VASCULAR AND METABOLIC RISK FACTORS, CAROTID ATHEROSCLEROSIS AND VASCULAR COGNITIVE IMPAIRMENT IN A FIRST NATIONS POPULATION

by

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ABSTRACT

The objectives of the thesis were to examine the associations between vascular and metabolic risk factors, carotid atherosclerosis and cognitive function in a Canadian First Nations population. Eligible individuals were ≥18 years and with First Nations status who had undergone cognitive function assessment by the Clock Drawing Test (CDT) and the Trail Making Test Parts A and B. Parts A and B were combined into an executive function score (TMT-exec). Anthropometric, vascular and metabolic risk factors were assessed by interview, clinical examinations and blood tests. Doppler ultrasonography assessed carotid atherosclerosis (carotid stenosis, plaque volume). For the 190 individuals with TMT-exec scores, obese individuals were at a 4-fold increased risk for lowered cognitive performance compared to those who were not obese (odds ratio [OR]: 3.77, 95% confidence interval [CI]: 1.46-9.72). Those having an increased waist circumference also had 5 times the risk compared to those without an increased waist circumference (OR: 5.41, 95% CI: 1.83-15.99). Individuals having the metabolic syndrome were at a 4-fold increased risk compared to those without the metabolic syndrome (OR: 3.67, 95% CI: 1.34-10.07). No other cardiovascular risk factors were associated and no associations were shown for the CDT. For TMT-exec

only, individuals with elevated levels of left (LCS) and total carotid stenosis (TCS) were less likely to demonstrate lowered cognitive performance (LCS, OR: 0.47, 95% CI: 0.24-0.96; TCS, OR: 0.40, 95% CI: 0.20-0.80). In structural equation modeling, for every 1-unit change in the anthropometric factor in kg/m^2, there was a 0.86-fold decrease in the percent of TCS ($p<0.05$). The etiology of VCI is vascular and is affected by non-traditional risk factors such as obesity. The health effects of obesity beyond traditional disease endpoints warrants further study. Mild-moderate levels of carotid stenosis are not detrimental to cognitive functioning and may additionally include acting as a mediator.

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CHAPTER 1: RATIONALE AND STUDY OBJECTIVES

1.1 INTRODUCTION

Cognitive decline and dementia are associated with disability, institutionalization and shorter survival in older individuals. The frequency of cognitive decline and dementia increases with age and therefore as the population ages, the individual and societal costs will also rise(1). In Canada, there are approximately 4 million individuals, or 12.5% of the Canadian population aged 65 years and older(2), making cognitive decline and dementia a major upcoming challenge.

Three major neurological disorders leading to cognitive decline and dementia are stroke, Alzheimer's disease and vascular dementia. Well established modifiable risk factors have been identified for stroke in comparison to Alzheimer's disease and vascular dementia, therefore substantial primary prevention efforts have targeted stroke and its vascular risk factors(3). A neurological entity that is not overtly pathological but is showing mild deterioration and is at risk to progress to one of the three major neurological disorders has been identified as the "brain at risk" model(4). This shift in thinking has been the focus of early primary prevention of cognitive decline and dementia(3,4). Characterizing this neurological entity, whether it is vascular cognitive impairment, vascular cognitive impairment no dementia (Vascular CIND), cognitive impairment no dementia (CIND), or mild cognitive impairment (MCI), and its risk factors has been the focus of much debate in the literature(5). With the intention of identifying risk factors for the early primary prevention of cognitive decline and dementia, we introduce the Trail Making Test Executive Function score (TMT-exec), adapted from previous work(5,6).

New perspectives on the etiology of cognitive decline and dementia has led to the emergence of studies that have examined risk factors in midlife for diseases of cognition in late life, in contrast to earlier studies that focused on late life risk factors(7). The implications of this new way of thinking about the development of cognitive decline and dementia are that the time point at which risk factors are assessed and how risk factors interact across the life course are important to consider. Reviews have shown a consistent relationship between hypertension and cognitive decline and dementia(8), and growing evidence of a relationship between obesity and cognitive decline and dementia(9). The evidence for the relationships of a number of vascular and metabolic risk factors is not yet clear. For this thesis, we examined the relationships between a number of cardiovascular risk factors and cognitive decline. Atherosclerosis, a pathological condition of the blood vessel wall that begins in early adolescence has been implicated as a factor in vascular-sensitive cognitive decline(10), such as executive function(11). We further examined the role of carotid atherosclerosis in association with cognitive performance on a test of executive function, and whether carotid atherosclerosis is a mediating factor between anthropometric risk factors and cognitive function.

The similarities between the cerebral and retinal circulations suggest that evaluating the health of an individual's eye may provide insight to their cognitive health(12). Few studies have examined this concept, and we will contribute to the body of literature by also examining this relationship. If we see a relationship between the two, then the retinal microenvironment may serve as a surrogate to tests of cognitive function.

The First Nations population is an under-studied population. Although they comprise only a small proportion of the total Canadian population, they experience a larger proportion of deleterious risk factors and chronic disease compared to their Canadian counterparts(13). If the

associations between the factors under study in this thesis exist, then given the increased burden of risk factors and disease in the First Nations population, the associations should be detected. Therefore, the First Nations population offered a unique opportunity for study.

1.2 STUDY OBJECTIVES

The study objectives were divided into primary and secondary objectives to guide study feasibility. The primary objectives were used as the basis for the power calculations (see Chapter 4).

1.2.1 Primary Objectives

1) To examine the associations of vascular and metabolic risk factors with cognitive decline among a Canadian First Nations population.

A. The relationships will be examined while accounting for the underlying correlational structure of the vascular and metabolic risk factors under study.

B. The inter-rater reliability of the Clock Drawing Test will be examined.

2) To examine whether carotid atherosclerosis is the mechanism by which metabolic risk factors affect cognitive decline among a Canadian First Nations population.

A. Carotid atherosclerosis will be examined by measures of carotid stenosis (right, left, and total), and plaque volume (right, left, and total).

1.2.2 Secondary Objectives

1) To examine the association of carotid atherosclerosis measured by plaque volume of the right and left carotid arteries and total plaque volume with cognitive decline among a Canadian First Nations population.

2) To examine the association of retinal microvasculature abnormalities with cognitive decline among a Canadian First Nations population.

3) To examine whether carotid atherosclerosis measured as total plaque volume is the mechanism by which metabolic risk factors affect retinal microvasculature abnormalities among a Canadian First Nations population.

1.3 STUDY HYPOTHESES

1) The presence of hypertension, a history of cardiovascular disease, having dyslipidemia, being obese, having the metabolic syndrome, insulin resistance, or diabetes, and an increased duration of diabetes are positively associated with cognitive decline. A gradient of risk according to the severity of vascular and metabolic dysfunction is hypothesized. For vascular risk factors, individuals with a history of cardiovascular disease will have a higher magnitude of risk than those with hypertension. For metabolic risk factors, individuals with an increased duration of diabetes will have a higher magnitude of risk compared to those with diabetes, those with diabetes will have a higher magnitude of risk compared to those with insulin resistance, those with insulin resistance will have a higher magnitude of risk compared to those with the metabolic syndrome, those with the metabolic syndrome will have a higher magnitude of risk

compared to those who are obese, and those who are obese will have a higher magnitude of risk compared to those with dyslipidemia.

2) When adjusting for the positive effect of carotid atherosclerosis measured as right, left, and total carotid stenosis or right, left, and total plaque volume on cognitive decline, the positive association between metabolic risk factors and cognitive decline will be attenuated.

3) Any increased plaque volume is positively associated with cognitive decline.

4) Any retinal microvasculature abnormality is positively associated with cognitive decline and thus may serve as an appropriate surrogate measure to cognitive decline.

5) When adjusting for the positive effect of carotid atherosclerosis measured as total plaque volume on retinal microvasculature abnormalities, the positive association between metabolic risk factors and retinal microvasculature abnormalities will be attenuated.

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CHAPTER 2: LITERATURE REVIEW

2.1 COGNITIVE DECLINE AND DEMENTIA

Individuals with abnormalities of the heart, blood vessels and circulation are at an approximately 2 to 5-fold increased risk for three major deleterious cognitive outcomes including stroke(1), Alzheimer's disease(2), and vascular dementia(3). Stroke increases with age (50-59 years: 177/100,000 vs. 70-79 years: 1,266/100,000)(4), and occurs when a blood clot blocks a blood vessel in the brain, thereby rapidly and dramatically interrupting the supply of blood and oxygen to the brain. This leads to neuronal cell death and loss of cognitive functioning. Behavioural and personality changes, language impairments, and difficulty learning and remembering new information are common cognitive effects of stroke(5). Alzheimer's disease is a progressive, irreversible neurodegenerative disease of the brain that leads to changes in cognitive function including alterations of memory, language, judgment, reasoning, and abstract thinking. It largely effects Canadians over the age of 80 years and represents 65% of all dementias in Canada(6, 7). In contrast to both stroke and Alzheimer's disease, vascular dementia may develop due to a single instance or multiple instances of inadequate blood flow to the brain. Decreased cognitive function is heterogeneous depending on the location of the brain infarct but may include decrements in language, vision and memory. It is characterized by a stepwise progression and may be halted with improved brain blood flow. It is the second most common form of dementia after Alzheimer's disease, predominately effects a broader range of ages than Alzheimer's disease and represents 19% of all dementias in Canada(6, 7). Therefore, the way in

which the blood supply affects cognitive function is complex, particularly for stroke and vascular dementia (Table 1).

A common vascular pathology among neurological disorders, particularly stroke and vascular dementia suggests environmental or lifestyle-related modifiable risk factors. Major modifiable risk factors for ischemic and hemorrhagic stroke are well known(8), and although the contribution of vascular pathology to sporadic Alzheimer's disease has received increased attention, generally established risk factors for Alzheimer's disease are not modifiable(9). Identifying preventable risk factors for vascular dementia has received much attention, though research has been limited due to uncertainty surrounding its diagnosis(10, 11). Risk factors for vascular dementia include age, high blood pressure, heart disease, and diabetes(7). Alzheimer's disease and vascular dementia often co-occur, therefore cerebrovascular disease is clinically heterogeneous(12). Overall, there is a need to identify risk factors associated with early onset decreases in cognitive function since modifiable risk factors present in midlife may be predictive of future neurological disorders(13) and early onset decreases in cognitive function may proceed future neurological disorders(14). In response to this growing awareness, Hachinski (1994) proposed the concept of vascular cognitive impairment(15). Vascular cognitive impairment represents a number of cerebral disease subtypes in which vascular pathology is a central feature(16). The main disease subtype is vascular dementia, and reviewing its origin has been a starting point for alternate ways of conceptualizing early onset decreases in cognitive function.

Evidence of a relationship between cerebral infarction and dementia led to the use of the term, multi-infarct dementia, which implicated thromboembolic events rather than chronic cerebral ischemia as the main etiological factor. Vascular dementia has since replaced the term

multi-infarct dementia. A number of vascular mechanisms and variations of the site and size of brain infarcts in the pathology of vascular dementia led to discordance in its clinical presentation and therefore uncertainty surrounding its diagnosis(17-20). Commonly used diagnostic criteria for vascular dementia include the National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et L'Enseignement en Neurosciences (NINDS-AIREN), the International Classification of Diseases 10th revision (ICD-10), and the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV). The underlying limitation in each of the abovementioned criteria is the analogy with Alzheimer's disease and the clinical feature of memory loss required in the definition of dementia(21, 22). For epidemiology studies, the inclusion of a group of individuals defined by diagnostic criteria in which memory loss is the hallmark feature would result in a study population confined to older ages for which early primary prevention would be least relevant(23), since the initial phase of dementia, as typically found in Alzheimer's disease, is characterized by memory loss(24). Age-related decreases in cognitive function have been hypothesized to be associated with executive function(25), therefore executive function has been the focal point for early primary prevention.

Table 1. Comparison of Three Major Neurological Disorders in Canada

Factors	Stroke	Alzheimer's disease	Vascular dementia
Burden	177/100,000, 50-59 years	65%	19%
Characteristic	Vascular	Degenerative	Vascular
Age	Increases with age	Elderly	Middle age to elderly
Deficits	Heterogeneous	Heterogeneous	Heterogeneous
Brain location	Depends on brain infarct	Multiple domains	Depends on brain infarct
Risk factors	Well established, many	Age, genes	Age, vascular

References are noted in the text.

2.1.1 Executive Function

Executive functions are higher-order cognitive abilities mediated primarily by the dorsolateral prefrontal cortex, otherwise known as the frontal lobe, and subcortical structures

linked to this region, together, referred to as the frontal-subcortical circuit. Syndromes associated with executive function include frontotemporal dementias and focal frontal lobe disorders. The frontal lobe is the largest lobe of the human brain, comprising approximately one-third of the total cortical volume. The frontal lobe is the focal point for the integration of information from the environment, the internal milieu of the body and the emotional state of the individual. The frontal lobe is divided into specialized regions, though it is common for more than one area to be compromised. Abstraction, insight, planning, and judgment are compromised in individuals with executive function deficits(26). Although there is no gold standard for assessing executive function, there are a number of measures for executive function, though not all test the same dimension. Formal tests include the California Card Sorting Test, Category Test, Concept Generation Test, Porteus Mazes, Raven's Progressive Matrices, Stroop Color-Word Interference Test, Tinker Toy Test, Tower of Hanoi, Tower of London, and the Wisconsin Card Sorting Test. Screening tests include the Behavioural Dyscontrol Scale, CLOX: An Executive Clock Drawing Task, Controlled Word Association Test, Design Fluency, Executive Interview (EXIT 25), Go/No-Go, and the Trail Making Test Part B. In contrast to the widely used screening test for dementia, the Mini-Mental State Examination (MMSE), tests of executive function can detect early and mild changes in cognitive function(27).

2.1.2 Epidemiology of Cognitive Decline and Dementia

Literature review of the descriptive epidemiology for cognitive decline and dementia has been a starting point for examining prevalence, risk factor trends and generating hypotheses. The Canadian Study of Health and Aging, a large study with cross-sectional and longitudinal components representative of both institutionalized and community-dwelling elderly individuals

found a prevalence of vascular cognitive impairment of 19.2%. The proportion of individuals reporting stroke, hypertension and diabetes was increased approximately 2-fold among individuals classified as having vascular cognitive impairment compared to the other subgroups including nonvascular cognitive impairment not demented, probable Alzheimer's disease and no cognitive loss(28). With respect to prognosis measured by time to institutionalization, individuals with vascular cognitive impairment had a statistically significant decreased time to institutionalization (e.g. less likely to be institutionalized) compared to individuals with probable Alzheimer's disease ($p<0.03$), a trend that was reversed in comparison to individuals without vascular cognitive impairment ($p<0.001$)(29). The Consortium to Investigate Vascular Impairment of Cognition, a Canadian multi-centred clinic-based study with cross-sectional and longitudinal components found a prevalence of vascular cognitive impairment of 24%. Compared to the overall study population, individuals with vascular cognitive impairment were more likely to have hypertension, lipids disorders and diabetes(30). The Canadian Cohort Study of Cognitive Impairment and Related Dementias, a multi-centred study of university-based ambulatory units with cross-sectional and longitudinal components found a prevalence of vascular cognitive impairment of 18.1%. Those classified as having cognitive impairment no dementia (CIND) performed at a lower level on the Trail Making Test Part B compared to those without cognitive impairment (CI) (CIND: Mean: 135.9, Standard deviation [SD]: 77.2 vs. No CI: Mean: 99.2, SD: 58.2 seconds)(31). A major limitation to the descriptive studies is the lack of diagnostic criteria. There is no generally accepted cognitive test and no standard criteria for defining vascular cognitive impairment(32). Previous studies have grouped together three diagnostic subtypes to define vascular cognitive impairment including vascular cognitive impairment that does not meet the definition of dementia (Vascular CIND e.g. cognitive

impairment no dementia), combined or mixed dementia, and vascular dementia(28-30). Differences in demographic characteristics and performance on neuropsychological tests for these diagnostic subgroups(33) suggest that a homogeneous definition is needed for future epidemiological studies. The recognition that lower levels of performance for specific cognitive domains, such as executive function, may serve as early markers of cognitive decline and dementia(34) led a number of studies to measure cognitive function by performance on standard neuropsychological tests.

A number of vascular and metabolic risk factors have been identified in association with cognitive decline and dementia. In a study examining generalized atherosclerosis measured as peripheral arterial disease and the development of Alzheimer's disease with or without vascular dementia, a 2-fold increased risk was shown when adjusted for age at baseline, ethnicity, education, income, apolipoprotein e-4 allele, and baseline scores on the MMSE (odds ratio [OR]: 2.4, 95% confidence interval [CI]: 1.4-4.2)(35). This is one of the few studies that have examined the contribution of vascular disease to cognitive decline and dementia. Hypertension, a major vascular risk factor for cerebrovascular disease has been extensively studied in relation to cognition and dementia and the results have been compiled in a review paper. Consistent results for midlife blood pressure, particularly systolic blood pressure and lowered cognition assessed by the MMSE or standard neuropsychological tests have been shown(36). Hypertension may stimulate the proliferation of smooth muscle cells in the media layer of the artery, thereby promoting the development of atherosclerosis(37).

The Three-City Study, a population-based longitudinal study of adults aged 65 years and older showed in the cross-sectional component that total cholesterol ≥6.2 mmol/L was associated with a 1.8-fold increased risk for dementia (OR: 1.76, 95% CI: 1.05-2.96) compared

to those with <6.2 mmol/L total cholesterol when adjusted for age, sex, education level, study centre, hypertension, low and high density lipoproteins, body mass index, daily alcohol consumption, smoking status, depressive symptoms, psychotropic drug intake, history of vascular disease, and self-perceived health(38). Women enrolled in the Heart Estrogen/Progestin Replacement Study trial were used to examine the associations between lipoproteins and cognitive impairment assessed by the MMSE. Using the three lowest quartiles as the referent group, individuals in the fourth quartile for total cholesterol (TC) and low density lipoprotein (LDL) were associated with an increased risk for cognitive impairment when adjusted for age, educational level, treatment group, diabetes status, health status, coronary bypass surgery, and aspirin use (TC, OR: 1.77, 95% CI: 1.06-2.97; LDL, OR: 1.76, 95% CI: 1.04-2.97)(39). The Leiden 85-Plus Study, a population-based study of adults aged 85 years and older living in the Netherlands showed in their cross-sectional study, that individuals classified in the lowest tertile of high density lipoprotein were associated with a 2.6-fold increased risk for cognitive impairment assessed by the MMSE when adjusted for level of education (OR: 2.6, 95% CI: 1.0-6.6), compared to those in the highest tertile. For these results, those with cardiovascular disease and stroke were excluded. Using the same model and exclusions, individuals in the lowest tertile of high density lipoprotein had a 3.7-fold increased risk for dementia (OR: 3.7, 95% CI: 1.3-10.1)(40). Overall, there appears to be a role for lipids and lipoproteins in cognitive decline and dementia. The underlying mechanism may be indirect via atherosclerosis(41).

Anthropometric risk factors identified include body mass index, waist circumference and waist-to-hip ratio. An international cross-sectional study showed associations between obesity measured by body mass index and tests of attention including choice reaction time, Trail Making Test Part A and span of visual memory tests, and tests of executive function including a

modified version of the Stroop test and the Austin Maze test, however aside from age, additional confounders were not considered in their multivariate analysis(42). The Framingham Heart Study and the Framingham Offspring Cohort Study were two prospective cohort studies that examined changes in cognitive function using standard neuropsychological tests. The original study found that among men only, performance decreased for those who were obese measured by body mass index on tests of visual reproductions (-0.23, $p<0.05$) and the digit span backwards test (-0.31, $p<0.01$) when adjusted for age, cardiovascular risk factors including obesity, hypertension, total cholesterol, alcohol consumption, and cigarettes per day, education, occupation, and native language as English. These were two of the eight subtests taken from the Kaplan-Albert neuropsychological test battery that assesses a number of cognitive skills simultaneously, including executive function(43). For the Offspring Cohort Study, performance decreased for those who were obese, defined as individuals classified in the highest quartile of waist-to-hip ratio compared to those in the first three quartiles combined on tests of visual reproductions (immediate: -0.154, $p<0.005$; delayed: -0.157, $p<0.004$) and the Trail Making Test Part B (-0.156, $p<0.004$), an effect which was not shown for the remaining six tests or those who were obese defined by body mass index(44). These results were unadjusted for any confounders. Previous studies that have examined midlife obesity measured by body mass index(45-47) or waist circumference(48) and risk of dementia have identified 2- to 5-fold increased risks. Results were adjusted for a number of demographic and cardiovascular risk factors. The relationship with obesity is complex, with those who are underweight having an approximately 1.5-fold increased risk for dementia, including Alzheimer's disease and vascular dementia (OR: 1.36, 95% CI: 1.07-1.73) and those who are classified as obese, having a 2-fold increased risk for Alzheimer's disease (OR: 1.80, 95% CI: 1.00-3.29). This pattern of results is

suggestive of a U-shaped relationship(49). The role of inflammatory factors such as adipocytokines or adipokines produced by abdominal fat have been suggested as mechanisms by which excess adiposity exerts its effect on cognitive function(50).

The Sacramento Area Latino Study of Aging, a longitudinal cohort study of individuals aged 60 years of age and older and self-designated as Latino who were followed for three years, showed in multivariable analysis that individuals with the metabolic syndrome had a decrease in cognition [0.39 times, p=0.04] measured by the Modified Mini-Mental State Exam (3MS) when adjusted for age, sex, education, place of birth, depression, history of stroke or myocardial infarction, smoking, and alcohol. At baseline, approximately 44% of the population was classified as having the metabolic syndrome as defined by the National Cholesterol Education Program (NCEP) criteria(51). In the Health, Aging and Body Composition Study, a five year prospective study of community individuals aged 70 to 79 years, a 1.2-fold increased risk for cognitive impairment defined as a change of five or more points on the 3MS was shown in multivariable analysis adjusted for age, education, race, baseline cognitive score, depression, alcohol use, stroke, and statin use (OR: 1.20, 95% CI: 1.02-1.41). At baseline, the prevalence of the metabolic syndrome was 56% according to the NCEP criteria(52). Whether the individual components of the metabolic syndrome act individually or jointly is not clear. The metabolic syndrome most likely contributes to cognitive decline through its effect on atherosclerosis and the inflammatory response(53). With the high prevalence of the metabolic syndrome as determined from existing studies, a consensus on the association with lowered cognitive function would have a substantial public health impact(54).

The InCHIANTI Study, a prospective population-based study of individuals aged 22 to 93 years without diabetes showed that among those less than 65 years of age, increasing levels

of insulin resistance as determined by the homeostasis model of assessment (HOMA) was associated with increased times to completion for the Trail Making Test Part B (TMT-B) [22.9 times, $p<0.001$], and the Trail Making Test Part A (TMT-A) [9.8 times, $p<0.001$]. For those 65 years of age and older, increasing levels of insulin resistance as determined by HOMA was associated with increased times to completion for TMT-B and TMT-A [TMT-B: 28.5, $p<0.001$; TMT-A: 10.9, $p=0.023$]. All results were multivariable including adjustment for age, sex, education, high-density lipoprotein, triglycerides, total insulin-like growth factor, hypertension, drug intake, physical activity, body mass index, waist-to-hip ratio, depression, and history of stroke(55). The Honolulu-Asia Aging Study, a population-based longitudinal study of Japanese-American men showed that low and high levels of insulin were associated with an increased risk of dementia (Low, OR: 1.54, 95% CI: 1.11-2.11; High, OR: 1.54, 95% CI: 1.05-2.26) when adjusted for age, education, fasting glucose, apolipoprotein e-4 allele, midlife blood pressure, cholesterol, smoking status, alcohol intake, % weight change from mid- to late-life, late-life depression, ankle brachial index, history of type 2 diabetes, stroke, and coronary heart disease(56). The Atherosclerosis Risk in Communities study, a six year longitudinal study of middle-aged adults aged 45 to 64 years showed that hyperinsulinemia, determined as ≥75th percentile and measured by HOMA, was associated with decreased performance on the delayed word recall (DWR) test at follow-up [0.0245, $p<0.05$] and the digit symbol subtest (DSS) and the word fluency (WF) tests at baseline [DSS: 0.0218, $p<0.05$; WF: 0.0471, $p<0.05$], compared to those without hyperinsulinemia. Results were adjusted for age, sex, race, marital status, education level, smoking status, alcohol use, depression score, history of hypertension, and history of hyperlipidemia. Individuals with dementia, type 2 diabetes and stroke were also excluded(57). Insulin resistance frequently precedes type 2 diabetes, and the underlying

mechanism linking them to decreased cognitive function may be abnormalities in endothelial-dependent vasodilation resulting in functional hypoglycemia. The hippocampus is more susceptible to damage due to hypoglycemia, therefore cognitive deficits are typically related to recent memory(58).

There have been a number of reviews on the relationship between diabetes and cognitive decline and dementia, with a general consensus that there is an association(59-61). One systematic review that confined its study selection criteria to 25 well-designed prospective cohort studies showed that diabetes was associated with a 1.7-fold increased risk for cognitive decline as assessed by the digit symbol substitution test (OR: 1.7, 95% CI: 1.3-2.3), a 1.2-fold increased risk for cognitive impairment as assessed by the MMSE (OR: 1.2, 95% CI: 1.1-1.4), and a 1.6-fold increased risk for dementia (OR: 1.6, 95% CI: 1.4-1.8) in pooled analysis. Study differences that prevailed included: (1) the definition of clinically meaningful decline in cognitive function, (2) identification of people with and without diabetes, (3) age groups that were studied, (4) the cognitive assessment tools used, (5) degree of adjustment for confounders, (6) degree of cognitive impairment permitted in studied participants at baseline, and (7) the completeness of follow-up, that ranged from 73-92%. Additionally, use of global measures of cognitive function such as the MMSE may underestimate the effect of diabetes on specific cognitive domains(60). Since diabetes is commonly under-diagnosed by approximately 30%, a number of individuals may be classified as not having diabetes thereby attenuating associations in longitudinal studies(59). Current studies should include fasting blood glucose tests to establish the presence of undiagnosed diabetes. The absence of information on confounders such as alcohol use and smoking, the absence of information on depression which may also affect cognitive functioning, and the learning effect, a phenomenon where some cognitive tests appear

more vulnerable to decline than others due to increased performance on some tests from repeated administration have been identified as study limitations(61). In spite of a number of study differences and limitations, the results for an association between diabetes and cognitive decline and dementia have been robust, supporting an association. The Atherosclerosis Risk in Communities Study involved cross-sectional and longitudinal components to examine the baseline correlates of cognitive function and change in cognitive function over a six year time period among individuals aged 45-64 years from four U.S. communities. The neuropsychological tests used to measure cognitive function included the DWR test, the DSS and the WF test. Among 10,963 individuals at follow-up, a statistically significant 3.4 and 1.1 decrease in adjusted mean change in cognition was observed for those reporting diabetes compared to those who did not for the DSS and WF tests respectively, an effect not shown for those having hyerlipidemia, hypertension, increased intimal-media thickness, reporting current or former smoking, and use of non-steroidal anti-inflammatory drugs. When stratified by age, the effect for diabetes was attenuated for those less than 58 years of age and increased for those more than 58 years of age for both the DSS and WF, however all remained statistically significant(62). The results indicate the reduction or improved control of diabetes as modifiable risk factors.

The role of carotid atherosclerosis as a preventative risk factor for vascular cognitive impairment has been extensively studied due to its thromboembolic ability, though a number of study limitations exist which has prevented an overall consensus on the relationship to date. A review of 14 epidemiological studies published between 1996 and 2000 on the role of carotid stenosis for vascular cognitive impairment could not provide a definite conclusion regarding the association due to a number of study design limitations including the lack of the use of an

appropriate control group, the absence of information on the cutpoint used to define the degree of carotid stenosis, and the use of symptomatic individuals undergoing carotid endarterectomy(63). In another systematic review of 18 epidemiological studies published between 1980 and 1999 on the role of carotid artery disease in cognitive disorders, results indicated that 14 studies supported an association between carotid artery disease and lowered cognition however a number of study limitations were identified. Methodological limitations included a lack of a sampling scheme or too few studies that described if the study population was consecutively sampled, lack of comprehensive assessment of risk factors, and lack of the exclusion of patients with concomitant psychiatric and/or neurological diseases that may also effect cognitive functioning(64).

A recent study found an approximately 4-fold statistically significant increased risk for combined right, left, and bilateral carotid stenosis of ≥35% associated with poor performance on tests of sustained attention (TMT-A, OR: 3.9, 95% CI: 1.9-8.1), and executive function (TMT-B, OR: 4.2, 95% CI: 1.9-9.1), when adjusted for age, sex and years of education(65). In a study that created five cognitive domain scores including: (1) global cognitive functioning, (2) language, (3) visual-spatial ability, (4) memory, and (5) attention-executive-psychomotor functions, an increase in intima-media thickness was associated with poorer performance in the attention-executive-psychomotor domain [0.23 times, $p<0.05$] in multivariate models when adjusted for age, education, sex, cardiovascular risk factors including hypertension, hypercholesterolemia, diabetes, tobacco use, and current systolic blood pressure, an effect not shown for the four other cognitive domains. The cognitive tests that comprised the attention-executive-psychomotor domain included: TMT-A, TMT-B, Stroop Test, WF test, letter search time to completion and errors, digit symbol subtest, the digit span subtest, and the Grooved

Pegboard Dominant Hand test(66). In the Rotterdam Study, a single centre prospective longitudinal study of adults aged 55 years and older, peripheral arterial disease, the presence of plaques, wall thickness, and a composite atherosclerosis score were examined in association with all dementia (ALL) and its subtypes, Alzheimer's disease (AD) and vascular dementia (VaD). The results here refer to the cross-sectional component of 284 with dementia and 1,698 without dementia. Increased risks were observed for peripheral arterial and ALL (OR: 1.5, 95% CI: 1.1-2.0), and VaD (OR: 2.5, 95% CI: 1.3-4.8); for the presence of plaques and AD (OR: 1.8, 95% CI: 1.2-2.7), VaD (OR: 3.2, 95% CI: 1.6-6.8), and ALL (OR: 1.9, 1.3-2.7); and wall thickness and VaD (OR: 1.9, 95% CI: 1.3-2.8) and ALL (OR: 1.3, 95% CI: 1.1-1.6). For the atherosclerosis score, the highest risk was shown for those with all three characteristics and VaD (OR: 9.5, 95% CI: 3.0-30.0). Results were adjusted for age and sex only. Overall, when increased risks were shown, they were the highest among individuals with more advanced atherosclerosis disease and cognitive decline of a vascular origin(67).

Inconsistent results across studies may be due to the use of different measures of carotid atherosclerosis, which may reflect different disease endpoints or stages of atherosclerosis(68, 69). In spite of a number of anthropometric(70-74), vascular(75-78) and metabolic(76, 79-81) risk factors associated with carotid atherosclerosis, previous studies have not included these risk factors as confounders in their multivariable analysis(65-67). The Cardiovascular Health Study is the only study to date that had detailed information on cardiovascular risk factors, carotid atherosclerosis and cognitive impairment. In the cross-sectional component, left sided carotid stenosis of ≥75% was associated with a 6.7-fold increased risk for cognitive impairment determined by the MMSE (OR: 6.7, 95% CI: 2.4-18.0), when adjusted for contralateral stenosis, demographic factors and vascular risk factors including hypertension or use of antihypertensive

medications, low density cholesterol, high density lipoprotein, the presence of diabetes, history of coronary artery disease or congestive heart failure, and current smoking(82).

Risk factors commonly not assessed but shown to affect cognitive functioning include exercise(83), alcohol(84) and smoking(85). Based on the potential for compromised cognitive functioning, individuals with traumatic brain injury(86), depression(87) and psychiatric(88) conditions should be excluded from studies. Lack of detailed assessment is a contributing factor, which in turn, affects the internal validity of previous studies.

Few studies of carotid atherosclerosis have been conducted among the Aboriginal population. Among a young population of 168 Oji-Cree residents in central Canada (mean age: 38.2 ± 0.49 years), multivariable linear regression models using normalized variable distributions found that intima-media thickness (IMT), an early stage measure of carotid atherosclerosis which may reflect medial wall hyperplasia or hypertrophy, was associated with hypertension (0.083 ± 0.029, p=0.004), an effect not shown for total plaque area or volume. Total plaque area, a more advanced stage of atherosclerosis than IMT was associated with current smoking (0.584 ± 0.221, p=0.009) and total cholesterol (0.194 ± 0.076, p=0.0012), effects which were not shown for IMT or total plaque volume. Total plaque volume, an alternate measure of carotid atherosclerosis which may also reflect a more advanced stage of atherosclerosis than IMT was associated with individuals classified as having diabetes (0.710 ± 0.221, p<0.0001), not shown for IMT or total plaque area(69). Only one additional study examined the relationship between risk factors and carotid atherosclerosis among Aboriginal people(89). This study found that increasing age, glycosylated hemoglobin levels, hypertension, and reporting current and former smoking were associated with increased levels of intimal

medial thickness. A summary of confounders identified from the literature is shown below (Table 2).

Table 2. Summary of Confounders From Literature Review I

Covariates Identified from the Literature									
	Study Covariates								
Thesis Covariates	Hypertension	Vascular Comorbidities	Elevated Lipids	Obesity	Metabolic Syndrome	Insulin/Insulin Resistance	Diabetes	Smoking	Other
Vascular									
Hypertension[1]	n/a	√	-	√	-	-	-	√	alcohol, occupation, depression, psychotropic drugs, AB index
Hx Pcvd[2]	-	n/a	-	-	-	-	-	-	ethnicity, education, income, ApoE e4 allele, MMSE
Metabolic									
Dyslipidemia[3]	√	√	√	√	-	-	√	√	education, HRT, aspirin use, alcohol, depressive symptoms, psychotropic drugs, self-perceived health
Obesity/BMI[4]	√	√	√	n/a	-	-	√	√	education, ethnicity, marital status, alcohol, occupation, native language, SES
MetS[5]	-	√	-	-	n/a	-	-	√	education, ethnicity, depression, alcohol, statin use
IR[6]	√	√	√	√	-	n/a	√	√	ethnicity, marital status, education, alcohol, depression score, drug intake, physical activity, waist-hip ratio, ApoE e4 allele, AB index, occupation
Diabetes[7]	√	√					n/a	√	education, depression, visual impairment, alcohol, ApoE e4 allele
Carotid measures									
CAD[8]	√		√				√	√	education, contralateral stenosis

Age and sex were considered in most studies as confounders, therefore are not listed here.

[1] Midlife blood pressure (Qui et al., 2005).

[2] Peripheral arterial disease (Newman et al., 2005).

[3] Total cholesterol (Dufouil et al., 2005), and LDL (Yaffe et al., 2002); HDL (Exel et al., 2002).

[4] Source (Whitmer et al., 2008; Whitmer et al., 2007; Elias et al., 2005; Rosengren et al., 2005; Whitmer et al., 2005).

[5] Source (Yaffe et al., 2007; Yaffe et al., 2004).

[6] Source (Young et al., 2006; Abbatecola et al., 2004; Peila et al., 2004).

[7] Source (Cukierman et al., 2005).

[8] Source (Haley et al., 2007; Johnston et al., 2004; Mathiensen et al., 2004).

AB index, ankle brachial index; MMSE, Mini Mental State Exam; SES, socioeconomic status; CAD, carotid atherosclerosis disease.

2.1.3 Summary of Literature Review

In summary, whether risk factors alone, carotid atherosclerosis or a combination is etiologically relevant for vascular cognitive impairment is not known, and less is known of the impact among the Aboriginal population. Differences in the assessment of risk factors, carotid atherosclerosis and cognitive function have made it difficult to make direct comparisons across studies. Methodological differences including different study populations, when risk factors have been assessed such as in midlife or late life and lack of adjustment for confounding factors have also limited direct comparisons across studies. Reviews have been conducted for hypertension and diabetes, with the results indicating an overall trend for an increased risk of cognitive decline and dementia. The associations for the remaining risk factors are not yet clear. Review studies on carotid stenosis have also been conducted, with the association not clear due to too many methodological limitations. There have been no population-based studies that have investigated the relationships between risk factors, plaque volume and cognitive function. This thesis will examine vascular and metabolic risk factors, carotid atherosclerosis and cognitive decline among a Canadian First Nations population. This study will contribute to the growing body of literature on cognitive decline and dementia. This study will use a population-based design, with detailed information on a number of risk factors. Careful statistical analysis will be performed to control for confounding variables. Application of a structural equation modeling approach will also be used in this thesis which will attempt to examine more carefully the interrelationships between vascular and metabolic risk factors, carotid atherosclerosis and cognitive decline. This will be the first study to examine plaque volume in relation to risk factors and outcomes. This study will be one of the few population-based epidemiology studies with detailed information on a number of risk factors that will focus on executive function.

2.2 VASCULAR AND METABOLIC RISK FACTORS

The rationale for investigating vascular and metabolic risk factors in relation to cognitive decline and dementia are multi-fold. First, over 50% of individuals who have experienced a stroke will have some degree of cognitive impairment and one-third of those individuals will develop dementia. Second, the risk factors that increase an individual's risk for cerebrovascular disease are the same as for cognitive impairment. Third, autopsy studies indicate vascular pathology in approximately one-third of dementia cases(90). Clearly, vascular and metabolic risk factors have a role in cognitive decline and dementia. Current available guidelines for the treatment of hypertension, cholesterol and diabetes, and recommendations to maintain a healthy body weight in response to the growing obesity epidemic offer methods for primary prevention(91). Identifying associations between vascular and metabolic risk factors and decreases in cognitive function, such as executive function, would permit early primary prevention.

2.3 ATHEROSCLEROSIS AND THE CAROTID ARTERIES

Atherosclerosis is a systemic vascular disease of the large arteries that develops through an insidious process, is initiated during early adolescence and requires prolonged exposure to predisposing factors, and is largely responsible for heart attacks and stroke. A normal artery consists of three layers: an innermost layer called the intima, a middle layer called the media, and an outer layer called the adventitia. At the lumen, a single layer of endothelial cells reside upon a basement membrane and internal elastic lamina. These endothelial cells regulate the functions of the arterial wall including thrombosis and vascular tone. The media consists of smooth muscle cells in an extracellular matrix. Separating the media from the adventitia is the

external elastic lamina. The adventitia consists of elastin, smooth muscle cells, fibroblasts, and collagen(92). Atherosclerosis can be viewed as a response to injury, with the accumulation of lipids as the prominent injurious event. Early atherosclerotic lesions are characterized by the accumulation of foam cells, which are macrophages with a cholesterol component. In abundance, these fatty streaks become the precursors of more advanced lesions. Developing lesions are characterized by a fibrous deposit, a lipid-rich necrotic particle in the media layer that begins to grow outwards towards the lumen. Calcification, ulceration at the luminal surface and haemorrhaging ensues. Advanced lesions are sufficient in size to obstruct blood flow. Rupture or erosion of an advanced lesion results in a thrombus or blood clot that in turn may cause an acute occlusion leading to a heart attack or stroke. A thromboembolism from an advanced lesion to the cerebral circulation results in ischemic stroke(93).

The degree of atherosclerosis can be determined in a number of different ways including assessment of stenosis, intima-media thickness (IMT), plaque area, and plaque volume. Stenosis, is the degree of narrowing in the artery(8). Severe stenosis is defined as narrowing of 70-99% of the lumen diameter and moderate stenosis as 50-69%, with 100% indicating complete artery occlusion. The implications for moderate and severe stenosis for carotid endarterectomy to prevent future stroke among symptomatic individuals(94) has precluded the study of stenosis for research purposes of etiology, with a majority of early studies focused on IMT. The usefulness of IMT and plaque area is their ability to identify asymptomatic individuals who may go on to develop cardiovascular disease independent of traditional risk factors, and thus identify individuals who would benefit from medical intervention. Increased IMT may be related to intima or media hypertrophy or both, and may be an adaptive response to changes in blood flow, wall tension or lumen diameter. IMT is not synonymous with

atherosclerosis and may occur in the absence of plaque(95). IMT is approximately 97.5% media and 2.5% intima, whereas atherosclerosis is a focal phenomenon that is confined to the intima. IMT assumes that atherosclerosis is distributed evenly along the length of the artery wall, which it is not, therefore plaque area emerged as a novel marker of cardiovascular disease given its good predictive ability. The literature on plaque volume as a novel marker is still developing(96). Different measures of atherosclerosis measure different stages of the disease and are therefore associated with risk factors differently. It is the association between these measures of atherosclerosis and traditional cardiovascular risk factors that would propel one of IMT, plaque area, or plaque volume towards the forefront in terms of identifying who may benefit from medical intervention. Early stages of atherosclerosis such as IMT are associated with hypertension, whereas more advanced stages of atherosclerosis such as plaque area and volume are associated with increasing vascular and metabolic dysfunction. For plaque area, cholesterol was strongly associated, while for plaque volume, the presence of diabetes was strongly associated. These relationships were specific for each measure of atherosclerosis, therefore it is not yet clear which measure of atherosclerosis is ideal(69)

The carotid arteries are common sites involved in atherosclerosis. The carotid arteries on each side of the neck (e.g. right and left sides) carry blood from the heart to various areas of the brain. Extending from the neck to the brain region are the right common carotid and the left common carotid arteries. At about the level of the thyroid, the common carotid arteries divide into internal and external segments, though are distally connected through the carotid ophthalmic triangle. The internal segments ascend into the brain region and divide into the anterior and middle cerebral arteries, at about the level of the optic nerve. The anterior cerebral arteries provide a majority of the blood supply to the forebrain, whereas the middle cerebral

arteries provide the blood supply to the frontal, temporal and parietal lobes. External segments provide the blood supply to the areas of the thyroid, face and eyes. The low level of redundancy to a given brain region is one factor that produces focal neurological dysfunction when blood flow is interrupted. The circle of Willis and the ophthalmic triangle offer two sources of collateral blood flow when there is an occlusion in the internal carotid artery, which is the source of blood supply to the frontal lobe(97). Brain damage due to the interruption of blood flow depends not only on the duration of the interruption but also on the capacity of the cerebral circulation to compensate(98). The destruction of brain tissue leading to cognitive decline can be attributed to small or large cerebral infarcts that block the blood supply to the brain(99). Silent infarcts (e.g. without clinical symptoms) are also implicated in the etiology of cognitive decline suggesting a role for small platelet aggregates or cholesterol microemboli shed from atherosclerotic lesions in the carotid arteries(100).

2.4 THE CANADIAN FIRST NATIONS POPULATION

The Aboriginal population is considered a special population due to their historical position within Canada. According to the 2006 Canadian census, approximately one million Canadians or 3.8% of the total population identified themselves as having an Aboriginal identity. The largest proportion of the total Aboriginal population is found in Nunavut (85%), followed by the Northwest Territories (50.3%) and the Yukon Territory (25.1%), Manitoba (15.5%) and Saskatchewan (14.9%), Alberta (5.8%) and British Columbia (4.8%), and Central and Eastern Canada (1.9%). First Nation people comprise approximately 60% of the total Aboriginal population(101). Reasons for First Nation people having a disproportionate burden of chronic disease despite having a younger population are not entirely clear, although may be

due in part to the complex effects of discrimination, poverty and social dislocation including the multigenerational effects of the residential school system. There is an increased proportion of First Nation (FN) people less than 55 years of age compared to the Canadian (CA) population (FN: 91.5% vs. CA: 78.6%), and an examination of the proportion of First Nation people less than 30 years of age indicates an age structure resembling a developing country (FN: 61.1% vs. CA: 38.8%)(102). First Nation people have an approximately two-fold increased age-standardized mortality rate of stroke compared to the rest of Canada, a trend that is less marked for acute myocardial infarction (AMI) (Stroke, FN: 71.5/100 000 vs. CA: 34.2/100 000; AMI, FN: 72.7/100 000 vs. CA: 52.1/100 000), and circulatory diseases (FN: 240/100 000 vs. CA:220/100 000)(103). Mortality differences, used as a health indicator in the absence of incidence data may in part be explained by an increased proportion of on-reserve First Nation people reported to have strong risk factors for stroke, such as diabetes (FN: 11.5% vs. CA: 4.3%)(102).

Few studies have been conducted among the First Nation people due to the absence of high quality health statistics data(104). There have been few analytic epidemiology studies, particularly among specific tribes(105), and which have included biomarkers of exposure and disease(106). Mortality estimates are limited by the effects of late reporting of deaths, misclassification of the cause of death from death certificates, incomplete census enumeration due to non-participation, misclassification of ethnic origin due to socially and culturally-based self-identification or a failure to self-identify with any census response category, and non-standardized data collection or processing with each census round. Morbidity estimates from hospital and physician care utilization records are limited to those who access health care services, the conditions for which access is sought and the level of detail recorded in

administrative files e.g. lack of ethnic-specific data. There have been a number of Aboriginal-based surveys with the major limitation being the reliance on self-report for conditions that may not have been previously diagnosed, such as diabetes and hypertension. Due to their historical position within Canada, increased burden of disease and prevalence of deleterious risk factors, the First Nation people provide a unique opportunity for study(107).

2.5 RETINAL MICROVASCULAR ABNORMALITIES AND RETINOPAHTY

Retinal microvascular abnormalities include all retinal microvascular pathology. Retinal arteriolar changes refer to abnormalities that are related to the retinal arterioles such as generalized and focal arteriolar narrowing, and arteriovenous nicking. Retinopathy is characterized by microvascular changes that are not arteriolar such as retinal haemorrhages, microaneurysms, cotton-wool spots, hard exudates, macular edema, and optic disk swelling. Retinal microvascular abnormalities reflect hypertension and other systemic processes including atherosclerosis and have been associated with cardiovascular disease, therefore have been suggested as markers for cardiovascular disease(108). The ability to non-invasively examine the retinal area, its association with vascular factors, atherosclerosis and cardiovascular disease, and its similar anatomy and physiology to the cerebral and coronary circulations makes it an ideal candidate marker to further our understanding of neurological disorders(109).

The definition of retinopathy used in population-based studies includes the presence of any of the following lesions such as: microaneurysms, retinal haemorrhages, soft or hard exudates, macular oedema, intraretinal microvascular abnormalities, venous bleeding, new vessels at the disc or elsewhere, vitreous haemorrhages, disc swelling, or laser photocoagulation scars(110). One of the microvascular complications of diabetes is retinopathy, therefore

retinopathy often appears in individuals with diabetes and is often referred to as diabetic retinopathy. It is estimated that at twenty years after the onset of diabetes, more than 80% of individuals taking insulin and over 60% of individuals not taking insulin will have some form of retinopathy. However, the proportion of individuals with the most severe form of retinopathy, proliferative retinopathy, is much less. Only 20% of individuals taking insulin will have proliferative retinopathy and about 10% of individuals not taking insulin will have proliferative retinopathy at twenty years after the onset of diabetes(111). With the elevated level of individuals with diabetes in the First Nation (FN) population compared to the rest of Canada (CA) (FN: 11.5% vs. CA: 4.3%)(102), further study of retinopathy and its relationship to cognitive function is needed.

In general, similarities between the retinal and cerebral areas prompted investigations of the relationship between retinal microvascular abnormalities and risk of cognitive decline and dementia. Only one systematic review has been conducted, which included six studies to date. Among those studies, two were prospective cohort studies and four were cross-sectional studies. Retinopathy was assessed in both prospective studies, which also included individuals with diabetes in their study populations, and in two of the four cross-sectional studies, which were population-based. Overall, the results were consistent with an association between retinal microvascular abnormalities and cognitive decline and dementia in diabetes populations and in the general population. Differences in the cognitive tests used and characterization of those tests make direct comparisons across studies difficult. In the cross-sectional component of the Atherosclerosis Risk in Communities study, individuals classified with any retinopathy were associated with a 2.6-fold increased risk of cognitive decline assessed by the digit symbol subtest (DSS) (OR: 2.6, 95% CI: 1.3-2.9) compared to those without retinopathy, when adjusted

for age, sex, race, field centre, education, occupation, diabetes, fasting glucose, hypertension, carotid intima-media thickness, cigarette smoking, alcohol consumption, fasting total cholesterol, high density lipoprotein, and triglyceride levels. The cutpoint used to dichotomize the DSS was 20 units, with ≤20 units indicating cognitive decline. In the cross-sectional component of the Cardiovascular Health Study, individuals classified with any retinopathy were more likely to have lower mean scores for the digit symbol subtest (39 vs. 42 units, $p<0.02$) compared to those without retinopathy when adjusted for age, sex, race, field centre, education level, internal carotid intima-media thickness, body mass index, hypertension, diabetes, and cigarette smoking. In studies that included individuals with diabetes, the grading of retinopathy differed and those prospective studies were susceptible to survival bias. Individuals with diabetes may more likely not attend follow-up visits which may be related to cognitive status and the presence of retinopathy leading to an underestimation of effects. The two prospective studies did not report the retinopathy status of the individuals with diabetes who were lost to follow-up. Different statistical adjustment for confounders is an additional study limitation which prevents conclusive findings for the association(110) (Table 3). Vision acuity is another potential limitation when using cognitive tests to assess cognitive function in individuals with diabetes, however only 3.6% of those with young onset diabetes and 1.6% of those with older onset diabetes have been estimated to be legally blind. Therefore, depending on the duration of the disease, less than 5% of the study population may be effected by lowered visual acuity(112). Overall, further investigations between retinopathy and cognitive function are needed.

The associations between vascular and metabolic risk factors and retinal microvascular abnormalities have been studied in some detail. A review of population-based studies concluded that there is an association between hypertension and retinal microvascular abnormalities,

though inconsistent relationships were concluded between atherosclerosis, the metabolic syndrome, diabetes, and retinal microvascular abnormalities. Risk factor associations for the components of retinopathy have been studied such as retinal haemorrhages, microaneurysms and cotton wool spots, though not for the presence of retinopathy per se(113). The relationships between vascular and metabolic risk factors, atherosclerosis and retinopathy require further study.

Table 3. Summary of Confounders From Literature Review II

Confounders Identified From Studies that Examined the Relationship Between Retinopathy and Cognitive Decline and Dementia	
Studies	Adjustment Criteria
Ryan et al.	Only mentioned exclusions: alcohol or drug abuse, head trauma, psychiatric disorder.
Kadoi et al.	Adjusted for autonomic neuropathy, distal symmetric polyneuropathy, overt nephropathy, coronary artery disease, peripheral vascular disease, systolic blood pressure, and duration of diabetes.
Wong et al.	Adjusted for age, sex, race, field centre, education, occupation, diabetes, fasting glucose, hypertension, carotid IMT, cigarette smoking, alcohol consumption, fasting total and HDL cholesterol and triglyceride levels.
Baker et al.	Adjusted for age, sex, race, field centre, education level, internal carotid IMT, BMI, hypertension, diabetes, and cigarette smoking.
Taken from Ding et al. (2008).	

2.6 CHAPTER SUMMARY

The associations between vascular and metabolic risk factors and cognitive decline and dementia are not clear, except for perhaps hypertension. The associations between carotid atherosclerosis and cognitive decline and dementia are also not clear. A number of study limitations have been identified and if addressed, future studies may provide more consistent results across studies. The proposed thesis offers a number of contributions to the knowledge of the associations between vascular and metabolic risk factors, carotid atherosclerosis and cognitive decline.

The First Nations population is an under-studied population. Given their increased burden of risk factors and disease, additional study on this population is needed. The

relationships under study for this thesis have not been examined in a First Nations population. Investigation of these relationships in a First Nations population is highly relevant given their increased burden of risk factors and disease compared to their Canadian counterparts. If the relationships truly exist, then they would most likely be detected in a First Nations population.

The similar anatomy and physiology between the retinal and cerebral microenvironments suggest that the health of an individual's eyes may be an indicator of their cognitive function. The relationship between retinopathy and cognitive decline and dementia has been reviewed, with only a few studies conducted to date and a number of study limitations identified. This thesis would provide additional evidence of the relationship, with a focus on executive function.

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CHAPTER 3: METHODS

3.1 STUDY POPULATION

The sampling frame and survey infrastructure previously developed for a population-based cross-sectional study on diabetes and diabetes complications were used for this thesis (CIHR, PI: Kue Young)(1). The previous study was conducted in a road-accessible First Nations community in southern Manitoba. Eligible individuals were 18 years of age and older, non-pregnant, and either community members of a First Nations band, or treaty[a] First Nations individuals from other bands having primary residence in the community for at least the preceding six months. Though, individuals who traveled outside of the community but had been back for less than six months were also included. Two local community residents recruited individuals by visiting each home. The recruiters explained the study and asked eligible individuals to participate. Details of the study were placed in the Health Centre newsletter, which is published monthly, posters were displayed around the community, and letters delivered to homes. In total, there were 1,640 eligible individuals including those who lived off-reserve[b]. A random sample in this community was not attempted because the exclusion of individuals is not appropriate. Individuals not selected in initial random sampling strategies who may have wanted to participate in the study may not participate if randomly selected at a later date. Family members may also not participate in the study in support of those who were excluded (personal communication, Dr. Bruce). Therefore, the sample was a volunteer sample. Demographic,

[a] Treaty First Nations people are those who signed 'treaties' with the British and Canadian governments regarding land entitlement.
[b] Off-reserve refers to 'reserves,' parcels of land held by Canada on behalf of the First Nations people. Individuals can be on- or off-reserve.

clinical, metabolic, and anthropometric data including fasting blood samples collected as part of the diabetes study were used to examine the relationships under study. Measures of carotid atherosclerosis, retinal microvascular abnormalities and cognitive function were collected as part of the Early Stroke Indicators Study (Canadian Stroke Network, PI: Sharon Bruce). The carotid and retinal measures were collected between May and June 2005, approximately one year after the tests of cognitive function were administered (August, 2004). The tests of cognitive function, measures of carotid atherosclerosis and measures of retinal microvascular abnormalities were collected after demographic, clinical, metabolic, and anthropometric data, including fasting blood samples. Figures 1a and 1b show the study population.

3.2 DATA COLLECTION

Data collection for risk factors, cognitive function, carotid atherosclerosis, and retinopathy was conducted in the context of the previously abovementioned studies. Additionally, data was collected on 20 volunteers from the U of T community as part of this thesis for two purposes: (i) to become familiar with the administration of the cognitive tests, (ii) to generate data for a sample of "healthy" individuals of a similar age as the First Nations population, e.g. young individuals without previous medical conditions. Ethics approval from U of T for this data collection was received on December 5th, 2007 (Protocol reference no. 20994), and is shown in Appendix 1.

3.2.1 Measurement of Risk Factors

The following information was collected through in-person questionnaires administered by trained personnel [IPQ], physical examination [PE], or analysis of fasting venous blood

samples [FBS] as part of the diabetes study. Fasting venous blood samples were collected and processed on site at the First Nations health centre and stored at -70 degrees Celsius. Approximately every two weeks, frozen samples were transported on dry ice to the Clinical Chemistry Lab at the Health Sciences Centre, Winnipeg for analysis. For each variable listed below, the method of ascertainment, its definition, a brief mention of its characterization, and the type of analysis where it will be used are provided. The instruments used for data collection are shown in Appendix 2 (Diabetes Complications Screening Questionnaire) and Appendix 3 (Cognitive Tests).

Age

Age was collected in response to the following question: what is your date of birth? Age in years was examined as a continuous and categorical variable in unadjusted analyses. Age was examined as a continuous variable in multivariable logistic regression and path analysis. [IPQ]

Sex

Sex was collected in response to checked categories and examined as a categorical variable for all analyses. [IPQ]

History of cardiovascular disease

A history of cardiovascular disease was determined from individuals reporting to have had at least one of the following conditions: myocardial infarction, angina, angiorevascularization or angioplasty, and claudication. It is a composite variable and was examined categorically (yes vs. no) in unadjusted and multivariable logistic regression. History of cardiovascular disease is a primary vascular risk factor. [IPQ]

Myocardial infarction

Ever having had a myocardial infarction was collected in response to the question: have you ever been told by a health care professional that you have a heart attack? Agreement between self-report and medical record review is strong for myocardial infarction (kappa: 0.80, 95% CI: 0.74-0.85)(2). Myocardial infarction was examined as a categorical variable (yes vs. no) when creating the composite variable, history of cardiovascular disease. [IPQ]

Angina, angiorevascularization or angioplasty, and claudication

Angina, angiorevascularization or angioplasty, and claudication were collected in response to the question: have you ever been told by a health care professional that you have other heart problems? Agreement between self-report and medical record review is moderate to strong for angina (kappa: 0.37, 95% CI: 0.35-0.39), angioplasty (kappa: 0.79, 95% CI: 0.77-0.81), and claudication (kappa: 0.53, 95% CI: 0.48-0.59)(3). Angina, angiorevascularization or angioplasty, and claudication were examined as categorical variables (yes vs. no) when creating the composite variable, history of cardiovascular disease. [IPQ]

Stroke

Stroke was collected in response to the question: have you ever been told by a health care professional that you have stroke? Agreement between self-report and medical record review is strong for stroke (kappa: 0.71, 95% CI: 0.63-0.79)(2). Stroke was examined as a categorical variable (yes vs. no), and responses were applied to the exclusion criterion. [IPQ]

Figure 1a. Study Population

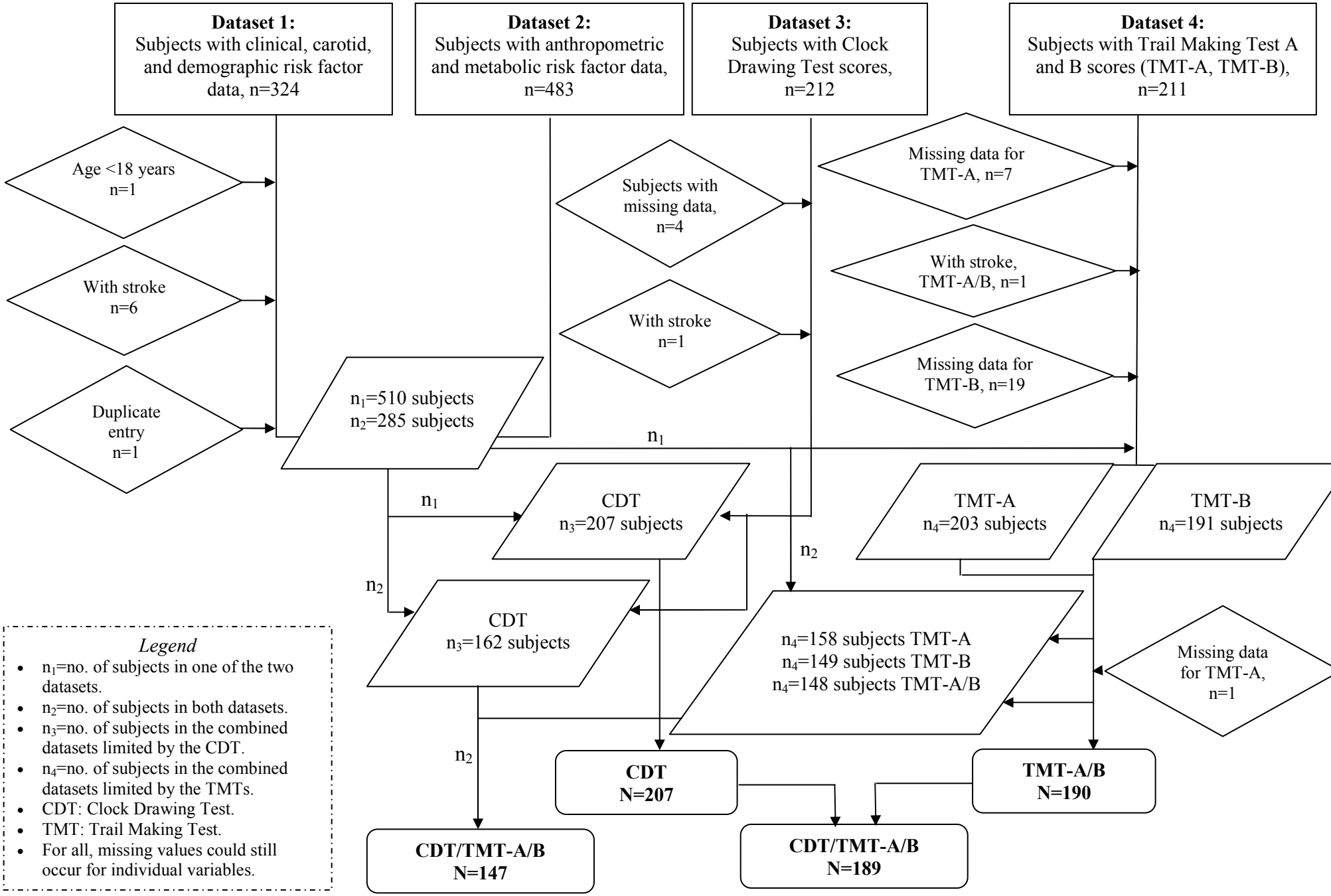
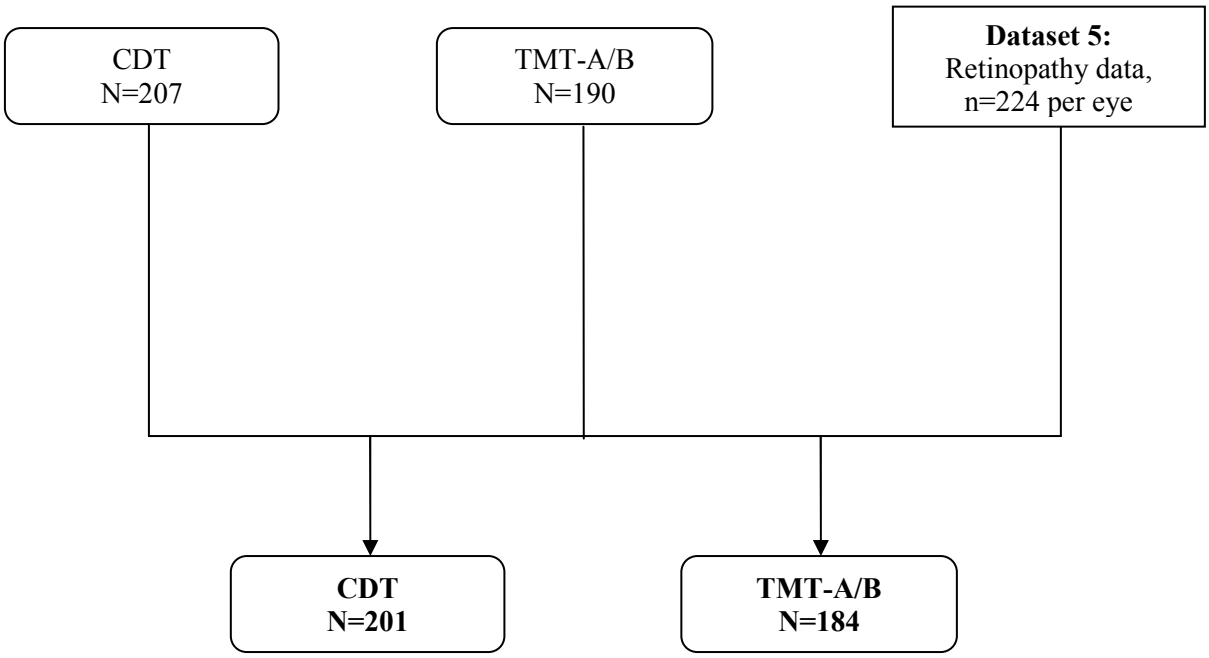


Figure 1b. Study Population (cont'd)



Legend

- CDT: Clock Drawing Test.
- TMT: Trail Making Test.
- For all, missing values could still occur for individual variables.

Diabetes

Ever having diabetes was collected in response to the question: have you ever been told by a health care professional that you have diabetes? Agreement between self-report and medical record review is strong for diabetes (kappa: 0.76, 95% CI: 0.70-0.82)(2). Diabetes (yes vs. no) was examined as a categorical variable in all analyses. Diabetes is a primary metabolic risk factor. [IPQ]

Duration of diabetes

Duration of diabetes was collected among those reporting diabetes by the following question: if yes, for how long have you had diabetes? Duration of diabetes in years was examined as a continuous and categorical variable in unadjusted analyses, and as a categorical variable in multivariable logistic regression. Duration of diabetes is a primary metabolic risk factor. [IPQ]

Blood pressure and hypertension

Blood pressure was collected by standard sphygmomanometer. Systolic and diastolic blood pressures in millimeters of mercury (mmHg) were examined as continuous variables in unadjusted and path analyses. Individuals with systolic blood pressure ≥135 mmHg and/or diastolic blood pressure ≥85 mmHg were classified as having hypertension(4). Hypertension was examined as a categorical variable (yes vs. no) in unadjusted and multivariable logistic regression. Hypertension is a primary vascular risk factor. [PE]

Weight, height, body mass index, and obesity

Weight and height were collected by standard anthropometry. Weight in kilograms (kg) and height in centimetres (cm) were examined as continuous variables in unadjusted analyses, and used to calculate body mass index. Body mass index (BMI) was defined as weight in kilograms divided by height in metres squared (m^2) and individuals were classified as underweight if they had a BMI of <18.5 kg/m^2, normal weight if they had a BMI of 18.5-24.9 kg/m^2, overweight if they had a BMI of 25.0-29.9 kg/m^2, and obese if they had a BMI of ≥30.0 kg/m^2(5). Body mass index was examined as a continuous variable in unadjusted and path analyses, and as a categorical variable unadjusted and multivariable logistic regression. Obesity is a primary metabolic risk factor. [PE]

Waist circumference

Waist circumference was collected by standard anthropometry to the nearest 0.5 cm. It was determined at the level of noticeable waist narrowing using an inelastic tape measure. For individuals in which waist narrowing was difficult to identify, an indeterminate waist was approximated by taking the girth at the estimated lateral level of the twelfth or lower floating rib. It was examined as a continuous variable in unadjusted and path analyses. According to the NCEP criteria, a high risk group was created that included individuals with a waist circumference: >102 cm (male) or >88 cm (female), and a low risk group for those with a waist circumference: <103 cm (male) or <89 cm (female)(4). Using these groups, waist circumference was further examined as a categorical variable in unadjusted and multivariable logistic regression. [PE]

Cholesterol, triglycerides, high density lipoprotein, and low density lipoprotein

Lipid levels were determined from fasting venous blood samples collected and processed using standard protocols. Cholesterol and triglycerides were determined from enzymatic colorimetric methods in millimoles per litre (mmol/L), and high density lipoprotein was determined by the direct enzymatic method, also in mmol/L. Low density lipoprotein was calculated according to the approximated Friedewald formula (low density lipoprotein = total cholesterol - high density lipoprotein - total triglycerides*0.46), and expressed in mmol/L. Low density lipoprotein was not calculated for samples with visible chylomicrons or triglycerides >4.52 mmol/L(6). Each lipid was examined as a continuous variable in unadjusted and path analyses. [FBS]

Dyslipidemia

According to the NCEP criteria, dyslipidemia was defined as triglycerides ≥1.7 mmol/L or high density lipoprotein <1.0 mmol/L (male) or <1.3 mmol/L (female)(4), and was examined as a categorical variable in unadjusted and multivariable logistic regression. Dyslipidemia is a primary metabolic risk factor. [FBS]

Metabolic syndrome

Metabolic syndrome was defined according to the NCEP criteria, which included three or more of the following: waist circumference >102 cm (male) or >88 cm (female), triglycerides ≥1.7 mmol/L, high density lipoprotein <1.0 mmol/L (males) or <1.3 mmol/L (females), hypertension defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg, and fasting blood glucose ≥6.1 mmol/L(4). Metabolic syndrome was examined as a

categorical variable in unadjusted and multivariable logistic regression. Metabolic syndrome is a primary metabolic risk factor. [IPQ, FBS, PE]

Homocysteine

Homocysteine was determined from fasting venous blood samples using standard high performance liquid chromatography with fluorometric detection in μmol/L. It was examined as a continuous variable in unadjusted and path analyses. Homocysteine was examined as a categorical variable in unadjusted logistic regression. [FBS]

Glucose and insulin

Glucose and insulin were determined from fasting venous blood samples using standard protocols. Glucose in mmol/L was determined using the Hexokinase/G6P-DH assay and insulin in *p*mol/L by immunoassay. Insulin resistance was calculated using the homeostasis model of assessment (HOMA) [(fasting plasma insulin (*p*mol/L) x 0.144 uU/ml x fasting plasma glucose (mmol/L))/(22.5)](7,8). Glucose and insulin resistance were examined as continuous variables in unadjusted analyses, insulin resistance was examined as a continuous variable in path analysis, and insulin resistance was examined as a categorical variable in unadjusted and multivariable logistic regression. Insulin resistance is a primary metabolic risk factor. [FBS]

Ever smoked

Ever smoked was collected in response to the question: have you ever smoked cigarettes, pipes, or cigars on an approximately daily basis? Agreement between self-reported smokers and serum cotinine levels >15 ng/ml was 92.5% (95% CI: 91.3-93.7)(9). Ever smoked (yes vs. no) was examined as a categorical variable in unadjusted and multivariable logistic regression. [IPQ]

Smoking duration

Smoking duration was collected among those reporting to have smoked by the following question: if yes, how long did you smoke? Smoking duration in years was examined as a continuous variable in unadjusted and path analyses, and as a categorical variable in unadjusted logistic regression. [IPQ]

3.2.2 Measurement of Cognitive Function

Three non-verbal neuropsychological tests were administered as part of the stroke study by two graduate research assistants at the research study site. The three cognitive tests included the Clock Drawing Test (CDT) and the Trail Making Test Parts A (TMT-A) and B (TMT-B). Data were ascertained alternating the presentation order of the Clock Drawing Test and the Trail Making Tests.

Clock Drawing Test

The Clock Drawing Test is a simple and rapidly administered test of cognitive function. It has been proposed as a screening test for the early signs of dementia and characterizes deficits in visuospatial abilities and abstract thinking(10). In brief, the Clock Drawing Test involves individuals having to draw in the numbers of a clock face on a given piece of paper with a preformed circle. Individuals are typically asked to place the hands of the clock to read "10 past 11" although this was not requested. Scoring followed the Watson method. According to the Watson method, the circle is divided into four equal quadrants by drawing one line through the centre of the circle and the number 12 and a second line perpendicular to and bisecting the first. The clock number in each quadrant is counted in a clockwise direction beginning with the

number 12. Each clock number is counted only once and if a clock number crosses one of the reference lines it is included in the adjacent quadrant in a clockwise direction. Three clock numbers in a quadrant are considered correct. Errors in the first to third quadrants are assigned a score of one and errors in the fourth quadrant are assigned a score of four, for a maximum total score of seven. The scoring system was developed by evaluating the errors in the positioning of the clock numbers. Data indicated moderate to strong sensitivity (62-88%), specificity (76-82%), and moderate agreement (kappa: 0.42-0.69) for errors in the number of digits in quadrants one to four compared to a diagnosis of probable dementia according to the NINCDS-ADRDA criteria. Errors in the fourth quadrant were weighted higher to account for its higher sensitivity, specificity and agreement compared to the other three quadrants (sensitivity: 87.5%; specificity: 82.3%; kappa: 0.69). Using a total score summed across the four quadrants, the cutpoint of a total score of four or greater was validated in comparison to a probable diagnosis of dementia with measures of sensitivity, specificity and kappa of 87%, 82% and 0.70, respectively. Reliability was evaluated by test re-test methods on a sample of 55 randomly selected non-demented adults from diverse clinical settings e.g. nursing home, outpatient clinic and rehabilitation ward. The reliability was shown to be strong ($r_{Spearman}$=0.76). In the thesis, scoring of the placement of the hands of the clock was not relevant, but may be omitted to remove the educationally-sensitive component(11). In the thesis, a total score of four or greater was used as the cutpoint to separate individuals based on performance. Scores were determined by two individuals, one individual was the same individual who administered the tests and another individual was a research technician. Preliminary data analysis showed that the inter-rater reliability of scoring for the Clock Drawing Test was substantial (κ=0.68, 95% CI: 0.57-0.78).

Trail Making Test Parts A and B

The Trail Making Test requires alertness and concentrated attention to the task at hand and is a useful screening test for several types of cognitive impairment regardless of the location of the brain lesion. Part A is a clinical assessment of motor and visual control and processing speed and Part B is a clinical assessment of executive function independent of test length and visual interference(12). Executive function broadly encompasses a set of cognitive skills that are responsible for the planning, initiation, sequencing, and monitoring of complex goal-directed behaviours associated with the frontal lobe(13).

In brief, Part A consists of 25 circles on a sheet of paper numbered from 1 through 25. The numbered circles are not placed in numerical sequence. Participants are asked to connect the circles as quickly as possible, beginning with one and continuing in ascending sequence. The test begins with a sample test containing circled numbers from 1 through 8. The individual is instructed to draw lines connecting the numbers as fast as they can. If the individual makes an error, they are stopped and the error is pointed out, the test re-explained, and they are directed to begin again. If the individual makes another error, their hand is guided through the test and instructed to begin again. The actual test was given with the following instructions: "On this page are numbers from 1 to 25. Do this the same way. Begin at number 1 (point) and draw a line from 1 to 2 (point 2), 2 to 3 (point 3), 3 to 4 (point 4) and so on, in order, until you reach the end (point). Remember to work as fast as you can. Start now." Errors were noted but not recorded and individuals were instructed to continue from the point of error with the timer continuing during the correction process. Time in seconds to test completion was recorded and a maximum score of 90 seconds was applied to individuals who could not complete the test.

Part B involves the subject having to draw a line alternating between numbers (1 to 13), and letters (A to L) in ascending sequence as quickly as possible. Numbers and letters are not placed in order. This test also begins with a sample test, similarly described as for Part A, except alternating between numbers (1-4) and letters (A-D). The same correction process was applied. For the actual test, similar instructions were provided as for Part A. Time in seconds to test completion was recorded. A maximum of 300 seconds was applied to individuals who could not complete the test. The same correction process as for Part A was applied. Validity has previously been shown in which Parts A and B were administered individually to 200 individuals with verified brain damage and 84 individuals without amnesia or clinical evidence of brain damage. The brain-damaged group included heterogeneous diagnoses and the control group comprised individuals who had entered the hospital with neurological complaints. Mean transformed scores were statistically significantly different between the two groups for Parts A and B ($p<0.001$), indicating strong discriminatory ability(14). In this study, characterization of the Trail Making Test Parts A and B were examined in detail and is discussed in Chapter 4.

3.2.3 Measurement of Carotid Atherosclerosis

Plaque volume and carotid stenosis were collected as part of the stroke study.

Plaque Volume

For each individual, a series of parallel two dimensional images were collected using Doppler ultrasonography and used to reconstruct a three dimensional image. Three dimensional images were acquired with a mechanical linear 3D scanning system (Life Imaging Systems Inc). In brief, images were produced by a motorized mechanism that translates a transducer at a

distance of 4.0 cm across the skin of an individual's neck. Both sides of the neck were quantified. Two dimensional images were acquired at regularly spaced 0.15 mm intervals and at a constant angle for approximately eight seconds. The resulting two dimensional images were used to reconstruct a three dimensional image for plaque volume measurement. Plaque volume on each side of the neck is the total of all plaque in the common, internal and external carotid arteries. The three dimensional scans from both sides of the neck were saved on CD for shipment to the core plaque volume laboratory in London, Ontario (Robarts Research Institute, Canada). The three dimensional images of plaque volume were measured by manual planimetry, a method that has been validated on vascular phantoms[c] with a mean accuracy of 3.1 ± 0.9%(15). Three dimensional images were quantified with an interslice distance of 1.0 mm from one end of the plaque to the other. Plaque boundaries were traced by using a mouse-driven cursor. The areas measured in each slice were summed and multiplied by the interslice distance to calculate the total plaque volume. Reliability data from a repeated-scan study found the coefficient of variation to range from 3.9 to 15.1%. Data from a multiple-observer study found an inter-rater reliability of 0.91 and intra-rater reliability of 0.87(16). Values for right, left and total plaque volume in millimeters cubed (mm^3) were used as continuous variables in unadjusted and path analyses, and as categorical variables in unadjusted and multivariable logistic regression.

Carotid Stenosis

Peak systolic velocity of the right and left internal carotid arteries measured using Doppler ultrasonography was used to estimate the percent stenosis of each side of the neck(17).

[c] Validity was demonstrated by determining the volume of agar plaques of various heights and lengths using water displacement and then imaging these simulated plaques *in situ* with 3D ultrasonography methods. Using phantoms with known plaque volume, the mean error was calculated.

Based on a validation study, peak systolic velocity was shown to highly correlate with carotid stenosis as measured by angiography. Percent carotid stenosis of the right and left carotid arteries as well as total carotid stenosis (sum of right and left) were used as continuous variables in unadjusted and path analyses, and as categorical variables in unadjusted and multivariable logistic regression.

3.2.4 Measurement of Retinal Microvascular Abnormalities

Assessment of retinal microvascular abnormalities provides a non-invasive measure of silent microvascular disease. As part of the stroke study, digital images were taken to assess retinal microvascular abnormalities and the presence of retinopathy using a portable camera. Trained technologists traveled to the specified First Nations community and digital fundus photographs of both eyes with no pharmacological papillary dilation were taken at a 45 degree angle in a darkened room. Six fields were photographed (three for each eye) using a nonmydriatic retinal camera(18). Retinopathy was defined as any one of the following conditions present for one of two eyes: (1) hard or soft exudates, or intraretinal microvascular abnormalities without microaneurysms, (2) haemorrhages without microaneurysms, (3) microaneurysms only, (4) early non-proliferative diabetic retinopathy, (5) moderate non-proliferative diabetic retinopathy, or (6) severe non-proliferative diabetic retinopathy. Grading for retinopathy was performed at the Ocular Epidemiology Reading Center, Department of Ophthalmology and Visual Sciences at the University of Wisconsin School of Medicine and Public Health (Madison, WI) according to standard protocols(19,20). Reproducibility of assessment of retinopathy has shown to be strong (kappa range: 0.80 to 0.99)(21).

3.2.5 Volunteer Sample

Data was collected on 20 volunteers from the U of T community for two purposes: (i) to become familiar with the administration of the Clock Drawing Test and the Trail Making Test Parts A and B, (ii) to generate data for a sample of "healthy" individuals of a similar age as the First Nations population, e.g. young individuals without previous medical conditions. Eligible individuals provided informed consent (Appendix 4). The results of this sub-study are shown and discussed in Chapter 4.

3.3 POWER CALCULATIONS

Power calculations were performed according to two selected risk factor variables, metabolic syndrome, defined according to the NCEP criteria, and obesity, defined as body mass index $\geq$30 kg/m^2. Appendix 5 shows the power calculations for proportions. Given a prevalence of 16% for the metabolic syndrome, 83 cases of vascular cognitive impairment and 125 control subjects according to the Clock Drawing Test, the study will have approximately 75% power to detect a magnitude of effect of 2.4, consistent with effect sizes reported for stroke. For a prevalence of 50% for obesity and a similar ratio of case to control subjects for the Clock Drawing Test, more than 90% power is available to detect a range of effect sizes (1.6-2.4). Appendix 6 shows the power calculations for means. For the Trail Making Test Part A, there will be sufficient power (70 to >90%) to detect a range of mean differences (5-20 seconds) given a standard deviation of 13.0, sample size of 53, and a control-to-case ratio of unequal groups of 3:1 for the metabolic syndrome. Similarly, there will be sufficient power (>90%) with increasing mean difference for body mass index having a sample size of 119 and a ratio of 1:1. For the Trail Making Test Part B, there is moderate power (~60%) to detect a mean difference

of 20 seconds or greater for the metabolic syndrome and body mass index. For a structural equation modeling approach, approximately 190 to 230 subjects are required to estimate a model with 19 to 23 variables. The theoretical model is over-identified, with 1 degree of freedom remaining. Sample size calculation for a structural equation modeling approach is shown in detail in Appendix 7.

3.4 MULTIVARIATE TECHNIQUES

Multivariate statistics were used for this thesis. Examples include structural equation modeling and principal components analysis. Both were used for this thesis and therefore will be described in brief below.

3.4.1 Structural Equation Modeling

Structural equation modeling (SEM) is a multivariate statistical modeling technique that allows complex theoretical relationships to be modeled as causal mechanisms. Structural equation modeling is not a method for identifying the cause of a disease. It uses statistics to test theoretical relationships. Reasons for its use include that it allows for a number of sophisticated modeling techniques simultaneously in contrast to classical regression analysis including flexibility of the nature of variables that can be included in the statistical models such as latent variables, the ability to account for measurement error, and the ability to estimate direct and indirect effects. Latent variables are not directly observed. They are inferred constructs that are interpreted with respect to the underlying observed variables that define them(22).

SEM includes path and measurement models. The absence of a measurement model in SEM is often referred to as path analysis, and uses similar statistics. The first step in SEM is

generating the theoretical model (SEM model) to be tested and includes a comprehensive literature review of the variables and interrelationships under study. Testing whether your theoretical model is in agreement with the observed data is examined through chi-square statistics and/or fit statistics. The rule of thumb is that a large p-value from a chi-square test is desirable, indicating that the null hypothesis is not rejected and your theoretical model is consistent with the observed data. Similarly, standard guidelines for fit statistics should indicate good model fit(22, 23).

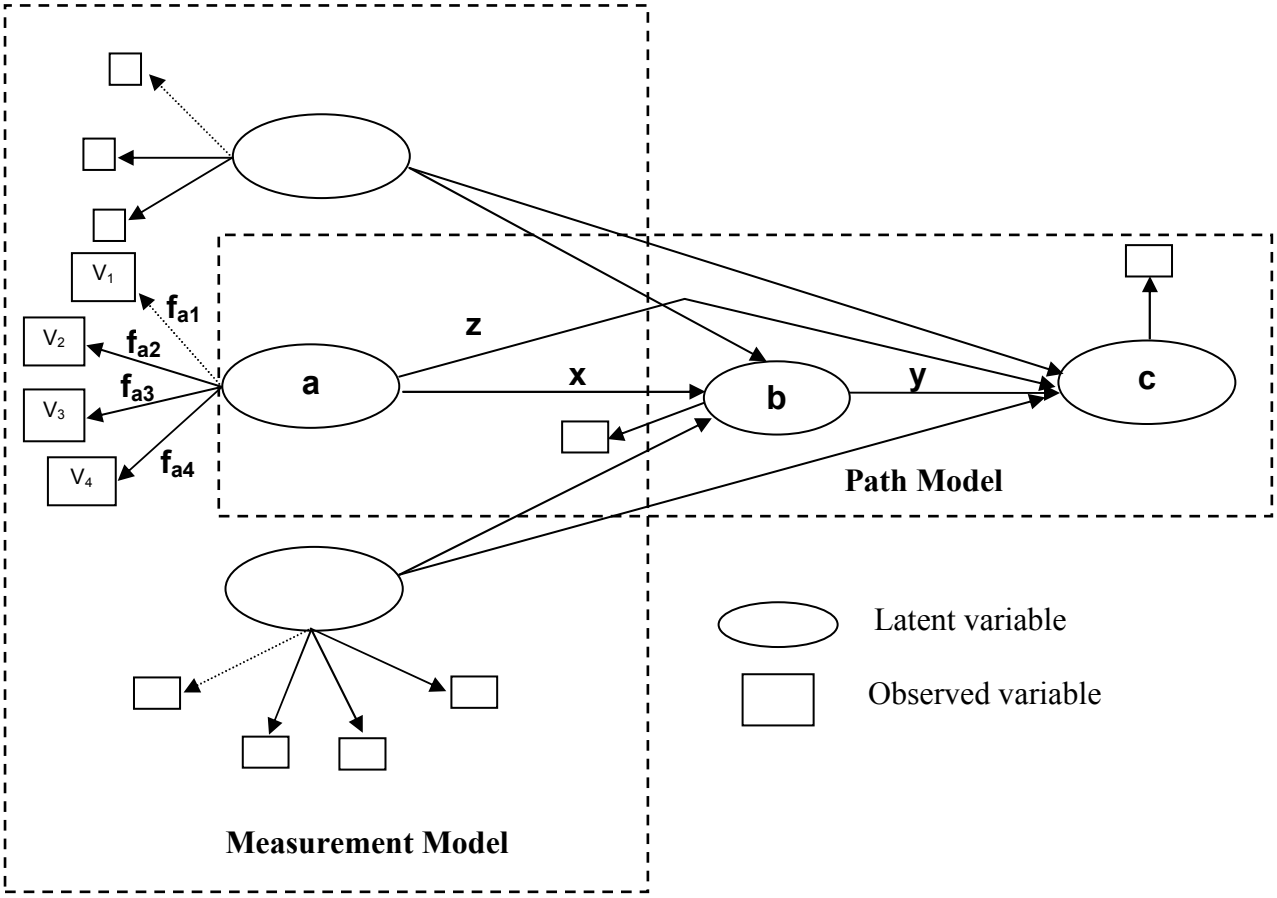
The notation and terminology for describing the SEM model may vary(24), however in general, for the schematic diagram of the SEM model, observed variables are represented by boxes and latent unobserved variables are represented as ovals. Figure 2 displays some key features of a SEM model (simplified), but depicts only a recursive model (unidirectional paths). The path model is displayed to the right-hand side of the simplified SEM model. The path model includes straight single-headed arrows between variables exerting the causal influence (antecedent variables) and the affected variable (consequent variable), representing unidirectional causal paths. In terms of statistics, the antecedent variables are the independent variables and the consequent variable is the dependent variable in linear regression equations (Equation 1). Estimated are path coefficients with corresponding standard errors and p-values. Interpretation of the path coefficient is analogous to linear regression modeling, the value given equates to the magnitude of change in the dependent variable for every 1-unit change in the independent variable, while holding constant all other independent variables. The measurement model is displayed to the left-hand side of the simplified SEM model. The measurement model also includes straight single-headed arrows. They signify which observed variables comprise the latent variable. In terms of statistics, the latent variable is the independent variable and the

observed variables are the dependent variables in linear regression equations (Equation 2). Estimated are factor loadings with their corresponding standard errors and p-values. A factor loading is analogous to a path coefficient from a latent variable to an observed variable. Its magnitude is reviewed and a factor loading that is not significant suggests that the observed variable is not representative of the underlying factor. All coefficients are estimated simultaneously and parameter estimation is maximum likelihood, which assumes multivariate normality of all variables. Overall, the SEM model represented by boxes, ovals and arrows is actually a set of linear regression equations. For this thesis, SEM was performed in SAS v 9.1 (SAS Institute, NC). In SAS, data input included both a correlation matrix and standard deviations. From this, a covariance matrix was created which formed the basis of the SEM analysis(23).

The complexity of SEM modeling includes mediator variables, which is a variable that mediates or intervenes between antecedent variables and a consequent variable (Figure 3). Therefore, an antecedent variable can have both direct and indirect effects on the consequent variable. The total effect is calculated as the sum of the direct (a->c) and indirect effects (a->b and b->c)(25, 26). The amount of mediation is calculated as the difference of direct effects (z-z') (Figures 3 and 4)(26). The concepts and calculation of total effects and mediation can be applied to more complicated SEM models. Therefore, SEM separates out the coefficients for the relationships of interest whereas classical regression analysis models all variables together. Similarly, adjusting for a mediating effect using classical regression analysis is not the same as subtracting the coefficients in SEM. Additionally, adjusting for a variable as a confounder when it should be handled as a mediation effect may result in an attenuation of the coefficient for the relationship of interest(25). These are the advantages of SEM with respect to statistical

modeling and understanding complex relationships. Similar to classical regression methods, measurement error is addressed by inclusion of all measured nontrivial variables and bias is reduced by inclusion of confounding variables of the relationship(s) of interest.

Figure 2. Simplified SEM Model



c = az + by, where z and y are path coefficients **(Equation 1)**

$V_1 = af_{a1}$, where f_{a1} is the factor loading between a and V_1 **(Equation 2)**
$V_2 = af_2$, where f_{a2} is the factor loading between a and V_2
$V_3 = af_{a3}$, where f_{a3} is the factor loading between a and V_3
$V_4 = af_{a4}$, where f_{a4} is the factor loading between a and V_4

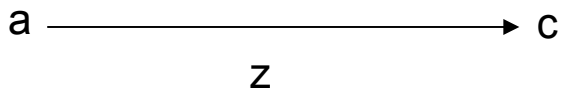


Figure 3. Direct Effects (z or a->c)

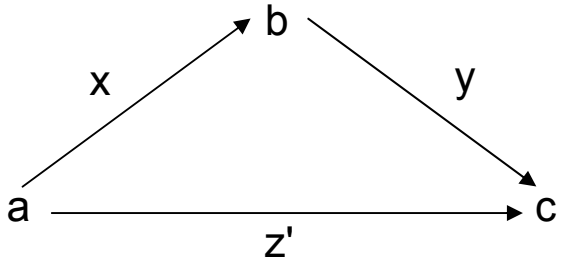


Figure 4. Total Effects (z' + xy) and Mediation (z-z')

3.4.2 Principal Components Analysis

Principal components analysis (PCA) is used when there are a large number of observed or measured variables (e.g. more than 10) that are highly correlated to one another and there is an interest to examine these variables simultaneously using regression methods. To circumvent the regression modeling limitations of SEM or classical regression methods due to multicollinearity of these variables, a principal components analysis can be used that reduces the number of variables to a smaller subset, which are referred to as factors or components. These factors or components are then interpreted to represent the clustering of the original variables among the factors or components(23).

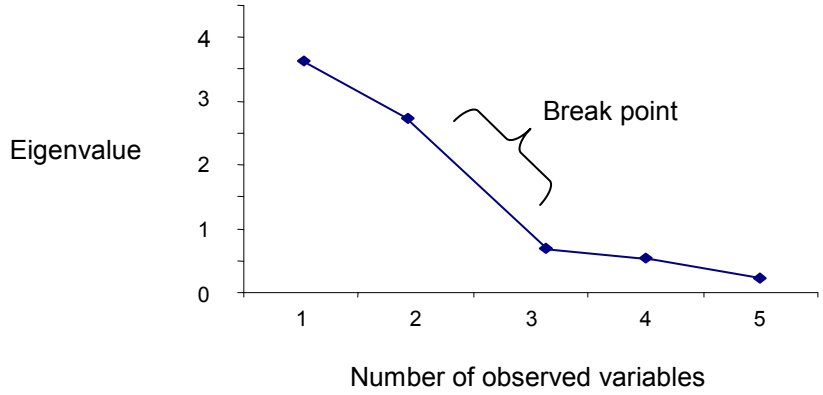
Unlike factor analysis, PCA does not assume an underlying causal structure between the factors or components and observed variables. Following from that, PCA uses an unadjusted correlation matrix in which all observed variables are standardized (e.g. $\sigma^2=1$) and the retained

components represent the partitioning of the total amount of variance provided by the observed variables, which has been maximized. Factor analysis uses an adjusted correlation matrix with communalities on the diagonal that have been estimated using prior communality estimates (h^2). In this way, factor analysis differs from PCA in that it accounts for the common variance of an observed variable and leaves the unique variance, otherwise known as the systematic or random error unanalyzed. Therefore, factor analysis and PCA have different functions although overlapping methodology(23).

Standard methods of PCA follow a sequence of analytical steps. In preliminary analysis, each continuous variable should be examined for its distribution and normality, since PCA assumes a normal distribution for the variables to be analyzed. A principal components analysis is then performed to identify the number of components that account for the maximum amount of total variance. Determination of the number of components to be retained is multi-fold and includes the following: (1) eigenvalue criterion, with components having an eigenvalue >1 such that a retained component accounts for a greater amount of variance than contributed by one variable alone; (2) scree plot analysis, with components above the eigenvalue break point (Figure 5); (3) proportion of total variance >5% for each component and calculated as the eigenvalue for a given component divided by the total number of observed variables being analyzed, and the highest cumulative total variance; (4) interpretability criterion. Determination or "extraction" of the components follows a one or two-step process. In the first step, components are extracted uncorrelated, known as a varimax rotation. For some datasets, PCA stops here and the results are interpreted. However, a promax or oblique rotation may be used which allows the extracted components to be correlated if there is belief that the observed variables are interrelated. Therefore, in a subsequent analysis step, the orthogonality or

uncorrelated requirement may be relaxed and the results are generated for a promax or oblique rotation. The results of a promax rotation compared to a varimax rotation should indicate greater differences for the factor loadings of an observed variable across the different components. Standard regression coefficients are interpreted for a promax rotation whereas results for the rotated factor pattern are interpreted for a varimax rotation. Determination of the number of components to be retained is multi-fold and includes the criteria mentioned above.

Figure 5. Scree Plot Analysis Example



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CHAPTER 4: DATA MANAGEMENT

4.1 PRELIMINARY DATA ANALYSIS

Preliminary data analysis was used to guide the operational definition of decreased cognitive function for Trail Making Test Parts A and B (TMT-A and TMT-B). The preliminary analysis included examining the distribution of times to completion for TMT-A (A) and TMT-B (B), and the linear relationship between the two tests. Derived scores were calculated and their distributions were also examined. The derived scores included: [B-A] (Difference), [B/A] (Ratio), and [(B-A)/A] (Combined), (TMT-D, TMT-R and TMT-C, respectively). The relationships between age and TMT-A and TMT-B, and the derived scores [B-A], [B/A] and [(B-A)/A] were examined. All descriptive statistics were calculated in SAS v. 9.1 (SAS Institute, NC).

Figure 6a-b displays the distributions for times to completion for the TMT-A and TMT-B. Among 203 individuals with times to completion for TMT-A, the average time was 28.4 seconds (Standard deviation [SD]: 13.0), and more than 50% of individuals had times greater than 24 seconds (Range: 10.0-97.0 seconds). Among 191 individuals with times to completion for TMT-B, the average time was 90.3 seconds (SD: 55.8), and more than 50% of individuals had times greater than 78 seconds (Range: 14.0-494.0 seconds). The relationship between TMT-A and TMT-B was strong. The Spearman correlation was 0.54, and very highly statistically significant ($p<0.0001$). Among 190 individuals with times to completion for both TMT-A and TMT-B, the average time for [B-A] was 63.3 seconds (SD: 48.8), and more than 50% of individuals had times greater than 52.0 seconds (Range: -5.0-397.0 seconds); the average score

for [B/A] was 3.4 units (SD: 1.7), and more than 50% of individuals had a score greater than 3.1 units (Range: 0.7-13.7 units); and the average score for [(B-A)/A] was 2.4 units (SD: 1.7), and more than 50% of individuals had a score greater than 2.1 units (Range: -0.3-12.7 units). For the derived scores, increased scores indicate a lower level of cognitive function (Figure 7a-c).

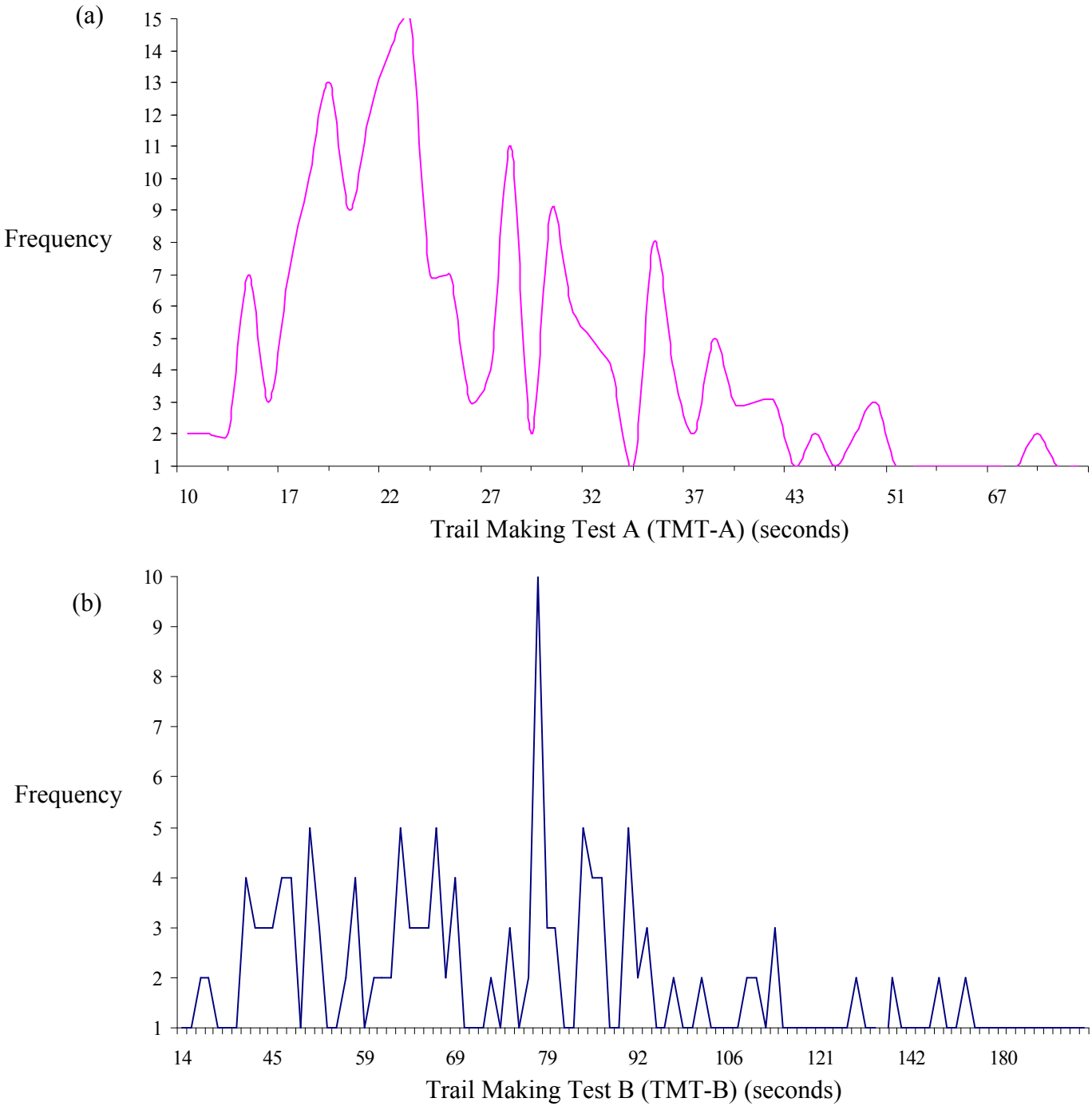
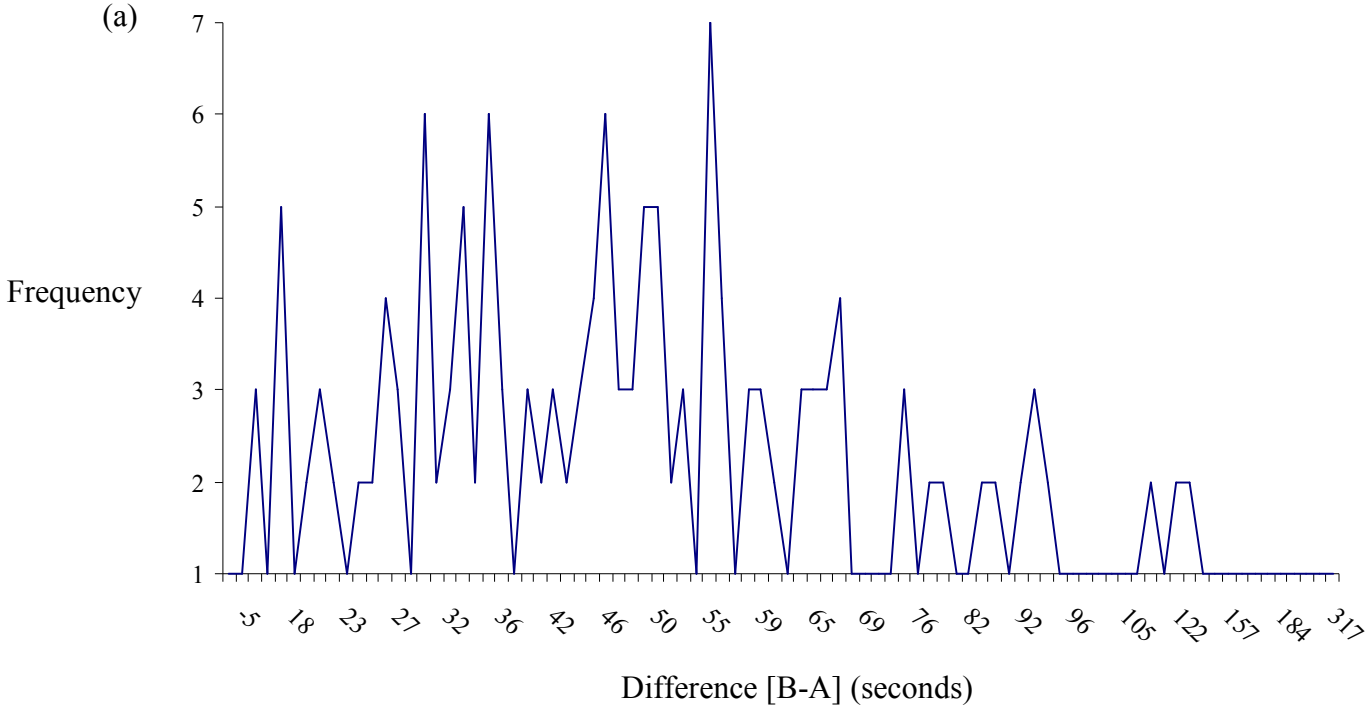
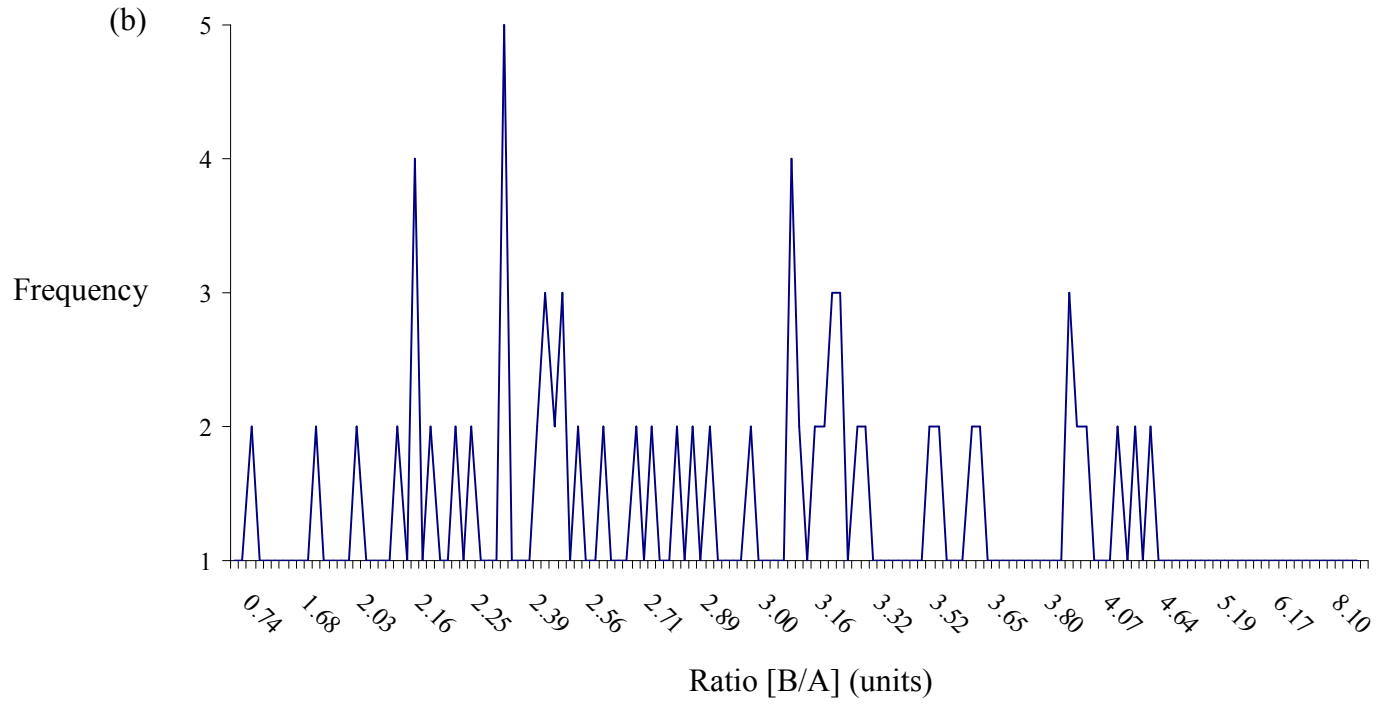


Figure 6. Distribution of Times to Completion (a) TMT-A, (b) TMT-B.

(a)


Frequency
7
6
5
4
3
2
1
5
18
23
27
32
36
42
46
50
55
59
65
69
76
82
92
96
105
122
157
184
317
Difference [B-A] (seconds)

(b)


Frequency
5
4
3
2
1
0.74
1.68
2.03
2.16
2.25
2.39
2.56
2.71
2.89
3.00
3.16
3.32
3.52
3.65
3.80
4.07
4.64
5.19
6.17
8.10
Ratio [B/A] (units)

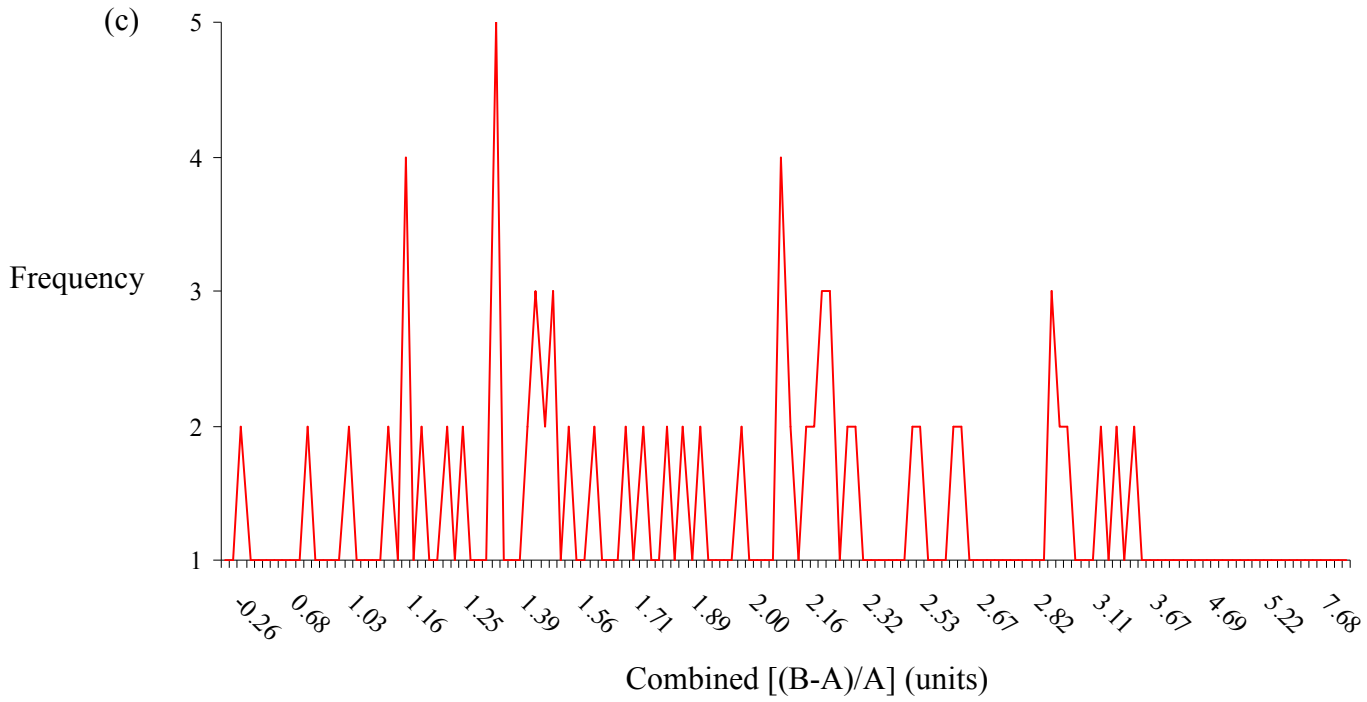


Figure 7. Distribution of Times to Completion for Derived Scores (a) [B-A], (b) [B/A], (c) [(B-A)/A].

Among 203 individuals with times to completion for TMT-A and 191 individuals with times to completion for TMT-B, there were 158 and 149 individuals who also had information on age (Note: there is a reduced sample size due to incomplete age information for some individuals). The Spearman correlations between age and times to completion for TMT-A and TMT-B were moderate and very highly statistically significant (TMT-A, r=0.29, p=0.0001; TMT-B, r=0.26, p=0.0015). Among the 190 individuals with times to completion for both TMT-A and TMT-B, there were 149 individuals who also had information on age (Note: there is a reduced sample size due to incomplete age information for some individuals). The Spearman correlations between age and the combined and ratio derived scores were similar, close to zero, and not statistically significant (TMT-C and TMT-R, r=0.08, p=0.3411), whereas the correlation between age and the difference score was moderate and highly statistically significant (TMT-D,

r=0.23, *p*=0.0042). When age was examined as a categorical variable, the display of data indicated increasing times to completion with increasing age for TMT-B. A similar trend was observed for the difference score and TMT-A, though less marked, and there was no effect for [B/A], and [(B-A)/A] (Figure 8). Overall, the median age was 38.0 years (Interquartile range [IQR]: 16.0 years) among the 149 individuals with both TMT-A and TMT-B times.

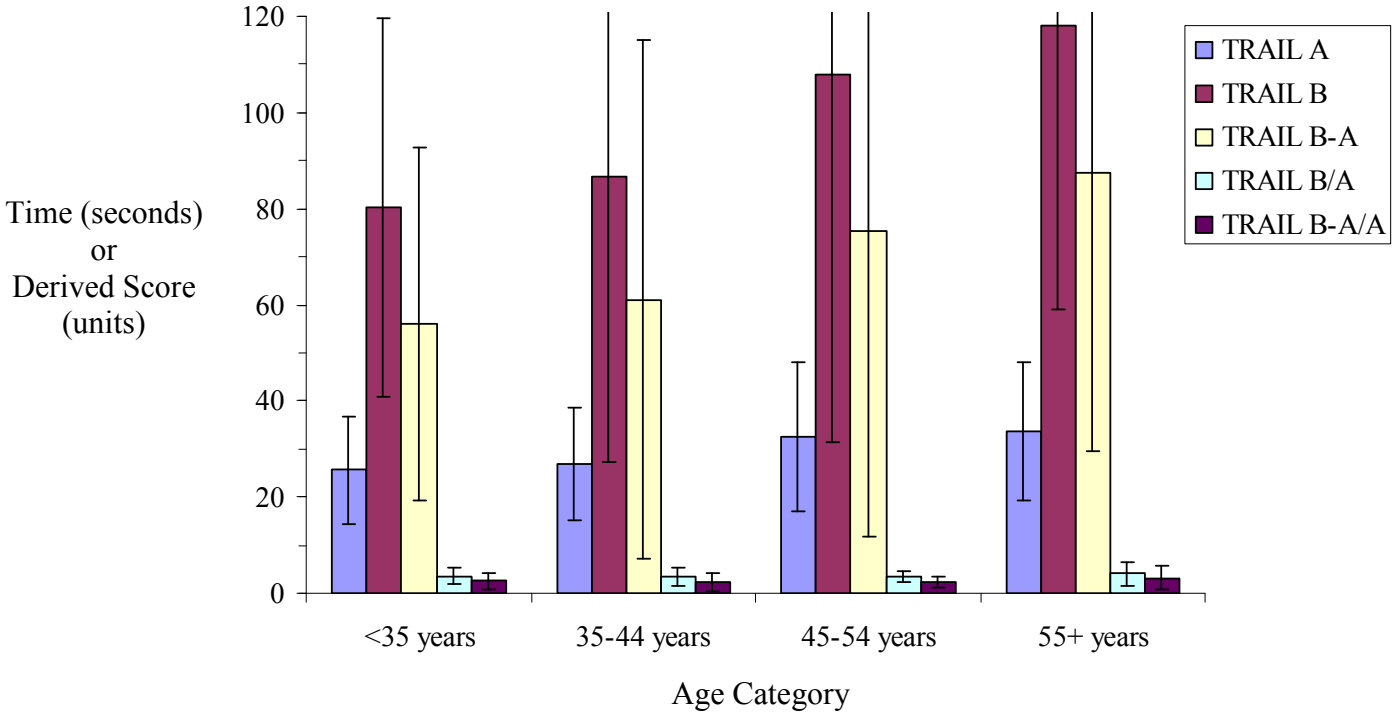


Figure 8. Mean Trail Making Test Times or Derived Scores By Age

The Trail Making Test requires alertness and concentrated attention and is a useful screening test for several types of cognitive impairment regardless of the location of the brain lesion. Part A is a clinical assessment of processing speed and Part B is a clinical assessment of executive function(1). Unlike the Clock Drawing Test, the Trail Making Test has not been dichotomized and fewer studies have included younger age groups. In comparison to 285 North American (NA) adults aged 18-90 years without a history of substance abuse, psychiatric or

neurological disorders, or receiving psychotropic medications, the mean time to completion for the TMT-B in our study was comparable to their 40-49 year old age group (FN: 90.3, SD: 55.8 vs. NA: 81.3, SD: 23.7 seconds); [B-A] in our study was comparable to their 50-59 and 60-69 year old age groups (FN: 63.3, SD: 48.8 vs. NA 50-59 years: 67.2, SD: 39.4 and NA 60-69 years: 65.6, SD: 33.8 seconds); and [B/A] in our study was comparable to their 70-79 year old age group (FN: 3.4, SD: 1.7 vs. NA 70-79 years: 3.5, SD: 1.8 units). Normative data for the [(B-A)/A] does not exist, however a difference of one between the ratio and combined scores allow inferences to be made. Mean time to completion for [(B-A)/A] in our study was comparable to their 70-79 year old age group (FN: 2.4, SD: 1.7 vs. NA 70-79 years: 2.5, SD: n/a units). Mean time to completion for the TMT-A in our study was similar to their 30-39 year old age group (FN: 28.4, SD: 13.0 vs. NA: 28.0, SD: 8.8 seconds)(2). In comparison to another North American population with normative data and additional exclusions for head injury and stroke, the First Nations population was performing similar to their 35-44 year old age group for the TMT-A (NA: 28.5, SD: 10.1 seconds), however our study had an increased average time to completion for TMT-B (NA: 58.5, SD: 16.4 seconds)(3). The strong correlation between times to completion for TMT-A and TMT-B suggests derived measures are appropriate. Consistent with normative data, in our study, there were increased times to completion with increasing age for TMT-A, a relationship that was similar though less marked for TMT-B, a further reduced effect for [B-A], and the absence of an effect for [B/A]. There was no normative data for the relationship between age and [(B-A)/A](4). A similar trend was observed when age was examined as a categorical variable. The overall pattern suggests that the individuals who participated in our study are a representative sample of cognitive function, though given the volunteer nature of the sampling scheme those who participated were less healthy.

Data collected by the Trail Making Test Parts A and B offer a unique opportunity to improve the detection of individuals as having lowered cognitive performance. Theoretically, the combined score provides additional information beyond TMT-A and TMT-B alone, the difference, and ratio scores. TMT-A evaluates processing speed and TMT-B contributes an added component of cognitive flexibility or executive function. The usefulness of the difference and ratio scores are that they attempt to adjust for individual variability by using the individual as their own control(2). The difference score attempts to isolate the cognitive processing effects related to TMT-B and the ratio score attempts to account for the effect of general slowing due to the aging process(5). The combined score utilizes concepts from the difference and ratio scores (e.g. combined score= (difference score) / (ratio score) = [(B - A) / A]), and follows that increased scores reflect poorer cognitive performance. The distribution of the combined score illustrates a bimodal distribution. One break point is observed at 2.33, and another break point at 2.70 units. If a cutoff score of ≥2.33 units is used to classify individuals as having lowered cognitive performance, there are 72 individuals (37.9%), similar to the prevalence noted for the Clock Drawing Test of 43.5% examined in preliminary analysis. If a cutoff of ≥2.70 units is used, there are 52 individuals (27.4%) who are classified as having lowered cognitive performance, slightly lower than the prevalence noted for the Clock Drawing Test. Based on the preliminary analysis, where a break point was detected, a cutpoint of 2.33 will be used for TMT-C.

4.2 VOLUNTEER SAMPLE

Among the 20 volunteers from the U of T community taken as a convenience sample, there were 11 (55%) males and 9 (45%) females who participated. For the Clock Drawing Test,

there were 15 (75%) individuals who scored zero and 5 (25%) individuals who had a score of two, indicating no cognitive decline for the volunteer population. For the Trail Making Test Part A, the average time to completion was 22.6 seconds (SD: 7.0), and 50% or more individuals had times greater than 22.4 seconds (Range: 13.5-45.2 seconds). For the Trail Making Test Part B, the average time was 51.9 seconds (SD: 14.5), and 50% or more individuals had times greater than 48.1 seconds (Range: 34.0-82.2 seconds). The average time for [B-A] was 29.3 seconds (SD: 11.9), and 50% or more individuals had times greater than 25.4 seconds (Range: 16.1-56.8 seconds); the average score for [B/A] was 2.4 units (SD: 0.7), and 50% or more individuals had scores greater than 2.2 units (Range: 1.7-4.2 units); the average score for [(B-A)/A] was 1.4 units (SD: 0.7), and 50% or more individuals had scores greater than 1.2 units (Range: 0.7-3.2 units). There were only three individuals (15%) who scored above 2.33, our designated cutpoint. For the volunteer sample, data indicated that the averages, medians and standard deviations were lower and the ranges were narrower compared to the data for First Nations population examined in preliminary analysis. In conclusion, our cutpoint of 2.33 is acceptable.

4.3 MISSING OR INCOMPLETE DATA

Missing or incomplete data for specific variables was considered in detail and was evaluated through careful statistical analysis in preliminary analyses. The final strategy for handling missing data was as follows: (i) for continuous risk factors, individuals were assigned to the mean value from among the total eligible population (N=510), except where the mean and median values were quite different and then the median value was assigned (Table 4), (ii) for categorical risk factors, individuals with incomplete data were assigned to the referent group (see final tables in Chapters 5 and 6). This method for handling individuals with missing or

incomplete data for specific variables was based on cross-examination of three different datasets for the Clock Drawing Test and the Trail Making Test Combined Score including: (iii) missing or incomplete data at random (Tables 5-8), (iv) individuals with missing or incomplete data deleted (Tables 9 and 10), and (v) individuals with missing or incomplete data assigned (Tables 11 and 12). When primary risk factors were examined across these three different datasets (iii, iv, v) for the Clock Drawing Test and the Trail Making Test Combined Score, the descriptive statistics and odds ratios were similar. Therefore, the dataset that maximized the sample size was chosen for subsequent multivariable analyses and included assigning individuals with missing or incomplete data for a specific variable to a value (see above description (i) and (ii), see final Tables 11 and 12, and final tables in Chapters 5 and 6). The following list of tables review the results for missing or incomplete data for the Clock Drawing Test (CDT) and the Trail Making Test Combined Score (TMT-C, shown below as TMT):

(i) Total population

Table 4. Descriptive Statistics for the Total Population (N=510)

(ii) *see final tables for categorical risk factors in Chapters 5 and 6.

(iii) Missing or incomplete data at random

Table 5. Distribution of Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (N=207)

Table 6. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (N=207)

Table 7. Distribution of Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (N=190)

Table 8. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and

Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (N=190)

(iv) Individuals with missing or incomplete data deleted

Table 9. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (N=154)

Table 10. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (N=141)

(v) Individuals with missing or incomplete data assigned (continuous risk factors)

Table 11. Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (N=207)

Table 12. Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (N=190)

In Table 4, the mean and median values were similar for most variables and therefore the mean value was assigned to individuals with missing or incomplete data. The median value was used for the following variables: triglycerides, glucose, insulin, duration of diabetes, total carotid stenosis, right plaque volume, left plaque volume, and total plaque volume. For the CDT in Table 5, there were no significant differences between CDT(+) and CDT(-) groups for any continuous risk factor. The results were similar for the CDT in Table 11, in which individuals with missing or incomplete data were assigned. Comparisons between groups for continuous risk factors when individuals with missing or incomplete data were deleted are not shown, as the results would be identical to Table 5. For categorical risk factors, results were similar between Table 6 vs. 9, except for sex, in that there were no effects for categorical risk factors when

comparing the different datasets. Similar results are shown in Chapters 5 for the CDT. For the TMT in Table 7, significant differences between TMT(+) and TMT(-) groups were shown for waist circumference, body mass index, left carotid stenosis, and total carotid stenosis. There were no differences for any other continuous risk factor. The results were similar for the TMT in Table 12, in which individuals with missing or incomplete data were assigned. Additional differences in Table 12 were shown for systolic blood pressure and right carotid stenosis. Comparisons between groups for continuous risk factors when individuals with missing or incomplete data were deleted are not shown, as the results would be identical to Table 7. For categorical risk factors, results were similar in that there were effects shown for waist circumference, body mass index, left carotid stenosis, and total carotid stenosis when comparing the different datasets, Table 8 vs. 10. Metabolic syndrome was additionally shown to have an effect in Table 8, though was only borderline in Table 10. Similar results are shown in Chapters 5 and 6 for the TMT. [Note: CDT(+) and TMT(+) refer to lowered cognitive performance, whereas CDT(-) and TMT(-) refer to not having lowered cognitive performance]

Table 4. Descriptive Statistics for the Total Population (N=510)

Variable	Total Population (N=510)
Age (years)	
Mean (SD)	38.5 (12.0)
Median (IQR)	37.0 (18.0)
Min-max	18.0-75.0
Missing	194 (38.0)
Diastolic blood pressure (mmHg)	
Mean (SD)	76.4 (10.4)
Median (IQR)	74.0 (10.0)
Min-max	57.0-128.0
Missing	199 (39.0)
Systolic blood pressure (mmHg)	
Mean (SD)	127.7 (17.2)
Median (IQR)	125.0 (22.0)
Min-max	90.0-200.0
Missing	199 (39.0)
Cholesterol (mmol/L)	
Mean (SD)	4.9 (1.2)
Median (IQR)	4.8 (1.6)
Min-max	2.2-8.4
Missing	224 (43.9)
Triglycerides (mmol/L)	
Mean (SD)	2.2 (2.1)
Median (IQR)	1.7 (1.4)
Min-max	0.3-26.2
Missing	224 (43.9)
Low density lipoprotein (mmol/L)	
Mean (SD)	2.8 (0.9)
Median (IQR)	2.7 (1.1)
Min-max	0.7-5.9
Missing	243 (47.7)
High density lipoprotein (mmol/L)	
Mean (SD)	1.2 (0.3)
Median (IQR)	1.2 (0.4)
Min-max	0.6-2.5
Missing	224 (43.9)

Table 4. *(cont'd)* **Descriptive Characteristics for the Total Population (N=510)**

Variable	Total Population (N=510)
Homocysteine (μmol/L)	
Mean (SD)	9.0 (4.7)
Median (IQR)	8.2 (2.5)
Min-max	4.3-65.8
Missing	224 (43.9)
Glucose (mmol/L)	
Mean (SD)	6.9 (3.5)
Median (IQR)	5.4 (2.1)
Min-max	3.4-20.6
Missing	31 (6.1)
Insulin (*p*mol/L)	
Mean (SD)	122.7 (121.4)
Median (IQR)	96.0 (98.0)
Min-max	7.0-1224.0
Missing	33 (6.5)
Waist circumference (cm)	
Mean (SD)	103.8 (16.4)
Median (IQR)	104.0 (23.0)
Min-max	67.0-196.0
Missing	49 (9.6)
Weight (kg)	
Mean (SD)	88.6 (20.3)
Median (IQR)	86.2 (27.9)
Min-max	43.4-160.0
Missing	44 (8.6)
Height (cm)	
Mean (SD)	167.8 (8.8)
Median (IQR)	167.8 (13.0)
Min-max	141.0-192.0
Missing	44 (8.6)
Duration of diabetes (years)	
Mean (SD)	9.3 (7.6)
Median (IQR)	7.0 (11.0)
Min-max	0.5-35.0
Missing	424 (83.1)
Smoking duration (years)	
Mean (SD)	16.1 (10.1)
Median (IQR)	15.0 (11.0)
Min-max	1.0-55.0
Missing	242 (47.5)

Table 4. *(cont'd)* **Descriptive Statistics for the Total Population (N=510)**

Variable	Total Population (N=510)
Right carotid stenosis (%)	
Mean (SD)	22.5 (10.8)
Median (IQR)	20.0 (10.0)
Min-max	0-50.0
Missing	194 (38)
Left carotid stenosis (%)	
Mean (SD)	22.7 (10.9)
Median (IQR)	20.0 (20.0)
Min-max	0-50.0
Missing	194 (38.0)
Total carotid stenosis (%)	
Mean (SD)	45.1 (19.3)
Median (IQR)	40.0 (30.0)
Min-max	0-100.0
Missing	194 (38)
Right plaque volume (mm^3)	
Mean (SD)	10.2 (15.6)
Median (IQR)	4.6 (12.6)
Min-max	0-112.4
Missing	199 (39)
Left plaque volume (mm^3)	
Mean (SD)	8.5 (10.8)
Median (IQR)	4.6 (9.9)
Min-max	0-59.6
Missing	200 (39.2)
Total plaque volume (mm^3)	
Mean (SD)	18.7 (21.7)
Median (IQR)	11.4 (22.8)
Min-max	0-135.3
Missing	205 (40.2)

Note: SD, standard deviation; IQR, inter-quartile range.

Table 5. Distribution of Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=207)

Variable	CDT(+) (n=90)	CDT(-) (n=117)	*p-value*[1]
Age (years)			
Median (IQR)	40.0 (14.5)	38.0 (19.0)	0.6470
Min-max	19.0-66.0	19.0-65.0	
Missing	22 (24.4)	22 (18.8)	
Diastolic blood pressure (mmHg)			
Median (IQR)	73.0 (12.5)	76.6 (11.0)	0.9177
Min-max	58.0-103.0	60.0-106.0	
Missing	22 (24.4)	22 (18.8)	
Systolic blood pressure (mmHg)			
Median (IQR)	127.5 (14.5)	126.0 (24.0)	0.6149
Min-max	108.0-195.0	92.0-200.0	
Missing	22 (24.4)	22 (18.8)	
Triglycerides (mmol/L)			
Median (IQR)	1.8 (1.8)	1.6 (1.3)	0.2081
Min-max	0.7-9.9	0.4-11.3	
Missing	22 (24.4)	22 (18.8)	
High density lipoprotein (mmol/L)			
Median (IQR)	1.1 (0.4)	1.1 (0.4)	0.6042
Min-max	0.6-2.3	0.7-2.5	
Missing	22 (24.4)	22 (18.8)	
Homocysteine (μmol/L)			
Median (IQR)	8.1 (2.6)	8.4 (2.5)	0.3085
Min-max	4.5-25.8	4.9-30.0	
Missing	22 (24.4)	22 (18.8)	
Glucose (mmol/L)			
Median (IQR)	5.6 (2.8)	5.6 (2.8)	0.7077
Min-max	3.4-19.5	3.5-19.6	
Missing	0 (0)	1 (0.9)	
Insulin resistance[2] (units)			
Median (IQR)	4.6 (5.4)	4.3 (5.0)	0.1857
Min-max	1.0-67.4	0.5-28.8	
Missing	0 (0)	1 (0.9)	

Table 5. *(cont'd)* **Distribution of Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=207)**

Variable	CDT(+) (n=90)	CDT(-) (n=117)	*p-value*
Waist circumference (cm)			
Median (IQR)	106.0 (22.0)	104.0 (26.0)	0.6319
Min-max	67.0-144.0	69.0-150.0	
Missing	3 (3.3)	8 (6.8)	
Body mass index (kg/m^2)			
Median (IQR)	31.8 (10.4)	31.4 (10.2)	0.5428
Min-max	19.2-53.3	19.8-51.1	
Missing	2 (2.2)	4 (3.4)	
Duration of diabetes (years)			
Median (IQR)	5.0 (8.0)	7.0 (8.0)	0.3759
Min-max	0.5-26.0	0.5-27.0	
Missing	69 (76.7)	90 (76.9)	
Smoking duration (years)			
Median (IQR)	16.5 (12.5)	15.0 (14.0)	0.3983
Min-max	1.0-40.0	1.0-40.0	
Missing	34 (37.8)	34 (29.1)	
Right carotid stenosis (%)			
Median (IQR)	20.0 (10.0)	20.0 (20.0)	0.1882
Min-max	10.0-50.0	0-50.0	
Missing	22 (24.4)	22 (18.8)	
Left carotid stenosis (%)			
Median (IQR)	20.0 (20.0)	20.0 (20.0)	0.0974
Min-max	0-50.0	0-50.0	
Missing	22 (24.4)	22 (18.8)	
Total carotid stenosis (%)			
Median (IQR)	40.0 (20.0)	40.0 (30.0)	0.0772
Min-max	10.0-100.0	0-90.0	
Missing	22 (24.4)	22 (18.8)	
Right plaque volume (mm^3)			
Median (IQR)	8.3 (15.6)	5.4 (12.6)	0.5653
Min-max	0-58.9	0-112.4	
Missing	22 (24.4)	24 (20.5)	

Table 5. *(cont'd)* **Distribution of Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=207)**

Variable	CDT(+) (n=90)	CDT(-) (n=117)	*p-value*
Left plaque volume (mm^3)			
Median (IQR)	4.4 (10.6)	5.6 (10.4)	0.3169
Min-max	0-31.8	0-51.6	
Missing	23 (25.6)	24 (20.5)	
Total plaque volume (mm^3)			
Median (IQR)	13.0 (22.0)	12.3 (22.4)	0.7394
Min-max	0-71.1	0-133.1	
Missing	23 (25.6)	26 (22.2)	

[1] *p-value* based on group differences for non-normally distributed variables by the Wilcoxon rank-sum test.
[2] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
Note: CDT, Clock Drawing Test; IQR, inter-quartile range.

Table 6. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=207)

Variable	CDT(+) (n=90)	CDT(-) (n=117)	OR (95% CI)[1]
Age			
<35 years	21	37	1.00
35-44 years	26	24	1.91 (0.88-4.13)
45-54 years	15	25	1.06 (0.46-2.44)
55+ years	6	9	1.18 (0.37-3.76)
Missing	22	22	-
Sex			
Males	33	67	1.00
Females	57	50	**2.31 (1.32-4.07)**
Missing	0	0	-
Hypertension[2]			
No	59	81	1.00
Yes	9	14	0.53 (0.16-1.77)
Missing	22	22	-
History of cardiovascular disease[3]			
No	58	76	1.00
Yes	10	19	0.69 (0.30-1.60)
Missing	22	22	-
Dyslipidemia[4]			
No	18	37	1.00
Yes	50	58	1.77 (0.90-3.49)
Missing	22	22	-
Homocysteine[§]			
<13 mmol/L*	65	91	1.00
≥13 mmol/L	3	4	1.05 (0.23-4.85)
Missing	22	22	-
Glucose[§]			
<7 mmol/L*	66	84	1.00
≥7 mmol/L	24	32	0.96 (0.51-1.77)
Missing	0	1	-
Waist circumference[5]			
Low risk	23	37	1.00
High risk	64	72	1.43 (0.77-2.66)
Missing	3	8	-
Body Mass Index[6]			
Normal	10	18	1.00
Overweight	24	29	1.49 (0.58-3.83)
Obese	54	66	1.47 (0.63-3.46)
Missing	2	4	-

Table 6. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=207)**

Variable	CDT(+) (n=90)	CDT(-) (n=117)	OR (95% CI)
Obese[6]			
No	34	47	1.00
Yes	54	66	1.13 (0.64-2.00)
Missing	2	4	-
Metabolic syndrome[7]			
No	59	77	1.00
Yes	31	40	1.01 (0.57-1.80)
Missing	0	0	-
Insulin resistance[8¥]			
<4.3 units	39	58	1.00
≥4.3 units	51	58	1.31 (0.75-2.27)
Missing	0	1	-
Ever diabetes			
No	47	68	1.00
Yes	21	27	1.13 (0.57-2.22)
Missing	22	22	-
Duration of diabetes[¥]			
<7 years	13	13	1.00
≥7 years	8	14	0.57 (0.18-1.82)
Missing	69	90	-
Ever smoked			
No	10	12	1.00
Yes	58	83	0.84 (0.34-2.07)
Missing	22	22	-
Smoking duration[¥]			
<15 years	22	41	1.00
≥15 years	34	42	1.51 (0.76-3.00)
Missing	34	34	-
Right carotid stenosis[¥]			
<20.0 %	24	28	1.00
≥20.0 %	44	67	0.77 (0.39-1.49)
Missing	22	22	-
Left carotid stenosis[¥]			
<20.0 %	26	30	1.00
≥20.0 %	42	65	0.75 (0.39-1.43)
Missing	22	22	-

Table 6. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=207)**

Variable	CDT(+) (n=90)	CDT(-) (n=117)	OR (95% CI)
Total carotid stenosis[¥]			
<40.0 %	30	35	1.00
≥40.0 %	38	60	0.74 (0.39-1.39)
Missing	22	22	-
Right plaque volume[¥]			
<5.4 mm^3	30	46	1.00
≥5.4 mm^3	38	47	1.24 (0.66-2.32)
Missing	22	24	-
Left plaque volume[¥]			
<5.6 mm^3	38	46	1.00
≥5.6 mm^3	29	47	0.75 (0.40-1.41)
Missing	23	24	-
Total plaque volume[¥]			
<12.3 mm^3	32	45	1.00
≥12.3 mm^3	35	46	1.07 (0.57-2.01)
Missing	23	26	-

[1] Odds ratio (OR); confidence interval (CI).
[2] Hypertension: ≥130/85 mmHg.
[3] History of cardiovascular disease: individuals self-reporting a history of angina, angioplasty/revascularization, myocardial infarction, or claudication. Missing category includes individuals with missing values for all four component variables.
[4] Dyslipidemia: triglycerides ≥1.7 mmol/L or high density lipoprotein <1.0 mmol/L (male) and <1.3 mmol/L (female).
[5] Low risk: <103 cm (male) or <89 cm (female); high risk: >102 cm (male) or >88 cm (female).
[6] Normal weight: 18.5-24.9 kg/m^2; Overweight: 25.0-29.9 kg/m^2; Obese: ≥30.0 kg/m^2.
[7] Metabolic syndrome: three or more of the following including waist circumference: >102 cm (male) or >88 cm (female), triglycerides: ≥1.7 mmol/L, high density lipoprotein: <1.0 mmol/L (male) or <1.3 mmol/L (female), hypertension: ≥130/85 mmHg, fasting plasma glucose: ≥6.1 mmol/L. Missing category includes individuals with missing values for all five component variables.
[8] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
[§] Categorization based on standard clinical criteria.
[¥] Categorization based on median value among CDT(-) individuals.
***** Optimal values according to clinical standards.
Note: CDT, Clock Drawing Test.

Table 7. Distribution of Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=190)

Variable	TMT(+) (n=72)	TMT(-) (n=118)	*p-value*[1]
Age (years)			
Median (IQR)	40.0 (18.0)	37.0 (15.0)	0.5998
Min-max	19.0-66.0	19.0-62.0	
Missing	12 (16.7)	29 (24.6)	
Diastolic blood pressure (mmHg)			
Median (IQR)	79.0 (13.5)	72.0 (10.0)	0.0818
Min-max	60.0-106.0	58.0-106.0	
Missing	12 (16.7)	29 (24.6)	
Systolic blood pressure (mmHg)			
Median (IQR)	130.0 (22.0)	122.0 (18.0)	0.0558
Min-max	98.0-200.0	92.0-190.0	
Missing	12 (16.7)	29 (24.6)	
Triglycerides (mmol/L)			
Median (IQR)	1.9 (1.9)	1.6 (1.4)	0.1994
Min-max	0.7-8.9	0.4-11.3	
Missing	12 (16.7)	29 (24.6)	
High density lipoprotein (mmol/L)			
Median (IQR)	1.1 (0.3)	1.1 (0.3)	0.7998
Min-max	0.7-2.5	0.8-2.3	
Missing	12 (16.7)	29 (24.6)	
Homocysteine (µmol/L)			
Median (IQR)	8.1 (3.0)	8.4 (2.4)	0.8678
Min-max	4.5-20.1	4.9-30.0	
Missing	12 (16.7)	29 (24.6)	
Glucose (mmol/L)			
Median (IQR)	5.7 (3.1)	5.5 (1.7)	0.5483
Min-max	3.7-19.5	3.4-19.6	
Missing	1 (1.4)	0 (0)	
Insulin resistance[2] (units)			
Median (IQR)	5.0 (4.7)	4.4 (5.7)	0.1303
Min-max	1.0-67.4	0.5-33.1	
Missing	1 (1.4)	0 (0)	

Table 7. *(cont'd)* **Distribution of Demographic and Cardiovascular Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=190)**

Variable	TMT(+) (n=72)	TMT(-) (n=118)	*p-value*
Waist circumference (cm)			
Median (IQR)	110.4 (15.3)	102.0 (22.0)	**0.0014**
Min-max	74.0-114.0	67.0-150.0	
Missing	5 (6.9)	5 (4.2)	
Body mass index (kg/m^2)			
Median (IQR)	33.7 (9.4)	30.1 (10.3)	**0.0015**
Min-max	19.8-53.3	19.2-51.1	
Missing	3 (4.2)	2 (1.7)	
Duration of diabetes (years)			
Median (IQR)	5.0 (8.0)	6.0 (10.0)	0.5945
Min-max	0.5-26.0	0.5-25.0	
Missing	53 (73.6)	95 (80.5)	
Smoking duration (years)			
Median (IQR)	15.0 (16.5)	16.0 (11.0)	0.5221
Min-max	2.0-40.0	1.0-40.0	
Missing	24 (33.3)	40 (33.9)	
Right carotid stenosis (%)			
Median (IQR)	20.0 (10.0)	20.0 (20.0)	0.0607
Min-max	0-50.0	0-50.0	
Missing	12 (16.7)	29 (24.6)	
Left carotid stenosis (%)			
Median (IQR)	20.0 (20.0)	20.0 (10.0)	**0.0096**
Min-max	0-40.0	0-50.0	
Missing	12 (16.7)	29 (24.6)	
Total carotid stenosis (%)			
Median (IQR)	30.0 (30.0)	40.0 (30.0)	**0.0070**
Min-max	10.0-80.0	0-100.0	
Missing	12 (16.7)	29 (24.6)	
Right plaque volume (mm^3)			
Median (IQR)	7.6 (18.3)	5.3 (10.4)	0.0528
Min-max	0-112.4	0-102.4	
Missing	13 (18.1)	30 (25.4)	

Table 7. *(cont'd)* **Distribution of Demographic and Cardiovascular Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=190)**

Variable	TMT(+) (n=72)	TMT(-) (n=118)	*p-value*
Left plaque volume (mm^3)			
Median (IQR)	5.3 (13.4)	4.8 (8.4)	0.3951
Min-max	0-42.6	0-51.6	
Missing	12 (16.7)	32 (27.1)	
Total plaque volume (mm^3)			
Median (IQR)	16.5 (31.8)	10.4 (15.8)	0.0956
Min-max	0-133.1	0-103.6	
Missing	13 (18.1)	33 (28.0)	

[1] *p-value* based on group differences for non-normally distributed variables by the Wilcoxon rank-sum test.
[2] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
Note: TMT, Trail Making Test Combined Score; IQR, inter-quartile range.

Table 8. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=190)

Variable	TMT(+) (n=72)	TMT(-) (n=118)	OR (95% CI)[1]
Age			
<35 years	22	33	1.00
35-44 years	17	30	0.85 (0.31-1.90)
45-54 years	16	19	1.26 (0.54-2.97)
55+ years	5	7	1.07 (0.30-3.81)
Missing	12	29	-
Sex			
Males	27	61	1.00
Females	45	57	1.78 (0.98-3.24)
Missing	0	0	-
Hypertension[2]			
No	49	81	1.00
Yes	11	8	2.27 (0.86-6.04)
Missing	12	29	-
History of cardiovascular disease[3]			
No	48	74	1.00
Yes	12	15	1.23 (0.53-2.86)
Missing	12	29	-
Dyslipidemia[4]			
No	16	33	1.00
Yes	44	56	1.62 (0.79-3.32)
Missing	12	29	-
Homocysteine[§]			
<13 µmol/L*	57	86	1.00
≥13 µmol/L	3	3	1.51 (0.29-7.74)
Missing	12	29	-
Glucose[§]			
<7 mmol/L*	47	91	1.00
≥7 mmol/L	24	27	1.72 (0.90-3.31)
Missing	1	0	-
Waist circumference[5]			
Low risk	12	44	1.00
High risk	55	69	**2.92 (1.41-6.07)**
Missing	5	5	-

Table 8. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=190)**

Variable	TMT(+) (n=72)	TMT(-) (n=118)	OR (95% CI)
Body Mass Index[6]			
Normal	5	21	1.00
Overweight	12	36	1.40 (0.43-4.53)
Obese	52	59	**3.70 (1.30-10.52)**
Missing	3	2	-
Obese[6]			
No	17	57	1.00
Yes	52	59	**2.96 (1.53-5.70)**
Missing	3	2	-
Metabolic syndrome[7]			
No	40	83	1.00
Yes	32	35	**1.87 (1.03-3.49)**
Missing	0	0	-
Insulin resistance[8¥]			
<4.4 units	28	59	1.00
≥4.4 units	43	59	1.54 (0.85-2.79)
Missing	1	0	-
Ever diabetes			
No	41	66	1.00
Yes	19	23	1.33 (0.65-2.74)
Missing	12	29	-
Duration of diabetes[¥]			
<6 years	10	11	1.00
≥6 years	9	12	0.83 (0.24-2.79)
Missing	53	95	-
Ever smoked			
No	10	11	1.00
Yes	50	78	0.71 (0.28-1.78)
Missing	12	29	-
Smoking duration[¥]			
<16 years	27	39	1.00
≥16 years	21	39	0.78 (0.38-1.60)
Missing	24	40	-
Right carotid stenosis[¥]			
<20.0 %	24	23	1.00
≥20.0 %	36	66	0.52 (0.26-1.05)
Missing	12	29	-

Table 8. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=190)**

Variable	TMT(+) (n=72)	TMT(-) (n=118)	OR (95% CI)
Left carotid stenosis¥			
<20.0 %	26	22	1.00
≥20.0 %	34	67	**0.43 (0.21-0.87)**
Missing	12	29	-
Total carotid stenosis¥			
<40.0 %	32	26	1.00
≥40.0 %	28	63	**0.36 (0.18-0.72)**
Missing	12	29	-
Right plaque volume¥			
<5.3 mm^3	25	44	1.00
≥5.3 mm^3	34	44	1.36 (0.70-2.64)
Missing	13	30	-
Left plaque volume¥			
<4.8 mm^3	29	43	1.00
≥4.8 mm^3	31	43	1.07 (0.55-2.07)
Missing	12	32	-
Total plaque volume¥			
<10.4 mm^3	23	42	1.00
≥10.4 mm^3	36	43	1.53 (0.78-3.00)
Missing	13	33	-

[1] Odds ratio (OR); confidence interval (CI).
[2] Hypertension: ≥130/85 mmHg.
[3] History of cardiovascular disease: individuals self-reporting a history of angina, angioplasty/revascularization, myocardial infarction, or claudication. Missing category includes individuals with missing values for all four component variables.
[4] Dyslipidemia: triglycerides ≥1.7 mmol/L or high density lipoprotein <1.0 mmol/L (male) and <1.3 mmol/L (female).
[5] Low risk: <103 cm (male) or <89 cm (female); high risk: >102 cm (male) or >88 cm (female).
[6] Normal weight: 18.5-24.9 kg/m^2; Overweight: 25.0-29.9 kg/m^2; Obese: ≥30.0 kg/m^2.
[7] Metabolic syndrome: three or more of the following including waist circumference >102 (male) or >88 (female), triglycerides: ≥1.7 mmol/L, high density lipoprotein: <1.0 mmol/L (male) or <1.3 mmol/L (female), hypertension: ≥130/85 mmHg, fasting plasma glucose: ≥6.1 mmol/L. Missing category includes individuals with missing values for all five component variables.
[8] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
§ Categorization based on standard clinical criteria.
¥ Categorization based on median value among TMT(-) individuals.
* Optimal values according to clinical standards.
Note: TMT, Trail Making Test Combined Score.

Table 9. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=154)

Variable	CDT(+) (n=66)	CDT(-) (n=88)	OR (95% CI)[1]
Age			
<35 years	21	34	1.00
35-44 years	24	24	1.62 (0.74-3.55)
45-54 years	15	23	1.06 (0.45-2.47)
55+ years	6	7	1.39 (0.41-4.69)
Sex			
Males	26	48	1.00
Females	40	40	1.85 (0.97-3.53)
Hypertension[2]			
No	58	76	1.00
Yes	8	12	0.87 (0.34-2.28)
History of cardiovascular disease[3]			
No	56	71	1.00
Yes	10	17	0.75 (0.32-1.76)
Dyslipidemia[4]			
No	18	35	1.00
Yes	48	53	1.76 (0.88-3.51)
Homocysteine[§]			
<13 µmol/L*	63	84	1.00
≥13 µmol/L	3	4	1.00 (0.22-4.63)
Glucose[§]			
<7 mmol/L*	45	61	1.00
≥7 mmol/L	21	27	1.05 (0.53-2.10)
Waist circumference[5]			
Low risk	17	26	1.00
High risk	49	62	1.21 (0.59-2.48)
Body Mass Index[6]			
Normal	8	15	1.00
Overweight	17	22	1.45 (0.50-4.21)
Obese	41	51	1.51 (0.58-3.90)
Obese[6]			
No	25	37	1.00
Yes	41	51	1.19 (0.62-2.29)
Metabolic syndrome[7]			
No	32	47	1.00
Yes	34	41	1.22 (0.64-2.31)

Table 9. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=154)**

Variable	CDT(+) (n=66)	CDT(-) (n=88)	OR (95% CI)
Insulin resistance[8¥]			
<4.2 units	29	43	1.00
≥4.2 units	37	45	1.32 (0.69-2.51)
Ever diabetes			
No	45	64	1.00
Yes	21	24	1.24 (0.62-2.50)
Duration of diabetes[¥]			
Never			1.00
<7 years	13	13	1.42 (0.60-3.36)
≥7 years	8	11	1.03 (0.39-2.78)
Ever smoked			
No	10	11	1.00
Yes	56	77	0.80 (0.32-2.01)
Smoking duration[¥]			
<15 years	33	50	1.00
≥15 years	33	38	1.32 (0.69-2.50)
Right carotid stenosis[¥]			
<20.0 %	23	27	1.00
≥20.0 %	44	64	0.81 (0.41-1.59)
Left carotid stenosis[¥]			
<20.0 %	26	28	1.00
≥20.0 %	41	63	0.70 (0.36-1.36)
Total carotid stenosis[¥]			
<40.0 %	29	33	1.00
≥40.0 %	38	58	0.75 (0.39-1.42)
Right plaque volume[¥]			
<5.4 mm^3	29	44	1.00
≥5.4 mm^3	38	47	1.23 (0.65-2.31)
Left plaque volume[¥]			
<5.6 mm^3	38	46	1.00
≥5.6 mm^3	29	45	0.78 (0.41-1.47)
Total plaque volume[¥]			
<12.3 mm^3	32	45	1.00
≥12.3 mm^3	35	46	1.07 (0.57-2.01)

[1] Odds ratio (OR); confidence interval (CI).
[2] Hypertension: ≥130/85 mmHg.
[3] History of cardiovascular disease: individuals self-reporting a history of angina, angioplasty/revascularization, myocardial infarction, or claudication. Missing category includes individuals with missing values for all four component variables.

[4] Dyslipidemia: triglycerides ≥1.7 mmol/L or high density lipoprotein <1.0 mmol/L (male) and <1.3 mmol/L (female).
[5] Low risk: <103 cm (male) or <89 cm (female); high risk: >102 cm (male) or >88 cm (female).
[6] Normal weight: 18.5-24.9 kg/m^2; Overweight: 25.0-29.9 kg/m^2; Obese: ≥30.0 kg/m^2.
[7] Metabolic syndrome: three or more of the following including waist circumference: >102 cm (male) or >88 cm (female), triglycerides: ≥1.7 mmol/L, high density lipoprotein: <1.0 mmol/L (male) or <1.3 mmol/L (female), hypertension: ≥130/85 mmHg, fasting plasma glucose: ≥6.1 mmol/L.
[8] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
[§] Categorization based on standard clinical criteria.
[¥] Categorization based on median value among CDT(-) individuals.
* Optimal values according to clinical standards.
Note: CDT, Clock Drawing Test.

Table 10. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Combined Score (TMT) (N=141)

Variable	TMT(+) (n=55)	TMT(-) (n=86)	OR (95% CI)[1]
Age			
<35 years	21	31	1.00
35-44 years	17	29	0.87 (0.38-1.96)
45-54 years	14	19	1.09 (0.45-2.64)
55+ years	3	7	0.63 (0.15-2.73)
Sex			
Males	21	43	1.00
Females	34	43	1.62 (0.81-3.22)
Hypertension[2]			
No	46	78	1.00
Yes	9	8	1.91 (0.69-5.29)
History of cardiovascular disease[3]			
No	45	71	1.00
Yes	10	15	1.05 (0.44-2.54)
Dyslipidemia[4]			
No	32	15	1.00
Yes	54	40	1.58 (0.76-3.30)
Homocysteine[§]			
<13 µmol/L*	52	83	1.00
≥13 µmol/L	3	3	1.60 (0.31-8.21)
Glucose[§]			
<7 mmol/L*	35	62	1.00
≥7 mmol/L	20	24	1.48 (0.72-3.04)
Waist circumference[5]			
Low risk	9	30	1.00
High risk	46	56	**2.74 (1.18-6.35)**
Body Mass Index[6]			
Normal	4	17	1.00
Overweight	10	24	1.77 (0.48-6.60)
Obese	41	45	**3.87 (1.20-12.46)**
Obese[6]			
No	14	41	1.00
Yes	41	45	**2.67 (1.27-5.59)**
Metabolic syndrome[7]			
No	22	48	1.00
Yes	33	38	1.90 (0.95-3.77)

Table 10. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=141)**

	Trail Making Test Executive Function Score (n=141)		
Variable	TMT(+) (n=55)	TMT(-) (n=86)	OR (95% CI)
Insulin resistance[8¥]			
<4.4 units	22	41	1.00
≥4.4 units	33	45	1.37 (0.69-2.71)
Ever diabetes			
No	39	63	1.00
Yes	16	23	1.12 (0.53-2.39)
Duration of diabetes[¥]			
<6 years	10	11	1.00
≥6 years	6	12	0.55 (0.15-2.02)
Ever smoked			
No	9	11	1.00
Yes	46	75	0.75 (0.29-1.95)
Smoking duration[¥]			
<16 years	37	48	1.00
≥16 years	18	38	0.62 (0.30-1.25)
Right carotid stenosis[¥]			
<20.0 %	23	22	1.00
≥20.0 %	36	63	0.55 (0.27-1.12)
Left carotid stenosis[¥]			
<20.0 %	25	21	1.00
≥20.0 %	34	64	**0.45 (0.22-0.91)**
Total carotid stenosis[¥]			
<40.0 %	31	24	1.00
≥40.0 %	28	61	**0.36 (0.18-0.71)**
Right plaque volume[¥]			
<5.4 mm^3	25	41	1.00
≥5.4 mm^3	34	44	1.27 (0.65-2.47)
Left plaque volume[¥]			
<4.7 mm^3	29	42	1.00
≥4.7 mm^3	30	43	1.01 (0.52-1.96)
Total plaque volume[¥]			
<10.4 mm^3	23	42	1.00
≥10.4 mm^3	36	43	1.53 (0.78-3.00)

[1] Odds ratio (OR); confidence interval (CI).
[2] Hypertension: ≥130/85 mmHg.
[3] History of cardiovascular disease: individuals self-reporting a history of angina, angioplasty/revascularization, myocardial infarction, or claudication. Missing category includes individuals with missing values for all four component variables.
[4] Dyslipidemia: triglycerides ≥1.7 mmol/L or high density lipoprotein <1.0 mmol/L (male) and <1.3 mmol/L (female).
[5] Low risk: <103 cm (male) or <89 cm (female); high risk: >102 cm (male) or >88 cm (female).

[6] Normal weight: 18.5-24.9 kg/m^2; Overweight: 25.0-29.9 kg/m^2; Obese: ≥30.0 kg/m^2.
[7] Metabolic syndrome: three or more of the following including waist circumference >102 (male) or >88 (female), triglycerides: ≥1.7 mmol/L, high density lipoprotein: <1.0 mmol/L (male) or <1.3 mmol/L (female), hypertension: ≥130/85 mmHg, fasting plasma glucose: ≥6.1 mmol/L.
[8] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
[§] Categorization based on standard clinical criteria.
[¥] Categorization based on median value among TMT(-) individuals.
* Optimal values according to clinical standards.
Note: TMT, Trail Making Test Combined Score.

Table 11. Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=207)

Variable	CDT(+) (n=90)	CDT(-) (n=117)	*p-value*[1]
Age (years)			
Mean (SD)	39.5 (9.4)	38.8 (10.4)	0.6163
Median (IQR)	38.5 (9.0)	38.5 (14.0)	
Min-max	19.0-66.0	19.0-65.0	
Diastolic blood pressure (mmHg)			
Mean (SD)	76.3 (8.9)	76.5 (8.6)	0.9220
Median (IQR)	76.4 (10.0)	76.4 (10.0)	
Min-max	58.0-103.0	60.0-106.0	
Systolic blood pressure (mmHg)			
Mean (SD)	127.1 (12.4)	129.5 (18.0)	0.7451
Median (IQR)	127.7 (10.0)	127.7 (16.0)	
Min-max	108.0-195.0	92.0-200.0	
Triglycerides (mmol/L)			
Mean (SD)	2.2 (1.5)	2.1 (1.7)	0.2046
Median (IQR)	1.7 (0.9)	1.7 (0.7)	
Min-max	0.7-9.9	0.4-11.3	
High density lipoprotein (mmol/L)			
Mean (SD)	1.2 (0.3)	1.2 (0.3)	0.6596
Median (IQR)	1.2 (0.2)	1.2 (0.3)	
Min-max	0.6-2.3	0.7-2.5	
Homocysteine (μmol/L)			
Mean (SD)	8.7 (2.8)	8.9 (3.0)	0.4602
Median (IQR)	8.6 (1.8)	8.9 (1.5)	
Min-max	4.5-25.8	4.9-30.0	
Glucose (mmol/L)			
Mean (SD)	7.1 (3.7)	7.0 (3.2)	0.7009
Median (IQR)	5.6 (2.8)	5.5 (2.7)	
Min-max	3.4-19.5	3.5-19.6	
Insulin resistance[2] (units)			
Mean (SD)	7.0 (9.4)	5.4 (4.8)	0.1760
Median (IQR)	4.6 (5.4)	4.2 (4.8)	
Min-max	1.0-67.4	0.5-28.8	
Waist circumference (cm)			
Mean (SD)	106.0 (14.8)	105.0 (16.2)	0.6155
Median (IQR)	105.0 (21.0)	103.8 (23.0)	
Min-max	67.0-144.0	69.0-150.0	
Body mass index (kg/m^2)			
Mean (SD)	32.8 (7.0)	32.1 (6.9)	0.5397
Median (IQR)	31.7 (10.2)	31.5 (10.0)	
Min-max	19.2-53.3	19.8-51.1	

Table 11. *(cont'd)* **Demographic and Cardiovascular Risk Factors and Cognitive Function by the Clock Drawing Test (CDT) (N=207)**

Variable	CDT(+) (n=90)	CDT(-) (n=117)	*p-value*
Duration of diabetes (years)			
Mean (SD)	7.1 (3.8)	7.4 (3.6)	0.4535
Median (IQR)	7.0 (0)	7.0 (0)	
Min-max	0.5-26.0	0.5-27.0	
Smoking duration (years)			
Mean (SD)	16.5 (7.5)	15.9 (8.9)	0.3608
Median (IQR)	16.1 (5.0)	16.1 (10.0)	
Min-max	1.0-40.0	1.0-40.0	
Right carotid stenosis (%)			
Mean (SD)	20.1 (7.7)	21.8 (10.7)	0.2559
Median (IQR)	20.0 (12.5)	20.0 (10.0)	
Min-max	10.0-50.0	0-50.0	
Left carotid stenosis (%)			
Mean (SD)	20.3 (8.6)	22.6 (10.7)	0.1258
Median (IQR)	20.0 (12.7)	22.7 (20.0)	
Min-max	0-50.0	0-50.0	
Total carotid stenosis (%)			
Mean (SD)	39.1 (14.5)	43.4 (18.4)	0.0703
Median (IQR)	40.0 (10.0)	40.0 (30.0)	
Min-max	10.0-100	0-90.0	
Right plaque volume (mm^3)			
Mean (SD)	8.7 (10.1)	10.9 (18.8)	0.6001
Median (IQR)	4.6 (10.7)	4.6 (6.5)	
Min-max	0-58.9	0-112.4	
Left plaque volume (mm^3)			
Mean (SD)	6.8 (7.3)	8.4 (9.8)	0.3138
Median (IQR)	4.6 (7.0)	4.6 (6.2)	
Min-max	0-31.8	0-51.6	
Total plaque volume (mm^3)			
Mean (SD)	16.0 (14.5)	19.4 (22.8)	0.9286
Median (IQR)	11.4 (13.5)	11.4 (13.6)	
Min-max	0-71.1	0-133.1	

[1] *p-value* based on group differences for non-normally distributed variables by the Wilcoxon rank-sum test.
[2] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
Note: CDT, Clock Drawing Test; IQR, interquartile range; SD, standard deviation.

Table 12. Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=190)

Variable	TMT(+) (n=72)	TMT(-) (n=118)	*p-value*[1]
Age (years)			
Mean (SD)	39.3 (10.9)	38.4 (9.2)	0.4497
Median (IQR)	38.5 (14.5)	38.5 (10.0)	
Min-max	19.0-66.0	19.0-62.0	
Diastolic blood pressure (mmHg)			
Mean (SD)	77.3 (9.1)	75.3 (7.9)	0.0636
Median (IQR)	76.4 (11.0)	76.4 (7.0)	
Min-max	60.0-106.0	58.0-106.0	
Systolic blood pressure (mmHg)			
Mean (SD)	130.6 (18.3)	126.3 (13.8)	**0.0429**
Median (IQR)	127.7 (20.0)	127.7 (10.0)	
Min-max	98.0-200.0	92.0-190.0	
Triglycerides (mmol/L)			
Mean (SD)	2.3 (1.6)	2.1 (1.7)	0.2078
Median (IQR)	1.7 (1.3)	1.7 (0.7)	
Min-max	0.7-8.9	0.4-11.3	
High density lipoprotein (mmol/L)			
Mean (SD)	1.2 (0.3)	1.2 (0.3)	0.4523
Median (IQR)	1.2 (0.2)	1.2 (0.2)	
Min-max	0.7-8.9	0.6-2.3	
Homocysteine (µmol/L)			
Mean (SD)	8.7 (2.3)	9.0 (3.3)	0.6163
Median (IQR)	8.5 (2.1)	9.0 (1.5)	
Min-max	4.5-20.1	4.9-30.0	
Glucose (mmol/L)			
Mean (SD)	7.2 (3.5)	6.9 (3.4)	0.5613
Median (IQR)	5.6 (3.1)	5.5 (1.7)	
Min-max	3.7-19.5	3.4-19.6	
Insulin resistance (units)[2]			
Mean (SD)	7.2 (9.9)	5.6 (5.2)	0.1438
Median (IQR)	5.0 (4.4)	4.4 (5.7)	
Min-max	1.0-67.4	0.5-33.1	
Waist circumference (cm)			
Mean (SD)	110.0 (14.9)	102.7 (15.6)	**0.0016**
Median (IQR)	109.0 (20.0)	102.5 (21.3)	
Min-max	74.0-114.0	67.0-150.0	
Body mass index (kg/m^2)			
Mean (SD)	34.3 (6.4)	31.4 (7.1)	**0.0015**
Median (IQR)	33.6 (8.5)	30.2 (10.2)	
Min-max	19.8-53.3	19.2-51.1	

Table 12. *(cont'd)* **Demographic and Cardiovascular Risk Factors and Cognitive Function by the Trail Making Test Combined Score (TMT) (N=190)**

Variable	TMT(+) (n=72)	TMT(-) (n=118)	*p-value*
Duration of diabetes (years)			
Mean (SD)	7.2 (3.9)	7.3 (3.4)	0.7352
Median (IQR)	7.0 (0)	7.0 (0)	
Min-max	0.5-26.0	0.5-25.0	
Smoking duration (years)			
Mean (SD)	15.8 (8.1)	16.6 (7.9)	0.4257
Median (IQR)	16.1 (10.0)	16.1 (6.0)	
Min-max	2.0-40.0	1.0-40.0	
Right carotid stenosis (%)			
Mean (SD)	19.3 (8.6)	22.0 (9.6)	**0.0327**
Median (IQR)	20.0 (12.5)	22.5 (10.0)	
Min-max	0-50.0	0-50.0	
Left carotid stenosis (%)			
Mean (SD)	19.5 (9.0)	23.5 (10.3)	**0.0061**
Median (IQR)	20.0 (12.7)	22.7 (10.0)	
Min-max	0-40.0	0-50.0	
Total carotid stenosis (%)			
Mean (SD)	38.0 (15.3)	44.2 (17.2)	**0.0047**
Median (IQR)	40.0 (10.0)	40.0 (10.0)	
Min-max	0-40.0	0-100	
Right plaque volume (mm^3)			
Mean (SD)	13.3 (19.7)	7.8 (12.5)	0.0960
Median (IQR)	4.6 (15.0)	4.6 (6.)	
Min-max	0-112.4	0-102.4	
Left plaque volume (mm^3)			
Mean (SD)	8.5 (10.5)	6.6 (8.0)	0.5226
Median (IQR)	4.6 (10.5)	4.6 (4.0)	
Min-max	0-42.6	0-51.6	
Total plaque volume (mm^3)			
Mean (SD)	22.2 (24.6)	14.7 (15.5)	0.0810
Median (IQR)	11.4 (24.2)	11.4 (7.4)	
Min-max	0-133.1	0-103.6	

[1] *p-value* based on group differences for non-normally distributed variables by the Wilcoxon rank-sum test.
[2] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
Note: TMT, Trail Making Test Combined Score; IQR, interquartile range; SD, standard deviation.

4.4 METHODOLOGICAL CONSIDERATIONS

This section describes a number of sensitivity analyses that were performed to examine the robustness of the results for the Clock Drawing Test (CDT) and the Trail Making Test Executive Function Score (TMT-exec). Also, this section provides additional data and describes the rationale for the presentation of results within the manuscript chapters 5 and 6.

To determine the robustness of our criteria for classifying whether an individual has lowered cognitive performance by the CDT, we examined the CDT using different classification strategies. If individuals defined by extreme discordant scores (n=31), e.g. where one rater scored an individual as positive and the other as negative are classified as not having lowered cognitive performance [CDT(-)], the prevalence of lowered cognitive performance by the CDT is reduced to 29% (60/207) from 43.5% (90/207), and if these 31 individuals are excluded from the analysis, the prevalence of lowered cognitive performance by the CDT is reduced to 33.5% (59/176) from 43.5% (90/207). Normative population-based data for the CDT for comparison does not exist and this is the first study that has characterized a First Nations population by the CDT using the Watson scoring method. When continuous risk factors were examined across these different datasets, there were no differences shown between those classified as having lowered cognitive performance and those that were classified as not having lowered cognitive performance. The results were similar to what we used for the main analysis. Therefore, classifying individuals as having lowered cognitive performance [CDT(+)] when one of two raters scored an individual as positive was acceptable and robust.

To determine the robustness of our cutpoint for classifying whether an individual has lowered cognitive performance by the TMT-exec, we examined the TMT-exec using a different cutpoint. To our knowledge, this is the first epidemiology study that characterized a non-clinical

population using the derived score based on the TMT Parts A and B [(B-A)/A, TMT-exec]. In preliminary analysis, we determined 2.33 as an acceptable cutpoint since a break point was shown here when we examined the TMT-exec distribution. Alternatively, a cutpoint of 2.7 was considered. Using a 2.33 cutpoint, there are 37.9% (72/190) individuals classified as having lowered cognitive performance by the TMT-exec. Using a 2.7 cutpoint, there are 27.4% (52/190) individuals classified as having lowered cognitive performance by the TMT-exec. When continuous risk factors were examined across groups, individuals classified as having lowered cognitive performance [TMT-exec(+)] compared to those classified as not having lowered cognitive performance [TMT-exec(-)] using the 2.7 cutpoint, risk factor differences across subgroups were shown for body mass index, waist circumference, left carotid stenosis, and total carotid stenosis. The magnitude and direction of differences were similar to what we used for the main analysis when using a 2.33 cutpoint. Therefore, classifying individuals as TMT-exec(+) using a 2.33 cutpoint was acceptable and robust.

For Chapter 6, the multivariable and SEM results utilize only carotid stenosis as a risk factor and the TMT-exec as the outcome. When plaque volume as a risk factor was examined with TMT-exec as the outcome in unadjusted analysis, there were no associations. Similarly, when carotid atherosclerosis (stenosis and plaque volume) as a risk factor was examined with the CDT as the outcome in unadjusted analysis, there were no associations. In the same chapter, the multivariable results showed associations for left and total carotid stenosis. However, for the SEM analysis, we presented the results for total carotid stenosis (TCS) only, since the results were similar to the results for left carotid stenosis (LCS) using the same SEM model (Model 2, LCS: -0.75 (0.44); TCS: -0.86 (0.40), $p<0.05$ for both). The strong Spearman correlation between left and total carotid stenosis may account for the similar results ($r=0.87$, $p<0.0001$).

The lack of significant results for right carotid stenosis precluded its examination in SEM analysis.

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CHAPTER 5: MANUSCRIPT 1

The following manuscript examines the relationships between cardiovascular risk factors and cognitive function. It has been accepted at Obesity.

Obesity and Lowered Cognitive Performance in a Canadian First Nations Population

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Running Title: Obesity and Cognitive Function

ABSTRACT

The association between obesity, other cardiovascular risk factors and cognitive function in a Canadian First Nations population was investigated using a cross-sectional design. Eligible individuals were aged 18 years or older, without a history of stroke, non-pregnant, with First Nations status, and who had undergone cognitive function assessment by the Clock Drawing Test and Trail Making Test Parts A and B. Parts A and B were combined into an executive function score (TMT-exec). Hypertension, a previous history of cardiovascular disease, dyslipidemia, metabolic syndrome, insulin resistance, and the presence and duration of diabetes were examined in addition to obesity. For TMT-exec only, obese individuals were at an approximately 4-fold increased risk for lowered cognitive performance compared to those who were not obese in multivariable models (odds ratio [OR]: 3.77, 95% confidence interval [CI]: 1.46-9.72) whereas there was no effect for overweight individuals compared to those with a normal weight in unadjusted analysis. Those having an increased waist circumference also had 5 times the risk compared to those without an increased waist circumference (OR: 5.41, 95% CI: 1.83-15.99). Adjusted for age, sex, and insulin resistance, individuals having the metabolic syndrome were at an approximately 4-fold increased risk compared to those without the metabolic syndrome (OR: 3.67, 95% CI: 1.34-10.07). No other cardiovascular risk factors were associated. Obesity and metabolic syndrome were associated with lowered cognitive performance. These results highlight the importance of studying the health effects of obesity beyond traditional disease endpoints, even in a relatively youthful population.

INTRODUCTION

The rising prevalence of obesity is a major public health concern in most developed countries, and increasingly also in many developing countries and societies undergoing rapid social transitions, such as indigenous peoples(1). In addition to the well recognized adverse health effects of obesity(2), emerging evidence points to the fact that cognitive function is also compromised in the presence of obesity(3). Specifically, many studies demonstrate an increased risk of cognitive dysfunction or dementia in obese individuals(4-10). Complicating the understanding of a direct effect of obesity is the fact that underlying vascular and metabolic complications prevalent in obesity likely contribute indirectly in obese individuals since hypertension(11), hyperlipidemia(12-14), metabolic syndrome(15), insulin resistance(16-19), and diabetes(20), are all risk factors for both cognitive decline and dementia. Nevertheless, some studies continue to observe increased risk in obese individuals even after adjustment for these metabolic and vascular factors(8-10).

Current longer term prospective studies indicate that obesity in mid adulthood contributes to dementia risk in older age(8-10). These data are consistent with a life course approach where midlife cerebrovascular damage may be evident in cognitive functions, such as executive function, but with persistent exposure to the neuropathological effects of being overweight or obese, cerebral damage is exacerbated and progression to dementia occurs(21). This chronic model of early dysfunction with progression to dementia is especially disturbing given the increasing prevalence of early onset obesity in many populations.

While many different cognitive domains are compromised in the presence of obesity(22), this study focused on executive function, as early deficits can be associated with progression to dementia, especially vascular dementia(23). The objective of this study was to

examine the association between anthropometric, vascular and metabolic risk factors and cognitive function in a Canadian First Nations population. A positive association between cardiovascular risk factors and lowered cognitive performance was hypothesized, with a gradient of increased risk according to the level of obesity, vascular and metabolic dysfunction.

METHODS AND PROCEDURES

This is a cross-sectional study conducted in a road-accessible First Nations community in southern Manitoba, Canada. The study was approved by the Human Ethics Boards of the University of Manitoba and the University of Toronto, with the approval of, and in partnership with, the particular First Nations community. Eligible individuals were non-pregnant community residents, aged 18 years or older, without a history of stroke, and designated with First Nations status. Recruitment occurred through home visits to each home in the community without sampling and advertisements in the local Health Centre newsletter. All eligible community members were invited to participate(24). Anthropometric, vascular and metabolic data were collected as part of a larger cross-sectional study on diabetes and diabetes complications. After exclusions, there were 510 eligible individuals. Cognitive function was assessed in the context of another study that examined vascular abnormalities and cognitive function. Three cognitive tests were administered by two graduate student research assistants at the research study site. Among individuals with risk factor data, there were 207 eligible individuals with Clock Drawing Test scores and 190 eligible individuals who completed Trail Making Test Parts A and B.

The Clock Drawing Test (CDT) involves individuals having to draw in the numbers of a clock face. According to the Watson scoring method, three clock numbers in a quadrant are

considered correct. Errors in the first to third quadrants are assigned a score of one and errors in the fourth quadrant are assigned a score of four, for a maximum total score of seven(25). Scoring was performed by two raters. Individuals with a CDT score of ≥4 by one of two raters were classified as having lowered cognitive performance and categorized as cases, whereas individuals with a CDT score of <4 by both raters were classified as not having lowered cognitive performance and categorized as controls.

Trail Making Test Part A consists of 25 circles on a sheet of paper. Participants are asked to connect the circles as quickly as possible, beginning with one and continuing in ascending sequence. Time in seconds to test completion is recorded and a maximum of 90 seconds is applied to individuals who cannot complete the test. Trail Making Test Part B involves the subject having to draw a line alternating between numbers and letters in ascending sequence as quickly as possible. Time in seconds to test completion is recorded and a maximum of 300 seconds is applied to individuals who cannot complete the test(26). A derived score was calculated which was then used to classify individuals. Alternative derived scores such as the difference: [B-A] and ratio: [B/A] have also been used in other studies. They attempt to isolate executive functioning related to Part B and account for processing speed related to Part A(27). We designated [(B-A)/A] the Trail Making Test Executive Function Score (TMT-exec) as it combines previous concepts and therefore may be more indicative of executive function. Individuals with TMT-exec≥2.33 were classified as having lowered cognitive performance and categorized as cases, whereas individuals with TMT-exec<2.33 were classified as not having lowered cognitive performance and categorized as controls. The cutpoint was determined from preliminary analysis in which the distribution of TMT-exec was bimodal (see Chapter 4).

Information on risk factors was ascertained through clinical examination and in-person questionnaires administered by trained personnel. Hypertension was defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg(28). Self-report of at least one of the following was used to define a previous history of cardiovascular disease: angina, history of angioplasty or revascularization, myocardial infarction, and peripheral arterial disease. Fasting venous blood samples were collected, stored at -20ºC, and analyzed for triglycerides, high density lipoprotein (HDL), glucose, and insulin. Weight and height were collected and used to calculate body mass index (BMI). Individuals were classified in four categories: underweight, BMI<18.5 kg/m^2; normal weight, BMI: 18.5-24.9 kg/m^2; overweight, BMI: 25.0-29.9 kg/m^2; and obese, BMI≥30.0 kg/m^2(29). Waist circumference to the nearest 0.5 cm was determined at the level of noticeable waist narrowing using an inelastic tape measure. For individuals in which waist narrowing was difficult to identify, an indeterminate waist was approximated by taking the girth at the estimated lateral level of the twelfth or lower floating rib. Metabolic syndrome was defined as ≥3 of the following: waist circumference >102 cm (male) or >88 cm (female), triglycerides ≥1.7 mmol/L, HDL <1.0 mmol/L (male) or <1.3 mmol/L (female), hypertension defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg, and fasting blood glucose ≥6.1 mmol/L(28). Having dyslipidemia and an increased waist circumference was determined according to the NCEP criteria(28). Insulin resistance was examined by the homeostasis model of assessment(30), and previously diagnosed diabetes and duration of diabetes were determined by self-report.

The proportion of individuals classified as cases and controls and who had complete information for cardiovascular risk factors were examined. Incomplete data for continuous cardiovascular risk factors were assigned to the mean value from among the total eligible

population (n=510), except where the mean and median values were quite different and then the median value was assigned. Descriptive statistics and risk estimates using this conservative approach for handling missing data were compared to the strategy of deleting individuals with incomplete information. The results were similar and the former was used for subsequent multivariable analysis. For categorical cardiovascular risk factors, individuals with incomplete data were assigned to the referent group. Unadjusted odds ratios with corresponding 95% confidence intervals were calculated using logistic regression to examine the associations between categories of cardiovascular risk factors and cognitive function defined by the CDT and TMT-exec. Cardiovascular risk factors with corresponding standard criteria were designated into categories based on existing cutpoints, whereas continuous risk factors without standard criteria were categorized according to their median value. The median value was determined from among controls. Multivariable logistic regression models were used to examine the associations between cardiovascular risk factors including hypertension, history of cardiovascular disease, dyslipidemia, obesity, metabolic syndrome, insulin resistance, diabetes, and duration of diabetes and cognitive function defined by the CDT and TMT-exec, while accounting for confounding variables. Potential confounding variables included all cardiovascular risk factors in addition to self-report of ever having smoked (no/yes). Statistical models were built for each risk factor separately with age and sex considered in all models. Confounding variables were included in the final models if $p<0.2$ and/or they were identified in the literature as confounders. Diabetes and duration of diabetes were entered in separate models. When examining the effects of dyslipidemia, obesity, metabolic syndrome, and insulin resistance, those having diabetes or fasting plasma glucose levels ≥7 mmol/L (e.g. undiagnosed diabetes) were excluded. Multivariable models were used to estimate the adjusted odds ratios,

95% confidence intervals, and p-values for each cardiovascular risk factor, while controlling for confounding variables. All analyses were calculated in SAS v. 9.1 (SAS Institute, NC).

RESULTS

For the CDT, 90 of 207 individuals were classified cases (CDT(+): 43.5%), while 117 individuals were classified as controls (CDT(-): 56.5%). Incomplete information for age, hypertension, history of cardiovascular disease, dyslipidemia, diabetes, and smoking predominated among the cardiovascular risk factors and occurred equally often by CDT status (CDT(+), n=22; CDT(-), n=22). A comparison of risk factor values for continuous variables showed no group differences (data not shown, $p>0.05$ for all), and for the 207 individuals, the median age was 39 years (Range: 19-65 years). Table 1 shows the unadjusted odds ratios. Females had a 2-fold increased risk for lowered cognitive performance by the CDT compared to males (odds ratio [OR]: 2.31, 95% confidence interval [CI]: 1.32-4.07). No other risk factors were shown to be significantly associated with the CDT. Table 2 shows the multivariable adjusted odds ratios. All associations were not statistically significant. Covariates for duration of diabetes were similar to those for diabetes, with covariates shown for diabetes only.

For the TMT-exec, 72 of 190 individuals were classified as cases (TMT-exec(+): 37.9%), while 118 individuals were classified as controls (TMT-exec(-): 62.1%). Incomplete information for age, hypertension, history of cardiovascular disease, dyslipidemia, diabetes, and smoking predominated among the cardiovascular risk factors and for TMT-exec(-) (TMT-exec(+), n=12; TMT-exec(-), n=29). For continuous risk factor variables, TMT-exec(+) individuals had a higher median body mass index (TMT-exec(+): 33.6, Interquartile range [IQR]: 8.5 vs. TMT-exec(-): 30.2 kg/m^2, IQR: 10.2, $p=0.0015$) compared to TMT-exec(-)

individuals. There were no individuals who were underweight (Range, TMT-exec: 19.2-53.3 kg/m^2). Group differences were also observed for waist circumference (TMT-exec(+): 109.0, IQR: 20.0 vs. TMT-exec(-): 102.5 cm, IQR: 21.3, p=0.0016) and systolic blood pressure (TMT-exec(+): 127.7, IQR: 20.0 vs. TMT-exec(-): 127.7, IQR: 10.0 mmHg, p=0.0429). No other continuous risk factors showed group differences, and for the 190 individuals, the median age was 38 years (Range: 19-62 years). Examining cardiovascular risk factors across tertiles for TMT-A showed no differences for all continuous variables, except age and systolic blood pressure (p<0.05) (see Appendix 8). Table 3 shows the unadjusted odds ratios. Individuals categorized as having an increased waist circumference were more likely to demonstrate lowered cognitive performance by TMT-exec compared to those without an increased waist circumference (OR: 2.97, 95% CI: 1.44-6.13). Individuals classified as obese (OR: 3.02, 95% CI: 1.57-5.81), and having the metabolic syndrome (OR: 2.58, 95% CI: 1.41-4.73) were more likely to have lowered cognitive performance by TMT-exec compared to those who were not obese and who did not have the metabolic syndrome respectively. When those who were overweight were removed from the referent group, the effect on lowered cognitive performance by TMT-exec for obesity increased and remained statistically significant (OR: 3.79, 95% CI: 1.34-10.73) compared to those with a normal weight. There was no effect for individuals categorized as overweight compared to those with a normal weight. When individuals categorized as having one or two components of the metabolic syndrome were removed from the referent group, those classified with ≥3 components of the metabolic syndrome were at an approximately 4.5-fold increased risk for lowered cognitive performance by TMT-exec (OR: 4.47, 95% CI: 1.21-16.59), compared to those with zero components. The unadjusted odds ratios for all other risk factors were not statistically significant. Table 4 shows the multivariable

adjusted odds ratios. Among individuals without diabetes, being obese was associated with an approximately 4-fold increased risk for lowered cognitive performance by TMT-exec compared to those who were not obese (OR: 3.77, 95% CI: 1.46-9.72, p=0.0061). When individuals categorized with an increased waist circumference were substituted for those who were classified as obese, and waist circumference was examined in the same multivariable model, there was a 5-fold increased risk for lowered cognitive performance by TMT-exec compared to those without an increased waist circumference (OR: 5.41, 95% CI: 1.83-15.99, p=0.0023). Metabolic syndrome was the only other cardiovascular risk factor found to be associated with TMT-exec (OR: 3.67, 95% CI: 1.34-10.07, p=0.0115). Categorizing individuals with an increasing number of components for the metabolic syndrome showed that when those with one or two components were removed from the referent group, there was an 8.5-fold increased risk for those classified with ≥3 components compared to those with zero components, however the confidence interval was wide (OR: 8.49, 95% CI: 1.57-45.86, p=0.0129).

In stratified analysis, an interaction effect for age and obesity and age and waist circumference were examined. Among those without diabetes, a statistically significant increased risk for lowered cognitive performance by TMT-exec was shown for the younger age groups (<39 years), a magnitude of effect that was slightly higher than for the older age groups (≥39 years) for both obesity and waist circumference. However, we were unable to find a significant interaction term due to inadequate power.

DISCUSSION

Our study found that obesity, expressed as body mass index or as a component of the metabolic syndrome was associated with lowered cognitive performance of an executive origin.

The pervasiveness of the obesity epidemic and its link to early onset decreases in cognitive function among a youthful First Nations population is a reason for concern.

This is the first study to shown an association between obesity and lowered cognitive performance of an executive origin. Heterogeneous study designs limit a direct comparison of our results to previous studies. Studies which included global measures of cognitive function were mixed, with no association for obesity in a recent meta-analysis(3). Similarly, in our study, no effect for obesity was shown with the CDT. Our results would have been strengthened had we observed similar results for both cognitive tests however it was expected given moderate agreement between the two tests for case-control cross-classification. Previous studies have shown approximately 2- to 5-fold increased risks for midlife obesity measured by body mass index(8-10) or waist circumference(4) and risk of dementia. Other studies supporting the association did not include fully adjusted analysis(5,7) or examined changes in cognitive performance(6). Metabolic syndrome was also associated and it appears that the increased risk largely operated through obesity.

The underlying biological mechanism leading to lowered cognitive performance of an executive origin is not clear. The contribution of atherosclerosis to silent infarcts(31) and the role of inflammatory factors such as adipocytokines or adipokines produced by abdominal visceral fat to the progression of endothelial dysfunction to atherosclerosis(32) are possibilities. If midlife obesity represents cumulative exposure to altered hormonal, metabolic and inflammatory states from birth to adult life that then predicts cognitive decline and dementia in late life(33), then similar risks observed in our study suggest the accelerated effects of an obesity milieu on lowered cognitive performance, given the youthfulness of the TMT-exec population. The lack of underweight individuals in our study did not allow us to examine the U-

shaped phenomenon of both underweight and obese individuals and an increased risk of dementia as shown in previous studies(3).

The absence of an effect for hypertension, a history of cardiovascular disease, and dyslipidemia, all linked to atherosclerosis(34) highlight the need to include more proximal risk factors. The increased risk for females in both unadjusted and multivariable models is not clear, though is likely as complex as the cardiovascular effects of sex steroids(35). The absence of an increased risk for diabetes and its metabolic counterpart insulin resistance is consistent with glucose dysfunctions affecting cognitive tasks determined by the hippocampus(36), compared to those that are vascular sensitive.

This study has several limitations, including the absence of information on past history of traumatic brain injury(37), and other psychiatric(38), or depressive conditions(39) that may affect cognitive functioning. Use of prevalent cases in the study of obesity cannot rule out reverse causality(40), however a number of prospective studies using dementia(8-10) as an end point would support forward directionality. The generalizability of the results is limited due to the volunteer nature of the population. Analysis of times to completion for Part A of the Trail Making Test with age was consistent with normative data(41), however times were shifted towards poorer performance. For the Trails, the potential for malingering exists(42), whereas population-based estimates for the CDT does not exist.

In conclusion, this study showed an association between obesity, expressed as body mass index or as a component of the metabolic syndrome and lowered cognitive performance in a First Nations population. The results highlight the importance of cognitive function assessment even in a relatively youthful population and the need to investigate the health effects of obesity

beyond traditional disease endpoints but in intermediate states such as decreasing cognitive function.

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DISCLOSURE

The author declared no conflict of interest.

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Table 1. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Lowered Cognitive Performance by the Clock Drawing Test (CDT) (N=207)

	CDT(+), n=90	CDT(-), n=117	
Covariates	n	n	OR (95% CI)
Age			
<35 years	21	37	1.00
35-44 years	48	46	1.84 (0.94-3.60)
45-54 years	15	25	1.06 (0.46-2.44)
55+ years	6	9	1.18 (0.37-3.76)
Sex			
Males	33	67	1.00
Females	57	50	**2.31 (1.32-4.07)**
Hypertension			
No	81	103	1.00
Yes	9	14	0.82 (0.34-1.98)
History of cardiovascular disease			
No	80	98	1.00
Yes	10	19	0.65 (0.28-1.47)
Dyslipidemia			
No	16	32	1.00
Yes	74	85	1.74 (0.89-3.42)
Waist circumference			
Low risk	23	37	1.00
High risk	67	80	1.35 (0.73-2.49)
Body Mass Index			
Normal	10	18	1.00
Overweight	24	29	1.49 (0.58-3.83)
Obese	56	70	1.44 (0.62-3.37)
Obese			
No	34	47	1.00
Yes	56	70	1.11 (0.63-1.94)
Metabolic syndrome			
No	41	65	1.00
Yes	49	52	1.49 (0.86-2.60)

Table 1. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Lowered Cognitive Performance by the Clock Drawing Test (CDT) (N=207)**

	CDT(+), n=90	CDT(-), n=117	
Covariates	n	n	OR (95% CI)
No. metS components			
0	5	14	1.00
1	15	27	1.56 (0.47-5.17)
2	21	24	2.45 (0.76-7.95)
3+	49	52	2.64 (0.88-7.87)
Insulin resistance			
<4.2 units	36	57	1.00
≥4.2 units	54	60	1.43 (0.82-2.49)
Ever smoked			
No	32	34	1.00
Yes	58	83	0.74 (0.41-1.34)
Ever diabetes			
No	69	90	1.00
Yes	21	27	1.01 (0.53-1.95)
Duration of diabetes			
Never	69	90	1.00
<7 years	13	13	1.30 (0.57-2.99)
≥7 years	8	14	0.75 (0.30-1.88)

OR, odds ratio; CI, confidence interval; metS, metabolic syndrome.

Table 2. Multivariable Adjusted Odds Ratios and 95% Confidence Intervals for Cardiovascular Risk Factors and Lowered Cognitive Performance by the Clock Drawing Test

	Total population, n=207	
Model 1	OR (95% CI)	p-value
Age[a]	1.02 (0.99-1.05)	0.3422
Sex	2.52 (1.40-4.53)	*0.0020*
Hypertension	0.80 (0.31-2.09)	0.6513
CVD[b]	0.57 (0.24-1.38)	0.2126
Obese	0.97 (0.53-1.76)	0.9117
Ever smoked	0.71 (0.38-1.33)	0.2867
Model 2		
Age	1.01 (0.98-1.04)	0.3773
Sex	2.42 (1.37-4.28)	*0.0024*
CVD	0.54 (0.23-1.29)	0.1662
Model 3		
Age	1.01 (0.98-1.04)	0.4281
Sex	2.42 (1.37-4.28)	*0.0024*
CVD	0.54 (0.22-1.29)	0.1659
Diabetes	1.05 (0.51-2.19)	0.8873
Duration of diabetes[c]		
<7 years	1.47 (0.60-3.60)	0.3981
≥7 years	0.67 (0.24-1.88)	0.4444
	Among those without diabetes, n=142	
Model 4	OR (95% CI)	p-value
Age	1.03 (0.99-1.08)	0.1541
Sex	3.62 (1.64-8.03)	*0.0015*
Hypertension	2.03 (0.38-10.76)	0.4038
CVD	0.16 (0.03-0.84)	*0.0304*
Dyslipidemia	2.09 (0.78-5.60)	0.1408
Obese	0.55 (0.21-1.41)	0.2119
Insulin resistance	2.13 (0.82-5.59)	0.1225
Ever smoked	0.80 (0.33-1.92)	0.6180
Model 5		
Age	1.03 (0.99-1.07)	0.1317
Sex	3.19 (1.48-6.86)	*0.0031*
CVD	0.15 (0.03-0.78)	*0.0239*
Metabolic syndrome	1.49 (0.61-3.59)	0.3801
Insulin resistance	1.57 (0.69-3.56)	0.2842

Table 2. *(cont'd)* **Multivariable Adjusted Odds Ratios and 95% Confidence Intervals for Cardiovascular Risk Factors and Lowered Cognitive Performance by the Clock Drawing Test**

	Among those without diabetes, n=142	
Model 6	OR (95% CI)	p-value
Age	1.03 (0.99-1.07)	0.1544
Sex	3.06 (1.40-6.69)	*0.0051*
CVD	0.16 (0.03-0.80)	*0.0256*
No. metS components		
1	1.03 (0.27-3.91)	0.9626
2	1.31 (0.34-5.10)	0.6984
3+	1.77 (0.40-7.80)	0.4481
Insulin resistance	1.45 (0.60-3.50)	0.4129

[a] All variables are categorized as specified in Table 1, except for age, which is a continuous variable in years.
[b] CVD, history of cardiovascular disease.
[c] Duration of diabetes was entered in model 3 separately from diabetes. Never having diabetes is the referent group. Covariates not shown.
OR, odds ratio; CI, confidence interval; metS, metabolic syndrome.

Table 3. Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Lowered Cognitive Performance by the Trail Making Test Executive Function Score (TMT-exec) (N=190)

	TMT-exec(+), n=72	TMT-exec(-), n=118	
Covariates	n	n	OR (95% CI)
Age			
<35 years	22	33	1.00
35-44 years	29	59	0.74 (0.37-1.48)
45-54 years	16	19	1.26 (0.54-2.97)
55+ years	5	7	1.07 (0.30-3.81)
Sex			
Males	27	61	1.00
Females	45	57	1.78 (0.98-3.24)
Hypertension			
No	61	110	1.00
Yes	11	8	2.48 (0.95-6.49)
History of cardiovascular disease[3]	60	103	1.00
No	12	15	1.37 (0.60-3.13)
Yes			
Dyslipidemia			
No	13	30	1.00
Yes	59	88	1.55 (0.75-3.21)
Waist circumference			
Low risk	12	44	1.00
High risk	60	74	**2.97 (1.44-6.13)**
Body Mass Index			
Normal	5	21	1.00
Overweight	12	36	1.40 (0.43-4.53)
Obese	55	61	**3.79 (1.34-10.73)**
Obese			
No	17	57	1.00
Yes	55	61	**3.02 (1.57-5.81)**
Metabolic syndrome			
No	26	70	1.00
Yes	46	48	**2.58 (1.41-4.73)**

Table 3. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Demographic and Cardiovascular Risk Factors and Lowered Cognitive Performance by the Trail Making Test Executive Function Score (TMT-exec) (N=190)**

	TMT-exec(+), n=72	TMT-exec(-), n=118	
Covariates	n	n	OR (95% CI)
No. metS components			
0	3	14	1.00
1	10	29	1.61 (0.38-6.79)
2	13	27	2.25 (0.55-9.22)
3+	46	48	**4.47 (1.21-16.59)**
Insulin resistance			
<4.4 units	29	59	1.00
≥4.4 units	43	59	1.48 (0.82-2.68)
Ever smoked			
No	22	40	1.00
Yes	50	78	1.17 (0.62-2.19)
Ever diabetes			
No	53	95	1.00
Yes	19	23	1.48 (0.74-2.97)
Duration of diabetes			
Never	53	95	1.00
<7 years	10	12	1.49 (0.61-3.69)
≥7 years	9	11	1.47 (0.57-3.77)

OR, odds ratio; CI, confidence interval; metS, metabolic syndrome.

Table 4. Multivariable Adjusted Odds Ratios and 95% Confidence Intervals for Cardiovascular Risk Factors and Lowered Cognitive Performance by the Trail Making Test Executive Function Score

	Total population, n=190	
Model 1	OR (95% CI)	p-value
Age[a]	1.00 (0.97-1.04)	0.8760
Sex	1.69 (0.89-3.22)	0.1101
Hypertension	2.46 (0.85-7.13)	0.0964
CVD[b]	1.27 (0.52-3.14)	0.6008
Obese	3.05 (1.43-6.52)	*0.0039*
Insulin resistance	0.73 (0.35-1.51)	0.3939
Ever smoked	0.99 (0.50-1.96)	0.9774
Model 2		
Age	1.00 (0.97-1.03)	0.9688
Sex	1.73 (0.91-3.28)	0.0958
Hypertension	2.39 (0.84-6.81)	0.1035
CVD	1.20 (0.49-2.95)	0.6932
Obese	3.11 (1.45-6.63)	*0.0034*
Diabetes	1.37 (0.60-3.11)	0.4538
Insulin resistance	0.68 (0.32-1.44)	0.3068
Duration of diabetes[c]		
<7 years	1.60 (0.57-4.43)	0.3703
≥7 years	1.14 (0.38-3.43)	0.8179
	Among those without diabetes, n=132	
Model 3	OR (95% CI)	p-value
Age	1.02 (0.97-1.06)	0.4926
Sex	1.99 (0.87-4.56)	0.1027
Hypertension	3.50 (0.52-23.58)	0.1975
CVD	1.24 (0.30-5.05)	0.7648
Dyslipidemia	1.32 (0.46-3.80)	0.6119
Obese	**3.77 (1.46-9.72)**	*0.0061*
Insulin resistance	0.52 (0.20-1.39)	0.1913
Ever smoked	0.79 (0.32-1.99)	0.6209
Model 4		
Age	1.02 (0.98-1.06)	0.4219
Sex	1.47 (0.66-3.26)	0.3438
Metabolic syndrome	**3.67 (1.34-10.07)**	*0.0115*
Insulin resistance	0.61 (0.23-1.61)	0.3155

Table 4. *(cont'd)* **Multivariable Adjusted Odds Ratios and 95% Confidence Intervals for Cardiovascular Risk Factors and Lowered Cognitive Performance by the Trail Making Test Executive Function Score**

	Among those without diabetes, n=132	
Model 5	OR (95% CI)	p-value
Age	1.01 (0.97-1.06)	0.5355
Sex	1.29 (0.56-2.93)	0.5489
No. metS components		
1	1.13 (0.24-5.20)	0.8785
2	3.32 (0.74-14.91)	0.1178
3+	**8.49 (1.57-45.86)**	*0.0129*
Insulin resistance	0.42 (0.15-1.19)	0.1024

[a] All variables are categorized as specified in Table 3, except for age, which is a continuous variable in years.
[b] CVD, history of cardiovascular disease.
[c] Duration of diabetes was entered in model 2 separately from diabetes. Never having diabetes is the referent group. Covariates not shown.
OR, odds ratio; CI, confidence interval; metS, metabolic syndrome.

CHAPTER 6: MANUSCRIPT 2

The following manuscript examines the relationships between the carotid measures and cognitive function and the interrelationships between cardiovascular risk factors, carotid atherosclerosis and cognitive function. It has been accepted to Neuroepidemiology.

Carotid Atherosclerosis and a Reduced Likelihood for Lowered Cognitive Performance in a Canadian First Nations Population

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Background: We investigated the associations among cardiovascular risk factors, carotid atherosclerosis and cognitive function in a Canadian First Nations population.

Methods: Individuals ≥18 years, without stroke, non-pregnant and with First Nations status were assessed by Trail Making Test Parts A and B. Results were combined into an executive function score (TMT-exec). Doppler ultrasonography assessed carotid stenosis and plaque volume. Anthropometric, vascular and metabolic risk factors were assessed by interview, clinical examinations and blood tests.

Results: For 190 individuals with TMT-exec scores, the median age of the population was 39 years. Compared to the referent group, individuals with elevated levels of left carotid stenosis (LCS) and total carotid stenosis (TCS) were less likely to demonstrate lowered cognitive performance (LCS, odds ratio [OR]: 0.47, 95% confidence interval [CI]: 0.24-0.96; TCS, OR: 0.40, 95% CI: 0.20-0.80). No effect was shown for plaque volume. In structural equation modeling, we found that for every 1-unit change in the anthropometric factor in kg/m^2, there was a 0.86-fold decrease in the percent of TCS ($p<0.05$).

Conclusions: Individuals with elevated levels of LCS and TCS were less likely to demonstrate lowered performance. There was some suggestion that TCS mediates the effect of anthropometric risk factors on cognitive function.

Key Words: cognitive function, executive, carotid stenosis, anthropometric, Native Americans

Introduction

An aging population is associated with an increased frequency of cognitive decline and dementia.[1] An early indication of cognitive decline and dementia is lowered performance on tests of executive function,[2,3] however measurement of executive function in pre-dementia disorders is still developing.[4] The association between a number of cardiovascular risk factors measured at midlife and diseases of cognition in late life suggest a common mediating biological mechanism, such as the apolipoprotein E ε4 allele.[5] The role for small platelet aggregates or cholesterol microemboli shed from atherosclerotic lesions in the carotid arteries implicated among symptomatic and asymptomatic individuals in the etiology of stroke,[6] may also affect cognitive function. Atherosclerosis is a systemic vascular disease of the arteries that develops through an insidious process, is initiated during early adolescence and requires prolonged exposure to predisposing factors.[7] It is not known whether cardiovascular risk factors exert their effects on cognitive function by mechanisms involving carotid atherosclerosis.[8] Recent studies have linked carotid atherosclerosis with a number of anthropometric and vascular,[9-11] and metabolic[12-14] risk factors. In epidemiological studies, the relationship between carotid atherosclerosis and cognitive decline and dementia remains unclear. Two large reviews of the relationship could not provide a definite conclusion due to a number of methodological limitations including the use of symptomatic populations, lack of a control group and poorly characterized measures of atherosclerosis,[15] and lack of a well-defined study population.[16] Recent investigations have also been limited in that they have not included cardiovascular risk factors as confounders in their multivariable analysis.[17-19] Only one previous study had information on cardiovascular risk factors, carotid atherosclerosis and cognitive function. In this

study, carotid stenosis was associated with an approximately 7-fold increased risk for cognitive impairment.[20]

The objectives of this study were to investigate the interrelationships between cardiovascular risk factors, carotid atherosclerosis and cognitive function. We hypothesize that an increased burden of carotid atherosclerosis would be associated with an increased risk for lowered cognitive performance and that carotid atherosclerosis mediates the effect of anthropometric risk factors on cognitive function.

Materials and Methods

This was a cross-sectional study conducted in a road-accessible Plains Ojibwa First Nations community in Southern Manitoba, Canada. Eligible individuals were community residents 18 years of age or older, without stroke, non-pregnant, and designated with First Nations status. Recruitment occurred through home visits to each home in the community without sampling and advertisements in the local Health Centre newsletter. All eligible community members were invited to participate. Demographic, anthropometric, vascular, and metabolic data were collected as part of a larger cross-sectional study on diabetes and diabetes complications.[21] After exclusions, there were 510 eligible individuals. Measures of carotid atherosclerosis and cognitive function were assessed among a subset of the participants. Cognitive tests were administered by two graduate student research assistants at the research study site. A total of 190 individuals completed the Trail Making Test Parts A and B, a majority of whom (n=148) had information on risk factors and carotid atherosclerosis. The study was approved by the Human Ethics Boards of the University of Manitoba and the University of

Toronto. The study was conducted with the approval of, and in partnership with, the particular First Nation community. Individuals who participated provided informed consent.

Trail Making Test Parts A and B were administered according to standard protocols.[22] A measure of executive function was calculated [(B-A)/A],[23] which we designated the Trail Making Test Executive Function Score (TMT-exec). Those with TMT-exec≥2.33 were classified as having lowered cognitive performance [TMT-exec(+)], whereas individuals with TMT-exec<2.33 were classified as not having lowered cognitive performance [TMT-exec(-)]. The cutpoint was determined from preliminary analyses in which the distribution of TMT-exec was bimodal (see Chapter 4).

For carotid plaque volume, a series of parallel two dimensional images were collected and used to reconstruct a three dimensional image.[24,25] Values for the right and left sides of the neck were determined in millimeters cubed (mm^3) and total plaque volume was calculated as the sum of both sides. Peak systolic velocity of the right and left internal carotid arteries was used to estimate the percent stenosis.[26] Percent carotid stenosis was determined for the right and left sides of the neck and total carotid stenosis was calculated as the sum of both sides.

Information on risk factors was ascertained through clinical examination and in-person questionnaires administered by trained personnel. Hypertension was defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg.[27] Self-report of at least one of the following was used to define a previous history of cardiovascular disease: angina, history of angioplasty or revascularization, myocardial infarction, and peripheral arterial disease. Weight and height were collected and used to calculate body mass index (BMI). Individuals were classified as obese if BMI ≥30 kg/m^2.[28] Waist circumference was determined at the level of noticeable waist narrowing using an inelastic tape measure. For individuals in whom waist

narrowing was difficult to identify, an indeterminate waist was approximated by taking the girth at the estimated lateral level of the twelfth or lower floating rib. An increased waist circumference and the presence of dyslipidemia were defined according to standard criteria.[27] Fasting venous blood samples were collected, stored at -20ºC and analyzed for triglycerides, cholesterol, high and low density lipoproteins, glucose, insulin, and total homocysteine (tHcy). Insulin resistance was examined by the homeostasis model of assessment,[29] and ever having smoked and duration of smoking from self-reports.

The proportion of individuals classified as having lowered cognitive performance was examined. For risk factor information and carotid atherosclerosis, individuals lacking complete data for specific variables were reassigned a value for each variable. Continuous variables were assigned to the mean value from among the total eligible population (n=510), except where the mean and median values were quite different and then the median value was assigned. Descriptive statistics and risk estimates using this approach for handling missing data were compared to the strategy of deleting individuals with incomplete information. The results were similar and the former was used for subsequent analyses. For categorical variables, individuals were assigned to the referent group. For descriptive analyses, each continuous variable was examined for its distribution and normality, and non-normal variables were log-transformed using the natural logarithm, when appropriate, for the structural equation modeling analysis. Descriptive analysis showed similar results for smoking duration when those who never smoked were assigned a smoking duration of zero years compared to those assigned to the mean value from among the total population, with the latter used for subsequent analysis and log-transformed for structural equation modeling analysis. Unadjusted odds ratios with corresponding 95% confidence intervals were calculated using logistic regression to examine

the associations between categories of carotid atherosclerosis and TMT-exec. Categories of risk factors were also examined in unadjusted analysis. Categories were based on cutpoints according to standard criteria (e.g. BMI≥30 kg/m^2 for obese) or the median value among TMT-exec(-) individuals (e.g. carotid measures), as determined from preliminary analysis. Multivariable logistic regression models were used to examine the associations between right (RCS), left (LCS) and total (TCS) carotid stenosis and right (RPV), left (LPV) and total (TPV) plaque volume and TMT-exec, while accounting for confounding variables that have been identified in the literature. Potential confounders included hypertension, a history of cardiovascular disease, dyslipidemia, obesity, insulin resistance, diabetes, homocysteine, and ever smoke. Statistical models were built for the right and left sides and the total, with age and sex considered in all models. Multivariable models were used to calculate the adjusted odds ratios, 95% confidence intervals and p-values.

A structural equation modeling (SEM) analysis was conducted to examine the *a priori* specified network of interrelationships between cardiovascular risk factors, TCS and TMT-exec as shown in a SEM model (Figure 1). This multivariate modeling technique is a useful tool that allows complex theoretical relationships to be statistically modeled.[30,31] Path coefficients and factor loadings with their corresponding standard errors and p-values were determined for the SEM model. Chi-square statistics were reviewed to examine model fit. The latent variables included in the model and labeled as anthropometric, pro-atherogenic and vascular were pre-determined using a data-driven approach of principal components analysis.[31] The amount of

mediation was determined from two different SEM models.[32] All analyses were calculated in SAS v. 9.1 (SAS Institute, NC).

Results

The descriptive characteristics are shown in Table 1. The median age of the sample was 38.5 years and 54% were female. Except for waist circumference, all risk factors were non-normally distributed. Overall, the data indicated that while the median values were not exceptional, the range of values exceeded optimal levels according to standard criteria,[28] for example, BMI was as high as 53.3 kg/m^2. Examining the median and interquartile range (IQR) for RCS and LCS suggested mild stenosis (RCS: 21.0, IQR: 9.3%; LCS: 22.0, IQR: 10.0%), and for TCS, moderate stenosis (TCS: 41.8, IQR: 16.7%).[6]

There were 72 individuals who were classified as TMT-exec(+) (37.9%), while 118 individuals were classified as TMT-exec(-) (62.1%). Table 2 shows the unadjusted odds ratios for risk factors and carotid atherosclerosis in relation to TMT-exec. Individuals classified as having LCS of ≥22.7% were less likely to demonstrate lowered cognitive performance compared to those with LCS of <22.7% (unadjusted odds ratio [OR]: 0.47, 95% confidence interval [CI]: 0.26-0.85). A similar effect was shown for TCS, where individuals classified as having TCS of ≥40% were less likely to demonstrate lowered cognitive performance compared to those with TCS of <40% (OR: 0.35, 95% CI: 0.19-0.67). There were no effects for RCS or plaque volume. Among TMT-exec(-), there were a number of individuals with TCS values defined by the 40% cutpoint. When we changed who was included in the referent group and examined the association for TCS of >40% compared to those with TCS of ≤40%, there was no effect. For the covariates, those classified as obese had a 3-fold increased risk for lowered

cognitive performance (OR: 3.02, 95% CI: 1.57-5.81). Increased waist circumference was the only other risk factor that was significantly associated with the outcome (OR: 2.97, 95% CI: 1.44-6.13).

Table 3 shows the multivariable odds ratios for right, left and total carotid stenosis and TMT-exec. Individuals with elevated levels of LCS and TCS were less likely to demonstrate lowered cognitive performance (LCS, OR: 0.47, 95% CI: 0.24-0.96; TCS, OR: 0.40, 95% CI: 0.20-0.80). There were no effects for RCS. There were also no effects for plaque volume (data not shown).

Table 4 shows the SEM results for the interrelationships between cardiovascular risk factors, total carotid stenosis and TMT-exec. All variables were log-transformed except age, TMT-exec and TCS. The chi-square statistic for model 1 was 234.3 ($p<0.0001$) and for model 2, it was 256.5 ($p<0.0001$) suggesting that our theoretical model was not consistent with our dataset. In model 1, no factor was associated with TMT-exec. For TMT-exec in model 2, no associations were shown. For TCS in model 2, the anthropometric factor was associated with a decrease (0.86 times, $p<0.05$) in the percent of TCS. Therefore, for every 1-unit change in the anthropometric factor standardized to kg/m^2, there was a 0.86-fold decrease in the percent of TCS when adjusted for age, and pro-atherogenic and vascular factors. No other factor was associated with TCS. Factor loadings were similar in magnitude and significant across models. The total effect of the anthropometric factor on TMT-exec was 65% and the amount of mediation was 31%, though not all path coefficients were significant. No modifications were performed. The final model with standardized estimates is shown in Figure 1.

Discussion

Our study found that individuals with elevated levels of LCS and TCS were less likely to demonstrate lowered performance on a test of executive function suggesting that carotid stenosis is not detrimental to cognitive functioning. The role of TCS in cognitive functioning may additionally include acting as a mediator, whereby anthropometric risk factors affect the levels of TCS, which in turn have the potential to influence cognitive functioning.

This study showed that higher levels of LCS and TCS were associated with a decreased likelihood for lowered cognitive performance of an executive origin. The absence of an association between carotid measures and the Trail Making Test Part A also suggest that the results for TMT-exec are attributed to executive function and not processing speed. The Cardiovascular Heart Study is the only previous study that also had detailed information on carotid atherosclerosis, cardiovascular risk factors and cognitive function.[20] For the cross-sectional component, they showed that LCS of ≥75% was associated with an approximately 7-fold increased risk for cognitive impairment in multivariable analysis. This study differs from ours in the use of no stenosis as the referent group, a higher cutpoint, an older population, and a global measure of cognitive function (i.e. MMSE). In our study, there was only one individual with zero TCS and only eight individuals with TCS above 80%. Information for both sides of the neck suggested that the effect shown for TCS was predominately accounted for by LCS. The lack of consistent results between carotid stenosis and plaque volume was expected given their weak correlation. Also contributing to the different results is the advanced nature of stenosis compared to plaque volume.[33]

We anticipated that increased levels of carotid atherosclerosis would be associated with an increased risk for lowered cognitive performance. Our results therefore are contradictory to

our *a priori* hypothesis. One reason we observed the opposite effect may be due to the phenomenon of compensatory vessel enlargement, where up to 40% stenosis, the lumen area and vessel wall increases to maintain a normal diameter in response to increases in plaque.[34] In our study population, carotid stenosis values at or slightly above this threshold may be better at triggering compensatory enlargement than lower values and may explain why no effect was shown for TCS of >40% relative to ≤40%, compared to ≥40% relative to <40%. Also contributing to the paradoxical results is the youthfulness of our study population, which precluded individuals with severe stenosis (≥75%) and those who would be symptomatic. Inclusion of a predominately asymptomatic study population suggests that our results are consistent with the notion that individuals with mild to moderate stenosis may have a healthier vascular microenvironment than individuals with higher values, therefore would be expected to perform better on tests of cognitive function of an executive origin. Age-associated changes in brain structure and function beginning at midlife with concomitant effects shown for midlife cardiovascular risk factors on diseases of cognition in late life highlight the presence of a latency effect, with their interrelationships across the life course not known.[5] Had we conducted a longitudinal study with an increasing aging population, an association between moderate-severe levels of carotid stenosis and an increased risk for lowered cognitive performance may have been detected. Although our results were in the opposite direction from anticipated, our study supports the role of vascular factors and disease in cognitive function of an executive origin.

The associations shown for the anthropometric risk factors were varied depending on the outcome involved and may have resulted due to a number of methodological reasons. First, our observation that the anthropometric factor was negatively associated with TCS is in contrast to

previous findings that showed a positive association.[11] Reasons include the use of different measures of atherosclerosis, which reflect different stages of the disease.[35] Excess adiposity may have a role in the very early stages of atherosclerosis such as with intima-media thickness and not carotid stenosis due to the inflammatory effects of adipokines.[36] Second, the First Nations population provided unique data, unlike previous studies. As in other First Nations populations in Canada undergoing rapid socioeconomic and lifestyle changes leading to increasingly prevalent health problems and chronic diseases such as diabetes, this population included individuals who were extremely obese. Therefore, increased levels of BMI and lower levels of TCS resulted in an overall negative correlation in our study. Third, consistent with a number of previous studies,[37] obesity and waist circumference and an increased risk for lowered cognitive performance was shown in our unadjusted analysis. Similar risks suggest the accelerated effect of an obesity milieu,[38] given the median age was 39 years in our study population. Finally, obesity influences atherosclerosis through a number of complex mechanisms including diabetes, hypertension and lipoproteins,[36] therefore alternative SEM models may be needed.

This study has several limitations, including the absence of information on past history of traumatic brain injury,[39] psychiatric[40] or depressive conditions,[41] and alcohol use[42] that may affect cognitive functioning. Temporality is an issue when using a cross-sectional design to examine etiology, though the influence of lowered cognitive performance on carotid stenosis is unlikely. Limitations to the generalizability of the results include the social and cultural context of the Manitoba First Nations population. Analysis of times to completion for Part A of the Trail Making Test with age was consistent with normative data,[43] suggesting our sample is representative. However, in comparison to a similarly aged population, times for Trail Making

Test Part B were slightly higher than for the general population, indicating that our volunteer sample may be less healthy.[44] There is no normative data for TMT-exec.

Conclusion

In conclusion, this study showed that individuals with elevated levels of LCS and TCS were less likely to demonstrate lowered cognitive performance. Our results do not support the hypothesis of carotid atherosclerosis being associated with an increased risk for lowered cognitive performance. However, given a plausible biological mechanism, our results support the role of vascular factors and disease in cognitive function of an executive origin. Also, this study showed that anthropometric risk factors were associated with a decrease in the percent of TCS but an increased risk for lowered cognitive performance. Our findings highlight the importance of careful consideration when examining complex biological relationships.

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Table 1. Descriptive Characteristics (N=190)

	Mean	SD	Median	IQR	Range	%
Age (years)	38.8	9.9	38.5	12	19-66	-
Sex (female)	-	-	-	-	-	53.7
Systolic blood pressure (mmHg)	127.9	15.7	127.7	12	92-200	
Diastolic blood pressure (mmHg)	76.1	8.4	76.4	9	58-106	
Body mass index (kg/m^2)	32.5	7	31.5	9.8	19.2-53.3	-
Waist circumference (cm)	105.4	15.7	104	23	67-150	-
Insulin resistance (units)	6.2	7.3	4.5	5.2	0.5-67.4	-
Triglycerides (mmol/L)	2.2	1.7	1.7	1	0.4-11.3	-
Cholesterol (mmol/L)	5	1.1	4.9	1	2.2-8.4	-
High density lipoprotein (mmol/L)	1.2	0.3	1.2	0.2	0.6-2.5	-
Low density lipoprotein (mmol/L)	2.8	0.8	2.8	0.5	0.8-5.9	-
Smoking duration (years)	16.3	8	16.1	10	1-40	-
Homocysteine (μmol/L)	8.9	2.9	8.9	1.7	4.5-30	-
Right carotid stenosis (%)	21	9.3	20	2.5	0-50	-
Left carotid stenosis (%)	22	10	22.7	20	0-50	-
Total carotid stenosis (%)	41.8	16.7	40	20	0-100	-
Right plaque volume (mm^3)	9.9	15.8	4.6	8.6	0-112.4	-
Left plaque volume (mm^3)	7.3	8.7	4.6	5.9	0-51.6	-
Total plaque volume (mm^3)	17.5	19.8	11.4	11.6	0-133.1	-

SD, standard deviation; IQR, interquartile range.

Table 2. Unadjusted Odds Ratios and 95% Confidence Intervals for Risk Factors and Carotid Atherosclerosis with TMT-exec

Covariates	TMT-exec(+), n=72	TMT-exec(-), n=118	OR (95% CI)
Age			
<35 years	22	33	1.00
35-44 years	29	59	0.74 (0.37-1.48)
45-54 years	16	19	1.26 (0.54-2.97)
55+ years	5	7	1.07 (0.30-3.81)
Sex			
Males	27	61	1.00
Females	45	57	1.78 (0.98-3.24)
Hypertension			
No	61	110	1.00
Yes	11	8	2.48 (0.95-6.49)
History of cardiovascular disease			
No	60	103	1.00
Yes	12	15	1.37 (0.60-3.13)
Dyslipidemia			
No	13	30	1.00
Yes	59	88	1.55 (0.75-3.21)
Obese			
No	17	57	1.00
Yes	55	61	3.02 (1.57-5.81)*
Ever smoked			
No	22	40	1.00
Yes	50	78	1.17 (0.62-2.19)
Right carotid stenosis			
<22.5%	46	58	1.00
≥22.5%	26	60	0.55 (0.30-1.00)
Left carotid stenosis			
<22.7%	44	50	1.00
≥22.7%	28	68	0.47 (0.26-0.85)*
Total carotid stenosis			
<40.0%	32	26	1.00
≥40.0%	40	92	0.35 (0.19-0.67)*
Right plaque volume			
<4.6 mm^3	24	40	1.00
≥4.6 mm^3	48	78	1.03 (0.55-1.91)
Left plaque volume			
<4.6 mm^3	29	42	1.00
≥4.6 mm^3	43	76	0.82 (0.45-1.50)

Table 2. *(cont'd)* **Unadjusted Odds Ratios and 95% Confidence Intervals for Risk Factors and Carotid Atherosclerosis with TMT-exec**

Covariates	TMT-exec(+), n=72	TMT-exec(-), n=118	OR (95% CI)
Total plaque volume			
<11.4 mm^3	24	46	1.00
≥ 11.4 mm^3	48	72	1.28 (0.69-2.36)

*$p<0.05$; TMT-exec, Trail Making Test Executive Function Score; OR, odds ratio; CI, confidence interval.

Table 3. Multivariable Adjusted Odds Ratios and 95% Confidence Intervals for Carotid Stenosis and TMT-exec

	OR (95% CI)	p-value
Age*	1.00 (0.97-1.03)	0.9321
Sex	1.67 (0.88-3.17)	0.1194
Right carotid stenosis	0.61 (0.30-1.24)	0.1739
Hypertension	2.27 (0.80-6.42)	0.1236
CVD	1.21 (0.50-2.94)	0.6743
Obesity	2.46 (1.25-4.84)	*0.0093*
Ever smoked	0.81 (0.38-1.72)	0.5837
Age	1.00 (0.97-1.04)	0.9010
Sex	1.87 (0.97-3.62)	0.0625
Left carotid stenosis	0.47 (0.24-0.96)	0.0368
Hypertension	2.20 (0.77-6.29)	0.1399
CVD	1.13 (0.46-2.79)	0.7919
Obesity	2.30 (1.16-4.56)	*0.0173*
Ever smoked	0.73 (0.35-1.55)	0.4182
Age	1.00 (0.97-1.03)	0.9084
Sex	1.68 (0.88-3.23)	0.1193
Total carotid stenosis	0.40 (0.20-0.80)	0.0092
Hypertension	2.05 (0.71-5.90)	0.1847
CVD	1.17 (0.47-2.90)	0.7431
Obesity	2.38 (1.20-4.72)	0.0129
Ever smoked	0.83 (0.41-1.68)	0.5973

*Age is a continuous variable. Remaining variables are categorical as in Table 2.
TMT-exec, Trail Making Test Executive Function Score; OR, odds ratio; CI, confidence interval; CVD, history of cardiovascular disease.

Table 4. Structural Equation Models

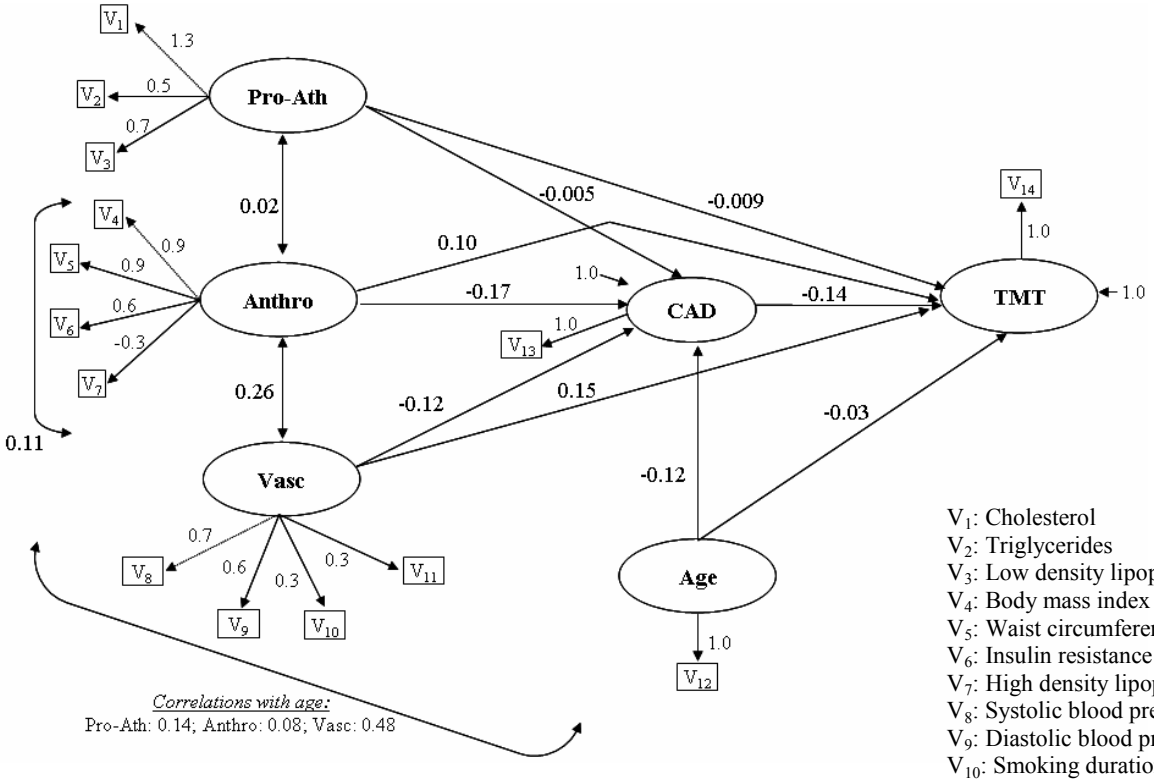
	Model 1	Model 2
Path Models	Path Coefficients (SE)	
TMT-exec (units)		
Age (years)	0.02 (0.04)	-0.003 (0.009)
Anthropometric (kg/m^2)	0.84 (0.78)	0.53 (0.42)
Pro-atherogenic (mmol/L)	0.06 (0.17)	0.03 (0.17)
Vascular (mmHg)	-3.26 (11.73)	1.82 (1.29)
TCS (%)	-	-0.14 (0.08)
TCS (%)		
Age (years)	-	-0.01 (0.009)
Anthropometric (kg/m^2)	-	-0.86 (0.40)*
Pro-atherogenic (mmol/L)	-	-0.02 (0.17)
Vascular (mmHg)	-	-1.44 (1.39)
Measurement Model	Factor Loadings (SE)	
Anthropometric (kg/m^2)		
Body mass index	1.00	1.00
Waist circumference	0.71 (0.04)***	0.72 (0.04)***
Insulin resistance	2.69 (0.25)***	2.70 (0.26)***
High density lipoprotein	-0.35 (0.08)***	-0.36 (0.08)***
Pro-atherogenic (mmol/L)		
Cholesterol	1.00	1.00
Triglycerides	0.93 (0.18)***	0.94 (0.18)***
Low density lipoprotein	0.72 (0.10)***	0.72 (0.10)***
Vascular (mmHg)		
Systolic blood pressure	1.00	1.00
Diastolic blood pressure	0.57 (0.23)*	0.73 (0.15)***
Smoking duration	9.40 (2.21)***	2.14 (0.75)**
Homocysteine	1.65 (0.57)**	0.82 (0.28)**

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. Estimates shown are not standardized.
SE, standard error; TMT-exec, Trail Making Test Executive Function Score; TCS, total carotid stenosis.

Figure Legends

V_1: Cholesterol
V_2: Triglycerides
V_3: Low density lipoprotein
V_4: Body mass index
V_5: Waist circumference
V_6: Insulin resistance
V_7: High density lipoprotein
V_8: Systolic blood pressure
V_9: Diastolic blood pressure
V_{10}: Smoking duration
V_{11}: Homocysteine
V_{12}: Age
V_{13}: Total carotid stenosis (CAD)
V_{14}: Trail Making Test Executive Function Score (TMT)

Pro-Ath, Pro-atherogenic; Anthro, Anthropometric; Vasc, Vascular.



Pro-Ath, Pro-atherogenic; Anthro, Anthropometric; Vasc, Vascular.

Legend

V_1: Cholesterol
V_2: Triglycerides
V_3: Low density lipoprotein
V_4: Body mass index
V_5: Waist circumference
V_6: Insulin resistance
V_7: High density lipoprotein
V_8: Systolic blood pressure
V_9: Diastolic blood pressure
V_{10}: Smoking duration
V_{11}: Homocysteine
V_{12}: Age
V_{13}: Total carotid stenosis (CAD)
V_{14}: Trail Making Test Executive Function Score (TMT)

Figure 1. SEM Model

CHAPTER 7: MANUSCRIPT 3

The following manuscript examines the relationship between retinopathy and cognitive function by the Clock Drawing Test and the Trail Making Test Executive Function Score.

Retinopathy and Lowered Cognitive Performance in a Canadian First Nations Population

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Key words: cognitive function, Clock Drawing Test, Trail Making Test, retinopathy, cardiovascular, risk factors

Abstract

Purpose: Among studies of retinal microvascular abnormalities, the assessment of retinopathy as a novel screening method to predict cognitive health is limited. The objective of this study was to examine the association between retinopathy and lowered cognitive performance in a Canadian First Nations population.

Methods: Eligible individuals aged 18 years or older, without stroke, non-pregnant and with First Nations status were assessed by the Clock Drawing Test (CDT) and the Trail Making Test Parts A and B, with the two parts combined into an executive function score ((B-A)/A, TMT-exec). Digital fundus photographs were taken for both eyes to assess retinopathy. Anthropometric, vascular and metabolic risk factors were assessed by interview, clinical examinations and blood tests. Carotid atherosclerosis was assessed by Doppler ultrasonography.

Results: Retinopathy was detected in 7.1% of the population. Individuals classified as having a previous history of cardiovascular disease, insulin resistance and diabetes were more likely to have retinopathy. No other cardiovascular risk factors were associated. In unadjusted analysis, there were no associations between retinopathy and lowered cognitive performance (CDT, odds ratio [OR]: 0.86, 95% confidence interval [CI]: 0.30-2.53; TMT-exec, OR: 1.79, 95% CI: 0.60-5.33). Multivariable adjusted analysis showed no associations for retinopathy and lowered cognitive performance (CDT: OR, 0.77, 95% CI: 0.25-2.31, p=0.6365; TMT-exec, OR: 1.35, 95% CI: 0.42-4.40, p=0.6149).

Conclusions: Retinopathy was not associated with lowered cognitive performance. Associations for microvascular risk factors suggest a panel of cognitive tests is needed for future studies.

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Introduction

The associations between a number of cardiovascular risk factors such as hypertension(1), obesity(2) and diabetes(3) and cognitive decline and dementia suggest a vascular etiology for neurological disorders. A number of cognitive functions that span different brain regions may be elicited by standard neuropsychological tests, such as frontal and non-frontal regions on measures of executive function(4). Therefore alternative ways of assessing cognition are needed. The relationship between retinopathy and stroke(5), which is often followed by cognitive impairment(6) suggests that the presence of retinopathy may predict who is at a high risk for deficits in cognitive function. Similarities in the microvasculature between the retinal and cerebral areas(7) suggest that retinal abnormalities may serve as a surrogate measure of cerebral pathology and an indicator of cognitive decline and dementia. The risk factors for retinopathy are similar to the risk factors for cognitive decline and dementia(8). If a relationship exists between retinopathy and cognitive decline and dementia, then this high risk group could be targeted for primary prevention.

One systematic review examined the association between retinal microvascular abnormalities, including retinopathy and cognitive decline and dementia(9). Overall, the results were consistent with an association for retinopathy. The two prospective studies included diabetes populations which limits the generalizability of the results and the two cross-sectional studies, although population-based, are limited since the number of studies was few and different cognitive tests and characterization of those tests were used. Therefore, the objective of this study was to examine the association between retinopathy and cognitive function in a Canadian First Nations population. A positive association between the presence of retinopathy

and lowered cognitive performance was hypothesized suggesting that retinopathy may be an appropriate surrogate measure or predictor of cognitive performance.

Materials and Methods

This was a cross-sectional study conducted in a road-accessible First Nations community in Southern Manitoba, Canada. Eligible individuals were community residents 18 years of age or older, without a history of stroke, non-pregnant, and designated with First Nations status. Recruitment without sampling occurred through home visits to each home in the community and advertisements in the local Health Centre newsletter. All eligible community members were invited to participate. Demographic, anthropometric, vascular, and metabolic data were collected as part of a larger cross-sectional study on diabetes and diabetes complications(10). Measures of carotid atherosclerosis, retinopathy and cognitive function were assessed among a subset of the participants. Cognitive tests were administered by two graduate student research assistants at the research study site. A total of 207 individuals completed the Clock Drawing Test and 190 the Trail Making Test Parts A and B, a majority whom (n=144) also had information on risk factors, carotid atherosclerosis and retinopathy.

The Clock Drawing Test (CDT) involves individuals having to draw in the numbers of a clock face. According to the Watson scoring method, three clock numbers in a quadrant are considered correct. Errors in the first to third quadrants are assigned a score of one and errors in the fourth quadrant are assigned a score of four, for a maximum total score of seven(11). Scoring was performed by two raters. Individuals with a CDT score of ≥ 4 by one of two raters were classified as having lowered cognitive performance [CDT(+)], whereas individuals with a

CDT score of <4 by both raters were classified as not having lowered cognitive performance [CDT(-)].

Trail Making Test Parts A and B were administered according to standard protocols(12). A measure of executive function was calculated [(B-A)/A], which we designated the Trail Making Test Executive Function Score (TMT-exec). Those with TMT-exec≥2.33 were classified as having lowered cognitive performance [TMT-exec(+)], whereas individuals with TMT-exec<2.33 were classified as not having lowered cognitive performance [TMT-exec(-)]. The cutpoint was determined from preliminary analyses in which the distribution of TMT-exec was bimodal (see Chapter 4).

Digital images using a portable camera were taken to examine retinopathy and were assessed at the Ocular Epidemiology Grading Center at the University of Wisconsin (Madison, WI). Trained technologists traveled to the specified First Nations community and digital fundus photographs of both eyes were taken(13). Grading for each eye was performed according to standard methods(14,15). Each eye was examined for the presence of the following retinal microvascular conditions: hard or soft exudates and intraretinal microvascular abnormalities without microaneurysms, haemorrhages without microaneurysms, microaneurysms only, early non-proliferative diabetic retinopathy, moderate non-proliferative diabetic retinopathy, and severe non-proliferative diabetic retinopathy. Retinal arteriolar changes such as generalized and focal arteriolar narrowing and arteriovenous nicking were not considered. The presence of any one of the above retinal microvascular conditions identified an individual eye (i.e. right or left) as having retinopathy otherwise the eye was classified as not having retinopathy. Possible retinopathy was assigned to eyes that had a complicated presentation and not gradable was assigned to eyes that could not be evaluated due to poor image quality. When the right or left

eye had missing information, the eye was classified as not having retinopathy. Therefore, each eye was classified in one of four categories: no retinopathy, possible retinopathy, retinopathy, or not gradable. Overall retinopathy was examined at the individual level as a dichotomous variable by combining retinopathy information from both eyes. Individuals were classified as having retinopathy [RT(+)] if retinopathy was present for one of two eyes or both eyes, otherwise individuals were classified as not having retinopathy [RT(-)].

Information on risk factors was ascertained through clinical examination and in-person questionnaires administered by trained personnel. Fasting venous blood samples were collected, stored at -20ºC and analyzed for triglycerides, high density lipoprotein, glucose, and insulin. Hypertension, dyslipidemia and metabolic syndrome were examined according to standard criteria(16). Self-report of at least one of the following was used to define a previous history of cardiovascular disease: angina, history of angioplasty or revascularization, myocardial infarction, and peripheral arterial disease. Weight and height were collected and used to calculate body mass index (BMI). Individuals were classified as obese if BMI $\geq$30 kg/m^2(17). Insulin resistance was examined by the homeostasis model of assessment(18), and ever having smoked from self-reports. Carotid atherosclerosis was determined by plaque volume and carotid stenosis. For carotid plaque volume, a series of parallel two dimensional images were collected using Doppler ultrasonography and used to reconstruct a three dimensional image(19, 20). For plaque volume, values for the right and left sides of the neck were determined in millimeters cubed (mm^3) and total plaque volume was calculated as the sum of both sides. Peak systolic velocity of the right and left internal carotid arteries was used to estimate the percent carotid stenosis(21). Percent carotid stenosis was determined for the right and left sides of the neck and total carotid stenosis was calculated as the sum of both sides.

The proportion of individuals classified as having retinopathy, and who also had lowered cognitive performance by the CDT and TMT-exec was examined. For risk factor information and carotid atherosclerosis, individuals lacking complete data for specific variables were reassigned a value for each variable. Continuous variables were assigned to the mean value from among the total eligible population (n=510), except where the mean and median values were quite different and then the median value was assigned. Descriptive statistics and risk estimates using this approach for handling missing data were compared to the strategy of deleting individuals with incomplete information. The results were similar and thus the former was used for subsequent analyses. For categorical variables, individuals were assigned to the referent group. Unadjusted odds ratios with corresponding 95% confidence intervals were calculated using logistic regression to examine the associations between retinopathy and lowered cognitive performance by the CDT and TMT-exec. Multivariable logistic regression models were used to examine the associations between retinopathy and lowered cognitive performance by the CDT and TMT-exec, while accounting for confounding variables that have been identified in the literature. Potential confounders included hypertension, a history of cardiovascular disease, dyslipidemia, obesity, insulin resistance, diabetes, ever smoked, and carotid atherosclerosis. Statistical models were built for CDT and TMT-exec, with age and sex considered in all models. Multivariable models were used to calculate the adjusted odds ratios, 95% confidence intervals and p-values. The relationships between categories of risk factors and retinopathy were also examined in unadjusted analysis. The associations were calculated separately for CDT and TMT-exec due to slight differences in their sample sizes. Risk factor categories were based on

cutpoints according to standard criteria (e.g. BMI$\geq$30 kg/m^2 for obese) or the median value

among those without retinopathy (e.g. carotid measures), as determined from preliminary analysis.

Results

There were 16 individuals with retinopathy (7.1%) and 208 individuals without retinopathy (92.9%) (Table 1). When retinopathy was cross-classified by the CDT or TMT-exec, there were 201 individuals who had CDT scores and were evaluated for retinopathy and 184 individuals who had TMT-exec scores and were evaluated for retinopathy. When the relationships between retinopathy and CDT and TMT-exec were examined, there were no statistically significant associations (Table 2). When we examined CDT scores between those with retinopathy and without retinopathy, there was no significant median difference (RT(+): 2.0, Interquartile range (IQR): 5.0 vs. RT(-): 0, IQR: 2.0 score, p=0.0924). When we examined TMT-exec scores between those with retinopathy and without retinopathy, there was also no median difference (RT(+): 2.4, IQR: 1.1 vs. RT(-): 2.0, IQR: 1.4 units, p=0.4842). Multivariable results are shown in Table 3. There were no associations between retinopathy and either CDT or TMT-exec. When retinopathy was examined in association with the Trail Making Test Part A as a continuous outcome measure adjusted for age and systolic blood pressure, there were no effects.

Tables 4 and 5 show the unadjusted odd ratios for cardiovascular risk factors with retinopathy, separately for the CDT and TMT-exec respectively. Increased risks for retinopathy were shown for those who were classified as having a previous history of cardiovascular disease compared to those who did not have a previous history (CDT, OR: 3.54, 95% CI: 1.11-11.29; TMT-exec, OR: 3.94, 95% CI: 1.21-12.89), for those who were classified as having insulin

resistance compared to those who did not have insulin resistance (CDT, OR: 6.50, 95% CI: 1.43-29.61; TMT-exec, OR: 5.72, 95% CI: 1.24-26.35), and for those classified as having diabetes or glucose ≥7 mmol/L compared to those who did not have diabetes and glucose <7 mmol/L (CDT, OR: 10.59, 95% CI: 2.87-30.07; TMT-exec, OR: 10.19, 95% CI: 2.72-38.18). The median age of those with retinopathy was approximately 43 years (IQR: 18.0), whereas those without retinopathy the median age was approximately 38.5 years (IQR: 13.0, p=0.5118) (n=201). The proportion of individuals who had diabetes and retinopathy was approximately 80% (CDT: 12/15, TMT-exec: 11/14).

To explore the association further, we relaxed the definition of retinopathy by reassigning the right and left eyes with possible retinopathy or not gradable to having retinopathy. When we did this, there were 99 individuals with retinopathy (44.2%), and 125 individuals without retinopathy (55.8%). Using this alternative approach, the results from unadjusted analysis were similar. When retinopathy was examined with CDT and TMT-exec, there were no effects. In multivariable analysis, individuals classified as having retinopathy were less likely to have lowered cognitive performance by the CDT (OR: 0.54, 95% CI: 0.30-0.99, p=0.0446). There was no association between retinopathy and lowered cognitive performance for the TMT-exec. When the cardiovascular risk factors were examined in relation to retinopathy, there was a significant association shown between diabetes and retinopathy (CDT, OR: 2.27, 95% CI: 1.24-4.17; TMT-exec, OR: 2.21, 95% CI: 1.17-4.19), smoking (CDT, OR: 1.99, 95% CI: 1.05-3.75; TMT-exec, OR: 2.19, 95% CI: 1.13-4.26), and total carotid stenosis (CDT, OR: 0.49, 95% CI: 0.26-0.90; TMT-exec, OR: 0.51, 95% CI: 0.27-0.96). No other risk factors were associated.

Discussion

Retinopathy was not associated with lowered cognitive performance by the CDT or the TMT-exec in a Canadian First Nations population. When an alternative definition of retinopathy was used, individuals with retinopathy were less likely to have lowered cognitive performance by the CDT however these results should be interpreted with caution. Overall, our data were not consistent with our *a priori* hypothesis or with the limited research to date.

This study is one of the few population-based studies that examined the association between retinopathy and lowered cognitive performance. In the cross-sectional component of the Cardiovascular Health Study in which 9.8% of the population was classified as having retinopathy, they found 3- to 4-fold differences in the mean scores for the digit symbol substitution test between those with retinopathy and those without retinopathy in multivariable and unadjusted analyses respectively(22). Individuals with retinopathy had lower mean scores indicating lowered cognitive function. The digit symbol substitution test is a measure of psychomotor performance(23), whereas the CDT requires a number of cognitive skills(24), and the TMT-exec is a measure of executive function(25). Different cognitive skills required by the three tests and different scoring systems, e.g. the derived score of TMT-exec and the scoring algorithm for the CDT may account for the conflicting results. The absence of an effect shown for Trail Making Test Part A, a measure of processing speed(12) further supports that the nature of the retinal-cognitive function association is not clear. In the cross-sectional component of the Atherosclerosis Risk in Communities Study in which 5.9% of the population was classified as having retinopathy, they found 0.2- to 1.1-fold differences in the mean scores for the delayed word recall test, the digit symbol subtest and the word fluency test between those with retinopathy and those without retinopathy in multivariable analysis. Individuals with retinopathy

had lower scores indicating lowered cognitive function. Using cutpoints to dichotomize the scores for the three cognitive tests, retinopathy was associated with an approximately 2-fold increased risk for lowered cognitive performance for all three tests(26). The larger study population in the previous study which allowed small mean differences to be detected, the absence of information on alcohol use in our study, and the different cognitive tests and characterization of those tests may account for the different study results. Among previous studies, only the digit symbol substitution test was used in both studies. Given the non-invasive nature of retinopathy assessment and its potential to be a rapid screening tool of cognitive function, future studies using a panel of neuropsychological tests is likely needed.

The prevalence of retinopathy in our study is consistent with previous population-based studies, which suggests that our data are representative(27). Individuals having had a previous cardiovascular disease, insulin resistance and diabetes were associated with retinopathy. Our results are consistent with previous studies that showed control of glycemia and blood pressure reduces the incidence of diabetic retinopathy(8). Insulin resistance is often a precursor to diabetes(28), and retinopathy has been shown in individuals with a range of glucose levels(29), therefore the increased risks observed between insulin resistance and retinopathy in our study are consistent with previous work. The absence of associations for smoking, dyslipidemia and obesity with retinopathy are similar to inconsistent findings reported in previous studies(8, 30, 31). Only one previous study has examined the metabolic syndrome(32), which showed an association and no study has examined our measures of carotid atherosclerosis. Retinal imaging is less susceptible to the limitations of different methods of assessment of cognitive function, therefore given the overlapping risk factor profile between retinopathy and cognitive decline and

dementia, retinal imaging as a non-invasive method of assessment of cognitive health warrants further investigation.

Strengths of this study include the population-based design and use of digital images to assess retinopathy. Limitations include the absence of information on past history of traumatic brain injury(33), and other psychiatric(34), or depressive conditions(35) that may affect cognitive functioning. Visual acuity may have affected performance on our cognitive tests however no individuals were diagnosed with the most likely condition related to blindness, advanced proliferative retinopathy(36). The generalizability of the results is limited due to the volunteer nature of the population. Analysis of times to completion for Part A of the Trail Making Test with age was consistent with normative data(37), however times for Part B were shifted towards poorer performance. For the Trails, the potential for malingering exists(38), whereas population-based estimates for the CDT does not exist.

In summary, we did not see an association between retinopathy and lowered cognitive performance as anticipated. Different cognitive tests and characterization of cognitive tests will likely contribute to inconsistent results across studies. A panel of cognitive tests is needed to fully examine the association. Similar microvascular risk factors for retinopathy and cognitive decline and dementia and the non-invasive method of assessment of the retinal area highlight the need for future investigations as the application of retinal status to cognitive health is tremendous for our aging population.

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Table 1. Descriptive Statistics for Retinopathy

Retinopathy	n (%)
Right eye	
No	154 (68.8)
Possible	7 (3.1)
Yes	14 (6.3)
Not gradable	48 (21.4)
Missing	1 (0.4)
Total	224 (100)
Left eye	
No	138 (61.6)
Possible	7 (3.1)
Yes	11 (4.9)
Not gradable	67 (29.9)
Missing	1 (0.4)
Total	224 (100)
Either eye	
No	208 (92.9)
Yes	16 (7.1)
Total	224 (100)

Table 2. Unadjusted Odds Ratios and 95% Confidence Intervals for Retinopathy and Cognitive Function by the Clock Drawing Test and Trail Making Test Executive Function Score

	Clock Drawing Test (CDT) [n (%)]		
Retinopathy	CDT(+), n=87	CDT(-), n=114	OR (95% CI)
No	81 (93.1)	105 (92.1)	1.00
Yes	6 (6.9)	9 (7.9)	0.86 (0.30-2.53)
	Trail Making Test Executive Function Score (TMT-exec) [n (%)]		
Retinopathy	TMT-exec(+), n=68	TMT-exec(-), n=116	OR (95% CI)
No	61 (89.7)	109 (94)	1.00
Yes	7 (10.3)	7 (6)	1.79 (0.60-5.33)

OR, odds ratio; CI, confidence interval.

Table 3. Multivariable Adjusted Odds Ratios and 95% Confidence Intervals for Retinopathy and Cognitive Function by the Clock Drawing Test and the Trail Making Test Executive Function Score

	OR (95% CI)	*p-value*
	Clock Drawing Test (N=201)	
Age[1]	1.01 (0.99-1.04)	0.3339
Sex	2.53 (1.42-4.51)	*0.0017*
Retinopathy	0.77 (0.25-2.31)	0.6365
	Trail Making Test Executive Function Score (N=184)	
Age	1.00 (0.97-1.04)	0.8489
Sex	1.62 (0.85-3.10)	0.1440
Retinopathy	1.35 (0.42-4.40)	0.6149
Obesity	2.65 (1.33-5.28)	*0.0055*
Total carotid stenosis	0.42 (0.21-0.84)	*0.0147*

[1] Age is a continuous variable. Remaining variables are categorized as in Tables 4 and 5.
OR, odds ratio; CI, confidence interval.

Table 4. Unadjusted Odds Ratios and 95% Confidence Intervals for Associations of Cardiovascular Risk Factors with Retinopathy Among those with Clock Drawing Test Scores (N=201)

Variable	RT(+)=15	RT(-)=186	OR (95% CI)
Age			
<45 years	10	138	1.00
≥45 years	5	48	1.44 (0.47-4.42)
Sex			
Male	6	93	1.00
Female	9	93	1.50 (0.51-4.38)
Hypertension			
No	12	167	1.00
Yes	3	19	2.20 (0.57-8.49)
Previous history of cardiovascular disease			
No	10	163	1.00
Yes	5	23	**3.54 (1.11-11.29)**
Dyslipidemia			
No	3	45	1.00
Yes	12	141	1.28 (0.35-4.73)
Obesity			
No	4	76	1.00
Yes	11	110	1.90 (0.58-6.19)
Metabolic syndrome			
No	4	100	1.00
Yes	11	86	3.20 (0.98-10.41)
Insulin resistance			
No	2	93	1.00
Yes	13	93	**6.50 (1.43-29.61)**
Diabetes			
No	3	135	1.00
Yes	12	51	**10.59 (2.87-30.07)**
Ever smoked			
No	3	59	1.00
Yes	12	127	1.86 (0.51-6.83)
Total carotid stenosis			
<40.0%	8	53	1.00
≥40.0%	7	133	0.35 (0.12-1.01)
Total plaque volume			
<11.4 mm^3	5	69	1.00
≥11.4 mm^3	10	117	1.18 (0.39-3.59)

RT, retinopathy; OR, odds ratio; CI, confidence interval.

Table 5. Unadjusted Odds Ratios and 95% Confidence Intervals for Associations of Cardiovascular Risk Factors with Retinopathy Among those with Trail Making Test Executive Function Scores (N=184)

Variable	RT(+)=14	RT(-)=170	OR (95% CI)
Age			
<45 years	10	129	1.00
≥45 years	4	41	1.26 (0.38-4.23)
Sex			
Male	6	81	1.00
Female	8	89	1.21 (0.40-3.65)
Hypertension			
No	12	154	1.00
Yes	2	16	1.60 (0.33-7.81)
Previous history of cardiovascular disease			
No	9	149	1.00
Yes	5	21	**3.94 (1.21-12.89)**
Dyslipidemia			
No	3	40	1.00
Yes	11	130	1.13 (0.30-4.24)
Obesity			
No	4	69	1.00
Yes	10	101	1.71 (0.52-5.67)
Metabolic syndrome			
No	4	90	1.00
Yes	10	80	2.81 (0.85-9.32)
Insulin resistance			
No	2	83	1.00
Yes	12	87	**5.72 (1.24-26.35)**
Diabetes			
No	3	125	1.00
Yes	11	45	**10.19 (2.72-38.18)**
Ever smoked			
No	3	55	1.00
Yes	11	115	1.75 (0.47-6.54)
Total carotid stenosis			
<40.0%	7	47	1.00
≥40.0%	7	123	0.38 (0.13-1.15)
Total plaque volume			
<11.4 mm^3	5	65	1.00
≥11.4 mm^3	9	105	1.11 (0.36-3.47)

RT, retinopathy; OR, odds ratio; CI, confidence interval.

CHAPTER 8: DISCUSSION AND CONCLUSIONS

8.1 THESIS SUMMARY

The overall aim of the thesis was to examine the associations between vascular and metabolic risk factors, carotid atherosclerosis and cognitive function in a First Nations population. The focus of the thesis on vascular and metabolic risk factors developed from a review of previous descriptive epidemiology studies on vascular cognitive impairment. These descriptive epidemiology studies showed that individuals with vascular cognitive impairment were more likely to display hypertension, dyslipidemia and diabetes(1, 2). Since the concept of vascular cognitive impairment incorporates a number of brain pathologies and disease subtypes, the underlying mechanism of disease for vascular cognitive impairment and the focus of this thesis with respect to the study of carotid atherosclerosis was largely extrapolated from vascular dementia(3). However, carotid atherosclerosis has not been studied in detail as a risk factor for vascular cognitive impairment(4, 5). Microvascular processes may also be involved in the etiology of vascular cognitive impairment(3), and review of previous epidemiology studies showed a relationship between retinopathy and cognitive function(6). The high prevalence of deleterious risk factors among the Canadian First Nations population offered a unique opportunity to study the outlined associations.

The main thesis results were two-fold. First, the thesis results showed an association between obesity and lowered cognitive performance, where individuals who were classified as obese were associated with an increased risk of lowered cognitive performance by the TMT-exec. Second, the thesis results showed an association between carotid stenosis and lowered

cognitive performance, although the effect was in the opposite direction than hypothesized. Individuals with increased levels of carotid stenosis were less likely to have lowered cognitive performance. Additional inquiry showed some evidence that carotid stenosis mediated the effect of anthropometric risk factors on lowered cognitive performance, and no association was found between retinopathy and lowered cognitive performance. In summary, the etiology of lowered cognitive performance has a vascular origin, which is predominately affected by non-traditional risk factors such as obesity. There appears to be no involvement of the microvascular environment in cognitive function as measured by the cognitive tests used in the thesis and for the First Nations population.

8.2 VASCULAR AND METABOLIC RISK FACTORS

The role of cardiovascular risk factors as investigated in the thesis including hypertension, history of cardiovascular disease, dyslipidemia, obesity, metabolic syndrome, insulin resistance, and diabetes can be divided into two categories; risk factors that exert their effect on cognitive function by macrovascular processes and those that exert their effect on cognitive function through the microvascular environment.

Hypertension, dyslipidemia, obesity, metabolic syndrome, insulin resistance, and diabetes affect the macrovascular environment by promoting the development of atherosclerosis(7-11). Despite atherosclerosis as the mechanism by which these risk factors affect cognitive function, only obesity was shown to be associated with lowered cognitive performance in the thesis. Visceral fat produces a number of factors including factors for energy homeostasis and the inflammatory response such as adiponectin, leptin, resistin, visfatin, vascular endothelial growth factor, and angiotensinogen; pro-inflammatory cytokines such as

tumor necrosis factor alpha and interleukins 1, 6, 8, and 10; acute phase inflammatory proteins such as C-reactive protein; lipid-related factors such as low density lipoproteins, apolipoproteins, free fatty acids, and glycerol; fibrinolytic factors such as tissue factors and plasminogen activator inhibitor-1; hormones, such as estrogen from the conversion of androgens; and other factors including angiotensinogen involved in vascular homeostasis and cortisol, from the conversion of cortisone. The release of a number of inflammatory factors from adipose tissue promotes the development of atherosclerosis. Visceral fat is a constitutively active endocrine and paracrine organ, generates a chronic inflammatory state in individuals, and produces an amount of adipokines involved in the inflammatory response in proportion to its mass(12). Therefore, the biological influence of obesity on the development of atherosclerosis is much greater than for the other cardiovascular risk factors and as a consequence, obesity was associated with an increased risk for lowered cognitive performance whereas no associations were shown for a majority of the remaining risk factors.

To investigate the role of adipose tissue more closely, a number of different strategies were used in the thesis. First, the obesity variable was re-categorized, with individuals who were overweight removed from the referent group. As expected, the association increased for those who were obese compared to those of a normal weight. Second, waist circumference was examined using similar multivariable models to obesity, and as expected, an increased risk for lowered cognitive performance was shown. Third, the metabolic syndrome was re-examined by categorizing individuals with an increasing number of components for the metabolic syndrome. As expected, an effect was shown for individuals who had three or more components of the metabolic syndrome, owing to the presence of obesity.

The role of microvascular factors in cognitive function was evaluated by examining the association between retinopathy and lowered cognitive performance. (This concept is separate from the thesis objective of studying retinopathy and cognitive function to determine whether retinopathy is a good surrogate measure for cognitive function). The lack of an association between retinopathy, a measure of microvascular disease and the Clock Drawing Test and the Trail Making Test Executive Function Score suggest that deficits on our tests of cognitive function were not due to processes within the microvascular environment. Returning to the concept of vascular cognitive impairment which is largely represented by vascular dementia, a subtype of vascular dementia known as subcortical ischemic vascular dementia incorporates small vessel disease which is predominately affected by microvascular pathologies (e.g. lacunar infarcts and ischemic white matter lesions)(13), and for which hypertension is associated and not carotid disease(14). Therefore, the underlying mechanism by which cardiovascular risk factors exert their effect on lowered cognitive performance as examined in the thesis was predominately by macrovascular processes such as atherosclerosis and not microvascular processes.

Potential microvascular effects of vascular and metabolic risk factors on cognitive function cannot be excluded, a process that involves neurovascular coupling. Neurovascular coupling is the regulation of cerebral blood flow in response to neural activity. Increased neuronal activity places demands on glucose and oxygen requirements, such as during completion of a cognitive test. Glucose and oxygen is delivered through an increase in the blood supply, a phenomenon termed functional hyperaemia. The neurovascular unit coordinates the need for an increased blood supply. The neurovascular unit is comprised of neurons, glia including astrocytes, microglia and oligodendrocytes, and vascular cells including endothelium,

smooth muscles cells or pericytes and adventitial cells. Factors released from neurons and astrocytes to the arteriolar vessel wall within specialized contact regions elicit a vasodilation response, thereby increasing the blood supply. The neurovascular coupling response is attenuated among individuals with hypertension, Alzheimer's disease and stroke during tests of cognitive function, which may lead to ischemia(15, 16). The neurovascular coupling response may also be impaired among obese individuals due to impaired endothelium-dependent vasodilatation and capillary recruitment, and the pro-inflammatory state. The availability of nitric oxide to induce its vasodilation effect may be compromised among obese individuals and in the presence of insulin resistance(17, 18). The microvascular effects of diabetes include alterations to the aldose reductase and hexosamine pathways, protein kinase activation and increased oxidative stress, and protein glycation leading to impaired endothelium function(19). Therefore, the relationship between cardiovascular risk factors and neurovascular coupling suggests that microvascular effects on cognitive function cannot be ruled out, however microvascular effects did not predominate with the tests of cognitive function used in the thesis.

8.3 BIOLOGICAL MECHANISM

The role of cardiovascular risk factors in stroke and vascular dementia support the hypothesis that both neurological disorders arise from a "common soil." The common underlying biological mechanism involved in both disorders is a thromboembolism from a developing atherosclerotic plaque resulting in one or more brain infarcts(20, 21). The role of small platelet aggregates or cholesterol microemboli shed from carotid plaques is suggested as one mechanism by which cerebral infarcts affect cognitive functioning without producing symptoms or clinically overt stroke, otherwise known as silent infarcts(22). Population-based

estimates of silent cerebral infarcts range from 8-28% in elderly populations, however this estimate is based predominately on the presence of lacunar infarcts on imaging(23). Results from the Framingham Offspring study showed that the risk factors for stroke are similar to the risk factors for silent cerebral infarction (SCI). In that study, carotid stenosis was also shown to be associated with SCI(24). Therefore, a vascular model of etiology for cognitive function with the involvement of risk factors, atherosclerosis and silent cerebral infarcts is supported.

In the thesis, carotid stenosis was associated with a reduced likelihood of lowered cognitive performance which was counter-intuitive to the thesis hypothesis. We provided an explanation that involved compensatory vessel enlargement in the face of a growing atherosclerotic plaque. Without additional neuroimaging data such as magnetic resonance imaging information, the role of the number of infarcts or the location of infarcts cannot help clarify whether our case and control groups were homogeneous with respect to underlying pathology(25).

Analogous to the impact of a cerebral infarct, is brain damage due to neuroinflammation. Neuroinflammation occurs as a natural part of aging. Although the First Nations population was predominately young, they were also obese. The youthfulness of the First Nations population did not protect cognitive function from the neuroinflammatory effects of a largely obese population. A further explanation regarding the associations found for obesity includes a process that involves a developing pro-neuroinflammatory state before the occurrence of a silent brain infarct due to inflammatory factors released from adipose tissue, which may have compromised the integrity of the brain and contributed to lowered cognitive performance after a silent brain infarct(26).

8.4 INTEGRATIVE MODEL

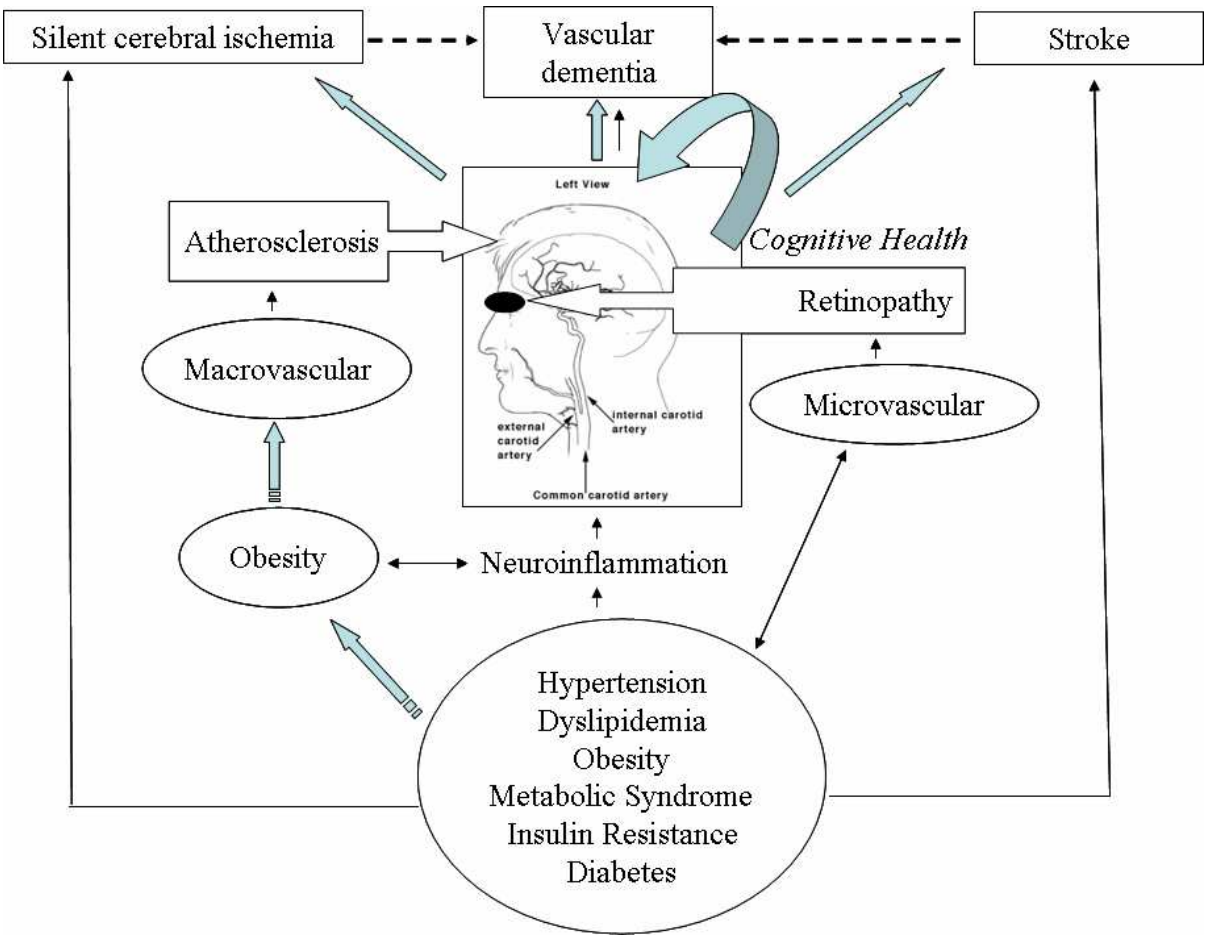
An integrative model linking cardiovascular risk factors and carotid atherosclerosis to lowered cognitive performance is shown in Figure 9. Silent cerebral ischemia can be considered along the continuum of cerebrovascular disease(27), which also includes vascular dementia and stroke. Risk factors operate at the macrovascular level by promoting atherosclerosis. Silent cerebral infarctions due to small platelet aggregates shed from atherosclerotic plaques exert their effect on cognitive function. Risk factors also interact with neuroinflammatory processes to negatively impact the integrity of the brain. Microvascular processes that compromise brain tissue also occur due to exposure to deleterious risk factors. Overall, the etiology of lowered cognitive performance is complex, with origins in vascular pathology. The effect of risk factors is likely not linear, and consequently, we did not observe increasing risk with an increasing level of vascular and metabolic dysfunction as hypothesized in the thesis.

8.5 COGNITIVE MEASURES

Assessment of executive function was a main focus of the thesis since deficits in vascular cognitive impairment surround executive function, whereas memory is not affected(14). Executive function represents high-level cognitive functions including goal formation, planning, carrying out goal-directed plans, and effective performance. For example, in a constantly changing environment, executive abilities allow individuals to change their thinking strategy, adapt, and inhibit inappropriate behaviours. Four distinct and most popularly studied executive functions include attention, planning, set-shifting, and verbal fluency(28).

How to measure executive function continues to be debated. Three popular executive function measures include the Wisconsin Card Sorting Test, a sorting methods test; Phonemic

Figure 9. Integrative Model



***Note: arrows do not imply causality**

Verbal Fluency, a task that requires individuals to say (or write) as many words as possible beginning with a specific letter; and the Stroop Color Word Interference Test, which is a test of slowed reaction time. Executive functioning has traditionally been linked to the functioning of the frontal lobes however lesion and neuroimaging studies have shown that both frontal and non-frontal regions of the brain may impact performance on tests of executive function. Therefore, tests of executive function activate a widespread network of brain regions, especially when the test involves a number of cognitive skills and integrated functioning(29).

The Clock Drawing Test was included in the thesis due to its ease of administration(30)

and ability to diagnose mild but clinically relevant dementia using a simple and objective protocol(31). The additional benefits of the Clock Drawing Test are that it is applicable across cultures and language groups, and when the test does not require the placement of the hands of the clock, such as in the thesis, the educational component is reduced(32). The limitations of the Clock Drawing Test are that it may be influenced by intelligence and there are no population-based estimates among healthy individuals, though age is the strongest predictor of cognitive decline(33).

The many cognitive skills required for the Clock Drawing Test may have prevented the detection of associations. Skills necessary for clock completion can be observed or inferred including comprehension, planning, visual memory and reconstruction of a graphic image, visuospatial abilities, motor programming and execution, numerical knowledge, abstract thinking, and executive function(34, 35). Lowered cognitive performance based on the Clock Drawing Test is consistent with the underlying mechanism of silent cerebral infarctions. A silent cerebral infarction in the anterior cerebral artery would compromise the frontal lobe but consistent perfusion through the middle cerebral artery would sustain the integrity of a number of brain regions including the frontal, temporal and parietal lobes allowing the Clock Drawing Test to be completed satisfactorily. The complicated nature of the Clock Drawing Test in terms of the many cognitive functions it assesses suggests that it could be eliminated from future epidemiology studies investigating the etiology of cognitive decline and dementia but continued to be used as a brief cognitive test to help diagnose dementia in the clinical setting.

Trail Making Test Parts A and B were combined into the Trail Making Test Executive Function score, which was used as a more homogeneous measure to examine executive function than alternate derived indices. Normative data show a robust association between the different

characterizations of Trails with age, a less consistent association with education, and an even less consistent association with sex(36, 37). Less is known about the executive function score. Although not formally introduced, the executive function score was previously used in a study by Stuss et al. (2001)(38), and was referred to as the proportional score. Results from the thesis were similar to the frontal lesion groups in the previous clinic-based study. The effects shown in the thesis for the Trail Making Test Executive Function Score were most likely due to its ability to isolate the cognitive processes of executive functioning (i.e. attention, planning, set-shifting) involved in the task performance(38). Lowered cognitive performance based on the Trail Making Test Executive Function Score is consistent with the underlying mechanism of silent cerebral infarctions. A silent cerebral infarction to the frontal lobe via the anterior cerebral artery is more likely to lead to deficits in cognitive functioning of an executive origin and shown on a test of executive function. Overall, the ability of the Trail Making Test Executive Function Score to target specific cognitive functions makes it a good candidate for future epidemiological studies.

For future research using tests of executive function, what is clear is that executive function is not synonymous with frontal functioning. Other brain areas are involved, although frontal lobe functioning is a major part. And, what does it mean to perform well or poorly on a test of executive function, such as the Trail Making Test Executive Function Score? How does that translate into everyday activities? Future research needs to consider the emotional or motivational executive functions governed by the frontal lobe in addition to cognitive functioning and elicited by everyday interactions(39). Alternate measures of executive function which mimic real-life situations include the Multiple Errands Test, which is a shopping task. How to incorporate ecologically valid or real-life tests of executive function into

epidemiological research presents a major upcoming challenge in the study of cognitive decline and dementia.

8.6 BIOMARKERS

There are two considerations regarding the role of biomarkers in the study of cognitive function. First, a biomarker may be a better indicator of risk factor levels compared to a conventionally measured risk factor if it is etiologically relevant and if it can be measured with less error, thus the biomarker can serve as a proxy measure. Second, a biomarker may be an earlier sign of pathology in the natural history of disease, thus the biomarker can serve as a surrogate marker of disease(40).

In the thesis, biomarkers of inflammation such as C-reactive protein would have helped to clarify the results of obesity. Whether inflammatory factors arising from excess adipose tissue initiate a cascade of inflammatory events involved in the etiology of lowered cognitive performance could have been more fully addressed.

Retinopathy was examined as a potential surrogate biomarker to tests of cognitive function. In the thesis, there was no association between retinopathy and lowered cognitive performance indicating that retinopathy is not a good surrogate biomarker of cognitive health in this population. Previous studies showed an association between retinopathy and the digit symbol substitution test, a test of psychomotor performance(41) with an emphasis on perceptual speed and visual scanning(42). Had the thesis included a broader range of neuropsychological tests, associations with retinopathy may have been revealed. On the other hand, inclusion of additional retinal microvascular abnormalities such as retinal arteriolar narrowing and arteriovenous nicking, both associated with cerebral lesions(43) may have revealed associations

with the tests of cognitive function used in the thesis. Assessment of retinal microvascular abnormalities as a window to cognitive health warrants future investigation. Future studies that include a panel of neuropsychological tests and all possible retinal microvascular abnormalities may help to address this unanswered question. Additionally, study of the association between retinal microvascular abnormalities and cognitive function may provide additional knowledge as to the pathology of deficits for specific tests of cognitive function (i.e. arteriolar vs. non-arteriolar). A better understanding of etiology may lead to strategies for the primary prevention of cognitive decline and dementia.

8.7 METHODOLOGICAL INNOVATION

The unique contribution of the thesis was the structural equation modeling (SEM) approach. Although logistic regression will be a mainstay of modern epidemiology since it estimates risk, the advantage of SEM compared to logistic regression is that the statistical analysis can be more complex. Therefore, SEM more adequately reflects the biological complexity that it attempts to model statistically, compared to logistic regression. For that reason, SEM which has been traditionally confined to social research has entered into the field of epidemiology.

Causation and causal thinking are the cornerstones of epidemiology research. Within the field of epidemiology, biochemical techniques have provided molecular insights into the relationship between a particular exposure and disease, but have created the need for more complex statistics to account for the introduction of measured biological complexity. The increasing number of variables may pose a limitation, since it requires correct conceptualization of the relationships under study. For example, an association may be undetected or attenuated

due to modeling an intervening variable as a confounder using logistic regression. SEM is a powerful tool that allows complex theoretical relationships to be modeled as "causal mechanisms" using statistics. Careful consideration must be given to the characterization of variables, the specified relationships, temporal sequence, and extraneous factors (e.g. control of confounding). SEM is not a method for identifying the cause of a disease when risk factors are biochemical or molecular in nature, this is restricted to laboratory sciences, but instead SEM uses statistics to test theoretical relationships.

One interesting question, is whether every epidemiology study should include a SEM analysis component? SEM is useful when there are many variables that should be modeled and when there is some suggestion of temporality. The quantitative information abstracted from SEM is different than from logistic regression. Therefore, SEM can only contribute to traditional logistic regression methods for estimating risk. The methodological problems that face logistic regression are not automatically solved when using SEM. SEM is only as good as the variables that have been measured in terms of measurement error and whether all relevant variables have been measured, and the theoretical model which has been proposed. The choice of whether to perform logistic regression alone, or logistic regression plus SEM should be made with respect to the study objectives, hypothesis and design. The introduction of SEM to the field of epidemiology can only help to promote new and in-depth ways of thinking about exposure and disease relationships.

8.8 STUDY STRENGTHS AND WEAKNESSES

One of the main strengths of the thesis is the statistical analyses. Previous studies examining the associations between cardiovascular risk factors or atherosclerosis and cognitive

function have not accounted for confounding variables. The thesis is also the first to apply a structural equation modeling approach to examine complex biological and etiological relationships between cardiovascular risk factors, atherosclerosis and cognitive function. Additional strengths of the thesis include the population-based design, detailed information on a number of cardiovascular risk factors, and use of digital images to assess retinopathy. In comparison to the early descriptive epidemiology studies on vascular cognitive impairment which identified hypertension, dyslipidemia and diabetes as potential risk factors, the thesis used an analytic approach, identified obesity in association with lowered cognitive performance, and proposed a biological framework for the complex etiology of lowered cognitive performance.

The main limitation of the thesis is with respect to the study design and the lack of a sampling scheme to recruit participants into the study. Due to cultural reasons, use of a random sampling scheme may have compromised the entire study. In the First Nations community, exclusion of individuals is not appropriate. Therefore, individuals not selected in initial random sampling strategies who may have wanted to participate in the study may not participate if randomly selected at a later date. Family members may also not participate in the study in support of those who were excluded. Although the estimates for the cognitive tests and retinopathy were consistent with other studies, additional unmeasured factors that may have affected the likelihood of participation may have biased the associations. Additional limitations of the thesis include the absence of information on past history of traumatic brain injury(44), other psychiatric(45), or depressive conditions(46), and alcohol use(47) that may affect cognitive functioning. Use of prevalent cases cannot rule out reverse causality however a number of prospective studies for the relationships of interest in the thesis support forward directionality.

The thesis used a cross-sectional design. The cross-sectional design assessed exposure and outcome at approximately the same time. The design is appropriate for diseases of a slow onset, a long duration, and which are asymptomatic such as vascular cognitive impairment. One of the main assumptions when using a cross-sectional design is that current exposures reflect past exposures, that is, exposures are unalterable. Similarly, the study design assumes that current exposures reflect exposures during the etiologically relevant time window. If mid-life risk factors affect late onset disorders of cognition, then this assumption may not be met when using a cross-sectional design. Reasons we showed an association with obesity include the stable nature of diet over time and consequently the relatively stable or slow change in body weight over time, excess adiposity as a state of being resulting in chronic inflammation, and the nature of atherosclerotic plaques and silent infarcts.

The First Nations population is unique therefore evaluation of the generalizability of the results requires some considerations. The biological nature of the relationship between obesity and lowered cognitive performance renders it applicable to other non-First Nations populations. Limitations to the generalizability of the results include the social and cultural context of the Manitoba First Nations population. For example, although it is unlikely that acute solvent exposure may have been a factor during cognitive test performance, the long-term neurological effects of solvent abuse cannot be ruled out(48). Therefore, the magnitude of the associations may be different in other First Nations populations and in non-First Nations populations depending on unmeasured factors that have long-term effects on cognitive function.

8.9 PUBLIC HEALTH IMPLICATIONS

Obesity is related to a number of diseases including insulin resistance, impaired fasting glucose or glucose intolerance, type 2 diabetes, hypertension, dyslipidemia, and chronic inflammation, therefore its public health impact is substantial. To address the health impact of obesity at the population level, health promotion strategies need to address diet and exercise, especially in the First Nations population. There is not yet a consensus of the relationship between obesity and lowered cognitive performance. The results for obesity in the thesis contribute to the growing body of knowledge on the topic.

With our aging population, the need to detect early onset decreases in cognitive function that signal the beginning of a host of potentially progressive changes in cognition has gained increased awareness(49). The traditional diagnostic criteria of dementia include the presence of an acquired impairment in memory and impairment in one or more cognitive domains such as: (1) executive function, (2) language (expressive or receptive), (3) praxis (learned motor sequences), and (4) gnosis (ability to recognize objects, faces or other sensory information). The impairments in cognition must be severe enough to interfere with work, usual social activities or relationships with others. The diagnosis is a process which involves taking the patient's history, interviewing a caregiver or family member, clinical examination, brief cognitive tests, basic laboratory tests, and structural imaging for patients meeting certain criteria. The Mini-Mental State Examination (MMSE) remains the most widely used instrument to determine the presence and severity of memory and cognitive deficits, however the Clock Drawing Test can also be used. The MMSE focuses on memory, attention, construction, and orientation(50). The Clock Drawing Test was developed as a non-verbal neuropsychological tool to measure constructional

apraxia[d] and intended to be a more rapid and efficient screening test for dementia independent of cultural and language barriers. The value of the Clock Drawing Test is its ability to identify decreased executive function in individuals with a normal Mini-Mental State Examination score(51), thus potentially characterizing an earlier stage of dementia. In the thesis, a more homogeneous measure of executive function which is currently not used in clinical practice, the Trail Making Test Executive Function Score, was associated with obesity and carotid stenosis. If a simple and rapid screening test of executive function can be used in clinical practice as an early indicator of cognitive decline and dementia, then perhaps the natural course of cognitive decline and dementia can be halted or reversed by addressing its associated cardiovascular risk factors. Similarly, assessment of cognitive health could emerge as the responsibility of family medicine practitioners.

8.10 CONCLUSIONS

The primary prevention of cognitive decline and dementia is a major upcoming challenge with the increasing aging population. Characterizing a neurological entity that precedes stroke, vascular dementia and Alzheimer's disease is the current direction of the cognition literature. Deficits in executive function is a good candidate as this new neurological entity however future research needs to exclude other cognitive functions, if the emphasis is to be on executive function.

The etiology of lowered cognitive performance is complex. The risk factors involved in the etiology of lowered cognitive performance were non-traditional, since the risk factors implicated were not confined to classic vascular risk factors such as hypertension or dyslipidemia but included risk factors such as obesity, waist circumference and the metabolic

[d] Apraxia is disorders of purposeful movements.

syndrome. The etiology of lowered cognitive performance was shown to be vascular in nature, with an underlying biological mechanism of disease that emphasized macrovascular processes and not the microvascular environment. The complexity of the etiology of lowered cognitive performance was summarized in an integrative model, which included neuroinflammation and neurovascular coupling as contributing biological mechanisms. Theoretically, the role of carotid atherosclerosis in cognitive decline is clear, however the results in the thesis were unexpected and difficult to explain, therefore the results require replication in other populations with high quality study designs.

Future directions should include short- and long-term goals. In the short-term, future epidemiology studies need to incorporate a panel of neuropsychological tests that examine different but specific cognitive functions. Therefore, different components of brain pathology (i.e. macro- vs. micro) and the relative contribution of any one deficit of cognitive function to future neurological disorders may be evaluated. The way in which cognitive tests are characterized and analyzed needs to be standardized across studies to enable comparability of results, and all studies should control for confounding variables. In the long-term, epidemiology studies should include measures of the social determinants of health such as income, education and employment. The social determinants of health are the social and economic conditions that influence health. They are strong predictors of health as they serve as indicators of material advantage or disadvantage (e.g. quality of food, access to amenities). For the First Nations population, adverse individual-level exposures associated with material advantage may accumulate across the life course to influence disease such as cognitive function (e.g. lifestyle choices such as diet and exercise, coping strategies, psychological stressors, cognitive engagement)(52,53). Epidemiology studies should include measures of executive function that

mimic real-life situations. A starting point may include clinical populations that have experienced some form of brain damage and are undergoing rehabilitation therapy. A cognitive screening test based on executive function having ecologic validity may emerge as an early indicator of cognitive decline and dementia, and therefore address the challenge of early detection of declining cognitive health for aging populations.

The study population used in the thesis was a First Nations population. Given the high prevalence of deleterious risk factors in the First Nations population, if there are associations between cardiovascular risk factors and cognitive decline, then the likelihood of detection of associations would be favoured. The association for obesity and lowered cognitive performance suggests the accelerated effects of an obesity milieu in this youthful population. On the other hand, had an older population been used, more extreme values of carotid atherosclerosis and more individuals may have had retinopathy for study.

In conclusion, the thesis has contributed new knowledge by examining the relationships between vascular and metabolic risk factors, carotid atherosclerosis and vascular cognitive impairment in a Canadian First Nations population. Highlighted are the detrimental effects of obesity, the role of macro- and microvascular processes, and the strengths and limitations of the Clock Drawing Test and the Trail Making Test Executive Function Score. Future studies on this research question that include a panel of neuropsychological tests are needed using high quality population-based designs. With the increasing aging population, heightened awareness of cognitive health is needed and primary prevention. The First Nations population experience a number of social and cultural differences compared to their Canadian counterparts, making them a unique population from which study results can be extrapolated and who require additional research to address their unique health needs.

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Appendix 1

Ethics Approval

University of Toronto
Office of the Vice-President, Research
Office of Research Ethics

PROTOCOL REFERENCE #20994

December 5, 2007

Dr. Kue Young
Public Health Sciences
155 College St.
Toronto, ON M5T 3M7

Ms. Jennifer Fergenbaum
Public Health Sciences
155 College St.
Toronto, ON M5T 3M7

Dear Dr. Young and Ms. Fergenbaum:

Re: Your research protocol entitled "Vascular and Metabolic Risk Factors, Carotid Atherosclerosis and Vascular Cognitive Function In A First Nations Population"

ETHICS APPROVAL Original Approval Date: December 5, 2007; Expiry Date: December 4, 2008

We are writing to advise you that a member of the Health Sciences Research Ethics Board has granted approval to the above-named research study, for a period of one year, under the REB's expedited review process. Ongoing projects must be renewed prior to the expiry date.

The following consent documents (received December 3, 2007) have been approved for use in this study: Study Information and Informed Consent. Participants should receive a copy of their consent form.

During the course of the research, any significant deviations from the approved protocol (that is, any deviation which would lead to an increase in risk or a decrease in benefit to participants) and/or any unanticipated developments within the research should be brought to the attention of the Office of Research Ethics.

Best wishes for the successful completion of your project.

Yours sincerely,

Jenny Peto, Research Ethics Coordinator

Appendix 2

Diabetes Complications Screening Questionnaire

A) Identifying Information

1. Today's Date: _____/_____/_____
   dd mm yy

2. Study ID# ___ ___ ___ ___ ___

3. Treaty # ___ ___ ___ ___ ___ ___ ___ ___ ___

4. Last Name: _____

   First Name: _____ Middle Name(s):_____

5. Name as on band list: _____
   Last Name

   First Name: _____ Middle Name(s): _____

6. Has participant signed a consent form?	
General Consent	Yes(___) No (___)
Genetics Consent	Yes(___) No (___)

7. Have you ever been told by a health care professional that you have diabetes?

   Yes(___) No (___) Refused (___)
   If yes, for how long have you had diabetes? _____ years

8. Have you fasted for 12 hours?

   Yes (___) No (___) Refused (___)

9. Time (use 24 hour clock) of fasting blood sample ___ ___ ___ ___ hours.

B) Personal Information

As part of the Sandy Bay Diabetes and Diabetes Complications Screening Project, we are gathering some information from Sandy Bay residents. I will ask you some personal questions, such as your date of birth and marital status. I will also ask you some questions about your health and some questions on your feelings and thoughts during the last month. If there are any questions that you do not want to answer please tell me so and we will move on to the next question.After the questions we will complete a short physical exam.

10. Does participant require an interpreter to answer questionnaire?

    Yes (___) No (___)

11. Sex: Male (___) Female (___)

12. What is your date of birth? _____/_____/_____
    dd mm yy

13. What is your current marital status?

Never married	_____	Widowed	_____
Married (or remarried)	_____	Common-Law	_____
Separated	_____	Refused	_____
Divorced	_____		

14. How many years of education have you completed?

None	_____	
Some elementary school	_____	
Elementary school complete	_____	
Some secondary school	_____	
Secondary school complete	_____	
Some post-secondary	_____	
Post-secondary complete	_____	
Other	_____	Specify _____
Refused	_____	

15. Are you currently employed?

Yes	_____
No	_____ ** go to 15c.
Refused	_____ ** go to 16.

15b. If currently employed, are you employed:

Full-time	_____	Occasional job	_____
Part-time	_____	Seasonal	_____
Refused	_____		go to 16

15c. If **not** currently employed, are you retired from your occupation?

Yes	_____
No	_____
Refused	_____

16. Do you speak an Aboriginal language well enough to carry on a conversation?

Yes	_____
No	_____
Refused	_____

If yes, specify the Aboriginal language _____

C.1) Medical History

Now I will ask you some questions about your health including your smoking history and if you have ever been diagnosed with certain diseases that are associated with diabetes.

17. Smoking History

a. Have you **ever** smoked cigarettes, pipes, or cigars on an approximately daily basis?

Yes	_____
No	_____ ** go to 17e
Refused	_____

b. If yes, did you smoke:

Cigarettes	_____
Pipe	_____
Cigars	_____

How long did you smoke? _____ years

c. Are you a current smoker?

Yes _____
No _____ ** go to 17e
Refused _____

d. i) how many cigarettes do you smoke in a day _____
ii) how many cigars do you smoke in a day _____
iii) how many pipefuls of tobacco in a day _____

Go To 18.

e. Does anyone in your household smoke cigarettes, pipes, or cigars?

Yes _____
No _____
Refused _____

18. Does anyone in your family have diabetes? Yes (___) No (___)

Unsure (___) Refused (___)

If yes, Who in your family has diabetes?

	Yes	No
Mother	_____	_____
Father	_____	_____
Sister(s)	_____	_____
Brother(s)	_____	_____
Maternal Grandmother	_____	_____
Maternal Grandfather	_____	_____
Paternal Grandmother	_____	_____
Paternal Grandfather	_____	_____

19. ***Have you ever been told by a health care professional that you have***:

a. **High blood pressure?** Yes (___) No (___)

Unsure (___) Refused (___)

If yes, How old were you when you were first told by a health care professional that you had high blood pressure?

Age in years _____

Have you ever been told by a health care professional that you have:

b. **Heart attack?** Yes (___) No (___)

Unsure (___) Refused (___)

If yes, How old were you when you had your first heart attack?

Age in years _____

c. **Other heart problems**? Yes (___) No (___)

Unsure (___) Refused (___)

If yes, please specify _____

How old were you when you were told you had heart problems?

Age in years _____

d. **Stroke?** Yes (___) No (___)

Unsure (___) Refused (___)

If yes, How old were you when you had your first stroke?

Age in years _____

e. **Kidney disease?** Yes (___) No (___)

Unsure (___) Refused (___)

If yes, please specify _____

How old were you when you were told you had kidney disease?

Age in years _____

Have you ever been told by a health care professional that you have:

f. **Diabetes-related vision problems?** Yes (___) No (___)

Unsure (___) Refused (___)

If yes, please specify _____

How old were you when you were told you had diabetes related vision problems?

Age in years _____

C.2) Treatment

If participant has diabetes ask question 20; if not go to Section D (question 21).

20. What kinds of treatments are you currently receiving for your diabetes?

	Yes	No	Refused
Changes to your diet	_____	_____	_____
Exercise	_____	_____	_____
Pills	_____	_____	_____
Insulin by needle	_____	_____	_____
Traditional therapies	_____	_____	_____

List Medications (dosage, frequency, duration of treatment)

_____

_____

_____

_____

_____

D) Stress Questions (omitted)

No E section.

F) Blood Pressure Measurement

26. Resting pulse (beats per minute) __ __ __

27. Arm blood pressure (sitting)

	Right		Left	
Blood Pressure (nearest 2 mm Hg)	__ __ __	__ __ __	__ __ __	__ __ __
	systolic	diastolic	systolic	diastolic

G) Neuropathy (omitted)

H) Anthropometric Measurements

		First Measurement	Second Measurement
34.	Weight (nearest 0.1 kg)	_____kg	_____kg
35.	Height (nearest 0.5 cm)	_____cm	_____cm
36.	Waist (nearest 0.5 cm)	_____cm	_____cm
37.	Hip (nearest 0.5 cm)	_____cm	_____cm

I) Metabolic Measures

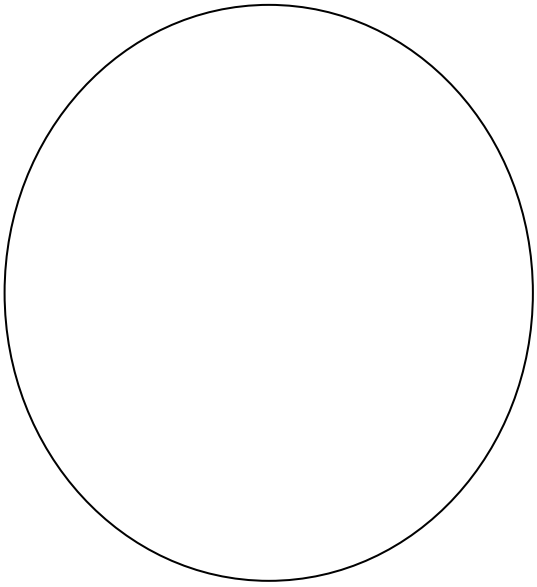
DCA 2000+ Analyzer

38.	Albumin	A = _____ mg/l
39.	Creatinine	C = _____ mmol/l
40.	A/C ratio	A/C = _____ mg/mmol

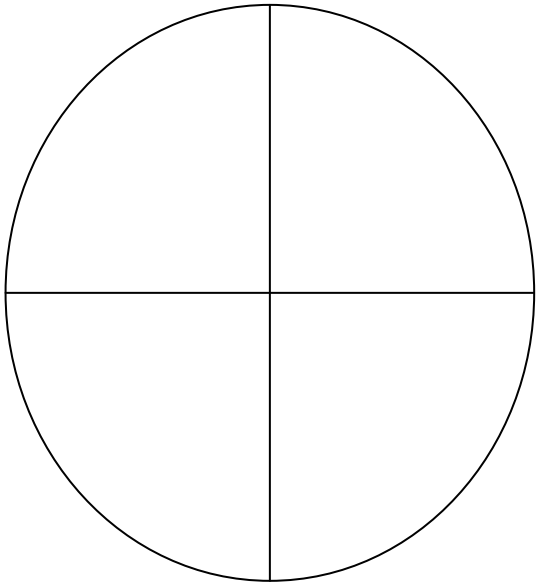
Appendix 3

Cognitive Tests

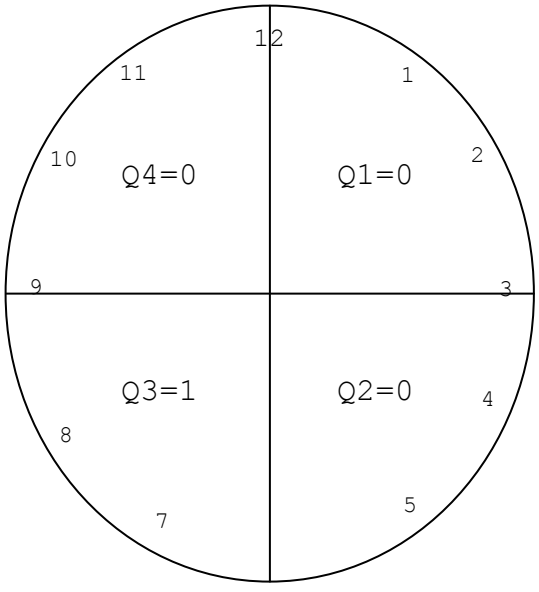
CLOCK DRAWING TEST



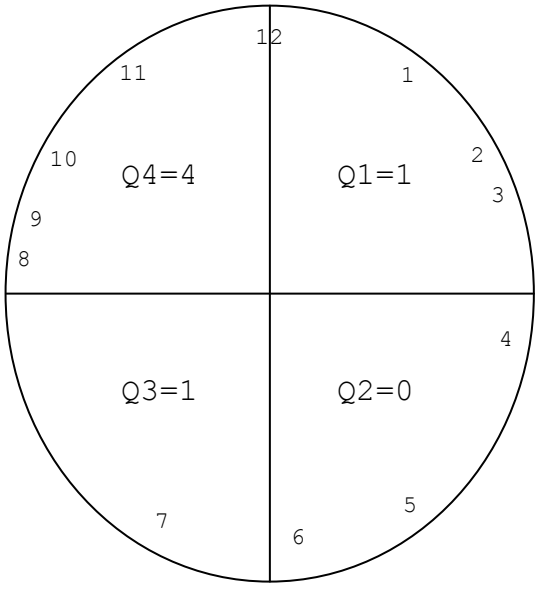
Sample pre-drawn circle provided to participants



Sample circle with pre-drawn bisecting line to facilitate scoring



Example 1:
Total score = 1
No cognitive decline



Example 2:
Total score = 6
Lowered cognitive performance

Appendix 3

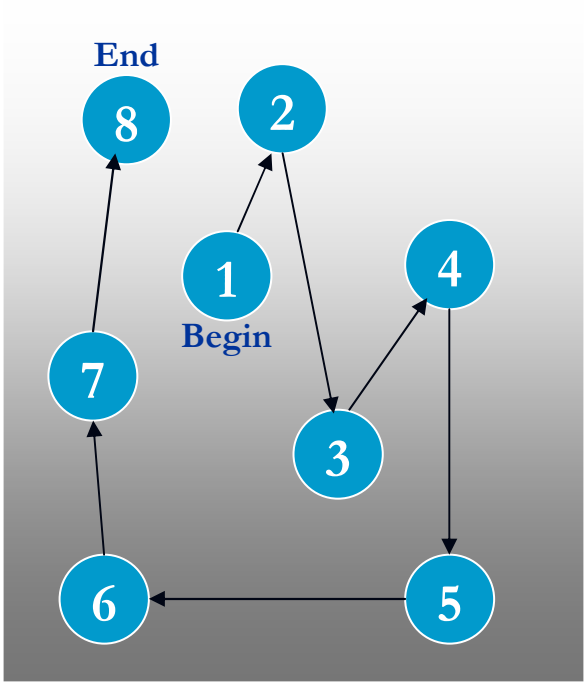
Study Sample: Clock Drawing Test

Appendix 3

Cognitive Tests

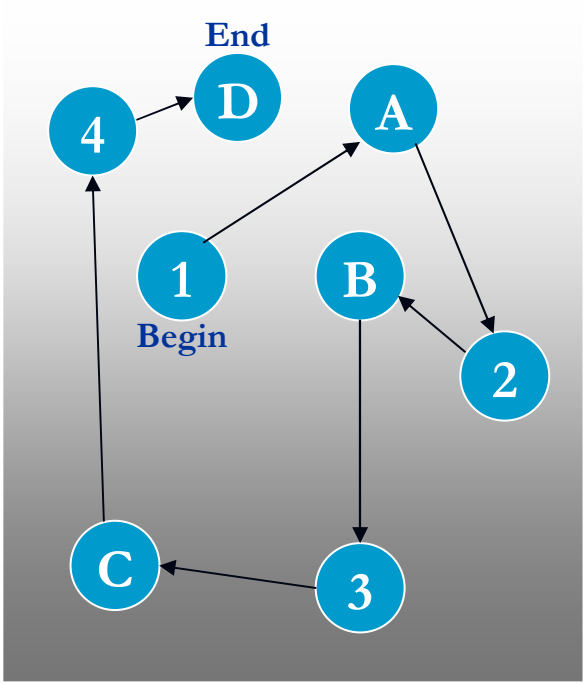
TRAIL MAKING TESTS

Part A (sample)



Numbers only

Part B (sample)



Numbers and letters

Appendix 4

Informed Consent Form

UNIVERSITY OF TORONTO
Department of Public Health Sciences, Faculty of Medicine

Study Information and Informed Consent
December 2007

VASCULAR AND METABOLIC RISK FACTORS, CAROTID ATHEROSCLEROSIS AND VASCULAR COGNITIVE FUNCTION IN A FIRST NATIONS POPULATION

This is an epidemiology study, a type of research study, which forms part of my PhD thesis research. In the larger study, a group of First Nations participants underwent a variety of blood tests, three pencil-and-paper drawing tests, and an ultrasound measurement of the arteries of the neck. Your participation will enable me to complete an important part of my PhD research.

You are being asked to take part in this portion of my PhD thesis because you are youthful and the results will provide information as to the performance of "healthy" subjects on three pencil-and-paper tests. Mainly, it will allow me to familiarize myself with the administration of the tests.

About 20 people will take part in this study: 10 men and 10 women.

If you are a current smoker, have a history of diabetes, high blood pressure, or have had a previous head injury requiring medical attention, you will not need to continue with this study and we thank you for the time. If you have none of these conditions, please continue with the consent form.

If you take part in this study, you will be required to complete three pencil-and-paper drawing tests. The tests will be completed immediately (e.g. no future appointment required), with my assistance (e.g. I will provide all the necessary materials and there is no cost or inconvenience to you), and I will be present to provide further instructions and for any additional questions (e.g. the tests should not take long/only a few minutes and two of the three tests will be timed by me).

There will be no follow-up.

You can decide to stop at any time.

There are no risks to your participation in this study.

There are no direct benefits to you.

Your information will be kept private and confidential including the use of a password protected laptop computer for the analysis and presenting only aggregate data. Nonetheless, no personal information is collected from you. Your drawings will be kept in a locked drawer, within locked office facilities at the Department of Public Health Sciences (e.g. 20 participants x 3 drawings each = 60 drawings in total).

There is no cost to you.

You may discuss your decision with your friends and family. If you have any questions, please feel free to ask me [see contact information below].

I understand the information and have had my questions answered. I agree to take part in this study.

Participant's Signature _____

Date _____

If you have any questions or concerns about your rights as a research participant, please contact Jill Parsons, Research Ethics Officer, Health Sciences jc.parsons@utoronto.ca or 416-946-5806

For further information, please contact:

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Appendix 5

Power Calculations - Proportions								
Clock Drawing Test, n=83, r=1.5								
Metabolic Syndrome								
OR	**P_0**	**P_1**	**P**	**N**	**D**	**N/D**	**(N/D)^1/2**	**Power**
1.60	0.16	0.234	0.189	0.674	0.384	1.756	-0.63	26.4
1.70	0.16	0.245	0.194	0.891	0.391	2.281	-0.45	32.6
1.80	0.16	0.255	0.198	1.131	0.397	2.848	-0.27	39.4
1.90	0.16	0.266	0.202	1.392	0.403	3.450	-0.10	46.0
2.00	0.16	0.276	0.206	1.671	0.409	4.082	0.06	52.4
2.20	0.16	0.295	0.214	2.279	0.421	5.418	0.37	64.4
2.40	0.16	0.314	0.221	2.942	0.431	6.825	0.65	74.2

Clock Drawing Test, n=83, r=1.5								
Body Mass Index								
OR	**P_0**	**P_1**	**P**	**N**	**D**	**N/D**	**(N/D)^1/2**	**Power**
1.60	0.50	0.615	0.546	25.818	0.620	41.664	4.49	>99.4
1.70	0.50	0.630	0.552	27.459	0.618	44.412	4.70	>99.4
1.80	0.50	0.643	0.557	29.027	0.617	47.058	4.90	>99.4
1.90	0.50	0.655	0.562	30.527	0.615	49.607	5.08	>99.4
2.00	0.50	0.667	0.567	31.961	0.614	52.062	5.26	>99.4
2.20	0.50	0.688	0.575	34.643	0.611	56.704	5.57	>99.4
2.40	0.50	0.706	0.582	37.099	0.608	61.014	5.85	>99.4

Formula: $Z_\beta=[(n(d^*)^2r)/((r + 1)p(1-p))]^{1/2} - Z_{\alpha/2}$

Where,
d* is the difference in proportions one wishes to detect ($d^*=p_1-p_0$).
n is the number of cases for a case-control study.
r is the ratio of controls to case (n=83 abnormal vs. n=125 normal according to the Clock Drawing Test).
p is the weighted average of p_1 and p_0, where $p_1 = (p_0OR)/(1 + p_0(OR-1))$ and $p=(p_1 + rp_0)/(1 + r)$.
p_0 is the proportion of exposed controls.
$Z_{\alpha/2}$ is the z-score using $\alpha=0.05$.
N is the numerator.
D is the denominator.

References: Kelsey JL et al. Methods in Observational Epidemiology, 2nd Edition. Oxford University Press 2000: New York, NY, USA.

Appendix 6

Power Calculations - Means						
Trail Making Test A, n=53, r=3						
Metabolic Syndrome						
Difference	**Std. Dev.**	**Numerator1**	**Numerator2**	**Numerator3**	**Z_β**	**Power**
5.0	13.0	0.38	39.8	6.3	0.46	67.7
10.0	13.0	0.77	39.8	6.3	2.89	>99.4
15.0	13.0	1.15	39.8	6.3	5.31	>99.4
20.0	13.0	1.54	39.8	6.3	7.74	>99.4
Trail Making Test B, n=50, r=3						
Metabolic Syndrome						
Difference	**Std. Dev.**	**Numerator1**	**Numerator2**	**Numerator3**	**Z_β**	**Power**
5.0	55.7	0.09	37.5	6.1	-1.41	<8.2
10.0	55.7	0.18	37.5	6.1	-0.86	19.5
15.0	55.7	0.27	37.5	6.1	-0.31	37.8
20.0	55.7	0.36	37.5	6.1	0.24	59.5
Trail Making Test A, n=119, r=1						
Body Mass Index, Obesity ≥30 kg/m^2						
Difference	**Std. Dev.**	**Numerator1**	**Numerator2**	**Numerator3**	**Z_β**	**Power**
5.0	13.0	0.38	95.5	9.8	1.80	96.4
10.0	13.0	0.77	95.5	9.8	5.56	>99.4
15.0	13.0	1.15	95.5	9.8	9.32	>99.4
20.0	13.0	1.54	95.5	9.8	13.07	>99.4
Trail Making Test B, n=112, r=1						
Body Mass Index, Obesity ≥30 kg/m^2						
Difference	**Std. Dev.**	**Numerator1**	**Numerator2**	**Numerator3**	**Z_β**	**Power**
5.0	55.7	0.09	56.0	7.5	-1.29	9.9
10.0	55.7	0.18	56.0	7.5	-0.62	26.8
15.0	55.7	0.27	56.0	7.5	0.06	52.4
20.0	55.7	0.36	56.0	7.5	0.73	76.7

Formula: Z_β=d*/SD[nr/r+1]^1/2 - $Z_{\alpha/2}$

Where,
d* is the difference in means one wishes to detect.
SD is the standard deviation.
n is the number of exposed individuals e.g. MetS$^+$ or obese.
r is the ratio of controls to case, or for unequal sample sizes (1+1/c), where c is the number of comparison subjects for each exposed subject or case.
$Z_{\alpha/2}$ is the z-score using α=0.05.
Power: the probability of detecting as statistically significant an association of a particular magnitude, and that any differences of importance are likely to be detected.

Note: solving for: $n=(1+1/c)(t_\alpha - t_\beta)\sigma^2/(u_1 - u_0)^2$, where 2=1+1/c for equal sample sizes, c=1 for 1:1 ratio.

References: Kelsey JL et al. Methods in Observational Epidemiology, 2nd Edition. Oxford University Press 2000: New York, NY, USA; Elwood M. Critical Appraisal of Epidemiological Studies and Clinical Trial, 2nd Edition. Oxford University Press 1998: New York, NY, USA.

Appendix 7

Power Calculations - Structural Equation Modeling

Step 1. Model Specification: prior research and theories to choose among plausible explanations
Step 2. Model Identification
Step 3. Model Estimation
Step 4. Model Testing (e.g. model fit)
Step 5. Model Modification

Step 1. Model Specification

Q. How to characterize metabolic, vascular and other risk factors in a measurement model?

Consider the number of latent constructs…

1.1. The scale for the latent construct is standardized. Therefore, $E(\xi_1,\xi_1) = Var(\xi_1) = 1$.

1.2. Measurement model in equation form: $\mathbf{x} = \mathbf{\Lambda_x}\xi + \delta$

X_1 = Cholesterol
X_2 = Low density lipoprotein
X_3 = Homocysteine
X_4 = Apolipoprotein A
X_5 = Apolipoprotein B
X_6 = Metabolic syndrome
X_7 = Type 2 diabetes
X_8 = Insulin resistance
X_9 = Smoking
X_{10} = History of cardiovascular disease

$\delta_{1\text{-}10}$ = error terms for the X's

$X_1 = \lambda_{11}\xi_1 + \delta_1$
$X_2 = \lambda_{21}\xi_1 + \delta_2$
$X_3 = \lambda_{31}\xi_1 + \delta_3$
$X_4 = \lambda_{41}\xi_1 + \delta_4$
$X_5 = \lambda_{51}\xi_1 + \delta_5$
$X_6 = \lambda_{61}\xi_1 + \delta_6$
$X_7 = \lambda_{71}\xi_1 + \delta_7$
$X_8 = \lambda_{81}\xi_1 + \delta_8$
$X_9 = \lambda_{91}\xi_1 + \delta_9$
$X_{10} = \lambda_{101}\xi_1 + \delta_{10}$

*Examine factor loadings (λ's) and statistical significance according to t-distribution.

*Examine fit statistics across the different models

Table 1. Comparison of Fit Statistics for Measurement Models

Fit Index	1-Factor	2-Factor	3- Factor	Final
χ^2Minimum Fit				
χ^2Normal Theory				
Df				
χ^2 / *df* Minimum Fit				
χ^2 / *df* Normal Theory				
NFI				
Model AIC				
RMSEA				
RMR				
GFI				

*Consider methods to increase model fit e.g. consider more than three latent constructs; free the error co-variances between the latent constructs; allow an indicator variable to load onto more than one latent construct; free the error co-variances between indicator variables.

Step 2. Model Identification

*Assuming a three-factor measurement model:

Rule: at least 10 subjects per variable are required. *Surrogate for sample size estimation.*

Therefore, having 23 variables, indicator and latent combined requires at least 230 subjects to estimate a structural equation with a measurement model component.

Number of known elements =[p(p + 1)]/2, where p is the number of observed variables, p=16
= [16(17)]/2
= 136

Therefore, there are 136 known elements.

Number of unknown elements: 10 γ path coefficients
1 β path coefficients
12 error variances of the exogenous variables
4 error variances of the endogenous variables

Therefore, there are a total of 27 unknown elements or free parameters in the model to estimate.

According to the t rule, a model is identified if $t \leq [(p + q)(p + q + 1)]/2$, where t is the number of unknown elements or free parameters, p is the number of endogenous variables, q is the number of exogenous variables. In this model, t=27, p=2, q=5.

Therefore, by the t rule: $t = 27 \leq [(p + q)(p + q + 1)]/2$
$= 27 \leq [(2 + 5)(2 + 5 + 1)]/2$
$= 27 \leq [(7)(8)]/2$
$= 27 \leq 28$

Therefore, this model is *over-identified*, and there is 1 degree of freedom remaining (27-28).

Step 3. Model Estimation

Measurement equations for risk factors: $\mathbf{x = \Lambda_x \xi + \delta}$

Atherogenic
$X_1 = \lambda_{11}\xi_1 + \delta_1$
$X_2 = \lambda_{21}\xi_1 + \delta_2$
$X_3 = \lambda_{31}\xi_1 + \delta_3$
$X_4 = \lambda_{41}\xi_1 + \delta_4$
$X_5 = \lambda_{51}\xi_1 + \delta_5$

Age
$X_{11} = \lambda_{114}\xi_4 + \delta_{11}$

Sex
$X_{12} = \lambda_{125}\xi_5 + \delta_{12}$

Metabolic
$X_6 = \lambda_{62}\xi_2 + \delta_6$
$X_7 = \lambda_{72}\xi_2 + \delta_7$
$X_8 = \lambda_{82}\xi_2 + \delta_8$

Vascular
$X_9 = \lambda_{93}\xi_3 + \delta_9$
$X_{10} = \lambda_{103}\xi_3 + \delta_{10}$

Measurement equations for vascular cognitive impairment and carotid atherosclerosis:
$\mathbf{y = \Lambda_y \eta + \varepsilon}$

$Y_1 = \lambda_{12}\eta_2 + \varepsilon_1$
$Y_2 = \lambda_{22}\eta_2 + \varepsilon_2$
$Y_3 = \lambda_{32}\eta_2 + \varepsilon_3$
$Y_4 = \lambda_{41}\eta_1 + \varepsilon_4$

Where,

Y_1 = Clock Drawing Test
Y_2 = Trail Making Test A
Y_3 = Trail Making Test B
Y_4 = carotid atherosclerosis
η_1 = Latent construct for carotid atherosclerosis
η_2 = Latent construct for vascular cognitive impairment
ε = Error terms for the Y's

Structural equation: $\boldsymbol{\eta = B\eta + \Gamma\xi + \zeta}$

$\eta_1 = \gamma_{11}\xi_1 + \gamma_{12}\xi_2 + \gamma_{13}\xi_3 + \gamma_{14}\xi_4 + \gamma_{15}\xi_5 + \zeta_1$
$\eta_2 = \gamma_{21}\xi_1 + \gamma_{22}\xi_2 + \gamma_{23}\xi_3 + \gamma_{24}\xi_4 + \gamma_{25}\xi_5 + \beta_{21}\eta_1 + \zeta_2$

Where,

η_1 = Latent construct for carotid atherosclerosis
η_2 = Latent construct for vascular cognitive impairment
ξ_1 = Latent construct for atherogenic risk factors
ξ_2 = Latent construct for metabolic risk factors
ξ_3 = Latent construct for vascular risk factors
ξ_4 = Latent construct for age
ξ_5 = Latent construct for sex
ζ = Error term for the η's

Step 4. Model Testing

	Dependent Variables	
Independent variables	Carotid Atherosclerosis	Vascular Cognitive Function
Metabolic risk factors	Estimate (SE)	Estimate (SE)
Vascular risk factors	Estimate (SE)	Estimate (SE)
Atherogenic risk factors	Estimate (SE)	Estimate (SE)
Age	Estimate (SE)	Estimate (SE)
Gender	Estimate (SE)	Estimate (SE)

From a t-distribution with $df \rightarrow \infty$, two-sided test: $*p<0.05$ at a critical value of 1.96, $**p<0.01$ at a critical value of 2.33[e]

Goodness of Fit Statistics
Chi-Sq Normal Theory:
Chi-Sq Minimum Fit:
df =
RMSEA:

Assessment of Mediation:

Direct effects: estimated from model (γ_{22})
Indirect effects: estimated from model (β_{21}*γ_{12})
Amount of mediation: c (γ_{22})- c' (γ_{22}) (direct effects model - mediation model)

Step 5. Model Modification
*Consider alternate theories, number of measurement models, freeing covariance, and other strategies to improve in model fit e.g. 1 remaining degrees of freedom.

[e] Reference: Rosner B. Fundamentals of Biostatistics, 5th Edition. Duxbury Thomson Learning 2000: Pacific Grove, CA.

Appendix 8

Descriptive Statistics for Trail Making Test A (N=203)

Variable	<22.0 seconds n=67 (32.8%)	22.0-30.0 seconds n=72 (35.3%)	>30.0 seconds n=64 (31.5%)
Age (years)[1]			
Mean (SD)	35.4 (8.7)	39.9 (9.7)	41.6 (10.6)
Median (IQR)	38.0 (9.5)	38.5 (10.5)	39.3 (13.5)
Min-max	19.0-62.0	19.0-65.0	19.0-66.0
Diastolic blood pressure (mmHg)			
Mean (SD)	75.6 (6.7)	76.2 (9.2)	77.2 (9.3)
Median (IQR)	76.4 (9.0)	76.4 (9.0)	76.4 (10.5)
Min-max	60.0-100.0	60.0-106.0	58.0-98.0
Systolic blood pressure (mmHg)[1]			
Mean (SD)	124.2 (13.8)	128.7 (15.4)	132.1 (17.1)
Median (IQR)	126.0 (17.0)	127.7 (12.0)	128.0 (14.5)
Min-max	98.0-195.0	92.0-180.0	110.0-200.0
Triglycerides (mmol/L)			
Mean (SD)	2.1 (1.4)	2.3 (1.6)	2.1 (1.9)
Median (IQR)	1.7 (1.1)	1.7 (1.0)	1.7 (0.8)
Min-max	0.6-6.8	0.6-9.0	0.4-11.3
High density lipoprotein (mmol/L)			
Mean (SD)	1.2 (0.3)	1.1 (0.2)	1.2 (0.3)
Median (IQR)	1.2 (0.2)	1.2 (0.3)	1.2 (0.3)
Min-max	0.7-2.5	0.6-1.9	0.6-2.3
Glucose (mmol/L)			
Mean (SD)	6.4 (2.9)	7.6 (3.8)	7.2 (3.5)
Median (IQR)	5.4 (1.2)	5.6 (4.2)	5.7 (2.8)
Min-max	3.7-18.2	3.4-19.5	3.5-19.6
Insulin (*p*mol/L)			
Mean (SD)	137.9 (132.4)	136.6 (121.6)	127.5 (157.5)
Median (IQR)	121.0 (95.0)	96.5 (114.5)	97.5 (93.0)
Min-max	19.0-1063.0	25.0-739.0	22.0-1224.0

Descriptive Statistics for Trail Making Test A (N=203) *(cont'd)*

Variable	<22.0 seconds n=67 (32.8%)	22.0-30.0 seconds n=72 (35.3%)	>30.0 seconds n=64 (31.5%)
Insulin resistance[2] (units)			
Mean (SD)	5.7 (5.9)	6.6 (6.7)	6.1 (9.0)
Median (IQR)	4.5 (4.3)	5.4 (6.1)	4.2 (4.5)
Min-max	0.5-36.1	0.7-44.5	0.8-67.4
Waist circumference (cm)			
Mean (SD)	106.9 (14.6)	103.4 (15.7)	106.1 (16.6)
Median (IQR)	107.0 (18.0)	103.4 (22.0)	105.0 (24.0)
Min-max	77.5-144.0	67.0-131.0	69.0-150.0
Body mass index (kg/m^2)			
Mean (SD)	33.7 (6.8)	31.6 (7.2)	31.9 (6.9)
Median (IQR)	32.2 (11.4)	30.7 (10.4)	31.2 (9.5)
Min-max	22.1-53.3	19.2-51.1	19.9-47.7
Duration of diabetes (years)			
Mean (SD)	6.8 (1.5)	7.6 (4.5)	7.2 (3.5)
Median (IQR)	7.0 (0)	7.0 (0)	7.0 (0)
Min-max	0.5-12.0	0.5-26.0	0.5-25.0

[1] $p<0.01$ based on group differences for non-normally distributed variables by the Wilcoxon rank-sum test.
[2] Insulin resistance defined by the homeostasis model of assessment: (insulin (*p*mol/L)*(0.144 uU/ml)*glucose (mmol/L))/(22.5).
Note: variables listed pertain to those considered in Manuscript 1 only.