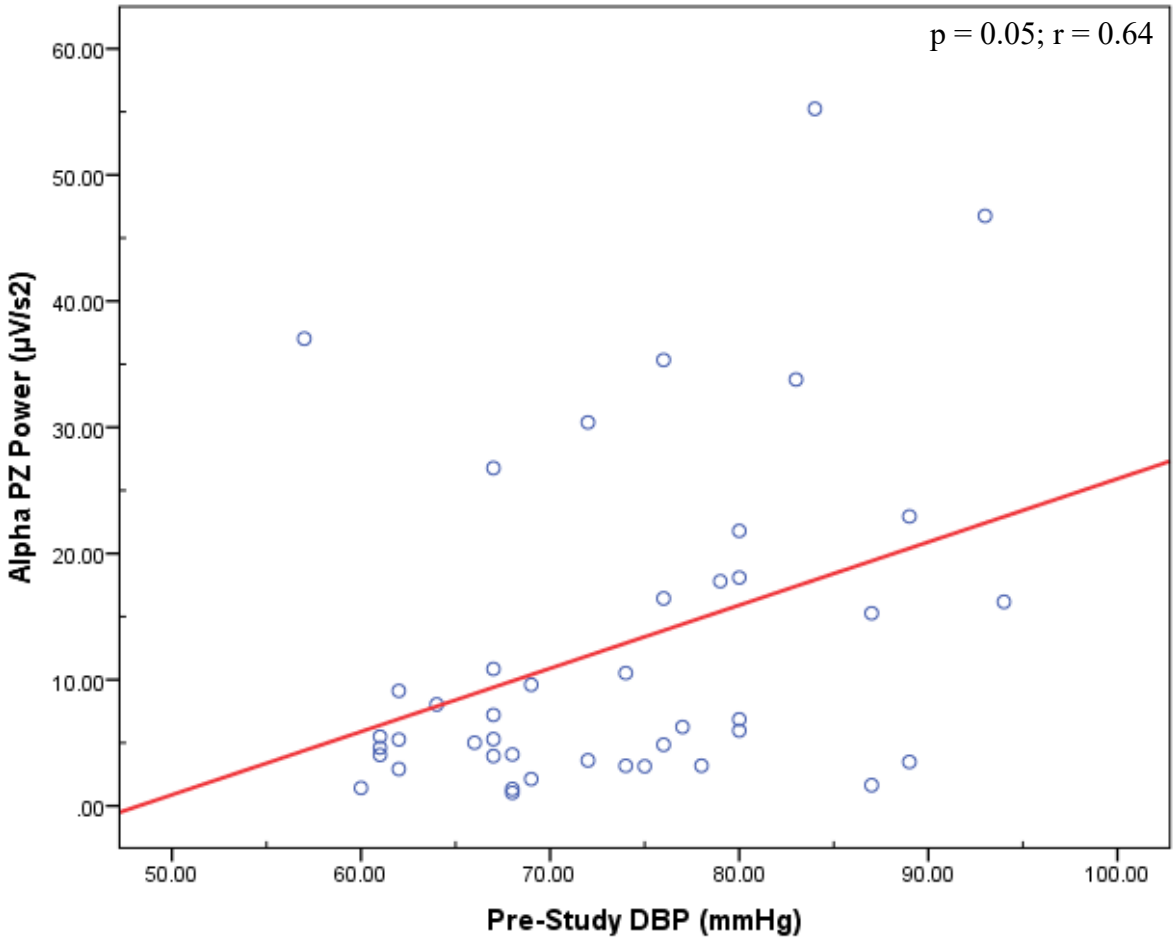


Figure 5.6. Positive correlation between pre-study diastolic blood pressure and alpha power at P_Z during the baseline phase for the non-clinical group.

Key:

DBP – diastolic blood pressure

P – Parietal

p – p-value

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

5.4 Associations between post-study DBP and EEG activity (non-clinical)

Several significant associations were found between post-study DBP and EEG activity during the baseline phase (Table 5.5). These links were found with gamma activity at multiple locations: F_4 ($p = 0.04$; $r = 0.40$), FC_4 ($p = 0.04$; $r = 0.39$), TP_7 ($p = 0.01$; $r = 0.46$), P_7 ($p = 0.02$; $r = 0.44$), TP_8 ($p = 0.01$; $r = 0.46$), and P_Z ($p = 0.01$; $r = 0.48$). No significant associations were found between post-study DBP and other EEG frequency bands (alpha, beta, theta, and delta) during the baseline phase.

Table 5.5. Associations between post-study DBP and EEG activity during the baseline phase for the non-clinical group.

Independent variable	**Dependent variable (Baseline)**	**Brain Area**	**p**	**r**
Post-Study DBP	Gamma (γ)	F_4	0.04*	0.40
		FC_4	0.04*	0.39
		TP_8	0.01*	0.46
		P_7	0.02*	0.44
		TP_8	0.01*	0.46
		P_Z	0.01*	0.48

Key:

DBP – diastolic blood pressure
C – Central
p – p-value
F – Frontal
P – Parietal
r – rho value
T – Temporal
***** – statistical significance

Multiple significant associations were found between post-study DBP and EEG variables during the active phase. These were mainly observed with fast-wave brain activities (alpha, beta, and gamma), although an association was also found with theta activity (Table 5.6). Partial Pearson's correlation revealed post-study DBP links with alpha activity at FC_3 ($p = 0.04$; $r = 0.32$) and O_2 ($p = 0.04$; $r = 0.33$), with beta activity at FC_3 ($p = 0.04$; $r = 0.32$) and FC_Z ($p = 0.02$; $r = 0.35$), and with gamma activity at FP_2 ($p = 0.03$; $r = 0.34$) (Figure 5.7) and FC_3 ($p = 0.04$; $r = 0.31$). Interestingly, for theta activity, an inverse link with post-study DBP was found at P_3 ($p = 0.01$; $r = - 0.39$). No significant associations were found between post-study DBP and delta activity.

Table 5.6. Associations between post-study DBP and EEG activity during the active phase for the non-clinical group

Independent variable	**Dependent variable (Active)**	**Brain Area**	**p**	**r**
Post-Study DBP	Alpha (α)	FC_3	0.04*	0.32
		O_2	0.04*	0.33
	Beta (β)	FC_3	0.04*	0.32
		FC_Z	0.02*	0.35
	Gamma (γ)	FP_2	0.03*	0.34
		FC_3	0.04*	0.31
	Theta (θ)	P_3	0.01*	- 0.39

Key:

DBP – diastolic blood pressure
F – Frontal
T – Temporal
C – Central
P – Parietal
O – Occipital
***** – statistical significance
p – p-value
r – rho value

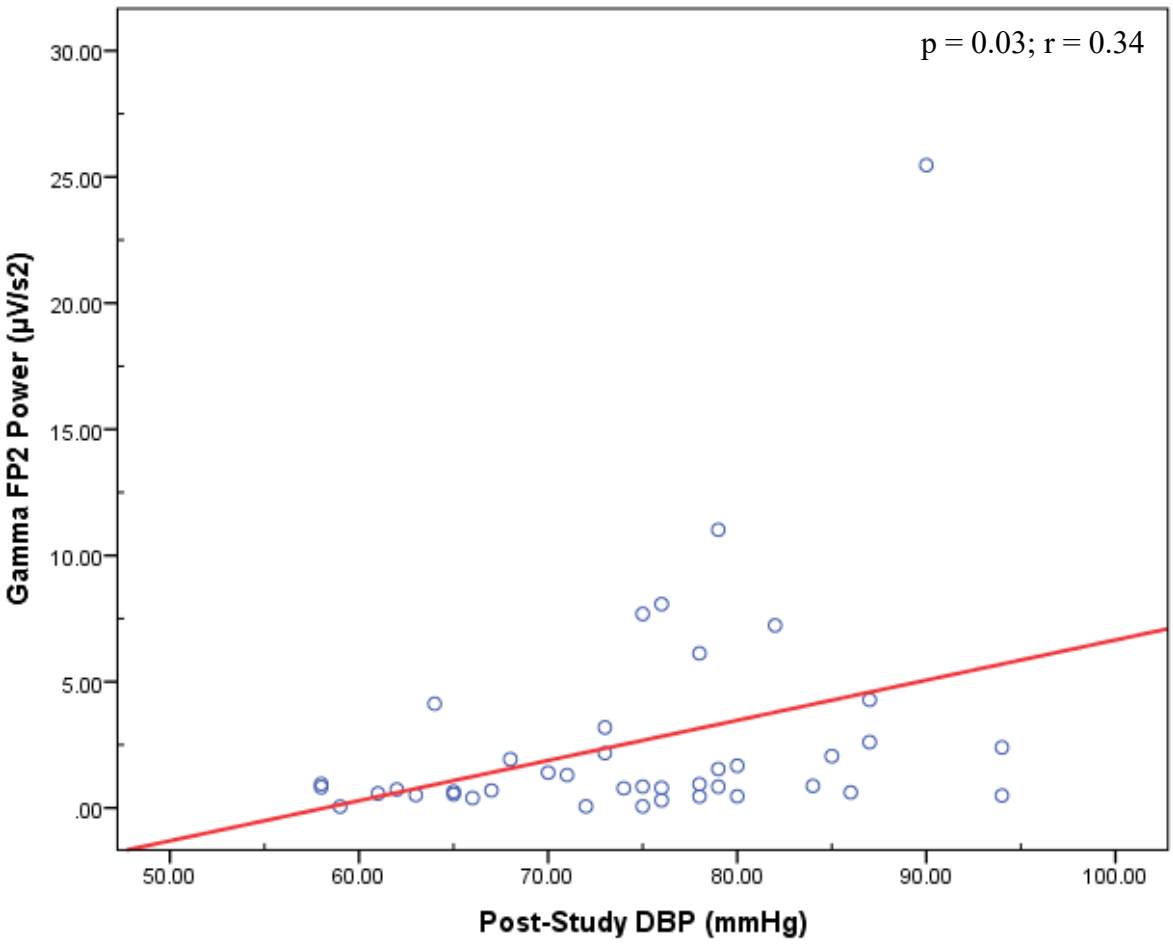


Figure 5.7. Positive correlation between post-study diastolic blood pressure and gamma power at FP_2 during the active phase for the non-clinical group.

Key:

DBP – diastolic blood pressure

F – Frontal

P – Parietal

r – rho value

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

p – p-value

5.5 Associations between pre-study BGL and EEG activity (non-clinical)

With respect to pre-study BGL and EEG activity, significant associations were found with beta activity at F_3 ($p = 0.03$; $r = 0.51$) (Figure 5.8) and F_4 ($p = 0.02$; $r = 0.53$) during the baseline phase (Table 5.7). However, there were no significant associations between pre-study BGL and other EEG variables (alpha, gamma, theta, and delta).

Table 5.7. Associations between pre-study BGL and EEG activity during the baseline phase for the non-clinical group.

Independent variable	**Dependent variable (Baseline)**	**Brain Area**	**p**	**r**
Pre-Study BGL	Beta (β)	F_3	0.03*	0.51
		F_4	0.02*	0.53

Key:

BGL – blood glucose level **F** – Frontal ***** – statistical significance

p – p-value **r** – rho value

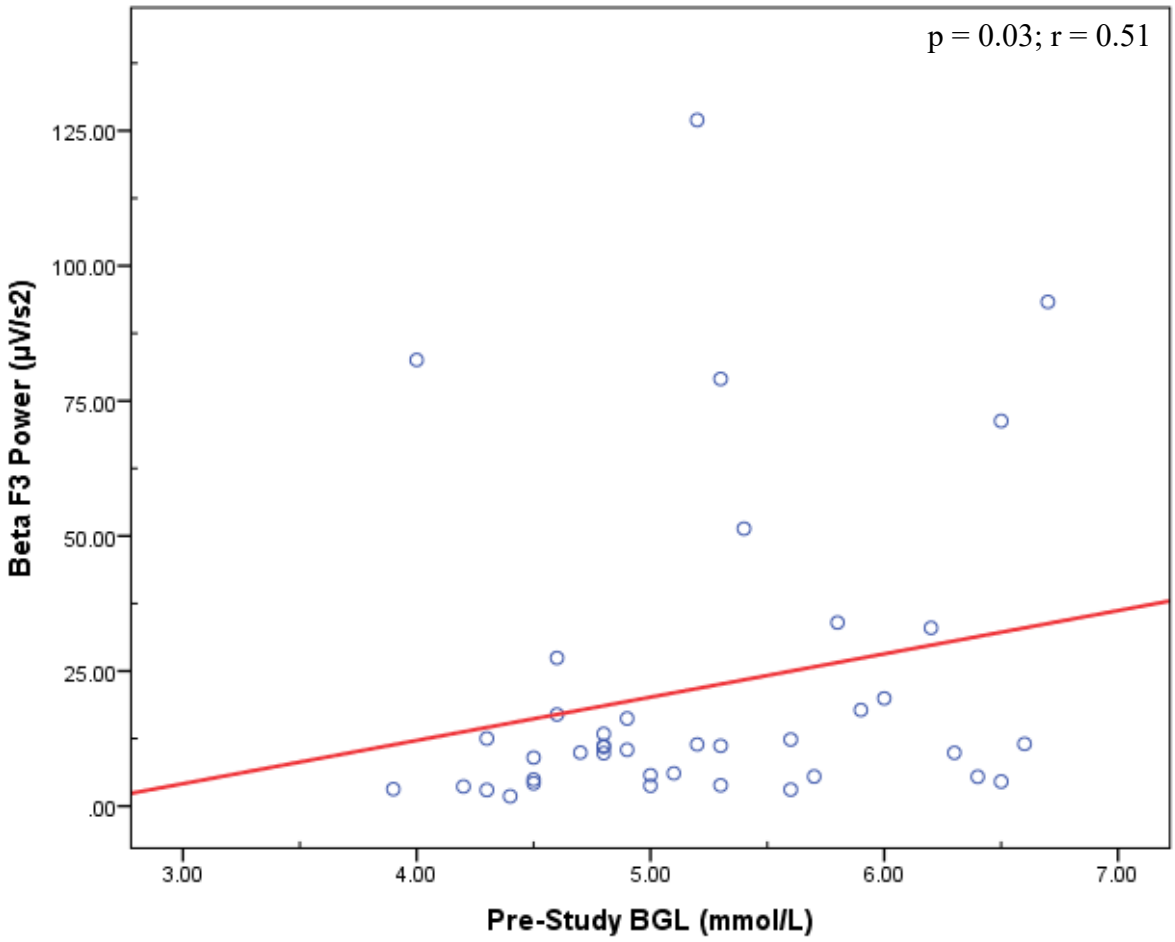


Figure 5.8. Positive correlation between pre-study blood glucose level and beta power at F_3 during the baseline phase for the non-clinical group.

Key:

BGL – blood glucose level

F – Frontal

p – p-value

mmol/L – millimoles per litre

µV/s² – microvolts per second squared

r – rho value

With respect to pre-study BGL and EEG activity during the active phase, significant inverse associations were identified with slow-wave brain activities (theta and delta) (Table 5.8). Pre-study BGL was inversely associated with theta activity at F_3 ($p = 0.04$; $r = -0.33$) and F_Z ($p = 0.02$; $r = -0.37$) (Figure 5.9), while for delta it was at P_7 ($p = 0.03$; $r = -0.34$). There were no significant associations between pre-study BGL and fast-wave brain activities (alpha, beta, and gamma).

Table 5.8. Associations between pre-study BGL and EEG activity during the active phase for the non-clinical group.

Independent variable	**Dependent variable (Active)**	**Brain Area**	**p**	**r**
Pre-Study BGL	Theta (θ)	F_3	0.04*	- 0.33
		F_Z	0.02*	- 0.37
	Delta (δ)	P_7	0.03*	- 0.34

Key:

BGL – blood glucose level **F** – Frontal **P** – Parietal

***** – statistical significance **p** – p-value **r** – rho value

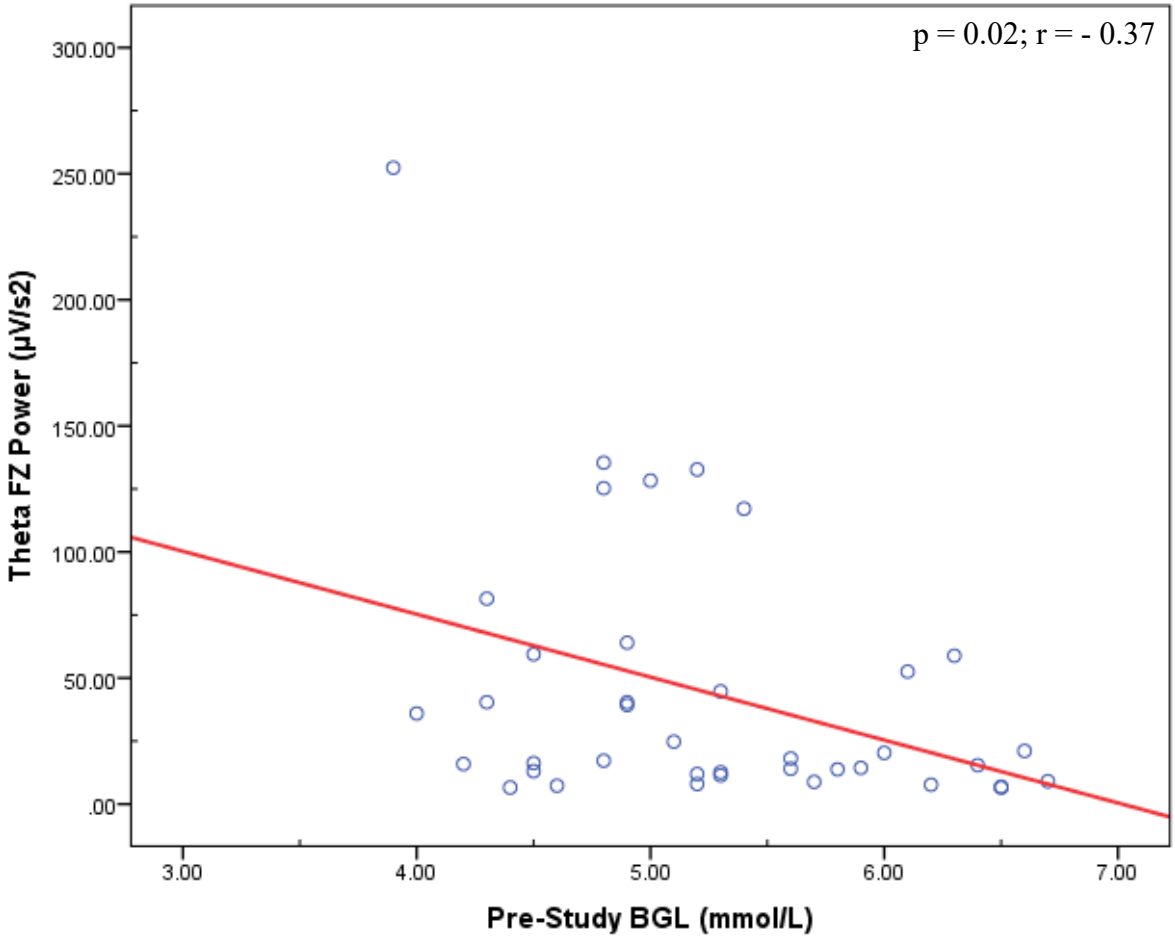


Figure 5.9. Negative correlation between pre-study blood glucose level and theta power at F_Z during the active phase for the non-clinical group.

Key:

BGL – blood glucose level

F – Frontal

p – p-value

mmol/L – millimoles per litre

µV/s² – microvolts per second squared

r – rho value

5.6 Associations between post-study BGL and EEG activity (non-clinical)

In contrast with pre-study BGL, no significant associations were found between post-study BGL and EEG variables during the baseline phase. However, during the active phase post-study BGL was significantly associated with theta activity at the following three locations: F_3 ($p = 0.01$; $r = -0.42$), F_Z ($p = 0.01$; $r = -0.43$), and C_Z ($p = 0.04$; $r = -0.34$) (Figure 5.10) (Table 5.9).

Table 5.9. Associations between post-study BGL and EEG activity during the active phase for the non-clinical group.

Independent variable	**Dependent variable (Active)**	**Brain Area**	**p**	**r**
Post-BGL	Theta (θ)	F_3	0.01*	- 0.42
		F_Z	0.01*	- 0.43
		C_Z	0.04*	- 0.34

Key:

BGL – blood glucose level **F** – Frontal **C** – Central

***** – statistical significance **p** – p-value **r** – rho value

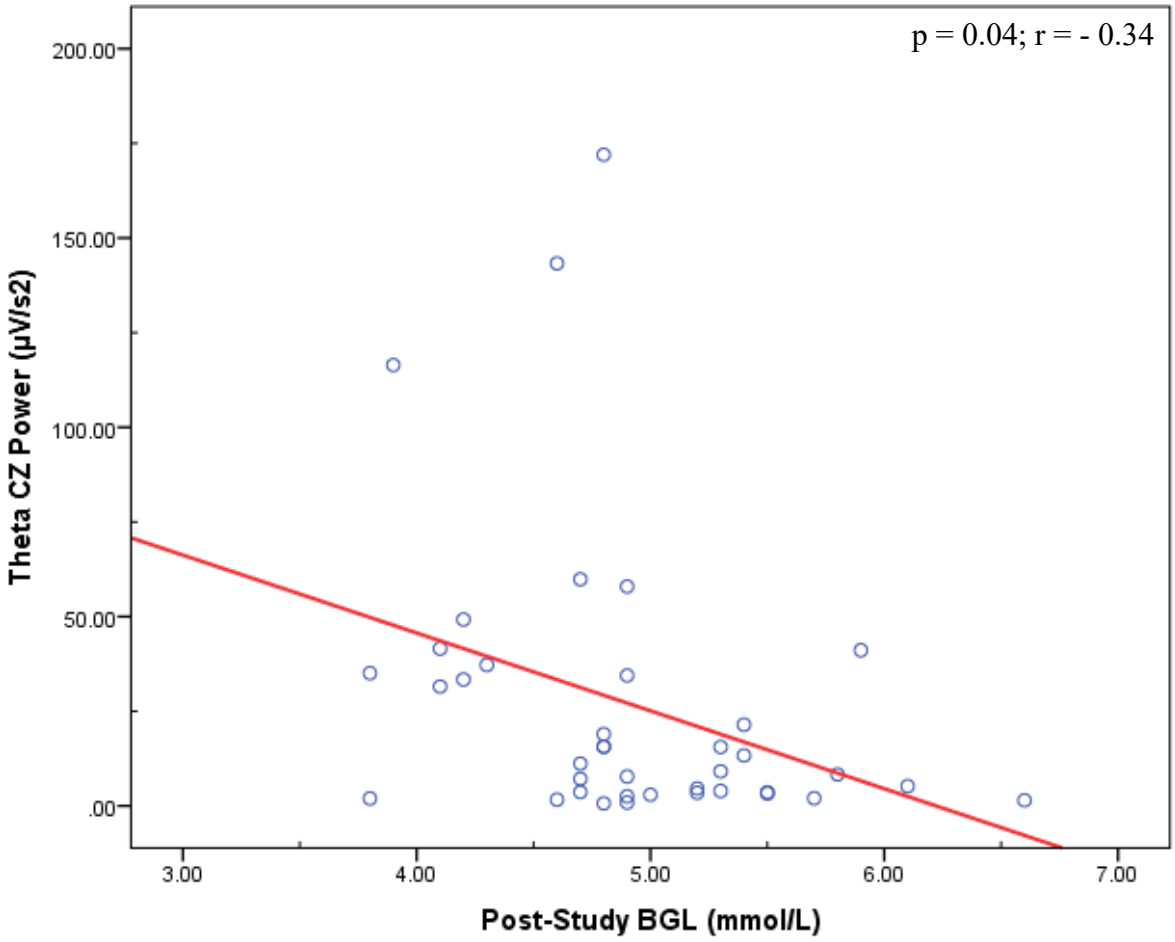


Figure 5.10. Negative correlation between post-study blood glucose level and theta power at C_Z during the active phase for the non-clinical group.

Key:

BGL – blood glucose level

C – Central

p – p-value

mmol/L – millimoles per litre

µV/s² – microvolts per second squared

r – rho value

5.7 Discussion: Associations between BP and BGL and Electroencephalography (EEG) (Non-Clinical)

This chapter discusses findings regarding the associations found between pre-study and post-study physiological variables (SBP, DBP, and BGL) and electroencephalography (EEG) data obtained during the baseline and active phases for the non-clinical group. The electroencephalography signal reflects ongoing electrical activity of underlying glucose and oxygen-dependent cortical pyramidal neurons, which are highly sensitive to small changes in BP and BGL (Jordan, 2004); therefore, the EEG represents a promising instrument for detecting changes in cognition linked to these variables.

5.7.1 Associations between BP and EEG

Data exploring associations between BP and EEG activity in normotensive individuals are lacking. This study is the first to report associations between BP (both SBP and DBP) and EEG activity across a broad age range in normotensive individuals using 32-channel EEG recording. It is also one of only a few investigations to explore associations between BGL and EEG activity during normoglycaemia. The main findings were: (1) BP was significantly correlated with fast-wave brain activities (alpha, beta, and gamma) over all cortical areas; and (2) BGL was significantly associated with fast-wave and slow-wave brain activities primarily over the frontal and central brain areas.

It is well established that synchronous neuronal activity reflects cognitive function (Uhlhaas & Singer, 2010; Hamm *et al.,* 2015; Modi & Sahin, 2017; Solomon *et al.,* 2017). The present analysis revealed that increasing SBP and DBP, which resided within the normotensive range (SBP: 112.5 ± 12.1 mm Hg; DBP: 73.7 ± 9.7 mm Hg), was associated with fast-wave brain activities (alpha, beta, and gamma), although more associations were identified for SBP. These associations for SBP and DBP were primarily observed over frontal brain regions, but others were found scattered across the cortex. This finding is novel and has not been reported previously. Although alpha activity is typically associated with idle brain states (Berger, 1929; Adrian & Matthews, 1954), several lines of evidence suggest they also underlie important cognitive processes, including attentional and perceptual tasks, information processing, semantic memory, and inhibitory control (Klimesch, 1997; Klimesch *et al.,* 2007; Zoefel *et al.,* 2011; Klimesch, 2012). Klimesch (1997) found increased alpha power correlated with memory and attention and suggested that alpha waves indicate proper thalamo-cortical function. It is

noteworthy that most of these cognitive processes are also subserved primarily by the frontal lobe (Cabeza & Nyberg, 1997; Wood & Grafman, 2003; Arnsten, 2009); thus, this may explain the alpha correlations observed predominantly in frontal brain areas in the present study.

Both SBP and DBP were also found to be significantly correlated with beta and gamma activity. These associations were localised chiefly over the frontal and central brain areas. This finding is novel but supports established literature that beta and gamma waves are cortically-generated rhythms (Fries, 2009; Campisi & LaRocca; Modi & Sahin, 2017). Beta and gamma rhythms have received considerable attention in the neurocognitive literature and several investigators have shown that beta and gamma waves reflect high-order cognitive processing, such as selective attention and consciousness (Fries, 2009; Campisi & LaRocca; Modi & Sahin, 2017). Conversely, declines in beta and gamma power have been reported in subjects with cognitive dysfunction and/or cognitive impairment (AD, dementia) (Jelic *et al.,* 1996; Huang *et al.,* 2000; Jeong, 2004). Thus, the significant associations identified between SBP and DBP and beta and gamma activity imply that BP in the normotensive range augments coordinated rhythmic activity of cortical neurons responsible for generating fast-wave activities, potentially resulting in enhanced cortical activation.

Interestingly, fewer associations were identified between DBP and electroencephalography activity than between SBP and EEG activity. This finding has not been reported in prior studies and potentially raises the possibility that SBP exerts stronger and more profound effects on cortical activity than DBP. In agreement with this, evidence obtained from cross-sectional and longitudinal studies has consistently shown that mid-life SBP is a strong predictor of long-term cognitive outcomes (Kilander *et al.,* 1998; Swan *et al.,* 1998; Yaffe *et al.,* 2014). However, in the seminal expert review of Iadecola *et al.* (2016), the authors acknowledge that not all studies have robustly compared evidence between SBP and DBP and cognition. The present analysis also found a weak, inverse association between DBP and parietal theta activity, suggesting that DBP, not SBP, may detrimentally affect vulnerable neuronal populations. Consistent with this observation, Taylor *et al.* (2011) assessed the independent effects of SBP and DBP on mortality in a large sample population (n = 13, 792, mean age: not reported) and found that DBP was a stronger predictor of mortality in younger adults than SBP. The mean age

of the non-clinical group in the present study was relatively young (31.3 ± 15.8 years); hence, this may have explained the outcome obtained. However, it is noteworthy that no other studies to date have examined associations between DBP and brain electrical activity in normotensive individuals. Therefore, this area of research warrants further investigation.

The literature is not particularly helpful in elucidating the mechanisms underlying the neurophysiological changes associated with BP. This is likely due to a lack of studies exploring links between BP and EEG activity. Although mechanisms have been proposed, multiple lines of evidence suggest that changes in cerebral blood flow (CBF) influence oscillatory activity. Cerebral blood flow, which is regulated by highly-specialised neurovascular units (NVUs), ensures underlying cortical pyramidal neurons receive a continuous, uninterrupted supply of both glucose and oxygen for optimum metabolic and electrical function (Hossmann, 1994; Foreman & Claassen, 2012; George & Steinberg, 2015; Sweeney *et al.,* 2018). Cerebral blood flow has also been closely linked to EEG activity (Figure 5.11); increased fast-wave EEG activity has been reported when CBF is in the normal range (35-50mL/100g/min), whereas declines in CBF have been correlated with increased slow-wave activity (Hossmann, 1994; Jordan, 2004). Other studies have also suggested that BP influences EEG activity *via* afferent fibres projecting to brain regions involved in high-order cognitive operations, such as the prefrontal cortex (PFC) and anterior cingulate (Goldstein & Silverman, 2006; Duschek *et al.,* 2007). These brain areas play important roles in orchestrating complex cognitive functions, such as attention and executive function, which have been consistently linked to fast-wave brain activities (Fries, 2009; Campisi & LaRocca, 2014; Modi & Sahin, 2017). Given the BP (SBP and DBP) of the non-clinical population assessed fell within the normal range, it is conceivable that BP in the normotensive range is associated with normal, uninterrupted CBF, which promotes reliable neuronal signalling and enhanced cortical activation.

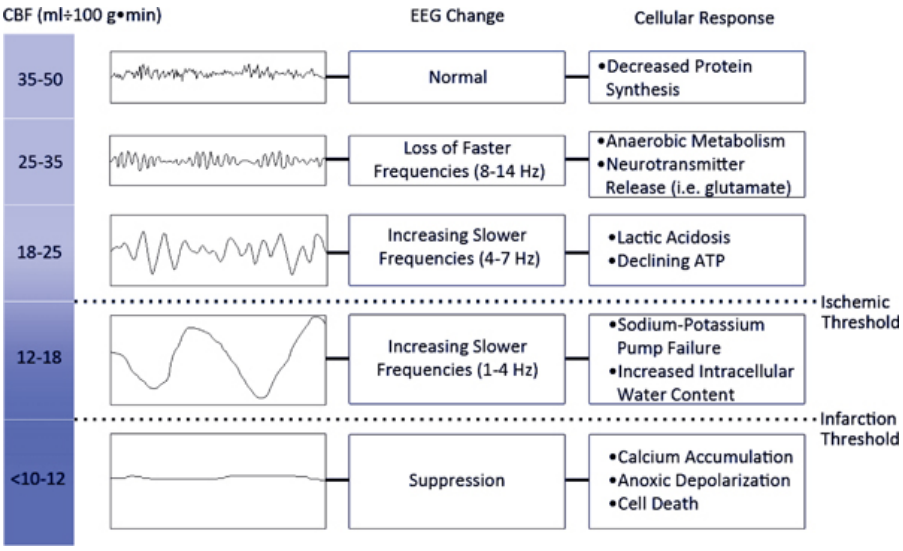


Figure 5.11. The relationship between cerebral blood flow and electroencephalography activity. Cerebral blood flow in the normal range is associated with predominantly normal EEG activity, whereas declines in CBF have been linked to increased slow-wave activity. Adapted from Foreman & Claassen, (2012, p 2).

5.7.2 Associations between BGL and EEG activity

Evidence that blood glucose levels (BGL) could influence EEG activity was first reported by Ross & Loeser (1951). Numerous studies have since demonstrated (primarily in clinical populations – T1DM, T2DM) that glycaemic fluctuations (*i.e.* hypo and hyperglycaemia) are associated with noticeable changes in cerebral electrical activity (Ross & Loeser, 1951; Tallroth *et al.,* 1990; Cox *et al.,* 2005; Rachmiel *et al.,* 2016). The main changes reported in these studies are (1) sharp increases in slow-wave activity (delta and theta) and (2) diminished fast-wave activity (alpha, beta, and gamma). These pronounced changes have been primarily observed over anterior brain regions and parieto-occipital areas, respectively, although some studies have found them diffusely distributed across the cortex. While the neurophysiological changes associated with glycaemic events have been explored, few studies have reported associations between BGL and EEG activity during euglycaemia in non-clinical groups.

The present analysis showed that increasing BGL, within the euglycaemic range, was significantly associated with beta activity in frontal brain regions. This finding is consistent with evidence obtained from similar studies (Tallroth *et al.,* 1990; Rachmiel *et al.,* 2016). The literature indicates that the EEG signal is dominated by fast-frequency, low amplitude oscillations (alpha, beta, gamma) during euglycaemia, suggesting continuous glucose supply to underlying cortical pyramidal neurons promotes enhanced fast-wave neural activity (Elsborg *et al.,*1990) (Figure 5.12). Cortical pyramidal neurons are known to be highly sensitive to perturbations in BGL and oxygen (Jordan, 2004) and it is well established that neural tissue requires a continuous, uninterrupted supply of glucose for optimal functioning (Sweeney *et al.,* 2018). Although beta oscillations typically underlie sensorimotor functions (Modi & Sahin, 2017), they have also been implicated in various high-order cognitive operations, including attention (Engel *et al.,* 2000; Campisi & LaRocca, 2014), language processing (Weiss & Muller, 2012), working memory, (Deiber *et al.,* 2007), and hedonic processing (Marco-Pallares *et al.,* 2015). Consensus exists in the literature that beta waves are also cortically generated oscillations, especially in frontal areas; hence, this may explain the correlations observed in the frontal region in the present study (Modi & Sahin, 2017).

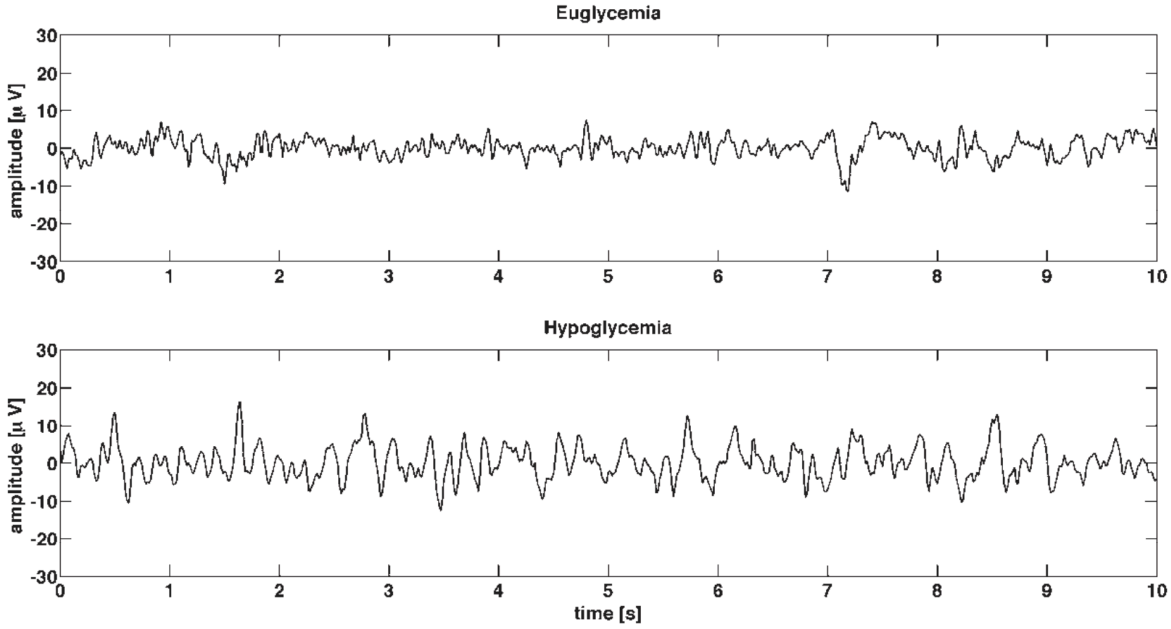


Figure 5.12. An example of an electroencephalogram tracing recorded during euglycaemia and hypoglycaemia in the same individual. Adapted from Elsborg *et al.,* (1990, p 277).

The finding that euglycaemia was inversely associated with slow-wave brain activities (theta and delta) in frontal and central brain regions in the present study also supports prior literature (Tallroth *et al.,* 1990; Brismar *et al.,* 2002; Rachmiel *et al.,* 2016) and implies that neuronal populations are not deprived of glucose. A global loss of slow-wave activity has been consistently reported during euglycaemia (Tallroth *et al.,* 1990; Bjorgaas *et al.,* 1998; Brismar *et al.,* 2002). Slowing of EEG activity has been attributed to a shift in energy substrate (*e.g.* from glucose to amino acids and/or lactate) (Beall *et al.,* 2012) and a reduction in cerebral glucose metabolism (Lewis *et al.,* 1974). Increased slow-wave activity has also been repeatedly correlated with cognitive dysfunction and cognitive impairment. In a 2.5-year longitudinal investigation, Coben *et al.* (1985) reported increased theta and delta activity and diminished alpha and beta activity in patients with Alzheimer's Disease (AD). Similarly, Giaquinto & Nolfe (1986) observed comparable changes in EEG activity in a cross-sectional investigation in subjects with dementia (n = 47, mean age 71 ± 5 yrs, 32 male, 15 female). Similar electrophysiological abnormalities have also been observed in patients with mild cognitive impairment (MCI) (Jelic *et al.,* 2000). Thus, data from the current study indicate that euglycaemia supports

optimal metabolic and electrical activities of sensitive underlying cortical pyramidal neurons, resulting in enhanced fast-wave brain activity.

The inverse association reported between euglycaemia and slow-wave actvities in the present study further supports the glycaemic thresholds proposed by prior studies. The EEG has been shown to be characterised by slow-wave activity when BGL is low (Pramming *et al.,* 1988; Tallroth *et al.,* 1990; Bjorgaas *et al.,* 1998; Hyllienmark *et al.,* 2005) or abnormally elevated (Rachmiel *et al.,* 2016). This pattern is widely thought to reflect cortical dysfunction (Elsborg *et al.,* 1990). Although hypoglycaemia is associated with a slowing of brain activity, the blood glucose threshold at which marked changes in EEG activity occur remains controversial. For example, some researchers have found detectable increases in slow-wave activity at 2 mmol/L (Pramming *et al.,* 1988), whereas others have reported changes at lower concentrations (1.8 mmol/L) (Tallroth *et al.,* 1990). Others suggest these changes vary significantly between individuals and occur between the concentration range of 1.6 – 3.4 mmol/L (Amiel *et al.,* 1991; Juhl *et al.,* 2010). However, the findings of the present study clearly indicate that the electroencephalography signal is sensitive to changes in BGL and can detect changes in cognitive activity linked to both BP and BGL.

5.8 Conclusions

The present analysis determined consistent associations between BP and BGL and oscillatory brain activity in a non-clinical population using non-invasive scalp electroencephalography. Various associations were found, suggesting that both variables, even in their normal range, influence ongoing cortical electrical activity. Several associations were also identified in post-study SBP, DBP, and BGL, indicating that small changes in these variables affect ongoing oscillatory brain activity. While changes in BGL are understood to directly affect the metabolic activities of sensitive glucose-dependent cortical pyramidal neurons, the mechanisms underlying BP-associated changes in EEG activity remain less clear and are yet to be fully understood. Current literature suggests that cerebral blood flow and reliable signalling to cognitively advanced brain regions, such as the prefrontal cortex and anterior cingulate cortex, may explain the observed EEG activity (Hossmann, 1994; Jordan, 2004). Neurochemical mechanisms have also been implicated (Gomèz *et al.,* 2004), although consensus in the literature is lacking. Another largely unexplored question is whether SBP or DBP elicits stronger effects on cortical activity. Therefore, this area warrants further investigation.

Although this study provides preliminary insight into the associations between BP, BGL and EEG activity, it is clear that larger, adequately-powered investigations (cross-sectional and longitudinal) are warranted for better elucidation of the EEG changes linked to these variables, especially BP. Ample evidence suggests the brain becomes vulnerable to metabolic insult/damage at specific glycaemic thresholds (Pramming *et al.,* 1988; Bjorgaas *et al.,* 1998; Tallroth *et al.,* 1990; Hyllienmark *et al.,* 2005) but thresholds for BP remain controversial. Given cognitive function broadly influences workplace productivity, public safety, mental wellbeing, and everyday activities, this could have broader implications, such as determining thresholds that support optimal cognitive performance and those that cause deterioration in cognitive function.

6. Associations between Blood Pressure, Blood Glucose Level and Electroencephalography (Clinical)

This chapter reports associations between BP (SBP and DBP), BGL and EEG variables (delta, theta, alpha, beta, and gamma) during the baseline and active phases for the clinical groups (T1DM, T2DM, and HTN). Associations found between disease-specific variables (disease duration and $HbA1_C$ level) and EEG activity, are also reported. The findings are presented commencing with links to pre-study variables followed by links to post-study variables. A Spearman's rank-order correlation was applied to determine associations between pre-study and post-study physiological variables (SBP, DBP, and BGL) and EEG activity (baseline and active phases) for all clinical groups.

6.1 Type 1 Diabetes Mellitus

6.1.1 Associations between pre-study SBP and EEG activity (T1DM)

Pre-study SBP was found to be significantly associated with beta and theta wave activity during the baseline phase (Table 6.1). For beta activity, these links with pre-study SBP were found at TP_8 ($p = 0.04$; $r = - 0.74$), whereas for theta activity it was found at CP_Z ($p = 0.04$; $r = 0.64$). There were no significant associations between pre-study SBP and the other EEG frequency bands (alpha, gamma, and delta).

Table 6.1. Associations between pre-study SBP and EEG activity during the baseline phase for the T1DM group.

Independent variable	Dependent variable (Baseline)	Brain Area	p	r
Pre-Study SBP	Beta (β)	TP_8	0.04*	- 0.74
	Theta (θ)	CP_Z	0.04*	0.64

Key:

SBP – systolic blood pressure
T – Temporal
P – Parietal
C – Central
p – p-value
r – rho value
* – statistical significance

Conversely, several significant associations were found between pre-study SBP and EEG variables during the active phase, mainly for slow-wave brain activities (theta and delta) (Table 6.2). Pre-study SBP links were inversely associated with theta activity at the following locations: F_4 ($p = 0.03$; $r = - 0.68$), FT_7 ($p < 0.05$; $r = - 0.83$) (Figure 6.1), T_7 ($p < 0.05$; $r = - 0.82$), P_7 ($p = 0.02$; $r = - 0.68$), and O_1 ($p = 0.01$; $r = - 0.80$). Inverse links were also found with delta activity at F_7 ($p = 0.02$; $r = - 0.72$), F_3 ($p < 0.001$; $r = - 0.90$), FT_7 ($p < 0.05$; $r = - 0.80$) (Figure 6.2), T_7 ($p < 0.001$; $r = - 0.85$), T_8 ($p = 0.02$; $r = - 0.73$), and O_1 ($p = 0.04$; $r = - 0.68$). No significant associations were found between pre-study SBP and fast-wave activities (alpha, beta, and gamma) during the active phase.

Table 6.2. Associations between pre-study SBP and EEG activity during the active phase in the T1DM group.

Independent variable	Dependent variable (Active)	Brain Area	p	r
Pre-Study SBP	Theta (θ)	F_4	0.03*	- 0.68
		FT_7	<0.05*	- 0.83
		T_7	<0.05*	- 0.82
		P_7	0.02*	- 0.68
		O_1	0.01*	- 0.80
	Delta (δ)	F_7	0.02*	- 0.72
		F_3	<0.001*	- 0.90
		FT_7	<0.05*	- 0.80
		T_7	<0.001*	- 0.85
		T_8	0.02*	- 0.73
		O_1	0.04*	- 0.68

Key:

SBP – systolic blood pressure
F – Frontal
T – Temporal
P – Parietal
C – Central
O – Occipital
* – statistical significance
p – p-value
r – rho value

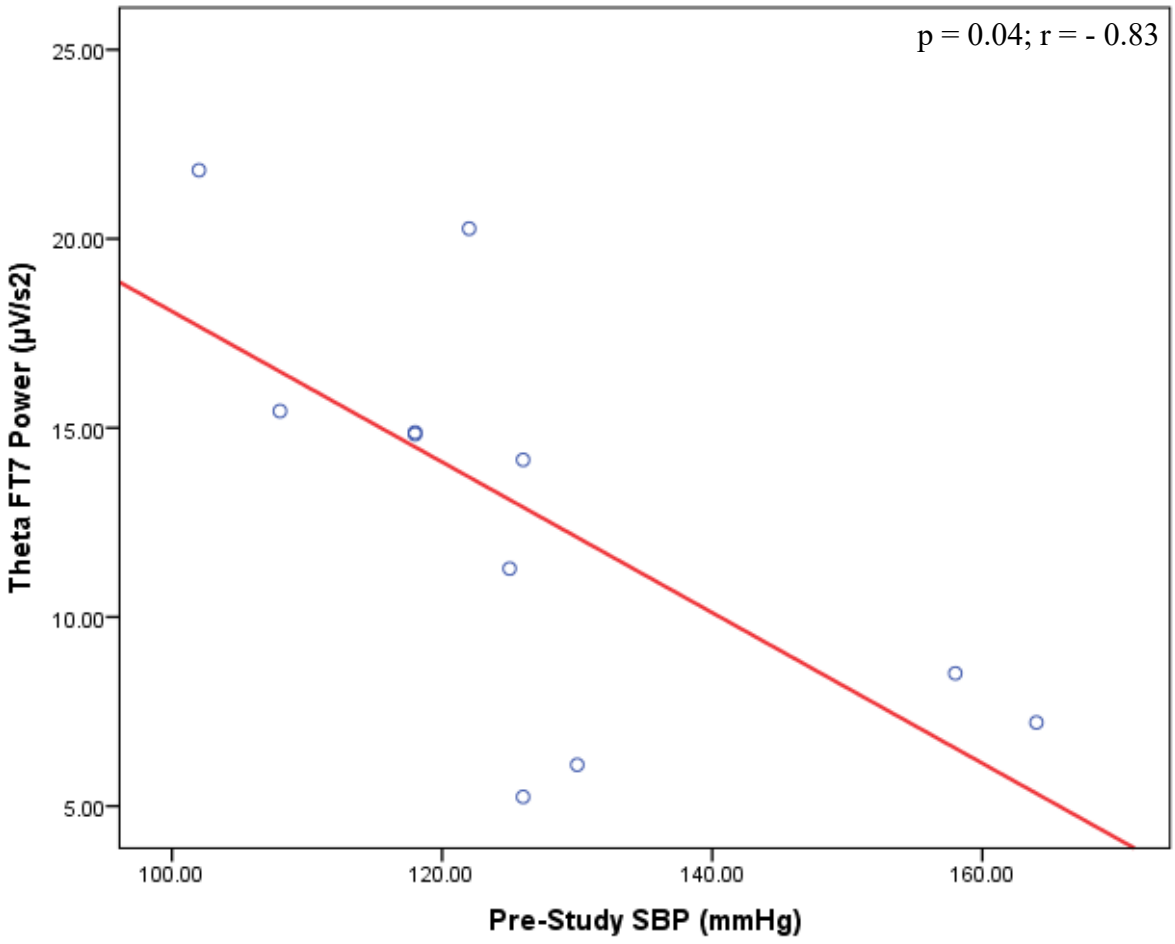


Figure 6.1. Negative correlation between pre-study systolic blood pressure and theta power at FT_7 during the active phase for the Type 1 diabetes mellitus group.

Key:

SBP – systolic blood pressure

F – Frontal

p – p-value

T – Temporal

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

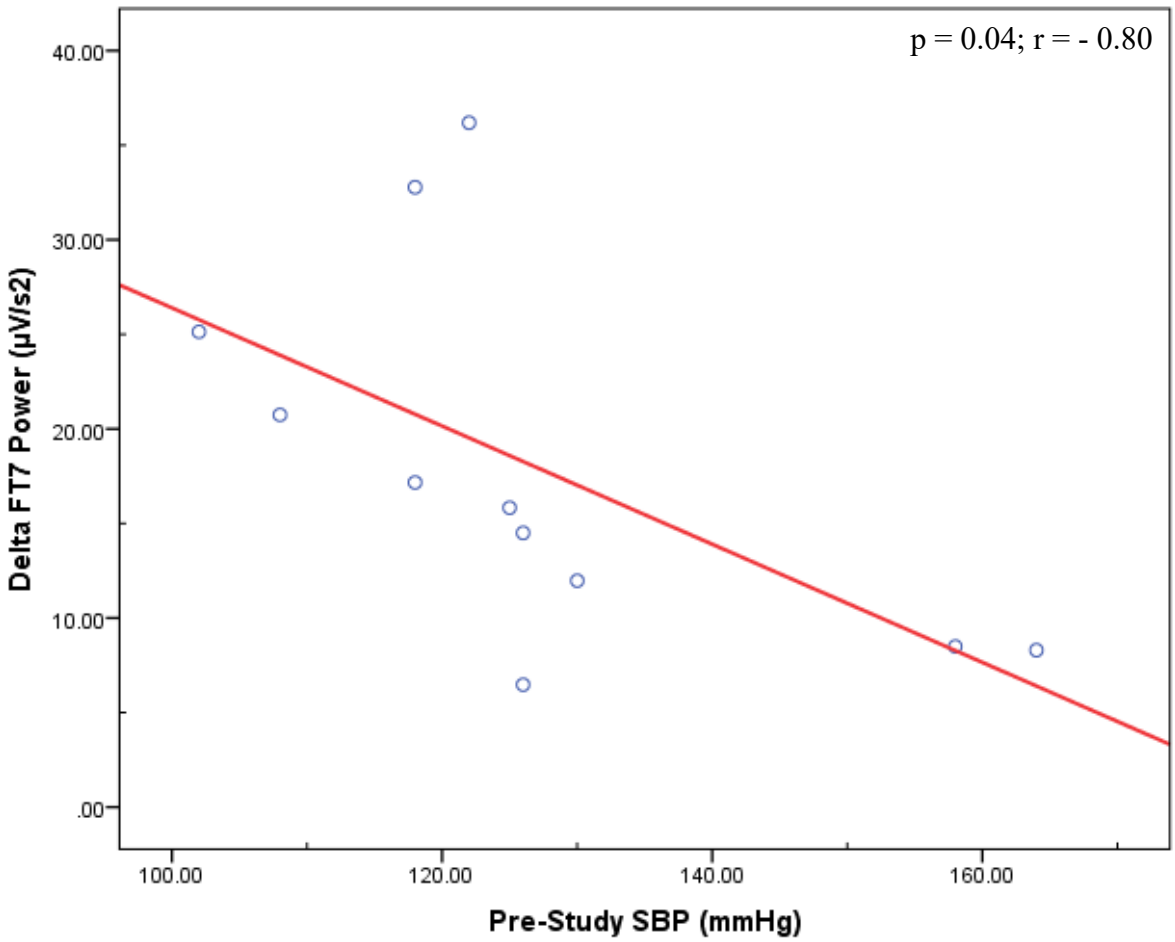


Figure 6.2. Negative correlation between pre-study systolic blood pressure and delta power at FT_7 during the active phase for the Type 1 diabetes mellitus group.

Key:

SBP – systolic blood pressure

F – Frontal

p – p-value

T – Temporal

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

6.1.2 Associations between pre-study DBP and EEG activity (T1DM)

There were several significant associations between pre-study DBP and EEG activity during the baseline phase (Table 6.3). For gamma activity, a link with pre-study DBP was found only at P_3 ($p = 0.01$; $r = 0.69$). In contrast, inverse associations with theta activity were found at FT_7 ($p = 0.01$; $r = -0.70$) (Figure 6.3), T_7 ($p = 0.03$; $r = -0.62$), CP_Z ($p = 0.03$; $r = -0.65$), and O_2 ($p = 0.01$; $r = -0.73$). No significant associations were found between pre-study DBP and the other EEG frequency bands (alpha, beta, and delta) during the baseline phase.

Table 6.3. Associations between pre-study DBP and EEG activity during the baseline phase for the T1DM group.

Independent variable	**Dependent variable (Baseline)**	**Brain Area**	**p**	**r**
Pre-Study DBP	Gamma (γ)	P_3	0.01*	0.69
	Theta (θ)	FT_7	0.01*	- 0.70
		T_7	0.03*	- 0.62
		CP_Z	0.03*	- 0.65
		O_2	0.01*	- 0.73

Key:

DBP – diastolic blood pressure
F – Frontal
T – Temporal
P – Parietal
C – Central
O - Occipital
* – statistical significance
p – p-value
r – rho value

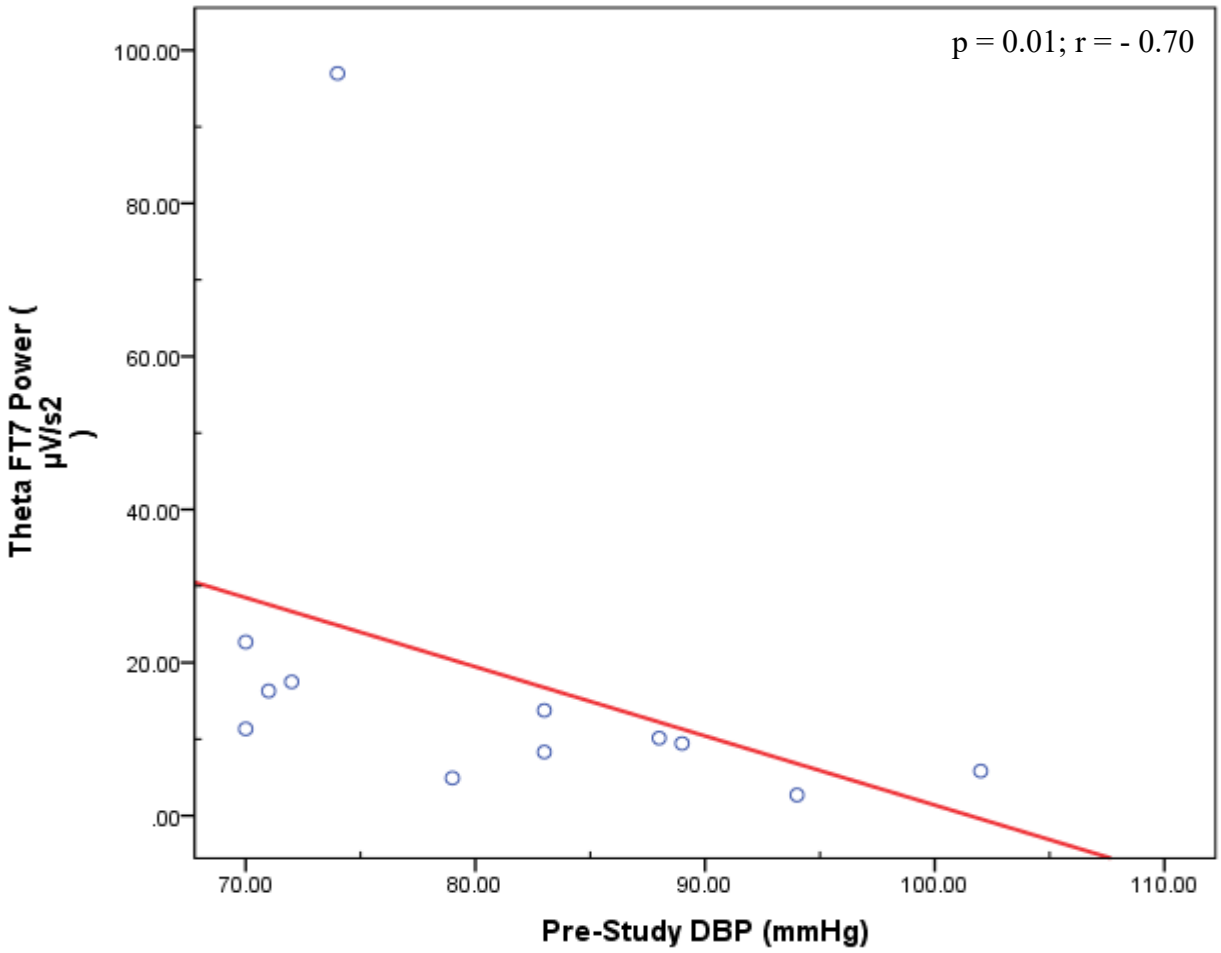


Figure 6.3. Negative correlation between pre-study diastolic blood pressure and theta power at FT_7 during the baseline phase for the Type 1 diabetes mellitus group.

Key:

DBP – diastolic blood pressure

mm Hg – millimetres of mercury

F – Frontal

T – Temporal

p – p-value

r – rho value

µV/s² – microvolts per second squared

In the active phase, a few significant associations were found between pre-study DBP and theta activity (Table 6.4). These pre-study DBP links were found at F_4 ($p = 0.01$; $r = -0.81$), O_Z ($p = 0.04$; $r = -0.66$), and O_2 ($p = 0.02$; $r = -0.68$). However, no significant associations were found between pre-study DBP and the other EEG frequency bands (alpha, beta, gamma, and delta) during the active phase.

Table 6.4. Associations between pre-study DBP and EEG activity during the active phase for the T1DM group.

Independent variable	**Dependent variable (Active)**	**Brain Area**	**p**	**r**
Pre-Study DBP	Theta (θ)	F_4	0.01*	- 0.81
		O_Z	0.04*	- 0.66
		O_2	0.02*	- 0.68

Key:

DBP – diastolic blood pressure
F – Frontal
O – Occipital
* – statistical significance
p – p-value
r – rho value

6.1.3 Associations between pre-study BGL and EEG activity (T1DM)

Significant associations were found between pre-study BGL and EEG activity during the baseline phase (Table 6.5). These pre-study BGL links were found with theta activity at FP_1 ($p = 0.02$; $r = 0.68$) (Figure 6.4) and O_Z ($p = 0.02$; $r = 0.64$). However, there were no significant associations between pre-study BGL and the other EEG variables (alpha, beta, gamma, and delta) during the baseline phase.

Table 6.5. Associations between pre-study BGL and EEG activity during the baseline phase for the T1DM group.

Independent variable	**Dependent variable (Baseline)**	**Brain Area**	**p**	**r**
Pre-Study BGL	Theta (θ)	FP_1	0.02*	0.68
		O_Z	0.02*	0.64

Key:

BGL – blood glucose level **F** – Frontal **P** – Parietal

O – Occipital **p** – p-value **r** – rho value

* – statistical significance

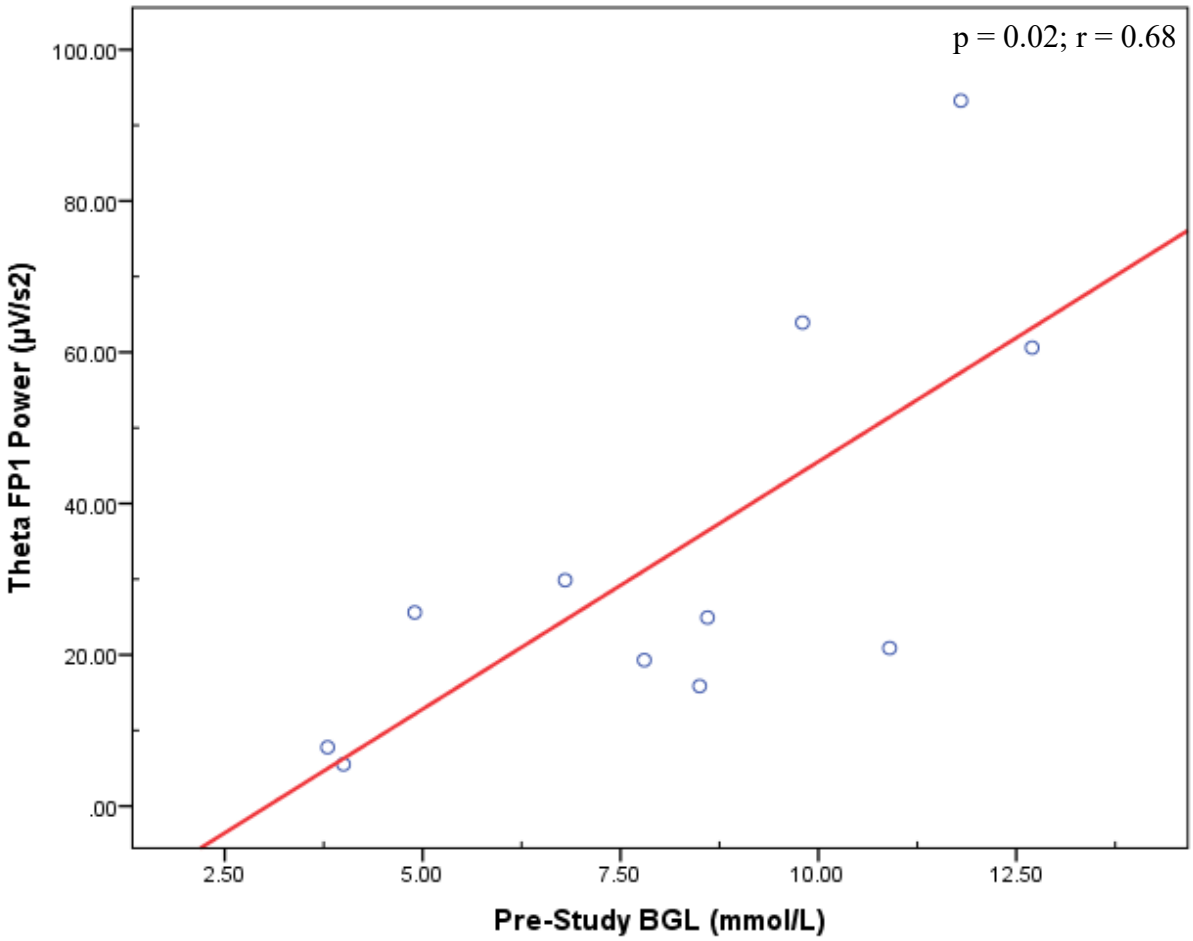


Figure 6.4. Positive correlation between pre-study blood glucose level and theta power at FP_1 during the baseline phase for the Type 1 diabetes mellitus group.

Key:

BGL – blood glucose level

F – Frontal

P – Parietal

r – rho value

mmol/L – millimoles per litre

µV/s² – microvolts per second squared

p – p-value

Similarly, pre-study BGL was significantly associated with theta activity during the active phase at the same two locations (Table 6.6) of FP_1 ($p = 0.03$; $r = 0.72$) and O_Z ($p = 0.02$; $r = 0.71$) (Figure 6.5). Pre-study BGL was also significantly associated with delta activity at TP_8 ($p = 0.02$; $r = 0.72$). No significant associations were found between pre-study BGL and fast-wave activities (alpha, beta, and gamma) during the active phase.

Table 6.6. Associations between pre-study BGL and EEG activity during the active phase for the T1DM group.

Independent variable	**Dependent variable (Active)**	**Brain Area**	**p**	**r**
Pre-Study BGL	Theta (θ)	FP_1	0.03*	0.72
		O_Z	0.02*	0.71
	Delta (δ)	TP_8	0.02*	0.72

Key:

BGL – blood glucose level
F – Frontal
P – Parietal
T – Temporal
O – Occipital
* – statistical significance
p – p-value
r – rho value

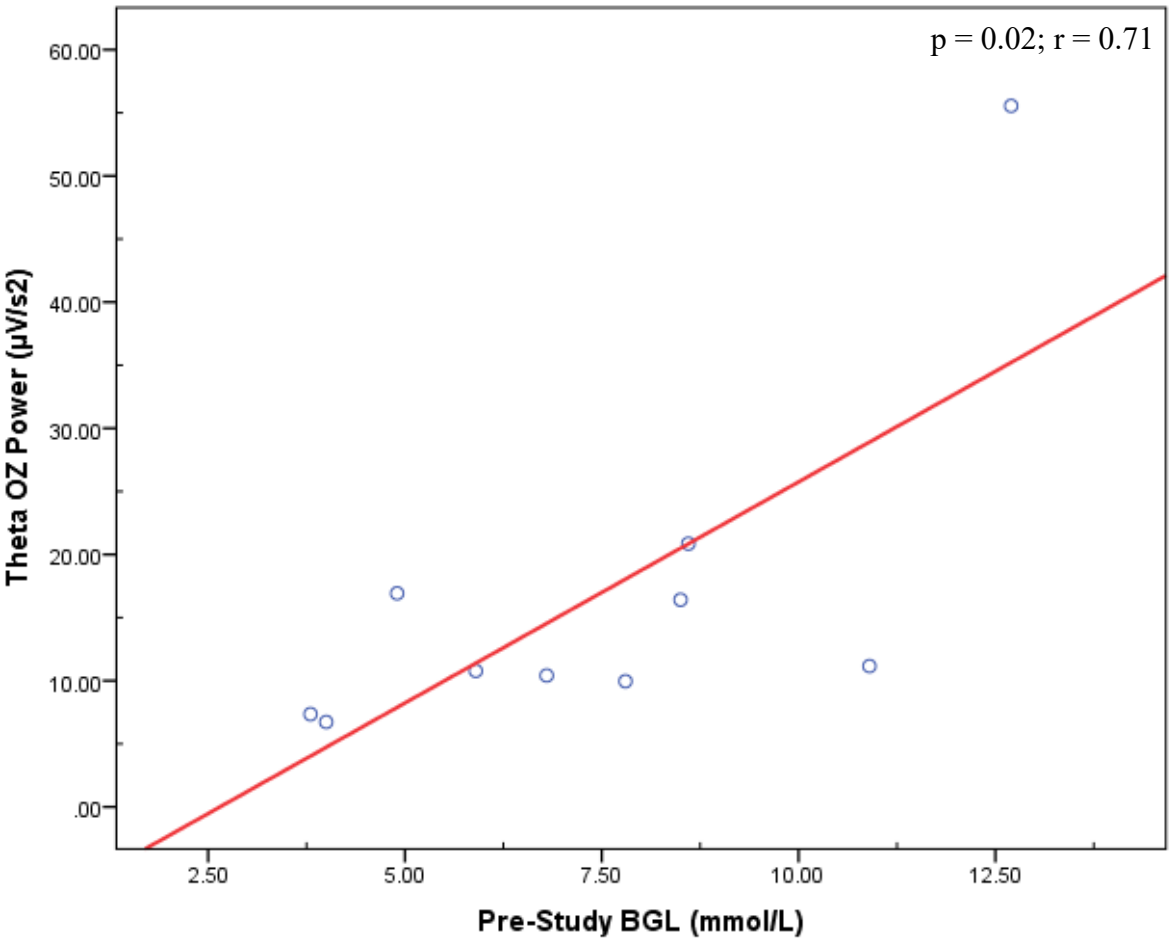


Figure 6.5. Positive correlation between pre-study blood glucose level and theta power at O_Z during the active phase for the Type 1 diabetes mellitus group.

Key:

BGL – blood glucose level

O – Occipital

p – p-value

mmol/L – millimoles per litre

µV/s² – microvolts per second squared

r – rho value

6.1.4 Associations between post-study SBP and EEG activity (T1DM)

A significant association was found between post-study SBP and EEG activity during the baseline phase (Table 6.7). An inverse link was observed between post-study SBP and theta activity at P_3 ($p = 0.02$; $r = -0.66$). No significant associations were found between post-study SBP and other EEG variables (alpha, beta, gamma, and delta) during the baseline phase.

Table 6.7. Associations between post-study SBP and EEG activity during the baseline phase for the T1DM group.

Independent variable	Dependent variable (Baseline)	Brain Area	p	r
Post-Study SBP	Theta (θ)	P_3	0.02*	- 0.66

Key:

SBP – systolic blood pressure **P** – Parietal * – statistical significance

p – p-value **r** – rho value

Conversely, there were several significant negative associations between post-study SBP and EEG activity during the active phase, mainly in slow-wave brain activities (theta and delta) (Table 6.8). For theta activity, the links were found at FT_7 ($p = 0.03$; $r = -0.67$), P_7 ($p = 0.04$; $r = -0.64$), and P_4 ($p = 0.03$; $r = -0.64$), while for delta they were at FT_7 ($p = 0.01$; $r = -0.75$), and C_3 ($p = 0.02$; $r = -0.68$). There were no associations between post-study SBP and fast-wave activities (alpha, beta, and gamma) during the active phase.

Table 6.8. Associations between post-study SBP and EEG activity during the active phase for the T1DM group.

Independent variable	Dependent variable (Active)	Brain Area	p	r
Post-Study SBP	Theta (θ)	FT_7	0.03*	- 0.67
		P_7	0.04*	- 0.64
		P_4	0.03*	- 0.64
	Delta (δ)	FT_7	0.01*	- 0.75
		C_3	0.02*	- 0.68

Key:

SBP – systolic blood pressure **F** – Frontal **P** – Parietal

C – Central **p** – p-value **r** – rho value

* – statistical significance

6.1.5 Associations between post-study DBP and EEG activity (T1DM)

Few significant associations were found between post-study DBP and EEG variables during the baseline phase (Table 6.9). For gamma activity, a negative association with post-study DBP was observed at FC_3 ($p = 0.01$; $r = - 0.69$), while for theta it was at P_3 ($p = 0.04$; $r = - 0.59$). There were no significant associations between post-study DBP and the other EEG variables (alpha, beta, or delta) during the baseline phase.

Table 6.9. Associations between post-study DBP and EEG activity during the baseline phase for the T1DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Post-Study DBP	Gamma (γ)	FC_3	0.01*	0.69
	Theta (θ)	P_3	0.04*	- 0.59

Key:

SBP – diastolic blood pressure **F** – Frontal **P** – Parietal

C – Central **p** – p-value **r** – rho value

* – statistical significance

Similarly, few significant associations were found between post-study DBP and EEG activity during the active phase. These links with post-study DBP were found with gamma, theta, and delta activities (Table 6.10). For gamma activity, the link with post-study DBP was found at FC_3 ($p = 0.04$; $r = 0.60$) (Figure 6.6). In contrast, association with theta was observed at O_2 ($p = 0.02$; $r = - 0.67$), and with delta at TP_8 ($p = 0.04$; $r = - 0.64$). There were no significant associations between post-study DBP and the other EEG variables (alpha and beta) during the active phase.

Table 6.10. Associations between post-study DBP and EEG activity during the active phase for the T1DM group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Post-DBP	Gamma (γ)	FC_3	0.04*	0.60
	Theta (θ)	O_2	0.02*	- 0.67
	Delta (δ)	TP_8	0.04*	- 0.64

Key:

SBP – diastolic blood pressure **F** – Frontal **C** – Central

O – Occipital **T** – Temporal **P** – Parietal

* – statistical significance **p** – p-value **r** – rho value

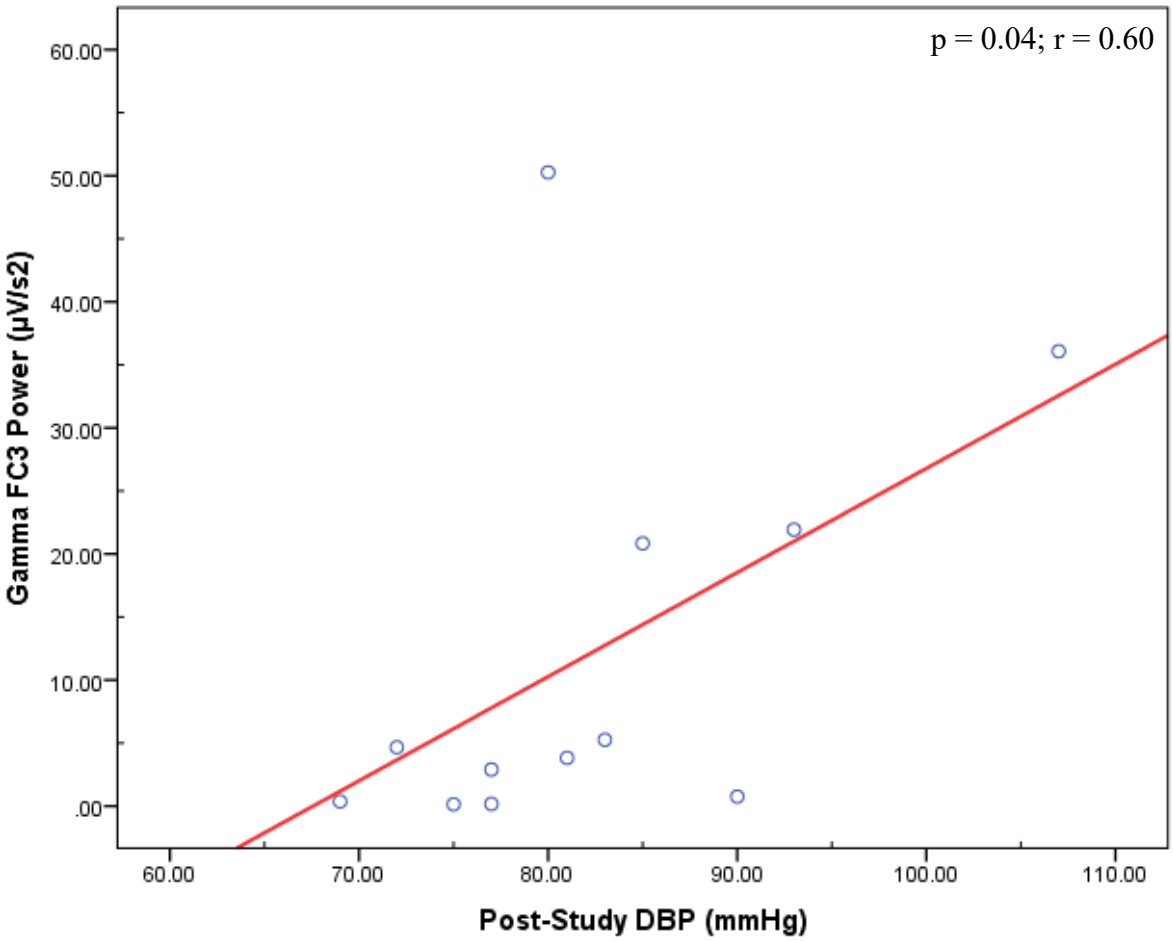


Figure 6.6. Positive correlation between post-study diastolic blood pressure and gamma power at FC_3 during the active phase for the Type 1 diabetes mellitus group.

Key:

DBP – diastolic blood pressure

mm Hg – millimetres of mercury

F – Frontal

C – Central

p – p-value

r – rho value

µV/s² – microvolts per second squared

6.1.6 Associations between post-study BGL and EEG activity (T1DM)

With respect to post-study BGL and EEG activity during the baseline phase, multiple significant inverse associations were found in alpha, beta, and theta activity (Table 6.11). For post-study BGL and alpha activity, this association was found at FP_1 ($p = 0.01$; $r = -0.62$), while for beta it was at both FP_1 ($p = 0.04$; $r = -0.70$) and TP_8 ($p = 0.02$; $r = -0.81$). In contrast, Spearman's rank-order correlation revealed post-study BGL links with theta activity at multiple locations as follows: FP_1 ($p = 0.04$; $r = -0.63$), FP_2 ($p = 0.01$; $r = -0.69$), F_7 ($p = 0.03$; $r = -0.65$), F_3 ($p = 0.02$; $r = -0.65$), F_4 ($p = 0.03$; $r = -0.62$), FC_4 ($p = 0.04$; $r = -0.59$), FT_8 ($p = 0.03$; $r = -0.63$), C_3 ($p = 0.04$; $r = -0.59$), TP_7 ($p = <0.05$; $r = -0.77$) (Figure 6.7), CP_3 ($p = 0.02$; $r = -0.64$), TP_8 ($p = 0.04$; $r = -0.59$), and P_8 ($p = 0.03$; $r = -0.64$). No significant associations were identified between post-study BGL and gamma or delta activity during the baseline phase.

Table 6.11. Associations between post-study BGL and EEG activity during the baseline phase for the T1DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Post-Study BGL	Alpha (α)	FP_1	0.04*	- 0.62
	Beta (β)	FP_1	0.04*	- 0.70
		TP_8	0.02*	- 0.81
	Theta (θ)	FP_1	0.04*	- 0.63
		FP_2	0.01*	- 0.69
		F_7	0.03*	- 0.64
		F_3	0.02*	- 0.65
		F_4	0.03*	- 0.62
		FC_4	0.04*	- 0.59
		FT_8	0.03*	- 0.63
		C_3	0.04*	- 0.59
		TP_7	<0.05*	- 0.77
		CP_3	0.02*	- 0.64
		TP_8	0.04*	- 0.59
		P_8	0.03*	- 0.64

Key:

BGL – blood glucose level
F – Frontal
C – Central
O – Occipital
T – Temporal
P – Parietal
* – statistical significance
p – p-value
r – rho value

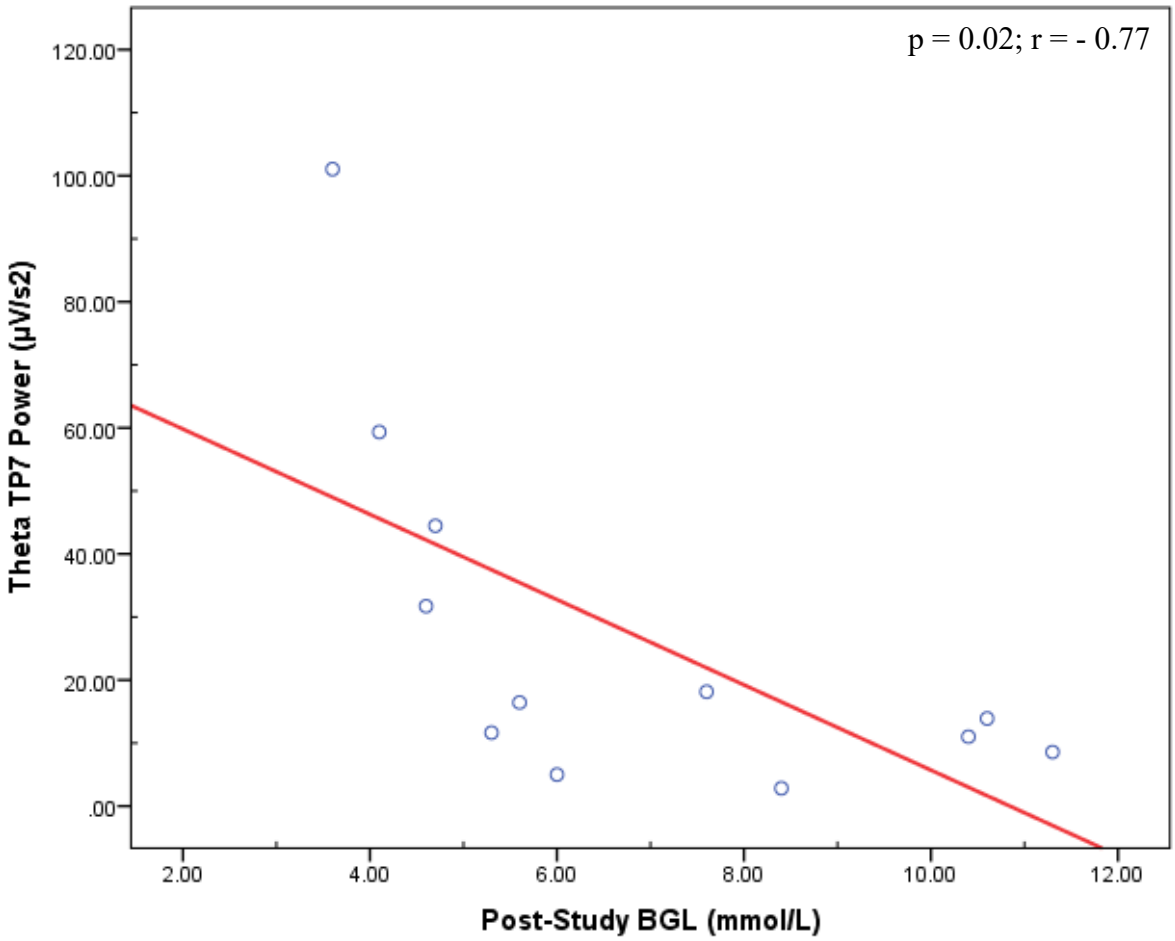


Figure 6.7. Negative correlation between post-study blood glucose level and theta power at TP_7 during the baseline phase for the Type 1 diabetes mellitus group.

Key:

BGL – blood glucose level

T – Temporal

P – Parietal

r – rho value

mmol/L – millimoles per litre

µV/s² – microvolts per second squared

p – p-value

Similarly, several significant inverse associations were found between post-study BGL and EEG activity during the active phase (Table 6.12). These were primarily observed in slow-wave brain activities (theta and delta), although one was found in beta. The link in post-study BGL with beta activity was observed at TP_8 ($p = 0.02$; $r = -0.81$). For theta activity, the links were found at F_3 ($p = 0.03$; $r = -0.65$), F_Z ($p = 0.02$; $r = -0.67$); TP_7 ($p = 0.02$; $r = -0.70$); TP_8 ($p = 0.01$; $r = -0.75$), and P_8 ($p < 0.05$; $r = -0.78$) (Figure 6.8). In contrast, associations of post-study BGL with delta activity were found at multiple locations as follows: F_Z ($p = 0.02$; $r = -0.70$), F_4 ($p = 0.02$; $r = -0.70$), FC_3 ($p = 0.04$; $r = -0.63$), P_8 ($p = 0.02$; $r = -0.69$), O_1 ($p = 0.01$; $r = -0.78$), and O_2 ($p = 0.04$; $r = -0.64$).

Table 6.12. Associations between post-study BGL and EEG activity during the active phase for the T1DM group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Post-Study BGL	Beta (β)	TP_8	0.02*	- 0.81
	Theta (θ)	F_3	0.03*	- 0.65
		F_Z	0.02*	- 0.67
		TP_7	0.02*	- 0.70
		TP_8	0.01*	- 0.75
		P_8	<0.05*	- 0.78
	Delta (δ)	F_Z	0.02*	- 0.70
		F_4	0.02*	- 0.70
		FC_3	0.04*	- 0.63
		P_8	0.02*	- 0.69
		O_1	0.01*	- 0.78
		O_2	0.04*	- 0.64

Key:

BGL – blood glucose level | **F** – Frontal | **C** – Central

O – Occipital | **T** – Temporal | **P** – Parietal

* – statistical significance | **p** – p-value | **r** – rho value

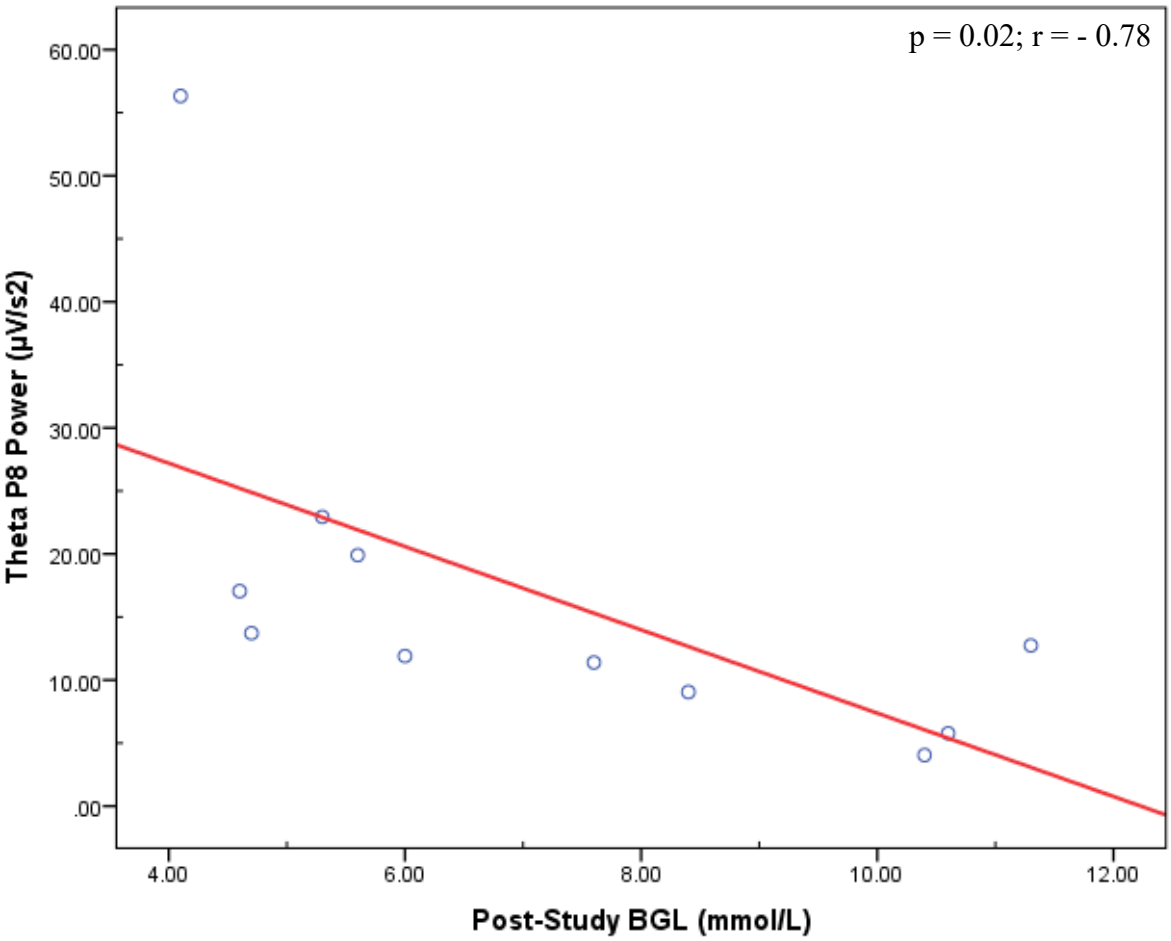


Figure 6.8. Negative correlation between post-study blood glucose level and theta power at P_8 during the active phase for the Type 1 diabetes mellitus group.

Key:

BGL – blood glucose level

P – Parietal

p – p-value

mmol/L – millimoles per litre

µV/s² – microvolts per second squared

r – rho value

6.1.7 Associations between disease-specific variables and EEG activity (T1DM)

As shown in Table 6.13 (below), several significant associations were found between disease-specific variables ($HbA1_C$ and disease duration) and EEG variables during the baseline phase for the T1DM group. Glycosylated haemoglobin was significantly associated with beta activity at C_3 ($p = 0.02$; $r = 0.79$) and TP_8 ($p = 0.04$; $r = 0.78$), and with theta activity at P_3 ($p = 0.03$; $r = 0.66$) and P_4 ($p = 0.02$; $r = 0.67$). In contrast, disease duration was inversely associated with gamma activity at FT_7 ($p = 0.03$; $r = -0.62$).

Table 6.13. Associations between disease-specific variables (HbA1c, disease duration) and EEG activity during the baseline phase for the T1DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
$HbA1_C$	Beta (β)	C_3	0.02*	0.79
		TP_8	0.04*	0.78
	Theta (θ)	P_3	0.03*	0.66
		P_4	0.02*	0.67
Disease Duration	Gamma (γ)	FT_7	0.03*	- 0.62

Key:

HbA1c – glycosylated haemoglobin **C** – Central **T** – Temporal
P – Parietal * – statistical significance **p** – p-value **r** – rho value

Similarly, multiple significant associations were found between $HbA1_C$ and slow-wave frequency brain waves (theta and delta) during the active phase (Table 6.14). For theta, these associations with $HbA1_C$ were found at the following locations: FT_7 ($p = 0.03$; $r = 0.69$), T_7 ($p = 0.01$; $r = 0.77$), P_7 ($p = 0.02$; $r = 0.73$), P_4 ($p = 0.04$; $r = 0.65$), O_1 ($p = 0.03$; $r = 0.72$), and O_2 ($p = 0.03$, $r = 0.70$), while links for delta were found at F_3 ($p = 0.01$; $r = 0.78$), FT_7 ($p = 0.02$, $r = 0.72$), T_7 ($p < 0.05$; $r = 0.86$) (Figure 6.9), C_3 ($p = 0.03$; $r = 0.69$), TP_7 ($p = 0.03$; $r = 0.70$), and CP_3 ($p = 0.04$; $r = 0.65$). There were no significant associations between disease duration and the EEG variables (delta, theta, alpha, beta, or gamma) during the active phase.

Table 6.14. Associations between disease-specific variables ($HbA1_C$, disease duration) and EEG activity during the active phase for the T1DM group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
HbA1c	Theta (θ)	FT_7	0.03*	0.69
		T_7	0.01*	0.77
		P_7	0.02*	0.73
		P_4	0.04*	0.65
		O_1	0.03*	0.72
		O_2	0.03*	0.70
	Delta (δ)	F_3	0.01*	0.78
		FT_7	0.02*	0.72
		T_7	<0.05	0.86
		C_3	0.03*	0.69
		TP_7	0.03*	0.70
		CP_3	0.04*	0.65

Key:

HbA1c – glycosylated haemoglobin **F** – Frontal **T** – Temporal

P – Parietal **O** – Occipital **C** – Central **p** – p-value

r – rho value * – statistical significance

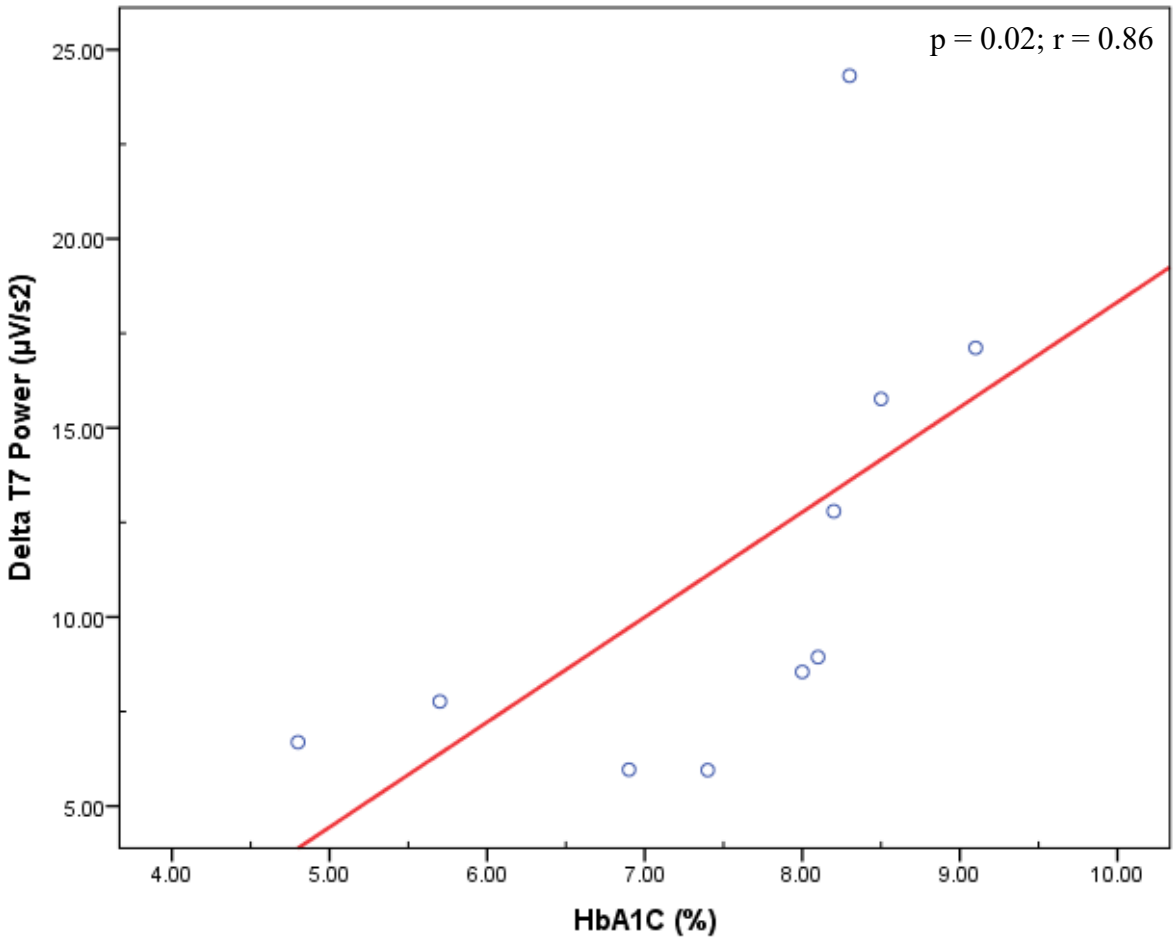


Figure 6.9. Positive correlation between glycosylated haemoglobin and delta power at T_7 during the active phase for the Type 1 diabetes mellitus group.

Key:

$HbA1_C$ – glycosylated haemoglobin

$\mu V/s^2$ – microvolts per second squared

r – rho value

T – Temporal

p – p-value

6.2 Type 2 Diabetes Mellitus (T2DM)

6.2.1 Associations between pre-study SBP and EEG activity (T2DM)

Multiple significant inverse associations were found between pre-study SBP and slow-wave activity during the baseline phase (Table 6.15). The link in pre-study SBP with theta activity was observed at FP_1 ($p = 0.01$; $r = -0.65$). In contrast, associations with delta activity were at F_Z ($p = 0.04$; $r = -0.58$), FT_8 ($p = 0.03$; $r = -0.66$), T_8 ($p < 0.01$; $r = -0.86$), CP_4 ($p = 0.01$; $r = -0.74$), TP_8 ($p = 0.04$; $r = -0.59$), and O_2 ($p = 0.01$; $r = -0.74$) (Figure 6.10). No significant associations were found between pre-study SBP and fast-wave frequencies (alpha, beta, and gamma) during the baseline phase.

Table 6.15. Associations between pre-study SBP and EEG activity during the baseline phase for the T2DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Pre-Study SBP	Theta (θ)	FP_1	0.01*	- 0.65
	Delta (δ)	F_Z	0.04*	- 0.58
		FT_8	0.03*	- 0.66
		T_8	<0.01*	- 0.86
		CP_4	0.01*	- 0.74
		TP_8	0.04*	- 0.59
		O_2	0.01*	- 0.74

Key:

SBP – systolic blood pressure
F – Frontal
T – Temporal
C – Central
P – Parietal
O – Occipital
***** – statistical significance
p – p-value
r – rho value

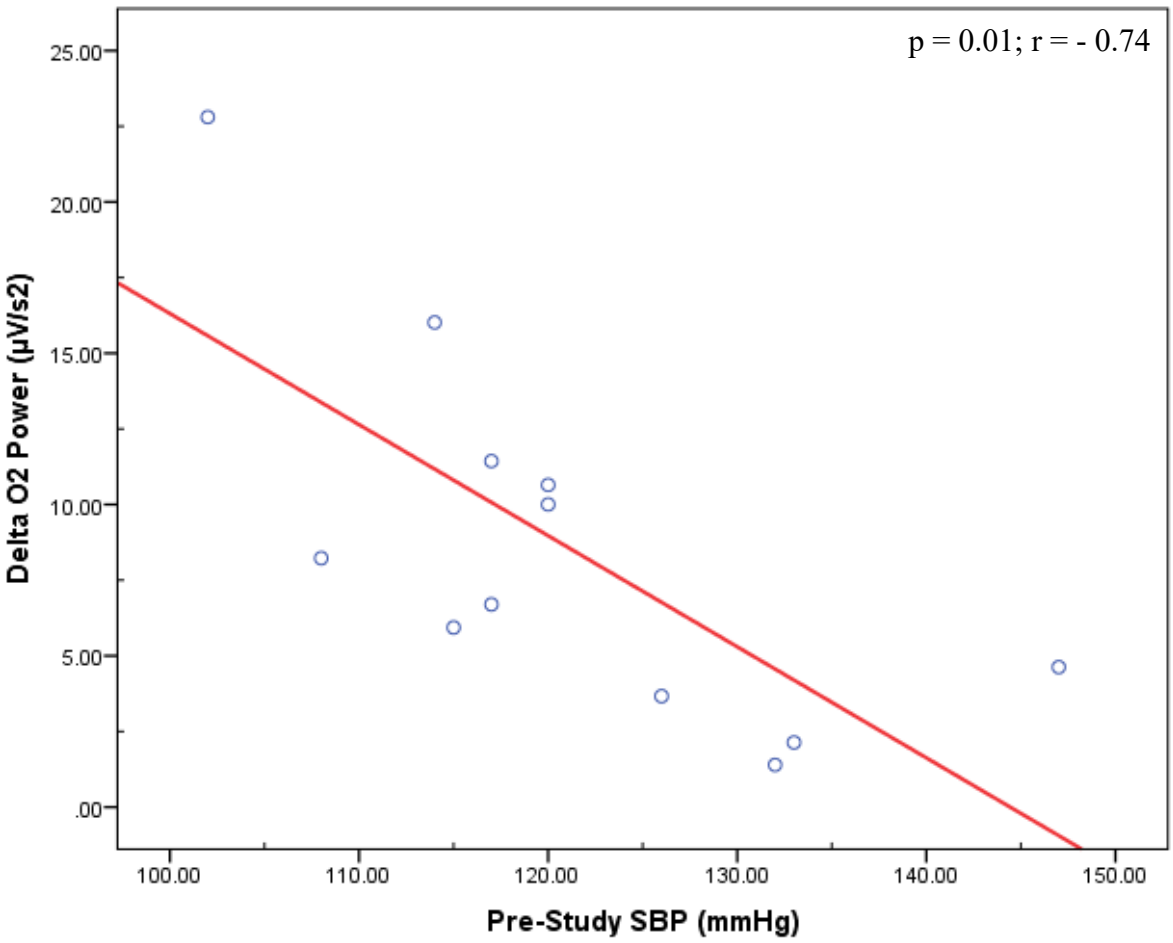


Figure 6.10. Negative correlation between pre-study systolic blood pressure and delta power at O_2 during the baseline phase for the Type 2 diabetes mellitus group.

Key:

SBP – systolic blood pressure

O – Occipital

p – p-value

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

Several significant associations were found between pre-study SBP and gamma, theta, and delta activity during the active phase (Table 6.16). For gamma, the links with pre-study SBP were observed at three locations: C_3 ($p = 0.02$; $r = 0.60$), CP_4 ($p = 0.03$; $r = 0.59$), and P_3 ($p = 0.02$; $r = 0.62$) (Figure 6.11), while for theta the links were found at FP_2 ($p = 0.04$; $r = -0.58$) and C_3 ($p = 0.02$; $r = -0.62$). For delta, a single link with pre-study SBP was found at TP_8 ($p = 0.02$; $r = -0.60$). Similarly, no significant associations were found between pre-study SBP and the fast-wave frequency bands (alpha and beta) during the active phase.

Table 6.16. Associations between pre-study SBP and EEG activity during the active phase for the T2DM group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Pre-Study SBP	Gamma (γ)	C_3	0.02*	0.60
		CP_4	0.03*	0.59
		P_3	0.02*	0.62
	Theta (θ)	FP_2	0.04*	- 0.58
		C_3	0.02*	- 0.62
	Delta (δ)	TP_8	0.02*	- 0.60

Key:

SBP – systolic blood pressure
C – Central
P – Parietal
F – Frontal
T – Temporal
***** – statistical significance
p – p-value
r – rho value

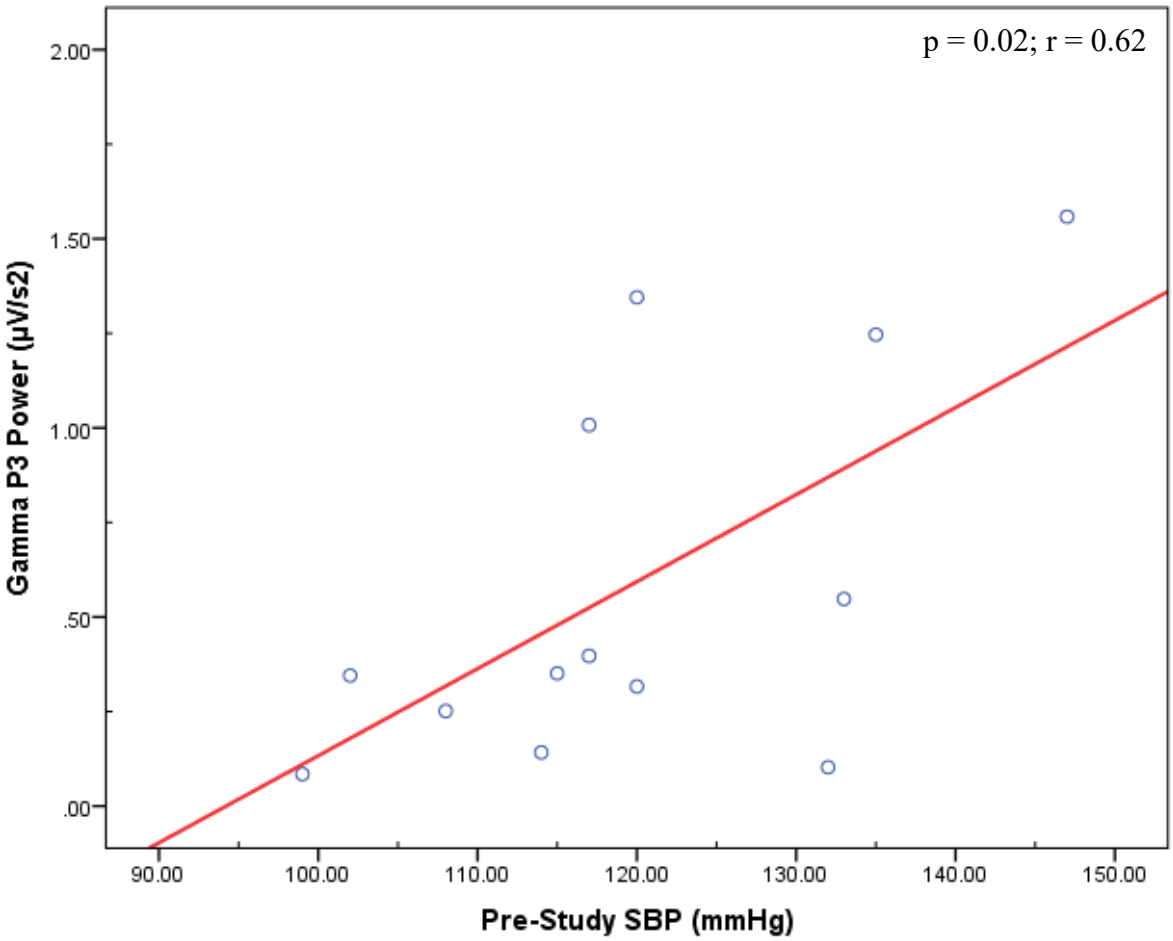


Figure 6.11. Positive correlation between pre-study SBP and gamma power at P_3 during the active phase for the Type 2 diabetes mellitus group.

Key:

SBP – systolic blood pressure

P – Parietal

p – p-value

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

6.2.2 Associations between post-study SBP and EEG activity (T2DM)

Multiple significant inverse associations were found between post-study SBP and EEG activity during the baseline phase (Table 6.17). Most associations were observed with slow-wave frequency bands (theta and delta), although some were found with the fast-wave activities (beta). For beta activity, these links with post-study SBP were found at F_4 ($p = 0.04$; $r = -0.54$), C_Z ($p = 0.02$; $r = -0.62$), and C_4 ($p = 0.04$; $r = -0.55$) (Figure 6.12), and for theta at C_Z ($p = 0.02$; $r = -0.63$). In contrast, associations with delta activity were found at the following locations: FP_1 ($p = 0.04$; $r = -0.65$), F_3 ($p = 0.03$; $r = -0.59$), C_Z ($p = 0.01$; $r = -0.71$), T_8 ($p = 0.01$; $r = -0.66$), CP_4 ($p = 0.04$; -0.59), and O_Z ($p = 0.04$; $r = -0.59$).

Table 6.17. Associations between post-study SBP and EEG activity during the baseline phase for the T2DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Post-Study SBP	Beta (β)	F_4	0.04*	- 0.54
		C_Z	0.02*	- 0.62
		C_4	0.04*	- 0.55
	Theta (θ)	C_Z	0.02*	- 0.63
	Delta (δ)	FP_1	0.04*	- 0.65
		F_3	0.03*	- 0.59
		C_Z	0.01*	- 0.71
		T_8	0.01*	- 0.66
		CP_4	0.04*	- 0.59
		O_Z	0.04*	- 0.59

Key:

SBP – systolic blood pressure
F – Frontal
C – Central
P – Parietal
O – Occipital
T – Temporal
***** – statistical significance
p – p-value
r – rho value

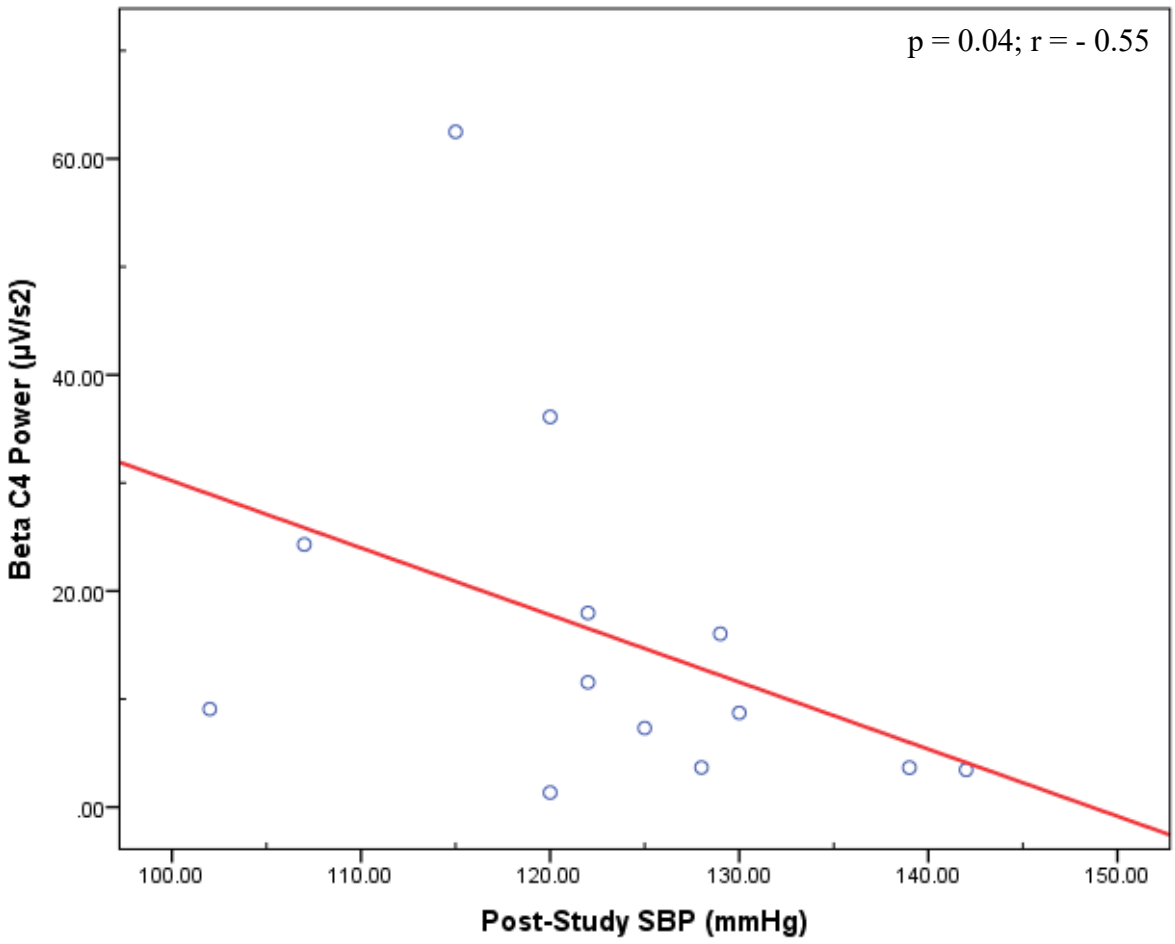


Figure 6.12. Negative correlation between post-study systolic blood pressure and beta power at C_4 during the baseline phase for the Type 2 diabetes mellitus group.

Key:

SBP – systolic blood pressure

C – Central

p – p-value

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

Similarly, several significant inverse associations were found between post-study SBP and EEG theta and delta activities during the active phase (Table 6.18). Spearman's rank-order correlation revealed links with post-study SBP for theta activity only at C_Z ($p = 0.04$; $r = -0.55$), while for delta inverse associations were found at FC_Z ($p = 0.04$; $r = -0.54$), C_Z ($p = 0.02$; $r = -0.61$), TP_8 ($p = 0.01$; $r = -0.65$), and O_1 ($p = 0.03$; $r = -0.59$). There were no significant associations between post-study SBP and fast-wave brain activities (alpha, beta, or gamma) during the active phase.

Table 6.18. Associations between post-study SBP and EEG activity during the active phase for the T2DM group.

Independent variable	**Dependent Variable (Active)**	**Brain Area**	**p**	**r**
Post-Study SBP	Theta (θ)	C_Z	0.04*	- 0.55
	Delta (δ)	FC_Z	0.04*	- 0.54
		C_Z	0.02*	- 0.61
		TP_8	0.01*	- 0.65
		O_1	0.03*	- 0.59

Key:

SBP – systolic blood pressure
C – Central
F – Frontal
T – Temporal
O – Occipital
* – statistical significance
p – p-value
r – rho value

6.2.3 Associations between pre-study and post-study DBP and EEG activity (T2DM)

Few significant inverse associations were found between pre-study DBP and theta and delta activity during the baseline phase (Table 6.19). Pre-study DBP was associated with theta activity at CP_Z ($p = 0.04$; $r = -0.54$), and with delta activity at CP_Z ($p = 0.04$; -0.58) and CP_4 ($p = 0.04$; $r = -0.60$). Similar to pre-study and post-study SBP, there were no significant associations between pre-study DBP and fast-wave brain activities (alpha, beta, or gamma) during the baseline and active phases. Further, no associations were found between post-study DBP and EEG activity during either the baseline or active phase.

Table 6.19. Associations between pre-study DBP and EEG activity during the baseline phase for the T2DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Pre-Study DBP	Theta (θ)	CP_Z	0.04*	- 0.54
	Delta (δ)	CP_Z	0.04*	- 0.58
		CP_4	0.04*	- 0.60

Key:

DBP – systolic blood pressure **C** – Central **P** – Parietal

***** – statistical significance **p** – p-value **r** – rho value

6.2.4 Associations between pre-study and post-study BGL and EEG activity (T2DM)

Few significant associations were found between pre-study BGL and slow-wave frequency bands (theta and delta) during the baseline phase (Table 6.20). Pre-study BGL was associated with theta activity at CP_Z ($p = 0.04$; $r = 0.54$) and with delta activity at O_1 ($p = 0.01$; $r = 0.69$).

Table 6.20. Associations between pre-study BGL and EEG activity during the baseline phase for the T2DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Pre-Study BGL	Theta (θ)	CP_Z	0.04*	0.54
	Delta (δ)	O_1	0.01*	0.69

Key:

BGL – blood glucose level **C** – Central **P** – Parietal

O – Occipital **p** – p-value

r – rho value * – statistical significance

Similarly, few significant associations were found between pre-study BGL and EEG activity during the active phase (Table 6.21). Pre-study BGL was linked with delta activity at TP_8 ($p < 0.05$; $r = 0.73$) (Figure 6.13), O_1 ($p = 0.02$; $r = 0.61$), and O_2 ($p = 0.01$; $r = 0.65$). There were no significant associations between pre-study BGL and the other EEG frequency bands (alpha, beta, gamma, or theta) during the active phase. No significant associations were also found between post-study BGL and EEG variables (delta, theta, alpha, beta, or gamma) during both the baseline and active phases.

Table 6.21. Associations between pre-study BGL and EEG activity during the active phase for the T2DM group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Pre-Study BGL	Delta (δ)	TP_8	<0.01*	0.76
		O_1	0.03*	0.61
		O_2	<0.01*	0.76

Key:

BGL – blood glucose level **T** – Temporal **P** – Parietal

O – Occipital **p** – p-value **r** – rho value

* – statistical significance

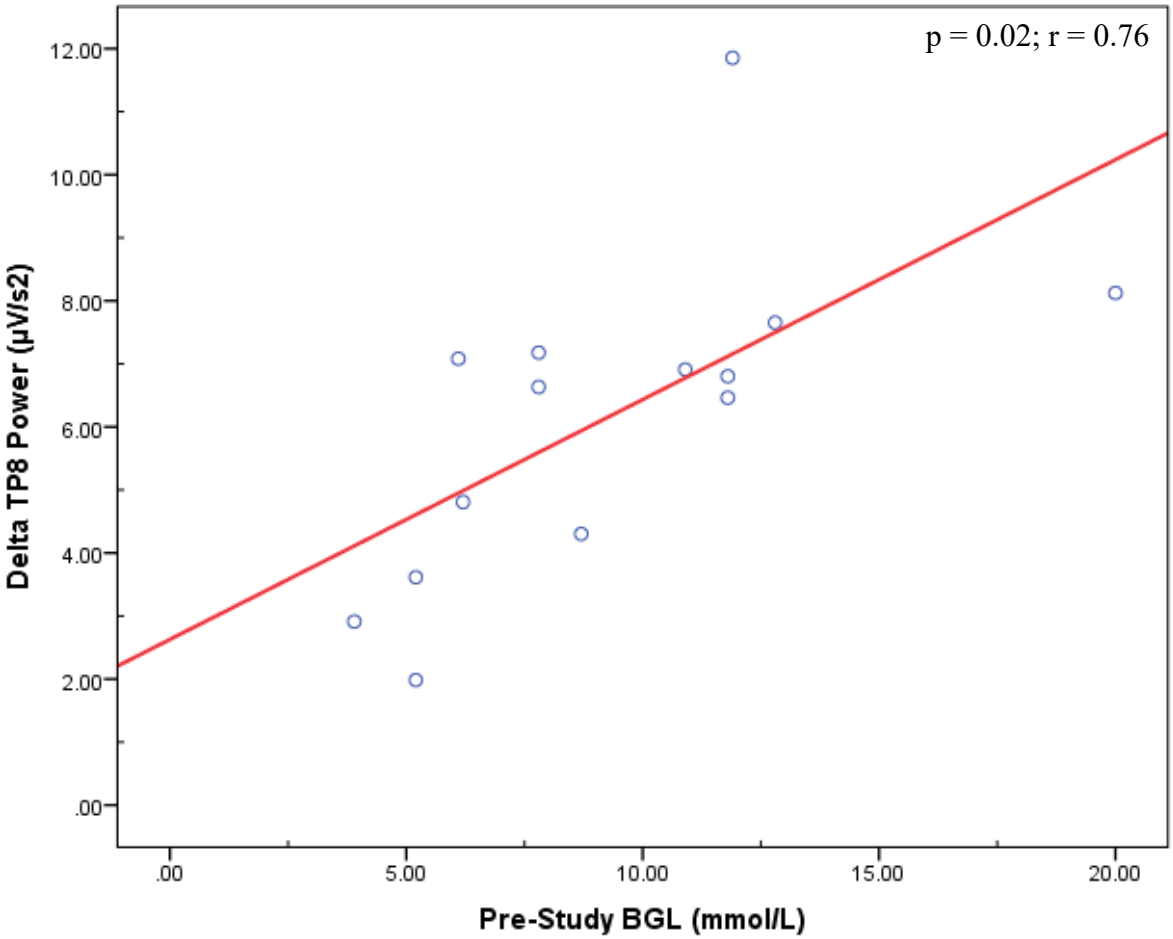


Figure 6.13. Positive correlation between pre-study blood glucose level and delta power at TP_8 during the active phase for the Type 2 diabetes mellitus group.

Key:

BGL – blood glucose level

T – Temporal

p – p-value

P – Parietal

mmol/L – millimoles per litre

µV/s² – microvolts per second squared

r – rho value

6.2.5 Associations between $HbA1_C$ and EEG activity (T2DM)

Several significant associations were identified between glycosylated haemoglobin ($HbA1_C$) and EEG activity during the baseline phase (Table 6.22). These links with $HbA1_C$ were found for gamma, theta, and delta, although most were observed for the slow-wave brain activities (theta and delta). Associations of $HbA1_C$ with gamma activity were found at CP_4 ($p = 0.01$; $r = -0.83$) and P_3 ($p = 0.04$; $r = -0.72$), whereas links with theta activity were at FP_1 ($p = 0.01$; $r = 0.80$), FC_Z ($p = 0.01$; $r = 0.90$), and P_Z ($p = 0.02$; $r = 0.85$). Associations with delta activity were observed at multiple locations as follows: FP_1 ($p = 0.04$; $r = 0.71$), F_7 ($p = 0.01$; $r = 0.81$), FT_7 ($p = 0.01$; $r = 0.87$), FC_Z ($p < 0.05$; $r = 0.91$), FC_4 ($p = 0.04$; $r = 0.74$), FT_8 ($p = 0.02$; $r = 0.79$), P_7 ($p = 0.01$; $r = 0.84$), P_3 ($p = 0.01$; $r = 0.87$), P_Z ($p = 0.01$; $r = 0.83$), P_8 ($p = 0.04$; $r = 0.74$), and O_2 ($p = 0.01$; $r = 0.81$).

Table 6.22. Associations between glycosylated haemoglobin ($HbA1_C$) and EEG activity during the baseline phase for the T2DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
$HbA1_C$	Gamma (γ)	CP_4	0.01*	- 0.83
		P_3	0.04*	- 0.72
	Theta (θ)	FP_1	0.03*	0.80
		FC_Z	0.01*	0.90
		P_Z	0.02*	0.85
	Delta (δ)	FP_1	0.04*	0.71
		F_7	0.01*	0.81
		FT_7	0.01*	0.87
		FC_Z	<0.05*	0.91
		FC_4	0.04*	0.74
		FT_8	0.02*	0.79
		P_7	0.01*	0.84
		P_3	0.01*	0.87
		P_Z	0.01*	0.83
		P_8	0.04*	0.74
		O_2	0.01	0.81

Key:

$HbA1_C$ – glycosylated haemoglobin
F – Frontal
C – Central
P – Parietal
T – Temporal
O – Occipital
p – p-value
r – rho value
***** – statistical significance

Conversely, only few significant associations were found between $HbA1_C$ and EEG activity during the active phase. (Table 6.23). Glycosylated haemoglobin was associated with theta activity at CP_4 ($p = 0.02$; $r = - 0.78$), O_1 ($p = 0.03$; $r = - 0.77$), and O_Z ($p = 0.03$; $r = - 0.77$). It was also associated with beta activity at P_Z ($p = 0.01$; $r = 0.87$).

Table 6.23. Associations between glycosylated haemoglobin ($HbA1_C$) and EEG activity during the active phase for the T2DM group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
HbA1c	Gamma (γ)	CP_4	0.02*	- 0.78
		O_1	0.03*	- 0.77
		O_Z	0.03*	- 0.77
	Theta (θ)	P_Z	0.01*	0.87

Key:

$HbA1_C$ – glycosylated haemoglobin **C** – Central **P** – Parietal

T – Temporal **p** – p-value **r** – rho value

***** – statistical significance

6.2.6 Associations between disease duration and EEG activity (T2DM)

No significant associations were found between disease duration and EEG activity during the baseline phase. However, several links were found between disease duration and EEG activity during the active phase, particularly with gamma and delta activities (Table 6.24). For gamma activity, these links with disease duration were found at F_7 ($p = 0.04$; $r = 0.56$), F_4 ($p = 0.02$; $r = 0.62$), FT_7 ($p = 0.03$; $r = 0.60$) (Figure 6.14), and TP_7 ($p = 0.03$; $r = 0.56$). In contrast, associations with delta activity were observed at CP_3 ($p = 0.04$; $r = 0.52$) and O_1 ($p = 0.01$; $r = 0.67$). No significant associations were found between disease duration and the other EEG activities (alpha, beta, or theta) during the active phase.

Table 6.24. Associations between disease duration and EEG activity during the active phase for the T2DM group

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Disease Duration	Gamma (γ)	F_7	0.04*	0.56
		F_4	0.02*	0.62
		FT_7	0.03*	0.60
		TP_7	0.03*	0.56
	Delta (δ)	CP_3	0.04*	0.52
		O_1	0.01*	0.67

Key:

F – Frontal **P** – Parietal **T** – Temporal **C** – Central

O – Occipital **p** – p-value **r** – rho value

***** – statistical significance

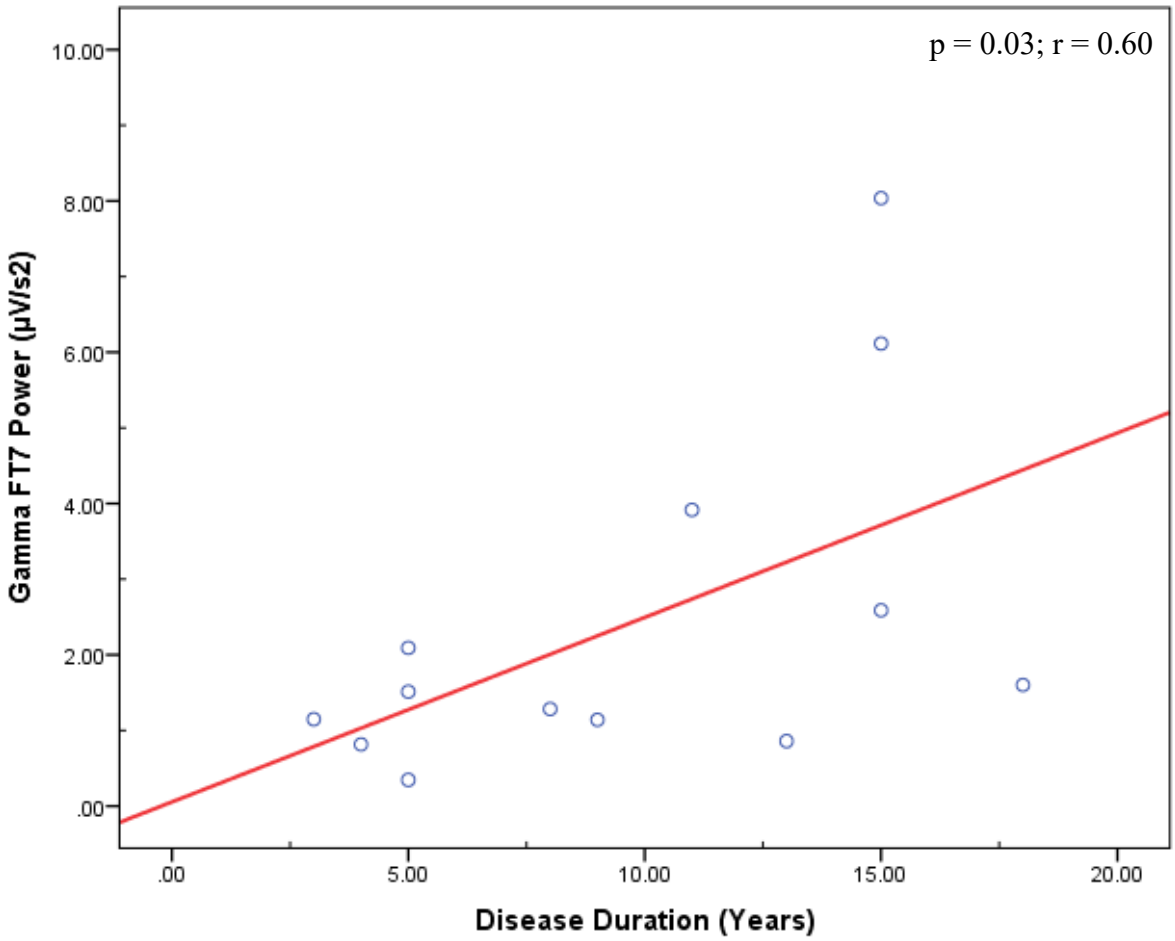


Figure 6.14. Positive correlation between disease duration and gamma power at FT_7 during the active phase for the Type 2 diabetes mellitus group.

Key:

F – Frontal **T** – Temporal **µV/s²** – microvolts per second squared

p – p-value **r** – rho value

6.3 Hypertension

6.3.1 Associations between pre-study SBP and EEG activity (HTN)

Only one significant association was found between pre-study SBP and EEG activity during the baseline phase (Table 6.25) for the HTN group. This link between pre-study SBP and theta activity was found at P_Z ($p = 0.03$; $r = 0.60$) (Figure 6.15). There were no significant associations between pre-study SBP and the other EEG frequency bands (delta, alpha, beta, or gamma) during the baseline phase.

Table 6.25. Associations between pre-study SBP and EEG activity during the baseline phase for the HTN group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Pre-Study SBP	Theta (θ)	P_Z	0.03*	0.60

Key:

SBP – systolic blood pressure
P – Parietal
***** – statistical significance
p – p-value
r – rho value

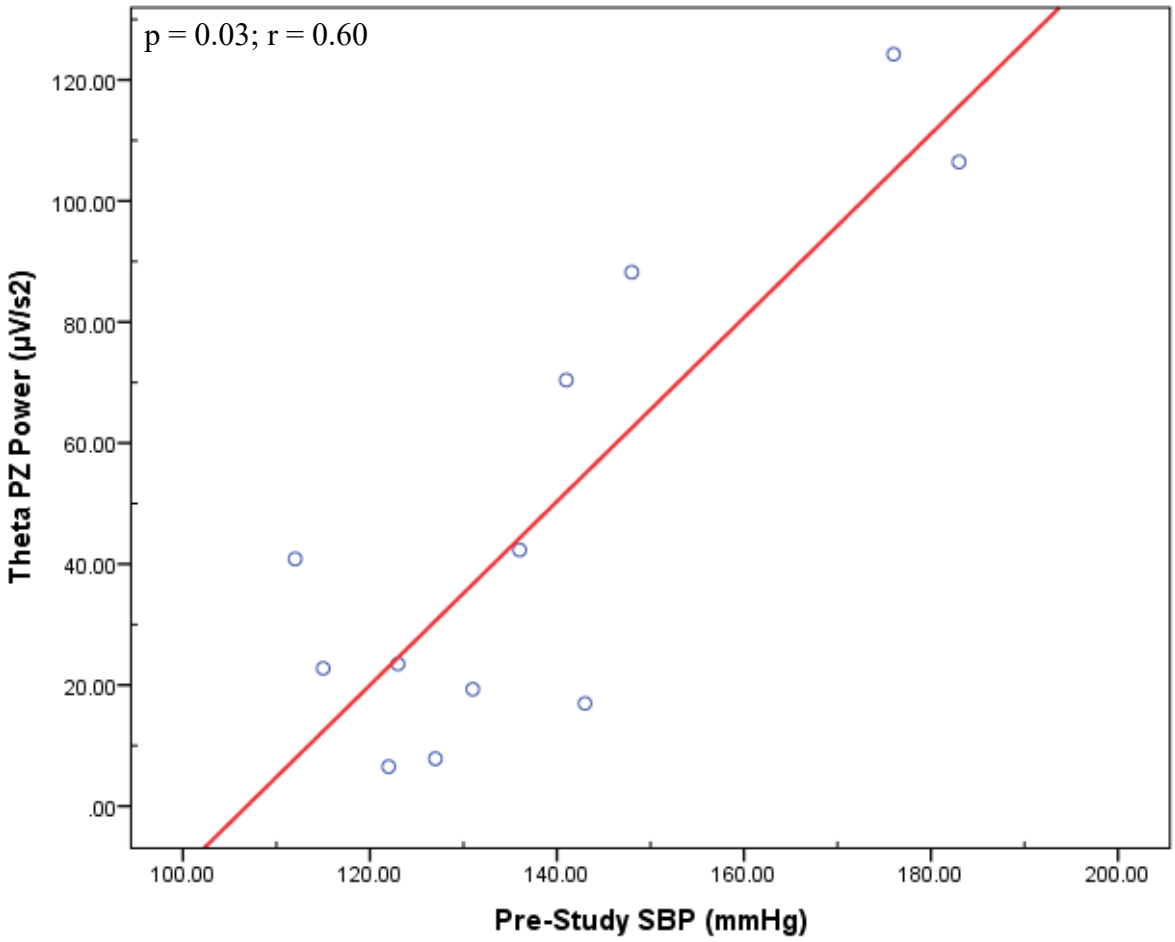


Figure 6.15. Positive correlation between pre-study systolic blood pressure and theta power at P_Z during the baseline phase for the hypertension group.

Key:

SBP – systolic blood pressure

P – Parietal

p – p-value

mm Hg – millimetres of mercury

µV/s² – microvolts per second squared

r – rho value

Significant associations were found between pre-study SBP and EEG activity during the active phase (Table 6.26). These active phase associations between pre-study SBP and beta activity were at F_3 ($p = 0.02$; $r = -0.77$) and P_Z ($p = 0.04$; $r = -0.70$). However, there were no significant associated between pre-study SBP and the other EEG variables (delta, theta, alpha, or gamma) during the active phase.

Table 6.26. Associations between pre-study SBP and EEG activity during the active phase for the HTN group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Pre-Study SBP	Beta (β)	F_3	0.02*	- 0.77
		P_Z	0.04*	- 0.70

Key:

SBP – systolic blood pressure **F** – frontal **P** – Parietal

*** –** statistical significance **p** – p-value **r** – rho value

6.3.2 Associations between post-study SBP and EEG activity (HTN)

As shown in Table 6.27, several significant associations between post-study SBP and EEG activity were found during the baseline phase. These links of post-study SBP were primarily found with alpha and slow-wave brain activities (theta and delta). Post-study SBP was associated with alpha activity at several locations as follows: FT_7 ($p = 0.04$; $r = 0.63$), FC_4 ($p = 0.01$; $r = 0.71$), FT_8 (p 0.01; $r = 0.83$), CP_3 ($p = 0.03$; $r = 0.64$), CP_4 ($p < 0.001$; $r = 0.74$), TP_8 ($p < 0.001$; $r = 0.80$), and P_7 ($p = 0.03$; $r = 0.63$). In contrast, associations with theta activity were at P_3 ($p = 0.03$; $r = 0.60$), P_4 ($p = 0.03$; $r = 0.60$), and O_2 ($p = 0.01$; $r = 0.66$), and with delta activity at FC_3 ($p = 0.04$; $r = 0.57$), C_Z ($p = 0.03$; $r = 0.59$), and P_4 ($p = 0.04$; $r = 0.57$). No significant associations were identified between post-study SBP and fast-wave oscillations (beta or gamma) during the baseline phase.

Table 6.27. Associations between post-study SBP and EEG activity during the baseline phase for the HTN group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Post-Study SBP	Alpha (α)	FT_7	0.04*	0.63
		FC_4	0.01*	0.71
		FT_8	0.01*	0.83
		CP_3	0.03*	0.64
		CP_4	<0.05*	0.74
		TP_8	<0.001*	0.80
		P_7	0.03*	0.63
	Theta (θ)	P_3	0.03*	0.60
		P_4	0.03*	0.60
		O_2	0.01*	0.66
	Delta (δ)	FC_3	0.04*	0.57
		C_Z	0.03*	0.59
		P_4	0.04*	0.57

Key:

SBP – systolic blood pressure
F – frontal
C – Central
P – Parietal
p – p-value
r – rho value
O – Occipital
* – statistical significance

Conversely, few significant associations were identified between post-study SBP and EEG activity during the active phase (Table 6.28). Post-study SBP was linked with theta activity at TP_7 ($p = 0.04$; $r = 0.90$) and CP_Z ($p = 0.04$; $r = 0.71$), and with delta activity at CP_Z ($p = 0.04$; $r = 0.62$). Similarly, there were no significant associations between post-study SBP and fast-wave oscillations (alpha, beta, or gamma) during the active phase.

Table 6.28. Associations between post-study SBP and EEG activity during the active phase for the HTN group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Post-Study SBP	Theta (θ)	TP_7	0.04*	0.90
		CP_Z	0.04*	0.71
	Delta (δ)	CP_Z	0.04*	0.62

Key:

SBP – systolic blood pressure **T** – Temporal **P** – Parietal

C – Central **p** – p-value **r** – rho value

* – statistical significance

6.3.3 Associations between pre-study DBP and EEG activity (HTN)

With respect to DBP, few significant associations were found between pre-study DBP and EEG activity during the baseline phase (Table 6.29). Pre-study DBP links with beta activity were observed at CP_3 ($p = 0.01$; $r = 0.71$), and with theta activity at TP_7 ($p = 0.02$; $r = 0.67$) (Figure 6.16) and O_Z ($p = 0.01$; $r = 0.76$). There were no significant associations between pre-study DBP and the other EEG frequency bands (delta, gamma, or alpha) during the baseline phase. Additionally, no significant associations were observed between pre-study DBP and EEG variables (delta, theta, alpha, beta, or gamma) during the active phase.

Table 6.29. Associations between pre-study DBP and EEG activity during the baseline phase for the HTN group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Pre-Study DBP	Beta (β)	CP_3	0.01*	0.71
	Theta (θ)	TP_7	0.02*	0.67
		O_Z	0.01*	0.76

Key:

DBP – diastolic blood pressure **C** – Central **T** – Temporal

P – Parietal **p** – p-value **r** – rho value

* – statistical significance

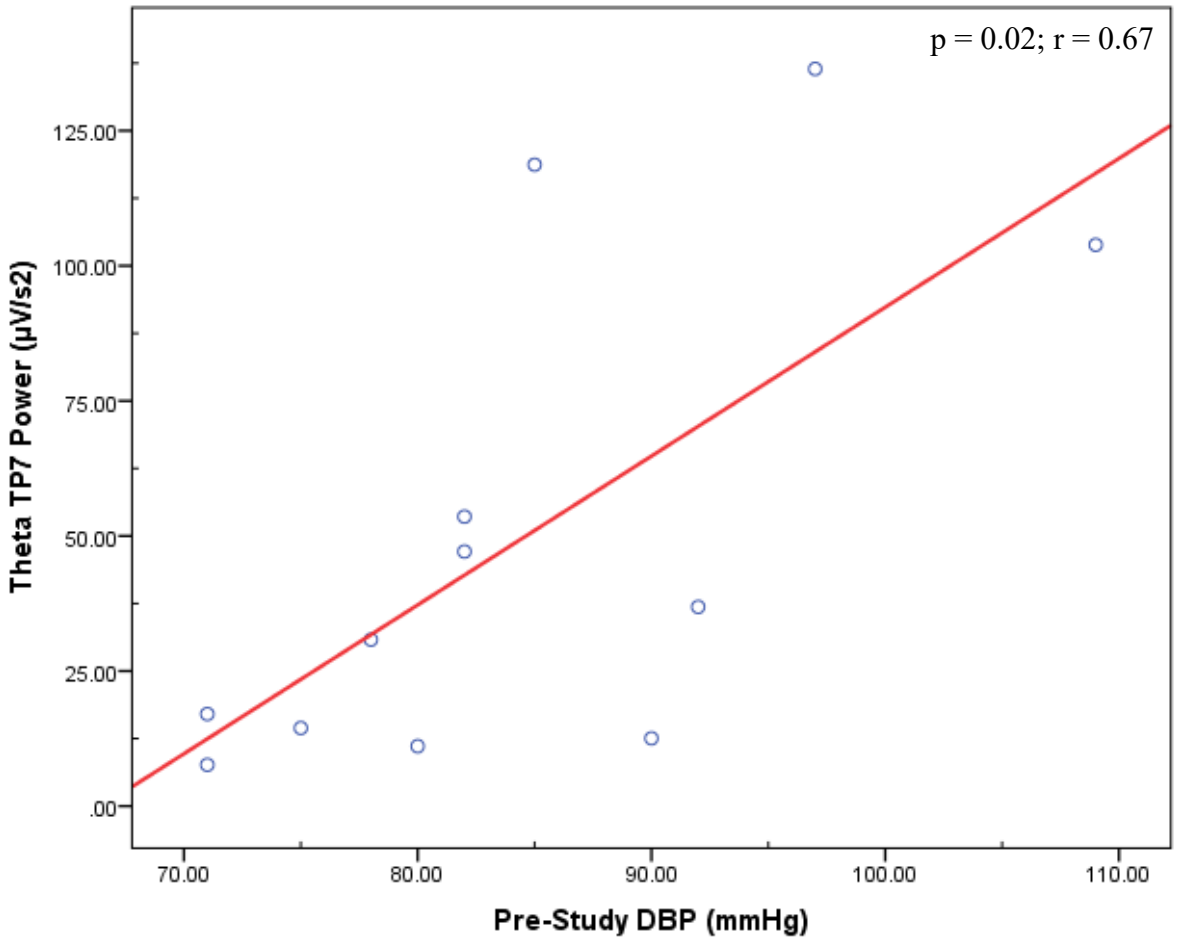


Figure 6.16. Positive correlation between pre-study diastolic blood pressure and theta power at TP_7 during the baseline phase for the hypertension group.

Key:

DBP – diastolic blood pressure

T – Temporal

µV/s² – microvolts per second squared

r – rho value

mm Hg – millimetres of mercury

P – Parietal

p – p-value

6.3.4 Associations between post-study DBP and EEG activity (HTN)

Two significant associations were identified between post-study DBP and EEG activity during the baseline phase (Table 6.30). Post-study DBP was associated with theta activity at TP_7 ($p = 0.04$; $r = 0.60$) and O_Z ($p = 0.01$; $r = 0.79$). However, there were no significant associations between post-study DBP and the other EEG variables (delta, alpha, beta, or gamma) during the baseline phase.

Table 6.30. Associations between post-study DBP and EEG activity during the baseline phase for the HTN group.

Independent variable	Dependent Variable (Baseline)	Brain Area	p	r
Post-DBP	Theta (θ)	TP_7	0.04*	0.60
		O_Z	0.01*	0.79

Key:

DBP – diastolic blood pressure **T** – Temporal **P** – Parietal

O – occipital **p** – p-value **r** – rho value

* – statistical significance

Conversely, several significant inverse associations were found between post-study DBP and EEG activity (beta and gamma) during the active phase (Table 6.31). Post-study DBP was linked with beta activity at F_3 ($p = 0.03$; $r = -0.72$), F_4 ($p = 0.04$; $r = -0.83$), and FC_Z ($p = 0.04$; $r = -0.70$), while associations with gamma activity were found at FT_7 ($p = 0.02$; $r = -0.65$) (Figure 6.17), FC_3 ($p = 0.02$; $r = -0.65$), T_7 ($p = 0.02$; $r = -0.70$), C_3 ($p = 0.04$; $r = -0.62$), CP_Z ($p = 0.03$; $r = -0.63$), and O_1 ($p – 0.03$; $r = -0.64$). No significant associations were found between post-study DBP and the other EEG variables (delta, theta, or alpha) during the active phase.

Table 6.31. Associations between post-study DBP and EEG activity during the active phase for the HTN group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Post-DBP	Beta (β)	F_3	0.03*	- 0.72
		F_4	0.04*	- 0.83
		FC_Z	0.04*	- 0.70
	Gamma (γ)	FT_7	0.02*	- 0.65
		FC_3	0.02*	- 0.65
		T_7	0.02*	- 0.70
		C_3	0.04*	- 0.62
		CP_Z	0.03*	- 0.63
		O_1	0.03*	- 0.64

Key:

DBP – diastolic blood pressure
F – Frontal
C – Central
P – Parietal
T – Temporal
O – Occipital
* – statistical significance
p – p-value
r – rho value

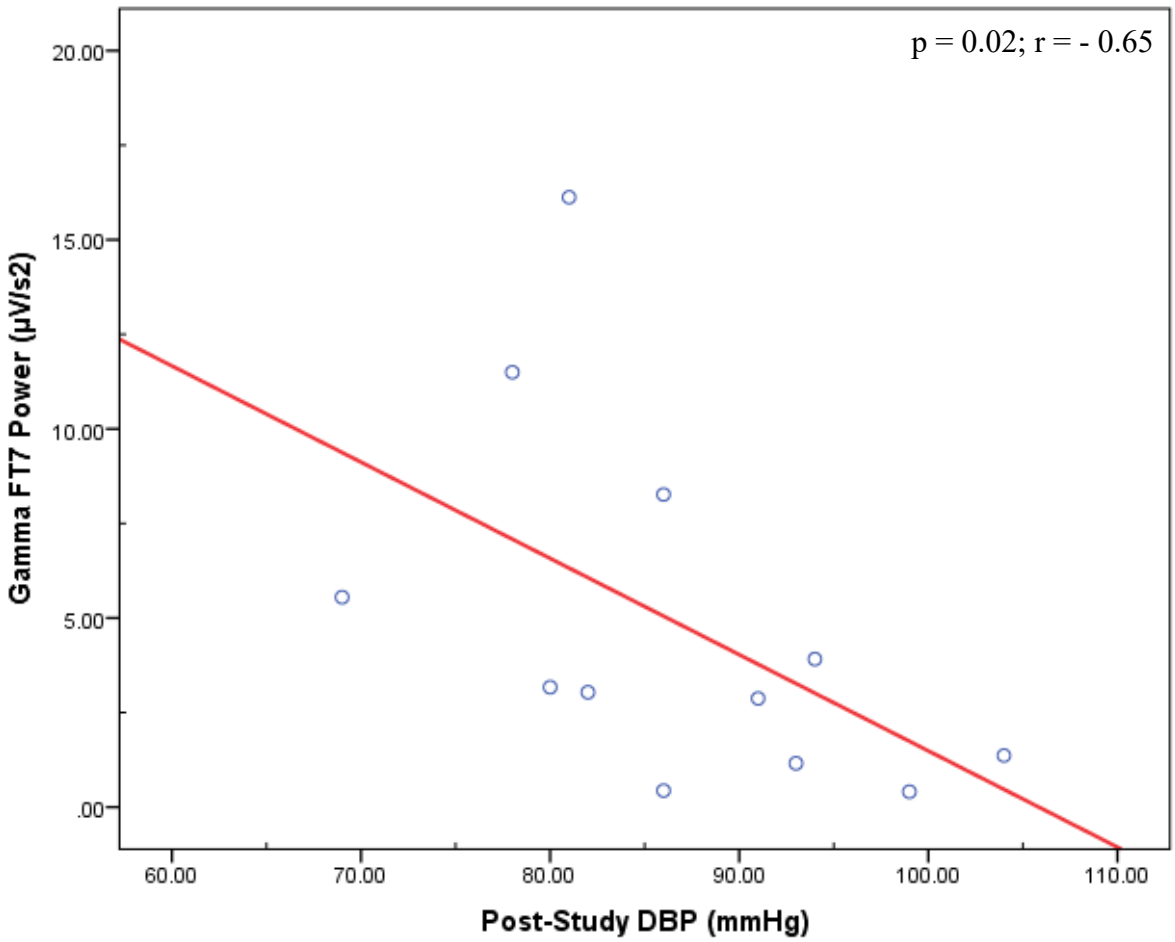


Figure 6.17. Negative correlation between post-study diastolic blood pressure and gamma power at FT_7 during the active phase for the hypertension group.

Key:

DBP – diastolic blood pressure

mm Hg – millimetres of mercury

F – Frontal

T – Temporal

µV/s² – microvolts per second squared

p – p-value

r – rho value

6.3.5 Associations between pre-study BGL and EEG activity (HTN)

No significant associations were found between pre-study BGL and EEG activity during the baseline phase. However, links between pre-study BGL and beta activity were identified at FT_7 ($p = 0.02$; $r = 0.77$) and FC_4 ($p = 0.04$; $r = 0.74$) during the active phase (Table 6.32). No significant associations were found between pre-study BGL and the other EEG variables (delta, theta, alpha, or gamma) during the active phase.

Table 6.32. Associations between pre-study BGL and EEG activity during the active phase for the HTN group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Pre-Study BGL	Beta (β)	FT_7	0.02*	0.77
		FC_4	0.04*	0.74

Key:

BGL – blood glucose level **F** – Frontal **T** –Temporal

C – Central **p** – p-value **r –** rho value

* – statistical significance

6.3.6 Associations between post-study BGL and EEG activity (HTN)

Similarly, no significant associations were found between post-study BGL and EEG activity during the baseline phase. However, associations during the active phase were observed between post-study BGL and beta activity at TP_7 ($p = 0.04$; $r = -0.79$) and P_8 ($p < 0.05$; $r = -0.93$) (Table 6.33). There were no associations between post-study BGL and the other EEG frequency bands (alpha, gamma, theta, or delta) during the active phase.

Table 6.33. Associations between post-study BGL and EEG activity during the active phase for the HTN group.

Independent variable	Dependent Variable (Active)	Brain Area	p	r
Post-Study BGL	Beta (β)	TP_7	0.04*	- 0.79
		P_8	<0.05*	- 0.93

Key:

BGL – blood glucose level **T** – Temporal **P** – Parietal

***** – statistical significance **p** – p-value **r** – rho value

6.4 Discussion: Associations between BP and BGL and Electroencephalography (EEG) (Clinical Samples)

This chapter explores the associations between pre-study and post-study physiological variables (SBP, DBP, and BGL) and EEG data for the clinical groups (T1DM, T2DM, and HTN). The findings are discussed sequentially, commencing with the T1DM group. Associations found between disease-specific variables (disease duration, $HbA1_C$) for each group, if any, are also discussed. The main findings are: (1) high BP is primarily correlated with slow-wave brain activities (theta and delta), (2) poor glycaemic control (T1DM and T2DM) is associated with slow-wave brain activities (theta and delta), and (3) disease duration in DM (both T1DM and T2DM) is associated with both fast-wave and slow-wave frequencies.

6.4.1 Associations between BP and EEG (T1DM and T2DM)

Literature exploring associations between BP and EEG activity is scarce. This longstanding neglect has been attributed to various factors described earlier (see Chapter 1, section 1.7.2). This study is the first to report associations between BP (SBP and DBP) and EEG activity in individuals with T1DM and T2DM. The present analysis revealed that increasing SBP, but within the normotensive range for the T1DM and T2DM groups, was inversely correlated with slow-wave EEG activities (theta and delta) in the frontal, temporal, and parietal brain regions. Although no studies have replicated this outcome in subjects with DM (T1DM and/or T2DM), this finding suggests that BP in the normotensive range supports optimal metabolic and electrical activities of underlying cortical pyramidal neurons, resulting in enhanced connectivity and fast-wave activity (Hossmann, 1994; Foreman & Claassen, 2012; George & Steinberg, 2015; Sweeney *et al.,* 2018). In accordance with this observation, neuropsychological studies consistently show that patients with normal BP perform better overall in neurocognitive assessments than those with hypertension (Harrington *et al.,* 2000; Wu *et al.,* 2016). Cortical pyramidal neurons are known to be sensitive to small changes in BP (Jordan, 2004) and are dependent on a continuous, uninterrupted supply of glucose and oxygen for optimal function (Sweeney *et al.,* 2018). While slow-wave activity has been linked to several cognitive processes (*e.g.* working and semantic memory) (Sauseng *et al.,* 2010) in healthy subjects, it has also been consistently reported in subjects with cognitive impairment (Jelic *et al.,* 1996; Jelic *et al.,* 2000) and is widely considered to reflect cortical network dysfunction (Uhlhaas & Singer, 2010). Others have suggested it may indicate neuronal

hypersynchronisation (Tomkins *et al.,* 2008). Thus, it is conceivable that BP in the normotensive range supports the metabolic and electrical activities of sensitive cortical neurons. This could be due to providing adequate CBF, which has been closely linked to EEG activity, and ensures activated cortical regions receive sufficient nutrients during metabolically-demanding cognitive tasks (Hossmann, 1994; Jordan, 2004; Foreman & Claassen, 2012; George & Steinberg, 2015; Sweeney *et al.,* 2018).

Similar to the non-clinical group, more correlations were identified between SBP and cerebral electrical activity than between DBP and cerebral electrical activity in the T1DM and T2DM groups. This finding has not been documented in any prior investigations and again potentially implies that SBP exerts stronger effects on brain oscillatory activity than does DBP. Clear evidence exists in the literature that SBP is associated with adverse long-term cognitive outcomes, particularly during mid-life (Kilander *et al.,* 1998; Swan *et al.,* 1998; Yaffe *et al.,* 2014). Interestingly, others have reported that slight elevations in DBP (10 mm Hg), but not SBP, are associated with an increased risk (7%) of cognitive impairment (Tsivgoulis *et al.,* 2009). Iadecola *et al.* (2016) advise that not all studies have compared evenly the evidence between SBP and DBP. There is also a lack of studies specifically investigating associations between DBP and brain electrical activity. Therefore, this area of research requires further exploration.

6.4.2 Associations between BP and EEG (Hypertension)

The present study showed that increasing SBP was significantly associated with slow-wave brain activities (theta and delta) over the central and parietal brain regions in the HTN group. This finding is novel and implies neuronal populations in central and parietal areas are preferentially damaged by high SBP. Aberrant oscillatory activity has been linked to disruptions in connectivity and coordinated rhythmic activity of underlying cortical neurons (Buzsaki & Draguhn, 2004; Babiloni *et al.,* 2011a; Hamm *et al.,* 2015; Modi & Sahin, 2017). Marked changes in BP, such as hypotension, have been associated with detectable and reversible changes in cortical electrical activity (Mani & Townsend, 1954; Weisz *et al.,* 2002; Duschek *et al.,* 2006), but no studies have investigated associations between hypertension and EEG activity. Chronic arterial hypertension is known to disrupt vulnerable arteries, such as the middle cerebral artery and lenticulostriate arteries (Sörös *et al.,* 2013), which supply crucial nutrients (glucose and O_2) to neurons in frontal, central, and parietal regions (Sörös *et al.,* 2013). Accumulating

evidence also indicates that hypertension damages neurovascular units (NVUs), which are critical vascular regulatory mechanisms responsible for regulating cerebral blood flow (Jennings *et al.,* 2005; Iadecola *et al.,* 2016). Interruptions in cerebral blood flow lead to hypoperfusion during periods of high metabolic demand, depriving sensitive cortical neurons of vital nutrients (glucose and O_2). Changes in CBF have also been closely linked to changes in oscillatory activity (Hossmann, 1994; Jordan, 2004). Therefore, it is plausible that the mechanisms described above may explain the increased slow-wave brain activity observed over the central and parietal regions.

Hypertension has also been linked to disrupting the integrity of the highly protective blood-brain barrier (BBB) (Rosenberg, 2012; Huisa *et al.,* 2015; Sweeney *et al.,* 2018). The mechanisms underlying this have yet to be clearly elucidated but are thought to involve pericyte degeneration (Armulik *et al.,* 2011) and hypoxia (Rosenberg *et al.,* 2016). It is known that damage to the BBB results in increased permeability and leakiness, allowing the unregulated entry of potentially toxic substances into the sensitive CNS parenchyma (Iadecola *et al.,* 2016; Sweeney *et al.,* 2018). This has been hypothesised to disturb reliable neuronal signalling and synaptic function, triggering neuroinflammation (Sweeney *et al.,* 2018). BBB disruption has also been associated with electrophysiological abnormalities, including observed increases in slow-wave activity (Tomkins *et al.,* 2008). Similar changes in EEG activity have been reported in patients with early cognitive impairment (MCI) and advanced cognitive dysfunction (AD and dementia) (Jelic *et al.,* 1996; Jelic *et al.,* 2000). As converging evidence suggests neuroinflammation is a clinical hallmark of both MCI and AD, it is conceivable that the increased slow-wave activity associated with SBP may potentially be an indicator of the early stages of neuroinflammation.

The increased slow-wave brain activity observed at BP ≥ 135/90 mm Hg in the HTN group in the present study also implies cerebral tissue becomes susceptible to the deleterious effects of high blood pressure early (*i.e.* before anti-hypertensive therapy is recommended). Although glycaemic thresholds for when changes in EEG activity occur have been established, consensus is lacking in the literature concerning thresholds for BP at which EEG is altered. It has been suggested that optimal BP in the brain is ≤ 130/80 mm Hg (Sörös *et al.,* 2013). This is corroborated by data from large-scale studies, which suggest that cortical tissue is more vulnerable than the cardiac system (Yusuf *et al.,* 2004;

O'Donnell *et al.,* 2010). In accordance with this, one large meta-analysis examining cross-sectional studies also concluded that the rate of vascular and overall mortality increases when BP exceeds 115/75 mm Hg (Lewington *et al.,* 2002). Based on the data obtained, the present study supports both Sörös *et al.* (2013) and Lewington *et al.* (2002), suggesting susceptibility to hypertension-associated cognitive dysfunction occurs earlier (BP ≥ 135/90 mmHg) than anti-hypertensive therapy is currently recommended (BP ≥ 140/90 mmHg) (Unger *et al.,* 2020). However, it is clear that further research investigating the electroencephalography changes in patients with HTN is required to more clearly define BP thresholds that detrimentally affect cognition. Such research will validate the electroencephalography changes observed in the present study and could have broader clinical implications, such as ascertaining the precise BP thresholds when the brain becomes vulnerable to damage from HTN or elevated BP. It could also determine the suitability of both BP and the EEG as non-invasive biomarkers of early cognitive dysfunction.

Interestingly, higher SBP was found to be significantly associated with increased alpha activity in frontal and central brain regions. This finding is novel. The literature generally suggests increased alpha power correlates strongly with attentional processes, working memory, and overall global cognitive status (Klimesch, 1996; Huang *et al.,* 2000; Knyazev, 2007), whereas declines in alpha activity reflect cortical dysfunction and/or cognitive decline (Huang *et al.,* 2000; Babiloni *et al.,* 2010; Babiloni *et al.,* 2011; van der Hiele *et al.,* 2011; Babiloni *et al.,* 2016). However, not all studies have replicated these findings: Alexander *et al.* (2006) reported increased alpha power in healthy subjects with subjective memory complaints (n = 100, mean age: 64.9 years, age range: 52-88 years, gender breakdown not reported) across the cortex from 26 electrode positions. On the other end of the BP spectrum, increased alpha power in hypotension has been theorised to reflect preparedness to react (Duschek *et al.,* 2006). It has been suggested that increases in the power of EEG frequency bands precede early stages of cognitive dysfunction and reflect compensatory/adaptive mechanisms to preserve cognitive function (Pijnenburg *et al.,* 2004; Smith *et al.,* 2007). Similar changes have been reported in neuroimaging studies (fMRI), where patients with MCI demonstrate enhanced cortical activation compared to those with AD (Sarter & Bruno, 2000). Thus, the present study data could suggest a potential early compensatory mechanism by cerebral tissue, at high SBP, to shield vulnerable neuronal populations from potential degeneration.

Strong inverse associations were observed between increasing DBP and fast-wave activity (beta and gamma). These were primarily localised to frontal and central brain regions. This finding is novel and suggests that high DBP also elicits detrimental effects on cortical electrical activity. Although SBP is considered a stronger predictor of adverse cognitive outcomes than DBP, prior studies have shown DBP is also associated with poor cognitive function, especially in younger populations (Tsivgoulis *et al.,* 2009). Potential neurodegeneration of brain areas responsible for generating beta and gamma-band oscillations may also explain the outcome obtained, as the synchronisation of neural oscillations depends upon the integrity of underlying synaptic connections (Uhlhaas & Singer, 2010). Hypertension is associated with reductions in both grey and white matter and global cortical neurodegeneration (Jennings *et al.,* 2012; Sörös *et al.,* 2013; Iadecola *et al.,* 2016; Iadecola *et al.,* 2019). Reductions in grey matter have been related to decreases in the amplitude of cognitive event-related potentials (ERPs) (McCarley *et al.,* 2002). Studies exploring resting EEG activity in subjects with neurodegenerative diseases (*e.g.* AD and dementia), which are characterised by widespread cortical atrophy, have also consistently demonstrated diminished beta and gamma power (Jelic *et al.,* 1996; Jelic *et al.,* 2000). Therefore, the reduced fast-wave brain activity may indicate disrupted cortico-cortical connections and early damage/neurodegeneration to brain areas responsible for generating these oscillations. At the neurotransmitter level, it may also suggest disturbances in GABA (γ-aminobutyric acid) -ergic interneurons (Cobb *et al.,* 1995). These have been implicated in generating cortical fast-wave oscillations (Cobb *et al.,* 1995) by acting as a pacemaker. Whether SBP or DBP elicits stronger effects on cortical electrical activity has not been explored in prior investigations and remains unknown; therefore, it should be assessed in future studies (cross-sectional and longitudinal).

The literature is not particularly helpful in elucidating the mechanisms underlying hypertension-associated electrophysiological abnormalities. This could be attributed to the longstanding neglect of research exploring the neurophysiological changes linked to hypertension. Unlike hypoglycaemia, which the literature suggests can cause permanent, irreversible changes in cerebral activity, studies suggest BP causes reversible changes in EEG activity (Mani & Townsend, 1954; Weisz *et al.,* 2002; Duschek *et al.,* 2006). This complicates ascertainment of the precise electroencephalography changes associated with hypertension. Current available literature suggests interruptions in CBF primarily account for the changes in EEG activity due to reduced perfusion to metabolically-active cortical regions (Hossmann, 1994; Jordan, 2004). Others implicate neurochemical mechanisms (Gomèz *et al.,* 2004). It is clear that future studies investigating the neurophysiological changes associated with hypertension are urgently warranted.

6.4.3 Associations between BGL and EEG (T1DM and T2DM)

Numerous studies have shown that changes in BGL (both hypoglycaemia and hyperglycaemia) are characterised by noticeable changes in EEG activity (Greenblatt, Murray, & Root, 1946; Izzo, Schuster, & Engel, 1953; Eeg-Olofsson, 1977; Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005). The main electrophysiological abnormalities reported are: (1) marked increases in slow-wave activity (theta and delta) and (2) sharp reductions in fast-wave brain activities (alpha, beta, and gamma) (Eeg-Olofsson, 1977; Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005). These changes have been primarily found over anterior and parieto-occipital brain regions, respectively (Izzo, Schuster, & Engel, 1953; Eeg-Olofsson, 1977; Brismar *et al.,* 2002; Hyllienmark *et al.,* 2005; Cooray *et al.,* 2011a).

The present analysis revealed that increasing BGL was associated with slow-wave theta power over anterior brain areas (FP_1). This result is consistent with existing evidence (Hauser *et al.,* 1995; Faigle, Sutter, & Kaplan, 2013; Rachmiel *et al.,* 2016). Available literature indicates there is a shift to slow-wave frequencies (theta and delta) during hyperglycaemia, with metabolically-demanding brain regions such as the prefrontal cortex being most vulnerable (Tallroth *et al.,* 1992; Frier, 2014); however, the blood glucose threshold at which marked changes in EEG activity occur remains unclear. Rachmiel *et al.* (2016) observed pronounced changes in EEG activity at a BGL of 11 mmol/L. Conversely, the present study found increases in slow-wave activity at BGL as

high as 8 mmol/L and a loss of slow-wave activity at BGL of 7 mmol/L. These findings imply a critical glucose threshold and provide suggestive evidence, requiring further confirmation, that vulnerable neuronal populations become susceptible to hyperglycaemia-induced damage within this narrow range. It is well established that cortical pyramidal neurons rely upon a continuous supply of glucose, with small changes in BP or BGL rapidly disrupting CNS homeostasis and impairing function (Jordan, 2004; Frier, 2014). The dissimilar outcome obtained between the present study and the investigation of Rachmiel *et al.* (2016) could be attributed to cerebral adaptation and a maladaptive counterregulatory response. Graveling *et al.* (2013) suggest the brain develops an adaptive mechanism in patients with poor metabolic control to attenuate the magnitude of damage to cortical tissue. Such adaptation could explain the electrophysiological abnormalities reported by Rachmiel *et al.* (2016) at higher BGL. It may also indicate potential damage to glucose-sensing neurons of the ventromedial hypothalamus (VMH), arcuate nucleus (ARC), and paraventricular nucleus (PVN), which play important roles in initiating the counterregulatory response (Song & Routh, 2006). Damage to these neurons results in maladaptive responses to high glucose, resulting in impaired awareness of hyperglycaemia.

The finding that glycosylated haemoglobin ($HbA1_C$) was correlated with slow-wave oscillations in both T1DM and T2DM groups is in agreement with available literature (Tsalikian *et al.,* 1981; Hauser *et al.,* 1995; Hyllienmark *et al.,* 2005) and contributes additional evidence that poor glycaemic control is associated with electrophysiological abnormalities. Poor glycaemic control is a well-established risk factor for cognitive dysfunction (Ryan *et al.,* 2003; Geijselaers *et al.,* 2017; Biessels & Despa, 2018) and has been previously correlated with increases in slow-wave activity (Tsalikian *et al.,* 1981; Hauser *et al.,* 1995; Hyllienmark *et al.,* 2005). Hauser *et al.* (1995) found poor metabolic control was correlated with increased theta/delta power in young subjects with T1DM (n = 44, mean age: not reported, mean $HbA1_C$: 8.3%, diabetes duration: 5.9 years). Similarly, Hyllienmark *et al.* (2005) found $HbA1_C$ correlated with increased delta activity and concluded that poor metabolic control (as measured using $HbA1_C$) is a risk factor for abnormalities in EEG activity (n = 35, mean $HbA1_C$: 7.2% (range: 4 – 10%), mean age: 17.1 ± 1.7 years (range: 14 – 19 years), disease duration: 7.6 ± 4.6 years, age of disease onset: 9.6 ± 4.6 years (range: 1.6 – 17 years), gender breakdown: 16 females, 19 males).

Not all studies have reported associations between glycaemic control and EEG activity. Soltész & Acsadi (1989) found no relationship between metabolic control and EEG slowing despite patients having high glycosylated haemoglobin level (mean $HbA1_C$: 11.3%). However, Soltész & Acsadi (1989) only visually inspected electroencephalography changes and this may have accounted for the discrepancy. While evidence from previous studies in our research unit and the broader literature indicates slowing of EEG activity is typically observed in drowsy and fatigued behavioural states (Lal & Craig, 2002; Campisi & LaRocca, 2014; Modi & Sahin, 2017), increases in slow-wave activity are consistently found in patients with cognitive dysfunction and/or cognitive impairment (Jelic *et al.,* 1996; Jelic *et al.,* 2000). This has led researchers to believe that slow-wave activity reflects underlying cortical disruption between distal regions and/or possible cognitive decline (Jelic *et al.,* 1996; Jelic *et al.,* 2000; Modi & Sahin, 2017). Collectively, these data suggest that (1) poor glycaemic control is a risk factor for electrophysiological abnormalities, and (2) the brain becomes vulnerable to insult from $HbA1_C$ concentrations as high as 7.2%; however, emerging evidence suggests patients with high $HbA1_C$, but not in the range diagnostic of diabetes, have an increased risk of dementia (Crane *et al.,* 2012). This should be investigated in subsequent studies. Importantly, the consistency of the data obtained in the present study raises the possibility that the EEG is a suitable non-invasive measure for detecting the subtle cognitive dysfunction triggered by diabetes.

The association between disease duration and electrophysiological abnormalities currently remains controversial. Inconsistent findings have been obtained: some investigators have shown it is associated with changes in evoked potentials related to cognitive function (P300 component) (Tallroth *et al.,* 1990), but most have found no relationship at all (Mooradian *et al.,* 1988; Hauser *et al.,* 1995; Hyllienmark *et al.,* 2005). The present analysis showed disease duration (T1DM: 17.8 ± 9.2 years, T2DM: 11.3 ± 5.9 years) was significantly correlated with beta and delta power. This result is novel. Increases in the power of EEG frequency bands have been suggested to precede early stages of cognitive dysfunction and reflect initial compensatory/adaptive mechanisms to preserve cognitive function (Pijnenburg *et al.,* 2004; Smith *et al.,* 2007). Comparable changes have been observed in studies assessing cortical activation in individuals with MCI and AD using functional magnetic resonance imaging (fMRI) (Sarter & Bruno, 2000). Hence, it is conceivable the increased beta power may represent an early

compensatory/adaptive mechanism by the brain to avert further damage to vulnerable neurons. Alternatively, it could suggest neurodegeneration of brain regions that generate beta-waves, such as cortico-cortical circuits (Uhlhaas & Singer, 2010; Modi & Sahin, 2017), due to repeated exposure to glycaemic events. On the other hand, the increased delta power likely reflects underlying cortical disruption. However, it is noteworthy that the duration of diabetes in the present study was greater than other studies; hence, this may explain the absence of correlations found in earlier investigations.

Little is known about how transient hyperglycaemia influences brain oscillatory activity. While it is associated with interruptions in cerebral blood flow (Morley, 2017), which have been previously linked to EEG activity, hyperglycaemia has also been implicated in disrupting the highly-regulated microenvironment of the CNS (Sweeney *et al.,* 2018). At the molecular level, data from animal studies indicate it results in impaired axonal transport, demyelination, and ion channel dysfunction (Tomlinson & Gardiner, 2008). There is also evidence that hyperglycaemia has direct inhibitory effects on orexigenic hypothalamic neurons involved in modulating wakefulness and vigilance (Burdakov *et al.,* 2006; Sakurai, 2014). Inhibition of these wakefulness-promoting neurons could explain the loss of fast-wave oscillations. Others ascribe the changes in EEG activity during hyperglycaemia to hyperosmolarity, electrolyte disturbances, and ketoacidosis (Misra & Kalita, 2018). Therefore, additional research is required to clarify the precise mechanisms by which hyperglycaemia causes the observed abnormal EEG activity.

An important unanswered question is whether the electrophysiological abnormalities commonly reported in patients with DM (T1DM and T2DM), and those found in HTN (discussed earlier), relate to the effects of medication use for the conditions or whether the effects can be reversed by therapy. Convincing evidence from a large, recently-published meta-analysis (n = 31,090, age: > 55 years, follow-up: 7 – 22 years) examining whether blood pressure-lowering medication reduces dementia risk showed anti-hypertensive therapy was associated with a 16% and 12% reduction in AD and dementia, respectively (Ding *et al.,* 2020). Similar effects were reported between different classes of anti-hypertensive medication, suggesting individual drug classes do not demonstrate clinical differences (Ding *et al.,* 2020). The risk of vascular cognitive impairment (VCI) was not assessed. Although medication use/type was not found to be significantly correlated with any variables, the possible confounding effects of medication cannot be

excluded. Interestingly, no studies have explored the impact of glucose-lowering medications on oscillatory brain activity in patients with T2DM. Most patients with T2DM in the present study were taking commonly-prescribed anti-diabetic medications (*e.g.* metformin, dipeptidyl peptidase 4-inhibitors (DPP4-is)). Observational studies suggest that these medications may exert beneficial effects on cognition by influencing critical brain processes (*e.g.* metabolism, inflammation, and regeneration) (Patrone *et al.,* 2014; Orkaby *et al.,* 2017), but no conclusive evidence exists that these therapies modify the risk of cognitive dysfunction in T2DM (De Galan *et al.,* 2009; Areosa Sastre *et al.,* 2017). Srikanth *et al.* (2020) suggest anti-hyperglycaemic agents that improve insulin sensitivity and glucose uptake in the brain (*e.g.* metformin, intranasal insulin, and glucagon-like peptide 1 receptor agonists (GLP-1RAs)) may be promising avenues for future research. Whether glucose-lowering medications can reverse aberrant oscillatory activity also remains unknown. It is clear this area of research requires further exploration.

6.5 Conclusions

The results of this study provide evidence to suggest that raised blood glucose concentrations and blood pressure (both SBP and DBP) are associated with changes in oscillatory brain activity, which can be consistently detected using non-invasive scalp electroencephalography. Several associations between the disease-specific variables ($HbA1_C$ and disease duration) were also found, not only supporting limited existing evidence, but also adding novel findings to the current literature. Collectively, the data from the present study indicate: (1) BP and BGL are risk factors for and contribute to electrophysiological abnormalities in both DM (T1DM and T2DM) and HTN, and (2) the EEG may be a promising non-invasive biomarker for reliably and accurately detecting early changes in cognition linked to DM and HTN. Prior studies have demonstrated the clinical potential of the electroencephalography signal in identifying early disturbances in cortical activity (Tallroth *et al.,* 1992; Hauser *et al.,* 1995; Jelic *et al.,* 2000; Hyllienmark *et al.,* 2005). While several mechanisms have been proposed to account for the abnormal EEG activity, the precise mechanisms remain poorly elucidated, especially with respect to hypertension. Given the established link between both DM and HTN and cognitive dysfunction, this area of research urgently requires further investigation.

Although novel data were obtained, as reported in this thesis, larger, adequately-powered studies (cross-sectional and longitudinal) are required to validate current data and provide a better understanding of the precise electrophysiological changes associated with both DM (T1DM and T2DM) and HTN. Such studies will validate the current preliminary abnormalities in EEG activity reported herein. The diagnostic specificity of altered neural oscillations should also be interpreted cautiously. Uhlhaas & Singer (2010) caution that aberrant oscillatory activity could reflect pathophysiological processes. Others implicate inflammation and oxidative stress as the cause of abnormal oscillatory brain activity (Mehvari *et al.,* 2016). Subsequent longitudinal studies investigating EEG activity in subjects with DM and HTN would clarify whether the altered neural activity indicates degeneration of underlying brain structures or early stages of these pathophysiological processes. Another limitation in the existing literature is the limited EEG montage systems utilised. Future studies should investigate brain oscillatory activity using the standardised international *10-20* system (Jasper, 1958) and a comprehensive montage system (*e.g.* 32-channel EEG), similar to the present investigation. This will enable meaningful comparisons between investigators and provide uniform coverage of the

scalp, better highlighting specific brain regions most susceptible to early deterioration. It is also critical that disease-specific variables (*e.g.* age of disease onset, disease duration, frequency of hypoglycaemic episodes) continue to be reported in as much detail as possible. Clear evidence exists in the literature that these variables influence cognitive outcomes (Munshi *et al.,* 2006, Roberts *et al.,* 2008, Roriz-Filho *et al.,* 2009), although the contribution of each is reportedly small (Biessels & Despa, 2018). Biessels & Despa (2018) and Biessels and Whitmer (2019) also suggest future randomised controlled trials (RCTs) should explore cognitive outcomes at least as a secondary endpoint. This may identify medications that, in addition to providing meaningful glycaemic benefits, elicit beneficial effects on cognition, which could contribute to reducing the substantial socioeconomic and emotional costs linked to treating diabetes-related complications.

7. Limitations, Future Directions, and Conclusions

7.1 Limitations and Future Directions

The present, cross-sectional investigation examined the relationship(s) between blood pressure (SBP and DBP) and blood glucose level (BGL) and cognitive function in clinical (T1DM, T2DM, and HTN) and non-clinical samples using scalp electroencephalography (EEG) and psychometric batteries (MMSE and the Cognistat). Although reliable and validated cognitive measures were used, and novel associations were identified, limitations need to be discussed to inform future directions. This could help advance the assessment of cognitive function in future studies.

Human physiological parameters are highly dynamic and influenced by many variables. Although decrements in cognitive function triggered by both diabetes and hypertension are observable in cross-sectional studies, the cross-sectional study design affords only a snapshot of cognitive function. This limits inferences about causality. While measures were taken to reduce variability in the data obtained (repetition of BP measurements, adequate rest periods between BP measurements, five-minute EEG recordings, administration of two neuro-psychometric batteries, enforcement of experimental constraints, noise and temperature-controlled and sound-attenuated laboratory, exclusion of participants drinking >16 standard drinks per day or taking psychotropic medication), future studies would benefit from longitudinal study designs obtaining 24-hour BP and BGL *via* ambulatory blood pressure monitors and continuous glucose monitoring (CGM) systems. This would reduce fluctuations in physiological variables and allow researchers to monitor long-term trajectories in cognitive function, enabling evaluation of any change in cognition. It could also allow more robust assessment and understanding of the pattern of decline of diabetes-associated cognitive decrements and hypertension-induced cognitive dysfunction.

The present study recruited participants with clinically-diagnosed diabetes mellitus (T1DM or T2DM; $HbA1_C \geq 6.5\%$) and HTN (BP > 140/90 mm Hg), with or without complications, controlled or uncontrolled with medication. These characteristics are representative of the general population, but future studies would benefit from recruiting individuals from high-risk populations (*e.g.* patients with pre-diabetes or who are pre-hypertensive). Epidemiological studies suggest an estimated 3-11% of patients with pre-diabetes ($HbA1_C \geq 5.7 - < 6.5\%$) convert to overt T2DM annually (DeFronzo *et al.,* 2015; ADA, 2020). Pre-diabetes is associated with an increased risk of developing T2DM and mortality (Abraham & Fox, 2013). It has also been linked to cognitive dysfunction: for example, in a large, population-based cohort of elderly subjects, Kalmijn *et al.* (1995) found elderly patients with impaired fasting glucose (IFG) performed worse in the MMSE compared to age-matched subjects with normoglycaemia. Data also revealed that patients with hyperinsulinaemia, a clinical pathophysiological hallmark of pre-diabetes, demonstrated sub-optimal cognitive performance (Kalmijn *et al.,* 1995). Therefore, screening patients with pre-diabetes for early, subtle cognitive dysfunction using objective cognitive measures and appropriate cognitive screening tools would be highly advantageous. Although current clinical guidelines discourage routine screening of patients with diabetes in the general population for potential cognitive dysfunction, due to this being labour-intensive and there being no disease-modifying therapies available to avert progression to dementia, early screening could enable early instatement of risk factor reduction countermeasures recommended to delay progression to overt, irreversible cognitive impairment (*e.g.* cardiovascular risk factor management and individualised diabetes care) (Kalmijn *et al.,* 1995; Biessels & Despa, 2018; Biessels & Whitmer, 2019).

It is estimated that approximately 45.8% of all diabetes cases in adults worldwide are undiagnosed (Beagley *et al.,* 2014). Given the link between pre-diabetes and cognitive dysfunction, studies exploring cognition using objective neurological measures in young individuals with pre-diabetes could also have significant implications for global health care. Such studies could determine whether pre-diabetes is associated with any early reversible changes in oscillatory brain activity or early deterioration in specific cognitive domains long before irreversible deficits in cognition have manifested. This could alert general practitioners of individuals at high risk of developing diabetes-associated cognitive decrements to commence early, aggressive therapeutic intervention to maintain

euglycaemia. It could also allow the initiation of robust risk-reduction countermeasures (outlined above), which may delay progression to overt T2DM and potentially diabetes-associated cognitive decrements. This may subsequently contribute to reducing the substantial socioeconomic burden linked to diabetes-related complications on individual patients, carers, and the healthcare system.

Glycosylated haemoglobin ($HbA1_C$) is widely considered the 'gold-standard' indicator of long-term glycaemic control; however, some researchers argue it does not accurately reflect minute-to-minute fluctuations in blood glucose concentrations (Kovatchev, 2017; ADA, 2020). It is also influenced by erythrocyte-related disorders and is insensitive to hypoglycaemic episodes (Kovatchev, 2017; ADA, 2020). Hypoglycaemic episodes – mild or severe – have been associated with permanent, irreversible changes in cognitive function (Frier*,* 2014). They have also been linked to predicting the development of cognitive impairment in the future (Yaffe *et al.,* 2013). The landmark Diabetes Control and Complications Trial (DCCT) found approximately 8% of severe hypoglycaemic episodes could be determined from $HbA1_C$ (DCCT, 1993). This issue is compounded by unreliable retrospective recall of severe and mild hypoglycaemic episodes, with recall lasting up to one year and one week, respectively (Frier, 2014). Emerging literature also suggests that glycaemic fluctuations in mid-life could contribute to cognitive decrements and dementia (Rawlings *et al.,* 2017). Therefore, future research may benefit from measuring real-time blood glucose concentrations *during* cognitive assessment (physiological and cognitive assessment) using continuous glucose monitoring (CGM). To date, few studies have evaluated cognitive outcomes in patients while recording BGL continuously using CGM. These studies may provide stronger visualisation of real-time changes in blood glucose concentration occurring during cognitive stimulation. They may also identify how minute-to-minute fluctuations in BGL influence cognitive function and could assist investigators to understand better the precise changes in EEG activity that occur in response to variations in these variables.

Acquisition of neuroimaging data *via* established neuroimaging modalities (*e.g.* functional magnetic resonance imaging (fMRI)) should be considered for future cognitive studies. These technologies are expensive but would facilitate the identification of brain regions activated during cognitive tasks and hence complement and validate any abnormalities observed in EEG recordings and the cognitive tools. Such data could support EEG as a potential non-invasive biomarker of early cognitive dysfunction and the MMSE and the Cognistat in clinical contexts as the preferred cognitive screening tools for screening the subtle cognitive decrements associated with DM and HTN. It could also afford prompt determination of brain areas vulnerable to early insult from both DM and HTN in susceptible individuals.

Scalp EEG recordings are the most cost-effective and common method for recording cortical activity; however, the signal may be attenuated by signal-distorting tissue, such as the skull and intermediary neural tissue (Ritter & Villringer 2006; Buzaki *et al.,* 2012). It also cannot adequately detect electrical activity generated by neuronal populations in deep-brain structures (*e.g.* the hippocampus). Neuroimaging data consistently indicate that the hippocampus is detrimentally affected by T2DM (Ritter & Villringer 2006; Buzaki *et al.,* 2012). One modified version of the EEG that addresses these limitations and could be deployed in future studies is electrocorticography (ECoG). This technique records brain activity directly from the cerebral cortex *via* stainless-steel electrodes implanted subdurally, bypassing underlying signal-distorting tissue and improving spatial resolution (Buzaki *et al.,* 2012). Therefore, future investigations may consider implementing this technique to more accurately record brain activity in patients with DM and HTN. The improved spatial resolution may also assist researchers to pinpoint earlier the brain regions susceptible to insult, enabling earlier therapeutic and lifestyle risk factor management intervention.

At the time of writing, this study was the first to report associations between BP and brain oscillatory activity; thus, the reproducibility of the EEG signal to consistently identify electrophysiological abnormalities associated with diabetes and hypertension, chiefly the latter, should be strongly considered in future longitudinal investigations. Brismar *et al.* (2005) found the EEG demonstrated high test-retest reliability in detecting reductions in fast-wave brain activity (beta and gamma) over repeat measurements at three and nine months post-baseline (correlation coefficient: 0.91 (alpha) and 0.92 (beta) between first and second visit in adolescent subjects with T1DM receiving multiple insulin therapy

(MIT). Further investigation of this reproducibility will assist investigators determine the appropriateness of the EEG as a screening instrument for monitoring the subtle changes in cognition linked to both diabetes and hypertension.

Numerous cognitive batteries are available to assess cognitive function, but no clear consensus exists in the literature concerning the suitable cognitive screening tools to detect the subtle cognitive decrements triggered by DM and HTN. These are commonly undetected by formal neurocognitive testing until frank and irreversible damage has occurred. The diverse range of cognitive assessments available also complicates comparability of data between studies. The Mini-Mental State Examination (MMSE) (Folstein *et al.,* 1975) and the Cognistat (Kiernan *et al.,* 1987) are established cognitive tools routinely deployed in clinical practice for screening cognitive impairment; however, they do not robustly assess all cognitive domains (such as executive function and long-term memory) or all modalities affected adversely by both DM and HTN (Srikanth *et al.,* 2020). This can result in potential cognitive decrements being overlooked and an incomplete representation of broader cognitive performance. One cognitive tool sensitive to executive function and recommended by the US National Institute of Neurological Disorders and Stroke, is the Montreal Cognitive Assessment (MoCA) (Nasreddine *et al.,* 2005). Previous investigators using this tool have reliably identified executive dysfunction, a cognitive domain detrimentally impacted by both DM and HTN (Dong *et al.,* 2010; Pendlebury *et al.,* 2010). Thus, the present study could have also benefited from investigating diabetes or hypertension-associated cognitive dysfunction using this assessment tool. However, it should be stressed that the modern, around-the-clock healthcare system permits little time for comprehensive assessment of cognition in day-to-day practice. It has also been suggested that screening all patients with diabetes for potential early cognitive dysfunction would be labour-intensive (Biessels & Whitmer, 2019) and impractical (Srikanth *et al.,* 2020), as prevention strategies for dementia in middle-aged individuals with diabetes mirror those with known cardiovascular risk factors (*i.e.* optimising glycaemic control, lipid concentrations, BP, and diet and exercise). Therefore, neuro-psychometric batteries sensitive to the subtle cognitive dysfunction associated with DM and HTN with a high negative predictive value should be prioritised. Consistency in neuropsychological assessments administered in future studies will also improve the comparability of data between investigators (Geijselaers *et al.,* 2017).

Although novel findings were reported in this thesis, it is noteworthy that this was a preliminary/pilot study. The sample sizes of the clinical populations were also small. This limits the generalisability of the findings and the number of adjustments performed during data analysis. It also may have potentially given rise to chance findings (Type I and Type II error). However, appropriate statistical analyses (non-parametric) were conducted to accommodate the smaller sample sizes (clinical populations) and consistent findings were obtained, likely reflecting true results. The recruited population may also not necessarily reflect a true representation of the general population. Recruitment from the local Sydney community could have inadvertently introduced bias, as this would have attracted participants in close proximity to the study location. Future studies should aim to recruit larger sample sizes, preferably age and BMI-matched, from as many geographical locations as possible. This would strengthen the translatability of the data and would also facilitate meaningful and direct between-group comparisons.

Several disease-specific (diabetes and hypertension linked) variables were obtained in the present study (*e.g.* glycosylated haemoglobin (HbA_{1C}), disease duration (chronicity), age of disease onset, medication, *etc.*). Current literature indicates these factors moderate the relationship between these conditions and cognitive function (Munshi *et al.,* 2006; Roberts *et al.,* 2008; Roriz-Filho *et al.,* 2009). One diabetes-related variable that may have been imprecisely reported by study participants was frequency and severity of hypoglycaemia. Hypoglycaemia is a common, reversible, adverse effect of glucose-lowering therapy in patients with diabetes (T1DM and T2DM) associated with noticeable changes in cognitive function (EEG activity and cognitive performance) (Frier, 2014). However, retrospective recall of hypoglycaemic events is often poor: severe hypoglycaemic episodes can be recalled accurately for up to one year, whereas mild episodes can only be recalled with accuracy for no longer than one week (Frier, 2014). This complicates precise ascertainment of the contribution of hypoglycaemia to cognitive dysfunction and may explain the controversial relationship currently reported between hypoglycaemia and adverse cognitive outcomes. Improvement in recall of hypoglycaemic episodes can be achieved by monitoring blood glucose concentrations continuously using CGM. Analysis of such data could reveal dynamics of blood glucose fluctuations, such as the type of hypoglycaemic event experienced (mild or severe). It could also enable potential prediction of upcoming adverse glycaemic events based on

patterns from previous glycaemic events (Kovatchev, 2017) and help researchers understand better the relationship between hypoglycaemia and cognitive dysfunction.

Blood pressure was obtained using a reliable and validated automated non-invasive blood pressure monitor (see Chapter 2, section 2.7) in accordance with recommendations outlined by the International Society of Hypertension Global Hypertension Practice Guidelines (*i.e.* quiet sitting position, five-minute rest period, repeat measurements, determination of average BP) (Unger *et al.,* 2020). While brachial blood pressure is the preferred method of blood pressure measurement in clinical practice and research, some researchers suggest it may not accurately reflect cerebral blood pressure, as the brain is located upstream from the measurement point (Cohen & Townsend, 2017). Thus, cerebral blood pressure may vary slightly from BBP. Future investigations exploring cognitive function in patients with hypertension would benefit from measuring cerebral perfusion pressure (CPP) in addition to BBP. This could help researchers determine the variability in BP that exists between the CNS and systemic circulation and understand better when the brain becomes vulnerable to hypertension-associated cognitive dysfunction.

The present investigation assessed cognitive function in the more prevalent and common forms of diabetes mellitus (T1DM and T2DM) and HTN (essential hypertension). However, various other types of diabetes and hypertension exist, including monogenic diabetes syndromes (maturity-onset diabetes of the young), gestational diabetes, drug or treatment-induced diabetes (*e.g.* prolonged glucocorticoid use), and treatment-resistant diabetes/hypertension (Oparil *et al.,* 2018; ADA, 2020). No studies to date have examined cognitive function in these forms. Although less common than the well-established sub-types, future studies investigating cognitive function in these rarer sub-types may provide important novel insights into cognitive dysfunction. It could also assist researchers to develop a profile of cognitive disposition unique to each form and enable comparisons of patterns of cognitive decline between the different types.

Numerous glucose-lowering agents are available to assist clinicians to optimise glycaemic control and to treat and manage T2DM. Although the anti-hyperglycaemic benefits of these medications are well established, it is currently unknown whether they confer neuroprotective effects. Sodium glucose co-transporter 2 inhibitors (SGLT2is, *e.g.* empagliflozin, dapagliflozin, and ertugliflozin) and glucagon-like peptide-1 receptor agonists (GLP-1RAs, *e.g.* semaglutide, dulaglutide, and liraglutide) are new classes of anti-hyperglycaemic agents that have demonstrated favourable cardio-renal outcomes independent of glycaemic control, including clinically-meaningful blood pressure reductions and weight loss (Zinman *et al.,* 2015). Current clinical guidelines recommend cardiovascular risk factor management and individualised diabetes management regimens, suitable for the patient and their capabilities, as standard of care for patients suspected of having diabetes-associated cognitive decrements (Biessels & Despa, 2018; Biessels & Whitmer, 2019; Srikanth *et al.,* 2020). Given these medications have demonstrated favourable cardio-renal outcomes independent of glycaemic control in large randomised controlled trials (RCTs), it is plausible these therapies may confer potential beneficial effects on cognition. Large, prospective, long-duration RCTs exploring whether these medications preserve cognitive function, perhaps as a secondary endpoint, would be invaluable. They could also contribute to guiding global health care decisions concerning choice of anti-diabetic medication for patients with diabetes, particularly those with established cognitive decrements.

Finally, the present study could have benefited from recording sensory-evoked event-related potentials (ERPs). These are EEG-derived recordings reflective of cortical activity in response to specific stimuli (*e.g.* auditory and visual) thought to indicate function of neural circuits (Modi & Sahin, 2017). The P300 wave (positive spike in brain activity 300 milliseconds (ms) after presentation of stimulus), which is elicited by auditory stimuli, is the most commonly investigated ERP and is posited to underlie attention and auditory processing (Mulert *et al.,* 2004; Howe, Bani-Fatemi, & De Luca, 2014). Appreciable evidence suggests that abnormalities in the P300 waveform, notably decreased amplitude or increased latency, reflect disrupted neuronal connectivity in frontal and/or parietal brain circuits and inattentiveness (Howe, Bani-Fatemi, & De Luca, 2014; Modi & Sahin, 2017). Early studies have shown that patients with DM (T1DM and T2DM) demonstrate prolonged P300 wave latencies and decreased amplitude, especially those with longstanding, poorly-controlled diabetes (Tsalikian *et al.,* 1981; Mooradian *et al.,* 1988;

Tallroth *et al.,* 1990). Similar P300 abnormalities have also been reported in subjects with hypertension, with higher BP associated with more marked changes (de Quesada-Martinez *et al.,* 2005). Given both DM and HTN are associated with noticeable abnormalities in the P300 brain potential, future investigations exploring cognitive function in subjects with DM or HTN could benefit from recording and analysing other ERPs linked to cognitive processes. The present study also recruited study participants across a broad age range (18-80 years). The impact of ageing on underlying neural architecture and hence neuronal oscillations is well established (Vlahou *et al.,* 2014); therefore, it should be controlled in future investigations, as in this study, to reduce its moderating effects on EEG activity.

7.2 Conclusions

Diabetes mellitus (T1DM and T2DM) and hypertension (HTN) are prevalent, chronic diseases associated with subtle cognitive dysfunction and an increased risk of cognitive impairment (65% increased risk of dementia for T1DM (Smolina *et al.,* 2015); 1.5 to 2.5 times increased risk for T2DM (Biessels, 2006; Cheng *et al.,* 2014); risk of dementia in hypertension unknown). These subtle cognitive decrements, which affect all age groups differently and progress insidiously, can interfere with and complicate daily disease self-management tasks (*e.g.* managing meals and medication, recognising hypoglycaemia, *etc.*), especially in elderly populations (> 65 years of age). Although cognitive dysfunction is being increasingly recognised in clinical practice as a complication of both DM and HTN, with recommendations for managing patients reporting cognitive complaints now included in professional clinical guidelines, clinicians still have difficulty addressing diabetes and hypertension-associated cognitive complaints with patients (Biessels & Whitmer, 2019). Awareness of cognitive dysfunction still also reportedly lags behind that of other well-known diabetes and hypertension-linked complications (*e.g.* retinopathy, nephropathy, neuropathy, stroke, *etc.*) but guidelines for evaluating and diagnosing cognitive dysfunction in DM are developing, especially for T2DM (Figure 7.1). Important questions also remain, such as the selection of cognitive screening tools to be used to detect the subtle cognitive dysfunction triggered by both conditions and the target groups that should be screened. The frequency of screening and when screening should commence, and whether early screening programmes could avert adverse cognitive outcomes, also remain unclear.

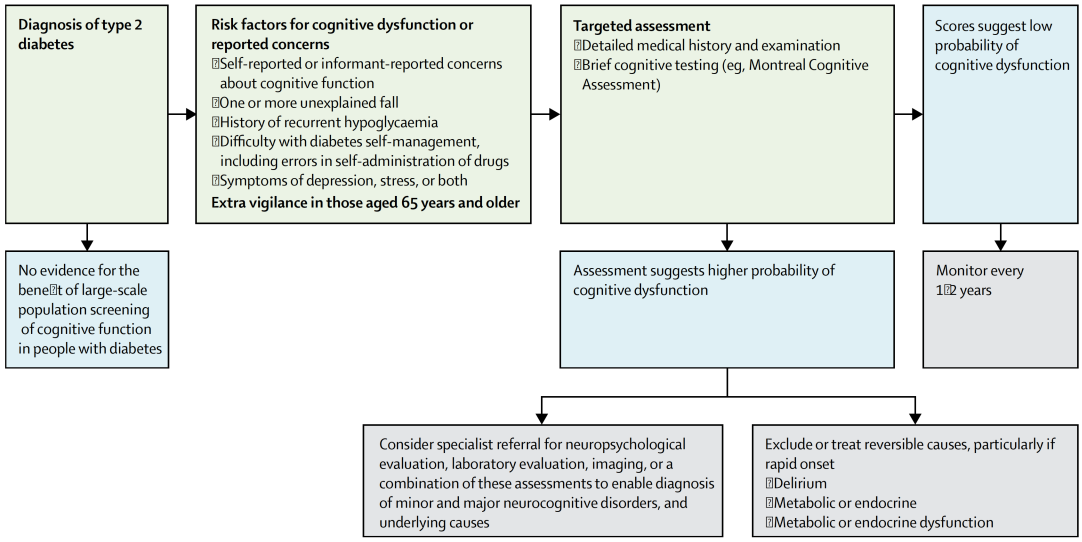


Figure 7.1. Framework for diagnosing and evaluating cognitive dysfunction in Type 2 diabetes mellitus. Adapted from Srikanth *et al.,* (2020, p 537).

Current global estimates suggest approximately 135.6 million (19.3%) people worldwide, over the age of 65 years, have diabetes (IDF, 2019). This number is expected to double by 2045, rising to 276.2 million (19.6%), primarily attributable to an ageing population (IDF, 2019). Similar patterns in prevalence have been reported for hypertension. Given the trends in prevalence predicted for both DM and HTN, and the increased co-occurrence of both diabetes and hypertension and cognitive impairment due to increasing numbers of elderly patients, it is clear that objective non-invasive biomarkers of early cognitive dysfunction are urgently required. Identification of suitable screening tools to allow screening for the subtle cognitive dysfunction linked to these conditions would also be advantageous. Such measures could help clinicians identify patients at increased risk of developing diabetes-associated cognitive decrements and hypertension-associated cognitive dysfunction. It will also allow early stringent glycaemic and blood pressure control, adequate cardiovascular risk factor management, and individualised diabetes care. Current literature and guidelines recommend these strategies to reduce the development of long-term micro- and macrovascular complications and to improve quality of life (QoL) (Biessels & Despa, 2018; Biessels *et al.,* 2020; ADA, 2020). This may contribute to reducing not only the substantial socioeconomic burden linked to diabetes and hypertension-related complications on carers, individual patients, and the healthcare system, but also delay progression to progressive, irreversible neurodegenerative diseases, such as AD and dementia.

The present investigation revealed that BP (SBP and DBP) and BGL are associated with observable changes in oscillatory brain activity, which could be consistently detected using non-invasive scalp EEG. The main changes found in EEG activity in response to BP and BGL were: (1) noticeable increases in slow-wave brain activity (theta and delta), marked over frontal and parietal brain regions, and (2) reductions in fast-wave brain activities (alpha, beta, and gamma), which were evident over central and parietal brain areas. These changes in cortical activity were particularly pronounced when BP and BGL reached certain thresholds (BP: > 135/90 mm Hg; BGL: > 8mmol/L), potentially suggesting the brain becomes vulnerable to insult from diabetes and hypertension-associated cognitive dysfunction at these thresholds. Multiple associations were also found between disease-specific variables ($HbA1_C$, disease duration, and age of disease onset) in the clinical groups and slow-wave activities (theta and delta). While Uhlhaas & Singer (2010) advise the diagnostic specificity of altered neuronal oscillations should be interpreted cautiously, the data from the present study suggest: (1) the EEG signal can reliably and accurately detect changes in ongoing brain oscillatory activity linked to small changes in BGL and BP, and (2) that the EEG could be a suitable neurophysiological measure for detecting the subtle and slowly-progressing cognitive decrements linked to both conditions, potentially identifying EEG as a non-invasive biomarker of early cognitive dysfunction. These are commonly undetected by formal cognitive assessment until frank and irreversible, due to their pernicious nature.

Although novel data were obtained in the present study, it is clear that larger, adequately-powered investigations (cross-sectional and longitudinal) are required to understand better the precise electrophysiological abnormalities that occur in patients with DM (T1DM and T2DM) and HTN, especially the latter. Such studies will validate the preliminary changes in EEG activity reported herein and help determine the main disease-specific variables (*e.g.* disease duration, $HbA1_C$, frequency of hypoglycaemia, *etc.*) that contribute to the electrophysiological abnormalities commonly observed in patients with DM and HTN. Continued investigation of the changes in EEG activity associated with these conditions may enable early recognition of brain regions susceptible to insult, allowing initiation and continuation of robust risk-reduction measures (*e.g.* cardiovascular risk factor management, reduced dietary salt intake, *etc.*) currently suggested by professional clinical guidelines. This may subsequently delay progression

to progressive, irreversible neurodegenerative conditions, such as AD and dementia, and reduce the substantial socioeconomic costs linked to these conditions.

8. Appendices

8.1 Consent Form (Non-clinical and Clinical)

UNIVERSITY OF TECHNOLOGY, SYDNEY
CONSENT FORM

I _____agree to participate in the research project ***'Investigating cognitive function in clinical and non-clinical samples using electroencephalography (EEG) and psychometric assessment: A comparative study (***Approval no: UTS HREC REF NO. 2014000110) being conducted by George Kalatzis in the Neuroscience Research Unit, University of Technology, Sydney (UTS). Funding for this research project has been provided by the School of Life Sciences (UTS).

I understand the purpose of this study is to explore associations between chronic medical disorders and cognitive function. This has implications for identifying disease states that profoundly impair cognitive function, those that accelerate cognitive decline, prompting larger human-based studies, and potentially identifying EEG as a non-invasive biomarker of early cognitive decline.

I understand that participation in this research will involve resting (quiet sitting) and cognitive measures (active mental stimulation). I am also aware that study participation will involve measurements of blood pressure and blood glucose level and brain activity through non-invasive techniques, as well as the completion of questionnaires on lifestyle factors, brain (cognitive) function. I understand this experimental protocol will inflict minimal risk and/or inconvenience.

I also understand the study will involve screening for blood pressure. If classified as normal (non-clinical), under circumstances I may be found to have high blood pressure (>160/100mmHg) throughout any period of the study, involvement in the study will discontinue and I will be offered to be escorted to a doctor and/or advised to consult a medical professional. If my blood pressure is found to be greater than 140/90 mmHg, I will be notified to consult a doctor. If my blood pressure is found to be greater than 160/100 mmHg prior to testing, I understand I will be excluded from further study participation.

Lastly, I also understand the study will involve measurement of blood glucose levels. This will be achieved using a sterile, single-use lancet and reliable and validated blood glucometre and will ONLY be measured before and after cognitive testing and will inflict minimal injury/pain.

I am aware I can contact the investigator (George Kalatzis) on [redacted] or the principal supervisor (Associate Professor Sara Lal) (02) 9514-1592 or Sara.Lal@uts.edu.au) if I have any concerns regarding the research. I am also aware that I am able to withdraw my participation from this research project at any time, without consequences, and without providing a reason.

I agree that George Kalatzis has answered my questions.

I agree that the data collected in this project may be published in a form that does not identify me in any way.

_____ ____/____/____
Signature (participant)

_____ ____/____/____
Signature (researcher or delegate)

NOTE:
This study has been approved by the University of Technology, Sydney Human Research Ethics Committee (HREC). If you have any complaints or reservations about any aspect of your participation in this research which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer (ph: 02 9514 9772; Research.Ethics@uts.edu.au) and quote the UTS HREC reference number. Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

8.2 Emergency Protocol

General Emergency Protocol
ALWAYS CALL SECURITY FIRST

UTS Contacts

1. Dial/call UTS Security: dial "6" on an internal UTS phone or 9514 1192
2. Dial/call 000
3. Dial/call student medical services (9514 1177)
4. Dial/contact principal supervisor (Sara Lal – 9514 1592)

If required:

UTS Medical Centre
Student Services Unit
Tower Building 1, Level 6, UTS
Ph: (02) 9514 1177

Opening Hours:
Monday: 8:30am – 5:30pm
Tuesday: 8:30am – 5:15pm
Wednesday: 8:30am – 5:00pm
Thursday: 8:30am – 3:45pm
Friday: 8:30am – 4:45pm
Saturday and Sunday: Closed)
(Note: opening hours are approximate)

Broadway General Practice (External medical centre)
Level 1, Broadway Shopping Centre,
Bay Street, NSW, 2007
Ph: (02) 8245 1500

Opening Hours:
Monday – Wednesday: 8:30am – 7:00pm
Thursday: 8:30am – 8:00pm
Friday: 8:30am – 7:00pm
Saturday: 9:00am – 6:00pm
Sunday: 10:00am – 6:00pm

Student/Researcher Protocol
Inclusion Criteria

The inclusion criteria for the present study was based on the Lifestyle Appraisal Questionnaire (LAQ) (Craig, Hancock, & Craig, 1996). Participants must meet the following inclusion criteria to be eligible for participation: no severe concomitant disease, no history of alcoholism and drug abuse, and no psychosis, psychological or intellectual problems likely to limit compliance.

Before commencement of any human-related research study, after the participant/volunteer has had a 10-minute sitting (rest) period, record three (3) sitting BP measurements from the participant's right arm. A standard sphygmomanometre or reliable and validated digital BP monitor (Omron, *etc.*) should be used to record BP measurements.

After the measurements, if the average of the three BP readings are >160/100mmHg or >160mmHg for systolic alone or >100mmHg for diastolic BP alone, the participant will not be included in the research study (see consent form in section 9.1 above) and will be thanked for their time and offer to be escorted to the nearest medical centre. The student/researcher must advise participant of their BP and encourage them to seek medical attention.

In the clinical samples (see section 8.1 above) if refused to be escorted to a medical centre, the participant may continue with the study (so long as they feel well enough to do so); however, they are still advised to see a GP regarding their elevated BP. Similarly, three BP readings are to be recorded at the end of the study (if the participant qualified and underwent the study). If BP readings are >160/100mmHg or >160mmHg for systolic alone or >100mmHg for diastolic BP alone, the participant is offered to be escorted to the nearest medical centre and advised to see a GP regarding their BP.

Note: In any case BP is >140/90mmHg, advise the participant to consult their GP.

NOTE:
According to the Australian Heart Foundation (AHF) (www.heartfoundation.org.au) new hypertension guidelines (2008):
Normal BP: < 120/80 mmHg
High to normal BP: 120-139/80-89 mmHg
Grade 1 (mild) hypertension: 140-159/90-99 mmHg
Grade 2 (moderate) hypertension: 160-179/100-109 mmHg
Grade 3 (severe) hypertension: > 180/110 mmHg

8.3 Chronic Disease Questionnaire (Diabetes Mellitus)

Neuroscience Research Unit
Diabetes Questionnaire

Name: Age:
Gender: Ethnicity:

1. Please circle which type of diabetes you have:

a) Type 1
b) Type 2

2. How long have you been diagnosed with diabetes?

3. Does anyone in your immediate family such as your siblings, parents or grandparents have a confirmed diagnosis of diabetes mellitus?

4. How regularly do you monitor your blood glucose levels?

5. In the last 3 months, have you had your haemoglobin a1c/glycosylated haemoglobin ($HbA1_C$) measured by your doctor? If so, please list.

6. Do you at present take any medication(s) to control your diabetes? If so, please list these medication(s).

7. How frequently do you take the medications listed above?

8. Over the years you have been diagnosed with diabetes have you experienced any severe hypoglycaemic (low blood glucose) episodes that have caused disturbance to your daily activities?

9. Do you engage in any physical/recreational activity to manage better your diabetes? If so, please list all physical activities you undertake along with a rough approximation of the time you spend on each activity per week.

10. What other measures do you undertake to control your blood glucose levels (BGL) (*e.g.* diet, stringent glucose monitoring, limit alcohol consumption *etc.)*

11. Have you developed any other medical issues (blindness, kidney issues, tingling in extremities, heart attack, stroke) from your diabetes?

12. On the scale below, please indicate how well you think you manage your diabetes (0= poor, 10= excellent)

1 2 3 4 5 6 7 8 9 10

8.4 Chronic Disease Questionnaire (Hypertension)

Neuroscience Research Unit
Hypertension Questionnaire

Name: Age:
Gender: Ethnicity:

1. Based on Australian hypertension guidelines, please circle which category best describes your degree of hypertension.

a) ≤120mmHg/80mmHg
b) ≥120mmHg/80mmHg (High-normal)
c) ≥140mmHg/90mmHg (Grade 1)
d) ≥160mmHg/100mmHg (Grade 2)
e) ≥180mmHg/110mmHg (Grade 3)

2. How regularly do you monitor your blood pressure?

3. How do you measure your blood pressure (*e.g.* manual sphygmomanometre, self-reported automatic BP monitor, measured by physician?)

4. How often do you have your blood pressure measured and examined by your doctor?

5. Does anyone in your immediate family such as your siblings, parents or grandparents have a confirmed diagnosis of hypertension?

6. How long have you been diagnosed with hypertension?

7. Do you at present take any medication(s) to control your blood pressure? If so, please list these medication(s).

8. How frequently do you take the medications listed above?

9. What other measures (*e.g.* physical activity, meditation exercises, restrict sodium intake, limit smoking) do you undertake to control your blood pressure?

10. Have you developed any adverse outcomes (*e.g.* stroke, coronary artery disease) from your hypertension? If others, please list.

11. On the scale below, please indicate how well you think you manage your high blood pressure (0= poor, 10= excellent)

1 2 3 4 5 6 7 8 9 10

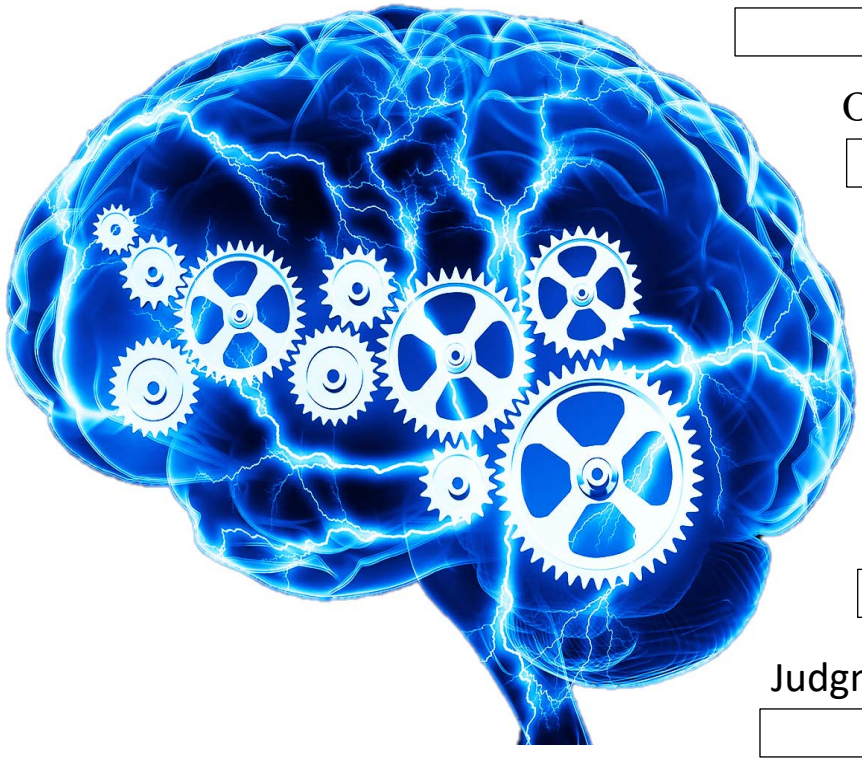
8.5 Cognitive Profile

Cognitive Profile

Name:

Age:

Date:



Attention

Orientation

Memory

Calculation

Construction

Judgment

*The visual cognitive profile summary above is NOT a validated representation of cognition and is intended only to provide a snapshot of your performance in specific cognitive domains based on responses provided in cognitive assessments today. Please consult your medical professional for a comprehensive cognitive evaluation.

8.6 Study Summary Sheet

Summary of Research
(to be completed immediately after each lab study)

Date: _____
Researcher: _____
Participant: _____

1. Provide a brief summary of the study (tick one of the following):
   - □ The study went smoothly
   - □ There were some issues
   - □ There were major issues

2. General account and summary of the study (detail in a few lines or more):

_____
_____
_____
_____

3. Were there any 'out-of-the-ordinary' events or issues in this lab study? Yes / No
If yes, provide more details:

_____
_____
_____
_____

4. Was there an emergency situation in the lab? Yes / No
If yes, provide more details:

_____
_____
_____
_____

Note:

If you answered YES to Question 3, you must notify a senior researcher and/or responsible academic/deputy responsible academic immediately. If you answered YES to Question 4, you SHOULD have followed the emergency protocol and you MUST report the incident using HIRO (Hazard and Incident Reporting Online) *via* the UTS Safety and Wellbeing website (https://www.uts.edu.au/about/safety-wellbeing/hazard-and-incident-response/hiro-support). Subsequently you must then notify a senior researcher or responsible/deputy responsible academic as soon as possible.

8.7 Recruitment Poster

NEUROSCIENCE RESEARCH UNIT

UNIVERSITY OF TECHNOLOGY SYDNEY

Do **YOU** have **DIABETES**?

Do **YOU** want to contribute to important research?

Criteria: 18-80, Diabetes mellitus (Type 1 or Type 2)

George Kalatzis

[redacted]

George.Kalatzis@student.uts.edu.au

**ALL data obtained is treated confidentially, remains secure and completely anonymous*

NOTE: This study has been approved by the University of Technology, Sydney Human Research Ethics Committee (HREC: 2014000110). If you have any complaints or reservations about any aspect of your participation in this research, which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer (ph: 02 9514 9615, Research.Ethics@uts.edu.au) and quote the UTS HREC reference number. Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

8.8 Participant Remuneration Form (Clinical)

Monetary payment of $50 AUD for participation in the study conducted by PhD candidate, George Kalatzis, of the Neuroscience Research Unit (NRU), School of Life Sciences (SoLS), University of Technology, Sydney (UTS). This research project is being supervised by Associate Professor Sara Lal.

Date of Participation	
Address	
Account Name	
BSB	
Account Number	
Financial Institution	

Payment will be deposited into the account above *via* UTS Financial Services Unit fortnightly.

Signed: _____
(Participant)

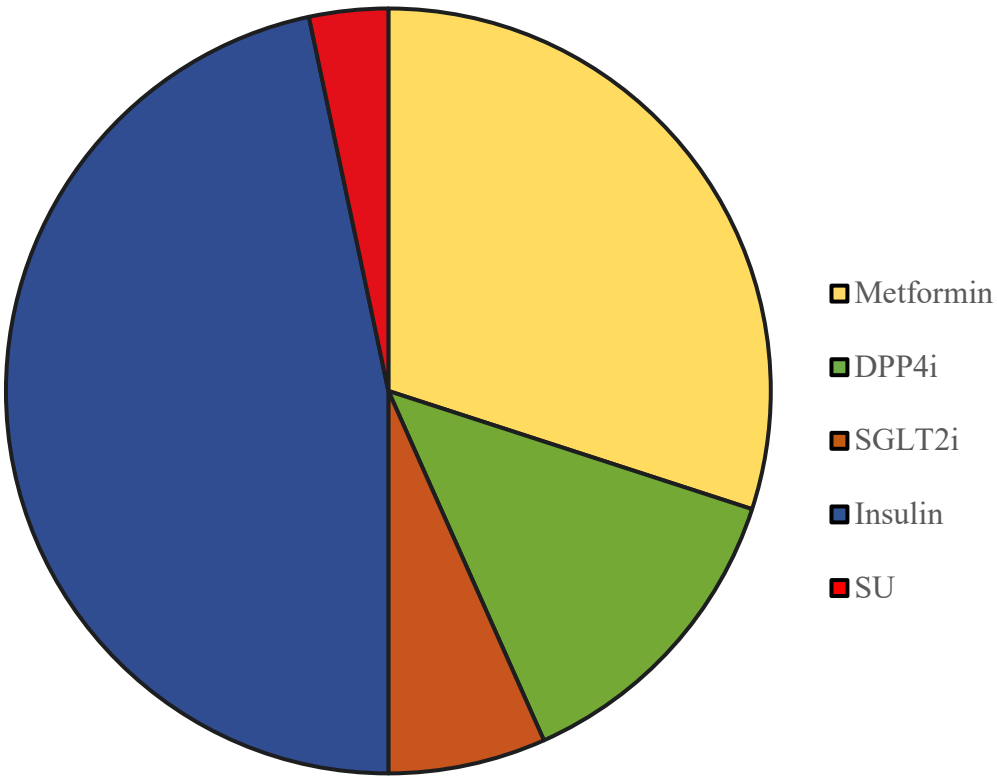
_____
Researcher (George Kalatzis)

_____

Financial Delegate (Deanne Koelmeyer) (School Manager, School of Life Sciences)

INTERNAL USE

8.9 Breakdown of glucose-lowering therapies (n = 30)



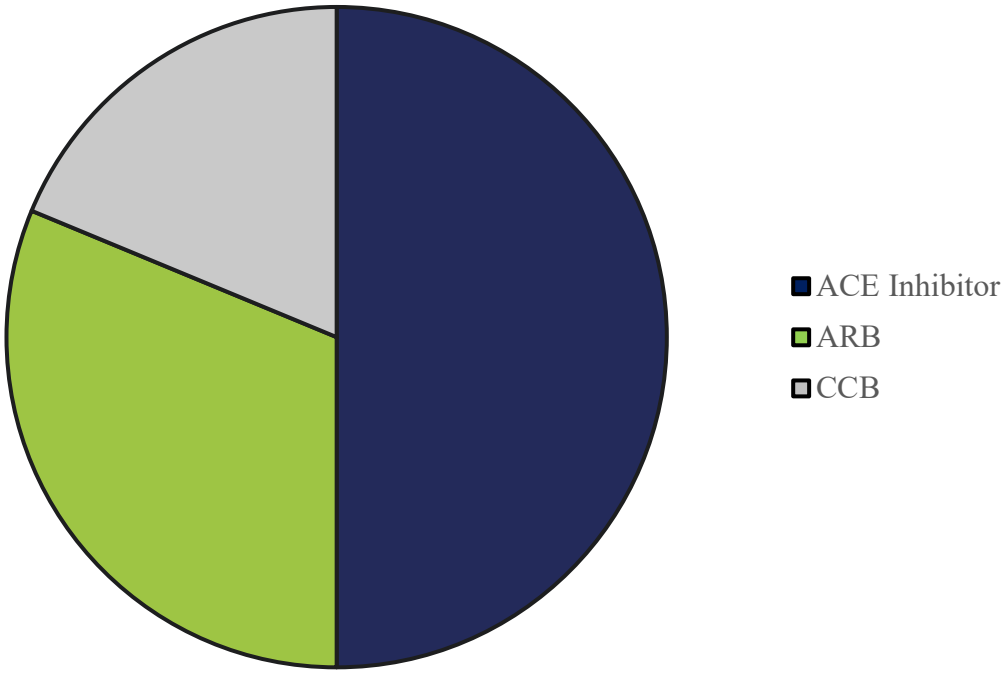
Key:

DPP4i – Dipeptidyl peptidase-4 inhibitor

SGLT2i – Sodium Glucose Co-Transporter 2 inhibitor

SU – Sulfonylurea

8.10 Breakdown of anti-hypertensive medication (n = 15)



Key:

ACE – Angiotensin Converting Enzyme Inhibitor

ARB – Angiotensin Receptor Blocker

CCB – Calcium Channel Blocker

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