COMPARISON OF AUTONOMIC AND CARDIOVASCULAR DYSFUNCTION TO COGNITIVE DECLINE IN PERSONS WITH TYPE 2 DIABETES

OLIVIA RAMRAJ

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

GRADUATE PROGRAM IN KINESIOLOGY AND HEALTH SCIENCE
YORK UNIVERSITY
TORONTO, ONTARIO

August 2023

Abstract

This thesis investigated the relationship between autonomic and cardiovascular dysfunction and cognitive decline in type 2 diabetes. Autonomic indices such as heart rate, blood pressure, pulse wave velocity and cardiac output response to exercise were measured as well as flow mediated dilation and pulse wave reactive hyperemia index. Global cognitive function, executive function and verbal learning tests were administered. Linear regression and correlations were performed between cognitive scores, and vascular and autonomic parameters. Microvascular function was inversely related to verbal learning (n=10, r=-0.86, p=0.0013) and executive function (n=11, r=-0.64, p=0.033). Resting cardiac output was associated with verbal learning (n=8, r=0.77, p=0.025). Heart rate during exercise was associated with global function (n=9, r=0.84, p=0.0020). The second minute of heart rate recovery was inversely related to global function (n=9, r=-0.71, p=0.030) and verbal learning (n=8, -0.71, p=0.037). Overall, hypoperfusion and reduced parasympathetic function were predictors of cognitive decline in type 2 diabetes.

Acknowledgements

Firstly, thank you to my supervisor Dr. Heather Edgell for all that you have done. I am so grateful for your support, encouragement, and the opportunities you have given me. You are an exceptional educator and supervisor, and I am excited to continue working with you.

Thank you to my committee members Dr. Michael Riddell and Dr. Dale Stevens for your support and enthusiasm for this project. Thank you to Dr. Walter Swardfager and his team at Sunnybrook hospital for providing me this research opportunity and supporting me throughout this project, it is a pleasure to work with you.

Thank you to my friend and lab mate Tania for your guidance, humor, and support for the past two years. In times of trouble and stress you were there to motivate and advise me and I look forward to continuing working with you and seeing you accomplish great things. Additionally, thank you to my current and former lab mates Jenan, Concetta, Smriti, Serah, and Eli, for making the lab a warm and welcoming space.

Thank you to my mother and father for encouraging me to pursue graduate school and to work to the best of my ability. You were selfless in raising me and I will not take for granted your countless sacrifices and struggles. I hope that you are proud of what I have accomplished with your love and support for all these years. I always work with you in mind. Thank you to my sister Celine, my best friend. You inspire me to work hard, and to support others around me. You are my pillar of strength and I could not have made it this far without you. Near or far, you have always helped me through anything and showed me that there is no problem too great to be

solved. Thank you to my grandmother for your unconditional love and support. Your loving nature and laughter always bring me happiness when life is difficult. Thank you to my Aunty Annie who inspires me with her strength, generosity, and dedication to family. I would also like to thank my late Aunt Doris who was like a second mother to me. Although she could not be here to see me complete this degree, I am truly grateful for the values she instilled in me and the time she gave to help raise me. Thank you to my friends and family who have supported me throughout this process: Uncle Shastri, Aunty Radica, Uncle Shakar and Aunty Devi, Chacho, and Aunty Lelo, my loving cousins Christopher, Andrea, Cindy, Emily, and Vidya, my friends Aliye, Ekkam, Dayna, and Fidan. Finally thank you to the friends and colleagues I have made at York. I am so grateful for each and every one of you.

Table of Content

List of Tables

List of Figures

List of Abbreviations

ANS: Autonomic Nervous System

AUC: Area Under the Curve

BDAE: Boston Diagnostic Aphasia Examination

BP: Blood Pressure

cf-PWV: Carotid-Femoral Pulse Wave Velocity

CVLT-II: California Verbal Learning Test Second Edition

DPP-4: dipeptidyl peptidase 4

DSST: Digit Symbol Substitution Test

ECG: Electrocardiogram

ELISA: Enzyme-Linked Immunosorbent Assay

eNOS: Endothelial Nitric Oxide Synthase

FMD: Flow Mediated Dilation

GLP-1: Glucagon-like peptide-1 receptor

HBA1c: Hemoglobin A1c, or Glycated Hemoglobin

HF: High Frequency

HR: Heart Rate

HRR: Heart Rate Recovery

HRV: Heart Rate Variability

LF: Low Frequency

MAP: Mean Arterial Pressure

MMSE: Mini Mental State Exam

MVC: Maximal Voluntary Contraction

NF-kB: Nuclear Factor kappa B

NO: Nitric Oxide

PNA: Parasympathetic Nerve Activity

PNS: Parasympathetic Nervous System

PTT: Pulse Transit Time

PWRHI: Pulse Wave Reactive Hyperemia Index

PWV: Pulse Wave Velocity

Q: Cardiac Output

RAAS: Renin- Angiotensin- Aldosterone- System

RHI: Reactive Hyperemia Index

ROS: Reactive Oxygen Species

SA: Sinoatrial Node

SCWT: Stroop Colour and Word Test

SERCA: Sarco-Endoplasmic Reticulum Calcium ATPase

SDRR: Standard Deviation of the RR Interval

SGLT2: sodium-glucose cotransporter

SNA: Sympathetic Nerve Activity

SNS: Sympathetic Nervous System

TPR: Total Peripheral Resistance

VST: Stroop Neuropsychological Test -Victoria Version

WAB: Western Aphasia Battery

Chapter 1: Literature Review

1.1. Understanding the Autonomic Nervous System

The autonomic nervous system (ANS) is responsible for unconscious and involuntary physiological processes such as the regulation of ventilation, heart rate (HR), blood pressure (BP), digestion, and sexual arousal. The sympathetic nervous system (SNS) prepares the body for physical activity by redirecting oxygenated blood towards exercising muscles, commonly referred to as the "fight or flight" response. The parasympathetic nervous system (PNS) has the opposite effect and promotes a state of relaxation, known as "rest and digest".

The ANS plays a significant role in the cardiovascular system by regulating HR and BP. Sensory information is relayed to the ANS through afferent systems that connect to the hypothalamus and tegmental nuclei in the brain through the vagus nerve. For example, baroreceptors in the aortic arch and carotid artery respond to deformation of the vessel wall during changes in BP (Haus et al., 1949). Additionally, chemoreceptors in the carotid bodies found at the bifurcation of the carotid artery and aortic arch, detect oxygen and carbon dioxide concentrations by sensing their partial pressures (Lahiri et al., 1981). When a change in BP is detected, the ANS modulates the balance between sympathetic nerve activity (SNA) and parasympathetic nerve activity (PNA). SNA regulates BP through cardiac and vascular noradrenergic sympathetic nerves (Esler et al., 1995), epinephrine synthesized by the adrenal medulla (Berecek & Brody, 1982), and activation of the renin- angiotensin-aldosterone- system (RAAS) (Tigerstedt & Bergman, 1898). As reviewed by Charkoudian and Rabbitts (2009), SNA influences HR by stimulating the sinoatrial (SA) node, and stroke volume by increasing contractility of cardiac muscle. The sympathetic system also promotes vasoconstriction in the peripheral vasculature to raise BP (reviewed in Charkoudian & Rabbitts, 2009). The ANS responds to short term increases in BP by reducing SNA to lower vasoconstriction, stroke volume, and HR which in turn reduce BP back to homeostatic levels (reviewed in Charkoudian and Rabbitts, 2009). Meanwhile, the PNS is simultaneously recruited to reduce HR (reviewed in Charkoudian and Rabbitts, 2009).

1.2. Measuring Autonomic Nervous System Function

1.2.1. Response to Isometric Hand Grip Exercise

Response to isometric hand grip exercise can be used to evaluate the autonomic control over the cardiovascular system. Sustained hand grip exercise increases HR and BP to provide adequate blood supply to working muscle (Martin et al., 1974). Hand grip exercise increases mean arterial pressure (MAP) (Alam & Smirk, 1937; Lind & McNicol, 1967) by activating the exercise pressor reflex. Mechanically sensitive group III afferent fibers, and metabolite sensitive IV afferent fibers are stimulated (Kaufman et al., 1983; J. H. Mitchell et al., 1983) resulting in increased SNA and vagal withdrawal (Arai et al., 1989; Kamiya et al., 2001). The rise in HR during hand grip exercise is attributed to vagal withdrawal (Martin et al., 1974; Freyschuss et al., 1970), and the continued stimulation of HR is maintained by sympathetic activation (Leon et al., 1970; Martin et al., 1974; Robinson et al., 1966). Recovery of resting BP and HR following exercise cessation is due to reactivation of the PNS (Imai et al., 1994). Healthier individuals return to resting values faster than those with pathological conditions such as hypertension (Jackson et al., 1973; Martin et al., 1974) . Furthermore, heart rate recovery (HRR) following exercise represents autonomic function (Savin et al., 1982) and is a predictor of mortality (Cole & Lauer, 1999).

1.2.2. Pulse Wave Velocity

Large artery stiffness is an important indicator of cardiovascular risk as it is the earliest sign of mechanical stress and vessel remodeling, a process associated with cardiovascular disease. Pulse wave velocity (PWV) is the gold standard method for non-invasively determining arterial stiffness and represents the velocity of pulse pressure waves created by the systolic contraction of the heart. Studies show that PWV is a predictor of cardiovascular events (Matsuoka et al., 2005; Mattace-Raso et al., 2006; G. F. Mitchell et al., 2010; Sutton-Tyrrell et al., 2005) and mortality (Cruickshank et al., 2002; Laurent et al., 2001; Shokawa et al., 2005). The current study will use PWV as an index of sympathetic activity as sympathetic innervation has a vasoconstrictive influence on the vasculature (Charkoudian et al., 2005) and reduces arterial wall distensibility (Failla et al., 1999; Gerová & Gero, 1969). The current study will use a previously established technique using pulse pressures recorded at the levels of the finger and toe to calculate PWV as an index of SNA (Edgell et al., 2016; Fouladi et al., 2019), as described in the methods.

1.2.3. Heart Rate Variability

Heart rate variability (HRV) is considered the gold standard technique for determining cardiac autonomic balance. HRV is a valid measure of autonomic function in diabetes (Benichou et al., 2018) and is capable of predicting hypertension and cardiovascular risk (Schroeder et al., 2003; Tsuji et al., 1996). HRV represents the variation between successive heart beats and is predominately controlled by vagal tone while at rest (Montano et al., 1998; Pomeranz et al., 1985), which produces greater variation. Low HRV and reduced baroreceptor sensitivity are indicators of higher resting sympathetic tone and cardiac autonomic dysfunction (Rosengård-Bärlund et al., 2011). The current study will determine the relative contributions of the SNS and PNS using spectral analysis and frequency domain parameters such as low frequency (LF; an index of combined SNS and PNS), high frequency (HF; an index of PNS), and LF/HF ratio (an index of SNS) (Malik et al, 1996), as described in the methods. A time domain parameter of HRV, standard deviation of the RR interval (SDRR) will also be calculated as described below.

1.3.Autonomic Function and Type 2 Diabetes

Type 2 diabetes is associated with arterial stiffness (Cruickshank et al., 2002) and cardiac autonomic dysfunction (Meyer et al., 2004; Tentolouris et al., 2003). In people with type 2 diabetes, arterial stiffness measured with PWV can be used to predict mortality (Cruickshank et al., 2002) and HRV is associated with autonomic dysregulation (Meyer et al., 2004) whereby individuals with type 2 diabetes exhibit reduced HRV (Benichou et al., 2018; Coopmans et al., 2020). Indeed, studies have shown that parasympathetic nerve fibers of the heart can be damaged in type 2 diabetes, leading to the development of cardiac autonomic neuropathy reducing HRV (Krishnaswamy et al., 2015).

1.4. Understanding Vascular Function

The vascular system is comprised of large vessels such as arteries and veins in addition to arterioles, capillaries and venules that are classified as the microvasculature (reviewed in Pugsley & Tabrizchi, 2000). These vessels form an elaborate network throughout the body to deliver vital nutrients and oxygen carried by the blood. Additionally, cellular and metabolic waste are transported by the vascular system for clearance. Arteries transport blood from the heart to arterioles and then continuously bifurcate into capillaries, the vessels with the smallest diameter, where substances are exchanged across the capillary walls. Blood flow transitions to the venous system at the level of the capillary due to connections with venules and veins connecting back to the heart. Borysenko and Beringer described three distinct layers of the vessel walls that differ in their proportion of smooth muscle and elastin content, giving rise to unique physiological functions (Borysenko and Beringer, 1984). The tunica intima is the thin innermost layer, formed by a single layer of endothelial cells anchored by the basal lamina (also known as basement membrane). Endothelial cell function is an important determinant of vascular health due to their role in promoting tissue homeostasis and regulation of vascular tone, a feature that will be discussed below. The tunica media is the intermediate layer, consisting of smooth muscle and elastin fibers, which give vessels their contractile properties. Lastly, the tunica adventitia is the outermost layer, consisting of fibro-elastic connective tissue for protection.

Blood vessels are capable of dilating and constricting in response to stimuli. Vasodilation is the outward expansion of blood vessel walls as vascular smooth muscle relaxes. This increase in vessel diameter accommodates a greater volume of blood and lowers total peripheral resistance (TPR) and BP. Vasoconstriction refers to the reduction in vessel diameter that occurs as the vascular smooth muscle contracts raising the TPR and BP. The sympathetic branch of the ANS regulates BP by influencing vascular tone (reviewed in Bruno et al., 2012). At rest, blood vessels are slightly vasoconstricted and may further contract or dilate if required.

1.5. Reactive Hyperemia

Reactive hyperemia is the compensatory physiological response to increase tissue reperfusion after arterial occlusion. Peripheral microvascular function can be determined non-invasively by activating reactive hyperemia and measuring an individual’s capacity to vasodilate (reviewed in Flammer et al., 2012). Experimental reactive hyperemia consists of a large influx of blood that occurs following the release of arterial occlusion to a limb (Bier, 1897) via a BP cuff wrapped around a segment of a limb inflated to suprasystolic pressures. For forearm occlusion, guidelines suggest inflating the BP cuff to at least 50 mmHg above systolic BP to occlude arterial blood flow to the limb for 5 minutes (reviewed in Corretti et al., 2002; reviewed in Thijssen et al., 2011). As blood flow is restricted, the tissue becomes ischemic and stimulates vasodilation through various physiological mechanisms such as metabolite accumulation (Koller & Kaley, 1990). Vasodilation resulting from increased blood flow during reperfusion is endothelium dependent (Nabel et al., 1990, Rubanyi et al., 1986) and regulated by endothelium-derived nitric oxide (NO) (Joannides et al., 1995). Shear stress is detected by stretch sensitive calcium channels on the surface of the endothelial cell membrane (Lansman et al., 1987) and the flow stimulus is transduced by calcium-activated potassium channels (Cooke et al., 1991; Miura et al., 2001; Olesen et al., 1988). The resulting hyperpolarization of the endothelial cell drives calcium entry into the cell and stimulates endothelial NO synthase (eNOS) by oxidizing L-arginine (Rubanyi et al., 1986; Stuehr, 2004). NO rapidly diffuses into the vascular smooth muscle cells where it triggers a cascade of events leading to vasodilation (Schultz et al., 1977). Calcium availability is reduced by inhibiting voltage gated calcium channels and stimulating the sarco-endoplasmic reticulum calcium ATPase (SERCA) to increase calcium reuptake (Van Hove et al., 2009). As a result, vascular smooth muscle contraction is prevented allowing vascular smooth muscle to relax and dilate.

1.6. Measuring Reactive Hyperemia:

1.6.1. Flow Mediated Dilation

Various techniques have been developed to measure the effects of reactive hyperemia. Early studies used venous occlusion plethysmography (Hewlett & Zwaluwenberg, 1909), a technique that uses a strain gauge around the limb to measure changes in volume. However flow mediated dilation (FMD), an ultrasound technique developed in 1992 by Celermajer and colleagues (Celermajer et al., 1992; Sorensen et al., 1995), is considered the gold standard non-invasive technique for detecting endothelial dysfunction. Using duplex ultrasound, FMD measures shear stress and the percent dilation from resting diameter in a conduit artery during the hyperemic response (typically the brachial artery). In this protocol, a BP cuff is wrapped around the forearm and inflated to restrict blood flow to the distal limb segment during the occlusion period and is rapidly deflated to produce reactive hyperemia. The arm is extended outward at heart level and the brachial artery is imaged using an ultrasound for at least 1 minute of baseline rest, 5 minutes of occlusion, and for at least 3 minutes of recovery (reviewed in Thijssen et al., 2011). The vasodilation observed during FMD is endothelium dependent (Miura et al., 2001, Lieberman 1994), and serves as a surrogate measure of NO bioavailability and endothelial function (Joannides et al., 1995).

FMD has clinical relevance as it has an inverse relationship with cardiovascular risk factors, as shown by the Framingham Heart Study (Miura et al., 2001). Endothelial dysfunction is an early predictor of cardiovascular disease and precedes the development of atherosclerotic plaque (Ludmer et al., 1986). In research settings, FMD has been used to examine the effects of interventions such as drugs (Anderson et al., 2000) or exercise training (Badrov et al., 2016) on vascular function and identify physiological mechanisms behind the responses.

FMD results can be influenced by a series of substances and activities. Participants undergoing FMD are required to refrain from ingesting high-fat foods, caffeine, and alcohol for at least 8 hours prior to testing. Further, participants may not engage in fatiguing exercise or smoking for 8 hours before testing. For repeated measurements or between-group studies, FMD tests should be performed at the same time of day due to diurnal variation (Järvisalo et al., 2006; Ringqvist et al., 2000).

1.6.2. Pulse Wave Reactive Hyperemia Index (PWRHI)

The EndoPAT device (Itamar, Israel) has been validated as a reliable non-invasive instrument for detecting endothelial dysfunction (Bonetti et al., 2004) and has been compared to FMD (Kuvin et al., 2003). A similar protocol of rest, 5 minutes of arterial occlusion, and reactive hyperemic recovery is used while arterial tonometry is measured at the level of the finger. The EndoPAT algorithm calculates the reactive hyperemia index (RHI) by obtaining a ratio of the average finger pulse wave amplitude for 60 seconds of reactive hyperemia compared to the average pulse wave amplitude for 3.5 minutes of baseline in the hand undergoing arterial occlusion (Kuvin et al., 2003). This ratio is then normalized to the non-occluded control arm that is measured concurrently to account for systemic changes in the microvasculature. Although EndoPAT and FMD both measure the blood flow response to reactive hyperemia, they measure distinct physiological mechanisms leading to vasodilation. Nardone and colleagues (2020) found that while EndoPAT is a measure of microvascular function and is correlated with dobutamine mediated coronary vasodilation (and thus dilation due to adrenergic stimulation), FMD is associated with acetylcholine and adenosine mediated coronary vasodilation.

Pulse Wave Reactive Hyperemia Index (PWRHI) is an experimental technique we developed measuring microvascular function. PWRHI is a proposed replacement technique for the EndoPAT and has been shown to have the best correlation when measuring a 10 second period of time around the peak hyperemic response ($r= 0.67$, $p=0.0002$, $R^2= 0.58$; Appendix 1). PWRHI uses the same protocol as the EndoPAT for inducing reactive hyperemia using piezoelectric pulse transducers (ADInstruments, USA) that are wrapped around the tip of the middle fingers rather than proprietary finger cuffs. These transducers are capable of detecting changes in force against its surface which occur with every systolic pulse, and the signal is recorded as a pulse wave signal similar to the first derivative of the waveform generated from the pneumatic finger probes of the EndoPAT that detect changes in plethysmographic pressure (Moerland et al., 2012). Therefore, the area under the curve (AUC) of the piezoelectric waveforms is determined as a surrogate for the EndoPAT signal.

1.7. Vascular Dysfunction and Type 2 Diabetes

Vascular function assessments such as FMD (Henry et al., 2004; Keymel et al., 2011; Maruhashi et al., 2013; Tsuchiya et al., 2007), and peripheral arterial tonometry (Gargiulo et al., 2013; Hahad et al., 2019; Hamburg et al., 2008) have verified that endothelium- dependent vasodilation is impaired in people with type 2 diabetes (Mäkimattila et al., 1999; Williams et al., 1996). Elevated production of reactive oxygen species (ROS) in diabetes contributes to the inactivation of endothelial-derived NO, limiting its ability to trigger vessel relaxation (Chowienczyk et al., 2000). Additionally, there is reduced enzymatic and non-enzymatic antioxidant content to counteract oxidative stress (Sundaram et al., 1996). This is supported by evidence that administering antioxidants can reverse endothelial dysfunction in people with type

2 diabetes (Chowienczyk et al., 2000). Furthermore, reduced NO content is associated with atherogenesis as endothelium-derived NO diminishes vascular lesions (Kolpakov et al., 1995), prevents leukocyte and platelet adhesion to the vessel wall (Radomski et al., 1987), as well as inhibits vascular smooth muscle cell migration to the intima (Sarkar et al., 1996). This, in addition to the increased expression of nuclear factor kappa B (NF-kB), a pro-inflammatory transcription factor, gives rise to foam cells and atherosclerotic plaques (Zeiher et al., 1995). Studies have found an inverse relationship between endothelial dysfunction and cardiovascular risk factors, supporting the use of endothelial dysfunction as a marker of vascular damage (Gokce et al., 2002; Rubinshtein et al., 2010; Yeboah et al., 2009).

1.8. Understanding Cognitive Function:

The term cognitive function encompasses multiple mental processes involved in acquiring and manipulating information, as well the ability to reason. The current study will focus on two domains that fall under cognitive function: executive function, and memory. Within these domains exist subdomains that are specific to a component ability (reviewed in Harvey, 2019). For instance, executive function contains reasoning, problem solving and component skills management, and requires the interaction of multiple basic and complex processes. Additionally, the memory domain contains the episodic/declarative subdomain containing a verbal component. This is of particular interest as daily experiences requiring verbal learning skills must be encoded and transferred in and out of long-term memory.

1.9. Measures of Cognitive Function

Cognitive function tests aim to evaluate an individual's ability to perform isolated cognitive skills and are commonly used to detect the severity of cognitive decline. The current study will use several cognitive tests in the form of verbal and written tests as well as questionnaires. The Mini Mental State Exam (MMSE) (Folstein et al., 1975) (Supplementary Information 1), California Verbal Learning Test Second Edition (CVLT-II) (Delis et al., 2000) (Supplementary Information 2), Trails Making Test Part A & B (Reitan, 1958) (Supplementary Information 3), Digit Symbol Coding (Supplementary Information 4), Stroop Neuropsychological Test- Victoria Version (VST) (Supplementary Information 5) (Spreen & Strauss 1998), and Verbal Fluency Test (FAS and Animal Naming) (Supplementary Information 6) will be administered as described in the methods. The MMSE measures degree of cognitive impairment by assessing awareness, attention and memory. The CVLT-II measures verbal learning by testing immediate recall, short delay free recall and long delay recall, which can be combined into one composite score for verbal learning as described in the methods. The Trails Making Test Part A & B measure executive function, cognitive flexibility, visual scanning capability, and working memory. Digit symbol coding assesses incidental memory recall. The SCWT measures an individual's ability to process stimulus attributes while there is another impeding attribute stimulus. And the Verbal Fluency Test determines semantic and phonetic fluency. A composite score for executive function can be calculated using scores from the Stroop Colours test, F-A-S test, Digit Symbol Coding test, and the Trails Making Test Part B, further described in the methods.

Previous studies have used several cognitive function tests together to associate cognitive decline and vascular dysfunction (Brant et al., 2018; Cohen et al., 2009; Kearney-Schwartz et al., 2009). For instance, Brant and colleagues (2018) used phonetic (F-A-S) and semantic (animal naming) verbal fluency tests, and the Trails Making Test Part B (all described below) to determine if there was an association between cognitive decline and endothelial function evaluated using the EndoPAT but did not find a significant correlation between endothelial function and the cognitive function tests when they adjusted for confounding factors. CVLT-II, Trails Making Part A, Digit Symbol-Coding, and the Stroop Colour Word Interference Test (all described below) have also been compared to EndoPAT scores (Saleem et al., 2019). Saleem and colleagues (2019) found a significant relationship between lower EndoPAT RHI and poor verbal memory performance but not overall cognition. However, they also found that higher RHI was associated with improved overall cognition and processing speed after 3 months of cardiac rehabilitation.

The relationship between autonomic function, specifically HRV, and cognitive decline has previously been investigated (Dalise et al., 2020; Mahinrad et al., 2016; Schaich et al., 2020; Zeki Al Hazzouri et al., 2018). Dalise and colleagues reported a relationship between global cognition measured by the MMSE and HRV sympathetic parameters and Mahinrad and colleagues (2016) found that reduced HRV was associated with poor executive function and processing speed. Further, Schaich and colleagues (2020) reported that measures of cardiac parasympathetic activity were associated with higher scores on the Digit Symbol Coding Test and Zeki Al Hazzouri and colleagues (2018) determined that higher standard deviation between R-R intervals (SDRR) was associated with better executive function and processing speed measured by the Stroop test and Digit Symbol Substitution Test (DSST). Additionally, resting PWV has been associated with cognitive impairment using the MMSE (Hanon et al., 2005; Scuteri et al., 2007; Zhong et al., 2014), as well as the Trails Making Test A & B, DSST, and

Verbal Fluency Test (F-A-S) (Zhong et al., 2014). For instance, Zhong and colleagues (2014) reported an association between arterial stiffness and poor executive function, memory, and psychomotor speed; Hanon and colleagues (2005) found a relationship between high PWV and cognitive impairment and Scuteri and colleagues (2007) determined that PWV was a strong predictor of cognitive decline. These relationships suggest that cognitive function may be associated with both vascular and autonomic function in type 2 diabetes.

1.10. Cognitive Function and Type 2 Diabetes

Individuals with type 2 diabetes are at greater risk for cognitive impairment (Cholerton et al., 2019; Fontbonne et al., 2001; Gregg et al., 2000). For instance, a study by Gregg and colleagues (2000), used the MMSE, Trails Making Test Part B, and a Digit Symbols Test to investigate cognitive decline in older women, and found lower baseline scores among the women with diabetes compared to those without. The MMSE and Digit Symbols Test revealed that the women with diabetes were prone to accelerated decline compared to women without diabetes when tested 3 and 6 years later (Gregg et al., 2000). Furthermore, those with type 2 diabetes are more likely to develop dementia (Leibson et al., 1997).

Appropriate glucose control reduces the risk of cognitive decline (Gao et al., 2008; Shorr et al., 2006). There are several hypotheses explaining the incidence of cognitive decline in type 2 diabetes including lesions in the brain vasculature (Qiu et al., 2014), insulin dysregulation (Ekblad et al., 2017), inflammation (Marioni et al., 2010), and oxidative stress (Berr et al., 2000). For instance, inflammation and oxidative stress resulting from hyperglycemia may lead to neuronal injury and brain atrophy (Lin et al., 2000, Vincent et al., 2005).

Chapter 2: Proposed Study Design

2.1. Research Objectives

This cross-sectional study aimed to determine if there is a relationship between the impaired vascular integrity and autonomic function found in patients with Type 2 Diabetes Mellitus and cognitive decline.

2.2. Hypotheses

We hypothesized that

1) Cognitive decline and vascular damage are associated in individuals with type 2 diabetes.
2) Cognitive decline and impaired autonomic function are associated in individuals with type 2 diabetes.

2.3. Methods

2.3.1. Participants

A total of 6 women and 6 men were included in the study, 11 with type 2 diabetes, 1 with prediabetes, high Hemoglobin A1c (HBA1c), impaired fasting glucose, or impaired glucose tolerance were recruited to participate. Patients were excluded if they had type 1 diabetes, neurological or neurodegenerative disorders, active cancer, pregnancy, or substance use disorder (diagnosed within the last 5 years). Patients were ineligible if they had a psychiatric diagnosis such as schizophrenia or bipolar disorder. The study was conducted at the Sunnybrook Health Sciences Centre. Ethical approval was obtained from York University Research Ethics Board and Sunnybrook Health Sciences Centre Research Ethics Board. Written informed consent was required from all participants.

2.3.2. Experimental Protocol

2.3.2.1. Patient Anthropometrics

Prior to testing, participant anthropomorphic data was collected. Measurements of height, weight, systolic BP, diastolic BP were taken. Distance from the clavicle to tip of the middle finger as well as from the clavicle to the tip of the great toe were measured and recorded. Participants were fasted for 12 hours and required to avoid caffeine and nicotine consumption during that time. Age, ethnicity, history of smoking, cardiovascular and respiratory conditions, number of times of exercise per week (used as an estimation of VO_2 max) (Ainsworth et al., 1993) and current medications were self-reported. After a period of supine rest, participants were fitted with the necessary equipment required for the vascular and autonomic tests described below.

2.3.2.2. Vascular Health Measures:

Two vascular assessments, FMD and PWRHI, were conducted simultaneously while patients lay supine. A standard BP cuff with trigger release was wrapped around the right forearm. The right arm was extended outward in a supinated position and remained at heart level to allow for ultrasound imaging of the brachial artery. Piezoelectric pulse transducers (ADInstruments, USA) were wrapped around the distal phalanx of both middle fingers, with sensors facing the pad/underside of the finger. Following 3.5 minutes of baseline data acquisition, blood flow was occluded to the right arm by inflating the BP cuff to 40 mmHg above systolic BP for a 5-minute duration. Following the occlusion period, the BP cuff was deflated to induce reactive hyperemia and recording continued for a 3-minute recovery period.

Pulse Wave Reactive Hyperemia Index (PWRHI)

PWRHI was used to measure microvascular function as we have found a strong correlation with the EndoPAT device (Appendix 1). A PowerLab and LabChart software (ADInstruments, USA) was used to capture the pulse wave recordings detected by the piezoelectric transducers on both middle fingers. Pulse waves were analyzed in the test (occluded) and control (non-occluded) arm for 3.5 minutes of baseline (pre) and for the 10 seconds around the peak amplitude (post) following cuff deflation. The area under the curve (AUC) for each cardiac cycle was obtained for the test and control arms. The peak analysis module of LabChart was used to determine the mean AUC in the test and control hands for 3.5 minutes of baseline and 10 seconds around the peak response. The PWRHI score was calculated as the ratio of the AUC during the 10s peak response divided by the baseline AUC in the test arm, and normalized to the ratio in the control arm: PWRHI= (AUCtest peak/test baseline)/ (AUCcontrol peak/control baseline).

Flow Mediated Dilation (FMD)

Flow mediated dilation (FMD) of the right brachial artery was determined using a Phillips Epiq 7g ultrasound (Phillips, USA) and an L18-5 transducer attachment. Ultrasound imaging was performed in duplex mode for a total duration of 10 minutes to capture both vascular images and blood velocity. Video recording lasted for 2 minutes of resting baseline, 5 minutes of vascular occlusion, and 3 minutes of reperfusion following cuff deflation. Ultrasound video was captured using a video grabber device (AV.io HD, Epiphan Systems, USA) and video recording software (VirtualDub version 1.10.4 by Avery Lee). FMD video was analyzed using automated wall tracking software (Cardiovascular Suite, Quipu, Italy). FMD was calculated as the percent change in the brachial artery diameter from baseline to post occlusion. Shear rate was calculated using an automated software (Cardiovascular Suite, Quipu, Italy). The software determined the time to peak artery diameter, then calculated the area under the blood velocity curve during that time interval. Shear rate was calculated as: Shear rate = 8 x mean blood velocity/ internal diameter.

2.3.2.3. Autonomic Nervous System Function Tests

Hand Grip Exercise

The BP and HR responses to hand grip exercise was tested in participants to evaluate their ability to regulate sympathetic activity. Continuous BP was recorded using a Finometer Pro device (Finapres Medical Systems, Netherlands), which required participants to wear a standard BP cuff around their left bicep. Additionally, a finger cuff was placed around the intermediate phalanx of the middle finger of the left hand, and a height corrector was used to apply a hydrostatic correction to the BP. This is required to account for the effect of gravity on the BP in the arm compared to the heart. To further correct for hydrostatic pressure, the left arm was placed in an arm sling to keep the finger at heart level. Participants had electrocardiogram (ECG) electrodes placed on their left rib, and below each clavicle. A piezoelectric pulse transducer (ADInstruments, USA) was placed on the right great toe, with the sensor towards the pad of the toe. Participants held a hand grip dynamometer in their right hand (ADInstruments, USA). All devices were connected to a PowerLab data acquisition device (ADInstruments, USA) and responses were recorded using LabChart software (ADInstruments, USA).

Participants were asked to squeeze a hand grip dynamometer with maximal force for two contractions, one minute apart. The contraction with greater force was used to obtain the maximal voluntary contraction (MVC). Following 5 minutes of resting supine baseline measurements, participants performed hand grip exercise to 40% of their MVC for 2 minutes. A computer monitor displayed the force applied to the hand grip dynamometer as a percentage of their MVC, to allow participants to regulate the intensity of their exercise. Data was recorded before, during and for 3 minutes after hand grip exercise.

Heart Rate Variability

Heart rate variability (HRV) was collected using a single lead ECG and a PowerLab device. Five minutes of resting supine ECG signal was selected and HRV was determined using the HRV LabChart module to generate an automated report. The automated report contained parameters pertaining to time and frequency domains. The time domain was analyzed using the standard deviation of the RR interval (SDRR) obtained from the report. Frequency domain parameters such as low frequency (LF) measures, high frequency (HF) measures, and low frequency/ high frequency (LF/HF) ratio were determined. LF indicated combined sympathetic and parasympathetic activity, while HF and LF/HF ratio indicated parasympathetic and sympathetic contributions respectively.

Pulse Wave Velocity

Pulse wave velocity (PWV) was determined from the pulse waves from the piezoelectric pulse transducer around the great toe and the Finometer Pro BP cuff around the finger (previously described) that recorded during the entire hand grip protocol using LabChart software. PWV was acquired during the final minute of baseline and for the second minute of hand grip exercise. For each timeframe, ~20 heart beats were analyzed by identifying the foot of each waveform and calculating the time difference between the start of the waveform for the toe pulse (T start toe) and the start of the waveform for the finger BP (T start finger). Pulse transit time (PTT) was calculated as PTT= T start toe- T start finger. The measured distance from the clavicle to the tip of the finger was subtracted from the distance from the clavicle to the tip of the toe. The PWV was calculated using the formula PWV= (Distance toe- Distance finger)/Average PTT

2.3.2.4. Cognitive Function Tests

Verbal tests and questionnaires were administered to assess the cognitive ability of participants. The Mini Mental State Examination (MMSE) is a 30-point questionnaire developed by Folstein and colleagues in 1975 to evaluate global cognitive impairment (Folstein et al., 1975; Supplementary Information 1). A score of 23 or 24 is the recommended cut-off to identify cognitive decline or dementia. The current study required a score greater than or equal to 24 to participate. The California Verbal Learning Test Second Edition (CVLT-II) was used to measure episodic verbal learning and recall (Delis et al., 2000). This edition is shorter in duration than the original California Verbal Learning Test (Delis et al., 1987) and is better tolerated by patients (Supplementary Information 2). The Trails Making Test, Part A & B (Reitan, 1958) was used to measure executive function, cognitive flexibility, working memory and the ability to perform visual scanning. The test required participants to perform several time limited line drawing tasks (Supplementary Information 3). Digit Symbol Coding examinations were used to test incidental memory recall by evaluating an individual’s ability to match numbers to an assigned symbol. The Digit Symbol Substitution Test (DSST) by David Wechsler is sensitive to brain damage (Wechsler, 1936; Russell, 1972). The modified Digit Symbol Coding examination used in this study is similar in concept to the DSST but utilized alternative symbols (Supplementary Information 4). The Stroop Color and Word Test (SCWT) (Golden & Freshwater, 1978; Stroop, 1935) originally designed by Stroop in 1935 is the gold standard measure of attention. This test measures the brain's ability to process an attribute stimulus, a stimulus with a characteristic requiring selective attention, while there is a competing attribute stimulus, referred to as the Stroop interference effect. This can affect multiple subdomains, including processing speed (Jensen, 1965) and cognitive control (Spikman et al., 2001). The current study used the Stroop Neuropsychological Test- Victoria version (VST) (Spreen & Strauss 1998), which is shorter in length and tolerated by participants who are easily fatigued (Supplementary Information 5). The VST includes identifying the colour of dots, neutral words, and colour names printed using another colour (Spreen & Strauss 1998). Furthermore, the VST exhibits test retest reliability and is sensitive to several conditions including Alzheimer's disease (Joubert et al., 2010) and aging (Moroni & Bayard., 2009; Troyer et al., 2006). The Verbal Fluency Test (F-A-S and Animal Naming) is a time limited assessment of an individual's language skills that requires participants to list words in phonetic and semantic categories (Supplementary Information 6). Verbal Fluency Tests such as Thurstone's Word Fluency test (Thurstone, 1938, Thurstone & Thurstone 1949) have been modified to include a phonetic component requiring participants to list words beginning with F, A, and S in a one-minute period for each letter (Bechtoldt et al., 1962; Benton, 1968). To test semantic fluency, participants must list words associated with a category such as animal names. For instance, the Western Aphasia Battery (WAB; Kertesz 1982), Boston Diagnostic Aphasia Examination (BDAE; Goodlass & Kaplan 1983), and the Set Test (Isaacs & Kennie, 1973) include animal naming categories.

Cognitive function was determined by assessing executive function and verbal learning by calculating an average of the executive function and verbal learning scores taken from several tests that measure the same aspects to form a composite score. Z-scores that have been normalized to account for age and sex were used. An **executive function cognitive score** was calculated using the z scores of the Stroop Colours test, F-A-S Verbal Fluency Test, Digit Symbol Coding, and Trails Making Test Part B. The z scores of the number of correct trials of the immediate recall, the short delay free recall, and long delay free recall from the CVLT-II were used to obtain the **composite verbal learning score**.

2.3.3. Statistical Analysis

Correlation and linear regression outputs were generated using SigmaPlot software (Systat Software Inc., USA). These comparisons were made between each of the vascular tests and the MMSE score and composite scores of the executive function and verbal learning cognitive assessments. The MMSE scores and executive function and verbal learning composite scores were also compared to the autonomic variables. Data were tested for normality using the Shapiro-Wilk test. Pearson's correlations were performed on normal data and reported using mean ± standard deviation, nonparametric data were analyzed using Spearman's correlation and reported as median (interquartile range). Participant anthropometric data were calculated as mean ± standard deviation. Statistical significance was defined as $p<0.05$. A correlation of 0.00-0.199 and 0.2-0.399 were defined as very weak and weak relationships respectively. Moderate associations were defined as ranging from 0.4-0.599. Correlational values ranging from 0.6-0.799 were considered strong, while values ranging from 0.8-1.0 were considered very strongly associated.

Chapter 3: Results

3.1. Participant Characteristics

Between August 10 2022 and April 11 2023, 12 adult (6 women) participants were assessed. Participant self-identified ethnicity was reported as: Caucasian (n=9), Black (n=1), and Asian (n=2). Sex, age, weight, BMI, systolic and diastolic BP, predicted VO_2 max using the Ainsworth equation (Ainsworth, 1993) are described in Table 1. Equal amounts of males and females were included. Age ranged 70 (65.6-74.8). BMI was normally distributed (29.2 ± 6.1) as well as systolic BP (131.8 ± 15.6), diastolic BP (80.1 ± 9.6), predicted VO_2 max (24.0 ± 5.7) and years of education (18.3 ± 2.5). The majority of participants were diagnosed with type 2 diabetes, consisting of 11 participants which accounted for 92% of the total participants included in the study with 15.7 ± 14.6 years of duration of diabetes. 1 participant was diagnosed with prediabetes and represented 8% of the total participants. Participants had 6.6 ± 0.50 % HBA1c. Self-reported medication usage indicated sulfonylurea use in 2 participants, metformin use in 6 participants, SGLT2 inhibitor use in 3 participants, DPP-4 inhibitor use in 3 participants, GLP-1 agonist use in 1 participant, and insulin use in 4 participants. The majority of participants had hypertension (n=11, 92%), and dyslipidemia (n=9, 75%). There were no current smokers. Self-reported medication use indicated cardiovascular medication usage with 3 participants using aspirin (25%), 7 using angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB; 58%), 5 participants using beta- blockers (BB) (42%), 3 participants using calcium channel blockers (CCB; 25%), and 9 participants using statins (75%).

Table 1. Anthropometric measures, hemodynamic measures, and medication use of patients with type 2 diabetes

n	12
Sex (Male %)	6 (50)
Age (y)	70.0 (65.6-74.8)
Weight (kg)	81.8 ± 19.2
BMI (kg/m^2)	29.2 ± 6.1
SBP (mmHg)	131.8 ± 15.6
DBP (mmHg)	80.1 ± 9.6
Resting HR (BPM)	67.1 ± 9.1
Predicted VO$_2$ Max	24.0 ± 5.7
Years of Education (y)	18.3 ± 2.5
Diabetes Status (n,%)	
Type 2 Diabetes	11 (92)
Duration of Diabetes (y)	15.7 ± 14.6
Pre-Diabetes	1 (8)
HbA1c (%)	6.6 ± 0.50
Antidiabetic Medication (n, %)	
Sulfonylureas	2 (17)
Metformin	6 (50)
SGLT2 inhibitor	3 (25)
DPP-4 inhibitor	3 (25)
GLP-1 agonist	1 (8)
Insulin	4 (33)
Risk Factors (n, %)	
Hypertension	11 (92)
Dyslipidemia	9 (75)
Smoker	0 (0)
Medication use (n, %)	
Aspirin	3 (25)
ACE or ARB	7 (58)
BB	5 (42)
CCB	3 (25)
Statins	9 (75)

Parametric data are presented as mean ± standard deviation. Skewed parametric data are presented as median (interquartile range). Nonparametric data presented as count (percentage).

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; VO$_2$ Max, maximal rate of oxygen consumption during physical exercise; HbA1c, glycated hemoglobin; SGLT2 inhibitor, sodium-glucose cotransporter inhibitor; DPP-4, dipeptidyl peptidase; GLP-1 agonist, glucagon- like peptide-1 receptor agonists; ACE, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, beta- blocker, CCB- calcium channel blocker.

3.2. Comparison of Vascular Function to Cognitive Function

Negative correlations between microvascular function as measured by PWRHI and the verbal learning composite score (n=10, r=-0.86, p=0.0013; Figure 1) and between PWRHI and the executive function composite score (n=11, r=-0.64, p=0.033; Figure 2) were found. A strong positive relationship was found between Q at rest and verbal learning (n=8, r=0.77, p=0.025; Figure 3). PWRHI was not significantly correlated with MMSE (p>0.05; Table 2). Endothelial function as measured using FMD and shear rate was not associated with cognitive function measurements (p>0.05; Table 2). There was no relationship between Q at rest and global cognitive function or executive function (p>0.05; Table 2). Further, PWV, MAP, and HR measured at rest were not associated with cognitive function measurements (p>0.05; Table 2).

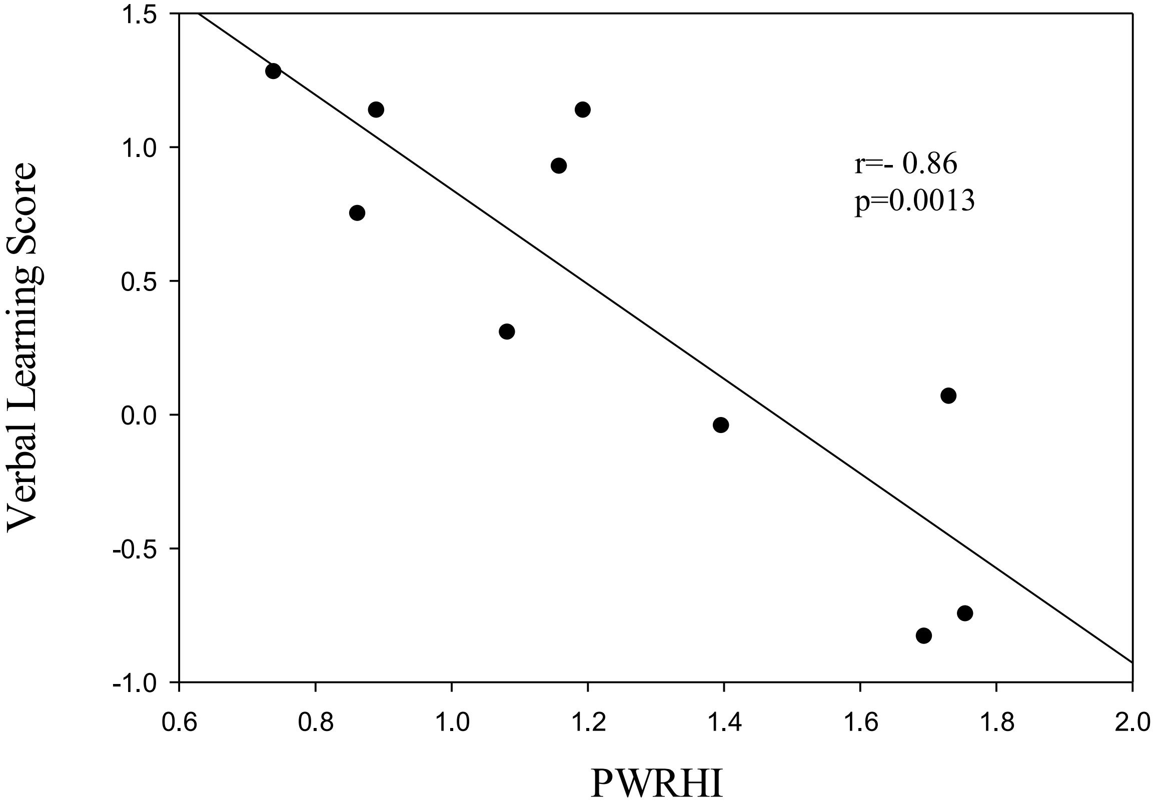


Figure 1. Pearson's correlation calculated between Pulse Wave Reactive Hyperemia Index (PWRHI) and the verbal learning composite score.

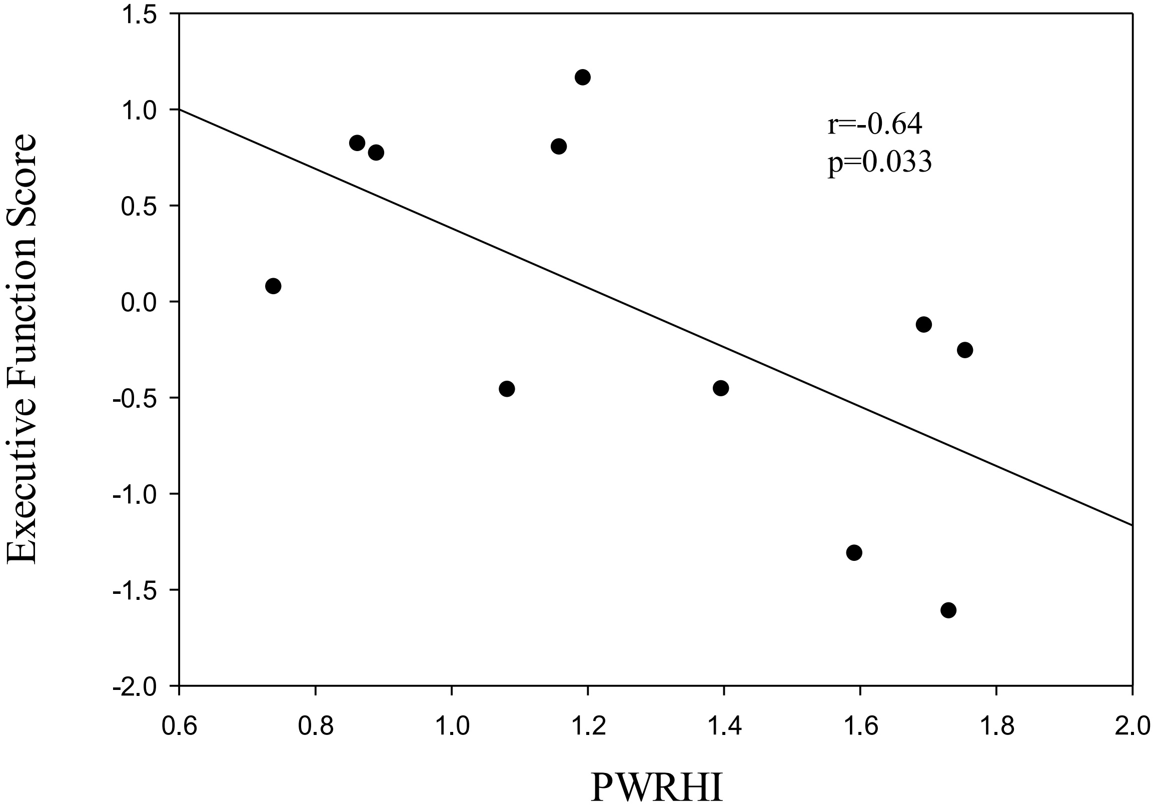


Figure 2. Pearson's correlation calculated between Pulse Wave Reactive Hyperemia Index (PWRHI) and the executive function composite score.

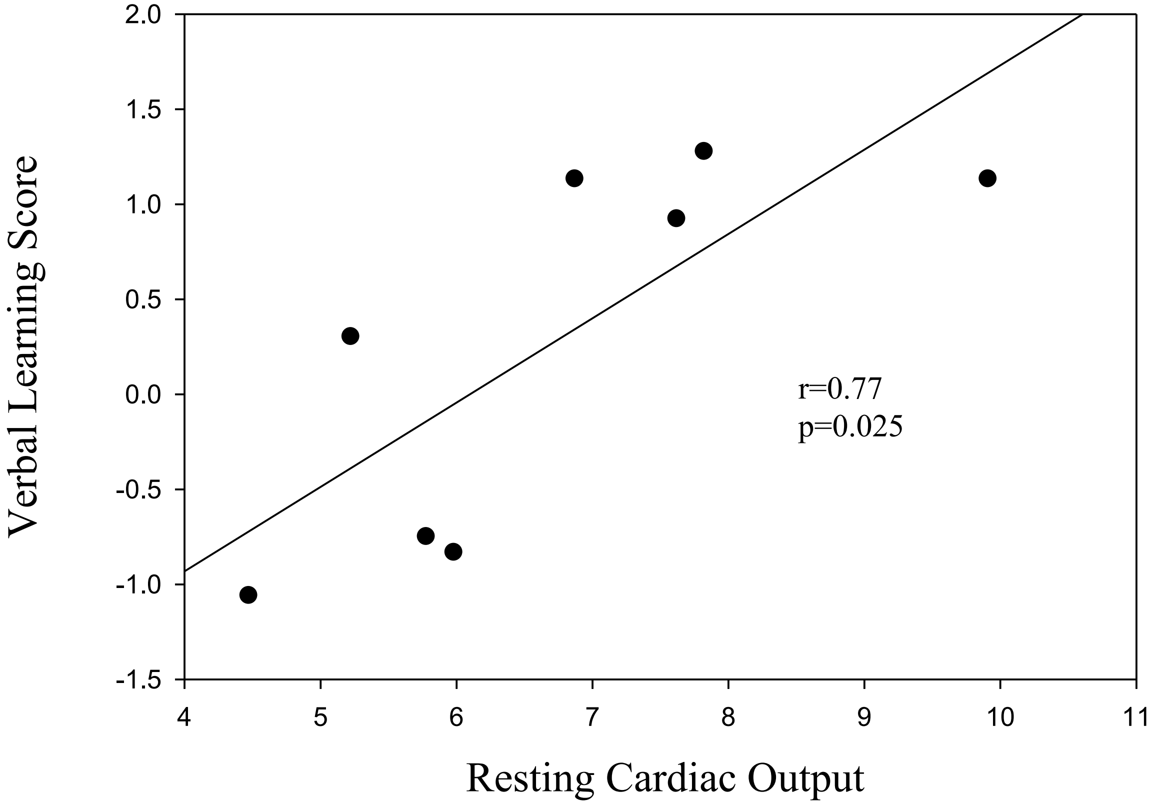


Figure 3. Pearson's correlation calculated between cardiac output at rest and the verbal learning composite score.

Table 2. Correlative data between cardiovascular measures and cognitive function as measured by MMSE, VLC and EFC scores.

Parameter	MMSE				VLC				EFC			
	n	r	p	R^2	n	r	p	R^2	n	r	p	R^2
PWRHI	11	-0.50	0.11	0.19	10	-0.86	**0.0013**	0.75	11	-0.64	**0.033**	0.41
FMD (%)	12	0.042	0.89	0.6	11	0.28	0.40	0.079	12	0.21	0.52	0.043
Shear Rate (s-1)	12	0.16	0.60	0.036	11	0.46	0.16	0.21	12	0.14	0.67	0.019
Resting PWV (m/s)	8	-0.44	0.26	0.048	8	-0.66	0.059	0.013	8	-0.20	0.65	0.039
MAP (mmHg)	10	-0.040	0.89	0.034	9	0.075	0.81	0.016	10	0.47	0.16	0.000074
HR (bpm)	10	-0.17	0.63	0.0060	9	0.28	0.46	0.080	10	-0.053	0.89	0.0028
Q (L/min)	9	0.26	0.49	0.28	8	0.77	**0.025**	0.59	9	-0.058	0.88	0.0033

MMSE, Mini Mental State Examination; VLC, verbal learning composite; EFC, executive function composite; PWRHI, pulse wave reactive hyperemia index; FMD, flow mediated dilation; PWV, pulse wave velocity; MAP, mean arterial pressure; HR, heart rate; Q, cardiac output

3.3. Comparison of Resting Autonomic Function to Cognitive Function

Table 3 found that resting autonomic function as measured using HRV analysis to obtain frequency domains such as HF to measure parasympathetic activation, and LF/HF as an index of sympathetic activation were not significantly correlated with MMSE (p>0.05; Table 3), verbal composite score (p>0.05; Table 3), and executive function composite score (p>0.05; Table 3). The time domain parameter SDRR obtained from HRV analysis was not associated with MSSE (p>0.05; Table 3), verbal learning (p>0.05; Table 3), or executive function (p>0.05; Table 3).

Table 3. Correlative data between resting autonomic measures and cognitive function as measured by MMSE, VLC, and EFC scores.

Parameter	MMSE				VLC				EFC			
	n	r	p	R^2	n	r	p	R^2	n	r	p	R^2
HF (power nu)	11	0.20	0.54	0.017	10	0.23	0.52	0.054	11	-0.41	0.20	0.16
LF/HF (power%)	11	-0.082	0.80	0.024	10	-0.091	0.79	0.0027	11	0.36	0.27	0.22
SDRR	11	0.015	0.95	0.034	10	-0.055	0.87	0.031	11	-0.15	0.65	0.032

MMSE, Mini Mental State Examination; VLC, verbal learning composite; EFC, executive function composite; HF, high frequency component of heart rate variability; LF/HF, low frequency/high frequency component of heart rate variability; SDRR, standard deviation of the R-R interval

3.4. Comparison of Response to Hand Grip Exercise vs Cognitive Function

The MAP response to isometric hand grip exercise was not significantly correlated with any cognitive function measurements (p>0.05; Table 4). HR response to isometric hand grip exercise was not significantly correlated with the verbal learning composite score (p>0.05; Table 4), or the executive function composite score (p>0.05; Table 4). Change in Q and PWV in response to hand grip exercise were not associated with cognitive function measurements (p>0.05; Table 4). Additionally, HRR in the first minute following the completion exercise was not associated with cognitive function measurements (p>0.05; Table 4). HRR in the second minute following exercise completion was not associated with executive function (p>0.05; Table 4). Importantly, a very strong positive correlation was found between HR response to handgrip exercise and MMSE (n=9, r=0.84, p=0.0020; Figure 4) and strong negative correlations were found between HRR in the second minute following exercise completion and MSSE (n=9, r=-0.71, p=0.030; Figure 5), and verbal learning (n=8, -0.71, p=0.037; Figure 6).

Table 4. Correlative data between response to hand grip exercise and cognitive function as measured by MMSE, VLC and EFC scores.

Parameter	MMSE				VLC				EFC			
	n	r	p	R^2	n	r	p	R^2	n	r	p	R^2
Delta MAP (mmHg)	10	-0.060	0.87	0.0025	9	-0.32	0.41	0.1	10	0.19	0.61	0.035
Delta HR (bpm)	9	0.84	**0.0020**	0.089	8	0.69	0.057	0.48	9	0.40	0.28	0.16
Delta Q (L/min)	9	0.12	0.74	0.018	8	0.17	0.68	0.030	9	0	0.98	0.000044
Delta PWV (m/s)	8	0.22	0.58	0.077	8	0.20	0.58	0.00031	8	0.048	0.89	0.24
HRR (min 1) (bpm)	9	-0.41	0.24	0.11	8	-0.48	0.22	0.23	9	-0.39	0.30	0.15
HRR (min 2) (bpm)	9	-0.71	**0.030**	0.052	8	-0.71	**0.037**	0.52	9	-0.37	0.31	0.026

MMSE, Mini Mental State Examination; VLC, verbal learning composite; EFC, executive function composite; MAP, mean arterial pressure; HR, heart rate; Q, cardiac output; PWV, pulse wave velocity; HRR, heart rate recovery

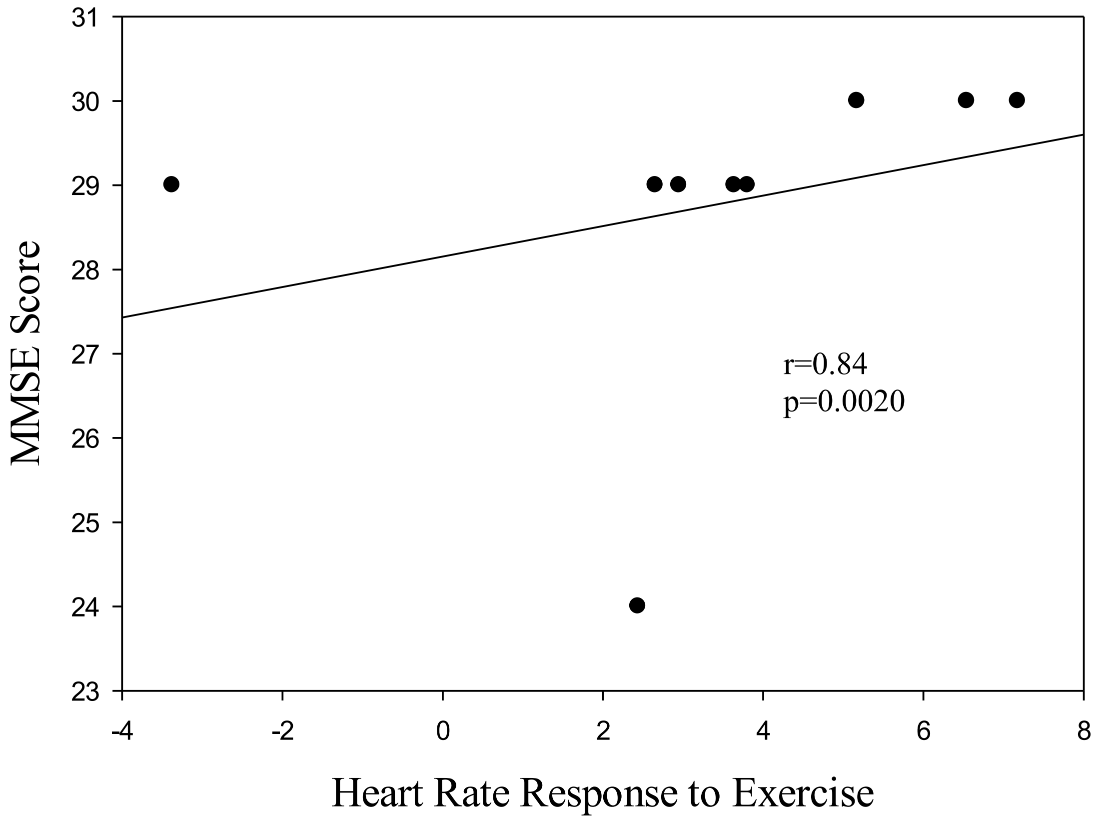


Figure 4. Spearman’s correlation calculated between heart rate response to exercise and Mini Mental State Examination (MMSE) score.

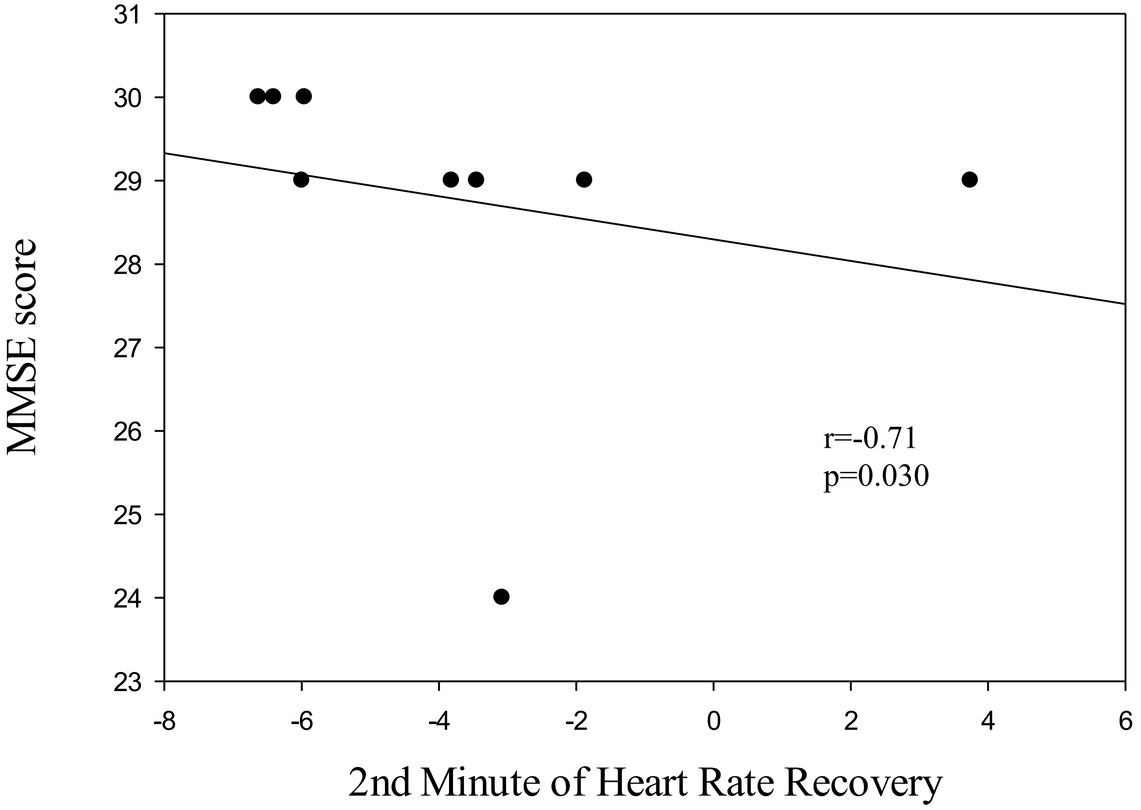


Figure 5. Spearman's correlation calculated between heart rate recovery in the second minute after exercise completion and Mini Mental State Examination (MMSE) score.

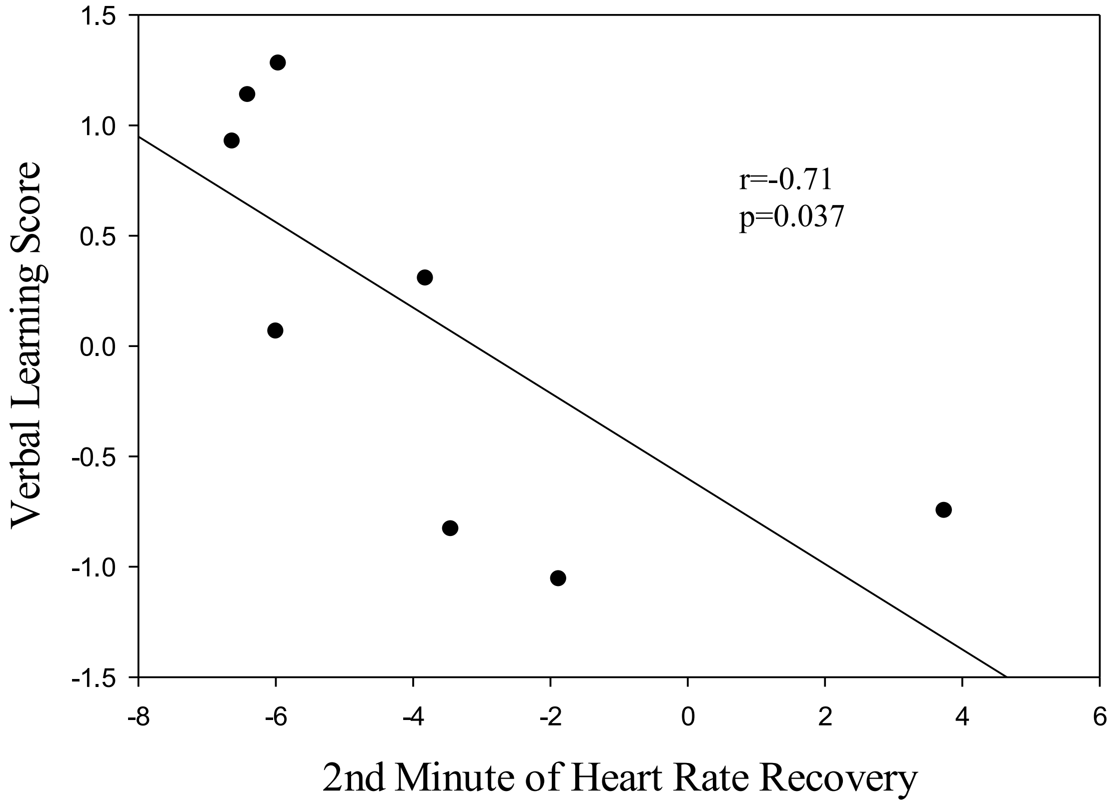


Figure 6. Spearman's correlation calculated between heart rate recovery during the second minute after exercise completion and the verbal learning composite score.

Chapter 4: Discussion

4.1. Summary

The current study aimed to determine if vascular and autonomic dysfunction were associated in persons living with type 2 diabetes. The results of this study indicate that endothelial function and shear rate as measured by the FMD protocol are not associated with cognitive function in these individuals. However, contrary to our hypothesis microvascular function measured using our novel PWRHI technique had a very strong inverse relationship with verbal learning and a strong inverse relationship with executive function. Resting Q had a strong positive relationship with verbal learning. Resting autonomic function, determined using HF, LF/HF and SDRR, was not associated with cognitive function, nor were resting BP, PWV and HR. HRR in the first minute post exercise was not correlated with cognitive function. On the other hand, HR response during exercise was very strongly related to global cognitive function, while HRR in the second minute post-exercise had a strong inverse relationship with both global cognitive function and verbal learning.

4.2. Cardiovascular Measures

Brachial FMD was expressed as a percent difference from baseline diameter, then compared to global cognitive function, verbal learning and executive function. FMD measures vasodilation in response to reactive hyperemia to estimate endothelial function and is an early marker of atherosclerosis. None of the global cognitive function, executive function, nor verbal learning scores were associated with FMD or shear stress in this study. The FMD response is dependent on NO bioavailability and an impaired response may be indicative of endothelial dysfunction (Anderson 1995), oxidative stress (Gurovich et al., 2014), and inflammation

(Kovacs et al., 2006). Previous studies have found increases in baseline diameter and lower FMD in type 2 diabetes compared to individuals with normal glucose metabolism (Henry et al., 2004) potentially due to diminished NO bioavailability due to oxidative stress from ROS (Su et al., 2008). However, our findings suggest that impaired global cognitive function, verbal learning, and executive function are not related to NO dependent vascular function. These findings contrast previously observed associations between FMD and cognitive function in other clinical populations such as stroke (Naiberg et al., 2016), Alzheimer's (Tachibana et al., 2016), and aging (Vendemiale et al., 2013). A meta-analysis conducted by Naiberg and colleagues (2016) concluded that most studies measuring FMD in stroke patients found that impaired vascular function predicts cognitive decline, especially executive function. Tachibana and colleagues (2016) found that FMD and global cognitive function measured by MMSE were significantly correlated and FMD was significantly predicted by MMSE score in Alzheimer's disease and vascular dementia. Vendemiale and colleagues (2013) found that endothelial dysfunction was associated with mild cognitive impairment in older adults. Importantly, there is evidence that markers of atherosclerosis such as FMD may not play a role in cognitive decline in the presence of insulin resistance. For example, Reijmer and colleagues (2011) found associations between metabolic syndrome and impaired cognitive function, however the decline in cognitive ability was not predicted by FMD. Our results suggest that brachial artery endothelial function is not correlated with cognitive dysfunction in type 2 diabetes. Brachial artery shear rate which elicits the FMD response was also not significantly correlated with measures of global cognitive function, verbal learning and executive function. However, studies measuring shear stress in arteries closer to the brain have found associations with cognitive function. For instance, a study by Liu and colleagues (2018) found low shear stress in the common carotid artery was associated with cognitive impairment measured with MMSE in a group of older adults consisting of 53% adults with type 2 diabetes.

Since we hypothesized microvascular dysfunction (i.e. PWRHI) would be associated with cognitive decline, the inverse relationships between microvascular function and verbal learning and executive function were unexpected as vascular damage has been implicated as a mechanism leading to cognitive decline in type 2 diabetes (Biessels et al., 2006). Additionally, individuals with type 2 diabetes have a higher risk of vascular dementia (Gudala et al., 2013; Hassing et al., 2002) and cerebral small vessel disease (Liu et al., 2018). In previous studies, poorer microvascular function was associated with cognitive impairment in overt cardiovascular disease (Fujiyoshi et al., 2018; Saleem et al., 2019). However, Lim and colleagues (2016) compared peripheral microvascular function and a battery of cognitive function tests in older adults and found no associations with MMSE or a verbal memory composite score but found a positive correlation ($p=0.045$) with executive function. Further, a study by Brant and colleagues (2018) on dementia-free older adults found that after adjusting for confounding variables, namely age, sex, and level of education, the association between peripheral microvascular function and global cognitive function, memory, and phonetic fluency lost significance. The authors suggested that as an early marker of atherosclerosis, peripheral microvascular dysfunction might occur prior to cognitive decline (Brant et al., 2018). The current study found that individuals with type 2 diabetes had lower average PWRHI scores compared to a previously collected cohort of healthy individuals (1.28 ± 0.37 vs 1.49 ± 0.68), confirming that peripheral microvascular function was blunted in these individuals. Considering it may be unlikely that greater microvascular function is truly correlated with impaired cognitive function, our results may indicate that the use of PWRHI is not suitable for use in type 2 diabetes. The PWRHI method requires further testing in clinical populations. To further illustrate, PWRHI was not correlated with the gold standard method, FMD in this study (Appendix 2). PWRHI may be less effective than FMD at capturing the vasodilatory response to arterial occlusion as it is measured in the vascular beds of the middle fingers which may be too far a distance from the forearm. It is also important to consider that the mechanisms of peripheral microvascular function differ considerably from the mechanisms of cerebral microvascular function (e.g. cerebral autoregulation).

Resting finger-toe PWV, resting MAP and resting HR were not correlated with cognitive function scores. Although the lack of association found between PWV and cognitive function scores was unexpected, it is worth noting that the inverse relationship observed between resting PWV and the verbal learning composite score was approaching significance and could show significance with greater sample size. In a previous study, arterial stiffness measured with the gold standard carotid femoral pulse wave velocity (cf-PWV) technique was found to be higher in individuals with type 2 diabetes compared to healthy controls, and negatively correlated with executive function and verbal memory in type 2 diabetes (Mehrabian et al., 2012). Another study by Laugesen and colleagues (2013) determined that the higher cf-PWV observed in type 2 diabetes was associated with greater prevalence of white matter lesions, further implicating elevated high PWV as a potential contributor to cognitive decline in type 2 diabetes. Therefore, our lack of associations between PWV and cognitive function could also be due to the location of the pulse tonometers on the finger and toe vs. the carotid and femoral arteries. The cf-PWV is indicative of stiffness of the large elastic conduit artery between the 2 locations (i.e. descending aorta) whereas finger-toe PWV is inclusive of both conduit artery stiffness as well as more muscular arteriolar stiffness.

Hypertension is a comorbidity associated with type 2 diabetes. Jamalnia and colleagues (2020) conducted a study on type 2 diabetes and found negative correlations between BP and MMSE, indicating that higher BP may play a role in cognitive decline. Hassing and colleagues (2004) found that greater cognitive decline occurred in people with both diabetes and hypertension than in the presence of either diseases alone. Similar findings were reported by Elias and colleagues (1997) as a part of the Framingham study which found individuals with both conditions are at risk of impaired cognitive function, specifically memory. Interestingly, the population-based Hoorn study found prolonged hypertension, even in the prediabetic stage, is related to cognitive decline in type 2 diabetes (Van Den Berg et al., 2009). Although we hypothesized resting MAP would be associated with cognitive decline, the present study had a very small sample size in comparison to the aforementioned studies that found statistically significant relationships. Moreover, all but one of our participants (92%) use anti-hypertensive medication which may have normalized their MAP response and prevented us from finding an association with cognitive function.

Resting HR, but not Q, is higher in those with type 2 diabetes compared to healthy individuals (Geijselaers et al., 2016; Mac Ananey et al., 2011; O'Connor et al., 2015; Regensteiner et al., 2009). Our results indicate that while resting HR may not be associated with cognitive decline, we found a strong positive association between resting Q and verbal learning. Longitudinal studies in older adults show that reduced Q negatively impacts language and episodic memory domains. Lower cardiac index, the left ventricular Q indexed by body surface area, correlates with lower resting blood flow to grey matter in older adults (Jefferson et al., 2017). Further, brain imaging studies support our findings as the left temporal lobe, associated with language comprehension and verbal memory (Elger et al., 1997), is particularly vulnerable to lower Q due to fewer collateral blood vessels in this region (Liebeskind, 2003). Overall, our findings support the hypothesis that blood flow is likely altered in type 2 diabetes and negatively impacts cognitive processes. Hypoperfusion in the brain is particularly alarming as neurovascular uncoupling can occur, leaving metabolic demands of neurons unmet resulting in a buildup of waste products, and may increase oxidative stress, putting this population at risk of cognitive decline (Fierstra & Mikulis, 2011).

4.3. Resting Autonomic Function

HR and Q are influenced by the ANS. To investigate autonomic regulation, we measured HRV parameters as indices of sympathetic and parasympathetic control of the heart. HRV data was measured in the time domain using SDRR as an index of parasympathetic activity. The frequency domain was evaluated using HF as an index of parasympathetic activity, and LF/HF ratio as an index of sympathetic activation. These HRV parameters were not associated with global cognitive function, verbal learning, or executive function in this study. Previous work by Dalise and colleagues (2020) found associations between global cognitive function and sympathetic HRV parameters such as SDRR, LF and LF/HF ratio, suggesting elevated sympathetic activation contributes to better cognitive function in older adults. Conversely, recent studies in clinical populations such as cerebral small vessel disease (Hu et al., 2023), and Alzheimer's disease (Nonogaki et al., 2017) found associations between higher sympathetic tone and cognitive decline. Hu and colleagues (2023) recorded 3 consecutive periods of 5-minute resting ECG using a 6-lead ECG in the seated position. Higher HF was associated with better MMSE performance whereas higher LF/HF ratio were associated with worse MMSE performance suggesting those with reduced parasympathetic tone and sympathetic dominance have worse cognitive function (Hu et al., 2023). Nonogaki and colleagues (2017) measured 5 minutes of ECG in the supine position and found that MMSE was negatively associated with LF/HF ratio, memory was positively associated with HF power, and negatively associated with LF/HF ratio even after adjusting for age, sex, years of education, hypertension, type 2 diabetes, and cholinesterase inhibitor use (Nonogaki et al., 2017). The findings of the present study suggest that resting HRV parameters are not associated with cognitive dysfunction in type 2 diabetes. Although sample size was small, correlations were low and p-values were far from significant. Sympathetic activity might have been more accurately assessed by invasive techniques such as muscle sympathetic nerve activity (MSNA) which is capable of recording sympathetic nerve firing rates directly at the peroneal nerve.

4.4. Response to Exercise

Those with type 2 diabetes are more likely to experience exercise intolerance, as indicated by impaired hemodynamic responses to exercise in comparison to healthy individuals (Regensteiner et al., 2010). We found that MAP response to exercise was not significantly correlated with our measures of cognitive function, however HR measured in the second minute of isometric hand grip exercise had a strong positive association with MMSE score, suggesting that the impaired autonomic response to exercise might play a role in cognitive decline. Similarly, a previous study measuring change in HR during 3 minutes of sustained isometric contraction to 30% of MVC found a smaller HR response in type 2 diabetes compared to healthy controls (Sucharita et al., 2011). In the current study, since the changes in Q and PWV in response to isometric handgrip exercise were not significantly correlated with cognitive function, yet there was a relationship with a smaller HR response, our findings imply that impaired parasympathetic withdrawal may contribute to cognitive dysfunction.

Post exercise HRR was measured as an index of parasympathetic activity. A greater reduction in HR in the first two minutes following exercise is representative of higher parasympathetic tone, and vagal reactivation typically starts to occur in the first 30 seconds post exercise (Imai et al., 1994). Although no significant relationships were found between cognitive function and HRR during the first minute post exercise in the present study, HRR in the second minute post exercise was inversely correlated with global cognitive function and verbal learning. HRR 1 minute post exercise may have been too soon following exercise to find a meaningful relationship with cognitive function, but our comparisons with cognitive function using HRR 2 minutes post exercise were consistent with the literature as this measure is an established index of parasympathetic activity in adults with (Lipinski et al., 2004) and without (Cole & Lauer, 1999) cardiovascular complications. Although studies on HRR and cognitive function appear scarce, HRR and global cognitive function have previously been associated in cardiovascular disease (Keary et al., 2012). There is also evidence that vagal stimulation is linked to memory, in particular memory consolidation, when assessed by verbal learning examinations (Ghacibeh et al., 2006).

4.5. Limitations and Future Directions

This study was limited by its cross-sectional design as causality could not be established. Future studies should aim to find a more direct relationship between vascular and autonomic dysfunction and cognitive decline using a longitudinal study. The current study was further limited by its small sample size. At most, comparisons consisted of 12 participants, and was as low as 8 participants in some comparisons. Due to its small sample size, the present study may not be generalizable to all individuals with type 2 diabetes, as there was not enough diversity in demographics such as age and ethnicity as well as sociocultural factors such as level of education. Additionally, significance is difficult to achieve in smaller sample sizes. For example, correlations between resting PWV and verbal learning, as well as between HR response to exercise and verbal learning, were approaching significance and could meet the threshold for significance with a larger sample size. Thus, the findings in the present study should be considered with caution.

Future studies should have sufficiently large sample sizes to carry out multiple linear regression analysis which would estimate the strength of several vascular and autonomic variables as predictors of cognitive performance. Additionally, covariate analysis should be considered as covariates such as age, sex, BMI, resting BP, and race among others are associated with cognitive function testing in type 2 diabetes (McFall et al., 2010). Confounding variables are also important to take into account considering significance may be attenuated when adjusting for confounders such as level of education, history of myocardial infarction or heart failure, as was found by Brant and colleagues (2018). Furthermore, the current study did not examine if cognitive function could be improved by targeting the parameters that were significantly associated with cognitive decline. Lifestyle interventions such as exercise training are useful in targeting vascular and autonomic impairments in other clinical populations and may promote better cognitive function in type 2 diabetes. Cardiac resynchronization therapy has been shown to improve cognitive function by increasing the left ventricular ejection fraction in heart failure (Hoth et al., 2010). Further, HRR is improved with exercise training such as cardiac rehabilitation (Jolly et al., 2011) which is also associated with enhanced cognitive function (Gunstad et al., 2005).

Additional vascular and autonomic reflex assessments should also be considered. For instance, the cardiovascular response to head up tilt, an orthostatic stress which stimulates the baroreflex, is impaired in type 2 diabetes (Jorge-Galarza et al., 2022). Previous work by Zhang and colleagues (2019) investigated the relationship between orthostatic intolerance and cognitive function and found cognitive function was reduced in those with both orthostatic intolerance and type 2 diabetes compared to those with type 2 diabetes alone. In the current study we assessed peripheral vascular function to make assumptions about brain blood flow. However, measuring cerebral brain flow is very complex. Our lab has previously used transcranial doppler as a non-invasive measure of brain blood velocity (Abidi et al., 2017; Joshi & Edgell, 2019; Pereira et al., 2022), and its addition to the current study could help determine if disturbed brain blood flow contributes to cognitive decline in this population.

Finally, whether all parameters are necessary to continue measuring in expansions of this study must be considered. Parameters that correlated poorly and were far from significant, such as those seen in the endothelial function testing may not yield a significant relationship with cognitive function in a greater sample size. FMD is both technically demanding and operator dependent, requiring an experienced technician to perform all measurements and analysis within a study to maintain consistency. Therefore, in a research or healthcare environment with limited resources, FMD testing may not realize important results as a marker of cognitive health. Additionally, our experimental index of microvascular function PWRHI may not be an be an appropriate vascular health index in this population, as previously discussed. HRV collected with single lead ECG yielded low correlational values that were far from statistical significance in the current study. Although HRV analysis may not be necessary, ECG collection is important to continue as HR response to exercise and HRR in the second minute following exercise cessation were related to cognitive function. Additionally, our study indicates that HR response to exercise might have been moderately associated with verbal learning as these findings were approaching statistical significance in the 8 participants collected (p= 0.057). The correlation between resting PWV and verbal learning was also approaching statistical significance (p= 0.059) and may produce an inverse relationship with greater sample size, indicating that faster blood flow might be associated with cognitive decline in those with type 2 diabetes. PWV measured between the finger and toe is an experimental technique. It is possible that other methods of measuring PWV such as the gold standard cf-PWV will be more accurate and yield a higher and more significant correlation with cognitive function.

4.6. Conclusions

Verbal learning was positively correlated with resting Q and HRR following exercise, and global cognitive function was associated with the HR response to isometric handgrip exercise and HRR in the second minute following exercise in people with type 2 diabetes. These findings suggest that hypoperfusion and impaired parasympathetic function may contribute to cognitive decline in this population. Future studies should further investigate the role of brain blood flow and autonomic reflexes as well as determine the effectiveness of lifestyle modifications that may protect against cognitive decline in this population.

References

Abidi, S., Nili, M., Serna, S., Kim, S., Hazlett, C., & Edgell, H. (2017). Influence of sex, menstrual cycle, and oral contraceptives on cerebrovascular resistance and cardiorespiratory function during Valsalva or standing. *Journal of Applied Physiology*, *123*(2), 375-386.

Ainsworth, B. E., Jacobs Jr, D. R., Leon, A. S., Richardson, M. T., & Montoye, H. J. (1993). Assessment of the accuracy of physical activity questionnaire occupational data. *Journal of occupational medicine*, 1017-1027.

Alam, M., & Smirk, F. H. (1937). Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *The Journal of physiology*, *89*(4), 372.

Anderson, T. J., Elstein, E., Haber, H., & Charbonneau, F. (2000). Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). *Journal of the American College of Cardiology*, *35*(1), 60-66.

Anderson, T. J., Uehata, A., Gerhard, M. D., Meredith, I. T., Knab, S., Delagrange, D., ... & Selwyn, A. P. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *Journal of the American College of Cardiology*, *26*(5), 1235-1241.

Arai, Y., Saul, J. P., Albrecht, P., Hartley, L. H., Lilly, L. S., Cohen, R. J., & Colucci, W. S. (1989). Modulation of cardiac autonomic activity during and immediately after exercise. *American Journal of Physiology-Heart and Circulatory Physiology*, *256*(1), H132–H141. https://doi.org/10.1152/ajpheart.1989.256.1.H132

Badrov, M. B., Freeman, S. R., Zokvic, M. A., Millar, P. J., & McGowan, C. L. (2016). Isometric exercise training lowers resting blood pressure and improves local brachial artery flow-mediated dilation equally in men and women. *European journal of applied physiology*, *116*, 1289-1296.

Bechtoldt, H. P., Benton, A. L., & Fogel, M. L. (1962). An Application of Factor Analysis in Neuropsychology. *The Psychological Record*, *12*(2), 147.

Benichou, T., Pereira, B., Mermillod, M., Tauveron, I., Pfabigan, D., Maqdasy, S., & Dutheil, F. (2018). Heart rate variability in type 2 diabetes mellitus: A systematic review and meta–analysis. *PLoS ONE*, *13*(4), e0195166. https://doi.org/10.1371/journal.pone.0195166

Benton, A. L. (1968). Differential behavioral effects in frontal lobe disease. *Neuropsychologia*, *6*(1), 53–60. https://doi.org/10.1016/0028-3932(68)90038-9

Berecek, K. H., & Brody, M. J. (1982). Evidence for a neurotransmitter role for epinephrine derived from the adrenal medulla. *American Journal of Physiology-Heart and Circulatory Physiology*, *242*(4), H593-H601.

Berr, C., Balansard, B., Arnaud, J., Roussel, A. M., Alpérovitch, A., & EVA Study Group. (2000). Cognitive decline is associated with systemic oxidative stress: the EVA study. *Journal of the American Geriatrics Society*, *48*(10), 1285-1291.

Bier, A. (1897). Die Enstehung des Collatereralkrieslauf. I, Die arterielle Collateral-kreislauf. *Archiv fihologische Anatomie und Physiologie, 147,* 257-293.

Biessels, G. J., Staekenborg, S., Brunner, E., Brayne, C., & Scheltens, P. (2006). Risk of dementia in diabetes mellitus: A systematic review. *The Lancet Neurology*, *5*(1), 64–74. https://doi.org/10.1016/S1474-4422(05)70284-2

Bonetti, P. O., Pumper, G. M., Higano, S. T., Holmes, D. R., Kuvin, J. T., & Lerman, A. (2004). Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *Journal of the American College of Cardiology*, *44*(11), 2137-2141.

Borysenko, M., & Beringer, T. (1984). *Functional histology*. Little Brown & Company. 195-208

Brant, L., Bos, D., Araujo, L. F., Ikram, M. A., Ribeiro, A. L., & Barreto, S. M. (2018). Microvascular endothelial function and cognitive performance: The ELSA-Brasil cohort study. *Vascular Medicine*, *23*(3), 212–218. https://doi.org/10.1177/1358863X18755004

Bruno, R. M., Ghiadoni, L., Seravalle, G., Dell'Oro, R., Taddei, S., & Grassi, G. (2012). Sympathetic regulation of vascular function in health and disease. *Frontiers in Physiology*, *3*. https://doi.org/10.3389/fphys.2012.00284

Celermajer, D. S., Sorensen, K. E., Gooch, V. M., Spiegelhalter, D. J., Miller, O. I., Sullivan, I. D., Lloyd, J. K., & Deanfield, J. E. (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *The Lancet*, *340*(8828), 1111–1115. https://doi.org/10.1016/0140-6736(92)93147-F

Charkoudian, N., Joyner, M. J., Johnson, C. P., Eisenach, J. H., Dietz, N. M., & Wallin, B. G. (2005). Balance between cardiac output and sympathetic nerve activity in resting humans: Role in arterial pressure regulation: Cardiac output, sympathetic activity and blood pressure in humans. *The Journal of Physiology*, *568*(1), 315–321. https://doi.org/10.1113/jphysiol.2005.090076

Charkoudian, N., & Rabbitts, J. A. (2009). Sympathetic Neural Mechanisms in Human Cardiovascular Health and Disease. *Mayo Clinic Proceedings*, *84*(9), 822–830. https://doi.org/10.4065/84.9.822

Cholerton, B., Omidpanah, A., Verney, S. P., Nelson, L. A., Baker, L. D., Suchy-Dicey, A., Longstreth, W. T., Howard, B. V., Henderson, J. A., Montine, T. J., & Buchwald, D. (2019). Type 2 diabetes and later cognitive function in older American Indians: The Strong Heart Study. *International Journal of Geriatric Psychiatry*, *34*(7), 1050–1057. https://doi.org/10.1002/gps.5108

Chowienczyk, P. J., Brett, S. E., Gopaul, N. K., Meeking, D., Marchetti, M., Russell-Jones, D. L., Änggård, E. E., & Ritter, J. M. (2000). Oral treatment with an antioxidant (raxofelast) reduces oxidative stress and improves endothelial function in men with Type II diabetes. *Diabetologia*, *43*(8), 974–977. https://doi.org/10.1007/s001250051478

Cohen, R. A., Poppas, A., Forman, D. E., Hoth, K. F., Haley, A. P., Gunstad, J., Jefferson, A. L., Tate, D. F., Paul, R. H., Sweet, L. H., Ono, M., Jerskey, B. A., & Gerhard-Herman, M. (2009). Vascular and cognitive functions associated with cardiovascular disease in the elderly. *Journal of Clinical and Experimental Neuropsychology*, *31*(1), 96–110. https://doi.org/10.1080/13803390802014594

Cole, C. R., & Lauer, M. S. (1999). Heart-Rate Recovery Immediately after Exercise as a Predictor of Mortality. *New England Journal of Medicine*. 341(18), 1351-1357

Cooke, J. P., Rossitch, E., Andon, N. A., Loscalzo, J., & Dzau, V. J. (1991). Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. *Journal of Clinical Investigation*, *88*(5), 1663–1671. https://doi.org/10.1172/JCI115481

Coopmans, C., Zhou, T. L., Henry, R. M. A., Heijman, J., Schaper, N. C., Koster, A., Schram, M. T., van der Kallen, C. J. H., Wesselius, A., den Engelsman, R. J. A., Crijns, H. J. G. M., & Stehouwer, C. D. A. (2020). Both Prediabetes and Type 2 Diabetes Are Associated With Lower Heart Rate Variability: The Maastricht Study. *Diabetes Care*, *43*(5), 1126–1133. https://doi.org/10.2337/dc19-2367

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., Deanfield, J., Drexler, H., Gerhard-Herman, M., Herrington, D., Vallance, P., Vita, J., & Vogel, R. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *Journal of the American College of Cardiology*, *39*(2), 257–265. https://doi.org/10.1016/S0735-1097(01)01746-6

Cruickshank, K., Riste, L., Anderson, S. G., Wright, J. S., Dunn, G., & Gosling, R. G. (2002). Aortic Pulse-Wave Velocity and Its Relationship to Mortality in Diabetes and Glucose Intolerance: An Integrated Index of Vascular Function? *Circulation*, *106*(16), 2085–2090. https://doi.org/10.1161/01.CIR.0000033824.02722.F7

Dalise, A. M., Prestano, R., Fasano, R., Gambardella, A., Barbieri, M., & Rizzo, M. R. (2020). Autonomic Nervous System and Cognitive Impairment in Older Patients: Evidence From Long-Term Heart Rate Variability in Real-Life Setting. *Frontiers in Aging Neuroscience*, *12*, 40. https://doi.org/10.3389/fnagi.2020.00040

Delis, D.C. (2000). California verbal learning test. *Adult version. Manual. Psychological Corporation*

Delis, D. C., Kramer, J. H., Kaplan, E., & Ober, B. A. (1987). California Verbal Learning Test-. *Assessment.*

Edgell, H., Stickland, M. K., & MacLean, J. E. (2016). A simplified measurement of pulse wave velocity is not inferior to standard measurement in young adults and children. *Blood Pressure Monitoring*, *21*(3), 192-195.

Ekblad, L. L., Rinne, J. O., Puukka, P., Laine, H., Ahtiluoto, S., Sulkava, R., ... & Jula, A. (2017). Insulin resistance predicts cognitive decline: an 11-year follow-up of a nationally representative adult population sample. *Diabetes care*, *40*(6), 751-758.

Elger, C. E., Grunwald, T., Lehnertz, K., Kutas, M., Helmstaedter, C., Brockhaus, A., Van Roost, D., & Heinze, H. J. (1997). Human temporal lobe potentials in verbal learning and memory processes. *Neuropsychologia*, *35*(5), 657–667. https://doi.org/10.1016/S0028-3932(96)00110-8

Elias, P. K., Elias, M. F., D'Agostino, R. B., Cupples, L. A., Wilson, P. W., Silbershatz, H., & Wolf, P. A. (1997). NIDDM and Blood Pressure as Risk Factors for Poor Cognitive Performance: The Framingham Study. *Diabetes Care*, *20*(9), 1388–1395. https://doi.org/10.2337/diacare.20.9.1388

Esler, M. D., Lambert, G. W., Ferrier, C., Kaye, D. M., Wallin, B. G., Kalff, V., ... & Jennings, G. L. (1995). Central nervous system noradrenergic control of sympathetic outflow in normotensive and hypertensive humans. *Clinical and experimental hypertension*, *17*(1-2), 409-423.

Failla, M., Grappiolo, A., Emanuelli, G., Vitale, G., Fraschini, N., Bigoni, M., Grieco, N., Denti, M., Giannattasio, C., & Mancia, G. (1999). Sympathetic tone restrains arterial distensibility of healthy and atherosclerotic subjects: *Journal of Hypertension*, *17*(8), 1117–1123. https://doi.org/10.1097/00004872-199917080-00011

Fierstra, J., & Mikulis, D. J. (2011). Neurovascular uncoupling in functional MR imaging. In *Functional Neuroradiology: Principles and Clinical Applications* (pp. 345-353). Boston, MA: Springer US.

Flammer, A. J., Anderson, T., Celermajer, D. S., Creager, M. A., Deanfield, J., Ganz, P., Hamburg, N. M., Lüscher, T. F., Shechter, M., Taddei, S., Vita, J. A., & Lerman, A. (2012). The Assessment of Endothelial Function: From Research Into Clinical Practice. *Circulation*, *126*(6), 753–767. https://doi.org/10.1161/CIRCULATIONAHA.112.093245

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state." *Journal of Psychiatric Research*, *12*(3), 189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Fontbonne, A., Berr, C., Ducimetière, P., & Alpérovitch, A. (2001). Changes in Cognitive Abilities Over a 4-Year Period Are Unfavorably Affected in Elderly Diabetic Subjects. *Diabetes Care*, *24*(2), 366–370. https://doi.org/10.2337/diacare.24.2.366

Fouladi, B., Joshi, H., & Edgell, H. (2019). Cardiovascular and autonomic responses to passive arm or leg movement in men and women. *European Journal of Applied Physiology*, *119*(2), 551-559.

Freyschuss U. (1970). Cardiovascular adjustment to somatomotor activation. The elicitation of increments in heart rate, aortic pressure and venomotor tone with the initiation of muscle contraction. *Acta physiologica Scandinavica. Supplementum*, *342*, 1–63.

Fujiyoshi, K., Yamaoka-Tojo, M., Minami, Y., Kutsuna, T., Obara, S., Kakizaki, R., Nemoto, T., Hashimoto, T., Namba, S., Shimohama, T., Tojo, T., & Ako, J. (2018). Endothelial Dysfunction Is Associated with Cognitive Impairment of Elderly Cardiovascular Disease Patients: A Reactive Hyperemia-Peripheral Arterial Tonometry Study. *International Heart Journal*, *59*(5), 1034–1040. https://doi.org/10.1536/ihj.17-610

Gao, L., Matthews, F. E., Sargeant, L. A., Brayne, C., & Cfas, M. (2008). An investigation of the population impact of variation in HbA1clevels in older people in England and Wales: From a population based multi-centre longitudinal study. *BMC Public Health*, *8*(1), 1-11. https://doi.org/10.1186/1471-2458-8-54

Gargiulo, P., Marciano, C., Savarese, G., D'Amore, C., Paolillo, S., Esposito, G., Santomauro, M., Marsico, F., Ruggiero, D., Scala, O., Marzano, A., Cecere, M., Casaretti, L., & Perrone Filardi, P. (2013). Endothelial dysfunction in type 2 diabetic patients with normal coronary arteries. *International Journal of Cardiology*, *165*(1), 67–71. https://doi.org/10.1016/j.ijcard.2011.07.076

Geijselaers, S. L. C., Sep, S. J. S., Schram, M. T., Van Boxtel, M. P. J., Van Sloten, T. T., Henry, R. M. A., Reesink, K. D., Kroon, A. A., Koster, A., Schaper, N. C., Dagnelie, P. C., Van Der Kallen, C. J. H., Biessels, G. J., & Stehouwer, C. D. A. (2016). Carotid stiffness is associated with impairment of cognitive performance in individuals with and without type 2 diabetes. The

Maastricht Study. *Atherosclerosis*, *253*, 186–193. https://doi.org/10.1016/j.atherosclerosis.2016.07.912

Gerová, M., & Gero, J. (1969). Range of the Sympathetic Control of the Dog Femoral Artery. *Circulation Research*, *24*(3), 349–359. https://doi.org/10.1161/01.RES.24.3.349

Ghacibeh, G. A., Shenker, J. I., Shenal, B., Uthman, B. M., & Heilman, K. M. (2006). The Influence of Vagus Nerve Stimulation on Memory: *Cognitive and Behavioral Neurology*, *19*(3), 119–122. https://doi.org/10.1097/01.wnn.0000213908.34278.7d

Gokce, N., Keaney, J. F., Hunter, L. M., Watkins, M. T., Menzoian, J. O., & Vita, J. A. (2002). Risk Stratification for Postoperative Cardiovascular Events via Noninvasive Assessment of Endothelial Function: A Prospective Study. *Circulation*, *105*(13), 1567–1572. https://doi.org/10.1161/01.CIR.0000012543.55874.47

Golden, C. J., & Freshwater, S. M. (1978). Stroop color and word test.

Goodglass, H., & Kaplan, E. (1972). *The assessment of aphasia and related disorders*. Lea & Febiger.

Gregg, E. W., Yaffe, K., Cauley, J. A., Rolka, D. B., Blackwell, T. L., Narayan, K. V., ... & Study of Osteoporotic Fractures Research Group. (2000). Is diabetes associated with cognitive impairment and cognitive decline among older women?. *Archives of internal medicine*, *160*(2), 174-180.

Gudala, K., Bansal, D., Schifano, F., & Bhansali, A. (2013). Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. *Journal of Diabetes Investigation*, *4*(6), 640–650. https://doi.org/10.1111/jdi.12087

Gunstad, J., MacGregor, K. L., Paul, R. H., Poppas, A., Jefferson, A. L., Todaro, J. F., & Cohen, R. A. (2005). Cardiac Rehabilitation Improves Cognitive Performance in Older Adults With Cardiovascular Disease: *Journal of Cardiopulmonary Rehabilitation*, *25*(3), 173–176. https://doi.org/10.1097/00008483-200505000-00009

Gurovich, A. N., Avery, J. C., Holtgrieve, N. B., & Braith, R. W. (2014). Flow-mediated dilation is associated with endothelial oxidative stress in human venous endothelial cells. *Vascular Medicine*, *19*(4), 251-256.

Hahad, O., Wild, P. S., Prochaska, J. H., Schulz, A., Hermanns, I., Lackner, K. J., Pfeiffer, N., Schmidtmann, I., Beutel, M., Gori, T., Deanfield, J. E., & Münzel, T. (2019). Endothelial Function Assessed by Digital Volume Plethysmography Predicts the Development and Progression of Type 2 Diabetes Mellitus. *Journal of the American Heart Association*, *8*(20), e012509. https://doi.org/10.1161/JAHA.119.012509

Hamburg, N. M., Keyes, M. J., Larson, M. G., Vasan, R. S., Schnabel, R., Pryde, M. M., Mitchell, G. F., Sheffy, J., Vita, J. A., & Benjamin, E. J. (2008). Cross-Sectional Relations of Digital Vascular Function to Cardiovascular Risk Factors in the Framingham Heart Study. *Circulation*, *117*(19), 2467–2474. https://doi.org/10.1161/CIRCULATIONAHA.107.748574

Hanon, O., Haulon, S., Lenoir, H., Seux, M.-L., Rigaud, A.-S., Safar, M., Girerd, X., & Forette, F. (2005). Relationship Between Arterial Stiffness and Cognitive Function in Elderly Subjects With Complaints of Memory Loss. *Stroke*, *36*(10), 2193–2197. https://doi.org/10.1161/01.STR.0000181771.82518.1c

Harvey, P. D. (2019). Domains of cognition and their assessment. *Dialogues in Clinical Neuroscience*, *21*(3), 227–237. https://doi.org/10.31887/DCNS.2019.21.3/pharvey

Hassing, L. B. (2004). Comorbid type 2 diabetes mellitus and hypertension exacerbates cognitive decline: Evidence from a longitudinal study. *Age and Ageing*, *33*(4), 355–361. https://doi.org/10.1093/ageing/afh100

Hassing, L. B., Johansson, B., Nilsson, S. E., Berg, S., Pedersen, N. L., Gatz, M., & McClearn, G. (2002). Diabetes Mellitus Is a Risk Factor for Vascular Dementia, but Not for Alzheimer's

Disease: A Population-Based Study of the Oldest Old. *International Psychogeriatrics*, *14*(3), 239–248. https://doi.org/10.1017/S104161020200844X

Hauss, W. H., Kreuziger, H., & Asteroth, H. (1949). Über die Reizung der Pressorezeptoren im Sinus caroticus beim Hund. *Z. Kreisl.-Forsch.*, *38*, 28.

Henry, R. M. A., Ferreira, I., Kostense, P. J., Dekker, J. M., Nijpels, G., Heine, R. J., Kamp, O., Bouter, L. M., & Stehouwer, C. D. A. (2004). Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not. *Atherosclerosis*, *174*(1), 49–56. https://doi.org/10.1016/j.atherosclerosis.2004.01.002

Hewlett, A. W., & Van Zwaluwenburg, J. G. (1909). Method for estimating the blood flow in the arm: preliminary report. *Archives of Internal Medicine*, *3*(3), 254-256.

Hoth, K. F., Poppas, A., Ellison, K. E., Paul, R. H., Sokobin, A., Cho, Y., & Cohen, R. A. (2010). Link Between Change in Cognition and Left Ventricular Function Following Cardiac Resynchronization Therapy. *Journal of Cardiopulmonary Rehabilitation and Prevention*, *30*(6), 401–408. https://doi.org/10.1097/HCR.0b013e3181e1739a

Hu, G., Collet, J.-P., Zhao, M., Lu, Y., & Wang, Y. (2023). Associations between Autonomic Function and Cognitive Performance among Patients with Cerebral Small Vessel Disease. *Brain Sciences*, *13*(2), 344. https://doi.org/10.3390/brainsci13020344

Imai, K., Sato, H., Hori, M., Kusuoka, H., Ozaki, H., Yokoyama, H., Takeda, H., Inoue, M., & Kamada, T. (1994). Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *Journal of the American College of Cardiology*, *24*(6), 1529–1535. https://doi.org/10.1016/0735-1097(94)90150-3

Isaacs, B., & Kennie, A. T. (1973). The Set Test as an Aid to the Detection of Dementia in Old People. *British Journal of Psychiatry*, *123*(575), 467–470. https://doi.org/10.1192/bjp.123.4.467

Jackson, D. H., Reeves, T. J., Sheffield, L. T., & Burdeshaw, J. (1973). Isometric effects on treadmill exercise response in healthy young men. *The American Journal of Cardiology*, *31*(3), 344–350. https://doi.org/10.1016/0002-9149(73)90266-X

Jamalnia, S., Javanmardifard, S., Akbari, H., Sadeghi, E., & Bijani, M. (2020). Association Between Cognitive Impairment and Blood Pressure Among Patients with Type II Diabetes Mellitus in Southern Iran. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *Volume 13*, 289–296. https://doi.org/10.2147/DMSO.S238247

Järvisalo, M. J., Jartti, L., Marniemi, J., Rönnemaa, T., Viikari, J. S. A., Lehtimäki, T., & Raitakari, O. T. (2006). Determinants of short-term variation in arterial flow-mediated dilatation in healthy young men. *Clinical Science*, *110*(4), 475–482. https://doi.org/10.1042/CS20050333

Jefferson, A. L., Liu, D., Gupta, D. K., Pechman, K. R., Watchmaker, J. M., Gordon, E. A., Rane, S., Bell, S. P., Mendes, L. A., Davis, L. T., Gifford, K. A., Hohman, T. J., Wang, T. J., & Donahue, M. J. (2017). Lower cardiac index levels relate to lower cerebral blood flow in older adults. *Neurology*, *89*(23), 2327–2334. https://doi.org/10.1212/WNL.0000000000004707

Jensen, A. R. (1965). Scoring the Stroop test. *Acta Psychologica*, *24*, 398–408. https://doi.org/10.1016/0001-6918(65)90024-7

Joannides, R., Haefeli, W. E., Linder, L., Richard, V., Bakkali, E. H., Thuillez, C., & Lüscher, T. F. (1995). Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation*, *91*(5), 1314-1319.

Jolly, M. A., Brennan, D. M., & Cho, L. (2011). Impact of Exercise on Heart Rate Recovery. *Circulation*, *124*(14), 1520–1526. https://doi.org/10.1161/CIRCULATIONAHA.110.005009

Jorge-Galarza, E., Torres-Tamayo, M., Martínez-Alvarado, M. D. R., Peña-Aparicio, B., González-Salazar, C., Reyes-Barrera, J., Sierra-Beltrán, M., Fajardo-Flores, E., Kostin, A., & González-

Hermosillo, J. A. (2022). Cardiovascular autonomic responses during head-up tilt test in newly diagnosed type 2 diabetes. *Irish Journal of Medical Science (1971 -)*, *191*(5), 2077–2084. https://doi.org/10.1007/s11845-021-02825-7

Joshi, H., & Edgell, H. (2019). Sex differences in the ventilatory and cardiovascular response to supine and tilted metaboreflex activation. *Physiological Reports*, *7*(6), e14041.

Joubert, S., Brambati, S. M., Ansado, J., Barbeau, E. J., Felician, O., Didic, M., Lacombe, J., Goldstein, R., Chayer, C., & Kergoat, M. J. (2010). The cognitive and neural expression of semantic memory impairment in mild cognitive impairment and early Alzheimer's disease. *Neuropsychologia*, *48*(4), 978–988. https://doi.org/10.1016/j.neuropsychologia.2009.11.019

Kamiya, A., Michikami, D., Fu, Q., Niimi, Y., Iwase, S., Mano, T., & Suzumura, A. (2001). Static handgrip exercise modifies arterial baroreflex control of vascular sympathetic outflow in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *281*(4), R1134–R1139. https://doi.org/10.1152/ajpregu.2001.281.4.R1134

Kaufman, M. P., Longhurst, J. C., Rybicki, K. J., Wallach, J. H., & Mitchell, J. H. (1983). Effects of static muscular contraction on impulse activity of groups III and IV afferents in cats. *Journal of Applied Physiology*, *55*(1), 105–112. https://doi.org/10.1152/jappl.1983.55.1.105

Kearney-Schwartz, A., Rossignol, P., Bracard, S., Felblinger, J., Fay, R., Boivin, J.-M., Lecompte, T., Lacolley, P., Benetos, A., & Zannad, F. (2009). Vascular Structure and Function Is Correlated to Cognitive Performance and White Matter Hyperintensities in Older Hypertensive Patients With Subjective Memory Complaints. *Stroke*, *40*(4), 1229–1236. https://doi.org/10.1161/STROKEAHA.108.532853

Keary, T. A., Galioto, R., Hughes, J., Waechter, D., Spitznagel, M. B., Rosneck, J., Josephson, R., & Gunstad, J. (2012). Reduced Heart Rate Recovery Is Associated with Poorer Cognitive Function in Older Adults with Cardiovascular Disease. *Cardiovascular Psychiatry and Neurology*, *2012*, 1–6. https://doi.org/10.1155/2012/392490

Kertesz, A. (1982). *Western Aphasia Battery*. San Antonio, TX. The Psychological Corporation.

Keymel, S., Heinen, Y., Balzer, J., Rassaf, T., Kelm, M., Lauer, T., & Heiss, C. (2011). Characterization of macro-and microvascular function and structure in patients with type 2 diabetes mellitus. *American journal of cardiovascular disease*, *1*(1), 68.

Koller, A. K. O. S., & Kaley, G. A. B. O. R. (1990). Role of endothelium in reactive dilation of skeletal muscle arterioles. *American Journal of Physiology-Heart and Circulatory Physiology*, *259*(5), H1313-H1316.

Kolpakov, V., Gordon, D., & Kulik, T. J. (1995). Nitric Oxide–Generating Compounds Inhibit Total Protein and Collagen Synthesis in Cultured Vascular Smooth Muscle Cells. *Circulation Research*, *76*(2), 305–309. https://doi.org/10.1161/01.RES.76.2.305

Kovacs, I., Toth, J., Tarjan, J., & Koller, A. (2006). Correlation of flow mediated dilation with inflammatory markers in patients with impaired cardiac function. Beneficial effects of inhibition of ACE. *European journal of heart failure*, *8*(5), 451-459.

Krishnaswamy, P. S., Egom, E. E., Moghtadaei, M., Jansen, H. J., Azer, J., Bogachev, O., ... & Rose, R. A. (2015). Altered parasympathetic nervous system regulation of the sinoatrial node in Akita diabetic mice. *Journal of Molecular and Cellular Cardiology*, *82*, 125-135.

Kuvin, J. T., Patel, A. R., Sliney, K. A., Pandian, N. G., Sheffy, J., Schnall, R. P., Karas, R. H., & Udelson, J. E. (2003). Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *American Heart Journal*, *146*(1), 168–174. https://doi.org/10.1016/S0002-8703(03)00094-2

Lahiri, S., Mokashi, A., Mulligan, E., & Nishino, T. (1981). Comparison of aortic and carotid chemoreceptor responses to hypercapnia and hypoxia. *Journal of Applied Physiology*, *51*(1), 55-61.

Lansman, J. B., Hallam, T. J., & Rink, T. J. (1987). Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers? *Nature*, *325*(6107), 811–813. https://doi.org/10.1038/325811a0

Laugesen, E., Høyem, P., Stausbøl-Grøn, B., Mikkelsen, A., Thrysøe, S., Erlandsen, M., Christiansen, J. S., Knudsen, S. T., Hansen, K. W., Kim, W. Y., Hansen, T. K., & Poulsen, P. L. (2013). Carotid-Femoral Pulse Wave Velocity Is Associated With Cerebral White Matter Lesions in Type 2 Diabetes. *Diabetes Care*, *36*(3), 722–728. https://doi.org/10.2337/dc12-0942

Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., Ducimetiere, P., & Benetos, A. (2001). Aortic Stiffness Is an Independent Predictor of All-Cause and Cardiovascular Mortality in Hypertensive Patients. *Hypertension*, *37*(5), 1236–1241. https://doi.org/10.1161/01.HYP.37.5.1236

Leibson, C. L., Rocca, W. A., Hanson, V. A., Cha, R., Kokmen, E., O’Brien, P. C., & Palumbo, P. J. (1997). Risk of Dementia among Persons with Diabetes Mellitus: A Population-based Cohort Study. *American Journal of Epidemiology*, *145*(4), 301–308. https://doi.org/10.1093/oxfordjournals.aje.a009106

Leon, D. F., Shaver, J. A., & Leonard, J. J. (1970). Reflex heart rate control in man. *American Heart Journal*, *80*(6), 729–739. https://doi.org/10.1016/0002-8703(70)90133-X

Lieberman, E. H., Gerhard, M. D., Uehata, A., Walsh, B. W., Selwyn, A. P., Ganz, P., ... & Creager, M. A. (1994). Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. *Annals of internal medicine*, *121*(12), 936-941.

Liebeskind, D. S. (2003). Collateral Circulation. *Stroke*, *34*(9), 2279–2284. https://doi.org/10.1161/01.STR.0000086465.41263.06

Lim, S. L., Gao, Q., Nyunt, M. S. Z., Gong, L., Lunaria, J. B., Lim, M. L., Ling, A., Lam, C. S.-P., Richards, A. M., Ling, L. H., & Ng, T. P. (2016). Vascular Health Indices and Cognitive Domain Function: Singapore Longitudinal Ageing Studies. *Journal of Alzheimer's Disease*, *50*(1), 27–40. https://doi.org/10.3233/JAD-150516

Lin, B., Ginsberg, M. D., Busto, R., & Li, L. (2000). Hyperglycemia triggers massive neutrophil deposition in brain following transient ischemia in rats. *Neuroscience letters*, *278*(1-2), 1-4.

Lind, A. R., & McNicol, G. W. (1967). Circulatory responses to sustained hand-grip contractions performed during other exercise, both rhythmic and static. *The Journal of Physiology*, *192*(3), 595–607. https://doi.org/10.1113/jphysiol.1967.sp008319

Lipinski, M. J., Vetrovec, G. W., & Froelicher, V. F. (2004). Importance of the first two minutes of heart rate recovery after exercise treadmill testing in predicting mortality and the presence of coronary artery disease in men. *The American Journal of Cardiology*, *93*(4), 445–449. https://doi.org/10.1016/j.amjcard.2003.10.039

Liu, J., Rutten-Jacobs, L., Liu, M., Markus, H. S., & Traylor, M. (2018). Causal Impact of Type 2 Diabetes Mellitus on Cerebral Small Vessel Disease: A Mendelian Randomization Analysis. *Stroke*, *49*(6), 1325–1331. https://doi.org/10.1161/STROKEAHA.117.020536

Ludmer, P. L., Selwyn, A. P., Shook, T. L., Wayne, R. R., Mudge, G. H., Alexander, R. W., & Ganz, P. (1986). Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *New England Journal of Medicine*, *315*(17), 1046-1051.

Mac Ananey, O., Malone, J., Warmington, S., O'Shea, D., Green, S., & Egaña, M. (2011). Cardiac Output Is Not Related to the Slowed O2 Uptake Kinetics in Type 2 Diabetes. *Medicine & Science in Sports & Exercise*, *43*(6), 935–942. https://doi.org/10.1249/MSS.0b013e3182061cdb

Mahinrad, S., Jukema, J. W., van Heemst, D., Macfarlane, P. W., Clark, E. N., de Craen, A. J. M., & Sabayan, B. (2016). 10-Second heart rate variability and cognitive function in old age. *Neurology*, *86*(12), 1120–1127. https://doi.org/10.1212/WNL.0000000000002499

Mäkimattila, S., Liu, M. L., Vakkilainen, J., Schlenzka, A., Lahdenperä, S., Syvänne, M., Mäntysaari, M., Summanen, P., Bergholm, R., Taskinen, M. R., & Yki-Järvinen, H. (1999). Impaired endothelium-dependent vasodilation in type 2 diabetes. Relation to LDL size, oxidized LDL, and antioxidants. *Diabetes Care*, *22*(6), 973–981. https://doi.org/10.2337/diacare.22.6.973

Malik, M. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use: Task force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology. *Annals of Noninvasive Electrocardiology*, *1*(2), 151-181.

Marioni, R. E., Strachan, M. W., Reynolds, R. M., Lowe, G. D., Mitchell, R. J., Fowkes, F. G. R., ... & Price, J. F. (2010). Association between raised inflammatory markers and cognitive decline in elderly people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes*, *59*(3), 710-713.

Martin, C. E., Shaver, J. A., Leon, D. F., Thompson, M. E., Reddy, P. S., & Leonard, J. J. (1974). Autonomic Mechanisms in Hemodynamic Responses to Isometric Exercise. *Journal of Clinical Investigation*, *54*(1), 104–115. https://doi.org/10.1172/JCI107731

Maruhashi, T., Soga, J., Fujimura, N., Idei, N., Mikami, S., Iwamoto, Y., Kajikawa, M., Matsumoto, T., Hidaka, T., Kihara, Y., Chayama, K., Noma, K., Nakashima, A., Goto, C., Tomiyama, H., Takase, B., Yamashina, A., & Higashi, Y. (2013). Relationship between flow-mediated vasodilation and cardiovascular risk factors in a large community-based study. *Heart*, *99*(24), 1837–1842. https://doi.org/10.1136/heartjnl-2013-304739

Matsuoka, O., Otsuka, K., Murakami, S., Hotta, N., Yamanaka, G., Kubo, Y., Yamanaka, T., Shinagawa, M., Nunoda, S., Nishimura, Y., Shibata, K., Saitoh, H., Nishinaga, M., Ishine, M., Wada, T., Okumiya, K., Matsubayashi, K., Yano, S., Ichihara, K., … Ozawa, T. (2005). Arterial stiffness independently predicts cardiovascular events in an elderly community—Longitudinal Investigation for the Longevity and Aging in Hokkaido County (LILAC) study. *Biomedicine & Pharmacotherapy*, *59*, S40–S44. https://doi.org/10.1016/S0753-3322(05)80008-3

Mattace-Raso, F. U. S., van der Cammen, T. J. M., Hofman, A., van Popele, N. M., Bos, M. L., Schalekamp, M. A. D. H., Asmar, R., Reneman, R. S., Hoeks, A. P. G., Breteler, M. M. B., & Witteman, J. C. M. (2006). Arterial Stiffness and Risk of Coronary Heart Disease and Stroke: The Rotterdam Study. *Circulation*, *113*(5), 657–663. https://doi.org/10.1161/CIRCULATIONAHA.105.555235

McFall, G. P., Geall, B. P., Fischer, A. L., Dolcos, S., & Dixon, R. A. (2010). Testing covariates of Type 2 diabetes-cognition associations in older adults: Moderating or mediating effects? *Neuropsychology*, *24*(5), 547–562. https://doi.org/10.1037/a0019246

Mehrabian, S., Raycheva, M., Gateva, A., Todorova, G., Angelova, P., Traykova, M., Stankova, T., Kamenov, Z., & Traykov, L. (2012). Cognitive dysfunction profile and arterial stiffness in type 2 diabetes. *Journal of the Neurological Sciences*, *322*(1–2), 152–156. https://doi.org/10.1016/j.jns.2012.07.046

Meyer, C., Milat, F., McGrath, B. P., Cameron, J., Kotsopoulos, D., & Teede, H. J. (2004). Vascular dysfunction and autonomic neuropathy in Type 2 diabetes: Original article. *Diabetic Medicine*, *21*(7), 746–751. https://doi.org/10.1111/j.1464-5491.2004.01241.x

Mitchell, G. F., Hwang, S.-J., Vasan, R. S., Larson, M. G., Pencina, M. J., Hamburg, N. M., Vita, J. A., Levy, D., & Benjamin, E. J. (2010). Arterial Stiffness and Cardiovascular Events: The Framingham Heart Study. *Circulation*, *121*(4), 505–511. https://doi.org/10.1161/CIRCULATIONAHA.109.886655

Mitchell, J. H., Kaufman, M. P., & Iwamoto, G. A. (1983). The Exercise Pressor Reflex: Its Cardiovascular Effects, Afferent Mechanisms, and Central Pathways. *Annual Review of Physiology*, *45*(1), 229–242. https://doi.org/10.1146/annurev.ph.45.030183.001305

Miura, H., Wachtel, R. E., Liu, Y., Loberiza, F. R., Saito, T., Miura, M., & Gutterman, D. D. (2001). Flow-Induced Dilation of Human Coronary Arterioles: Important Role of Ca $^{2+}$ -Activated K $^{+}$ Channels. *Circulation*, *103*(15), 1992–1998. https://doi.org/10.1161/01.CIR.103.15.1992

Moerland, M., Kales, A. J., Schrier, L., van Dongen, M. G. J., Bradnock, D., & Burggraaf, J. (2012). Evaluation of the EndoPAT as a Tool to Assess Endothelial Function. *International Journal of Vascular Medicine*, *2012*, 1–8. https://doi.org/10.1155/2012/904141

Montano, N., Cogliati, C., Porta, A., Pagani, M., Malliani, A., Narkiewicz, K., Abboud, F. M., Birkett, C., & Somers, V. K. (1998). Central Vagotonic Effects of Atropine Modulate Spectral Oscillations of Sympathetic Nerve Activity. *Circulation*, *98*(14), 1394–1399. https://doi.org/10.1161/01.CIR.98.14.1394

Moroni, C., & Bayard, S. (2009). Inhibitory process: what evolution after the age of 50?. *Psychologie & NeuroPsychiatrie du vieillissement*, *7*(2), 121-129.

Nabel, E. G., Selwyn, A. P., & Ganz, P. (1990). Large coronary arteries in humans are responsive to changing blood flow: An endothelium-dependent mechanism that fails in patients with atherosclerosis. *Journal of the American College of Cardiology*, *16*(2), 349–356. https://doi.org/10.1016/0735-1097(90)90584-C

Naiberg, M. R., Newton, D. F., & Goldstein, B. I. (2016). Flow-Mediated Dilation and Neurocognition: Systematic Review and Future Directions. *Psychosomatic Medicine*, *78*(2), 192–207. https://doi.org/10.1097/PSY.0000000000000266

Nardone, M., Miner, S., McCarthy, M., Ardern, C. I., & Edgell, H. (2020). Noninvasive microvascular indices reveal peripheral vascular abnormalities in patients with suspected coronary microvascular dysfunction. *Canadian Journal of Cardiology*, *36*(8), 1289-1297.

Nonogaki, Z., Umegaki, H., Makino, T., Suzuki, Y., & Kuzuya, M. (2017). Relationship between cardiac autonomic function and cognitive function in Alzheimer's disease: Heart and cognition in AD. *Geriatrics & Gerontology International*, *17*(1), 92–98. https://doi.org/10.1111/ggi.12679

O'Connor, E., Green, S., Kiely, C., O'Shea, D., & Egaña, M. (2015). Differential effects of age and type 2 diabetes on dynamic vs. Peak response of pulmonary oxygen uptake during exercise. *Journal of Applied Physiology*, *118*(8), 1031–1039. https://doi.org/10.1152/japplphysiol.01040.2014

Olesen, S.-P., Claphamt, D., & Davies, P. (1988). Haemodynamic shear stress activates a K+ current in vascular endothelial cells. *Nature*, *331*(6152), 168–170. https://doi.org/10.1038/331168a0

Pereira, T. J., Wasef, S., Ivry, I., Assadpour, E., Adeyinka, B. O., & Edgell, H. (2022). Menstrual cycle and oral contraceptives influence cerebrovascular dynamics during hypercapnia. *Physiological Reports*, *10*(13), e15373.

Pomeranz, B., Macaulay, R. J., Caudill, M. A., Kutz, I., Adam, D., Gordon, D., Kilborn, K. M., Barger, A. C., Shannon, D. C., Cohen, R. J., & et, al. (1985). Assessment of autonomic function in humans by heart rate spectral analysis. *American Journal of Physiology-Heart and Circulatory Physiology*, *248*(1), H151–H153. https://doi.org/10.1152/ajpheart.1985.248.1.H151

Pugsley, M. K., & Tabrizchi, R. (2000). The vascular system An overview of structure and function. *Journal of Pharmacological and Toxicological Methods*. *44*(2), 333-340.

Qiu, C., Sigurdsson, S., Zhang, Q., Jonsdottir, M. K., Kjartansson, O., Eiriksdottir, G., ... & Launer, L. J. (2014). Diabetes, markers of brain pathology and cognitive function: the Age, Gene/Environment Susceptibility–Reykjavik Study. *Annals of neurology*, *75*(1), 138-146.

Radomski, M. W., Palmer, R. M. J., & Moncada, S. (1987). The role of nitric oxide and cGMP in platelet adhesion to vascular endothelium. *Biochemical and Biophysical Research Communications*, *148*(3), 1482–1489. https://doi.org/10.1016/S0006-291X(87)80299-1

Ravona-Springer, R., & Schnaider-Beeri, M. (2011). The association of diabetes and dementia and possible implications for nondiabetic populations. *Expert Review of Neurotherapeutics*, *11*(11), 1609–1617. https://doi.org/10.1586/ern.11.152

Regensteiner, J. G., Bauer, T. A., Reusch, J. E. B., Quaife, R. A., Chen, M. Y., Smith, S. C., Miller, T. M., Groves, B. M., & Wolfel, E. E. (2009). Cardiac Dysfunction during Exercise in Uncomplicated Type 2 Diabetes. *Medicine & Science in Sports & Exercise*, *41*(5), 977–984. https://doi.org/10.1249/MSS.0b013e3181942051

Reijmer, Y. D., Van Den Berg, E., Dekker, J. M., Nijpels, G., Stehouwer, C. D. A., Kappelle, L. J., & Biessels, G. J. (2011). The metabolic syndrome, atherosclerosis and cognitive functioning in a non-demented population: The Hoorn Study. *Atherosclerosis*, *219*(2), 839–845. https://doi.org/10.1016/j.atherosclerosis.2011.08.032

Reitan, R. M. (1958). Validity of the Trail Making Test as an indicator of organic brain damage. *Perceptual and motor skills*, *8*(3), 271-276.

Ringqvist, Å., Caidahl, K., Petersson, A.-S., & Wennmalm, Å. (2000). Diurnal variation of flow-mediated vasodilation in healthy premenopausal women. *American Journal of Physiology-Heart and Circulatory Physiology*, *279*(6), H2720–H2725. https://doi.org/10.1152/ajpheart.2000.279.6.H2720

Robinson, B. F., Epstein, S. E., Beiser, G. D., & Braunwald, E. (1966). Control of Heart Rate by the Autonomic Nervous System: Studies in Man on the Interrelation Between Baroreceptor Mechanisms and Exercise. *Circulation Research*, *19*(2), 400–411. https://doi.org/10.1161/01.RES.19.2.400

Rosengård-Bärlund, M., Bernardi, L., Sandelin, A., Forsblom, C., Groop, P.-H., & on behalf of the FinnDiane Study Group. (2011). Baroreflex Sensitivity and Its Response to Deep Breathing Predict Increase in Blood Pressure in Type 1 Diabetes in a 5-Year Follow-Up. *Diabetes Care*, *34*(11), 2424–2430. https://doi.org/10.2337/dc11-0629

Rubanyi, G. M., Romero, J. C., & Vanhoutte, P. M. (1986). Flow-induced release of endothelium-derived relaxing factor. *American Journal of Physiology-Heart and Circulatory Physiology*, *250*(6), H1145–H1149. https://doi.org/10.1152/ajpheart.1986.250.6.H1145

Rubinshtein, R., Kuvin, J. T., Soffler, M., Lennon, R. J., Lavi, S., Nelson, R. E., Pumper, G. M., Lerman, L. O., & Lerman, A. (2010). Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. *European Heart Journal*, *31*(9), 1142–1148. https://doi.org/10.1093/eurheartj/ehq010

Russell, E. W. (1972). WAIS factor analysis with brain-damaged subjects using criterion measures. *Journal of Consulting and Clinical Psychology*, *39*(1), 133.

Saleem, M., Herrmann, N., Dinoff, A., Mazereeuw, G., Oh, P. I., Goldstein, B. I., Kiss, A., Shammi, P., & Lanctôt, K. L. (2019). Association Between Endothelial Function and Cognitive Performance in Patients with Coronary Artery Disease During Cardiac Rehabilitation. *Psychosomatic Medicine*, *81*(2), 184–191. https://doi.org/10.1097/PSY.0000000000000651

Sarkar, R., Meinberg, E. G., Stanley, J. C., Gordon, D., & Clinton Webb, R. (1996). Nitric Oxide Reversibly Inhibits the Migration of Cultured Vascular Smooth Muscle Cells. *Circulation Research*, *78*(2), 225–230. https://doi.org/10.1161/01.RES.78.2.225

Savin, W. M., Davidson, D. M., & Haskell, W. L. (1982). Autonomic contribution to heart rate recovery from exercise in humans. *Journal of Applied Physiology*, *53*(6), 1572-1575.

Sessa, W. C. (2004). eNOS at a glance. *Journal of cell science*, *117*(12), 2427-2429.

Schaich, C. L., Malaver, D., Chen, H., Shaltout, H. A., Zeki Al Hazzouri, A., Herrington, D. M., & Hughes, T. M. (2020). Association of Heart Rate Variability With Cognitive Performance: The Multi-Ethnic Study of Atherosclerosis. *Journal of the American Heart Association*, *9*(7), e013827. https://doi.org/10.1161/JAHA.119.013827

Schroeder, E. B., Liao, D., Chambless, L. E., Prineas, R. J., Evans, G. W., & Heiss, G. (2003). Hypertension, Blood Pressure, and Heart Rate Variability: The Atherosclerosis Risk in Communities (ARIC) Study. *Hypertension*, *42*(6), 1106–1111. https://doi.org/10.1161/01.HYP.0000100444.71069.73

Schultz, K. D., Schultz, K., & Schultz, G. (1977). Sodium nitroprusside and other smooth muscle-relaxants increase cyclic GMP levels in rat ductus deferens. *Nature*, *265*(5596), 750-751.

Scuteri, A., Tesauro, M., Appolloni, S., Preziosi, F., Brancati, A. M., & Volpe, M. (2007). Arterial stiffness as an independent predictor of longitudinal changes in cognitive function in the older individual. *Journal of Hypertension*, *25*(5), 1035–1040. https://doi.org/10.1097/HJH.0b013e3280895b55

Shokawa, T., Imazu, M., Yamamoto, H., Toyofuku, M., Tasaki, N., Okimoto, T., Yamane, K., & Kohno, N. (2005). Pulse Wave Velocity Predicts Cardiovascular Mortality-Findings from the Hawaii-Los Angeles-Hiroshima Study-: Findings From the Hawaii-Los Angeles-Hiroshima Study. *Circulation Journal*, *69*(3), 259–264. https://doi.org/10.1253/circj.69.259

Shorr, R. I., de Rekeneire, N., Resnick, H. E., Yaffe, K., Somes, G. W., Kanaya, A. M., Simonsick, E. M., Newman, A. B., & Harris, T. B. (2006). Glycemia and cognitive function in older adults using glucose-lowering drugs. *The journal of nutrition, health & aging*, *10*(4), 297–301.

Sorensen, K. E., Celermajer, D. S., Spiegelhalter, D. J., Georgakopoulos, D., Robinson, J., Thomas, O., & Deanfield, J. E. (1995). Non-invasive measurement of human endothelium dependent arterial responses: Accuracy and reproducibility. *Heart*, *74*(3), 247–253. https://doi.org/10.1136/hrt.74.3.247

Spikman, J. M., Kiers, H. A. L., Deelman, B. G., & van Zomeren, A. H. (2001). Construct Validity of Concepts of Attention in Healthy Controls and Patients with CHI. *Brain and Cognition*, *47*(3), 446–460. https://doi.org/10.1006/brcg.2001.1320

Spreen O., Strauss, E. (1998). *A compendium of neuropsychological tests: Administration, norms, and commentary.*(2nd ed.). New York: Oxford University Press

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of experimental psychology, 18*(6), 643.

Stuehr, D. J. (2004). Enzymes of the L-Arginine to Nitric Oxide Pathway. *The Journal of Nutrition*, *134*(10), 2748S-2751S. https://doi.org/10.1093/jn/134.10.2748S

Su, Y., Liu, X. M., Sun, Y. M., Jin, H. B., Fu, R., Wang, Y. Y., ... & Luan, Y. (2008). The relationship between endothelial dysfunction and oxidative stress in diabetes and prediabetes. *International journal of clinical practice*, *62*(6), 877-882.

Sucharita, S., Bantwal, G., Idiculla, J., Ayyar, V., & Vaz, M. (2011). Autonomic nervous system function in type 2 diabetes using conventional clinical autonomic tests, heart rate and blood pressure variability measures. *Indian Journal of Endocrinology and Metabolism*, *15*(3), 198. https://doi.org/10.4103/2230-8210.83406

Sundaram, R. K., Bhaskar, A., Vijayalingam, S., Viswanathan, M., Mohan, R., & Shanmugasundaram, K. R. (1996). Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clinical science (London, England: 1979)*, *90*(4), 255-260.

Sutton-Tyrrell, K., Najjar, S. S., Boudreau, R. M., Venkitachalam, L., Kupelian, V., Simonsick, E. M., Havlik, R., Lakatta, E. G., Spurgeon, H., Kritchevsky, S., Pahor, M., Bauer, D., & Newman, A. (2005). Elevated Aortic Pulse Wave Velocity, a Marker of Arterial Stiffness, Predicts Cardiovascular Events in Well-Functioning Older Adults. *Circulation*, *111*(25), 3384–3390. https://doi.org/10.1161/CIRCULATIONAHA.104.483628

Tachibana, H., Washida, K., Kowa, H., Kanda, F., & Toda, T. (2016). Vascular Function in Alzheimer’s Disease and Vascular Dementia. *American Journal of Alzheimer’s Disease & Other Dementiasr*, *31*(5), 437–442. https://doi.org/10.1177/1533317516653820

Tentolouris, N., Liatis, S., Moyssakis, I., Tsapogas, P., Psallas, M., Diakoumopoulou, E., Voteas, V., & Katsilambros, N. (2003). Aortic distensibility is reduced in subjects with type 2 diabetes and cardiac autonomic neuropathy: Aortic distensibility and autonomic neuropathy. *European*

Journal of Clinical Investigation, *33*(12), 1075–1083. https://doi.org/10.1111/j.1365-2362.2003.01279.x

Thijssen, D. H. J., Black, M. A., Pyke, K. E., Padilla, J., Atkinson, G., Harris, R. A., Parker, B., Widlansky, M. E., Tschakovsky, M. E., & Green, D. J. (2011). Assessment of flow-mediated dilation in humans: A methodological and physiological guideline. *American Journal of Physiology-Heart and Circulatory Physiology*, *300*(1), H2–H12. https://doi.org/10.1152/ajpheart.00471.2010

Thurstone, L. L. (1938). Primary Mental Abilities. Chicago: University of Chicago Press.

Thurstone, L. L., & Thurstone, T. G. (1949). *Examiner Manual for the SRA primary mental abilities Test*. Chicago: Science Research Associates

Tigerstedt, R. & Bergman, P. Niere and Kreislauf. *Arch. Physiol. 8:* 223-271, 1898.

Troyer, A. K., Leach, L., & Strauss, E. (2006). Aging and Response Inhibition: Normative Data for the Victoria Stroop Test. *Aging, Neuropsychology, and Cognition*, *13*(1), 20–35. https://doi.org/10.1080/138255890968187

Tsuchiya, K., Nakayama, C., Iwashima, F., Sakai, H., Izumiyama, H., Doi, M., & Hirata, Y. (2007). Advanced Endothelial Dysfunction in Diabetic Patients with Multiple Risk Factors; Importance of Insulin Resistance. *Journal of Atherosclerosis and Thrombosis*, *14*(6), 303–309. https://doi.org/10.5551/jat.E525

Tsuji, H., Larson, M. G., Venditti, F. J., Jr, Manders, E. S., Evans, J. C., Feldman, C. L., & Levy, D. (1996). Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation*, *94*(11), 2850–2855.

Van Den Berg, E., Dekker, J. M., Nijpels, G., Kessels, R. P. C., Kappelle, L. J., De Haan, E. H. F., Heine, R. J., Stehouwer, C. D. A., & Biessels, G. J. (2009). Blood pressure levels in pre-diabetic stages are associated with worse cognitive functioning in patients with type 2 diabetes. *Diabetes/Metabolism Research and Reviews*, *25*(7), 657–664. https://doi.org/10.1002/dmrr.1009

Van Hove, C., Van der Donckt, C., Herman, A., Bult, H., & Fransen, P. (2009). Vasodilator efficacy of nitric oxide depends on mechanisms of intracellular calcium mobilization in mouse aortic smooth muscle cells: Vasodilator efficacy of nitric oxide. *British Journal of Pharmacology*, *158*(3), 920–930. https://doi.org/10.1111/j.1476-5381.2009.00396.x

Vendemiale, G., Romano, A. D., Dagostino, M., De Matthaeis, A., & Serviddio, G. (2013). Endothelial dysfunction associated with mild cognitive impairment in elderly population. *Aging Clinical and Experimental Research*, *25*(3), 247–255. https://doi.org/10.1007/s40520-013-0043-8

Vincent, A. M., Mclean, L. L., Backus, C., & Feldman, E. L. (2005). Short-term hyperglycemia produces oxidative damage and apoptosis in neurons. *The FASEB Journal*, *19*(6), 1-24.

Wechsler, D. (1939). *The measurement of adult intelligence*. The Williams & Wilkins Company.

Williams, S. B., Cusco, J. A., Roddy, M.-A., Johnstone, M. T., & Creager, M. A. (1996). Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *Journal of the American College of Cardiology*, *27*(3), 567–574. https://doi.org/10.1016/0735-1097(95)00522-6

Yeboah, J., Folsom, A. R., Burke, G. L., Johnson, C., Polak, J. F., Post, W., Lima, J. A., Crouse, J. R., & Herrington, D. M. (2009). Predictive Value of Brachial Flow-Mediated Dilation for Incident Cardiovascular Events in a Population-Based Study: The Multi-Ethnic Study of Atherosclerosis. *Circulation*, *120*(6), 502–509. https://doi.org/10.1161/CIRCULATIONAHA.109.864801

Zeiher, A. M., Fisslthaler, B., Schray-Utz, B., & Busse, R. (1995). Nitric Oxide Modulates the Expression of Monocyte Chemoattractant Protein 1 in Cultured Human Endothelial Cells. *Circulation Research*, *76*(6), 980–986. https://doi.org/10.1161/01.RES.76.6.980

Zeki Al Hazzouri, A., Elfassy, T., Carnethon, M. R., Lloyd-Jones, D. M., & Yaffe, K. (2018). Heart Rate Variability and Cognitive Function In Middle-Age Adults: The Coronary Artery Risk Development in Young Adults. *American Journal of Hypertension*, *31*(1), 27–34. https://doi.org/10.1093/ajh/hpx125

Zhang, J., Chi, H., Wang, T., Shen, T., Wang, H., Yuan, X., & Li, Z. (2019). Effects of orthostatic hypotension on cognition in type 2 diabetes mellitus. *Annals of Neurology*, *86*(5), 754–761. https://doi.org/10.1002/ana.25578

Zhong, W., Cruickshanks, K. J., Schubert, C. R., Carlsson, C. M., Chappell, R. J., Klein, B. E. K., Klein, R., & Acher, C. W. (2014). Pulse Wave Velocity and Cognitive Function in Older Adults. *Alzheimer Disease & Associated Disorders*, *28*(1), 44–49. https://doi.org/10.1097/WAD.0b013e3182949f06

Appendix 1

In Preparation for Journal of Vascular Research:

A novel method for assessment of endothelial function

O. Ramraj, B.Sc.[1]; S. Badhwar, Ph.D[1]., TJ. Pereira, M.Sc.[1], and H Edgell, Ph.D.[1,2]

[1]School of Kinesiology and Health Science, York University, Toronto, Ontario, Canada
[2]Muscle Health Research Centre, York University, Toronto, Ontario, Canada

Running Head: Novel method for assessing endothelial function

Address for Correspondence:
Heather Edgell, PhD
355 Bethune College, York University
4700 Keele St, Toronto, ON, Canada M3J 1P3
Tel: +1 (416) 736-2100 x22927
Fax: +1 (416) 736-5774
E-mail: edgell@yorku.ca
ORCID: 0000-001-8865-1921
Twitter: @heather_edgell

Abstract

Current non-invasive methods of determining endothelial function may not be practical or accessible due to requiring experienced personnel or expensive ongoing equipment costs. Piezoelectric transducers can reliably obtain blood pressure waves from the finger, similar to peripheral arterial tonometry devices; thus, their use could be extended to estimate endothelial function. The current study aims to determine the validity of using piezoelectric transducers as an alternative method of measuring endothelial function, as compared to an EndoPAT device. Twenty-five adults (aged 20-64 years) completed a reactive hyperemia test (5 minutes of forearm circulatory occlusion and 5 minutes of recovery) with piezoelectric transducers on the middle fingers and EndoPAT probes on the index fingers concurrently. Average area under the curve (AUC) of the pulse wave signal for both the occluded and control arms were determined at baseline, every 30secs post-occlusion for 3mins, and for 10secs around the peak response. The pulse wave reactive hyperemia index (PWRHI) was calculated as the ratio of AUC post-occlusion to AUC baseline in the test arm and was normalized to the same ratio in the control arm. The PWRHI at each timepoint was correlated with the reactive hyperemia index (RHI) from the EndoPAT. The highest significant correlation was found between the RHI and PWRHI calculated at 10secs around the peak response (r=0.67, p=0.0002). In conclusion, the PWRHI method is a reusable and user-friendly measure of endothelial function that could provide better access to vascular health screening.

Keywords: non-invasive, piezoelectric transducers, vascular health, clinical, reactive hyperemia, microvascular

Introduction

The vascular endothelium has several crucial roles in maintaining vascular function, including regulating blood vessel tone(Furchgott & Zawadzki, 1980). For example, shear stress acts on the endothelium causing vasodilation via nitric oxide release(Frame & Sarelius, 1996; Palmer et al., 1987; Rubanyi et al., 1986). Chronic conditions such as diabetes and obesity, overexpression of pro-inflammatory mediators, increased oxidative stress, and disturbed arterial flow patterns can all cause endothelial dysfunction(Alexander et al., 2021). Thus, a reduction in endothelial function can be used as a screening tool for estimating cardiovascular risk.

Indeed, endothelial dysfunction has been recognized as a potential predictive and prognostic marker for cardiovascular morbidity and mortality(Gokce et al., 2002b; Kinlay & Ganz, 1997; Sorensen et al., 1994). Various assessments have been developed to evaluate the endothelial function and the quality of an individual's vascular health. The gold standard method for assessing coronary endothelial function is an invasive test involving the infusion of adenosine and/or acetylcholine into the coronary artery to induce vasodilation(Hasdai & Lerman, 1999; Reriani et al., 2010). Comparatively, flow-mediated dilation (FMD) is a non-invasive ultrasound technique that involves imaging the brachial artery to capture increased diameter in response to reactive hyperemia after a period of circulatory occlusion(Corretti et al., 2002) and has been shown to correlate with coronary arterial function(Takase et al., 1998). Further, an operator-independent technique called peripheral arterial tonometry (PAT) using the EndoPAT device measures the reactive hyperemia index (RHI), where arterial pulse waves are recorded in both hands during and after reactive hyperemia. The RHI is the ratio of the pulse wave signal comparing the maximal hyperemic response to baseline while normalizing to the non-occluded hand. Several studies have established this method as a reliable measure of endothelial function(Bonetti et al., 2004; Hamburg

& Benjamin, 2009; Kuvin et al., 2003; Moerland et al., 2012; Nohria et al., 2006), and it has been shown to correlate with FMD measurements(Kuvin et al., 2003). However, in addition to equipment costs, each of these measurements requires hospital affiliation, skilled personnel and/or costly single use operational supplies.

In the current study, we propose using readily available reusable piezoelectric pulse transducers during reactive hyperemia as an affordable, user-friendly method for evaluating endothelial function. The pulse transducers detect changes in force against the piezoelectric crystals, which are translated into a pulse wave signal. Foo et al.(Foo & Lim, 2006) found that these pulse transducers could reliably obtain pulse transit time and heart rate when placed on the radial artery at the wrist compared to commercial ECG and pulse oximetry devices. Further, our lab has previously used pulse transducers to determine pulse wave velocity in conjunction with the pulse wave from a Finometer or Nexfin finger pulse(Edgell et al., 2016). Qananwah et al.(Qananwah et al., 2020) determined that there was a strong relationship and morphological similarities between piezoelectric plethysmograms and volumetric variations of blood circulation at the finger, suggesting that the pulse waves obtained from the piezoelectric pulse transducers are morphologically comparable to the waveforms recorded using the EndoPAT. Therefore, since the signal from the piezoelectric pulse transducer has been used to determine peripheral arterial stiffness and is structurally similar to the arterial tonometry signal from the EndoPAT device, we hypothesize that our new protocol, the pulse wave reactive hyperemia index (PWRHI) method, can be used in lieu of the EndoPAT to determine endothelial function.

Methods

Twenty-five adults (females, n=15; Table 1) were recruited to participate in the current study. Self-identified ethnicity was recorded: 17 Caucasian, 5 Asian/South Asian, and 3 Arab/Middle-Eastern. Ethical clearance was obtained by the York University Ethics Review Board. Written informed consent was taken from each participant before starting the study. Participants were asked to refrain from the consumption of caffeine, alcohol, and fatty foods, as well as smoking and strenuous exercise, for a duration of at least 12 hours prior to testing.

Reactive Hyperemia:

EndoPAT tonometry finger cuffs were placed on both the left and right index fingers. Participants were seated with hands and fingers in a relaxed position over the seat's armrests and palms facing down while feet were firmly planted on the ground. Piezoelectric pulse transducers (ADInstruments, Colorado Springs, USA) were wrapped around both the left and right middle fingertips such that the transducer surface was placed against the pad of the finger. Participants were asked to refrain from moving for the duration of the test to eliminate movement artifacts. A reactive hyperemia test was conducted, which consisted of 5 minutes of baseline, 5 minutes of suprasystolic circulatory occlusion to the left arm (i.e., +50 mmHg), and 5 minutes of recovery following the release of occlusion. Piezoelectric signals were acquired through a PowerLab device (ADInstruments, Colorado Springs, USA) and recorded using LabChart software (ADInstruments, Colorado Springs, USA) for the duration of the reactive hyperemia test to determine the PWRHI.

Data Analysis

LabChart software (ADInstruments, Colorado Springs, USA) was used to determine the continuous positive area under the curve (AUC) of the pulse transducer waveform for each cardiac cycle. In the test (occluded) and control (non-occluded) arms, the averaged AUC was calculated s for 3.5 minutes of baseline prior to occlusion (to match the baseline analysis used by the EndoPAT(Bonetti et al., 2004)), 10 seconds around the peak response, and 30-second averages starting at occlusion release until 3 minutes post-occlusion. For each time point following the occlusion release, the PWRHI was calculated as the ratio of AUC post-occlusion to AUC baseline in the test arm normalized to the same ratio in the control arm:

$$\text{PWRHI} = (\text{AUC}_{\text{test}}\ \text{post-occlusion}/\text{AUC}_{\text{test}}\ \text{baseline})/(\text{AUC}_{\text{control}}\ \text{post-occlusion}/\text{AUC}_{\text{control}}\ \text{baseline}).$$

The RHI was determined with proprietary software (EndoPAT, Itamar Medical, Israel) and was used as the independent variable to compare against the PWRHI method.

Statistical Analysis

Data was tested for normality using the Shapiro-Wilk test. Means and standard deviations were calculated to describe normally distributed data (e.g., height, weight, heart rate), while medians and interquartile ranges (IQR) were calculated to describe variables that failed normality (e.g., age, systolic and diastolic pressure, time to peak). One-way repeated measures analysis of variance (ANOVA) was performed to compare the temporal change in the AUC after release of circulatory occlusion to baseline. Univariate linear regression analysis and correlation analysis were performed between RHI (independent variable) and PWRHI for each time point using Pearson's test for normally distributed data and Spearman's test for non-parametric data using SigmaPlot software (Systat Software Inc., USA). Bland-Altman plots were constructed to compare the two methods. Significance was set at $p<0.05$.

Results

Participant characteristics, including resting cardiovascular variables, are described in Table 1. The AUC of the occluded arm increased during reactive hyperemia, where the median time to the peak AUC response was 72 seconds (IQR: 62-114s). At this time, the AUC was significantly higher than baseline (0.014au (0.010-0.025au) vs 0.010au (0.005-0.021au), p=<0.001). Compared to baseline, significant increases of the positive AUC in the test arm were observed after 90 sec [0.016au (0.010-0.025au), p < 0.001], 120 seconds [0.017au (0.010-0.025au), p=0.001] and 150 seconds [0.017au (0.010-0.024au), p<0.001].

There were significant positive correlations between RHI and PWRHI at every timepoint analyzed (Table 1); however, the highest correlation was observed at 10 seconds around the peak pulse amplitude in the hyperemic phase (Table 1; Figure 1). This was supported by the Bland-Altman plot, which shows good agreement between methods using the 10 seconds around the peak (Figure 2).

Discussion

The results of this study indicate that the PWRHI method using piezoelectric pulse transducers is a suitable and less expensive alternative for evaluating endothelial function compared to alternatives such as cardiac catheterizations, ultrasound imaging, and EndoPAT. It was found that PWRHI, calculated using a ratio of the AUC at the 10 seconds around the peak amplitude after cuff release to baseline and normalized to the control arm, had a strong correlation and good agreement with the RHI generated by the EndoPAT, a device that has been validated for the measurement of endothelial function.

The EndoPAT generates its RHI score by calculating a ratio of the average pulse wave amplitude during the 60-120 seconds following cuff release to the 3.5 minutes prior to inflation in the test arm and normalizing it to the control arm(Bonetti et al., 2004). The strong correlations between the two methods can be partly explained by the similar time frames utilized and the morphological similarities of the pulse waves generated by the two methods. For example, we used a similar baseline period compared to the EndoPAT algorithm (i.e. 3.5 minute average), and the time of maximal response in the current study overlapped the previously observed time of maximum response of the EndoPAT device (60-120s) Similarly, previous studies have also observed visual similarities between the two pulse waveform types used in this study(Qananwah et al., 2020) suggesting comparability between methods.

Although we observed good agreement between the two methods, the RHI and PWRHI scores were not perfectly correlated. Potential differences could stem from the fact that the inflatable EndoPAT probes measure blood volume in the entire tip of the index finger, and the piezoelectric sensors detect changes in pressure against the ventral surface of the middle fingertip, which is a much smaller surface area. Further, the EndoPAT probes exert a counterpressure (70mmHg) to prevent venous pooling(Kuvin et al., 2003), while the piezoelectric sensors are incapable of doing so.

Limitations

The current study is limited by its small sample size. However, the included study population was heterogenous and included individuals of varied ages, sexes, and ethnicities. Even so, this method should be repeated in larger and more diverse populations to identify any differences associated with age, sex, ethnic group and health status. Our participants were generally healthy, where none declared previously diagnosed cardiovascular disease, and only 3 declared having asthma. Future studies should investigate the ability of the PWRHI method to detect endothelial dysfunction in unhealthy populations. It would be expected that individuals with vascular damage or endothelial dysfunction would have a delayed or blunted hyperemic response, as observed with the EndoPAT. A larger sample size will also allow the determination of threshold values for the detection of endothelial dysfunction using the PWRHI method and the generation of receiver operating characteristic (ROC) curves, which demonstrate the sensitivity and specificity of a methodology for predicting disease risk. For example, Heiss et al.(Heiss et al., 2023) have previously obtained reference intervals from FMD that can be used to predict cardiovascular risk using ROC curves. Further, the repeatability and reliability of PWRHI across day-to-day measurements need to be investigated, as the current study conducted a single trial.

In the present study, testing was conducted in the seated position, and recent work suggests that the natural logarithm of RHI is suppressed in the upright posture(Habib et al., 2022); thus, our RHI values may be underestimated when compared to other studies and/or population averages. Similarly, our methodologies may further underestimate RHI due to the use of forearm occlusion to induce reactive hyperemia rather than upper arm occlusion as per EndoPAT recommendations. For consistency within our lab, forearm occlusion was chosen to replicate a previous lab study that demonstrated a relationship between dobutamine-induced coronary vasodilation and the EndoPAT response(Nardone et al., 2020).

Conclusion

The PWRHI method of evaluating endothelial function can be used instead of the EndoPAT RHI and is advantageous as its reusability and accessibility will allow researchers, clinicians, and their patients to have better access to vascular testing. The devices and the associated software are easy to use and make the analysis of the pulse wave recordings convenient.

Additionally, the simplicity of this method would allow it to be used as an effective demonstration of reactive hyperemia in undergraduate physiology labs that possess the required equipment.

References

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980 Nov;288(5789):373–6.

2. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. American Journal of Physiology-Heart and Circulatory Physiology. 1986 Jun 1;250(6):H1145–9.

3. Frame MDS, Sarelius IH. Endothelial cell dilatory pathways link flow and wall shear stress in an intact arteriolar network. Journal of Applied Physiology. 1996 Nov 1;81(5):2105–14.

4. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature. 1987 Jun;327(6122):524–6.

5. Alexander Y, Osto E, Schmidt-Trucksäss A, Shechter M, Trifunovic D, Duncker DJ, et al. Endothelial function in cardiovascular medicine: a consensus paper of the European Society of Cardiology Working Groups on Atherosclerosis and Vascular Biology, Aorta and Peripheral Vascular Diseases, Coronary Pathophysiology and Microcirculation, and Thrombosis. Cardiovascular Research. 2021 Jan 1;117(1):29–42.

6. Sorensen KE, Celermajer DS, Georgakopoulos D, Hatcher G, Betteridge DJ, Deanfield JE. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. J Clin Invest. 1994 Jan 1;93(1):50–5.

7. Kinlay S, Ganz P. Role of Endothelial Dysfunction in Coronary Artery Disease and Implications for Therapy. The American Journal of Cardiology. 1997 Nov;80(9):11I-16I.

8. Gokce N, Keaney JF, Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk Stratification for Postoperative Cardiovascular Events via Non-invasive Assessment of Endothelial Function: A Prospective Study. Circulation. 2002 Apr 2;105(13):1567–72.

9. Hasdai D, Lerman A. The assessment of endothelial function in the cardiac catheterization laboratory in patients with risk factors for atherosclerotic coronary artery disease. Herz. 1999 Nov;24(7):544–7.

10. Reriani MK, Lerman LO, Lerman A. Endothelial function as a functional expression of cardiovascular risk factors. Biomarkers in Medicine. 2010 Jun;4(3):351–60.

11. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. J Am Coll Cardiol. 2002 Jan;39(2):257–65.

12. Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, et al. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. The American Journal of Cardiology. 1998 Dec;82(12):1535–9.

13. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. American Heart Journal. 2003 Jul;146(1):168–74.

14. Hamburg NM, Benjamin EJ. Assessment of Endothelial Function Using Digital Pulse Amplitude Tonometry. Trends in Cardiovascular Medicine. 2009 Jan;19(1):6–11.

15. Moerland M, Kales AJ, Schrier L, van Dongen MGJ, Bradnock D, Burggraaf J. Evaluation of the EndoPAT as a Tool to Assess Endothelial Function. Int J Vasc Med. 2012;2012:1–8.

16. Bonetti PO, Pumper GM, Higano ST, Holmes DR, Kuvin JT, Lerman A. Non-invasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. Journal of the American College of Cardiology. 2004 Dec;44(11):2137–41.

17. Nohria A, Gerhard-Herman M, Creager MA, Hurley S, Mitra D, Ganz P. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. Journal of Applied Physiology. 2006 Aug;101(2):545–8.

18. Foo JYA, Lim CS. Pulse Transit Time Based on Piezoelectric Technique at the Radial Artery. J Clin Monit Comput. 2006 Jul 10;20(3):185–92.

19. Edgell H, Stickland MK, MacLean JE. A simplified measurement of pulse wave velocity is not inferior to standard measurement in young adults and children. Blood Pressure Monitoring. 2016 Jun;21(3):192–5.

20. Qananwah Q, Dagamseh A, Alquran H, Ibrahim KS, Alodat M, Hayden O. A comparative study of photoplethysmogram and piezoelectric plethysmogram signals. Phys Eng Sci Med. 2020 Dec;43(4):1207–17.

21. Heiss C, Rodriguez-Mateos A, Bapir M, Skene SS, Sies H, Kelm M. Flow-mediated dilation reference values for evaluation of endothelial function and cardiovascular health. Cardiovascular Research. 2023 Mar 17;119(1):283–93.

22. Habib K, Fallah B, Edgell H. Effect of Upright Posture on Endothelial Function in Women and Men. Front Physiol. 2022 Mar 24;13:846229.

23. Nardone M, Miner S, McCarthy M, Ardern CI, Edgell H. Non-invasive Microvascular Indices Reveal Peripheral Vascular Abnormalities in Patients With Suspected Coronary Microvascular Dysfunction. Canadian Journal of Cardiology. 2020 Aug;36(8):1289–97.

Table 1. Anthropometrics and resting cardiovascular variables. Data is presented as mean±SD or median with interquartile ranges.

Parameter (n = 25)	
Age (years)	27 (22- 38)
Height (cm)	169±11
Weight (kg)	78±22
Systolic blood pressure (mmHg)	110 (102- 126)
Diastolic blood pressure (mmHg)	76 (67- 80)
Heart Rate (bpm)	76±11

Table 2. Correlative data between endothelial function as measured by EndoPAT RHI versus the novel PWRHI method.

	10s around peak amplitude ratio	0-30s amplitude ratio	30-60s amplitude ratio	60-90s amplitude ratio	90-120s amplitude ratio	120-150s amplitude ratio	150-180s amplitude ratio
Spearman's r (p-value)	0.67 (p=0.0002)	0.49 (p=0.011)	0.57 (p=0.002)	0.63 (p=0.0007)	0.58 (p=0.002)	0.66 (0.0002)	0.56 (p=0.003)
Coefficient of Determination (R^2)	0.58	0.37	0.45	0.49	0.43	0.47	0.36

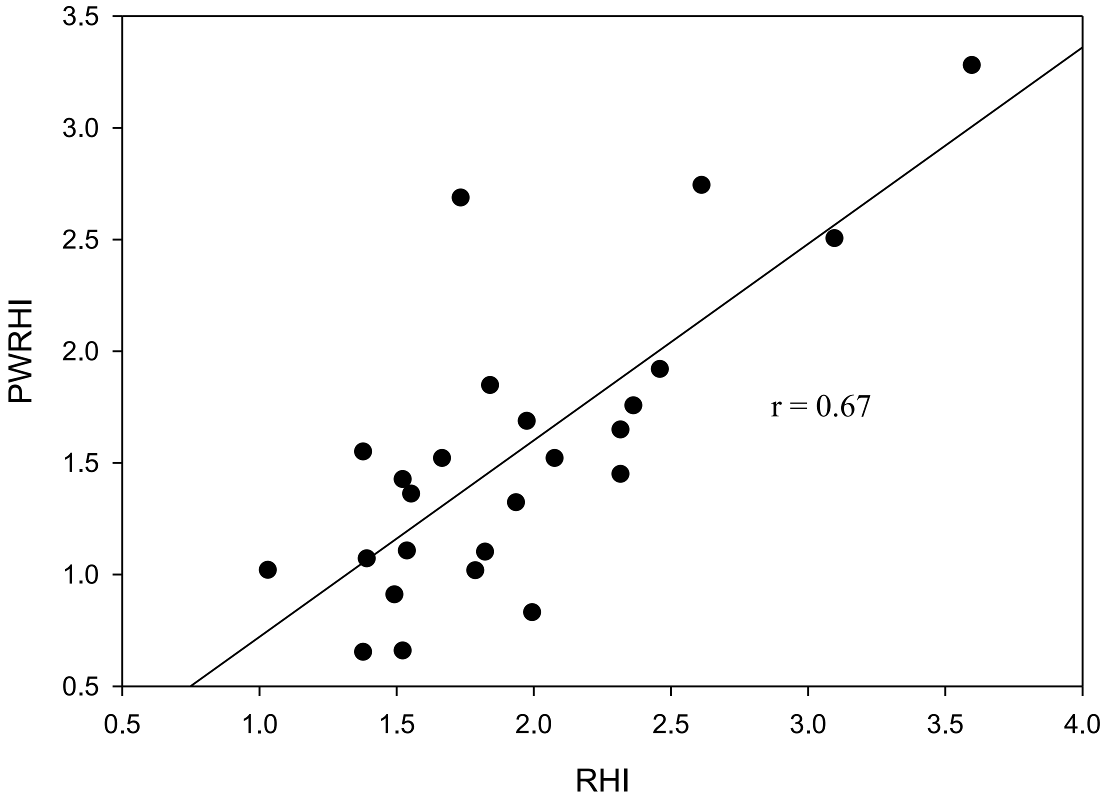


Figure 1. Spearman's R correlation calculated between EndoPAT RHI and PWRHI at 10sec surrounding the peak hyperemic response.

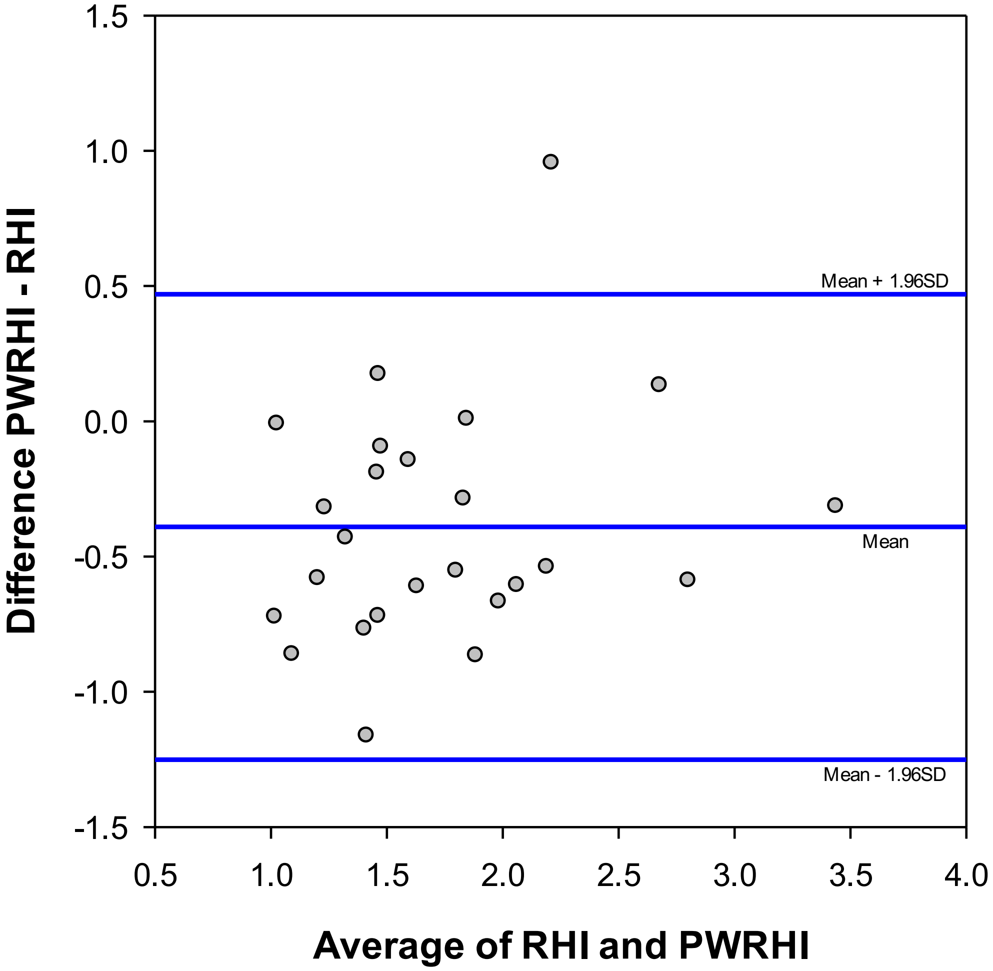
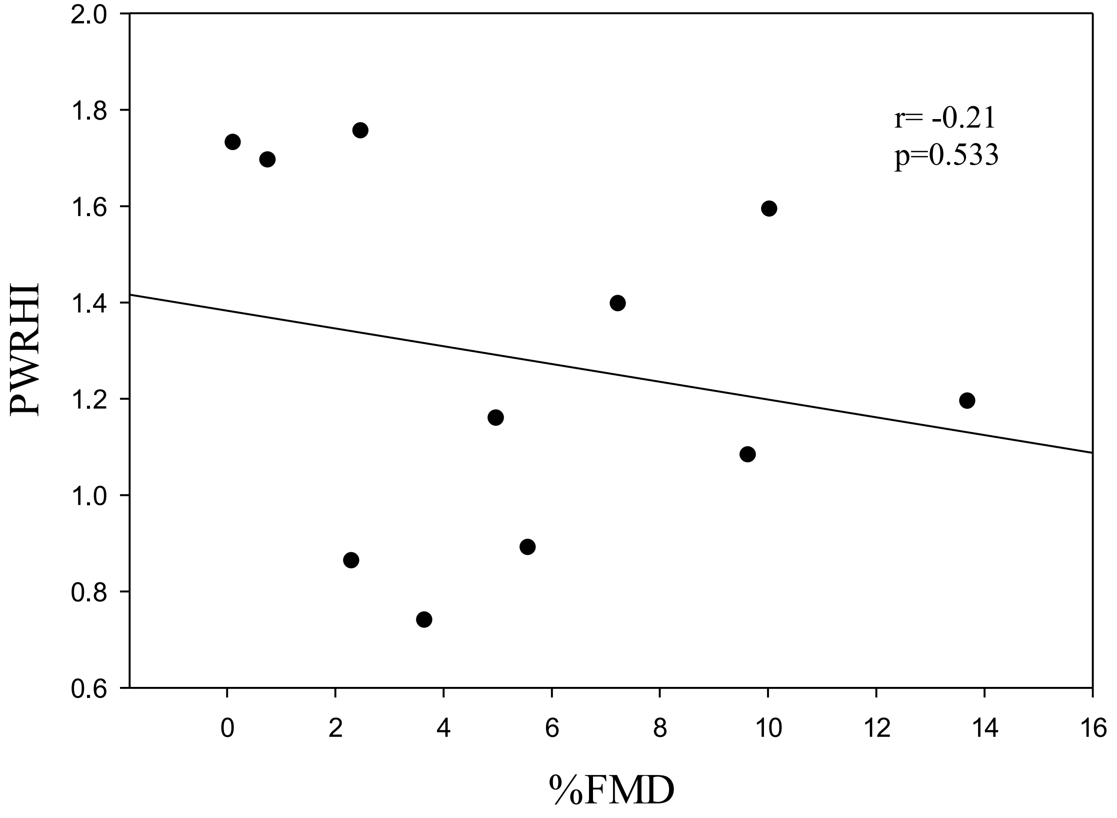


Figure 2. A Bland-Altman plot demonstrating the agreement between RHI and PWRHI at 10sec around peak. The difference between methods was plotted against the average of both methods. Lines represent the upper and lower limit of agreement, a good agreement is found between both methods since most values fall within this range.

Appendix 2



Appendix 2. Correlation between percent increase in Flow Mediated Dilation (FMD) and Pulse Wave Reactive Hyperemia Index (PWRHI).