CHANGES IN BRAIN STRUCTURE AND FUNCTION: UNDER-RECOGNIZED COMPLICATIONS OF TYPE 1 DIABETES

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ABSTRACT

Type 1 diabetes (T1D) develops in genetically susceptible individuals due to an auto-immune mediated destruction of pancreatic beta-cells. Disproportionately affecting non-Hispanic Caucasians, it is becoming more common among other races/ethnicities. With improvements in T1D management, patients can expect to live almost as long as people without T1D. Consequently, these patients will live longer with diabetes-associated complications, causing higher healthcare costs and reduced quality of life. When taken in consideration with a projected 3-5% annual increase in T1D incidence, preventing or delaying the onset and progression of diabetes complications deserves immediate public health attention.

While most T1D-related complications are well researched, its deleterious effects on the brain have received less attention. Because the majority of T1D brain studies have focused on pediatric and young adult populations, the complex interplay between increasing age and T1D-related brain abnormalities remains unclear. The goal of this research is to bring attention to the effects of childhood-onset T1D on brain structure and function in middle-aged adults who are also experiencing aging-induced cerebral insults. Data from a subset of middle-aged adults participating in the Pittsburgh Epidemiology of Diabetes Complications Study (EDC), diagnosed

with childhood-onset T1D between 1950-80, baseline 1986-88 and who underwent brain imaging and neuropsychological testing between 2010 and 2013 was used to address this goal, yielding some novel results.

First, the presence and severity of white matter hyperintensities was dramatically greater in those with vs. without T1D. These lesions developed at a younger-than-expected age and were independent of traditional cardiovascular risk factors. Skin intrinsic fluorescence and smoking were identified as factors deserving further exploration. Second, adults with vs. without T1D scored worse on IQ, executive function and psychomotor tasks. Chronic hyperglycemia, proliferative retinopathy and polyneuropathy were related to worse task scores, suggesting a systemic effect of chronic hyperglycemia. Third, among those with T1D, reduced blood flow to the prefrontal cortex and superior parietal lobes was related to cognitive dysfunction; these regions are important for information processing and executive function, tasks found to be impaired in T1D participants. These results suggest that altered brain structure and function should be considered a T1D complication with important public health relevance.

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PREFACE

I am writing this as I'm flying to the 74th ADA annual conference where I have been invited to give two oral presentations (papers 1 and 2 of this dissertation). We are over Denver now and I can see the Rocky Mountains ahead with snow still left on some of the peaks. Looking out the window at the city I left behind seven years ago, I am struck by how far I've travelled, both physically and metaphorically, and by how much I owe to the amazing group of people who have helped me make this journey.

First, I have to acknowledge my son, Lee. You are the reason I strive to be a better person. Your faith in me made it a little easier to get through all those times of self-doubt. It is my sincere hope that someday you and Becca will understand how much a parent loves their child! Along that vein, I of course have to thank my parents for their support, not just during these years at Pitt, but always; a girl couldn't ask for better parents! My grandmother, Ann Guthrie; I am honored to have you as a role model with your positive outlook and generous nature. My sisters, Katherin and Le'Anna, and my brothers, Mark and Perry – I could always count on you to make me laugh when I needed a break from all the rigors of doctoral studies, love you all!

And I have some of the greatest friends in the world! Melissa, Naomi, Lisa, Rose: hope you all know how much I treasure your friendship, wish you knew I was flying over your heads

right now! Dr. Shevock, if it wasn't for you, I wouldn't be Dr. Nunley; you are such an inspiration and I want you to know I would never have survived those long days in the Tom Collins building, or the training classes in Smyrna, or H1N1 and being activated for SHOCK!!

Then of course, I have to recognize my dissertation committee; without your guidance and patience, I would not be on this plane today. Dr. Boudreau, thanks so much for your statistical genius, I look forward to learning more from you! Dr. Costacau, you were invaluable in helping me with the EDC cohort data, and your "thumbs up" at my defense totally erased all my presentation anxiety, thanks! Dr. Aizenstein, everyone expressed their incredulity that you asked me such 'difficult questions' at my defense because they know you as such a nice and easy-going person, but I appreciate you for being a little 'tough' and holding me up to your high degree of expectations. Dr. Zgibor, I was saddened that our professional relationship "ended" after my first year of working with you and CAPH, then you joined as my dissertation committee chair! I was lucky to have your support as well as friendship during my time as a PhD student. You always said just what I needed to hear to keep me going and I will always be grateful for your help throughout this process. And Dr. Rosano, how do I express my thanks to you? You are such a wonderful mentor and friend! I feel so lucky that your interest in my depression and diabetes paper opened the door for us to work together, and I'm excited about starting my post-doc so I can keep learning from you. I cannot put into words my gratitude for all you've done to help me reach this goal!

Mandarq, you've been my constant companion along this journey from Denver to Dover to Pittsburgh. I promise more walks and play time now that this dissertation has been defended. I'm excited to see where we go from here…

1.0 INTRODUCTION

Diabetes mellitus (DM), a disease marked by impaired glucose regulation, has afflicted humans for thousands of years, with the earliest written reference made by Egyptian physicians circa 1500 B.C.[1] The disease is classified into two primary categories, type 1 and type 2, although lesser known types also exist. Type 1 diabetes (T1D), formerly known as juvenile onset or insulin dependent diabetes, develops due to autoimmune-mediated destruction of pancreas beta-cells, resulting in loss of insulin production. Diagnosis of T1D typically occurs before age 20 although it can be diagnosed at a more advanced age. Type 2 diabetes (T2D), formerly referred to as non-insulin dependent diabetes, develops due to the body's inability to properly utilize insulin. It is most commonly diagnosed among individuals aged 40 and older after a period of insulin resistance. An alarming trend, however, of increasing T2D incidence rates in teens and children is occurring as physical inactivity and obesity rates are also increasing in youth.

According to the Centers for Disease Control and Prevention (CDC), an estimated 1.7 million new cases of DM, the majority of those being T2DM, were diagnosed among Americans age 20 and older in 2012.[2] Improvements in diabetes management, combined with public health efforts to increase disease awareness and earlier diagnosis, have resulted in people living longer with the disease, as indicated by rising prevalence rates each year. Approximately 29 million American adults, 9.3% of the population, were living with the disease in 2012;

alarmingly, an estimated 8 million of these people (28%) were unaware they had developed the disease.[2] Type 1 diabetes makes up roughly 5% of all diabetes cases, with the overwhelming majority, 90-95%, being type 2.[3]

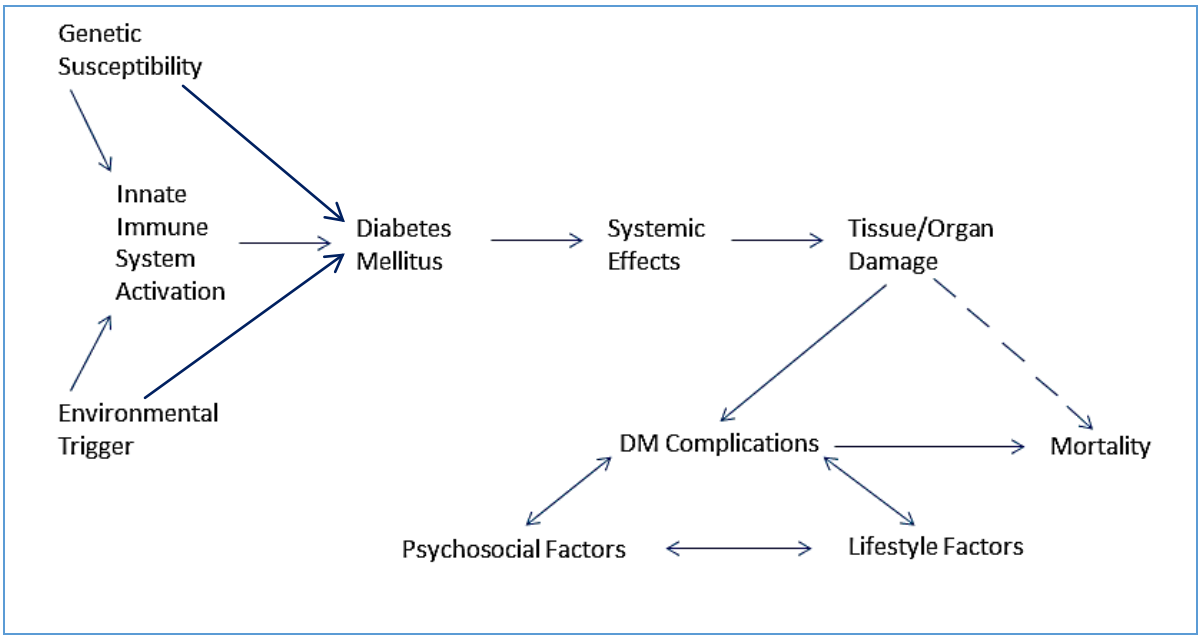

Figure 1.1. Conceptual framework of diabetes pathogenesis, effects and complications

Chronic hyperglycemia is associated with the development of specific complications in people with diabetes. These can be broadly categorized as microvascular and macrovascular complications. Diabetes is known to have negative effects on vision (retinopathy), kidney function (microalbuminuria, nephropathy and renal failure) and nerve function (neuropathy). It is also associated with higher rates of depression, certain cancers, infections (and delayed healing), non-traumatic amputations, especially of lower extremities, stroke, heart disease and death. Changes to brain structure and function, on the other hand, are less recognized complications of diabetes. Recent advances in neuroimaging techniques have greatly improved

researchers' ability to investigate the effects of diabetes on brain structure and function, yet these results remain under debate.

The primary aim of this paper is to demonstrate the deleterious effects of type 1 diabetes on brain structure and function in middle-aged adults. To achieve this aim, this research will (1) characterize structural changes in the brains of adults with T1D and (2) demonstrate that these structural changes are associated with poorer performance in neurocognitive tasks. A secondary aim is to identify factors related to changes in brain structure and function that may be amenable to interventions. Brain imaging and comprehensive neuropsychological test data from a well-characterized population of adults with childhood-onset T1D will be analyzed and compared to a similarly aged population without T1D to address these aims.

2.0 DIABETES MELLITUS

This section briefly introduces the types of diabetes. It also presents the current understanding of the epidemiology and etiology (i.e., genetics and risk factors) associated with the two major types of diabetes.

2.1 TYPE 1 DIABETES

Type 1 diabetes, previously known as juvenile or insulin-dependent diabetes, refers to the condition in which the pancreas beta cells lose their ability to secrete insulin in sufficient amounts to maintain glucose homeostasis.[3] Individuals with T1D require exogenous insulin to properly utilize glucose for energy. It is among the most frequently diagnosed metabolic disorders in children.

2.1.1 Epidemiology

Even though the majority of T1D cases are diagnosed before age 20, it can develop at any age.[3,4] Symptoms vary somewhat, depending on the degree of beta cell destruction. Common

symptoms include polydipsia, polyuria, gastrointestinal distress or pain, lethargy and weight loss. In younger children, these signs may go unnoticed, with T1D diagnosed only after the child suffers a ketosis-induced coma.

According to the Juvenile Diabetes Research Foundation (JDRF), as many as three million Americans are living with T1D, 85% of whom are adults (http://jdrf.org/about-jdrf/fact-sheets/jdrf-and-diabetes-statistics/). The JDRF website also reports that approximately 80 Americans are diagnosed with T1D each day, with an estimated 15,000 children and 15,000 adults diagnosed annually. On a global scale, the International Diabetes Foundation (IDF) estimates that the world-wide incidence of T1D increases by 3% annually.[5]

Type 1 diabetes is the only organ-specific autoimmune disease not occurring more frequently among females than males.[6] While the overall incidence rates are fairly equal in children diagnosed prior to age 15, a male to female ratio of 2:3 is seen among those of Western European descent diagnosed between the ages of 15-40 years.[7] Williams et al. (2002) tested 10,326 non-diabetic first-degree relatives of patients with T1D (diagnosed prior to age 20) for islet cell antibodies (ICA). This population of individuals, all younger than 40 years of age and representing eight European countries (Austria, Greece, Hungary, Norway, Poland, Russia, Turkey and the United Kingdom), displayed similar rates of islet autoimmunity even though T1D rates vary tremendously across these countries. Levels of ICA were similar for males and females until age 10. Among individuals aged 10-20 years, however, islet autoimmunity was almost 2.5 times higher among males than females. This provides some explanation as to why T1D rates begin to show a sex difference around age 10.[6]

Racial differences in T1D rates also exist. Based on data from 2002-2005, the SEARCH for Diabetes in Youth, a collaboration between the National Institutes of Health (NIH) and the CDC found that, in the US, non-Hispanic white youths had the highest incidence of T1D, 24.8/100,000 per year among those under age 10, and 22.6/100,000 per year among those age 10-19 years. Over this same time, incidence rates for non-Hispanic blacks under age 10 were 16.5/100,000 per year, followed by 15/100,000 per year among Hispanics, 7/100,000 per year among Asian-Pacific Islanders, and 4/100,000 per year among American Indians. The same trend was observed for those aged 10-19 years; the incidence rate among non-Hispanic blacks was 15/100,000 per year, followed by 13.5/100,000 per year among Hispanics, 7.7/100,000 per year among Asian-Pacific Islanders and 5.2/100,000 among American Indians (http://diabetes.niddk. nih.gov/dm/pubs/statistics/).[8]

2.1.2 Etiology

There is no single cause of type 1 diabetes; it is a complex disease with suspected environmental triggering of the condition that only occurs among individuals with a genetic predisposition. The lifetime risk of T1D in the general population is estimated to be 0.4%, with higher risk conferred to individuals who have a first degree relative with T1D. Interestingly, having a father with T1D increases a child's risk of developing the disease by 3-5% whereas having a mother with T1D increases the child's risk by only 1-2%.[9]

2.1.2.1 Genetics

Type 1 diabetes is a complex, multigenic disorder that is not yet fully understood. In the early 1970s, the human leukocyte antigen (HLA), located on the sixth chromosome, became the first gene to be associated with T1D.[10] Roughly half of all T1D cases are associated with HLA, an antigen which encodes glycoproteins that allow the immune system to differentiate self from non-self.[11] The HLA *DR3* and *DR4* alleles are most highly associated with the risk of developing T1D, with the highest susceptibility seen in those with the *DR3/DR4* combination. Children with this genotype have a 5% risk of developing T1D by the age of 15^{9} while the *DR2* allele is protective against T1D.[10,11]

In 1983, researchers found a genetic link between T1D and the insulin (INS) gene. This gene is found on the 11^{th} chromosome and as the name implies, is involved in coding for insulin production. The class I allele appears to increase the risk of T1D while the class III allele is protective.[10]

An additional six genes are now known to be related to T1D. Cytotoxic T-lymphocyte antigen (CTLA-4) gene on chromosome 2 leads to an increase in T-cell self-reactivity thereby increasing the risk of autoimmune disorders. Protein tyrosine phosphatase non-receptor 22 (PTPN22) on chromosome 1 appears to increase the negative regulation of T- cell activation. Interleukin 2 receptor alpha (IL2RA) on chromosome 10 encodes the IL-2 receptor complex and thereby controls the production of regulatory T-cells. Interferon induced with helicase C domain 1 (IFIH1) on chromosome 2 is believed to be involved in releasing interferon-gamma,

leading to apoptosis of virally-infected β cells; the GG genotype is associated with shorter time to overt diabetes than the GA or AA genotypes.[9-12]

2.1.2.2 Risk Factors

Type 1 diabetes has very few established risk factors. Having the genes for islet cell autoimmunity, and having a first degree relative with T1D, point to a strong genetic component, yet most cases of T1D are diagnosed among individuals with no family history of the disease.

Proposed environmental triggers include exposure to the Epstein-Barr virus, cytomegalovirus, coxsackievirus or the mumps virus. It is believed that infection with these agents either stimulates the body's autoimmune response to attack the islet cells, or it may be that these viruses directly infect the pancreas, leading to beta cell destruction. Low levels of vitamin D have been offered as a risk factor, supported by the increased rates in northern countries with lower sunlight. Contradicting this theory, however, is that early exposure to dairy products such as cow's milk, a good source of vitamin D, appears to be associated with an increased risk of T1D. Evidence seems to support an increased risk of T1D as maternal age increases, with the highest risk in children born to mothers age 45 and older.[13] According to the Mayo Clinic website, babies born with jaundice or who develop respiratory infections within the first week after delivery may also have an increased risk of developing T1D (http://www.mayoclinic.org/diseases-conditions/type-1-diabetes/basics/risk-factors/con-20019573).

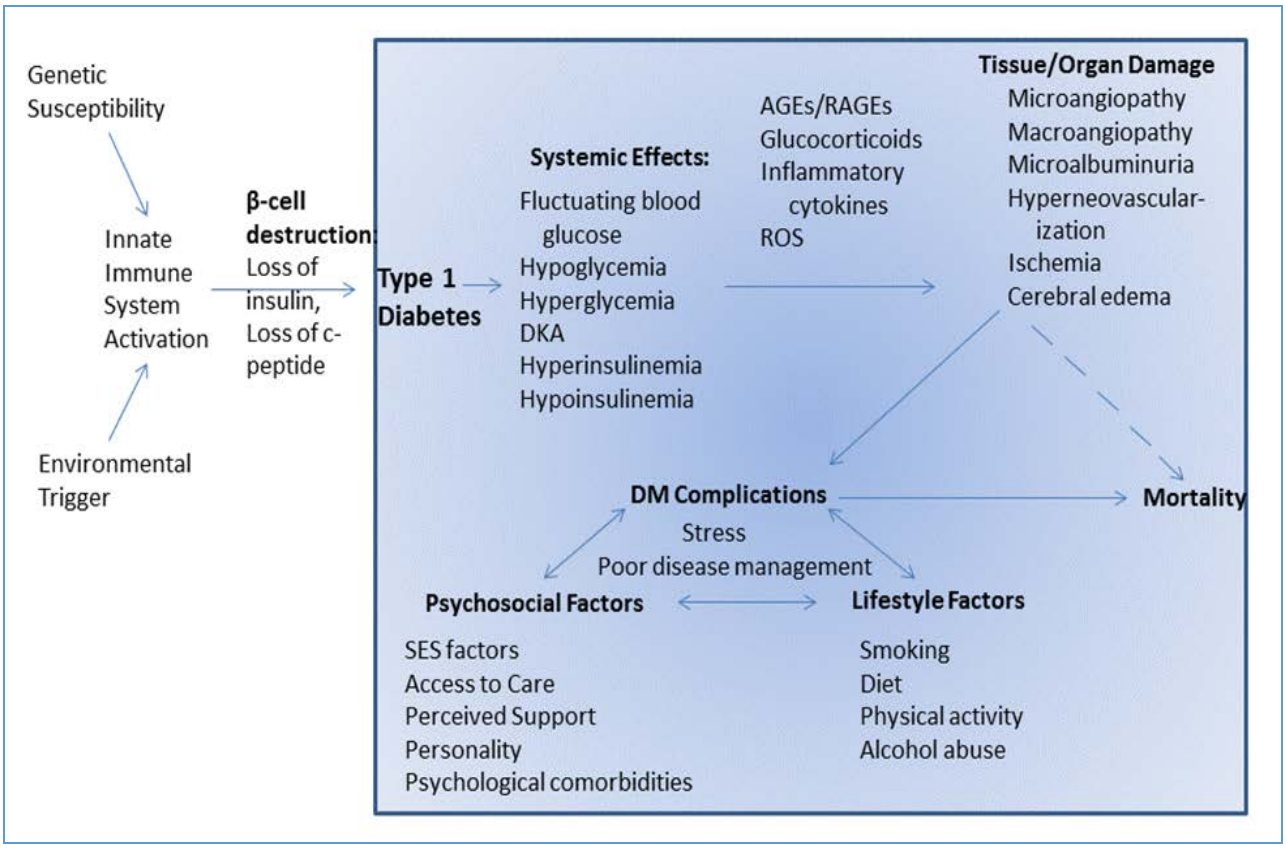

Figure 2.1.1 Conceptual framework of type 1 diabetes pathogenesis, effects and complications

2.2 TYPE 2 DIABETES

Previously known as non-insulin-dependent diabetes, type 2 diabetes (T2D) refers to the condition in which the body becomes resistant to insulin and fails to properly utilize glucose. Type 2 diabetes is by far the most commonly diagnosed type, accounting for 90-95% of all diabetes cases.[3] Even though it is most frequently diagnosed among adults age 45 and older, T2D is becoming increasingly common among younger adults, even among children. The rise in obesity and sedentary lifestyle in these age groups is blamed for this disturbing trend.

2.2.1 Epidemiology

Over the past thirty years, the number of newly diagnosed cases of T2D among adults aged 20 and older has tripled, increasing from 493,000 in 1980 to more than 1.9 million in 2012.[2] Data from the 1980 and 2010 Behavioral Risk Factor Surveillance System (BRFSS) show that adults age 18-44 have experienced the greatest percentage increase in newly diagnosed T2D cases, from 1.7/1000 in 1980 to 4.6/1000 in 2010. Adults over age 65 continue to have the highest overall T2D incidence rate, increasing from 6.2/1000 in 1980 to 12.4/1000 in 2010. The most recent national diabetes fact sheet estimates that half of all new T2D cases in 2010 were diagnosed in adults between 50 and 64 years of age, with roughly 21% of new cases diagnosed in adults aged 65 and older.[2] Type 2 diabetes affects males and females equally, with an estimated 13 million males (11.8%) and 12.6 million females (10.8%) over the age of 20 living with T2D.

In contrast to T1D, non-Hispanic whites have the lowest prevalence of T2D (7.1%), based on 2007-2009 US survey data.[2] Non-Hispanic blacks have the highest prevalence (12.6%) over this same time period. Prevalence among Hispanics approaches that of Non-Hispanic blacks (11.8%); prevalence is estimated at 8.4% among Asian Americans.[2,3]

2.2.2 Etiology

Like type 1 diabetes, type 2 diabetes is a complex disease with no single cause. T2D typically develops insidiously over a number of years. Both lifestyle behaviors, which are generally considered modifiable, and environmental factors, generally considered less-modifiable, contribute to the development of T2D.

2.2.2.1 Genetics

Type 2 diabetes is a complex disease with multiple risk factors and a strong genetic component. Identical twin studies of T2D generally show greater than 70% concordance, indicating a moderate genetic contribution to T2D risk.[11] Genome-wide association studies (GWAS) have identified more than 70 genes influencing development of T2D.[14] Even though a large number of genes are identified, their effect size is small (OR 1.1-1.2), indicating that more genes remain to be identified. Furthermore, most (96%) GWAS studies to date have concentrated on Caucasian populations, so the risks conferred by these genes cannot be generalized to other races/ethnicities; GWAS studies are underway to investigate the heritability of T2D in other populations.[14]

2.2.2.2 Risk Factors

Unlike T1D, multiple risk factors are known for T2D, with increasing age and overweight/obesity considered the primary risk factors. Other strong risk factors include race, family history of T2D, abdominal adiposity and physical inactivity or sedentary lifestyle. Women who develop gestational diabetes or who deliver a baby weighing more than nine pounds have an increased risk of developing T2D, as do these offspring. Having "pre-diabetes" or insulin resistance, impaired glucose tolerance, hypertension, dyslipidemia, cardiovascular disease and polycystic ovary syndrome (PCOS) also increase the risk of developing T2D. Recently, depression has become recognized as both a risk factor for, and a consequence of, type 2 diabetes.[15] Importantly, many of these risk factors tend to co-occur; individuals with higher combinations of these risk factors have a greater risk of developing T2D than does a person with only one or two factors.

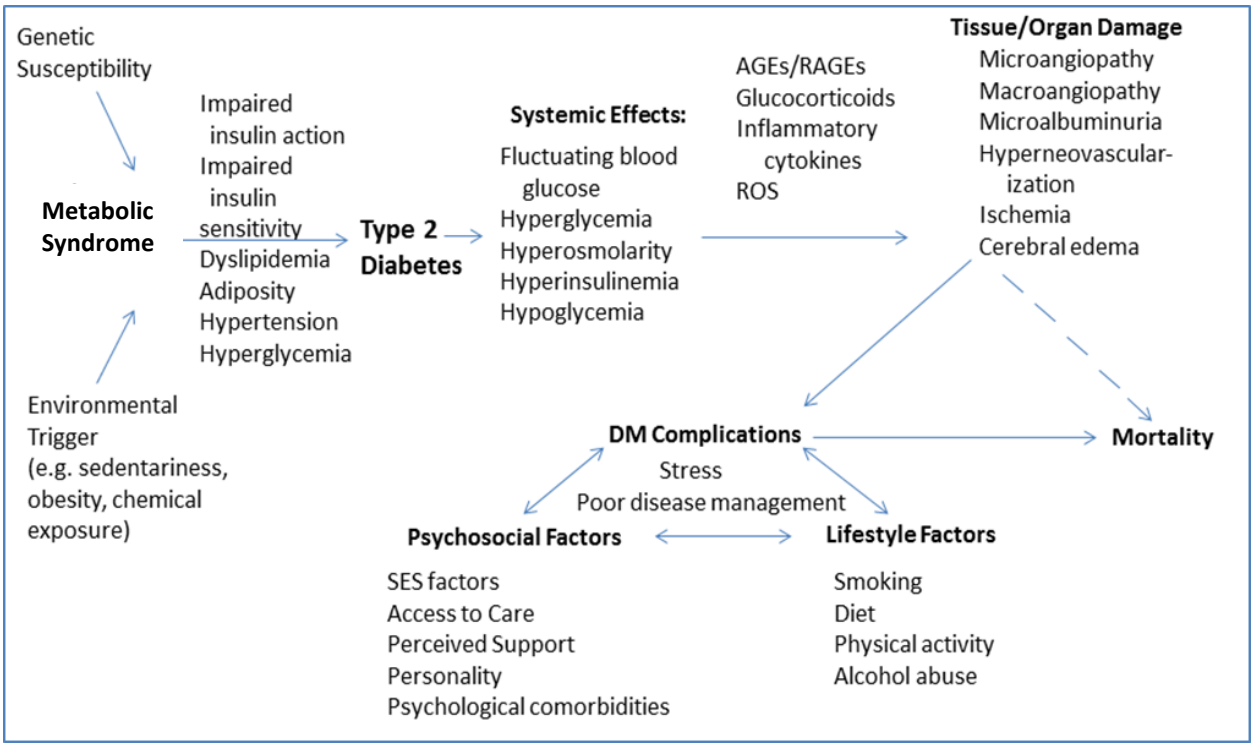

Figure 2.2. Conceptual framework of type 2 diabetes pathogenesis, effects and complications

2.2.3 Prevention and Intervention

Currently, type 1 diabetes can only be managed (although some may consider pancreatic transplantation a 'cure'), while T2D is considered to be a mostly preventable disease. Results from the Diabetes Prevention Program (DPP) and several other international intervention trials show that lifestyle changes, such as dietary changes, modest weight reduction and greater physical activity can prevent or delay the development of T2D among high-risk individuals.[16-19]

In some individuals with T2D, the disease can be managed through diet and lifestyle alone although most will eventually require oral agents to maintain glucose regulation. For a percentage of T2D cases, oral agents cease to adequately manage the disease after a number of

years. These individuals eventually require exogenous insulin in addition to or in place of their oral medication(s).[20] Interest is growing to determine if early insulin treatment in T2D patients significantly improves glycemic control and lowers long-term complication rates, with recent trials exploring whether early therapy with insulin or Exenatide® better protect against diabetes complications.[21-24]

2.3 OTHER TYPES OF DIABETES MELLITUS

The vast majority, approximately 95%, of diabetes mellitus cases fall in the type 1 or type 2 classifications. Gestational diabetes (GD) develops in 2-10% of pregnancies each year in the U.S., with African-American, Native American and Hispanic women more likely to develop the condition compared to non-Hispanic Caucasian women.[3] While GD usually resolves post-delivery, some 5-10% of women do not recover and are diagnosed with T2D shortly after their delivery. As mentioned earlier, GD is a risk factor for T2D, with between 35-60% of women diagnosed with GD going on to develop T2D within 20 years. Children of women with GD are also at higher risk of developing T2D.

Other rare types of DM are related to specific conditions or diseases. These make up only 1-5% of all diabetes cases. Examples include pancreatectomy, chronic pancreatitis, cystic fibrosis, pancreatic cancer, glucagonoma, pheochromocytoma, Cushing's Syndrome, Wolfram Syndrome and congenital lipoatrophic disorders.[25-31]

3.0 DIABETES MELLITUS COMPLICATIONS

People with T1D or T2D may develop the same complications, but the onset and progression differ by diabetes type as well as by individual variability. Complications can be classified as chronic or acute as well as by micro- or macrovascular nature. It is possible that the pathophysiology of complications differ by type but further studies are needed to determine the mechanisms contributing to the development of complications. This section will discuss the well-known complications related to diabetes mellitus.

3.1 CHRONIC COMPLICATIONS

Long-term exposure to hyperglycemia adversely affects numerous body systems although great variability exists between rates of these complications. Individual differences in disease control efforts, duration of disease, age at disease onset and presence/control of comorbidities (e.g. hypertension, dyslipidemia, depression and anxiety) all contribute to the development and severity of diabetes-related complications. These complications can be divided into microvascular and macrovascular conditions. Generally, T1D is more commonly associated with microvascular complications while macrovascular complications occur more frequently among those with T2D.

3.1.1 Microvascular Complications

Chronic hyperglycemia damages small blood vessels throughout the body. The most common diabetes-related microvascular complications are retinopathy, nephropathy and neuropathy. For individuals with T1D, these conditions are typically diagnosed many years after the onset of diabetes. For many with T2D, it is not uncommon for patients to show early signs of at least one of the three above-mentioned conditions at the time of their T2D diagnosis.

Diabetic retinopathy is the leading cause of blindness among adults ages 20-74 in the United States.[32] This is a progressive condition, characterized by growth of new blood vessels along the retina and often associated with a build-up of fibrous tissue between the vessels and the retina. These new blood vessels are prone to hemorrhaging, leading to macular edema and increased risk of glaucoma, all of which can lead to partial or complete permanent blindness. Diabetes duration appears to be the strongest predictor of retinopathy.[33] Within 15 years of their diagnosis, 25-50% of patients with T1D develop some degree of retinopathy; the condition affects virtually 100% of T1D patients within 30 years of diagnosis.[34] The prevalence of non-proliferative retinopathy is somewhat lower in T2D than in T1D, affecting approximately 20-30% of T2D patients within 15 years of diagnosis. According to Crawford et al. (2009), proliferative retinopathy was found in only 3% of T2D patients compared to 17% of T1D patients 11 years post-diagnosis.[34]

Diabetic nephropathy in the forms of intercapillary glomerulonephritis, nodular diabetic glomerulosclerosis or Kimmelstiel-Wilson syndrome occurs in 20-30% of those with T1D or T2D.[35] In its earliest stage, termed microalbuminuria, low but abnormal levels of albumin can

be detected in urine samples. Glomerular filtration function continues to decline at a variable rate, ranging from a loss of 2-20 ml/min/yr., depending on individual factors.[36] Confirmation of overt nephropathy (ON) occurs when urine protein levels surpass 300 mg/day.[35]

According to the 2004 American Diabetes Association (ADA) position statement, 80% of T1D patients with microalbuminuria will progress to ON within 10-15 years if they do not receive some type of intervention. Among T1D patients with ON, 50% will progress to end stage renal disease (ESRD) within 10 years, and in more than 75% within 20 years, unless they receive some type of targeted intervention.[35] Microalbuminuria is present in 20-25% of T2D patients at time of diagnosis, due to the long latency between the biological onset and diagnosis of T2D.[36] Without specific intervention, roughly 20-40% of T2D patients will progress from micro-albuminuria to ON over a 20 year period. Unlike T1D patients, however, only 20% of T2D patients with ON will progress to ESRD within 20 years of the onset of nephropathy. This may be due to the high mortality rate related to cardiovascular disease (CVD) among T2D patients; as T2D-related CVD mortality rates fall, there may be a related increase in ESRD in this population.[35]

Diabetic neuropathy is a common microvascular complication affecting nerve function. Several types exist, affecting different areas of the nervous system; these can be focal or diffuse. The most common types are distal symmetric polyneuropathy (DSP) and neuropathies involving the autonomic nervous system.[37] Roughly half of those with DSP are asymptomatic; these individuals are at a greater risk of unrecognized injuries to the extremities, resulting in greater risk for amputations. Symptomatic individuals describe burning or “electrical” pain or numbness in the feet, legs and/or hands, with intensification of pain at night.[32] Those with an

autonomic neuropathy are at an increased risk of morbidity and mortality, particularly those with cardiac autonomic neuropathy (CAN).[37] Other organ systems can also be affected, leading to "gastroparesis, constipation, diarrhea, anhidrosis, bladder dysfunction, erectile dysfunction, exercise intolerance, resting tachycardia, silent ischemia, and even sudden cardiac death."[32] The exact etiology of diabetic neuropathy remains unclear, but exposure to chronic hyperglycemia, tissue ischemia, oxidative stress and accumulation of advanced glycation end-products (AGEs) all potentially contribute to the development of diabetic neuropathy.[32]

3.1.2 Macrovascular Complications

Exposure to chronic hyperglycemia and resultant AGEs, as well as chronic inflammation and oxidative stress, damage large blood vessels throughout the body, primarily via atherosclerosis. Plaques accumulate throughout the arteries, leading to reduced blood flow and increased pressure against the arterial walls. An acute vascular event will occur should one of these lesions rupture.[32] Because people with T2D are more likely to also have metabolic syndrome with high risk factors for cardiovascular disease, such as central adiposity, dyslipidemia and hypertension, they have a greater risk of suffering from diabetes-related macrovascular complications compared to those with T1D.[38] Results from the United Kingdom Prospective Diabetes Study (UKPDS) show that tight control of cardiovascular risk factors decreased mortality from T2D-related conditions by 32%, two-thirds of which were related to cardiovascular disease.[39]

Coronary artery disease (CAD) is the leading cause of death in people with T2D.[40] In fact, the risk of a first myocardial infarction (MI) in those with T2D is equivalent to the risk of a second MI in people without diabetes. Those with T2D are also more likely to die from a first MI compared to people without diabetes.[41] Among T1D patients, CAD is highly associated with increased mortality, with evidence of an association between chronically elevated blood glucose and an increased risk of fatal CAD.[42] This same study also found that lower insulin dose, in addition to many standard risk factors for cardiovascular disease, was associated with a greater prevalence of non-fatal CAD.[42]

Cerebrovascular disease (CVD) includes stroke and transient ischemic attack (TIA). Data from the Baltimore-Washington Cooperative Young Stroke Study found more than a ten-fold higher risk for stroke among DM participants younger than age 44 compared to those without diabetes.[43] Additionally, those with DM are more likely to die from a stroke compared to individuals without diabetes. Furthermore, individuals with DM who survive a stroke are more likely to experience another stroke and have more than triple the risk of developing stroke-related dementia compared to individuals without diabetes.[44]

Peripheral artery disease (PAD) occurs due to reduced blood flow to the extremities. It is particularly common in the legs and is highly associated with intermittent claudication (pain when standing or walking).[45] Diabetes doubles to quadruples the risk of developing PAD and this reduced blood flow in the legs is associated with a 20-fold increased risk of lower extremity amputations among adults age 65-74 years.[44] Controlling glycemic levels and treating dyslipidemia and hypertension significantly lower the risk of PAD and other macrovascular complications in patients with DM.[44]

3.2 ACUTE COMPLICATIONS

Most of the acute DM complications result from severe hyperglycemia: diabetic ketoacidosis (DKA), hyperosmolar hyperglycemic state (HHS) and lactic acidosis (LA). HHS primarily affects those with T2D while DKA primarily occurs among those with T1D. Both types may be affected by LA. Hypoglycemic events are most common among T1D patients but can sometimes occur in T2D patients attempting tight glucose control.

Diabetic Ketoacidosis (DKA) occurs in the absence of insulin, e.g. in people with T1D, because the body cannot properly utilize glucose for energy. Starving tissues signal the liver to increase the production of glucose, leading to hyperglycemia. The kidneys work to remove the excess glucose through the urine, causing water and salts to also be excreted; this process is termed osmotic diuresis. This becomes a cycle leading to dehydration, polydipsia and polyuria. Eventually, the liver begins to burn fatty acids for fuel (lipolysis), with the end result being a build-up of ketone bodies in the blood, causing the blood pH to drop (acidosis). If left uncorrected, DKA is life-threatening, causing coma, seizures and cerebral edema. DKA is fairly common among those with T1D, often being the presenting symptom leading to a diagnosis of T1D in youth. The condition may occur on rare occasions in those with T2D, such as during periods of extreme stress or severe illness.

Hyperosmolar Hyperglycemic State (HHS) may present after prolonged periods of hyperglycemia among people with DM, particularly T2D. This condition was formerly known as hyperosmolar non-ketotic coma or non-ketotic hyperosmolar coma. HHS is similar to DKA in that hyperglycemia leads to dehydration and depletion of solutes. Unlike DKA, ketone bodies

are usually not present in HHS. Untreated, HHS can result in coma, seizures and death. Most cases of HHS occur in individuals suffering from an illness such as pneumonia, influenza, stroke or heart failure.

Lactic Acidosis refers to a type of metabolic acidosis defined by "a large anion gap, low pH of arterial blood, substantial reduction of bicarbonate levels and increased lactic acid levels" and is usually diagnosed when pH drops below 7.37 and lactic acid levels rise over 5 mmol/L.[46] Once a somewhat common and often fatal complication related to the medication phenformin, LA has become quite rare in DM patients, estimated at 3.3 cases per 100,000 patient years, and is now most commonly diagnosed among individuals using the oral antidiabetic drug metformin.[46] In rare instances, LA can co-occur with DKA in patients with T1D, and with HHS in patients with T2D. It seems to be more common among DM patients who abuse alcohol, perhaps due to liver damage, but the exact cause is unknown.[45]

Hypoglycemia refers to low blood glucose (< 70 mg/dL) and is a frequent complication among those using insulin or the oral drugs sulfonylurea and glinide.[47] DM patients able to recognize symptoms of hypoglycemia can usually reverse the low glucose level with no adverse effect. If, however, the individual is hypoglycemic unaware or cannot access food/drink or another source of glucose, the blood glucose can drop to dangerous levels, leading to "severe" hypoglycemic events. In such an event, the individual needs assistance to reverse the low glucose levels (e.g., glucagon injection). Some may lose consciousness and many require hospitalization to correct glucose levels. Hypoglycemia occurs much more frequently among those with T1D than with T2D, with the incidence of severe hypoglycemic events ranging from 115-320 per 100 patient years for those with T1D compared to 35-70 per 100 patient years for

those with T2D.[47] The most common complaint associated with hypoglycemia is short-term neurological impairment that appears to resolve shortly after establishing normoglycemia.[48,49]

4.0 TYPE 1 DIABETES AND BRAIN STRUCTURE

The brain parenchyma is comprised of two types of tissue, described by their color: gray and white matter.

White matter (WM) forms the bulk of the deep brain. It consists mainly of axon fibers and glial cells. Glial cells serve to support and protect neurons. Cerebral axons are enveloped in myelin, an insulating layer of oligodendrocytes. This white, fatty myelin sheath, from which WM derives its name, allows saltatory conduction of nerve impulses. Damaged myelin impedes impulse transmission with resultant deficits in cognitive and/or physical function (e.g., balance, speech) as evidenced by demyelinating neurodegenerative diseases such as multiple sclerosis.

Cerebral gray matter (GM) is comprised of neuronal cell bodies, dendrites, astrocytes and oligodendrocytes supplied by an intricate capillary network. It is distributed primarily along the brain surface although there are sub-cortical GM structures: the thalamus, hypothalamus, subthalamus, basal ganglia, hippocampus, amygdale and the olfactory nucleus. Gray matter structures are also found in the cerebellum: the dentate, fastigial, globose and emboliform nuclei, and in the brain stem: the substantia nigra, red nucleus, olivary and cranial nerve nuclei. Even though research has linked gray matter volume with a number of outcomes including mobility,[50] cognitive function,[51,52] addiction,[53] mental disorders[54] and mood disorders, GM damage remains an area of active research with many unknowns. For example, multiple

sclerosis research previously focused on WM damage and only in the past year have researchers determined it also affects cerebral GM.[55] This research hopes to contribute to understanding several of these unknowns. For example: does an association exist between gray matter volume and cognitive dysfunction in middle-aged adults with T1D?; if present, is the effect of GM volume global or focal?; could microstructural measures of gray matter integrity such as GM diffusivity and/or cerebral blood flow identify individuals at risk of cognitive dysfunction who may benefit from a pharmaceutical or lifestyle intervention?

4.1 TYPE 1 DIABETES AND CEREBRAL SMALL VESSEL DISEASE

As discussed previously, T1D causes both microvascular and macrovascular complications (see Sections 3.1.1, 3.1.2). These are systemic vascular effects; cerebral blood vessels are not immune to the effects of T1D. Indeed, cerebral macrovascular damage leading to an increased risk of hemorrhagic stroke in individuals with T1D is well recognized.[43,56] Conversely, cerebral microangiopathy in T1D is not well-studied and deserves further investigation.

Increasing age and hypertension have been established as the major risk factors for cerebral small vessel disease (SVD).[57] Hyperglycemia has also been identified as an important contributor to the development of SVD not only in the brain, but in the kidneys and eyes as well.[58] Even though hypertension and hyperglycemia cause impaired vascular tone (i.e., vessel stiffening), the mechanisms by which these factors damage cerebral small vessels are heterogeneous and not fully understood; Figure 4.1 presents a simplified proposed mechanistic pathway by which these factors affect brain structure and function. Hyperglycemia causes

oxidative stress which leads to endothelial dysfunction and resultant tissue hypoperfusion.[59] Similarly, hypertension results in vessel wall stiffening, endothelial dysfunction and hypoperfusion. Cerebral small vessel damage compromises the blood-brain-barrier. Chronic hypoperfusion leads to loss of neurons[60] and oligodendrocytes[61] and glial activation.[62] Hypoperfusion causes acute closing of pre- and post- capillary sphincters and in the longer term, causes microemboli to develop, clogging capillary beds and eventually a loss of capillaries.[57]

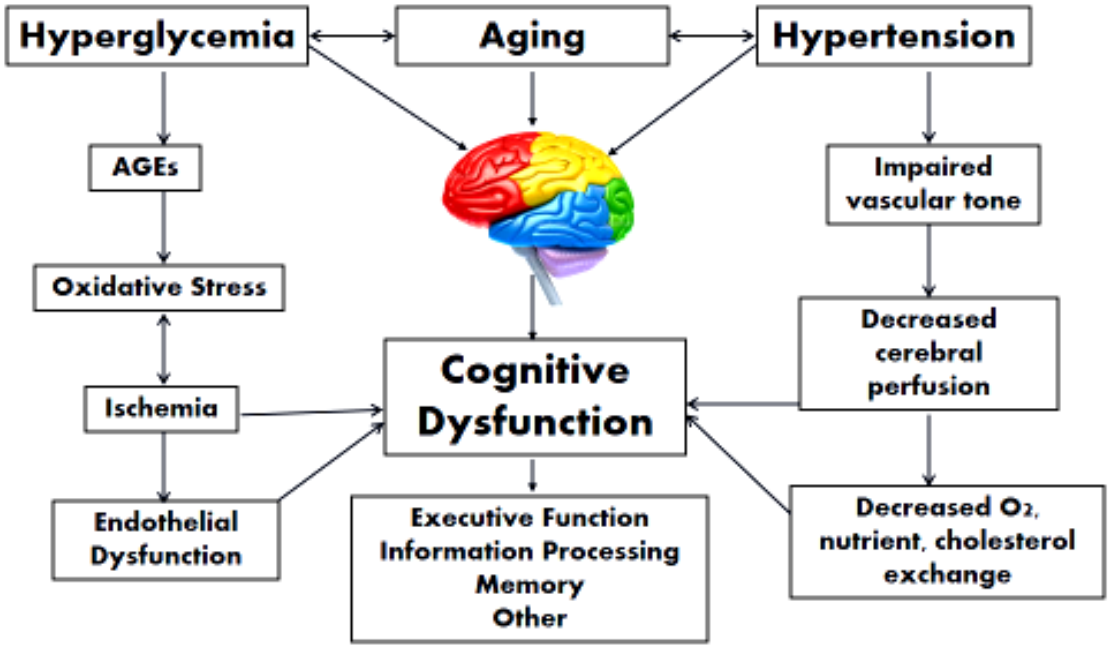

Figure 4.1 Proposed pathophysiological pathways for diabetes-related changes in brain structure and resultant changes in brain function.

4.2 ASSESSING BRAIN STRUCTURE USING NEUROIMAGING

The etiology of many important disorders of the central nervous system remains elusive. For some diseases, like stroke, the most important risk determinants have been identified but better prevention and therapeutic approaches are needed to reduce the continued high incidence and mortality, especially in selected racial, ethnic and socioeconomic groups. Evolving new technologies for studying disease etiology, pathologies and host susceptibility provide potential opportunities to define these conditions, to understand their etiology and to respond with effective prevention and treatments. Specifically, the application of advanced imaging methodologies can facilitate the phenotype's characterization earlier in the course of the disease, can improve causal inference in observational epidemiological studies and can enable accurate monitoring of response to therapy.

Evolving technologies alone, however, are not sufficient to advance our understanding of the etiology and pathogenesis of disorders of the central nervous system. It is essential to understand how to apply such technology in the context of carefully characterized populations, especially those with larger sample size and unique characteristics, i.e. higher or lower incidence of disease, that are followed in studies which are rigorously conducted and designed.

4.3 ASSESSING CEREBRAL WHITE MATTER HEALTH USING NEUROIMAGING

Studies show that T1D patients tend to have smaller WM volumes compared to their non-T1D peers.[63-68] White matter volume, however, is not the best indication of brain function. Other

neuroimaging measures provide a better assessment of white matter integrity at both the micro- and macro-structural levels.

4.3.1 White Matter Hyperintensities

White matter hyperintensities (WMH) serve as a macrostructural marker of cerebral small vessel disease. WMH appear as bright areas on T2-weighted fluid attenuated inversion recovery (FLAIR) images.[69,70] Although sometimes located deep in the white matter, they are more frequently noted at the periventricular caps and rims.[70] WMH are associated with advanced microvascular disease[70-72] and represent a heterogeneous set of conditions such as gliosis, demyelination, myelin pallor[70,72,73] or post-inflammatory water retention in the interstitial space.[74]

Cerebral WMH are common MRI findings among adults over age 65,[75] with a prevalence rate ranging from 60-100%.[76,77] Advancing age is the strongest predictor of WMH, with hypertension and hyperglycemia important contributors.[58,70,75,77] Even though the exact nature of WMH cannot be determined by MRI alone (e.g., demyelination vs. gliosis), these lesions are not benign. Brain imaging studies in elderly populations demonstrate that the presence of WMH is associated with increased rates of stroke, disability, depression, dementia and death.[70,72,78,79] The implications of WMH presence in adults ages 40-50 remains controversial, with prevalence estimates ranging from 0-50%.[80-82] Because they are not common findings in adults at this age range, few studies exist that investigate WMH in healthy adults younger than age 60.

4.3.2 Fractional Anisotropy

Diffusion tensor imaging (DTI), a neuroimaging technique based on the three-dimensional diffusion of water molecules, is useful in assessing WM integrity at the microstructural level.[83] In unrestricted environments, water molecules diffuse freely in random directions (isotropic diffusion). In restricted environments, diffusion is largely unidirectional; this is known as anisotropic diffusion.[83] Myelinated bundles of axons in cerebral white matter restrict water movement, with healthy myelin allowing diffusion parallel to the axons (axonal diffusivity) and prohibiting diffusion perpendicular to the axons (radial diffusivity). Axonal and radial diffusivity values are used to calculate the fractional anisotropy (FA) for each voxel of cerebral white matter.

FA is a scalar (0-1) measure that describes the magnitude, orientation and direction of diffusion. Low FA values are interpreted as areas of poor myelin integrity, low fiber density or axonal damage. Importantly, DTI assumes that all the fibers within each voxel travel in the same direction. In reality, fibers within voxels often cross, bend and otherwise change directions. This creates a challenge when interpreting changes in FA values; does a reduction in FA truly represent WM damage or is it merely an artifact caused by crossing or kissing fascicles in the same voxel?

DTI requires a minimum of six diffusion directions to estimate the diffusion tensor, from which FA is calculated. Researchers now prefer to use 32-64 directions to improve the signal to noise ratio, but this does not overcome the issue of crossing/bending fibers.[84] Alternative imaging protocols that do not rely on estimating a diffusion tensor, such as diffusion spectrum

imaging[85] and high angular resolution diffusion imaging (HARDI)[86] provide a solution to the crossing fiber issue. As researchers continue to refine and cross-validate these methods, they may replace the current DTI protocol as the standard tool to analyze cerebral WM health.

DTI also assumes that each voxel depicts only one tissue type. In reality, an estimated 30-50% of voxels classified as being WM also contain other tissue types, i.e., gray matter and/or cerebrospinal fluid (CSF).[87] The presence of multiple tissue types in the same voxel causes what is known as partial volume effects. Because of their isotropic diffusion, contamination by GM or CSF will cause a spurious reduction in FA.[88] To overcome partial volume effects on FA, image acquisition parameters can be adjusted to reduce the influence of partial volume effects on FA values. Using smaller voxel sizes and increasing directions, for a higher signal-to-noise ratio, are easily employed strategies to deal with partial volume effects.[87] In addition, use of a shorter repetition time (TR) and a minimum diffusion weight (b value) greater than zero have been shown to reduce the effects of CSF contamination in calculating FA values.[89]

4.3.3 Type 1 Diabetes and White Matter Hyperintensities

Relatively few MRI studies have examined the prevalence of WMH in middle aged adults with T1D [90-97] and these yield conflicting results. Out of the five case-control studies comparing WMH in participants with vs. without T1D,[90,92,95-97] only Dejgaard et al.[90] found a significant between-group difference in WMH prevalence.[92,93,95,97] In one study comparing WMH between patients with T1D and T2D, a significantly greater number of deep WMH were detected among T2D

compared to T1D participants.[98] While their presence does relate to suboptimal brain health, the exact effects of WMH on brain function remain to be determined.

4.3.4 Type 1 Diabetes and Fractional Anisotropy

Only three studies were found using DTI in T1D populations,[99-101] with two of these from the same group and using the same T1D population.[99,100] All three reported significantly lower fractional anisotropy (FA) among T1D participants compared to non-DM controls, suggesting reduced WM integrity in those with T1D. In a group of 73 children (mean age 16.8 years) with T1D, an increasing frequency of severe hyperglycemic episodes was significantly related to lower FA in the superior parietal lobe whereas FA in this same region significantly increased with increasing frequency of hypoglycemic episodes.[101] In a study of 25 adults with T1D, mean age 45 years, Kodl et al. (2008) reported a significant association between older age, longer diabetes duration and higher A1c and lower FA values in the optic radiation and posterior corona radiata; longer disease duration was also significantly related with lower FA in the splenium.[99] Moreover, reduced FA was associated with worse performance on the Rey - Osterreith Complex Figure, copy task, and the Grooved Peg Board test, both of which involve sensory motor integration and therefore provide some indication of cerebral white matter health.[99]

4.4 ASSESSING CEREBRAL GRAY MATTER HEALTH USING NEUROIMAGING

4.4.1 Gray Matter Volume

Loss of brain neurons and/or their connections results in smaller gray matter volume, known as GM atrophy. This can be visualized on MRI as enlarged ventricles, widening sulci and cortical thinning. Loss of GM can be global or focal and is highly associated with increasing age[102] although GM atrophy appears to occur earlier or at a faster rate in many conditions, such as multiple sclerosis,[103] hypertension[104] and migraines.[105] Despite being especially well-studied in relationship to dementia, with over 1000 articles returned from a recent Ovid search combining the terms "dementia" and "brain atrophy", the exact mechanisms and implications of GM atrophy are not yet fully understood.

4.4.2 Type 1 Diabetes and Gray Matter Volume

Studies differ regarding the relationship between T1D and GM. Seven studies report significantly smaller GM volume (overall and in specific regions) in T1D participants compared to non-DM controls.[66,67,98,100,106-108] One study reported larger hippocampal volumes in T1D participants compared to non-DM controls.[109] Five studies reported no difference in GM volume between T1D and non-DM controls[63,68,92,93,110] and one study found enlarged ventricles in T1D participants with early disease onset (diagnosed younger than age seven) compared to those with a T1D diagnosis after age seven.[94]

Severe hypoglycemic episodes were believed to be the major cause of reduced GM volume in people with T1D, but recent studies point to chronic hyperglycemia and DKA as being more detrimental to GM development.[63,66,93,107] Differences in population characteristics, such as age at time of T1D diagnosis, duration of T1D and exclusion of those with any T1D-related complications, may contribute to the lack of consistency between study results.

4.5 ASSESSING CEREBRAL BLOOD FLOW USING NEUROIMAGING

4.5.1 Arterial Spin Labeling

The brain requires a regulated flow of blood in order to provide oxygen and other nutrients to brain cells and to remove metabolic by-products. Cerebral blood flow (CBF) refers to the perfusion rate, usually in ml of blood/100g brain tissue/minute. Positron emission tomography (PET), single photon emission computed tomography (SPECT) and dynamic susceptibility contrast (DSC) have been historically utilized in CBF studies and clinical practice, but these invasive methods require use of a radiotracer. A newer method, arterial spin labelling (ASL) is rapidly gaining favor as a non-invasive method to analyze CBF without the use of radioisotopes and with no concerns of nephrotoxicity.[111]

Limitations of ASL must be considered, especially as it is a newer imaging technique. In particular, ASL has a relatively low signal-to-noise ratio which could cause detection problems in clinical settings, i.e., in populations suffering from diseases related to reduced cerebral blood flow.[111] Variations in image acquisition (i.e., pulsed vs. continuous vs. pseudo-continuous ASL)

and differences in pre- and post-processing software protocols make it difficult to compare results across studies. These can be minimized with the use of a validated automated pipeline; by using a 3-D fast spin echo rather than 2-D echo-planar single shot sequence, signal-to-noise ratio is maximized while distortions and artifacts are minimized.[112]

4.5.2 Type 1 Diabetes and Arterial Spin Labeling

Only one study was found utilizing ASL in a population of adults (mean age 40 yrs.) with T1D.[113] Mangia et al. (2012) report that hypoglycemic unaware T1D participants demonstrated a blunted increase in thalamic blood flow during early hypoglycemia (using hyperinsulinemic clamp) compared to individuals without T1D. They suggest that their findings implicate the thalamus in coordinating a counter regulatory response to hypoglycemia[113] but additional ASL studies are needed to corroborate this finding. Studies should also explore the effects of long-term T1D on global and regional CBF, its clinical significance and T1D-related risk factors related to reduced CBF. To date, no cognitive studies in adults with T1D utilizing ASL have been published.

In summary, a variety of modalities exist to examine changes in brain structure at the macro- and microstructural levels. It is theorized that microstructural changes precede macrostructural brain changes. That is, microstructural changes occur earlier in T1D cerebral pathology while macrostructural changes become detectable after years of damage. Other than acute insults such as stroke or traumatic brain injury, changes to cerebral structure that cause symptomatic changes in brain function (i.e., cognitive dysfunction) gradually progress over time

until such a point as they become visible on MRI, e.g., WMH. Additionally, markers of each technique are influenced by a complex interaction of T1D-specific risk factors (e.g., chronic hyperglycemia, severe hypoglycemia, insulinemia) and provide different implications regarding functional outcomes (Figure 5). No study has been identified that has conducted a thorough multi-modal investigation of brain structure and function among a population with long-standing T1D as is proposed in this research.

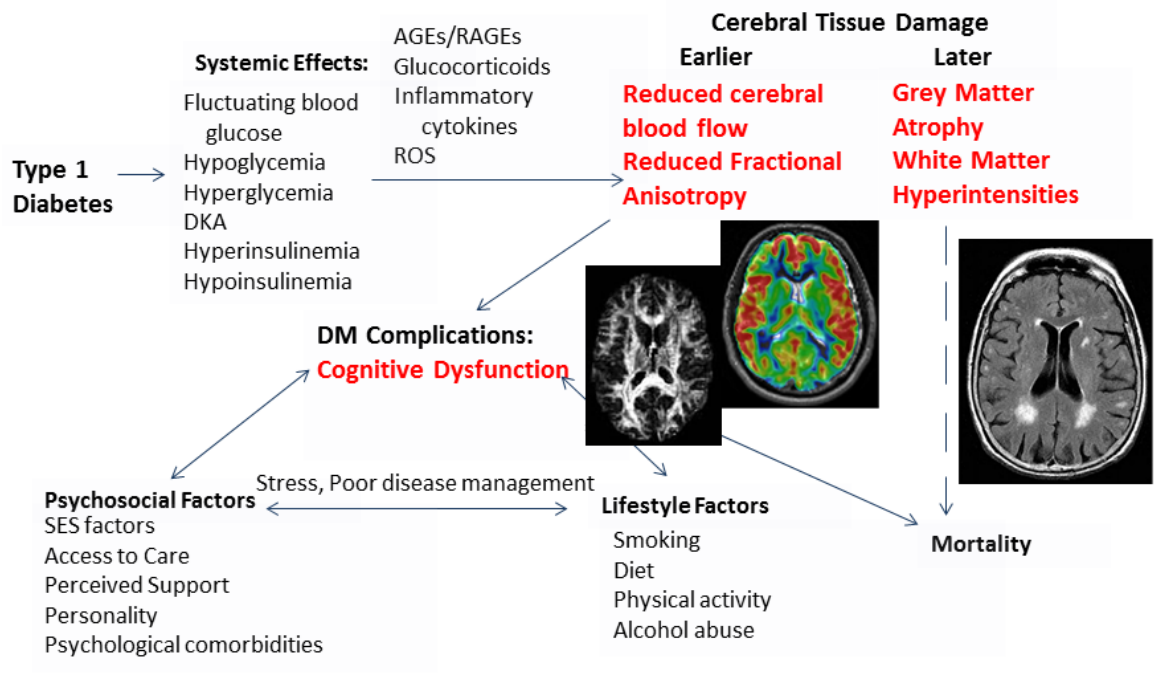

Figure 4.2.0. Conceptual framework of type 1 diabetes pathogenesis and its effects on the brain

5.0 TYPE 1 DIABETES AND BRAIN FUNCTION

In contrast to T2D, which is a recognized risk factor for dementia, less is known about the relationship between cognition and T1D.[114-117] Animal models of T1D demonstrate a deleterious effect of diabetes on the central nervous system[118-120] and studies report mild deficits in information/psychomotor processing test scores in youth with as compared to youth without T1D (note: these scores, while lower, are still within the "normal" range). A growing body of literature demonstrates that T1D negatively affects cognitive function in adults with T1D[68,92,94,99,107,121-125] but the mechanisms of this relationship remain unclear. Further complicating the matter is the effect of advancing age, a risk factor in itself for cognitive dysfunction. Identifying factors contributing to cognitive deficits in T1D remains an important area of research, especially as individuals with T1D are now living well into older age yet remain under-represented in T1D studies.

5.1 COGNITIVE DOMAINS AND TESTS

For the purposes of this paper, four broad areas (domains) of cognitive function will be considered: intelligence, executive function which encompasses information processing and psychomotor speed, memory and other (attention, visual perception, language). Researchers

often further divide or aggregate these domains, resulting in difficulties when attempting to compare cognitive function across studies. For instance, some studies differentiate between visual perception and visual-spatial ability[126] while others consider the two as a single domain.[127,128] Additionally, a multitude of neuropsychological tests exist to assess function in each domain, further complicating comparisons of results across different studies.[129] A complete listing of every neuropsychological test available would be impractical; rather, a list and short explanation of the instruments used for this research is presented in the appendix (Appendix A).

5.1.1 Type 1 Diabetes and Cognitive Dysfunction

Type 1 diabetes is most often associated with mild to moderate deficits in information processing speed[68,92,93,96,99,124,127] and attention.[68,93,96,124] Wessels et al. (2007) reported significantly poorer performance in the visual-spatial domain among T1D participants without retinopathy compared to the non-T1D control group[68] while Brands et al. (2006) found the opposite; they report better performance on the Rey-Osterrieth Complex Figure by T1D participants compared to non-T1D controls (mean difference 0.56; 95% CI 0.13, 0.98).[92] The participants in the Brands et al. (2006) study were 20 years older on average than those in the Wessels et al. (2007) study (61 yrs. vs. 42 yrs. of age, respectively) with equivalent levels of education.[68,98]

Only one study with brain imaging and neuropsychological testing published within the past 10 years[66] found a marginally significant difference in overall intelligence between T1D

participants and non-DM controls based on test scores from the Wechsler Abbreviated Scale of General Intelligence; the effect size was small (mean difference = 3.03; *p*=0.05). Two recent meta-analyses report similarly small yet statistically significant deficits in general intelligence in adults[127] and children[81] with T1D compared to non-T1D controls. Both reported significantly worse performance on tests of information processing speed, attention (visual and sustained), cognitive flexibility and visual perception domains, with no statistically significant between group differences noted regarding memory and learning domains.[127,128]

Brismar et al. (2007) conducted an extensive study to identify factors that might predict cognitive impairment in adults with T1D.[126] They found that T1D duration was significantly related to poorer performance in the following domains: psychomotor speed, memory, executive function (reflects control and management of other cognitive processes) and overall score.[126] Younger age of T1D onset was related to worse performance in these domains: general intelligence, verbal ability, working memory, attention and processing speed; hypertension was associated with worse performance in these domains: memory, visual perception; BMI was related to worse performance in these domains: general intelligence and processing speed. Female sex predicted poorer scores for psychomotor speed tasks. Nephropathy was related to worse performance on tasks of psychomotor speed (excluding Pegboard) and retinopathy was related to worse performance on tasks of executive function.[126]

6.0 SUMMARY

Worldwide incidence and prevalence rates of DM continue to increase annually. The International Diabetes Federation estimates that well over 30 million people in the U.S., and more than 220 million people in India and China, will be living with DM by the year 2030.[5,14] Many individuals with DM develop severe and often disabling complications, resulting in high medical, personal and societal costs. In addition, DM increases the risk of mortality; diabetes continually ranks as one of the top 10 leading causes of death in the U.S., with experts cautioning that DM is underreported on death certificates.[2,3,130]

Over the last few decades, advances in treatment and management of the disease have improved the life expectancy of DM patients, especially those with T1D. Whereas pre-19702, few T1D patients survived beyond middle age, those diagnosed after 1970 can expect to live almost as long as someone without diabetes (69 yrs. vs. 72 yrs., respectively).[131] These longer-lived T1D patients brought to light a previously under-recognized complication of DM – deleterious effects on the central nervous system (CNS) such as cerebral grey matter atrophy, compromised integrity of cerebral white matter and an increased prevalence of cognitive dysfunction in adults at a younger-than-expected age. Unlike the body of literature assessing these conditions in children and young adults, relatively few studies have investigated these conditions in older adults living with T1D since childhood, and the results remain equivocal.

Determining the prevalence of white matter hyperintensities and cognitive dysfunction in middle-aged adults with childhood onset T1D, identifying risk factors related to these outcomes and employing a multi-modal neuroimaging approach to comprehensively examine the effects of T1D on brain structure and function in an aging T1D population are the objectives of this research. Raising awareness about these CNS complications and identifying its risk factors should enable clinicians and patients with T1D to design strategies to better manage this condition and minimize the negative impacts of this disease.

7.0 METHODS

The research proposed herein seeks to heighten the awareness that T1D negatively impacts brain structure and function to such a degree that they gain the same attention as other well-recognized complications of T1D, e.g. blindness and kidney failure. To do so, the following gaps in knowledge will be addressed: (1) Is long-term exposure to T1D related to changes in cerebral white matter integrity as assessed by the presence of white matter hyperintensities in middle-aged adults with T1D since childhood, and if so, which factors contribute to the presence of WMH? (2) Does long-term exposure to T1D negatively impact cognitive function? If so, which cognitive tests/domains are most affected and which factors are associated with T1D-related cognitive dysfunction? (3) Do MRI measures explain the presence of cognitive dysfunction in middle-aged adults with childhood-onset T1D? How do these MRI measures relate to cognitive test scores? How do age, disease duration and other factors affect T1D-related cognitive dysfunction?

7.1 AIMS

Specific Aim 1: Determine if white matter hyperintensities (WMH), a measure of cerebral white matter health, occur more frequently among middle-aged adults with a childhood-onset of T1D compared to similarly-aged adults without T1D.

Hypothesis 1: Middle-aged adults with childhood-onset T1D will have a greater burden of WMH compared to similarly-aged adults without T1D.

Hypothesis 2: Among those with T1D, poor glycemic control, a history of high blood pressure and the presence of diabetes-related microvascular complications will be related to a greater burden of WMH.

Hypothesis 3 (exploratory): The associations described in H2 will be stronger among those younger than age 50 compared to those older than age 50 at time of MRI, with age being the strongest predictor of WMH in the older group.

Specific Aim 2: Investigate differences in neurocognitive task scores between middle-aged adults with childhood-onset T1D compared to similarly-aged adults without T1D.

Hypothesis 1: Participants with T1D will perform worse in tasks assessing executive function/information processing compared to similarly-aged adults without T1D.

Hypothesis 2: Between-group differences in task scores (effect sizes) will be larger in this population of middle-aged adults compared to effect sizes noted in pediatric and young adult T1D studies.

Hypothesis 3: Among T1D participants, poor glycemic control, longer disease duration, history of high blood pressure, prevalent depressive symptoms and prevalent microvascular complications will be associated with a higher prevalence of cognitive dysfunction.

Specific Aim 3: Examine the relationship between cognitive dysfunction and cerebral micro- and macrostructural measures in middle-aged adults with childhood-onset T1D.

Hypothesis 1: Measures of early changes to brain structure, i.e., reduced cerebral blood flow, will be related to the presence of cognitive dysfunction in this population of middle-aged adults with childhood-onset T1D.

Hypothesis 2: Poor glycemic control, history of high blood pressure, and prevalence of microvascular complications will be associated with lower CBF in middle-aged adults with childhood-onset T1D.

7.2 STUDY POPULATIONS

A cross-sectional design, comparing a population of middle-aged adults with T1D to a similarly-aged population without T1D was used to answer the research questions put forth in this document.

7.2.1 Type 1 Diabetes Population

Data from a subset of participants (N=106) from the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study will be analyzed to answer specific aims. Individuals with childhood-

onset T1D (≤ age 17 at diagnosis), diagnosed or seen within one year of their diagnosis at the Children's Hospital of Pittsburgh (all diagnosed between 1950 and 1980) were invited to participate in the EDC. A total of 658 patients, living within 100 miles of Pittsburgh, were enrolled in the EDC and underwent a baseline assessment between 1986 and 1988. For the first 10 years after their baseline exam, EDC participants underwent a complete physical exam and provided survey information every two years. After this time, participants completed mail-in questionnaires every two years for another eight years. A complete physical exam occurred in 2004-2006.

Notifications of the brain imaging study were sent to all EDC participants expected at the 2010-2012 EDC clinic visit. Out of 263 potential participants, 157 replied with interest in the MRI study. Of those, 112 were found to be eligible for MRI, 106 of whom were successfully scheduled and underwent brain imaging, neuropsychological testing and an abbreviated physical exam. Of the 106 with MRI data:

- nine had poor/no FLAIR image, resulting in N=97 for analyses of WMH (Aim 1).
- 11 did not complete the neuropsychological test battery, resulting in N=95 for analyses of cognitive dysfunction (Aims 2, 3).
- 13 had missing neuropsychological test scores or incomplete MRI sequences, resulting in N=93 for the multi-modal neuroimaging assessment of cognitive dysfunction (Aim 3).

7.2.2 Comparison Population without Type 1 Diabetes

Individuals from the University of Pittsburgh's MRI Study of Pre-Hypertensive adults (MR Hyper) study, free of T1D, served as a comparison group to address Aims 1 and 2. Details of the MR Hyper study have been previously described.[132] In brief, to study the effects of vascular risk factors on cerebral blood flow and cognition, 414 middle-aged adults living in the Pittsburgh area were screened to participate in this study. Of those, 110 did not meet blood pressure inclusion criteria (either too high or too low), 60 declined to participate and 14 withdrew, yielding a study population of 230 pre-hypertensive adults, mean age 46 years. Brain MRIs were acquired from 2010 - 2013. To mirror the EDC racial distribution, only Caucasians were included in these analyses. At time of MRI, biological and lifestyle data were collected for MR Hyper participants using methods comparable to those for EDC patients. SIF and HbA1c, however, were not available for these participants. Details of MR Hyper exclusion criteria are provided in Appendix C.

8.0 MANUSCRIPT 1: WHITE MATTER HYPERINTENSITIES IN MIDDLE-AGED ADULTS WITH CHILDHOOD-ONSET TYPE 1 DIABETES: PREVALENCE AND CONTRIBUTORS

8.1 ABSTRACT

Cerebral white matter hyperintensities (WMH), common features among adults age 65 and older, are indicative of microvascular diseases and increase the risk of stroke, depression, dementia, disability and death. Accrual of WMH significantly increases with older age, but it may be delayed by intervening on vascular factors. As patients with type 1 diabetes live longer, it is important to identify factors to prevent WMH. We determined prevalence and correlates of WMH in 97 middle-aged adults with childhood-onset type 1 diabetes participating in the Pittsburgh Epidemiology of Diabetes Complications Study since 1989 (age and duration: 49 and 41 years; 48% female), using high-resolution imaging at 3Tesla. Data from 81 adults without type 1 diabetes served as a comparison group (mean age 48 years; 53% female). Those with type 1 diabetes had significantly larger volumes of WMH than controls (0.214% vs. 0.003%, respectively) independent of age, blood pressure or lipid levels. In univariate logistic regression models among those with type 1 diabetes, significant predictors of WMH $\geq$ the median (0.001%

± .003%) were older age (OR=1.11, for each year), longer diabetes duration (OR=1.11, for each year), older age at diagnosis (OR=3.53 for age≥ 7), ever smoking cigarettes (OR=3.12), prevalent cardiac autonomic neuropathy (OR=2.52) and peripheral polyneuropathy (OR=2.94). Although these associations were independent of diabetes duration, only smoking and peripheral polyneuropathy remained significant after adjustment for age. Glycemic control (HbA1c current and historic, skin intrinsic fluorescence) did not significantly predict WMH among the full cohort. These findings indicate that childhood-onset diabetes contributes to earlier WMH development, and that older age and smoking may further accelerate WMH accrual among middle-aged patients with diabetes. Further studies to clarify the relationship between peripheral neuropathy and central nervous system disturbances are warranted.

8.2 INTRODUCTION

A striking feature of type 1 diabetes is that patients develop brain abnormalities commonly observed in much older adults without diabetes.[133] In addition to brain atrophy and infarcts, some children and young adults with type 1 diabetes develop cerebral small vessel disease, detected on magnetic resonance imaging (MRI) as white matter hyperintensities (WMH).[133] While cerebral WMH are common findings among non-diabetic adults over age 65,[75] with a prevalence ranging from 60-100%,[76,77] these lesions are not benign; they are associated with increased rates of depression, disability, stroke, dementia and death.[79,134-138] Uncommon before age 65, WMH are not routinely studied in younger adults. Initial evidence suggests that antihypertensive therapy may reduce progression of WMH.[72]. We contend that type 1 diabetes increases the risk of WMH development, and at a younger than expected age, thereby deserving greater attention in patients with type 1 diabetes, many of whom now survive well beyond age 50.

In addition to advancing age, many WMH risk factors overlap with characteristics of diabetes, particularly hypertension, hyperglycemia[72,139] and physical inactivity.[139,140] Chronic exposure to these shared conditions may predispose type 1 diabetes patients to develop WMH in excess of the 0-50% prevalence reported in middle-aged adults without diabetes.[80-82] Given patients' increased life expectancy[131] and the 3% annual increase in T1D incidence,[141] determining the prevalence of and risk factors for WMH related to this disease deserve prompt investigation.

Few studies have explored the prevalence of WMH in middle-aged adults with T1D[90-97] and the relationship between WMH with older age and cardiovascular risk factors remains unstudied.

Moreover, results to date have been inconsistent. In studies comparing WMH in participants with and without type 1 diabetes,[90,92,95-97] only one[90] found significantly greater WMH in cases than controls. A major limitation of these prior studies is their use of visual, semi-quantitative ratings of WMH, obtained at lower resolution, which best capture advanced WMH and may underestimate smaller lesions. Volumetric measures of WMH obtained at high resolution are needed to quantify WMH and identify correlated factors in type 1 diabetes patients.

This study's goals are to estimate the prevalence of WMH in middle-aged patients with type 1 diabetes and to characterize their WMH risk profile. Hypotheses include that adults with T1D have greater WMH compared to similarly-aged adults without T1D. We also tested whether blood pressure and other known WMH risk factors contribute to any between-group difference. Additionally, analyses explored relationships between WMH and diabetes-specific as well as general health factors collected over a 20-year span prior to MRI.

8.3 METHODS

All study procedures received local IRB approval prior to study initiation. All participants provided informed consent prior to undergoing procedures.

8.3.1 Study Populations

Type 1 diabetes participants were drawn from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC), an ongoing, prospective study of individuals with childhood-onset (diagnosed ≤ 17 years) type 1 diabetes. All EDC participants, diagnosed between 1/1/50 and 5/31/80 and seen within one year of diagnosis at Children's Hospital of Pittsburgh, underwent a baseline assessment (1986 – 1988) when the mean age was 28 years and the average diabetes duration was 19 years. After their baseline visit, EDC patients were followed with biennial exams for 10 years, thereafter completing biennial questionnaires. An additional physical exam occurred in 2004-2006 (18-yr follow-up). A total of 263 locally-resident EDC participants contacted for the 2010-2012 exam were invited to participate in this MRI study. Of these, 157 did not undergo imaging: 81 were not interested, 37 were ineligible for MRI and 39 did not reply or did not show for their MRI. The remaining 106 (mean age 48 years, 50% female, 98% Caucasian) underwent brain imaging between December 2010 and December 2012. Due to poor or no WMH imaging, nine were excluded from analyses, yielding an analytical sample of n=97 (Figure 8.1).

Non-type 1 diabetes population: Adults without type 1 diabetes from the University of Pittsburgh's MR Hyper Study served as a comparison group. Details of the MR Hyper Study have

been previously described.[132] In brief, to study the effects of vascular risk factors on cerebral blood flow, 414 middle-aged adults living in the Pittsburgh area were screened to participate in this study. Of those, 110 did not meet blood pressure inclusion criteria (either too high or too low), 60 declined to participate and 14 withdrew, yielding a study population of 230 pre-hypertensive adults, mean age 46 years; full exclusion criteria are presented in Appendix C. Brain MRIs were acquired from 2010 – 2013 when participants also completed a neuropsychological test battery with many of the same tasks as administered to the EDC participants. To mirror the EDC racial distribution, only Caucasians were included in these analyses. At time of MRI, biological and lifestyle data were collected for MR Hyper participants using methods comparable to those for EDC patients. SIF and HbA1c, however, were not available for these participants.

8.3.2 Covariates

Diabetes-specific variables: For EDC participants, the following were collected at each physical exam from baseline to time of MRI: insulin dose (average daily units/Kg body weight); HbA1c (using saline-incubated blood samples and cation-exchange microcolumn chromatography prior to October 1987, using high-performance liquid chromatography through 2004-2006, and thereafter using the DCA 2000 analyzer); estimated glucose disposal rate (an estimate of insulin sensitivity based on a regression equation derived from insulin clamp studies)[142]; use of insulin pump (yes/no); and fasting serum glucose.

Repeated assessment of HbA1c at every exam allowed the calculation of an average HbA1c over the duration of the EDC study. "A1c months", a variable combining cumulative degree and duration of glycemic exposure, was also assessed (see [143] for details).

Skin intrinsic fluorescence (SIF), a measure which partially reflects advanced glycation end products (AGEs) in the skin, was assessed at MRI. A skin fluorescence spectrometer (SCOUT DS®) noninvasively measured the skin of the left volar forearm. Details on the device and SIF calculations are discussed elsewhere.[144]

Severe hypoglycemia history was assessed in 1990-92, when patients self-reported loss of consciousness due to hypoglycemia over the previous two years.

Diabetes-related complications: Prevalence of complications was ascertained in 2004-2006. While this was an average of five years prior to MRI, these were the most recent EDC physical exam data available for analyses.

Renal disease refers to microalbuminuria (MA), overt nephropathy (ON) or End Stage Renal Disease. ON was defined as the presence of an albumin excretion rate >200 µg/min in at least 2 of 3 timed urine collections and MA as an albumin excretion rate between 20-200 µg/min in at least 2 of 3 urine samples.

Coronary artery disease (CAD) was defined as myocardial infarction (Minnesota Codes 1.1 or 1.2), fatal CAD (determined by review of death records and family interview) or angiographic evidence of 50% or more stenosis. CAD also included angina diagnosed by the clinic physician and/or ischemic ECG changes (Minnesota Codes: 1.3, 4.1-4.3, 5.1-5.3, 7.1).

Neuropathies: Distal symmetric polyneuropathy (DSP) was determined by medical history and clinical examination using established protocols, i.e. the symptoms and signs consistent

with DSP, and reduced deep tendon reflexes. DSP was further determined as 'confirmed' using the Vibratron II to detect vibratory threshold. Cardiac autonomic neuropathy (CAN) was determined by heart variation during deep breathing and by heart rate and blood pressure response to standing.

Retinopathy: Eye exams were done using three standard field stereo fundus photographs. Photographs were read at the Madison Reading Center in Wisconsin and graded using the Early Treatment Diabetic Retinopathy Study classification, with a grade of 60 or higher in one eye or a grade less than 60 but with panretinal photocoagulation scars consistent with laser therapy indicating proliferative retinopathy.[145]

Biological risk factors: Hypertension was defined as any blood pressure reading ≥140/90 mmHg or ever reporting use of antihypertensive medication from baseline through day of MRI.

Blood pressure: Three seated blood pressure readings were taken with a random-zero sphygmomanometer on day of MRI, with an average of the second and third readings used, per the Hypertension Detection and Follow-up Program Protocol.

Cholesterol: SYNCHRON CX® Systems was used to measure non-fasting total cholesterol and high-density cholesterol (HDLc) at time of MRI. Non-HDL cholesterol was calculated as (total cholesterol – HDLc).

Serum creatinine levels were measured at time of MRI.

Apolipoprotein E (ApoE) status was ascertained in 2004-2006.

Body mass index (BMI) was calculated based on the participants' weight and height at time of MRI (kg/m^2).

Lifestyle/Behavioral risk factors:

Smoking status was self-reported as current, past or never smoking 100 cigarettes and was dichotomized as ever vs. never smoking based on information obtained through 2004-2006.

Alcoholic beverage consumption was dichotomized as any vs. no alcohol in the past week based on participant self-report in 2004-2006.

Physical activity for the past week was determined via Paffenbarger self-report questionnaire in 2004-2006. Responses were used to estimate energy expenditure (kcal).

Brain MRI protocol. Both cohorts underwent brain MRI in a 3Tesla Siemens Trio TIM scanner located in the Magnetic Research Center at the University of Pittsburgh. A T1-weighted 3D sequence (MPRAGE: TR/TI/FA = 2300/900/9, Resolution 256, Grappa 2), acquisition time 7.5 minutes, was used to attain grey matter (GM) and white matter (WM) volumes. For WMH, a T2-weighted FLAIR sequence (TR/TE = 9002/56 ms Ef; TI = 2200 ms, NEX = 1) was used with an interleaved acquisition; 48 slices (3mm, no gap).

Markers of macro-structural measures: An automated WMH segmentation method was used.[69] First, skull and surrounding soft tissue were stripped from the underlying brain on T1-weighted images. This map was used to remove brain from skull in the corresponding T2 FLAIR images at the same voxel size. Clustered WMH, based on voxel intensities on T2 FLAIR images, were then derived. Total brain volume constituted the sum of volumes of GM, WM and cerebrospinal fluid, obtained using an automated labeling pathway. For EDC patients, normalized GM volume was computed as a percentage [(GM volume/intracranial volume)*100]; this variable was not calculated for MR Hyper participants.

8.3.3 Statistical Analyses

Group differences were assessed using t-test for normally-distributed continuous variables, Fisher exact test for categorical variables and Wilcoxon rank test for non-normal data. Demographic and other factors potentially related to WMH were compared between EDC patients by MRI status (Table 8.1), between EDC and MR Hyper participants (Table 8.2), between EDC patients by WMH burden (Table 8.3) and between EDC patients by age < or ≥ 50 (Supplementary Table 8.1). Tobit censored regression (data not shown) was performed on the combined populations (EDC and MR Hyper) to determine if case status predicted WMH volume independently of other factors.[146]

In analyses restricted to EDC patients, WMH volume was dichotomized as either < or ≥ the EDC median of 0.00111 ± 0.0031%, corrected for intracranial volume. Factors that differed significantly by WMH burden were tested using logistic regression models. Because older age is the strongest risk factor for WMH in aging studies, two approaches were applied to control for the effects of age on WMH in EDC patients. Models were first conducted adjusting for age at MRI then stratified by the EDC median age of 50, using age-group specific median values of WMH (M age group<50 = 0.00108 ± 0.0032%; M age group≥50 = 0.00166 ± 0.0028%).

To examine survival bias effects on these associations, analyses were repeated excluding the 18 EDC patients diagnosed before 1965, as mortality was relatively negligible for those diagnosed post-1965 (15%) compared to those diagnosed prior to 1965 (44%).

Analyses were conducted using SAS 9.3 and SPSS 21.

8.4 RESULTS

Compared to the 157 patients without MRI (Table 8.1), the 106 EDC patients with MRI were significantly younger, less likely to be female, with a lower prevalence of CAD, CAN or microalbuminuria and a lower BMI (all $p<0.05$).

Compared to MR Hyper participants (Figure 8.2, Table 8.2), EDC patients had almost sixty times greater WMH volume ($p<0.0001$). This difference remained significant ($p<0.0001$) after excluding the 32 EDC patients with hypertension (mean WMH = 0.0019%) or when excluding the prominent EDC outlier (mean WMH = 0.0019%). WMH risk profiles differed significantly between cohorts (Table 8.2). Compared to MR Hyper participants, EDC patients had significantly higher glucose and lower physical activity levels, but EDC patients also had lower diastolic blood pressure, total cholesterol, non-HDL cholesterol and were less likely to report consuming alcohol. No covariates modified the between-group difference in WMH; in Tobit censored regression models (not shown), having type 1 diabetes significantly predicted greater WMH volume independently of any factors that differed between the two cohorts (coefficient = 0.0021%, $p<0.0001$).

Among EDC patients, high WMH burden, defined as WMH $\geq$ 0.0011%, the EDC median, was significantly and positively associated with age, disease duration, SIF, prevalence of CAN, prevalence of DSP and smoking status (Table 3). WMH burden was not significantly associated with usual markers of glucose control, including any A1c measure or fasting glucose at MRI (all $p>0.50$; Table 8.3). Associations of WMH burden with other risk factors for age-related WMH (e.g., systolic blood pressure at MRI or averaged across EDC study years, diastolic blood

pressure, hypertensive history, non-HDL cholesterol, physical activity or alcohol consumption) were also non-significant (all $p>0.10$; Table 8.3).

Associations between WMH burden and SIF, the prevalence of CAN or DSP and ever smoking cigarettes were overall similar after adjustment for age (Table 4) or duration (Table 8.5), with the exception of SIF. The association of WMH burden with SIF was attenuated and non-significant ($p>0.10$) after adjustment for age (Table 8.4).

The interaction term of SIF with age predicting WMH burden was statistically significant ($p=0.008$) while interaction terms with age and CAN ($p=0.80$), DSP ($p=0.06$) and smoking ($p=0.70$) were non-significant.

In models stratified by age 50 at MRI, WMH burden was associated with SIF among those $\geq$ but not those < age 50 (duration-adjusted Odds Ratios, [95% confidence intervals]: 1.30 [1.06, 1.60], $p=.012$ and .98 [.84, 1.14], $p=.76$, respectively). Among those $\geq$ age 50, the association of WMH with SIF remained significant and substantially unchanged (1.29 [1.00, 1.66], $p=.048$) after adjustment for characteristics associated with older age in this sample, i.e. disease duration, prevalence of CAN, DSP and proliferative retinopathy, overall SBP and non-HDL cholesterol (Supplementary Table 8.1). Further adjustment for diastolic blood pressure, age at diagnosis or diagnosis pre/post 1965 did not modify these associations (not shown). In the subgroup $\geq$ age 50, WMH was not associated with CAN, DSP or smoking (all $p>0.05$). In contrast, WMH burden was significantly associated with CAN and smoking history among those < age 50 (Supplementary Table 2). Results for those diagnosed 1965 and later were similar to the subgroup < age 50; that is, associations were significant for CAN, DSP and smoking but not for SIF (Supplementary Table 3).

8.5 DISCUSSION

Applying high-resolution neuroimaging along with careful characterization of diabetes factors in this large sample of middle-aged adults revealed a remarkably high WMH prevalence in participants with type 1 diabetes (EDC patients). This study identified a profile of factors related to WMH, consisting of SIF, neuropathies and smoking, and the pattern of associations differed by age. These findings indicate that childhood-onset diabetes can accelerate WMH development and that cerebral WMH begin to accumulate in patients with type 1 diabetes earlier and/or faster than expected based on age alone. While WMH should be considered an important complication of diabetes, its accrual may be prevented or delayed via strict glycemic control, particularly for older adults, and possibly by tobacco avoidance. Preventing WMH could have beneficial effects on overall brain health, especially for patients entering their sixth decade of life in whom disease-related brain changes overlap with age-related brain changes. Brain imaging studies[147-149] show continued myelination and development of new WM tracts connecting cortical regions during adolescent years, with an additional late myelination wave in the fourth decade of life. Glycemic control during these important years may help compensate for WM abnormalities that had already developed earlier among diabetes patients.

WMH in adults with type 1 diabetes has received initial attention, but results have been inconsistent. One small case-control study[90] applied volumetric WMH measures similar to those of our study, finding WMH in 69% of type 1 diabetes cases vs. 12% of age-matched controls. Other case-control studies applying crude measures of WMH[92,95] found non-significant between-group differences while two other studies[96,97] reported an absence of WMH in both type 1

diabetes and control participants. Methodologies used to ascertain WMH varied across studies and may explain contradictory findings. Visual rating scales used in these prior studies are less accurate at detecting smaller lesions compared to volumetric assessments of WMH[150-152] as utilized in our study. In addition to neuroimaging methodologies, selective exclusion criteria may further explain the discrepant findings of WMH prevalence; two studies[95,96] excluded participants with complications such as CAD, neuropathy, nephropathy and hypertension. It is reasonable to expect that studies conducted among "healthier" type 1 diabetes patients would detect a fewer WMH, but such individuals unlikely represent the typical adult with long-standing diabetes.

Among EDC patients in this study, long-term hyperglycemia as indicated by higher SIF, was related to higher WMH burden, especially for older patients. SIF is a novel, non-invasive measure of AGEs in the skin, partially reflecting systemic glycosylation. The half-life of skin AGEs has been estimated at 15 years,[153] thus SIF provides glycation information in terms of years compared to months as seen with A1c values. The association between SIF and WMH may reflect the negative impact hyperglycemia may have on brain health, pointing to SIF as a potential biomarker of underlying WMH. Importantly, SIF increases with age even among those without diabetes;[154] we found that SIF was significantly higher in the older compared to the younger subgroup (26.61 ± 4.96 and 22.58 ± 3.83 respectively, $p<0.001$). We noted that older age modified the association between SIF and WMH and that the relationship between WMH and SIF differed by age, as indicated by the significant interaction term of SIF by age predicting WMH burden. SIF was the only factor significantly related to WMH burden in patients $\geq$ 50 but was not related to WMH among those < age 50. Since older age also predicts high WMH burden,[61,139] it is possible that longer exposure to hyperglycemia, as indicated by higher SIF,

combined with older age, can further compromise brain health in patients with type 1 diabetes entering their sixth decade of life. Another explanation for the stronger SIF WMH association in older adults is if older patients had been exposed to more severe hyperglycemia compared to the younger patients, which would have precipitated a more rapid WMH accrual. This, however, seems unlikely as all EDC patients exhibited fair to good glycemic control, with a 20-year A1c average of 8.25% (Table 8.1) and control was similar for the younger vs. older subgroups (20-year mean A1c: 7.93% ± 0.89 and 8.21% ± 1.05, p=0.16, respectively). These findings indicate the need to monitor SIF and myelination in type 1 diabetes patients via longitudinal neuroimaging studies and to consider using SIF as a biomarker of underlying WMH development.

The associations between neuropathies and WMH burden found in this study are also novel. Although there is a biological plausibility for associations between neuropathies in the central, peripheral or autonomic nervous systems, these associations have not been examined in detail. Most prior studies excluded patients with neuropathy[92,95,96] or had very low prevalence of neuropathy.[91] The clinical relevance and overall strength of these associations need further study, especially in relationship with other behavioral risk factors. For example, we noted that neither CAN nor DSP remained significantly associated with WMH after adjusting for smoking. In multivariable models (Tables 8.4, 8.5), smoking tripled the odds of high WMH independent of age, disease duration or neuropathies. These results were stronger for the younger versus the older subgroup. Studies indicate that smoking increases the risk of WMH in older adults without diabetes[155,156] yet only one study examined smoking and WMH in adults with type 1 diabetes,[95] finding no significant relationship between the two. The participants of

this prior work were younger (average age 32 years), with disease duration almost half that observed in our study, even when our observations were restricted to those < age 50. It is possible that smoking may contribute to higher WMH during a certain window of time, making age an important factor. It cannot be ignored that some risk factors exert a stronger effect on the development of brain abnormalities that differs by age groups.

This study's negative findings also deserve attention. First, no association between WMH and concurrently measured levels of blood pressure, lipids or fasting glucose levels were found. Moreover, the difference in WMH volume between EDC and MR Hyper participants was not explained by factors associated with higher prevalence of WMH in older populations, including blood pressure, lipids or glucose levels.[70,71,77,155,157-160] It is possible that long-term exposure to diabetes may have a greater effect on WMH than traditional vascular factors. Of note, these traditional factors were measured concurrently with WMH, whereas WMH develops over several years of "incubation". Once cerebral WMH become manifest, these factors may no longer play a substantial role. This is consistent with a recent animal study showing that diabetes has a greater effect than hypertension on neurodegeneration in the rat brain.[161] Negative findings may also reflect the small range of blood pressure and lipids in both studies, which were especially well controlled for the EDC patients; they presented with lower DBP and higher lipid levels, with lower alcohol consumption, compared to MR Hyper participants. Smoking history was similar between cohorts. While SIF predicted WMH burden, no other glycated hemoglobin measures were significantly related to WMH. This is consistent with previous studies[91,92,95,154] and underscores the importance of moving beyond A1c to measure glycemic control in diabetic patients. Similar to our negative finding, previous studies reported

no significant association between severe hypoglycemia and WMH,[92,93,95] [91-93,95-97] although animal studies show hypoglycemia inhibits oligodendrocyte development and apoptosis of oligodendrocyte precursor cells.[162] CAD, microalbuminuria and proliferative retinopathy were not significantly related to WMH burden, similar to results of earlier studies.[91,92,95,97] No association was detected between WMH and age at diagnosis. Two studies found associations with WMH and "early" age at diagnosis[92,94], with one defining early diagnosis as prior to age eight[94] while the other defined early diagnosis as prior to age 18.[92] It is possible that this sample's range of ages at diagnosis was insufficient to detect an association.

Strengths of this study include the use of a high resolution neuroimaging protocol and volumetric assessment of WMH. Patients were drawn from a well-defined cohort of adults with long-standing type 1 diabetes, with more than 20 years of follow-up data. Any EDC participant interested in and eligible for the MRI study was allowed to participate, making the study findings generalizable to patients with a similar duration of childhood-onset type 1 diabetes. Limitations of this study include the cross-sectional nature of the brain imaging and the verification of diabetes complications roughly four years prior to MRI. A survivor bias is present in that these patients have survived with diabetes and are therefore different from patients who died before the MRI study. The EDC patients participating in this MRI study are, in general, much healthier than the parent EDC cohort. These limitations, however, should underestimate the true prevalence of WMH in type 1 diabetes patients, making these findings even more important. Prospective brain imaging studies with accurate, early assessments of severe hypo- and hyperglycemic events are needed to better understand WMH in individuals with childhood-

onset type 1 diabetes. Studies with larger sample sizes and wider age ranges are needed to fully examine the contribution of chronological age and age of diabetes onset on WMH development.

In light of these findings, prevention of WMH in patients with childhood-onset type 1 diabetes should focus on long-term glycemic control and behavioral factors associated with vascular disease. In particular, efforts to deter the use of tobacco products could significantly delay the development and/or progression of WMH. In addition, with chronic poor glycemic control increasing the risk for diabetic neuropathies, improved patient education combined with early, intense interventions to lower blood glucose levels may also prevent or delay the development of WMH.

8.6 TABLES AND FIGURES

Table 8.6.1. Characteristics of Pittsburgh Epidemiology of Diabetes Complications Study (EDC) participants by MRI status. Data are from EDC exam cycle 10 (2004-2006) unless otherwise noted

		EDC no MRI n=157	EDC with MRI n=106	*p*-value
		Values presented are N (%) or Mean ± SD		
Brain imaging	WMH volume (mm^3) † 1	--	0.214±0.31	--
Demographic factors	Age (years)	45.8±7.6	43.7±7.1	**0.03**
	Female	99 (63.0%)	54 (50.9%)	**0.05**
Diabetes-specific variables	Duration (years)	37.0±7.1	35.6±6.4	0.12
	HbA1c monthly estimate (AU) 3	1040.0±484.2	957.0±378.0	0.15
	HbA1c (%) (mmol/mol)	7.4±1.3 (58±14.2)	7.4±1.5 (58±16.4)	0.99
	Mean HbA1c (%) (mmol/mol) 3	8.5±1.2 (70±13.1)	8.5±1.0 (70±10.9)	0.81
	Age at diagnosis (years)	8.8±4.0	8.1±4.1	0.16
	Diagnosis after 1/1/1965	113 (72.0%)	86 (81.1%)	0.09
	Severe hypoglycemia in 1988-1992 2	33 (29.0%)	21 (22.8%)	0.32
Diabetes complications	Coronary artery disease	47 (30.5%)	18 (17.0%)	**0.01**
	Cardiac autonomic neuropathy	57 (54.3%)	48 (45.7%)	**0.006**
	Distal symmetric polyneuropathy	85 (59.9%)	51 (49.0%)	0.09
	Microalbuminuria	96 (66.2%)	56 (53.3%)	**0.04**
	Proliferative retinopathy	87 (58.4%)	52 (49.5%)	0.16
Biological risk factors	Fasting glucose (mg/dL)	167.1±73.0	174.4±77.1	0.51
	Systolic blood pressure (mmHg)	116.6±17.1	113.3±16.3	0.15
	Diastolic blood pressure (mmHg)	64.9±9.5	66.4±11.4	0.31
	Study 20-yr average SBP (mmHg)3	114.6±10.8	111.9±11.6	0.06
	Ever high blood pressure #	29 (18.8%)	15 (14.2%)	0.32
	Body Mass Index (kg/m^2)	27.7±4.8	26.5±4.3	**0.04**
	ApoE4 allele status 24, 34, 44	32 (30.2%)	35 (23.2%)	0.21
Behavioral risk factors	History of ever smoking cigarettes 3	60 (38.2%)	30 (28.4%)	0.11
	Physical activity, past week (Kcal) ‡	784 (336-1663)	994 (420-1964)	0.18

† (WMH Volume/Total brain volume) * 100
‡ Data presented are median (Interquartile range)
# SBP ≥ 140 or DBP ≥ 90 or report of ever using antihypertensive medication at any EDC physical exam
1 = Measure taken 2101-2013; 2 = Measure taken 1990-1992; 3 = Ever, average or cumulative measure from baseline through cycle 10 exam (2004-2006)

Table 8.6.2 Characteristics of participants with type 1 diabetes (Pittsburgh Epidemiology of Diabetes Complications Study, T1D) and participants without type 1 diabetes (Pittsburgh MR Hyper Study, no T1D) at time of MRI (2010-2013) unless otherwise noted

		T1D N=97	**No T1D N=81**	***p*-value**
		Values presented are N (%) or Mean ± SD		
Brain imaging	WMH volume (%) †	0.214 ± 0.31	0.004 ± 0.01	**<0.0001**
Demographic factors	Age (years)	49.5 ± 6.9	48.1 ± 7.3	0.1996
	Female	47 (48%)	43 (53%)	0.5510
Biological factors	Fasting glucose (mg/dL)	176.3 ± 86.9	92.3 ± 20.4	**<0.0001**
	Systolic blood pressure (mmHg)	118 ± 15	117 ± 10	0.6848
	Diastolic blood pressure (mmHg)	65 ± 9	77 ± 7	**<0.0001**
	Ever high blood pressure #	32 (33%)	5 (6%)	**<0.0001**
	Body Mass Index (kg/m^2)	27.1 ± 4.6	27.7 ± 6.0	0.4849
Behavioral factors	History of ever smoking cigarettes [2]	33 (34%)	37 (46%)	0.1253
	Physical activity in past week (Kcal) ‡[1]	1092 (504-1999)	79 (616-3173)	**0.0253**

† (WMH Volume / Total brain volume)* 100

‡ Data presented are median (Interquartile range)

For T1D participants: SBP ≥ 140 or DBP ≥ 90 or report of ever using antihypertensive medication at any EDC physical exam; for MR Hyper participants without T1D, past history of hypertension based on self-report at time of MRI

FOR T1D (EDC) PARTICIPANTS: 1=measure taken 2004-06; 2=ever, cumulative or average from baseline through time of MRI

Table 8.6.3 Characteristics of participants with type 1 diabetes (Pittsburgh Epidemiology of Diabetes Complications Study), grouped by low vs. high white matter hyperintensity burden (WMH < or ≥ group median) at time of MRI (2010-2012) unless otherwise indicated

		Low WMH† < 0.1113 n=49	**High WMH† ≥ 0.1113 n=48**	***p*-value**
		Values presented are N (%) or Mean ± SD		
Brain imaging	WMH volume (%) †	0.072 ± .031	0.358±0.384	**<0.0001**
	Normalized grey matter volume (%) ^	33.80 ± 2.00	33.60 ± 2.51	0.6453
Demographic factors	Age at MRI (years)	47.3 ± 6.0	51.7 ± 7.1	**0.0014**
	Female	22 (45%)	25 (52%)	0.5446
Diabetes-specific variables	Duration (years)	39.5 ± 4.2	43.2 ± 7.6	**0.0041**
	Skin intrinsic fluorescence (AU) [1]	23.12 ± 4.01	25.67 ± 5.19	**0.0106**
	HbA1c monthly estimate (AU) [3]	1119.1 ± 466.1	1116.1 ± 461.1	0.9748
	HbA1c at time of MRI (%) (mmol/mol)	7.70±1.26 (61±13.8)	7.55±1.29 (59±14.1)	0.5934
	Mean HbA1c (%) (mmol/mol) [3]	8.14±1.02 (65±11.1)	8.03±0.96 (64±10.5)	0.6128
	Age at diagnosis (years)	7.9 ± 4.4	8.5 ± 4.0	0.4233
	Diagnosis after 1/1/1965	48 (98%)	31 (65%)	**<0.0001**
	Any severe hypoglycemia (1988-1992) [2]	6 (14%)	13 (30%)	0.1175
Diabetes complications	Coronary artery disease [2]	9 (18%)	6 (13%)	0.5759
	Cardiac autonomic neuropathy [2]	15 (31%)	28 (58%)	**0.0133**
	Distal symmetric polyneuropathy [2]	14 (29%)	30 (63%)	**0.0020**
	Microalbuminuria [2]	21 (43%)	29 (60%)	0.1053
	Proliferative retinopathy [2]	21 (44%)	31 (65%)	0.0647
Biological risk factors	Fasting glucose (mg/dL)	171.5 ± 83.36	181.0 ± 90.97	0.5991
	Systolic blood pressure (mmHg)	117.6 ± 15.5	118.6 ± 14.4	0.7305
	Diastolic blood pressure (mmHg)	66.7 ± 9.0	63.9 ± 9.5	0.1432
	Study 20-yr average systolic blood pressure (mmHg)[4]	110.7 ± 8.5	113.6 ± 10.2	0.1284
	Ever high blood pressure #	12 (252%)	20 (42%)	0.1290
	Body Mass Index (kg/m^2)	27.7 ± 4.9	26.5 ± 4.4	0.2274
	ApoE4 allele status 24, 34, 44	16 (33%)	11 (23%)	0.3660
Behavioral risk factors	History of ever smoking cigarettes [4]	11 (22%)	22 (46%)	**0.0189**
	Physical activity in past week (Kcal) ‡[2]	994 (504-1868)	1151 (504-2371)	0.4585

† (WMH volume / total brain volume) * 100 ‡ Data presented are median (Interquartile range)
^ (GMV/ICV)*100 # SBP ≥ 140 or DBP ≥ 90 or report of ever using antihypertensive medication at any EDC exam 1=measure taken 2006-2008; 2=measure taken 2004-06; 3=measure taken 1990-92; 4=ever, cumulative or average measure from baseline through time of MRI

Table 8.6.4 Logistic regression models for participants with type 1 diabetes (Pittsburgh Epidemiology of Diabetes Complications Study) showing the independent effects of diabetes-related factors on the odds of high white matter hyperintensity burden ($\geq$ cohort median of 0.1113%) when controlling for age at MRI and specified factor(s)

Model	**1**	**2**	**3**	**4**	**5**	**1+3+5**
	Odds Ratio (95% Confidence Limits) and Wald *p*-value					
Age at MRI (years)	1.12 (1.04, 1.20) .002	1.10 (1.02, 1.18) .015	1.10 (.1.02, 1.18) .013	1.08 (1.00, 1.17) .060	1.12 (1.04, 1.20) .003	1.10 (1.02, 1.18) .012
Skin Intrinsic Fluorescence		1.09 (.98, 1.21) .132				--
Cardiac Autonomic Neuropathy			2.61 (1.02, 6.71) .046			2.20 (0.82, 5.87) .116
Distal Symmetric Neuropathy				2.62 (0.95, 7.26) .064		--
Ever smoking 100+ cigarettes					2.98 (1.12, 7.91) .001	2.57 (.94, 7.02) .066

Table 8.6.5 Logistic regression models for participants with type 1 diabetes (Pittsburgh Epidemiology of Diabetes Complications Study) showing the independent effects of diabetes-related factors on the odds of high white matter hyperintensity burden (> cohort median of 0.1113%) when controlling for diabetes duration at MRI and specified factor(**s)**

Model	**1**	**2**	**3**	**4**	**5**	**1+3+4+5**
	Odds Ratio (95% Confidence Limits) and Wald *p*-value					
Diabetes duration (years)	1.11 (1.03, 1.19) .008	1.09 (1.01, 1.18) .033	1.09 (1.01, 1.18) .029	1.07 (.98, 1.16) .137	1.11 (1.03, 1.20) .009	1.07 (.98, 1.16) .143
Skin Intrinsic Fluorescence		1.10 (1.00, 1.22) .059				--
Cardiac Autonomic Neuropathy			2.96 (1.18, 7.44) .021			1.71 (0.58, 4.99) 0.329
Distal Symmetric Neuropathy				3.06 (1.14 8.21) .026		2.49 (.80, 7.80) .116
Ever smoking 100+ cigarettes					3.03 (1.16, 7.90) .023	2.84 (1.02, 7.88) .046

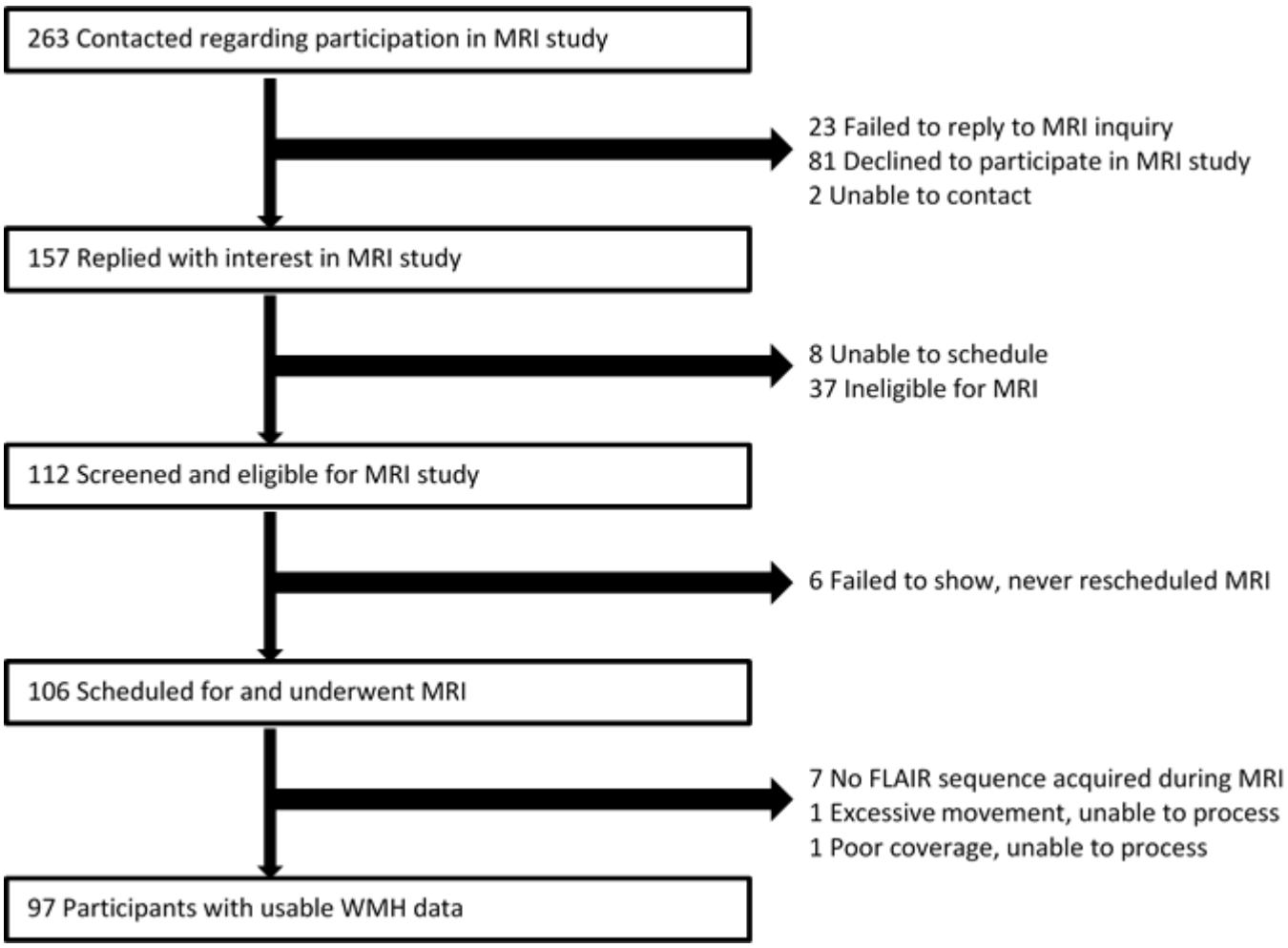

Figure 8.6.1 Flow chart showing recruitment of participants with type 1 diabetes from the Epidemiology of Diabetes Complications Study (EDC) parent cohort for the MRI study

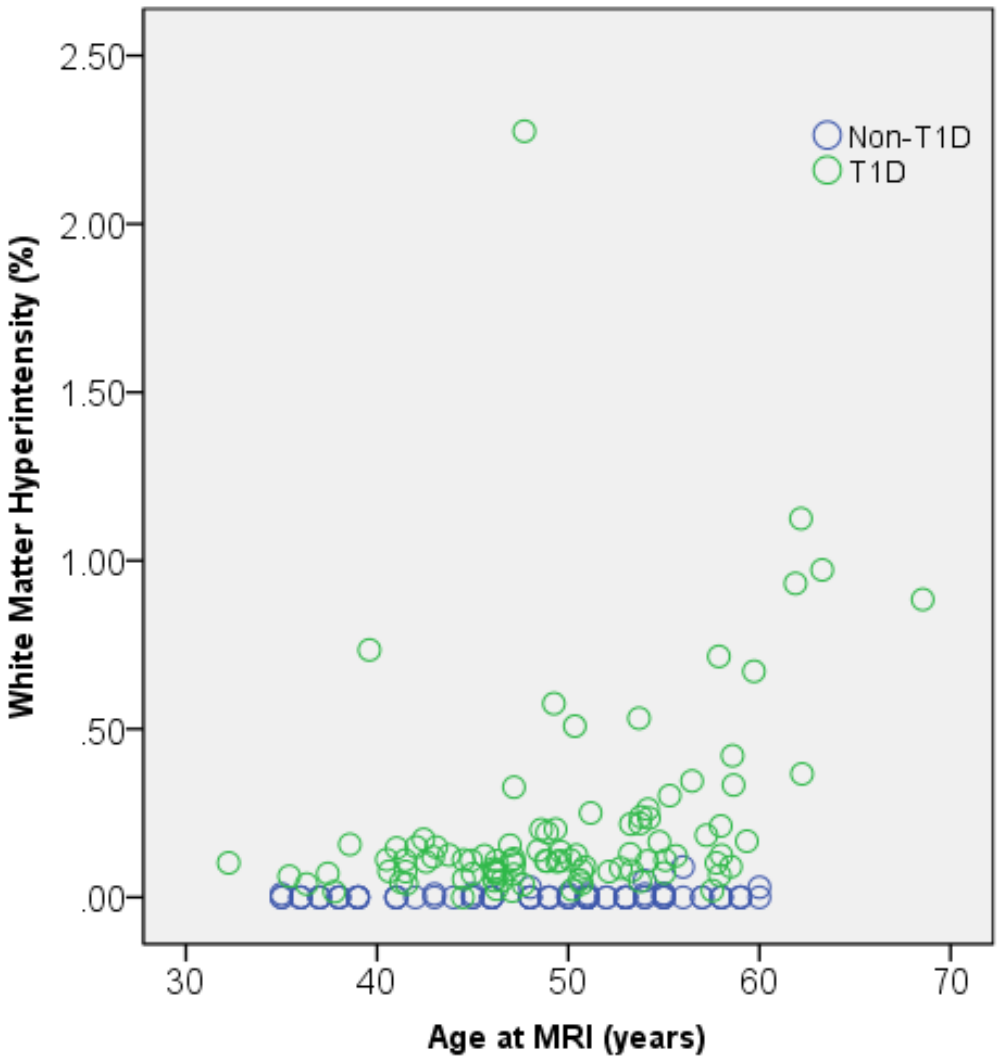

Figure 8.6.2 Plot of white matter hyperintensity volume (WMH, % of total brain volume), by age at MRI, for Pittsburgh Epidemiology of Diabetes Complications (EDC) participants with type 1 diabetes (green) and MR Hyper participants without type 1 diabetes (blue)

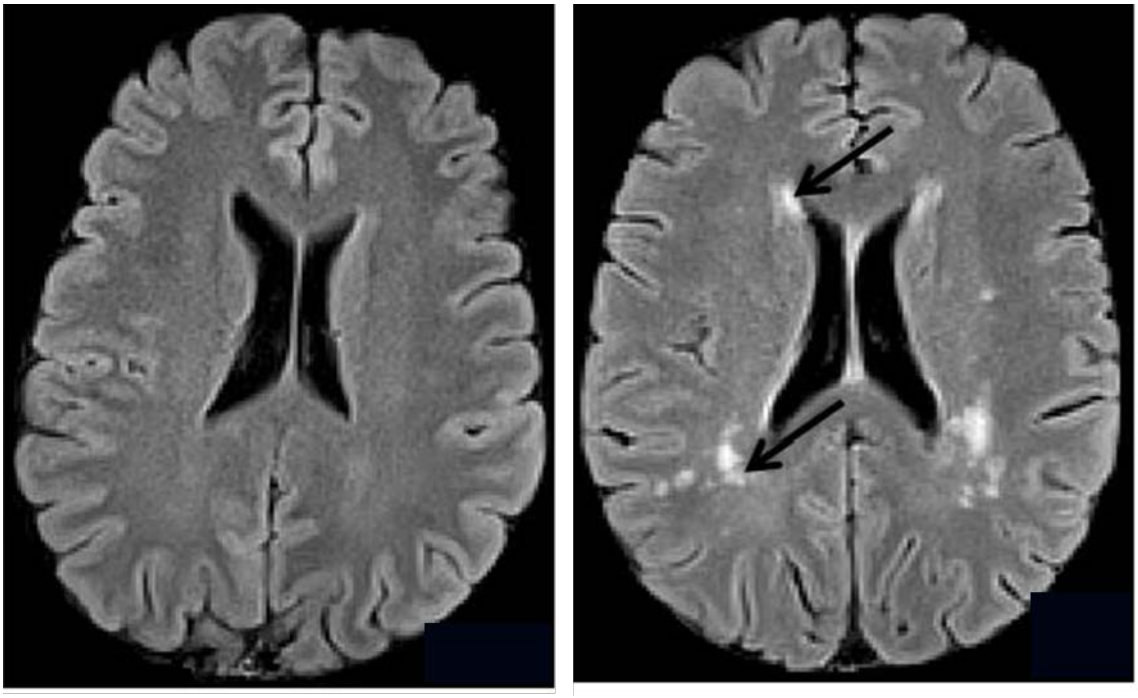

Figure 8.6.3 Left: FLAIR image of 49 y.o. female without type 1 diabetes, no visible WMH; **Right**: FLAIR image of 49 y.o. female with type 1 diabetes since age 10 with noticeable WMH (black arrows) at ventricular rims, caps as well as in deep tissue

Table 8.6.6 Characteristics of participants with type 1 diabetes (Pittsburgh Epidemiology of Complication Study) by age < or ≥ 50 years at time of MRI, 2010-2012, unless otherwise noted

		Age <50 at MRI n=53	Age ≥ 50 at MRI n=44	*p*-value
		N (%), Mean ± SD or Median (Interquartile Range)		
Brain Imaging	WMH volume (%)	0.164 ± .319	0.273 ± 0.279	0.0764
	Normalized grey matter (%)*	33.92 ± 2.30	33.42 ± 2.19	0.2831
Demographic factors	Age at MRI (years)	44.4 ± 4.0	55.6 ± 4.1	<0.0001
	Female	22 (42%)	25 (57%)	0.1563
Diabetes-specific variables	Duration (years)	37.6 ± 4.3	45.7 ± 5.6	<0.0001
	Skin intrinsic fluorescence (AU) †	22.58 ± 3.83	26.61 ± 4.96	<0.0001
	HbA1c monthly estimate (AU) ‡	1130.5 ± 470.1	1102.5 ± 455.4	0.7680
	HbA1c at time of MRI (%)(mmol/mol)	7.79 ± 1.30 (63±14.2)	7.43 ± 1.24 (56±13.6)	0.1771
	Mean HbA1c (%)(mmol/mol) ‡	8.21 ± 1.05 (66±11.5)	7.93 ± 0.89 (64±9.7)	0.1531
	Age at T1D diagnosis (years)	6.8 ± 4.0	9.9 ± 3.9	0.0002
	Diagnosis after 1/1/1965	52 (98%)	27 (61%)	<0.0001
	Any severe hypoglycemia	11 (26%)	8 (19%)	0.6040
Diabetes complications§	Coronary artery disease	7 (13%)	8 (18%)	0.5784
	Cardiac autonomic neuropathy	14 (27%)	29 (66%)	0.0002
	Distal symmetric polyneuropathy	11 (21%)	33 (75%)	<0.0001
	Microalbuminuria	26 (49%)	24 (55%)	0.6843
	Proliferative retinopathy	22 (42%)	30 (68%)	0.0141
Biological risk factors	Fasting glucose (mg/dL)	183.1 ± 97.1	168.1 ± 73.0	0.3979
	Systolic blood pressure (SBP)(mmHg)	115.8 ± 14.1	120.9 ± 15.5	0.1101
	Diastolic blood pressure (mmHg)	68.4 ± 7.9	61.5 ± 9.5	0.0003
	Mean SBP over EDC study (mmHg) ‡	110.2 ± 8.8	114.6 ± 9.7	0.0237
	Ever hypertensive ¶	13 (25%)	19 (44%)	0.0517
	Body Mass Index (kg/m^2)	27.9 ± 4.8	26.1± 4.3	0.0516
	ApoE allele status 24, 34, 44 §	14 (26%)	13 (30%)	0.8213
Behavioral risk factors	Ever smoking 100+ cigarettes ‡	15 (28%)	18 (41%)	0.2050
	Physical activity in past week (Kcal) §	1228 (756-2634)	784 (364-1860)	0.1632

* (Grey matter volume / Intracranial volume) * 100
† Measure taken at 2006-2008 exam
‡ "Ever", average or cumulate measure from EDC baseline through time of MRI
§ Measure taken at 2004-2006 exam
‖ Based on 1990-1992 exam questionnaire when participant self-reported experiencing any hypoglycemic events resulting in loss of consciousness over the previous two years
¶ SBP ≥ 140 or DBP ≥ 90 or report of ever using antihypertensive medication at any EDC physical exam

Table 8.6.7 Logistic regression models showing independent effects of diabetes-related factors on high white matter hyperintensity burden (> age-group specific WMH median) for participants with type 1 diabetes, stratified by age 50 at MRI, controlling for duration

Age ≥ 50 at MRI, WMH median=0.166 %	Model	1	2	3	4	5	
		Odds Ratio (95% Confidence Limits) and Wald *p*-value					
	Diabetes duration (years)	1.14 (1.01, 1.29) .040	1.15 (1.00, 1.33) .048	1.14 (1.00, 1.30) .045	1.11 (.98, 1.27) .113	1.15 (1.01, 1.31) .030	--
	Skin Intrinsic Fluorescence		1.30 (1.06, 1.60) .012				--
	Cardiac Autonomic Neuropathy			1.83 (.46, 7.24) .387			--
	Distal Symmetric Neuropathy				4.35 (.74, 25.61) .104		--
	Ever smoking 100+ cigarettes					2.40 (.60, 9.64) .219	--
Age < 50 at MRI, WMH median=0.108 %	Model	1	2	3	4	5	1+3+5
	Diabetes duration (years)	0.99 (.87, 1.13) .910	0.99 (.87, 1.13) .882	1.00 (.87, 1.15) .998	0.98 (.86, 1.13) .806	0.99 (.86, 1.14) .914	1.00 (.86, 1.16) .974
	Skin Intrinsic Fluorescence		.98 (.84, 1.14) .764				--
	Cardiac Autonomic Neuropathy			6.11 (1.40, 26.60) .016			6.15 (1.32, 28.63) .021
	Distal Symmetric Neuropathy				3.48 (.77, 15.74) .106		--
	Ever smoking 100+ cigarettes					4.38 (1.12, 17.04) .033	4.40 (1.03, 18.75) .045

Table 8.6.8 Logistic regression models among participants diagnosed with type 1 diabetes on or after 1/1/1965 from the Pittsburgh Epidemiology of Diabetes Complications Study showing the effects of diabetes-related measures and complications on high white matter hyperintensity burden (≥cohort median of 0.107%)

Model	1	2	3	4	5	1+3+4+5
	Odds Ratio (95% Confidence Limits) and Wald *p*-value					
Diabetes duration (years)	1.02 (.90, 1.20) .978	1.00 (.89, 1.11) .948	0.97 (.5, 1.09) .566	0.96 (.85, 1.08) .505	1.01 (.90, 1.13) .912	.96 (.84, 1.09) .491
Skin Intrinsic Fluorescence		1.08 (.97, 1.20) .158				--
Cardiac Autonomic Neuropathy			5.28 (1.84, 15.13) .002			2.82 (.81, 9.89) .105
Distal Symmetric Neuropathy				3.80 (1.31, 11.05) .014		2.41 (.65, 8.96) .189
Ever smoking 100+ cigarettes					3.54 (1.25, 9.98) .017	3.08 (.98, 9.63) .054

9.0 MANUSCRIPT 2: COGNITIVE FUNCTION IN LONG-TERM SURVIVORS OF CHILDHOOD-ONSET TYPE 1 DIABETES

9.1 ABSTRACT

Adults with type 1 diabetes (T1D) are now more likely to live longer, but the combined effects of older age and T1D on cognitive function remains unknown. We assessed the correlates of cognitive dysfunction among middle-aged adults with childhood-onset T1D compared to adults without T1D. During 2010-2013, 97 adults with childhood-onset T1D, participating in the Pittsburgh Epidemiology of Diabetes Complications Study (mean age 49 $\pm$ 7 years; mean duration 41 $\pm$ 6 years; 51% female, 98% Caucasian) and 138 similarly-aged adults without T1D (mean age 49 $\pm$ 7 years; 55% female, 100% Caucasian) completed an extensive neuropsychological evaluation to test performance in executive function, psychomotor speed, memory and other domains. After controlling for education, significant between-group differences were found for scores on the North American Adult Reading Test (NAART), Digit Symbol Substitution Test (DSST), Grooved Pegboard (peg) and Rey Osterrieth Complex Figure copy task (ROCF-c), with standardized effect sizes (Cohen's *d*) ranging from 0.6-0.9. Between-

group score differences were not explained by other factors (age, blood pressure, history of high blood pressure). Furthermore, compared to participants without T1D, a greater percentage of participants with T1D had test scores $\geq$ 1.5SD worse than "expected" (i.e., compared to published normative scores), with such scores indicative of clinically relevant dysfunction. Among those with T1D, linear regression models showed that worse performance on the DSST, peg and ROCF-c tests was associated with poorer glucose control (higher skin intrinsic fluorescence [SIF]) and with prevalent microvascular complications (distal symmetric polyneuropathy, proliferative retinopathy and overt nephropathy). No factors examined were related to NAART score. These results suggest that the early, small effects of T1D on information processing and executive control intensify with advancing age and that prevalent microvascular complications may indicate worse cognitive function in middle-aged adults with childhood-onset T1D.

9.2 INTRODUCTION

Individuals with childhood-onset type 1 diabetes (T1D) develop mild cognitive dysfunction[128,163-165] at a much earlier age than occurs among the general population.[166,167] Because of the increased life expectancy for individuals with T1D, they now suffer longer with this complication.[168] Despite the known association between increasing age and cognitive dysfunction,[169] the majority of T1D-related cognitive dysfunction studies focus on children and young adults. Consequently, the role of aging on cognitive dysfunction among middle-age adults with childhood-onset type 1 diabetes remains unknown. This issue deserves immediate attention as cognitive dysfunction negatively impacts quality of life for those with the condition as well as for their caregivers[170] with high direct and indirect costs.[171]

In their 2005 meta-analysis, Brands et al. report that adults with T1D demonstrate mild to moderate (Cohen's effect size, *d*, 0.2-0.7) deficits in tasks assessing executive function, attention, intelligence and psychomotor speed domains while learning and memory domains appear preserved.[127] Importantly, this meta-analysis included studies of individuals with adult-onset T1D. Since the influence of age at diagnosis on the development of cognitive dysfunction remains equivocal, including studies with mixed populations (i.e., childhood vs. adult onset) limits the ability to make conclusions regarding cognitive dysfunction in adults with childhood-onset T1D. Furthermore, the study populations tended to be relatively young; only seven out of 42 studies had a mean population $\geq$ 40 years of age. This under-representation of older individuals highlights the need for additional studies of aging adults with childhood-onset T1D to better understand the combined effects of diabetes and advancing age on cognitive function.

Additionally, the mechanisms underlying the development and progression of T1D-related cognitive dysfunction remain unclear. Current studies underscore an important relationship between chronic hyperglycemia and cognitive dysfunction.[165,172-174] One proposed mechanism is via chronic hyperglycemia leading to endothelial dysfunction[59] with resultant development of microvascular complications (e.g., retinopathy, neuropathy and nephropathy). These conditions are associated with an increased risk of cognitive dysfunction in people with type 1 diabetes.[126,127,175,176] Shared pathophysiology may explain this relationship; that is, chronic hyperglycemia may have the same deleterious effects on cerebral microvasculature as it has on small blood vessels elsewhere in the body (kidney, retina, etc.) thereby leading to T1D-related cognitive dysfunction.[163] An additional possibility is that sustained exposure to hyperglycemia at a level below that which would cause diabetic ketoacidosis (DKA) may result in a state of subclinical cerebral edema, with unknown consequences on cognitive function.[177]

This research aims to characterize cognitive function in middle-aged adults with childhood-onset T1D. Relationships between diabetes- and health-related factors and neurocognitive test scores will be explored. We hypothesize that overall cognitive test scores will be worse among participants with T1D compared to similarly-aged adults without T1D and that greatest between-group differences will be found in tasks assessing executive function and information processing. Among those with T1D, hyperglycemia, high blood pressure and prevalent microvascular complications will be associated with worse cognitive performance.

9.3 METHODS

All study procedures received local IRB approval prior to study initiation. All participants provided informed consent prior to undergoing procedures.

9.3.1 Study Populations

Type 1 diabetes participants were drawn from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC), an ongoing, prospective study of individuals with childhood-onset (diagnosed ≤ 17 years) T1D. All EDC participants, diagnosed between 1/1/50 and 5/31/80, were seen within one year of diagnosis at Children's Hospital of Pittsburgh and underwent a baseline assessment (1986 – 1988) when the mean age was 28 years and the average diabetes duration was 19 years. After their baseline visit, participants were followed with biennial exams for 10 years, thereafter completing biennial questionnaires. An additional physical exam occurred in 2004-2006. Out of 263 locally-resident participants invited to participate in this cognitive study, 159 replied with interest. Of these, 47 were ineligible for MRI (a requirement to participate in the neurocognitive study) due to metallic implants/objects or claustrophobia, leaving 112 eligible for MRI. Fifteen participants did not complete the neuropsychological battery, given between 2010 and 2013, yielding an analytical sample size of N=97 (Figure 9.6.2).

Non-type 1 diabetes comparison population: Adults without type 1 diabetes, from the University of Pittsburgh's MR Hyper Study, served as a comparison group. Details of the MR Hyper Study have been previously described.[132] In brief, to study the effects of vascular risk factors on cerebral blood flow, 414 middle-aged adults living in the Pittsburgh area were

screened to participate in this study. Of those, 110 did not meet blood pressure inclusion criteria (either too high or too low), 60 declined to participate and 14 withdrew, yielding a study population of 230 pre-hypertensive adults, mean age 46 years; full exclusion criteria are presented in Appendix C. Participants completed a neuropsychological battery (2011-2013) which included a large number of the same tests as used the EDC Study, with the exception of the Logical Memory and Block Design tasks. To mirror the EDC racial distribution, only Caucasians (n=138) were included in these analyses. At the time of cognitive testing, biological and lifestyle data were collected for MR Hyper participants using standard methods comparable to those for EDC participants. SIF and HbA1c, however, were not available for these participants.

9.3.2 Covariates

Diabetes-specific variables: The following variables were collected at each physical exam from EDC baseline to time of cognitive testing: fasting serum glucose and HbA1c (using saline-incubated blood samples and cation-exchange microcolumn chromatography prior to October 1987, using high-performance liquid chromatography through 2004-2006, and thereafter using the DCA 2000 analyzer). Repeated assessment of HbA1c at every exam allowed the calculation of an average HbA1c from EDC baseline through time of neurocognitive testing.

Skin intrinsic fluorescence (SIF), a measure which partially reflects advanced glycation end products (AGEs) in the skin, was assessed during the 2006-2008 EDC exam. A skin fluorescence spectrometer (SCOUT DS®) noninvasively measured the skin of the left volar forearm. Details on the device and SIF calculations are discussed elsewhere.[144]

Diabetes-related complications: Prevalence of complications was ascertained during the 2004-2006 exam; this occurred on average five years prior to neuropsychological testing.

Renal disease refers to microalbuminuria (MA), overt nephropathy (ON) or End Stage Renal Disease. ON was defined as the presence of an albumin excretion rate >200 μg/min in at least 2 of 3 timed urine collections and MA as an albumin excretion rate between 20-200 μg/min in at least 2 of 3 urine samples.

Coronary artery disease (CAD) was defined as myocardial infarction (Minnesota Codes 1.1 or 1.2), fatal CAD (determined by review of death records and family interview) or angiographic evidence of 50% or more stenosis. CAD also included angina diagnosed by the clinic physician and/or ischemic ECG changes (Minnesota Codes: 1.3, 4.1-4.3, 5.1-5.3, 7.1).

Neuropathies: Distal symmetric polyneuropathy (DSP) was determined by medical history and clinical examination using established protocols, i.e. the symptoms and signs consistent with DSP, and reduced deep tendon reflexes. DSP was further determined as 'confirmed' using the Vibratron II® to detect vibratory threshold. Cardiac autonomic neuropathy (CAN) was determined by heart variation during deep breathing and by heart rate and blood pressure response to standing.

Retinopathy: Eye exams were done using three standard field stereo fundus photographs. Photographs were read at the Madison Reading Center in Wisconsin and graded using the Early Treatment Diabetic Retinopathy Study classification, with a grade of 60 or higher in one eye or a grade less than 60 but with panretinal photocoagulation scars consistent with laser therapy indicating proliferative retinopathy.[145]

Biological risk factors: Hypertension was defined as any blood pressure reading ≥140/90 mmHg or ever reporting use of antihypertensive medication from EDC baseline through day of neurocognitive testing; for the MR Hyper participants, high blood pressure was defined as blood pressure ≥140/90 mmHg on the day of the MRI (those with a history of hypertension or chronic use of antihypertensive medication were ineligible for the MR Hyper Study). Use of hypertension medication is available from study entry to time of MRI for EDC participants.

Blood pressure: Three seated blood pressure readings were taken with a random-zero sphygmomanometer on day of neurocognitive testing, with an average of the second and third readings used, per the Hypertension Detection and Follow-up Program Protocol.

Cholesterol: SYNCHRON CX® Systems was used to measure non-fasting total cholesterol and high-density cholesterol (HDLc) at time of neurocognitive testing. Non-HDL cholesterol was calculated as (total cholesterol – HDLc).

Apolipoprotein E (ApoE) status was ascertained in 2004-2006 for EDC participants and at time of neurocognitive testing for MR Hyper participants.

Body mass index (BMI) was calculated based on the participants' weight and height at time of neurocognitive testing (kg/m^2).

Lifestyle/Behavioral risk factors:

Smoking status was self-reported as current, past or never smoking 100 cigarettes and was dichotomized as ever vs. never smoking based on information obtained through time of neurocognitive assessment.

Physical activity for the past week was determined via self-report from the Paffenbarger Physical Activity Questionnaire[178] in 2004-2006 for EDC participants and at time of

neurocognitive testing for the MR Hyper participants. Responses were used to estimate energy expenditure (kcal).

9.3.3 Neuropsychological Testing

The following cognitive tests were administered to participants from both studies. More information regarding each test is provided in Appendices A and B.

1. North American Adult Reading Test (NAART)
2. Rey Osterrieth Complex Figure (ROCF)
3. Rey Auditory Verbal Learning Test (RVLT)
4. Four Word Short Term Memory (4WSTM)
5. Digit Symbol Substitution Test (DSST), number complete in 90 seconds
6. Trail Making Test A and B (TMTA, TMTB), time to complete
7. Wechsler Adult Intelligence Scale (WAIS) Block Design
8. Verbal Fluency – FAS and Animals Tests
9. Stroop Color-Word Test (Golden version)
10. Grooved pegboard, dominant hand, time to insert pegs

Neuropsychological tests were administered by one trained study staff member to ensure consistency between participant experiences. Tests were scored by trained study staff unaware of participant cognitive status.

9.3.4 Statistical Analyses

Groups (T1D, no T1D) were assessed for differences in population characteristics using t-test for continuous variables, Fisher exact test for categorical variables and Wilcoxon rank sum test for highly skewed data. Mean and percent differences in raw cognitive test scores were compared by T1D status, controlling for years of education. Standardized effect sizes were computed using Cohen's *d* ($2t/\sqrt{df}$),[179] classified as small ($d \leq 0.2$), moderate ($0.21 \leq d < 0.5$) or large ($d \geq 0.51$).[180] Indicator variables were created for each task to show whether cognitive any test score was $\geq$ 1.5SD worse than published normative data.[27-30] Analyses were repeated excluding all participants with a history of high blood pressure (SBP $\geq$ 140 mmHg, DBP $\geq$ 90 mmHg) or self-report use of anti-hypertensive medication.

Cognitive test scores that differed significantly (education adjusted $p<0.001$) between groups (T1D, no T1D) were used as dependent variables (DV) in linear regression models. To test the contributions of T1D status and other factors on cognitive test scores, factors (with the exception of serum glucose due to its correlation with group status) that differed between groups ($p \leq 0.05$) entered step-wise regression models. Multicollinearity was deemed severe if the variance inflation factor (VIF) $\geq$ 2.5; any factor(s) reaching this VIF were not included in the same model. Adjusted R^2 was used to identify the most parsimonious models.

In order to identify diabetes-specific factors significantly related with cognitive function, further analyses were restricted to participants with T1D. Unadjusted standardized betas between each diabetes variable of interest with each DV were reported. Factors significantly

associated with each DV (p<0.05) were further tested to examine how first adjusting for years of education then further adjusting for age affected the relationship.

All analyses were conducted using SAS 9.3 and SPSS 21.

9.4 RESULTS

Groups did not significantly differ (p>0.05) regarding age at neurocognitive testing or sex distribution (Table 9.6.1). Serum glucose and history of high blood pressure were statistically significantly (p<0.05) higher among participants with T1D compared to those without T1D. Years of education, diastolic blood pressure and physical activity were statistically significantly lower for participants with T1D compared to those without T1D. The groups did not significantly differ in regards to systolic blood pressure, ApoE4 status or smoking history (Table 9.6.1).

Between group differences were observed for tasks assessing premorbid IQ, psychomotor speed, executive function and visuo-spatial construction, but not for memory (Table 9.6.2). In particular, participants with T1D performed significantly worse (education adjusted p<0.05) than those without T1D on NAART (number correct), DSST (number complete in 90 seconds), Grooved Pegboard (time to insert pegs, dominant hand), Trail Making Test B (time to complete), Trail Making Test B:A ratio and the ROCF-copy tasks. Effect sizes for these tasks were large, with d ranging from 0.5-0.9 (Table 9.6.2, Figure 9.6.3). Group differences in scores for other tasks were not statistically significant (education adjusted p>0.05), with small to moderate effect sizes, (d=0.1-0.3).

Between group differences were also observed when comparing test scores to published norms. Compared to participants without T1D, a higher percentage of participants with T1D scored at least 1.5SD worse than published normative data. Greater between-group differences were seen for the DSST, Grooved Pegboard and ROCF-copy tasks as compared to other tasks (Figure 9.6.1).

In linear regression models, having T1D (Table 9.6.3, unadjusted model) explained 8% of the between-group difference in NAART score, 11% of DSST score difference, 13% of pegboard score difference and 12% of ROCF-copy score difference. Years of education explained another 23% of difference in NAART score but only explained an additional 2-5% of group differences in scores for DSST and ROCF-copy; education did not contribute to group differences in Grooved Pegboard scores (Table 9.6.3, education adjusted model). History of high blood pressure was included in the most parsimonious model for each DV even though it was only weakly associated with the outcome. Diastolic blood pressure was included only in the parsimonious model for NAART score while physical activity was included in the most parsimonious models for Grooved Pegboard and ROCF-copy. The combination of factors explained an additional 5% of the variance in NAART score, 3.4% of the variance in Grooved Pegboard score and less than 1% of the variance in DSST or ROCF-copy scores over the education adjusted model (Table 9.6.3, most parsimonious model).

In further analyses restricted to participants with T1D, statistically significant (unadjusted $p \leq 0.05$) relationships were seen between DSST, Grooved Pegboard and ROCF-copy scores and prevalent distal symmetric polyneuropathy, overt nephropathy and proliferative retinopathy (Table 9.6.4). Scores on these three tasks were also significantly related to a

measure of chronic hyperglycemia, with worse DSST and ROCF-copy scores being significantly associated with higher SIF and worse Grooved Pegboard score being significantly related to higher A1c months. Worse DSST score was also significantly related to longer diabetes duration and with cardiac autonomic neuropathy. Worse NAART score was not statistically significantly related to any of the factors examined, although there was a borderline association with higher A1c months (p=0.06) (Table 9.6.4). Except for the relationship between ROCF-copy and proliferative retinopathy, all of the relationships remained statistically significant after adjustment for years of education. After further adjustment for age, DSST score remained significantly related to distal symmetric polyneuropathy and proliferative retinopathy, Grooved Pegboard score remained significantly related to A1c months, distal symmetric polyneuropathy, overt nephropathy and proliferative retinopathy, and ROCF-c score remained significantly related to SIF and overt nephropathy (Table 9.6.4). No statistically significant associations were found between task scores and estimated glomerular filtration rate (eGFR, per the Chronic Kidney Disease Epidemiology Collaboration formula,[181] data not shown). First order relationships between task scores and SIF were independent of smoking history and eGFR (data not shown).

9.5 DISCUSSION

Similar to studies involving children and young adults with T1D,[127,128,165] the largest score differences were seen in tasks assessing premorbid IQ, psychomotor speed, executive function and visuo-spatial construction. We found larger effect sizes, however, compared to previous

studies. In the Gaudieri et al. meta-analysis comparing children with and without T1D,[128] no effect sizes were greater than 0.2, a 'small' effect per Cohen's classification. The large effect sizes found in our study more closely resemble those reported a. meta-analysis of cognitive function in adults with T1D; Brands et al. reported significantly worse performance by T1D compared to non-T1D participants in overall cognition (Cohen's *d*~0.35), intelligence (*d* 0.4-0.8), psychomotor efficiency (*d*~0.6), attention (*d* 0.3-0.4), cognitive flexibility (*d*~0.5) and visual perception (*d*~0.4).[127] The studies included in this meta-analysis, however, were not limited to adults with childhood-onset T1D; this is, many of the participants in the meta-analyses were diagnosed in adulthood, thus obscuring the true effects of childhood-onset T1D on cognitive performance. Additionally, we cannot compare task scores directly as each study included in the meta-analyses employed a different battery of neurocognitive tasks. Differences in effect sizes reported for pediatric[128] and adult[127] T1D populations suggest that increasing age contributes to T1D-related cognitive decline at a faster rate than occurs in non-T1D populations.

We found no factors to explain the between group difference in NAART score. It is possible that the factors influencing NAART performance occurred much earlier in the disease physiology, near time of diagnosis, and thus do not appear in this analysis. The effect of these early, unknown factors may be so strong that the influence of traditional risk factors on cognitive decline (eg., hypertension, hyperglycemia) become non-significant. A life-course approach to cognitive function in childhood-onset T1D with repeated neuropsychological testing, brain imaging and demographic/biological risk factor assessment would enable us to better understand the complex nature of T1D and cognitive function.

Cognitive function is believed to steadily increase from childhood into the early 20s, remain fairly stable from the 20's until the 40's to early 50's, then begin to decline steadily into older age.[182] It remains unknown whether childhood-onset T1D prevents the brain from reaching its full cognitive abilities or if the decline in cognitive function occurring in late middle-aged adults occurs at a much earlier age in people with T1D, or possibly a combination of the two. Northam et al. demonstrate that in children, T1D exerts measurable effects on cognitive function within a few years of diagnosis.[183,184] Conversely, no significant declines in cognitive test scores (average of 18 years between neurocognitive testing) were reported among adults participants in the Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications study [48] even though smoking, hypertension and highest tertile A1c levels were found to negatively affect learning, memory, spatial information, psychomotor efficiency and motor speed.[185] While our study is cross-sectional and cannot assess cognitive change over time, the differences in cognitive task scores between participants with and without T1D suggest accelerated brain aging occurs in people with childhood-onset T1D.

The clinical importance of these results is demonstrated in the comparison of test scores to published norms (Figure 1). At least 10-15% of T1D participants scored $\geq$ 1.5SD worse than normative means on NAART, Trails B, Ratio Trails B:A and RVLT interference while almost 20% met this degree of impairment on ROCF-copy, 25% on DSST and 45% on Grooved Pegboard. Conversely, among non-T1D participants, approximately 12% met this level of impairment on NAART and approximately 10% on the Grooved Pegboard; for the remaining tasks, 1-7% of non-T1D participants scored $\geq$ 1.5SD from published norms. A score of 1.5SD worse than the

published norms is used in aging studies as indication of clinically relevant cognitive dysfunction or dementia. The fact that these middle-aged adults with T1D score comparable to older adults with dementia indicates an immediate need for intervention and/or assistance.

In conclusion, this study characterizes cognitive function among middle-aged adults with childhood-onset T1D. These exceptional patients have survived into middle age with T1D. They are under-represented in T1D studies but as they comprise a rapidly growing proportion of T1D patients, they deserve prompt attention in order to prevent or delay progression of complications. Other studies have shown that early glycemic control may prevent or delay the development of microvascular complications in people with type 1 diabetes. We extend the possibility that glycemic control may also benefit cognitive functioning and suggest that prevalent distal symmetric neuropathy, overt nephropathy and proliferative retinopathy may identify individuals suffering from executive function, psychomotor speed and visuo-spatial difficulties. While this study is limited by its cross-sectional nature, it does provide needed descriptive information about cognitive status in long-term T1D survivors, giving policy-makers an idea of the need for services for such individuals. From a public health perspective, reducing cognitive dysfunction in people with type 1 diabetes should correspond with a reduction in diabetes-related complications and improved self-care.[163] Longitudinal studies including the use of repeated brain imaging measures and precise ascertainment of hyper- and hypoglycemic events are needed to disentangle the complexities of type 1 diabetes-related cognitive dysfunction.

9.6 TABLES AND FIGURES

Table 9.6.1 Characteristics of participants with type 1 diabetes (T1D, from Pittsburgh's Epidemiology of Diabetes Complications study) and without type 1 diabetes (no T1D, from Pittsburgh's MR Hyper Study) at time of neurocognitive assessment (2010-2013) unless otherwise noted

		T1D N=97	No T1D N=138
Values presented are N(%), Mean ±Standard Deviation or Median (Interquartile Range)			
Demographic factors	Age (years)	49.1 ± 6.6	48.7 ± 7.2
	Female	49 (51%)	76 (55%)
	Education (years)	16 (13 - 17) **	16 (14 - 18)
Biological factors	Serum glucose pre-cognitive testing (mg/dL)	174.5 ± 86.3 ****	91.3 ± 16.3
	Systolic blood pressure(mmHg)	119.6 ± 15.6	119.4 ± 9.9
	Diastolic blood pressure (mmHg)	66 (60 - 70) ****	80 (74 - 84)
	History of high blood pressure†	37 (38%) ****	11 (8%)
	Apolipoprotein Ɛ4 status (24, 34, or 44)	29 (30%)	27 (21%)
Lifestyle factors	Ever smoking 100+ cigarettes	37 (38%)	58 (42%)
	Physical activity in past week (Kcal)#	1092 (420 - 1981) *	1628 (616 - 3008)

*p<0.05; ** p<0.01; *** p<0.001; ****p<0.0001

† For T1D participants: SBP $\geq$ 140 or DBP $\geq$ 90 or report of ever using antihypertensive medication at any EDC physical exam; for participants without T1D, history of high blood pressure based on self-report at time of neurocognitive assessment

For T1D participants, measure taken 2004-06

Table 9.6.2 Comparison on raw scores for each task in the neuropsychological test battery (controlling for years of education) between participants with type 1 diabetes (T1D, Pittsburgh Epidemiology of Diabetes Complications Study) and without type 1 diabetes (no T1D, Pittsburgh MR Hyper Study)

Domain	Test	T1D Raw Score	No T1D Raw Score	Raw Score Difference (no T1D – T1D)			Effect Size
		Mean ± SD		Mean	Percent	*p*-value*	*d*~
IQ^	North American Adult Reading Test Number correct	37.1 ± 9.4	43.3 ± 10.3	6.2	-15.0%	.0009	0.62
Psycho-motor Speed	Digit Symbol Substitution Test	54.5 ± 13.5	63.4 ± 11.8	8.9	-15.0%	<.0001	0.70
	Grooved Pegboard †	88.8 ± 33.2	69.0 ± 17.6	19.8	25.1%	<.0001	0.66
	Trail Making Test Part A †	27.6 ± 11.9	28.2 ± 13.9	0.6	-1.7%	.524	0.05
Executive Function	Stroop Color:Word	41.0 ± 9.5	44.2 ± 8.8	3.2	-7.5%	.071	0.33
	Trail Making Test Part B †	65.4 ± 38.5	53.4 ± 22.3	12.0	16.5%	.030	0.46
	Ratio B:A †	2.5 ± 1.4	2.0 ± 0.7	0.5	21.2%	.004	0.54
	Verbal Fluency: FAS	44.4 ± 13.8	48.2 ± 11.9	3.7	-7.4%	.114	0.29
	Letter Number Sequence	11.0 ± 2.9	11.9 ± 2.8	1.0	-8.2%	.055	0.33
Memory	Rey Auditory Verbal Learning Sum trials 1-5	54.1 ± 8.9	56.0 ± 9.0	2.0	-3.7%	.464	0.22
	Interference	6.4 ± 2.6	6.9 ± 1.9	0.5	-7.5%	.363	0.24
	Delayed recall	11.0 ± 3.0	11.4 ± 3.0	0.4	-4.0%	.664	0.15
	Rey Osterrieth Complex Figure Delayed recall	18.6 ± 6.7	19.9 ± 6.6	1.3	-6.6%	.410	0.20
	4-Word Short term memory 5 second list	15.2 ± 3.1	16.3 ± 3.2	1.1	-7.0%	.095	0.33
	15 second list	12.3 ± 4.1	13.4 ± 3.8	1.2	-9.2%	.172	0.29
	30 second list	10.5 ± 4.5	11.1 ± 4.7	0.6	-5.8%	.757	0.13
Other	Rey Osterrieth Complex Figure Copy	30.7 ± 5.4	33.9 ± 3.4	3.2	-9.9%	<.0001	0.87
	Verbal Fluency: Animals	21.7 ± 5.3	23.3 ± 5.5	1.5	-6.5%	.146	0.28

* *p*-value adjusted for years of education

~ Effect size = Cohen's d, calculated as $2t/\sqrt{df}$ to account for unequal variances

^ Considered an estimate of premorbid IQ

† Tasks in which a higher score = worse performance; for other tasks, higher scores = better performance

Table 9.6.3 Linear regression models showing the effects of type 1 diabetes and other covariates of interest on cognitive test scores

	Standardized Beta (SE) *p*-value			
Outcome: NAART	Unadjusted model	Adjusted for education	Most parsimonious model	Fully adjusted model
Type 1 diabetes	-.294 (1.33) <0.001	-.197 (1.17) <0.001	-.255 (1.61) <0.001	-.246 (1.71) 0.003
Years of education		.491 (0.21) <0.001	.531 (0.21) <0.001	.521 (0.22) <0.001
Diastolic blood pressure			-.118 (0.07) 0.092	-.101 (0.07) 0.167
History of high blood pressure/using antihypertensive medication			-.065 (1.53) 0.280	-.083 (1.58) 0.194
Physical activity (Kcal)				-.079 (.000) 0.170
Adjusted R^2	0.082	0.312	0.362	0.337
Outcome: DSST score	Unadjusted model	Adjusted for education	Most parsimonious model	Fully adjusted model
Type 1 diabetes	-.332 (1.67) <0.001	-.297 (1.70) <0.001	-.260 (1.82) <0.001	-.230 (2.49) 0.014
Years of education		.146 (0.31) 0.022	.149 (0.32) 0.019	.152 (0.33) 0.023
Diastolic blood pressure				-.051 (0.11) 0.543
History of high blood pressure/using antihypertensive medication			-0.103 (2.19) 0.120	-.121 (2.34) 0.101
Physical activity (Kcal)				.063 (.001) 0.350
Adjusted R^2	0.106	0.121	0.126	0.108

Table 9.6.3 Continued

Outcome: Pegboard	Unadjusted model	Adjusted for education	Most parsimonious model	Fully adjusted model
Type 1 diabetes	.365 (3.47) <0.001	0.347 (3.55) <0.001	.251 (3.68) <0.001	.338 (4.66) <0.001
Years education		-.078 (0.63) 0.233	-.146 (0.61) 0.027	-.139 (0.61) 0.038
Diastolic blood pressure				.116 (0.20) 0.161
History of high blood pressure/using antihypertensive medication			0.134 (4.33) 0.056	.081 (4.50) 0.269
Physical activity (Kcal)			-.124 (.001) 0.061	-.114 (.001) 0.093
Adjusted R^2	0.129	0.129	0.163	0.152
Outcome: ROCF copy	Unadjusted model	Adjusted for education	Most parsimonious model	Fully adjusted model
Type 1 diabetes	-.347 (0.58) <0.001	-0.297 (0.57) <0.001	-.214 (0.65) 0.002	-.151 (0.87) 0.103
Years education		.246 (0.10) <0.001	.272 (0.11) <0.001	.263 (0.11) <0.001
Diastolic blood pressure				.083 (0.04) 0.317
History of high blood pressure/using antihypertensive medication			-.119 (0.75) 0.081	-.139 (0.80) 0.056
Physical activity (Kcal)			.062 (.000) 0.328	.071 (.000) 0.268
Adjusted R^2	0.117	0.170	0.176	0.169

Table 9.6.4 Associations between characteristics of participants with type 1 diabetes (Pittsburgh Epidemiology of Diabetes Complications Study) and select cognitive test scores. Measures taken at time of cognitive assessment (2010-2013) unless otherwise noted

	NAART	DSST	Peg	ROCF
	Standardized Beta (SE), *p*-value			
Diabetes duration (years)	-.006 (0.16), 0.96	-.350 (0.21), <0.001*	.212 (0.63), 0.07	-.169 (0.09), 0.10
Diagnosed ≥ age 8 years	.039 (1.95), 0.71	-.178 (2.76), 0.09	.146 (7.62), 0.21	-.035 (1.12), 0.74
Skin Intrinsic Fluorescence (AU)[1]	-.072 (0.22), 0.51	-.245 (0.30), 0.02*	.229 (0.90), 0.06	-.278 (0.12), 0.01**
A1c months	-.198 (<0.01), 0.06	-.143 (<0.01), 0.17	.287 (<0.01), 0.01**	-.039 (<0.01), 0.71
Coronary artery disease[2]	.140 (2.64), 0.18	-.066 (3.92), 0.53	-.016 (10.50), 0.89	.099 (1.52), 0.35
Cardiac autonomic neuropathy[2]	-.151 (2.02), 0.16	-.275 (2.84), 0.01*	.106 (8.17), 0.38	-.161 (1.10), 0.14
Distal symmetric polyneuropathy[2]	-.070 (1.99), 0.52	-.388 (2.66), <0.001**	.369 (7.72), 0.002**	-.257 (1.16), 0.02*
Overt nephropathy[2]	.038 (2.52), 0.75	-.252 (3.40), 0.03*	.458 (9.55), <0.001**	-.241 (1.35), 0.04**
Proliferative retinopathy[2]	-.036 (1.95), 0.73	-.338 (2.64), <0.001**	.493 (6.68), <0.001**	-.205 (1.10), 0.05

NAART: North American Adult Reading Test, number correct (max score = 61); DSST: Digit Symbol Substitution Test (number correct in 90 seconds); Peg: Grooved Pegboard, time to insert pegs (seconds) using dominant hand; ROCF: Rey-Osterrieth Complex Figure (score on copy task); A1c months: cumulative glucose control score calculated by multiplying the number of HbA1c units above normal at each cycle by the number of months between the midpoints of the preceding and succeeding cycle intervals

1=measure taken 2006-2008; 2=measure taken 2004-2006

* Remained significant ($p \leq 0.05$) after adjusting for years of education

** Remained significant ($p \leq 0.05$) after adjusting for years of education and age at neurocognitive testing

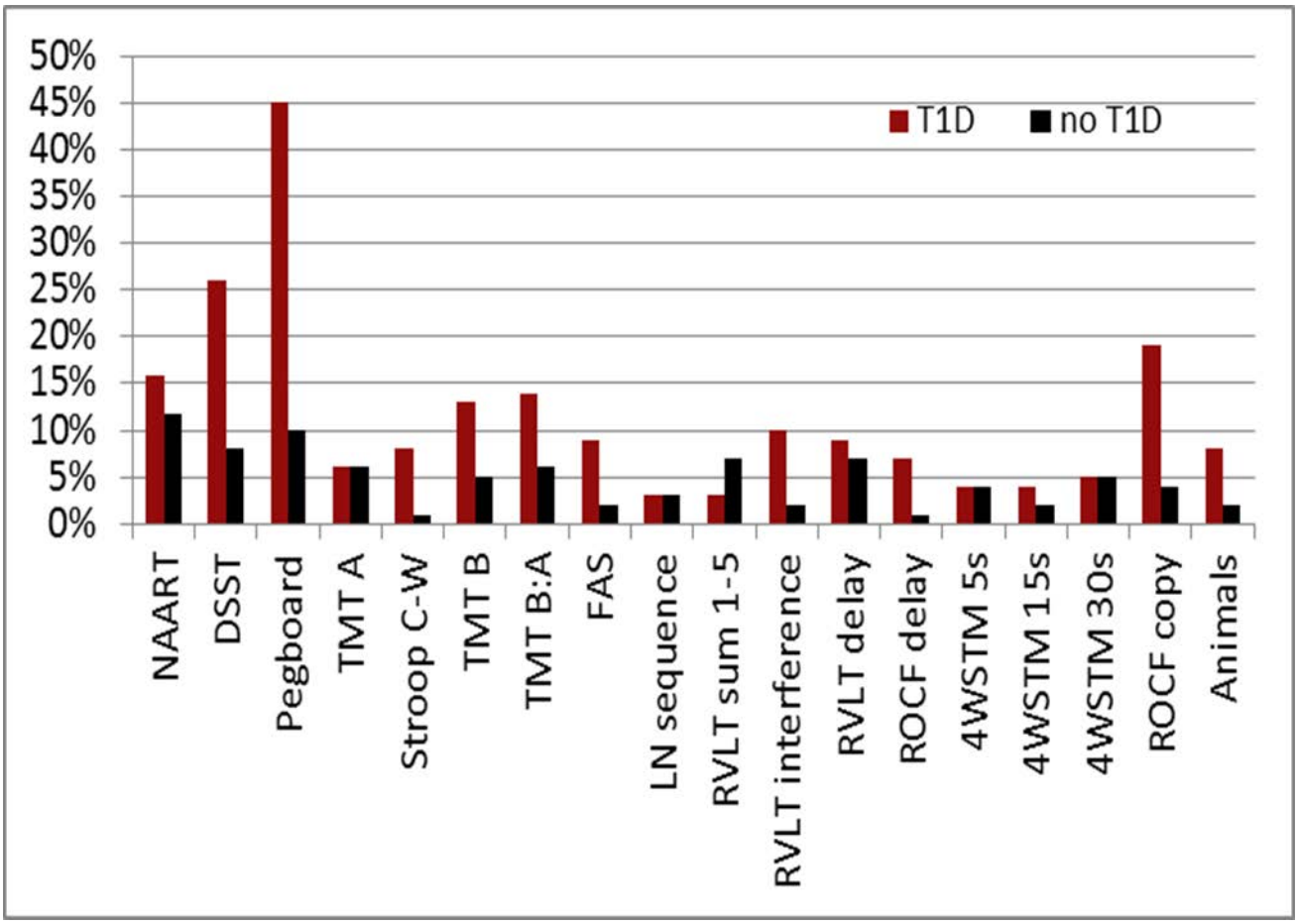

Figure 9.6.1 Percentage of participants with type 1 diabetes (red bars) and without type 1 diabetes (black bars) scoring 1.5 or more SD worse than published normative data.

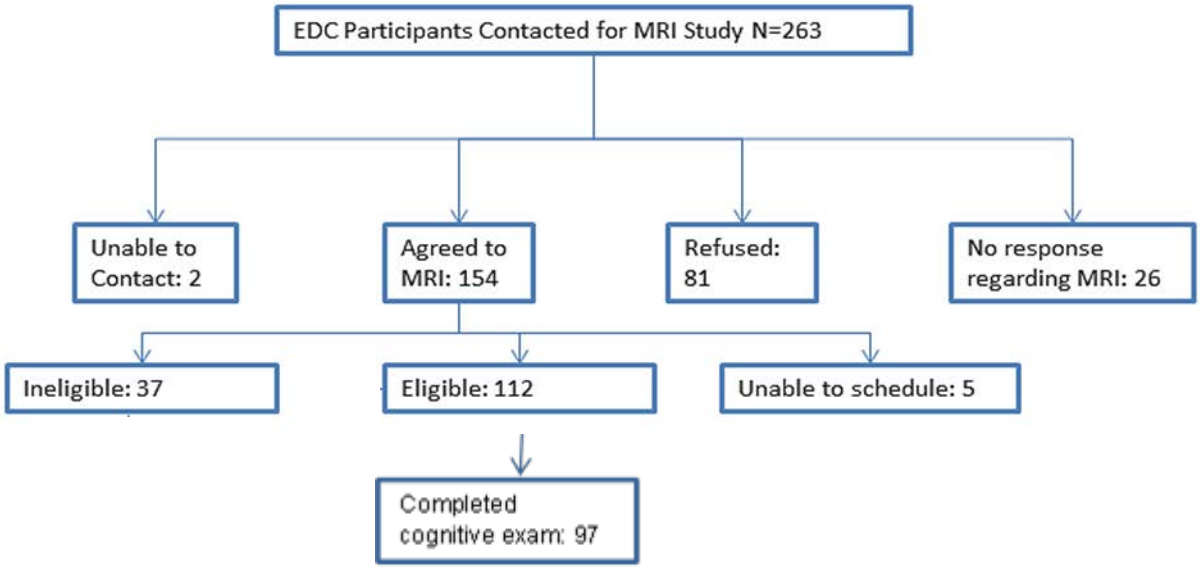

Figure 9.6.2 Flow chart showing recruitment of participants with type 1 diabetes from the parent Pittsburgh Epidemiology of Diabetes Complications (EDC) Study into the MRI/neurocognitive study

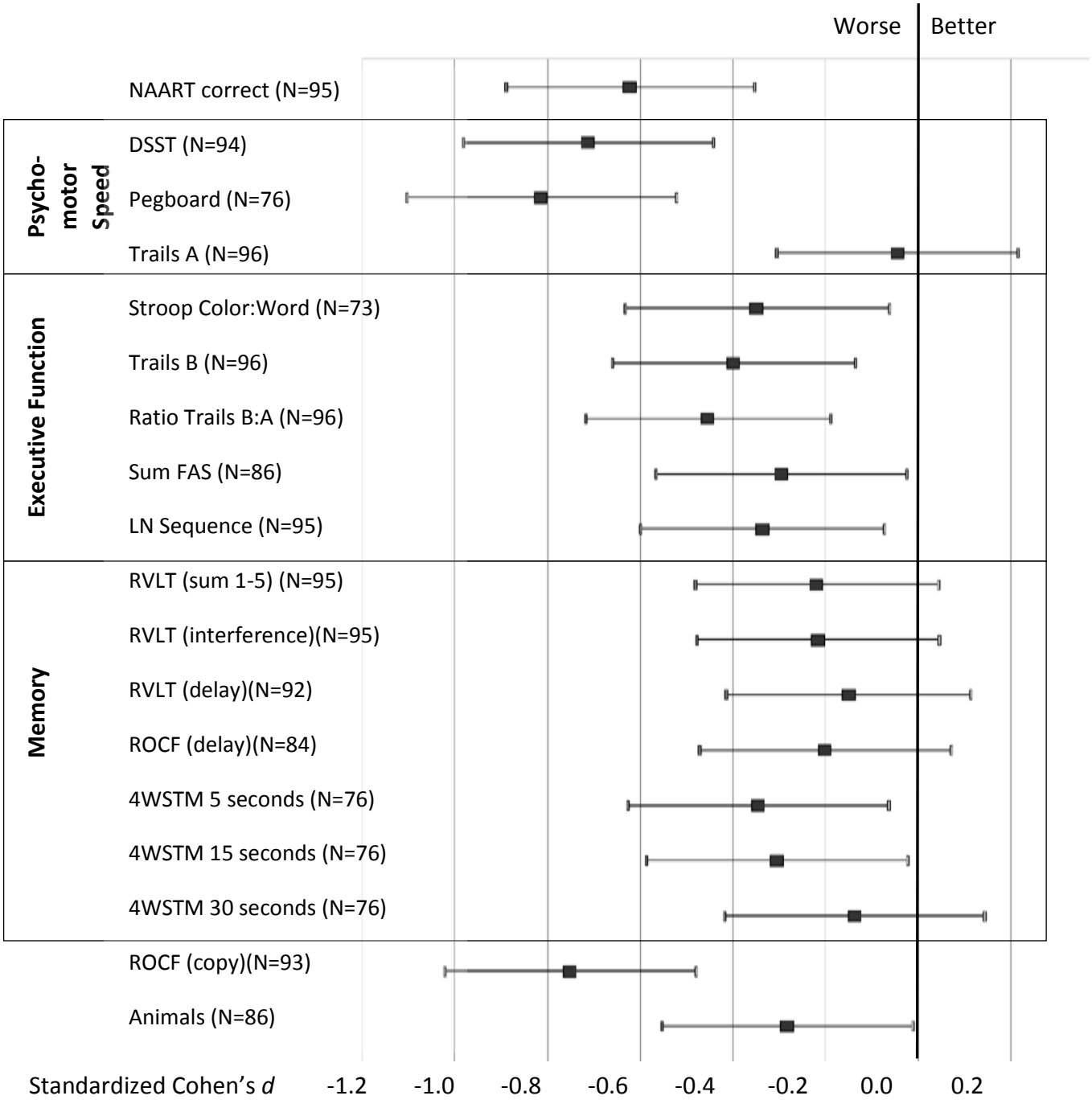

Figure 9.6.3 Standardized effect sizes of raw score differences between participants with type 1 diabetes (T1D, Pittsburgh Epidemiology of Diabetes Complications Study) and without type 1 diabetes (Pittsburgh MR Hyper Study). Sample size for T1D participants in parentheses (N=xx). For participants without T1D, N=138 for all tasks except NAART (NAART N=137)

10.0 MANUSCRIPT 3: REDUCED CEREBRAL BLOOD FLOW RELATED TO COGNITIVE DYSFUNCTION IN MIDDLE-AGED ADULTS WITH CHILDHOOD-ONSET TYPE 1 DIABETES

10.1 ABSTRACT

Objective. Employ a non-invasive neuroimaging approach to examine the relationship between brain perfusion and cognitive dysfunction in middle-aged adults with childhood-onset type 1 diabetes.

Methods. A subset of 93 adults (mean age 49 years, mean diabetes duration 41 years) from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC) underwent brain neuroimaging and completed a neuropsychological battery between December 2010 and January 2103. Cognitive status (cognitive normal vs. cognitive dysfunction) was determined by clinical adjudication. Between-group differences were examined using logistic regression models, controlling for IQ.

Results. 32% of participants were classified with cognitive dysfunction. Reduced cerebral blood flow to the right middle frontal gyrus and bilateral superior parietal lobes was significantly related to cognitive dysfunction; no other neuroimaging markers, either whole

brain or region of interest, were significantly related to cognitive dysfunction. Having an ankle-brachial index (ABI) $\geq$ 1.3 and being in the lowest vs. highest quartile of physical activity, as assessed in 2004-2006, both significantly increased the odds of cognitive dysfunction, however only high ABI remained significantly associated with cognitive dysfunction after adjusting for reduced cerebral blood flow.

Conclusion. Cognitive dysfunction in middle-aged adults with type 1 diabetes is related to reduced cerebral blood flow, vascular stiffness (as indicated by high ABI) and low physical activity. Further studies are needed to investigate the vascular mechanisms underlying these associations and whether interventions aimed at preventing vascular stiffness and increasing physical activity would reduce cognitive dysfunction in people with T1D.

10.2 INTRODUCTION

Adults with type 1 diabetes mellitus (T1D) have a higher prevalence of mild to moderate cognitive dysfunction compared to similarly-aged peers without diabetes.[133,163-165,177,186,187] Greatest decrements are noted in executive function, psychomotor speed, intelligence, attention and visual perception.[127,165] In addition, cognitive dysfunction appears at a younger age in those with T1D[128,164] compared to the general population.[166,167] The risk factors and neuroanatomical correlates that underlie T1D-related cognitive dysfunction remain unclear and deserve further investigation. Studying this relationship among older adults with T1D is particularly important since advancing age also negatively affects cognitive function. Considering the improved life expectancy of people with T1D,[131] research in this area is of public health urgency in order to develop strategies to minimize the development and/or progression of cognitive dysfunction in adults with childhood-onset type 1 diabetes.

Among the type 1 diabetes factors that correlate with brain imaging markers, the two main candidates are chronic hyperglycemia and high blood pressure because of their known effects on cerebral vasculature.[58] Both conditions lead to thickening of vessel walls and narrowing of the lumen, which causes diffuse ischemic damage, changes in myelin structure, loss of axons and necrosis of gray and/or white matter.[58] While severe hypoglycemia has long been considered a risk factor for the deleterious effects of T1D on the central nervous system,[188] some studies have failed to prove such an association.[126,163] Chronic hyperglycemia, however, negatively impacts endothelial function[58] and has emerged as a modifiable risk factor for T1D-related cognitive dysfunction.[189] Even though hypertension plays a major role in age-

related cognitive decline,[58,190] its contribution to T1D-related cognitive dysfunction remains unclear, with two studies reporting an association between hypertension and reduced cognitive function [126,176] while a third did not.[127]

Although gray matter volume and white matter hyperintensities are known correlates of cognitive impairment in older adults without T1D,[52,70,191] few studies have examined these relationships in adults with T1D. One study reports a relationship between reduced cognitive function and smaller cerebral white matter volume[68] in adults with vs. without T1D but other studies do not support this association.[92,95] While middle-aged adults with childhood-onset T1D have a higher prevalence of cerebral WMH compared to adults without T1D, (Nunley et al., 2014, unpublished), studies have not found an association between these lesions and T1D-related cognitive dysfunction.[93-95] The relationship between gray matter volume and cognitive function in adults with T1D also remains unknown. Musen et al. conducted a GM voxel based morphometry study in young adults with T1D but did not provide results showing correlations between cognitive test scores and GM density.[192] Other studies report cerebral atrophy (as suggested by larger ventricle size) in participants with vs. without T1D but found no significant correlations between this marker of atrophy and cognitive dysfunction.[92-94,96] One possible reason for these discrepancies is that advanced structural changes such as WMH and loss of GM volume may not be evident in younger people with T1D who have earlier/milder cognitive impairment.[193]

Recent advances in imaging technology can assess earlier changes in brain structure as compared to WMH or GM volume. Microstructural disruption of gray and white matter integrity, as assessed using diffusion tensor imaging (DTI), correlates with poor cognitive test

performance on executive function, memory and perception speed tasks in age-related cognitive decline.[194,195] Two DTI studies have examined the relationship between cognitive function and cerebral white matter microstructural integrity in adults with T1D, with both reporting lower fractional anisotropy (FA) values in participants with vs. without T1D.[99,125] Kodl et al. report that lower FA was related to poorer performance on the Rey-Osterreith Complex Figure Drawing and grooved pegboard tasks.[99] While Van Duinkerken et al. found an association between lower FA and lower overall cognitive performance, they did not report associations between FA values and individual neurocognitive tests.[125]

Cerebral blood flow (CBF) can also detect early indications of compromised brain integrity. Lower CBF is associated with mild cognitive impairment and dementia in elderly populations.[196-198] Unlike other CBF techniques (e.g., positron emission tomography, X-ray computed tomography),[199] ASL does not require the use of a radiotracer. Rather, radiofrequency irradiation is used to magnetically label arterial blood water molecules. Decay of the magnetic signal occurs with T1 relaxation.[200] The non-invasiveness of ASL makes it a more acceptable methodology to research study participants. It also allows CBF imaging in patient populations with contraindications for contrast agents. No studies were found that examined the relationship between CBF using ASL and cognitive function in adults with T1D.

Lack of consistency between studies (e.g. differences in duration of disease, glycemic control, brain tissue of interest and choice of neuropsychological tests) prevent a complete understanding of the effects T1D has on brain structure and function. In addition, most of these studies utilized low magnetic field strength (.5 or 1.0 Tesla) which may have limited the ability to detect early and/or small changes in the T1D brain. Lastly, only one study examined older

T1D participants[92] while the remaining studies examined younger adults with T1D. As such, the full effects of T1D on cerebral structure and function, combined with the known effects of advancing age on cerebral structure and function, remain to be ascertained.

This research will explore the possibility of using a non-invasive measure of CBF to explain the pathophysiology of early cognitive dysfunction in middle-aged adults with childhood-onset T1D. The aim of this research is to 1) determine if cerebral blood flow partially explains the presence of clinically-adjudicated cognitive dysfunction in middle-aged adults with childhood-onset T1D; and 2) identify potential risk factors for cognitive dysfunction using data collected over the 20 years preceding MRI and cognitive testing. The hypotheses are 1) clinically-adjudicated cognitive dysfunction will be correlated with reduced cerebral blood flow in this population of middle-aged adults with childhood-onset T1D; 2) in these participants with childhood-onset T1D, older age, poor glycemic control, diabetes duration and the presence of neuropathy and retinopathy will be associated with cognitive dysfunction.

10.3 METHODS

Each study protocol received local IRB approval prior to study initiation. All participants completed informed consent prior to undergoing study procedures.

10.3.1 Study Population

Participants for this study were drawn from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC), an ongoing, prospective study of individuals with childhood-onset (diagnosed $\leq$ 17 years) T1D. All EDC participants, diagnosed between 1/1/50 and 5/31/80 and seen within one year of diagnosis at Children's Hospital of Pittsburgh, underwent a baseline assessment (1986 – 1988) when the mean age was 28 years and the average diabetes duration was 19 years. After their baseline visit, EDC patients were followed with biennial exams for 10 years, thereafter completing biennial questionnaires. An additional physical exam occurred in 2004-2006. A total of 263 locally-resident EDC participants contacted for the 2010-2012 exam were also invited to participate in this MRI study. Of these, 157 did not undergo imaging: 81 were not interested, 37 were ineligible for MRI and 39 did not reply or did not show for their MRI. The remaining 106 (mean age 48 years, 50% female, 98% Caucasian) underwent brain imaging between December 2010 and December 2012. Due to poor or missing imaging sequences, nine individual were excluded from analyses. Another four participants did not undergo neurocognitive testing, yielding an analytical sample of n=93 (Figure 10.6.1).

10.3.2 Neuropsychological Testing

The following tasks comprised the neurocognitive test battery. Details of each test are provided in Appendix B.

1. North American Adult Reading Test (NAART)
2. Wechsler Memory Scale-Revised (WMS-R) Logical Memory, Story A
3. Rey Osterrieth Complex Figure (ROCF)
4. Rey Auditory Verbal Learning Test (RVLT)
5. Four Word STM
6. Digit Symbol Substitution Test (DSST), number complete in 90 seconds
7. Trail Making Test A and B (TMTA, TMTB), time to complete
8. Wechsler Adult Intelligence Scale (WAIS) Block Design
9. Verbal Fluency – FAS and Animals Tests
10. Stroop Color-Word Test (Golden version)
11. Grooved pegboard, dominant hand, time to insert pegs

Neuropsychological tests were administered by one trained study staff member to ensure consistency between participant experiences. Tests were scored by trained study staff unaware of participant cognitive status.

The clinical adjudication process has been previously described and validated.[166] For this study, a clinical psychologist with expertise in dementia and a clinical psychologist with expertise in cognitive functioning in people with T1D evaluated the cognitive tests and clinical history of each patient since the first available record. Using standardized diagnostic criteria

from neurocognitive test scores in conjunction with medical and personal histories and brain MRIs, they determined the presence of "Cognitive disorder not otherwise specified (NOS)" (DSM-IV Code 294.9) for each participant; this category is reserved for cognitive dysfunction resulting from the direct physiological effects of some general medical condition (i.e., type 1 diabetes) and that does not meet DSM-IV criteria for other types of cognitive disorders such as delirium or dementia.[201]

10.3.3 Covariates

The following were ascertained at each EDC visit, from baseline (1986-1988) to time of MRI: diabetes duration; HbA1c (using saline-incubated blood samples and cation-exchange microcolumn chromatography prior to October 1996, using high-performance liquid chromatography through 1996-98, and thereafter using the DCA 2000 analyzer); estimated glucose disposal rate (eGDR, an indicator of insulin sensitivity); use of insulin pump (yes/no); and serum glucose.

Hemoglobin A1c was also measured at time of MRI. An average HbA1c value was calculated over all visits. A measure known as "A1c months" that combines a cumulative degree and duration of glycemic exposure was also included (see [143] for specific details).

<u>Diabetes-related complications</u>

Prevalence of the following complications was ascertained at the 2004-2006 exam which occurred, on average, five to six years prior to MRI:

Renal disease refers to either microalbuminuria (MA), overt nephropathy (ON) or End Stage Renal Disease (ESRD; dialysis or renal transplantation). ON was defined as the presence of renal failure (serum creatinine >5 mg/dl and/or ESRD) or an albumin excretion rate >200 μg/min in at least 2 of 3 timed urine samples. In the absence of urine collections, a serum creatinine >2 mg/dl indicated ON. MA was defined as an albumin excretion rate between 20-200 μg/min in at least 2 of 3 urine samples.

Coronary artery disease (CAD) was defined as myocardial infarction (Minnesota Codes 1.1 or 1.2), fatal CAD (determined by review of death records and, where appropriate, family interview), or angiographic evidence of 50% or more stenosis. CAD also included angina diagnosed by the clinic physician and/or ischemic ECG changes (Minnesota Codes: 1.3, 4.1-4.3, 5.1-5.3, 7.1).

Neuropathy: Distal symmetric polyneuropathy (DSP) was determined by medical history and clinical examination using established protocols, i.e. the presence of two or more of: symptoms, signs consistent with DSP, or reduced deep tendon reflexes. DSP was further determined as 'confirmed' using the Vibratron II tester to detect a vibratory threshold above the age-specific normal range. Cardiac autonomic neuropathy (CAN) was determined by heart variation during deep breathing and by heart rate and blood pressure response to standing and was considered present if the mean expiration to inspiration ratio was $\leq$1.1.

Retinopathy: Eye exams were done using three standard field stereo fundus photographs. Photographs were read at the Madison Reading Center in Wisconsin and graded using the Early Treatment Diabetic Retinopathy Study classification, with a grade of 60 or higher in one eye or a

grade less than 60 but with panretinal photocoagulation scars consistent with laser therapy indicating proliferative retinopathy.[145]

Biological risk factors

Hypertension was defined as any study blood pressure reading ≥140/90 mmHg or ever reporting use of antihypertensive medication. Ever use of antihypertensive and lipid lowering medications were compiled using data from each EDC clinic visit and time of MRI.

Blood pressure: Three seated blood pressure readings were taken with a random-zero sphygmomanometer on day of MRI, with an average of the second and third readings, per the Hypertension Detection and Follow-up Program Protocol.[202]

Cholesterol: SYNCHRON CX® Systems was used to measure non-fasting total cholesterol and high-density cholesterol (HDLc). Non-HDL cholesterol was calculated as HDLc subtracted from total cholesterol.

Ankle-Brachial Index (ABI) was calculated as the ratio between systolic blood pressure measured at the posterior tibialis and the upper arm, using measures from the same side (usually the right side), during the 2004-2006 exam. ABI was dichotomized as below or above 1.3 as this has been previously shown to correlate with mortality[203] and arterial calcification[204] in people with T1D.

Apolipoprotein E (ApoE) status was ascertained at the 2004-2006 exam.

Body mass index (BMI) in kg/m^2 was calculated based on the participants' weight and height recorded at time of MRI.

Skin intrinsic fluorescence (SIF), a marker of advanced glycation end products (AGEs), was assessed among T1D participants in 2006-2008. This utilized a skin fluorescence

spectrometer (SCOUT DS®) to take a noninvasive measure from the skin of the left volar forearm. Details on the device and SIF calculations are discussed elsewhere. [144]

Lifestyle/Behavioral risk factors

Smoking status was determined at each EDC exam via self-report questionnaire as currently, formerly or never smoking 100 cigarettes. Responses were dichotomized as ever vs. never smoking using information obtained from baseline through the EDC 2004-2006 exam.

Physical activity was estimated using the Paffenberger questionnaire during the 2004-2006 exam. Results were used to calculate energy expenditure over the past week in Kcal.

10.3.4 Brain Imaging Protocols

All study participants underwent brain MRI scanning in a 3Tesla Siemens Trio TIM scanner located in the Magnetic Research Center at the University of Pittsburgh. A T1-weighted 3D sequence (MPRAGE: TR/TI/FA = 2300/900/9, Resolution 256, Grappa 2), acquisition time 7.5 min, was used to attain grey matter (GM) and white matter volumes. For WMH, a FLAIR sequence (TR/TE = 9002/56 ms Ef; TI = 2200 ms, NEX = 1) was used with an interleaved acquisition; 48 slices (3mm, no gap). Diffusion-weighted images were acquired using a single short spin-echo sequence (TR = 5300 ms, TE = 88 ms, TI = 2500 ms, 90° flip angle, 256 mm × 256 mm FOV, two diffusion values of b = 0 and 1000 s/mm, 12 diffusion directions, four repeats, 40 slices, 3 mm thick, 128 × 128 matrix size, 2 mm × 2 mm × 3 mm voxel size, and GRAPPA = 2). A pulsed arterial spin labeling (PASL) sequence was used for perfusion fMRI scans.

Interleaved images with and without labeling were acquired using a gradient echo planar imaging sequence (TR/TE/TI = 3000/20/1800 ms; flip angle = 90°). The tagging/control duration was 0.7 s. 19 oblique slices (thickness/gap = 5/1 mm, field of view = 224 mm × 224 mm, matrix = 70 × 70, voxel = 3.2 mm × 3.2 mm × 5 mm) covered the whole brain. All images were examined for neurologic abnormalities by a neuroradiologist.

Neuroimaging measures of interest included whole brain gray matter volume, gray matter mean diffusivity, cerebral blood flow, white matter hyperintensities and fractional anisotropy of normal appearing white matter. Gray matter diffusivity and cerebral blood flow were adjusted for normalized total brain gray matter, computed as (total gray matter / intracranial volume x 100). Gray matter volume, blood flow (via ASL) and mean diffusivity (via DTI) were extracted from left and right prefrontal cortex (middle frontal gyri), hippocampus, superior parietal lobes and anterior cingulated lobes. White matter hyperintensities and fractional anisotropy (via DTI) were extracted from left and right superior longitudinal and uncinate tracts. These regions and tracts were selected because they are known to be vulnerable to high blood pressure and because they are related to impaired executive function and memory in older populations without T1D.

10.3.5 Statistical Analyses

Participants were categorized by cognitive status (i.e. presence vs. absence of cognitive dysfunction) as determined by the adjudication process. For comparison purposes only, this classification was compared to an algorithm (Nunley et al. 2014, unpublished) that determined

the presence of cognitive dysfunction using published normative data.[205-207] Because the clinical adjudication process is the gold standard, validated and used in dementia studies, this definition of cognitive dysfunction was used for all analyses. For comparative purposes only, clinically adjudicated cognitive status was compared to cognitive status determined using an algorithm that defined cognitive dysfunction as having at least two test scores worse than 1.5SD from published normative data (Nunley et al. 2014, unpublished). The two methods agreed on 55/108 as being cognitively normal and 27/108 as having cognitive dysfunction (Table 10.6.1).

Group differences were assessed using t-test for continuous variables, Fisher exact test for categorical variables and Wilcoxon rank sum test for highly skewed data. For measures last assessed at the 2004-2006 EDC exam, models were controlled for the time interval between assessment and MRI. Logistic regression was conducted using a reduced sample size of N=68 with no missing measures (i.e., a subset with complete MRI and risk factors of interest) to test for effects of neuroimaging measures and diabetes-related factors on cognitive dysfunction, controlling for NAART. Analyses of neuroimaging data of GM volume, GM mean diffusivity and cerebral blood flow of gray matter were adjusted for normalized gray matter volume of total brain (total brain gray matter volume divided by intracranial volume).

Analyses were conducted using SAS 9.3 and SPSS 21.

10.4 RESULTS

In this population of middle-aged adults with childhood-onset type 1 diabetes, the clinical adjudication process identified 31 of 97 (32%) participants as having cognitive dysfunction. Cognitive dysfunction was related with significantly lower NAART scores, a measure of general intelligence, and significantly fewer years of education (Table 10.6.1). Participants classified with cognitive dysfunction were significantly more likely to have an ankle-brachial index (ABI) $\geq$ 1.3 and to report lower physical activity than participants classified as cognitively normal (Table 10.6.1). These differences remained significant after adjusting for the time interval from 2004-2006, when these measures were taken, and time of MRI. Trends were observed in CDSP, proliferative retinopathy, estimated glucose disposal rate and ever reporting the use of blood pressure medications although these differences did not reach statistical significance (Table 10.6.1). No significant between-group differences were found in age at MRI, hypertension history and/or any measure of poor glycemic control (A1c at time of MRI continuous or $\geq$ 7.5%, A1c average from 1998-2012 continuous or $\geq$ 7.5% or skin intrinsic fluorescence).

Cognitive dysfunction and brain imaging. No significant between-group differences were detected in whole brain gray matter atrophy, white matter hyperintensities, cerebral blood flow, fractional anisotropy of normal appearing white matter or gray matter mean diffusivity (Table 10.6.2). Higher blood flow measured from the right frontal middle gyrus, and left and right superior parietal lobes, was protective against cognitive dysfunction (*p*-values = 0.049, 0.041 and 0.037, respectively); further adjustment for sex did not significantly alter these relationships. Similar trends were observed for whole brain CBF as well as blood flow measured

from the left anterior cingulate, although these differences were not significant (*p*-values = 0.135, and 0.177, respectively). Cognitive dysfunction was not significantly related to regional gray matter volume, GM mean diffusivity, FA of normal appearing WM or WMH.

Risk factors, brain imaging and cognitive dysfunction. In logistic regression models controlling for NAART score, low physical activity and high ABI remained significantly associated with cognitive dysfunction independently of IQ and time interval between measure assessment and MRI. Having an ABI > 1.3 was associated with an eleven-fold increase in the odds of cognitive dysfunction (Table 10.6.3, Model 4). Being in the lowest vs. highest quartile of physical activity (PA) was associated with a six-fold increase in the odds of cognitive dysfunction (Table 10.6.3, Model 3). The effect of PA became non-significant (p=1.00) while high ABI remained stable and significant in the model including both factors (Table 10.6.3, Model 5). These associations remained unchanged when further adjustments were made for blood flow to the right middle frontal gyrus or the superior parietal lobe, bilaterally (Table 10.6.3, Model 5, adjusted for CBF).

10.5 DISCUSSION

In this study of middle-aged patients with T1D since childhood, we found that having a higher ABI (>1.3) is related to cognitive dysfunction. Higher ABI is a marker of subclinical vascular conditions including atherosclerosis, vessel stiffness and calcification, which are known risk factors for cognitive impairment in older adults without T1D.[208,209] Vascular stiffness may serve as an indicator of early changes to brain structure.[210] To our knowledge, the relationship of higher ABI with cognitive dysfunction in T1D has not been examined. People with T1D tend to have an ABI $\geq$ 1.3, an indicator of calcification in the lower extremities.[211] High ABI is also related to medial arterial calcification in adults with T1D, which is a risk factor for stiffness and stroke.[204] Vessel stiffening can lead to cerebral hypoperfusion, with diffuse subclinical ischemia and resultant damage to oligodendrocytes and focal necrosis in gray and white matter, offering a mechanistic explanation for the relationship with cognitive dysfunction.

We also found a relationship between low physical activity and cognitive dysfunction, albeit this was weaker. While this relationship is known in the aging literature,[212,213] it has not been explored with those with T1D. The exact mechanisms of physical activity's beneficial effect on cognition remain unknown but studies show increased neurogenesis, angiogenesis and increased growth factors related to cognition.[212] Another proposed mechanism is that physical activity improves insulin sensitivity, as occurs in type 2 diabetes, and insulin resistance is correlated with dementia.[214] The marginal relationship between lower estimated glucose disposal rate, a measure of insulin resistance, and cognitive dysfunction in this population of middle-aged adults with T1D lends support to this theory.

In this study, lower cerebral blood flow was related to greater odds of cognitive dysfunction, with associations being strongest for the right middle frontal gyrus and bilateral, superior parietal lobes. These regions are important for executive function[215], attention and decision making,[216] which are also severely affected in adults patients with T1D (Nunley et al, MS2, unpublished and Supplementary Table 2).

In conclusion, this study shows that utilizing a multi-modal approach to examine the neuroanatomical correlates of cognitive dysfunction can advance our understanding of cognitive dysfunction for this middle-aged population with long-term T1D. It also underscores the relevance of examining markers of cerebral blood flow in addition to more conventional markers of structural integrity. Without longitudinal data, however, we cannot determine if these structural changes appeared earlier than expected or whether they have progressed with age. Another limitation of the current study is the relatively small sample size and the lag time between ascertainment of T1D microvascular complications and brain imaging. While survival bias is present in this T1D population and limits the generalizability of these findings, it also allows us to study factors related to their ability to survive the damage caused by this disease. Further studies are needed to determine if intervening on vascular stiffness and physical inactivity may prevent or delay the development of cognitive dysfunction in people with T1D.

10.6 TABLES AND FIGURES

Table 10.6.1 Characteristics of study participants by clinically adjudicated cognitive dysfunction status at time of MRI (2010-2013) unless otherwise indicated

		Cognitive Dysfunction		
		Yes (N=31)	No (N=66)	p-value*
		Values are Mean ± SD, N (%) or Median (IQR)		
Population Characteristics	Age, years	49.6 ± 6.2	48.8 ± 6.8	0.5616
	Duration, years	41.8 ± 5.2	40.7 ±6.6	0.4149
	Age at diagnosis, years	7.9 ± 4.6	8.1 ± 4.0	0.7814
	Female	13 (42%)	36 (55%)	0.2814
	Education, years	13 (12-16)	16 (14-17)	0.0021
	NAART score	31.9 ± 10.4	39.5 ± 7.9	0.0009
	Depressive symptoms †	9 (32%)	12 (19%)	0.1670
	24, 34 or 44 allele †	11 (35%)	18 (27%)	0.4780
	Body Mass Index	27.1 ± 5.4	27.5 ± 4.4	0.4962
Glucose control	HbA1c monthly estimate	1034.7 ± 472.2	1154.0 ± 408.6	0.2055
	HbA1c at MRI $\geq$ 7.5%	20 (65%)	38 (59%)	0.6608
	HbA1c average 1998-2012 $\geq$ 7.5%	22 (71%)	41 (63%)	0.4979
	Serum glucose at MRI, mg/dL	182.6 ± 77.8	170.9 ± 90.3	0.5502
	Skin intrinsic fluorescence (AU) ‡	24.7 ± 4.2	24.5 ± 5.0	0.8716
Cardiovascular factors	Ankle Brachial Index > 1.3 †	9 (31%)	4 (7%)	0.0038
	Systolic blood pressure (mmHg)	118.1 ± 16.7	120.3 ± 15.2	0.5186
	Study 20-yr average SBP (mmHg) *	110.5 ± 10.9	113.3 ± 10.6	0.2392
	History of high blood pressure #	14 (45%)	23 (35%)	0.3742
Lifestyle Factors	Ever smoked 100+ cigarettes ~	12 (39%)	25 (38%)	0.9999
	Physical activity in past week (Kcal) †	476 (280-1488)	1228 (732-2379)	0.0132
Diabetes Complications	Coronary artery disease †	4 (13%)	11 (17%)	0.6392
	Cardiac autonomic neuropathy †	12 (41%)	29 (49%)	0.4645
	Confirmed distal symmetric polyneuropathy †	19 (61%)	27 (46%)	0.1713
	Microalbuminuria †	16 (55%)	33 (60%)	0.8260
	Overt nephropathy †	7 (26%)	14 (27%)	0.9440
	Proliferative retinopathy †	18 (58%)	28 (42%)	0.1301

† Measure taken 2004-2006; ‡ Measure taken 2006-2008; * average from 1986-88 through 2004-06; # Any SBP > 140 OR DBP > 90 OR ever using antihypertensive medication from EDC baseline through MRI; ~ "Any" or "Ever" based on self-report from 1986-88 through 2004-2006

Table 10.6.2 Results from univariate logistic regression models showing the independent effects of cerebral blood flow, after controlling for IQ (NAART score) and normalized gray matter volume (total GM volume/Intracranial volume) on the odds of clinically adjudicated cognitive dysfunction

Cerebral Blood Flow (ml/100g tissue/min)	**OR (95% CL) *p*-value**
Whole brain	0.94 (0.87, 1.02) 0.135
Middle Frontal Gyrus, left	0.98 (0.93, 1.05) 0.609
Middle Frontal Gyrus, right	0.93 (0.86, 1.00) 0.049
Superior Parietal Lobe, left	0.94 (0.88, 0.99) 0.041
Superior Parietal Lobe, right	0.93 (0.87, 1.00) 0.037
Anterior cingulate, left	0.96 (0.90, 1.00) 0.177
Anterior cingulate, right	0.97 (0.91, 1.02) 0.242

Table 10.6.3 Logistic regression models, controlling for NAART, and time interval between measure collected (2004-2006) to MRI (2010-2013) showing the independent effects of select risk factors on the odds of clinically adjudicated cognitive dysfunction

	1	**2**	**3 (1 and 2**	**3** adjusted for right middle frontal gyrus CBF	**3** adjusted for left superior parietal lobe CBF	**3** adjusted for right superior parietal lobe CBF
Odds Ratio (95% CL) *p*-value						
Low physical activity (q1 v q4)	6.07 (1.07, 34.56) 0.042		5.64 (0.72, 44.30) 0.100	7.13 (0.82, 62.18) 0.076	9.31 (0.96, 90.64) 0.055	7.46 (0.82, 68.06) 0.075
ABI $\geq$ 1.3		11.86 (2.55, 55.20) 0.002	12.03 (2.18, 66.57) 0.004	10.64 (1.65, 68.70) 0.013	11.16 (1.78 , 70.03) 0.010	10.41 (1.69, 64.21) 0.012

Table 10.6.4 Comparison of participants identified as having cognitive dysfunction using the clinical adjudication determination vs. the algorithm comparing participant test scores to published normative data

		Cognitive Dysfunction per Clinical Adjudication N(%)	
Cognitive Dysfunction per Normative Data N(%)		Not Present	Present
	Not Present	50 (77%)	8 (27%)
	Present	15 (23%)	22 (73%)
	Total	65 (68%)	30 (32%)

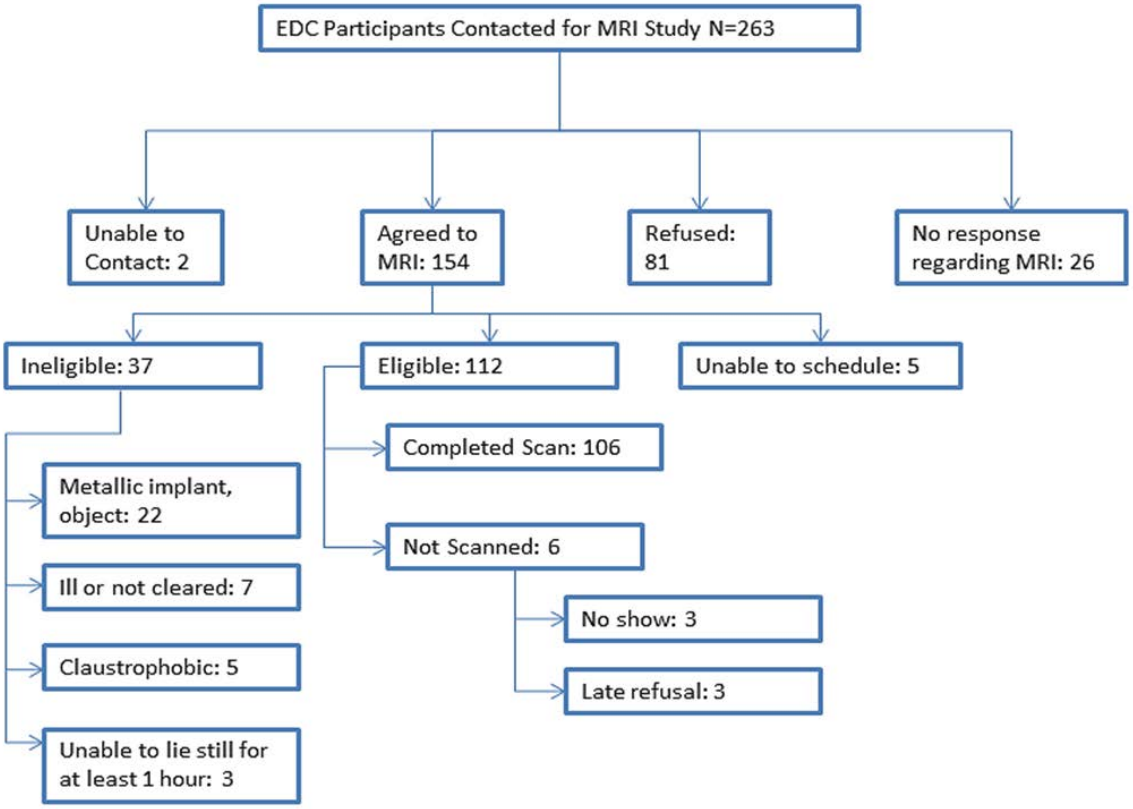

Figure 10.6.1 Recruitment flow chart of participants with type 1 diabetes from the parent EDC cohort for the MRI/neurocognitive study

11.0 DISCUSSION, PUBLIC HEALTH IMPORTANCE, FUTURE DIRECTIONS

This overall goal of this research was to provide evidence in support of deleterious effects of childhood-onset type 1 diabetes on brain structure and function. While T1D effects on the peripheral nervous system (e.g., distal symmetric polyneuropathy, autonomic neuropathy) are well recognized and studied, such is not the case regarding its effects on the central nervous system. While CNS effects were once difficult to study *in vivo*, advances in neuroimaging have overcome this obstacle. Even so, relatively few studies over the past two decades have included neuroimaging in studies of cognitive performance among adults with T1D. Even fewer studies have included middle-aged and older adults who have lived with T1D since childhood. These aging individuals with childhood-onset T1D are also experiencing the effects of advancing age on cognitive function. Now that people with T1D can expect to live almost as long as those without T1D, identifying factors that may help prevent or delay T1D-related cognitive dysfunction should be a public health priority.

To accomplish this goal, the research herein addressed three gaps in knowledge regarding brain structure and function in middle-aged adults with childhood-onset T1D: 1) the presence of cerebral WMH, a macrostructural measure of WM health; 2) the prevalence of cognitive dysfunction; and 3) the brain imaging markers that best correlate with cognitive dysfunction in these individual. Diabetes-specific, general health and lifestyle risk factors

related to each of these also remain unclear so these were also studied in relationship to each gap in knowledge.

White matter hyperintensities, as a percentage of total brain volume, were almost sixty times higher in this T1D population compared to similarly-aged adults without T1D. Among participants with T1D, older age, longer T1D duration, higher SIF, a history of smoking, prevalent cardiac autonomic neuropathy and distal symmetric polyneuropathy were significantly related to high WMH burden. Hypertension, a major contributor to age-related WMH, and poor glycemic control were not significantly related to WMH burden.

In this population of middle-aged adults, childhood-onset type 1 diabetes correlates with a higher prevalence of cerebral WMH than would be expected based on age alone. This could be due to earlier accrual and/or faster progression of cerebral WMH. The cross-sectional nature of this study does not allow investigation of WMH evolution; a longitudinal brain imaging study, with first MRI at time of diagnosis, would allow investigation into the development and progression of WMH in people with type 1 diabetes from childhood through middle-age. Formation of advanced glycation end products resulting from chronic hyperglycemia, as measured by SIF, may contribute to the development of WMH, especially in older adults with type 1 diabetes. Cigarette use may further exacerbate the development of these lesions. WMH deserve further study in people with type 1 diabetes and whether its accrual may be prevented or delayed via strict glycemic control, particularly for older adults, and possibly by tobacco avoidance.

Cognitive dysfunction is well-studied in children and young adults with T1D, but few studies have examined this condition in older adults with childhood-onset T1D. No studies have

yet taken a life-course approach to document the advent and progression of T1D-related cognitive dysfunction. Consequently, the best approximation of cognitive dysfunction progression in people with T1D is to compare results from pediatric to results from adult cognitive studies.

Gaudieri et al. conducted a meta-analysis on cognitive dysfunction in children.[128] Compared to controls, children with T1D scored worse on tasks assessing intelligence, psychomotor/information processing speed, attention/executive function and visual motor integration, with all effect sizes < 0.2. No between-group differences were detected in memory and learning. Similarly, Brands et al. conducted a meta-analysis on cognitive dysfunction in adults.[127] Compared to controls, adults with T1D scored worse on tasks assessing intelligence, psychomotor/information processing speed, attention, cognitive flexibility (executive function) and visual perception, with effect sizes ranging from 0.3 – 0.7. Again, no between-group differences were detected in memory and learning. From comparing these two meta-analyses, one could conclude that the mild cognitive dysfunction detected in childhood appears to progress to moderate dysfunction in young adulthood (most of the study populations included in the Brands et al. analysis ranged from early 20s to late 30s). These are not repeated measures from the same participants so several assumptions are inherent in making such conclusions, but similar results from the few longitudinal cognitive studies also suggest a slow progression of cognitive dysfunction.[66,173,176,217]

This cross-sectional study found that, compared to middle-aged adults without T1D, adults with childhood-onset T1D performed significantly worse on tasks assessing intelligence, executive function, information processing and attention. Effect sizes (see Table 9.2) were even

greater than those reported by Brands et al. in younger adults,[127] ranging from 0.5-0.9. In contrast to findings among children and young adults, this population of T1D participants scored significantly worse than those without T1D in two assessments of memory, with effect sizes > 1.5 (Table 9.2). Chronic hyperglycemia was significantly correlated with cognitive dysfunction, prevalent proliferative retinopathy and distal symmetric polyneuropathy, but only elevated A1c remained significantly associated with cognitive dysfunction in the fully adjusted model.

The prevalence of cognitive dysfunction in this population of middle-aged adults with childhood-onset T1D was twice that observed in adults without T1D. In fact, cognitive dysfunction is as prevalent in this population as mild cognitive dysfunction is in much older individuals (age 70 and older).[190,218] This suggests that advancing age interacts with the effects of T1D on cognitive function, with a gradual worsening over time. While memory was affected, it was only related to the ability to encode and retrieve complex information, not the type of memory deficits associated with Alzheimer's Disease. Improved glycemic control may prevent or delay the development of cognitive dysfunction in people with type 1 diabetes. From a public health perspective, reducing cognitive dysfunction in people with type 1 diabetes should correspond with a reduction in diabetes-related complications and improved self-care.[163] Longitudinal studies including the use of repeated brain imaging measures and precise ascertainment of hyper- and hypoglycemic events are needed to disentangle the complexities of type 1 diabetes-related cognitive dysfunction.

The risk factors and neuroanatomical correlates that underlie T1D-related cognitive dysfunction remain unclear and deserve further investigation. Studying this relationship among

older adults with T1D is particularly important since advancing age also negatively affects cognitive function. Considering the improved life expectancy of people with T1D,[131] research in this area is of public health urgency in order to develop strategies to minimize the development and/or progression of cognitive dysfunction in adults with childhood-onset type 1 diabetes. This is the first study to employ a multi-modal neuroimaging approach to examine T1D-relaetd cognitive dysfunction.

This is the first cognitive study to use ASL to measure cerebral blood flow in T1D participants. It is also the first study to report a relationship between high ABI (>1.3) and cognitive dysfunction as well as between low physical activity and cognitive dysfunction. Utilizing a multi-modal approach to examine the neuroanatomical correlates of cognitive dysfunction can help understand cognitive dysfunction in this middle-aged population with long-term T1D. It also underscores the relevance of examining markers of cerebral blood flow in addition to macrostructural integrity. Longitudinal studies, however, are necessary to determine if these structural changes appeared earlier than expected or whether they have progressed with age. In addition, further studies are needed to investigate the vascular mechanisms underlying these associations and whether interventions aimed at preventing vascular stiffness and increasing physical activity would reduce cognitive dysfunction in people with T1D.

APPENDIX A: BRIEF DESCRIPTIONS AND SCORING CRITERIA FOR NEUROCOGNITIVE TASKS COMMON TO BOTH GROUPS (TYPE 1 DIABETES, NO TYPE 1 DIABETES)

Details of each test administered in the neuropsychological battery.

1. North American Adult Reading Test (NAART) (measure of general intelligence). Maximum score = 61. Participant is asked to read a list of words aloud while the test administrator marks down incorrectly pronounced words. No time limit.
2. Rey Osterrieth Complex Figure (ROCF) copy (measure of visuospatial ability). Maximum score = 36. Test administrator gives participant a pencil and paper. Participant is told to look at a picture and copy it onto the paper as accurately as possible and that they will be asked to draw the same picture again later, from memory. No time limit.
3. Rey Osterrieth Complex Figure (ROCF) immediate recall (measure of memory). Maximum score = 36. As soon as participant finishes the ROCF copy, the administrator hands them a blank sheet of paper, saying "I just asked you to draw a design. The design was up here (pointing to top of page) and you copied it right here (pointing to lower half of page). I want you to do that again." No time limit, no other clues provided.
4. Rey Auditory Verbal Learning Test (RVLT) (measure of memory). Maximum score = 75 on trials 1-5, = 15 on interference trial, = 15 on recall. Test administrator reads a list of 15 words to the participant who then repeats the words back in any order. Administrator

marks down each word recalled correctly. This is repeated four more times for a total of five trials. After the fifth trial, administrator reads a different set of words and asks the participant to repeat back as many as they can remember, in any order, again marking down each correctly recalled word. After this trial, the administrator asks the participant to repeat as many words as they can remember from the first list (trials 1-5), marking down the number correctly repeated by the participant. No time limit on these tests.

5. Digit Symbol Substitution Test (DSST)(measure of psychomotor speed). No maximum score. Test administrator points to the top of the sheet and tells participant "Look at these boxes. Notice that each has a number in the upper part and a mark or symbol in the lower part. Every number has a different mark or symbol". The administrator points to the grid and shows the participant how to fill in the answer sheet by doing three number/symbols, then asks the participant to try. Administrator corrects any errors during the training. Once the participant clearly understands the task, they are told to start at a specific box and to fill out as many squares, in order, as possible until told to stop. Administrator marks the numbers completed at 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 second intervals, although number completed in 90 seconds was used for these analyses.

   After the grid has been filled in, the administrator takes away the key and hands the participant a box with all numbers (1-9) and asks the participant to fill in the matching symbols from memory.

6. Trail Making Test, time limit of 300 seconds for each test. For each test, participant is first shown a brief sample by test administrator.

a. Test A (TMTA)(measure of psychomotor speed) Test administrator has participant draw a line connecting dots, from 1 through 25, in numeric order and as quickly as possible.
b. Test B (TMTB)(measure of executive function). Test administrator has participant draw a line connecting dots, from 1 through 13 and A through L, alternating sequentially between number and letter (1-A-2-B-3-C…13) as quickly as possible.

7. ROCF delayed recall (measure of memory). Maximum score = 36. Test administrator gives participant a sheet of paper and asks them to remember the design they drew earlier and to draw it in the bottom half of the page as neatly and accurately as they can. No time limit.
8. RVLT delayed recall (measure of memory). Maximum score = 15. Test administrator asks participant to remember the list of 15 words read earlier and to repeat back as many as they can in any order. No time limit.
9. Block Design (measure of visuospatial skills). Maximum score = 51. Test administrator shows participant a group of blocks telling them “See these blocks? They are all alike; on some sides they are all red, on some all white and on some they are half red and half white.” After showing the participant how to put the blocks together to make a design and the participant indicates they understand the task, the participant is given a paper with xx different designs and asked to arrange the blocks to match each of these designs. Correct scores are given for completely matching each desired design. Trial is discontinued after three consecutive errors with no time limit.

10. Verbal Fluency (also known as FAS or Controlled Word Association Test)(measure of executive function). No maximum score. Participant is asked to name as many words as they can, excluding proper nouns and numbers, for each of the letters “F”, “A” and “S” then asked to name as many animals as they can. Each trial continues for 60 seconds before the administrator requests the next sequence.
11. Four Word Short Term Memory (4WSTM)(measure of verbal memory). Maximum score = 60. Test administrator reads aloud four unrelated words at the rate of one word per second. This is immediately followed by a three-digit number. As soon as the number is heard, the subject begins counting backwards by threes until the examiner says ‘stop.’ At that point, the subject is asked to recall the words.
12. Stroop Color Word Test (Golden version) (measure of executive function). No maximum raw score. Time limit of 45 seconds for each test component.
    a. Words: Participant is asked to read a list of words down a column (red, green, blue repeated randomly in five columns of 20 rows) as fast as possible, then move to the next column and the next column until time expires. No errors are marked. If the participant makes a mistake, the administrator interjects by saying “wrong” or ‘no” and the participant corrects them self then continues reading.
    b. Colors: A series of colored XXX in red, blue and green appear in five columns of 20 rows. Participants are to read down the columns, naming the color of the XXX, as quickly as possible. No errors are marked. If the participant makes a mistake, the administrator interjects by saying “wrong” or ‘no” and the participant corrects them self then continues reading.

c. Color-Word (sometimes called ‘interference’): The three words “red”, “green” and “blue” are printed in red, green and blue either matching or not matching the word. again in 5 columns of 20 words each. Participants are told to say the COLOR of the ink, not the printed WORD, reading down columns as quickly as possible. No errors are marked. If the participant makes a mistake, the administrator interjects by saying “wrong” or ‘no” and the participant corrects them self then continues reading.

APPENDIX B: NEUROPSYCHOLOGICAL BATTERY MANUAL OF OPERATIONS FOR TYPE 1 DIABETES MRI AND COGNITION STUDY

IMAGING BIOMARKERS OF ACCELERATED BRAIN AGING IN TYPE 1 DIABETES

MANUAL OF OPERATIONS

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09.20.10

Version 1.0 This includes all cognitive tests, instructions, and answer sheets

The order of tests is presented below.

Cognitive Test Battery Session:

Opening Monologue/Introduction to Cognitive Testing Session

North American Reading Test

Story Memory

Rey Complex Figure (Copy + Immediate Recall)

Rey Auditory Verbal Learning Test (Learning trials, interference and short recall)

Digit Symbol Substitution Test (with incidental recall)

Trail Making Test

Letter-Number Sequencing

Block Design Test

Story Memory – Delayed Recall

Rey Complex Figure – Delayed Recall

Rey Auditory Verbal Learning Delayed Recall

Four Word STM Test

Stroop Color Word Test

Verbal and Category Fluency

Grooved Pegboard

Opening Monologue/Introduction to the Cognitive Testing Session

"This session will take about an hour and half. Do you know anything about the tests we'll be doing today? We're going to do a number of tests examining things like attention, memory, eye-hand coordination, and overall mental and motor efficiency. I'm going to be asking you to do a number of different tasks---like matching symbols to numbers, remembering words from a list, and copying a design. You will find some of these tasks fairly easy, while others are quite difficult. The most important thing is for you to try and do your best on all of the tasks. These tasks have a kind of game-like quality to them, and most people find them interesting and challenging. I hope you will too".

Subject ID _____ Start Time: _____ Date _____

"BEFORE WE BEGIN, I'D LIKE TO ASK YOU A FEW GENERAL QUESTIONS."

1. How old are you now? _____ years
2. How far did you go through school? _____ (# years; BA; MA; post graduate)

3. Which hand do you prefer to write with? right ☐; left ☐; both equally well ☐

4. Do you have any problems right now using any of your fingers? Yes☐; No ☐

Left Right

[if yes] Which fingers? L R M I T T I M R L

What's the problem? _____

5. Can you move both wrists freely? Yes ☐; No ☐

[if not] Which one do you have trouble with? Right ☐; Left ☐

What's the problem? _____

6. Do you wear glasses or contact lenses? Yes ☐; No ☐

[if yes] Do you have them with you? Yes ☐; No ☐

7. Do you have any problems with your hearing? Yes ☐; No ☐

[if yes] What's the problem? _____

8. Have you taken any medications or drugs in the past 48 hours? [List drug, approximate quantity, reason for taking drug, and time]

_____

_____

9. Have you had any beer or other alcohol within the past 48 hours?

Beer: No ☐; Yes [when] _____ Quantity _____

Wine: No ☐; Yes [when] _____ Quantity _____

Hard Liquor: No ☐; Yes [when] _____ Quantity _____

10. Have you smoked any cigarettes within the past 48 hours? No ☐; Yes ☐: number ___

11. Have you ever had a head injury where you lost consciousness? No ☐; Yes ☐: If yes, what happened, and when? How long were you 'out'? ≥ 30 mins No ☐; Yes ☐

Has this happened on more than one occasion? Please explain.

_____

_____

_____

Subject ID _____ Date _____

NORTH AMERICAN ADULT

READING TEST (NAART)

This task requires subjects to read a list of 61 irregularly spelled words (debt; gauge; leviathan) and pronounce them. It provides an excellent estimate of premorbid verbal intelligence which is quite resistant to the effects of acquired brain damage. Raw scores can be converted into estimated verbal, performance, and full scale IQ scores [219].

Administration: Hand the subject the page consisting of 3 columns of words. Say "This page contains a list of words that I want you to read out loud, beginning here (point to 'debt') and continuing down this column and on to the next. I must warn you that there are many words that you probably won't recognize; in fact, most people don't know them, so just guess at these. Please read them loudly enough and slowly enough so that I can follow you. OK? Go Ahead!"

NOTE: Put a check by the word if the pronunciation is accurate; if wrong, put an x by the word.

Scoring: Add up the number of INCORRECT responses and use the Conversion Table for Calculation of NAART Score to get the NAART score.

NAART Number INCORRECT words _____

NAART Score _____

Subject ID _____ Date _____

STORY MEMORY – IMMEDIATE RECALL

Story recall is among the most widely used clinical measures of memory. For this study only the first story of the Logical Memory subtest of the WMS-R will be used. The examiner reads the story to the subject in a clear voice. Immediately after hearing the story, the subject is asked to retell the story from memory. The story should be read with adequate volume and clarity for the subject to understand during the presentation. No repetitions are permitted.

Administration:

"Now I am going to read you a little story. Listen carefully, because when I am through, I want you to tell the story back to me, just the way your heard it. Are you ready?"

As this is the first test in the session, the subject may be anxious or nervous. Please make sure the subject is concentrating and ready to attend to the story before you begin reading.

Story should be read (next page) slowly and naturally, and **not** in a monotone, and while the examiner is **looking down** at this page.

"Now tell the story back to me the very best you can."

[Record response verbatim: After the subject is apparently finished, if recall is not complete the examiner should probe, without making eye contact, by asking **"*Anything else*?"** and note (Q).

Then say: **“Later on I will ask you to tell me this story again, so try not to forget it”**

Scoring criteria:

Record the subject’s responses on the response form in a legible and decipherable manner. Scoring is deferred until after the examination is done. The story consists of 25 units. On the score sheet labeled ‘Logical Memory I’ underline or circle each unit that is successfully recalled. Then total the units recalled and enter the number (0-25) in the space provided.

Examples of acceptable responses are provided in the following pages.

Subject ID _____ Date _____

REY COMPLEX FIGURE COPY (with immediate and delay recalls)

Begin by giving the subject the test sheet and a pencil with an eraser.

Administration of Copy: ***"I want you to copy this design. Go ahead and draw it right here. Make it as neat and accurate as you can."*** [and point to the lower section of the answer sheet]. ***"Later on I will ask you to draw it again for me, from memory."*** There is no time limit, and erasing is permitted. If the subject asks "does it have to be perfect," say something to the effect that ***"You should try your best – try to make it look like the one on the page."***

Administration of Immediate Recall: After completion of the Copy, hand subject the answer sheet and point to the lower half of the page and say ***"I just asked you to draw a design. The design was up here*** [point to upper half of page] ***and you copied it right here*** [point to lower half of page]. ***I want you to do it again."*** Do not provide any other cues.

Copy Score _____ (0-36)

Immediate Recall Score _____ (0-36)

Subject ID _____ Date _____

REY AUDITORY VERBAL LEARNING TEST

"I am going to read a list of words. <u>Listen carefully</u>, because after I stop, I want you to say back to me as many words as you can remember. It doesn't matter in what order you repeat them. Just try to remember as many as you can."

Read words at the rate of 1 word / second. Following presentation of the list, ask the subject to recall as many words as they can remember and record them by checking under "recall 1."

Then say, ***"Now I'm going to read the same list again, and once again when I stop I want you to tell me as many words as you can remember, including words you said the first time. It doesn't matter in what order you say them. Just say as many words as you can remember, whether or not you said them before."***

Test Recall again (#2) and repeat for 3 more study/test trials

List A	Recall A1	Recall A2	Recall A3	Recall A4	Recall A5	List A
Dxxx						Dxxx
Cxxxxx						Cxxxxx
Bxxx						Bxxx
Cxxxxx						Cxxxxx
Sxxxx						Sxxxx
Pxxxxx						Pxxxxx
Mxxx						Mxxx
Gxxxxx						Gxxxxx
Hxx						Hxx
Fxxxxx						Fxxxxx
Nxxx						Nxxx
Txxxxx						Txxxxx
Cxxxx						Cxxxx
Hxxxx						Hxxxx
Rxxxx						Rxxxx

TOTAL A1_____ A2_____ A3_____ A4_____ A5_____

Subject ID_____ Date _____

Following Recall 5, say, ***"Now I'm going to read a second list of words. This time, again, you are to say back as many words of this second list as you can remember. Again, the order in which you say the words does not matter. Just try to remember as many as you can."***

List B	Recall B1
Dzzz	
Rzzzzz	
Bzzz	
Szzz	
Szzzz	
Mzzzzzzz	
Gzzzzzz	
Tzzzz	
Czzzz	
Bzzz	
Lzzz	
Gzz	
Pzzzzz	
Czzzzz	
Fzzz	

TOTAL _____

Following the recall of this list, say ***"Now I want you to tell me as many words as you can from the <u>first</u> list that I read to you"*** **(RecallA6)**

List A	Recall A6
Dxxx	
Cxxxxx	
Bxxx	
Cxxxxx	
Sxxxx	
Pxxxxx	
Mxxx	
Gxxxxx	
Hxx	
Fxxxxx	
Nxxx	
Txxxxx	
Cxxxx	
Hxxxx	
Rxxxx	

Total _____

Subject ID _____ Date _____

DIGIT SYMBOL SUBSTITUTION TEST

Administration: Point to the key at the top of the answer sheet and say ***"Look at these boxes. Notice that each has a number in the upper part and a mark or symbol in the lower part. Every number has a different mark or symbol."*** Point to the grid below and say, ***"Here just the numbers are written. I would like you to fill in the symbol that is paired with each number, like this…."*** Demonstrate the first 3 numbers and say, "***Now you try it up to here,"*** while pointing out the heavy dark line separating the sample area from the test items.

Correct any errors made on the sample. When the subject clearly understands the task, point to the "2" and say," ***"Now I want you to begin here [point] and fill in as many squares as you can without skipping any. When you get to the end of the row, just go on to the next row. Ready? Go!"*** If the subject stops at the end of the first line and does not immediately go on to the second line or row, say ***"Go on to the next row."*** If the subject omits any, say, ***"Do them in order"*** or "***Don't skip any.***" If the subject attempts to erase, say, **"Don't erase – just go on!"**

Number of symbols completed within each 30 second interval:

30" _____; 60" _____; 90" _____; 120" _____; 150" _____;

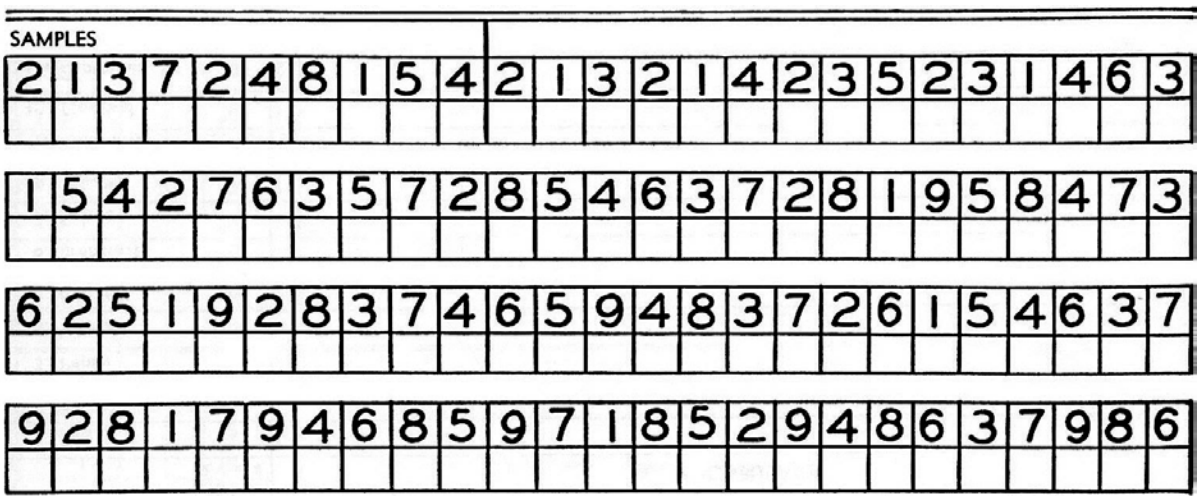
180" _____; 210" _____; 240" _____; 270" _____; 300" _____.

Total time to complete grid: _____ seconds. _____

[At the end of every 30 second interval, mark on grid below the item completed by subject.]

ADMINISTER ENTIRE TASK

Do Not Stop at End of 90 sec!!!



Subject ID _____ Date _____

DIGIT SYMBOL - INCIDENTAL RECALL

Incidental Recall: [Hand subject small grid] and say ***"Now I'd like to see how many of these symbols you can remember."***

1	2	3	4	5	6	7	8	9

Subject ID _____ Date _____

TRAIL MAKING TEST

Trails A – Sample: ***"I want you to draw a line connecting the numbers in order from 1 to 2 to 3 to 4"*** [trace path from number to number] "***and so on, until you reach the end"*** [point]***. "Do it as quickly as you can. Ready? Go."*** Begin timing promptly, watch for errors and correct them immediately.

TIME _____ ERRORS _____

Trails A – Test: ***"Now I want you to do the same thing. this time there are more numbers"*** [hand Trails A sheet to subject] ***"so you would connect 1 to 2 to 3 to 4"*** [very quickly trace path] ***"and so on all the way to the end of 25"*** [point]. ***"Neatness does not count. Remember, work as quickly as you can, and be sure to do the numbers in order. Ready? Go."***

TIME _____ ERRORS _____

Trails B – Sample: ***"This one is a little different. It has both numbers and letters and I want you to alternate – number, letter, number, letter, both in order. So you would start at 1 and draw a line from 1 to A",*** [trace path]**,** ***"from A to 2, from 2 to B, from B to 3, from 3 to C, and so on, until you reach the end"*** [point]. ***"Remember, go number, letter, number, letter as quickly as you can. Ready? Go!"***

TIME _____ ERRORS _____

Trails B – Test: *"Now I want you to do the same thing. This time there are more numbers and letters"* [hand Trails B sheet to subject]. "***Start here at 1 and draw a line from 1 to A, from A to 2, from 2 to B, from B to 3, from 3 to C"*** [trace path], "***and so on until you reach the end at 13"*** [point to 13**].** ***"Remember to do the numbers and letters in order by alternating number, letter, number, letter. Do this as quickly as you can. Ready? Go!"***

TIME _____ ERRORS _____

Correction Cues:

Step 1: ***"Stop! Put your pencil here!"***

Step 2: ***"What comes next?"***

Step 3: ***"After ____ comes ?????"***

Step 4: ***"Remember: number, letter, number, letter. What's next here?"***

_____

DISCONTINUE TESTS

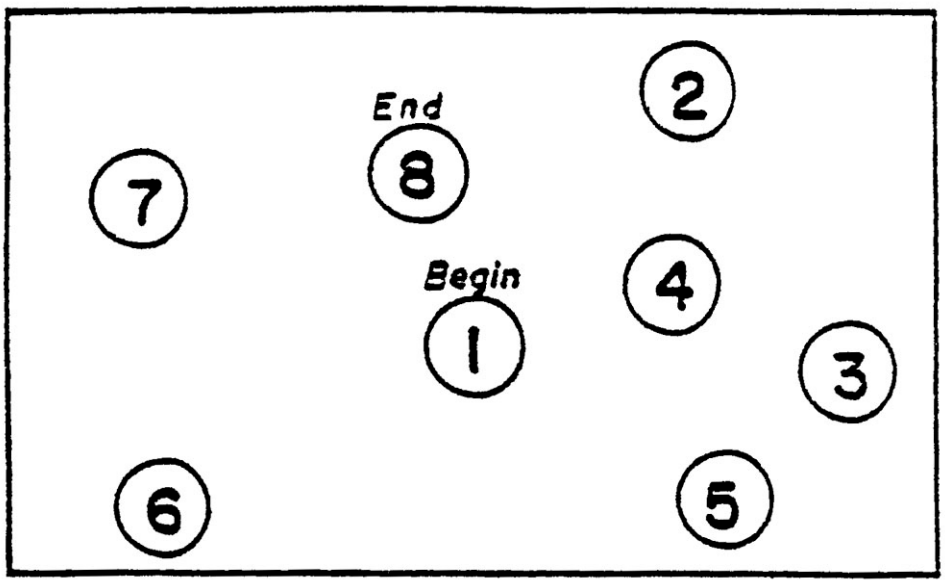
AFTER 300 SECONDS

_____

TRAIL MAKING

Part A

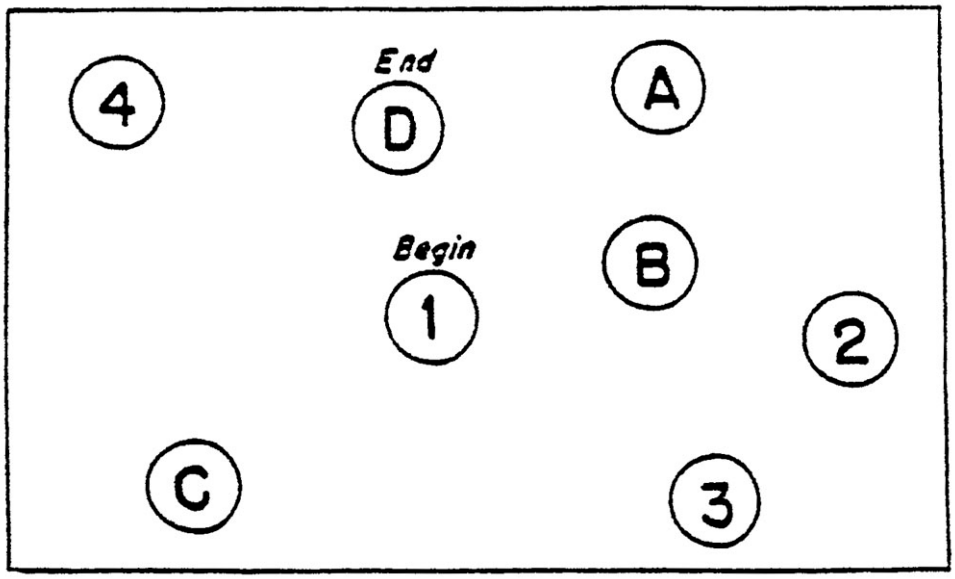
SAMPLE



TRAIL MAKING

Part B

SAMPLE



Subject ID _____ Date _____

LETTER NUMBER SEQUENCING

"I am going to say a group of numbers and letters. After I say them, I want you to tell me the numbers first, in order, starting with the lowest number. Then tell me the letters in alphabetical order. For example, if I say B-7, your answer should be 7-B. The number goes first, then the letter. If I say 9 –C – 3, then your answer should be 3 – 9 – C, the numbers in order first, then the letters in alphabetical order. Let's practice."

For each of the practice strings on answer sheet, first read the string at the rate of 1 number or letter per second, and then pause, waiting for the subject's response. If incorrect, first correct to ensure the subject understands, and then go to the next string. If correct, simply reinforce, and go on to next string.

6 – F	_____	6 – F
G – 4	_____	4 – G
3 – W - 5	_____	3 – 5 – W
T – 7 – L	_____	7 – L – T
1 – J – A	_____	1 – A – J

Following completion of the practice strings, proceed to test item 1. Record all responses on the answer sheet. Administer all 3 trials at each string length.

"Now let's try these…."

Subject ID _____ Date _____

BLOCK DESIGN TEST –WAIS-R

"Now I am going to ask you to make some designs. See these blocks? They are all alike: on some sides they are all red, on some all white, and on some they are half red and half white." (Turn blocks to show the different sides.) ***"I am going to put some blocks together to make a design. Watch me."*** (Make the first design. Give participant four scrambled blocks. Leave your design intact.) ***"Now, make one just like I did here. Tell me when you are finished."*** (If the subject rotates the design at all, correct them before moving on to the next trial. After that, any rotation of 30° or more makes the trial incorrect, even if placement is otherwise correct.)

"This time, I want you to put the blocks together to look like the designs I show you on these pictures. Tell me when you are finished with each one."

Discontinue after any three consecutive errors

12.0 Total Score _____ (0-24)

Subject ID _____ Date _____

STORY MEMORY – DELAY

Administration:

Delayed recall of the story should be about 20 minutes after the initial presentation. If 20 minutes has not elapsed, or if 30 minutes or more has elapsed, continue with the test but record this time on the score sheet.

"A while ago I read you a story. I want you to tell me that story again. As much as you can remember, even if you're not completely sure." [After recall is apparently complete, probe with **"Anything else?"** and note Q.] If the subject cannot remember anything from the story, the examiner should say "It was a story about a woman who was robbed", and indicate the cue on the answer sheet.

"Tell me everything you can about the story"

Scoring:

Follow the scoring criteria for Immediate Recall and record the score at the bottom of the score sheet labeled 'Logical Memory II'.

Subject ID _____ Date _____

DELAYED REY FIGURE

13.0

14.0 Hand subject the answer sheet and point to the lower half of the page and say *"A few minutes ago I asked you to draw a design. The design was up here* **[point to upper half of page]** *and you copied it right here* **[point to lower half of page].** *I want you to draw it again. Make it as neat and as accurate as you can."* **Do not provide any cues, except to say that they just copied it a few minutes ago.**

REY FIGURE DELAYED RECALL SCORE _____ (0-36)

Subject ID _____ Date _____

REY AUDITORY VERBAL LEARNING TEST - DELAY

"A while ago I read a list of words to you several times, and you had to repeat back the words. Tell me the words from that list." ***Do not cue the subject in any way; if asked, you can indicate that it was the first list of words."*** [If asked, subjects do NOT have to recall the words in order.]

List A	Recall A7
Dxxx	
Cxxxxx	
Bxxx	
Cxxxxx	
Sxxxx	
Pxxxxx	
Mxxx	
….	
….	

DELAYED

RECALL TOTAL _____

Subject ID _____ Date _____

STROOP TEST – ANSWER SHEET TASK 1 - WORDS

[Hand subject page 1 – words] ***"This is a test of how fast you can read the words on this page. After I say 'begin' I want you to read <u>down</u> the columns starting with the first one*** [point to the leftmost column] ***and then continue without stopping down the remaining columns in order*** [run hand down remaining columns], ***until I say STOP. If you make a mistake, I will let you know, by saying 'whoops' or 'wrong.' Correct yourself and continue without stopping. Remember to read the words as quickly and as accurately as you can. If you get to the end before I say STOP, go back to the beginning and start over. Ready? Begin!"***

Allow the subject 45 seconds. Mark <u>on your answer sheet</u> (by circling the word), just where they were when time was called.

RED	BLUE	GREEN	RED	BLUE
…	…	…	…	…

Stop after 45 seconds

STROOP WORDS SCORE = _____

Subject ID _____ Date _____

TASK 2 - COLOR

APPENDIX A*"This next task is a little different. Again, I want you to start in column one, but this time you will be identifying the colors red, green, and blue, as quickly as you can. Can you tell these colors apart? O.K. Remember, if you make a mistake, I will tell you. Correct yourself and then continue until I tell you to stop. Ready? Go!"* Begin timing.

BLUE	RED	BLUE	GREEN	RED
---	---	----	----	----

Stop after 45 seconds

STROOP COLOR SCORE = _____

Subject ID _____ Date _____

TASK 3 – COLOR/WORD

["Hand subject page three – Color/Word]. ***"This page is similar to the page you just finished, but this time I want you to name the <u>color of the ink</u> the words are printed in, ignoring the word that is printed. For example*** [point to the first item of the first column], ***this is the first item. What would you say?"***

[If incorrect, examiner should say] ***"No, that is the word that is spelled there. I want you to say the color of the ink the word is printed in. Try this one*** [point to the next item]."

[If correct, say] ***"Good. You will do this page just like the others, starting with the first column [point] and then going on to complete the page. Any questions? Get ready. Go!"***

BLUE	RED	BLUE	GREEN	RED
…	…	…	…	…

15.0 Stop after 45 seconds

STROOP INTERFERENCE SCORE = _____

Subject ID _____ Date _____

FOUR WORD SHORT-TERM MEMORY TEST

This test measures the ability of the subject to hold small amounts of verbal information in memory for several seconds. On each trial, four unrelated words are read to the subject at the rate of one word per second. This is immediately followed by a three-digit number. As soon as the number is heard, the subject begins counting backwards by threes until the examiner says 'stop.' At that point, the subject is asked to recall the words. The purpose of the mental arithmetic task is to prevent the subject from rehearsing the words during the retention interval. Three retention intervals will be used: 5, 15, and 30 seconds.

Administration: this test is introduced by saying, ***"I'm going to read you four words, which I would like you to try to remember. In order to make your task more difficult, however, after I read the fourth word, I'm going to read a 3-digit number, like 100. As soon as I read you that number, I want you to begin counting backwards from it <u>by threes</u> as rapidly and as accurately as you can. I want you to continue doing that until I tell you to stop. When I say STOP, you tell me what the four words are. Do you understand?"***

You should then ask, ***"How good are you at counting backwards by threes? Let's try it. Start from 100."*** Have the subject practice counting from 100 to 70. Correct the subject as necessary, and give encouraging feedback. More than just one counting-practice trial may be necessary; the memory task itself should not be initiated until you are certain that the subject is counting as efficiently as s/he can.

Before beginning the first test trial, you may wish to quickly review the instructions. Thus:

"Before we begin, I want to quickly review what you'll be doing. First, you'll hear 4 words – and I want you to try to remember those. Then you'll hear a number – and I want you to count backwards from it by threes. After a while, I'll tell you to stop, and you'll tell me the words."

Introduce the first test trial by saying, ***"Here are the first four words I want you to try to remember."*** and read them at a rate of 1 word per second. You should not make eye contact with the subject while reading the words or waiting for the subject's response. In reading these words, the pitch of the voice should drop somewhat with the last word in each series. Following the last word, the examiner pauses 2 seconds and says, ***"100…"*** and waits for the subject to begin counting. If the subject fails to begin, you should cue with ***"97…"*** If that fails, it may be necessary to cue again with ***"94…"*** At the end of the 5 second retention interval you should say **"Stop!"** and if the subject does not spontaneously produce the words, ask ***"What were the four words?"***

Following the subject's response, introduce trial 2 by saying ***"Let's try these. Ready?"*** and read the words.

Before beginning trial 4, warn the subject that different three-digit numbers will now be used: ***"Now we will be counting from a number other than 100. Whatever number I say, that's the one you start counting from. Ready?"*** Subsequent trials should be introduced with "Let's try these" or "ready?" or some similar warning.

FOUR WORD SHORT-TERM MEMORY TEST - page 3

It is important to monitor the subject while s/he is counting backwards. Subjects should be counting as quickly as possible (i.e., the rate should be such that you are convinced that they are not rehearsing the words). At the end of a trial you may want to say something like ***"I'd like you to try to count a little faster next time."*** During a trial, you may wish to say something like ***"Faster"*** and move your hands in an emphatic manner. While we ask the subject to count both rapidly and accurately, s/he is not penalized for making occasional counting errors. If many errors are made (often a sign that the subject is merely generating words randomly), you should make a comment to the effect that ***"You should try to count as accurately as you can."***

If no response has been made by the subject 5 seconds or so after you have said "Stop", ask ***"what are the words?"*** If no response is forthcoming, or if the subject reports that s/he doesn't know, that should be noted **(DK)**, and the next trial administered.

If the subject asks whether the words have to be recalled in order, you can say that that is not necessary.

If the subject says that s/he didn't hear one or more words, you should make a note on the answer sheet and say, ***"I'm sorry, I can't repeat the words."***

Similarly, if the subject misses one or more words on a trial and asks something like ***"what word(s) did I miss that time?"*** you cannot provide any information; you can only say something to the effect that ***"I'm sorry; I just can't say."***

This task is extremely difficult and frustrating for many subjects. If it is apparent that the subject is becoming upset or anxious, you have an obligation to reassure the subject that indeed, this is a difficult task and that many people have trouble, but that they should continue and try to do their best.

Recording: Correct responses should be circled, incorrect responses should be written on the line to the right, and the order of all responses indicated with small numbers written immediately above the responses.

Discontinuation: The entire task is given.

Subject ID _____ Date _____

FOUR WORD SHORT- TERM MEMORY TEST

"I'm going to read you four words, which I would like you to try to remember. In order to make your task more difficult, however, after I read the fourth word, I'm going to read a 3-digit number, like 100. As soon as I read you that number, I want you to begin counting backwards by threes as rapidly and as accurately as you can. I want you to continue doing that until I tell you to stop. At that point you'll tell me what the four words are."

"How good are you at counting backwards by threes?" Let's try it. Start from 100." [provide practice]

"Before we begin, I want to quickly review what you'll be doing. First, you'll hear 4 words – and I want you to try to remember those. Then you'll hear a number – and I want you to count backwards from it by threes. After a while, I'll tell you to stop, and you'll tell me the words." "Here are the first four words I want you to try to remember…"

_____

ADMINISTER ALL ITEMS DO NOT SKIP ANY ITEMS

_____

[Circle correct words; write incorrect words on line to right; indicate order of recall with small numbers above each response.] The score is the number of words correctly recalled after the interference.

Subject ID _____ Date _____

GROOVED PEGBOARD

"This is called the grooved pegboard. The purpose of it is to see how quickly and accurately you can work with your hands. Each peg has a 'key' on one side [show subject] ***which will fit into a groove on the hole when the peg is turned the right way"*** [demonstrate by inserting first peg; then remove it].

[Two trials are administered: first dominant, then non-dominant hand.] ***"I want you to do this with your*** _____ [right/left] ***hand."*** [give instructions for appropriate hand. When finished, introduce test with non-dominant hand by saying] ***"Now I want you to use your other hand."***

Right hand instructions: ***"When I say 'go,' begin here*** [point to top left hole from subject's side; sweep hand from left to right] ***and put the pegs in the holes one at a time, as fast as you can, using only your right hand. If you drop a peg and it goes on the floor, just grab another one and keep going. Any questions? Ready? Go!"***

[after all pegs have been inserted] ***"Now I would like you to take the pegs out, one at a time, with your right hand. Work as quickly as you can. Ready? Go."***

Left hand instructions: **"*When I say 'go,' begin here*** [point to top right hole from subject's side; sweep hand from right to left] ***and put the pegs in the holes one at a time, as fast as you can, using only your left hand. Any questions? Ready? Go!"***

[after all pegs have been inserted] ***"Now I would like you to take the pegs out, one at a time, with your left hand. Work as quickly as you can. Ready? Go!"***

In inserting pegs, subjects must pick up the pegs one at a time and should not pig up the first peg until the examiner says ***"go."*** Often a subject will pick up a peg while the examiner is giving the directions and will be holding the peg. Tell subjects that this is not allowed. Also, sometimes subjects begin to insert pegs helter-skelter, rather than in the order (left to right for right hand; reverse for left hand) specified in the instructions. Please encourage them to follow the instructions, perhaps by saying ***"no"*** and waving one's hand across the board in the specified order while saying something like ***"go this way"*** or ***"put the next one here."***

Which hand is dominant? Right ☐; Left ☐

Dominant Hand: Time to insert pegs (sec) _____

Pegs dropped _____

Time to remove pegs _____

<u>Non-Dominant Hand:</u> Time to insert pegs (sec) _____

Pegs dropped _____

Time to remove pegs _____

Subject ID _____ Date _____

VERBAL FLUENCY TEST

This test, also known as the "Controlled Word Association Test," or the "F A S" test, assesses the ability of subjects to spontaneously generate a list of words beginning with designated letters of the alphabet. Numerous clinical studies have found performance on this measure to be affected by frontal lobe dysfunction – particularly in the dominant hemisphere. The response measure is the number of words produced in 60 seconds. Three letters will be used: F, A, and S.

Administration: ***"I would like you to name as many words as you can beginning with the letter 'F.' Leave out proper names (like Fred), names of places (like France), and numbers (like five). OK? I want you to name as many words as fast as you can, till I tell you to stop. Ready? Go!"***

Begin timing promptly and write each word as it is given. 60 seconds is allowed per letter. At the end of 60 seconds say, "***Time's up! Now I would like you to name as many words as you can beginning with the letter _____. Again, no proper names, names of place, or numbers. Ready? Go!"***

Again, record all responses. At the end of 60 seconds, repeat the instructions for the letter 'S.'

If, for any letter, the subject begins by giving words that start with a non-designated letter (e.g., for 'F' the subject begins with a word like 'breakfast'), s/he should be corrected and reminded of the task by repeating the instructions. On the other h and, if the subject is carrying out the task correctly and spontaneously shifts to a different letter (e.g., fish, fin, flounder, cod…), you should not correct the subject, but continue to record all responses.

If the subject repeats words that could be homonyms (e.g., son/sun) yet doesn't indicate to the examiner that they are different words, you should ask – but only after that trial is completed – something like 'do you know you repeated the word 'son'? Hopefully, with this open-ended question the subject will indicate whether it was a true repetition or two different words.

Discontinuation: The entire task is given.

Subject ID _____ Date _____

VERBAL FLUENCY

(F, A, S, and Animals)

[Three 60 second trials are administered. Read the instructions for the letter 'F' and record each response. The instructions are subsequently repeated for the letters 'A' and 'S '. If the subject repeats words which could be homonyms, you should probe ["do you know you repeated _____?"] at the end of that trial.

[For 'F'] ***"I would like you to name as many words as you can beginning with the letter 'F.' Leave out proper names (like Fred), names of places (like France), and numbers (like five). OK? Ready? Go!"***

[For 'A' & 'S'] ***"Time's up! Now I would like you to name as many words as you can beginning with the letter _____. Again, no proper names, names of place, or numbers. Ready? Go!"***

['Animals'] ***"Now I want you to name all the animals you can think of. Ready? Go!"***

"F"	**"A"**	**"S"**	**Animals**
TOTALS F=_____	**A=_____**	**S=_____**	
Animals=_____			

Subject ID _____ Date _____

CLINICAL RATING

Examiner __ __ Date __ __/ __ __/ __ __ Clock Time: _____

1. How willing was this subject to try his or her best?

_____ very willing _____ somewhat willing

_____ not too willing _____ very unwilling

2. How well did this subject understand the instructions?

_____ completely _____ very well _____ fairly well

_____ not too well _____ not well at all

3. How much did this subject want to chat about matters unrelated to the session?

_____ a great deal – unusually talkative _____ quite a bit

_____ a moderate amount _____ not too much _____ very little

4. To what extent were you able to maintain control over the session?

_____ completely _____ quite a bit _____ a moderate amount

_____ not too much _____ very little

5. How smoothly did you and this subject work together?

_____ extremely smoothly – no strain _____ very smoothly

_____ fairly smoothly _____ not too smoothly _____ not smoothly at all

6. In general, what was the pace of the session?

_____ much faster than average _____ somewhat faster than average

_____ about average _____ somewhat slower than average

_____ much slower than average

7. Overall, how much did distractions and interruptions affect the session?

_____ very much _____ much _____ somewhat

_____ little _____ very little

Subject ID _____ Date _____

CLINICAL RATING Page 2

8. To what extent do you feel the information obtained is accurate?

_____ completely _____ mostly _____ moderately

_____ somewhat _____ not very

9. Overall, how would you rate this subject's anxiety level?

_____ not anxious at all

_____ somewhat anxious at the beginning, or only intermittently

_____ somewhat anxious during much of the session

_____ moderately anxious at the beginning, or only intermittently

_____ moderately anxious during much of the session

_____ extremely anxious at the beginning, or only intermittently

_____ extremely anxious during much of the session

10. Overall, how would you rate this subject's level of depression?

_____ not at all depressed

_____ mildly depressed

_____ moderately depressed

_____ severely depressed

11. On which particular tasks did this subject appear most anxious?

_____

12. On which particular tasks did this subject appear most apathetic?

_____

13. On which particular tasks did this subject appear most angry?

_____

14. Which particular tasks did this subject seem to enjoy the most?

_____

15. During debriefing, what concerns did the subject raise?

_____

16. How did you deal with these concerns?

_____

_____

APPENDIX C: EXCLUSION CRITERIA FOR MR HYPER STUDY

a. < 35 or > 60 yrs.

b. Non-english speaking

c. HTN history or taking medications for HTN.

d. Regular night shift.

e. Insulin dependent diabetic.

f. Any cardiac disease, insulin dependent diabetes, seizure disorder, unconscious > 30 mins, amnesia > 24hrs, more than 2 episodes of MTBI, any physical or psychological problem you deal with on a daily basis for 3+ months, chronic liver or kidney disease, treatment for cancer in the past 12 months, serious neuropsychiatric conditions, stroke, multiple sclerosis, brain tumor.

g. Metal in body, refused for an MRI or refused themselves – claustrophobia.

h. Current pregnancy.

i. >30% overweight by Metropolitan Life Insurance table standards

j. Medications – any cardiac medication on a routine basis – exclude and for certain categories on a regular basis: anti-anxiety**, narcotics*, antihistamines*, sleeping pills**, inhaled medications for asthma, COPD (chronic bronchitis,

emphysema) – exclude - */** exceptions are made for those taking less than half of the time, but they must abstain for *= 24hrs and ** = 72 hrs.

BIBLIOGRAPHY

1. Polonsky KS. The past 200 years in diabetes. *New England Journal of Medicine.* 2012;367(14):1332-1340.

2. Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. *Atlanta GA: U.S. Department of Health and Human Serivices.* 2014.

3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2014;37(Supplement 1):S81-S90.

4. Daneman D. Type 1 diabetes. *The Lancet.* 2006;367(9513):847-858.

5. Massi-Benedetti M. Type 1 diabetes, A very special issue. *Diabetes Voice.* 2011;56(Special Issue 2):1-52.

6. Williams AJK, Bingley PJ, Moore WPT, Gale EAM. Islet autoantibodies, nationality and gender: a multinational screening study in first-degree relatives of patients with Type I diabetes. *Diabetologia.* 2002/02/01 2002;45(2):217-223.

7. Gale EAM, Gillespie KM. Diabetes and gender. *Diabetologia.* 2001/01/01 2001;44(1):3-15.

8. Centers for Disease Control and Prevention. National diabetes fact sheet: National estimates and general information on diabetes and prediabetes in the United States. *Atlanta GA: U.S. Department of Health and Human Serivices.* 2011;Centers for Disease Control and Prevention, 2011.

9. Stanescu DE, Lord K, Lipman TH. The epidemiology of type 1 diabetes in children. *Endocrinology and Metabolism Clinics of North America.* 2012;41(4):679-694.

10. Mehers KL, Gillespie KM. The genetic basis for type 1 diabetes. *British Medical Bulletin.* 2008;88(1):115-129.

11. Dean L, McEntyre J. *The Genetic Landscape of Diabetes (Internet)*. Bethesda (MD): National Center for Biotechnology Information; 2004.

12. Winkler C, Lauber C, Adler K, et al. An interferon-inducedhHelicase (IFIH1) gene polymorphism associates with different rates of progression from autoimmunity to type 1 diabetes. *Diabetes.* 2011;60(2):685-690.

13. Bingley PJ, Douek IF, Rogers CA, Gale EAM. *Influence of maternal age at delivery and birth order on risk of type 1 diabetes in childhood: Prospective population based family study.* Vol 3212000.

14. Sanghera DK, Blackett PR. Type 2 diabetes genetics: Beyond GWAS. *J Diabetes Metab.* 2012;3(198).

15. Pan A, Keum N, Okereke OI, et al. Bidirectional association between depression and metabolic syndrome: A systematic review and meta-analysis of epidemiological studies. *Diabetes Care.* 2012;35(5):1171-1180.
16. Tuomilehto J, Lindström J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *New England Journal of Medicine.* 2001;344(18):1343-1350.
17. The Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New England Journal of Medicine.* 2002;346(6):393-403.
18. Uusitupa M, Louheranta A, Lindstrom J, et al. The Finnish Diabetes Prevention study. *British Journal of Nutrition.* 2000;83(Suppl. 1):S137-S142.
19. Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). *Diabetologia.* 2006;49(2):289-297.
20. Turner RC CC, Frighi V, Holman RR, for the U. K. Prospective Diabetes Study Group. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: Progressive requirement for multiple therapies (UKPDS 49). *JAMA.* 1999;281(21):2005-2012.
21. Inagaki N, Atsumi Y, Oura T, Saito H, Imaoka T. Efficacy and Safety Profile of Exenatide Once Weekly Compared With Insulin Once Daily in Japanese Patients With Type 2 Diabetes Treated With Oral Antidiabetes Drug(s): Results From a 26-Week, Randomized, Open-Label, Parallel-Group, Multicenter, Noninferiority Study. *Clinical Therapeutics.* 2012;34(9):1892-1908.e1891.
22. Diamant M, Van Gaal L, Stranks S, et al. Safety and efficacy of once-weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes over 84 weeks. *Diabetes Care.* 2012;35(4):683-689.
23. Bolli GB, Consoli A, Giaccari A. Early insulin treatment in type 2 diabetes: ORIGINal sin or valuable choice as ORIGINal treatment? An open debate on the ORIGIN study results. *Nutrition, Metabolism and Cardiovascular Diseases.* 2012;22(12):1007-1012.
24. Palumbo PJ. The case for insulin treatment early in type 2 diabetes. *Cleveland Clinic Journal of Medicine.* 2004;71(5):385-386.
25. d'Annunzio G, Minuto N, D'Amato E, et al. Wolfram syndrome (diabetes insipidus, diabetes, optic atrophy, and deafness): Clinical and genetic study. *Diabetes Care.* 2008;31(9):1743-1745.
26. Pivonello R, De Leo M, Vitale P, et al. Pathophysiology of diabetes mellitus in Cushing's Syndrome. *Neuroendocrinology.* 2010;92(suppl 1)(Suppl. 1):77-81.
27. Desbois-Mouthon C, Magré J, Amselem S, et al. Lipoatrophic diabetes: genetic exclusion of the insulin receptor gene. *Journal of Clinical Endocrinology & Metabolism.* 1995;80(1):314-319.
28. Sjoberg RJ, Kidd GS. Pancreatic diabetes mellitus. *Diabetes Care.* 1989;12(10):715-724.
29. Moran A, Brunzell C, Cohen RC, et al. Clinical care guidelines for cystic fibrosis–related diabetes: A position statement of the American Diabetes Association and a clinical

practice guideline of the Cystic Fibrosis Foundation, endorsed by the Pediatric Endocrine Society. *Diabetes Care.* 2010;33(12):2697-2708.

30. Chastain MA. The Glucagonoma Syndrome: A review of its features and discussion of new perspectives. *The American Journal of the Medical Sciences.* 2001;321(5):306-320.

31. Nakhla M, Polychronakos C. Monogenic and other unusual causes of diabetes mellitus. *Pediatric Clinics of North America.* 2005;52(6):1637-1650.

32. Fowler MJ. Microvascular and macrovascular complications of diabetes. *Clinical Diabetes.* 2008;26(2):77-82.

33. Fong DS, Aiello L, Gardner TW, et al. Retinopathy in diabetes. *Diabetes Care.* 2004;27(suppl 1):s84-s87.

34. Crawford TN, III DVA, Kerrison JB, Jablon EP. Diabetic retinopathy and angiogenesis. *Current Diabetes Reviews.* 2009;5:8-13.

35. American Diabetes Association. Nephropathy in diabetes. *Diabetes Care.* 2004;27(suppl 1):s79-s83.

36. Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? *Diabetologia.* 2008;51(5):714-725.

37. Boulton AJM, Vinik AI, Arezzo JC, et al. Diabetic neuropathies: A statement by the American Diabetes Association. *Diabetes Care.* 2005;28(4):956-962.

38. Bate KL, Jerums G. 3: Preventing complications of diabetes. *Med J Aust.* 2003;179(9):498-503.

39. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ.* 1998;317(7160):703-713.

40. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Phys Ther.* 2008;88(11):1322-1335.

41. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *New England Journal of Medicine.* 1998;339(4):229-234.

42. Conway B, Costacou T, Orchard T. Is glycaemia or insulin dose the stronger risk factor for coronary artery disease in type 1 diabetes? *Diabetes and Vascular Disease Research.* 2009;6(4):223-230.

43. Rohr J, Kittner S, Feeser B, et al. Traditional risk factors and ischemic stroke in young adults: The Baltimore-Washington Cooperative Young Stroke study. *Archives of Neurology.* 1996;53(7):603-607.

44. Lüscher TF, Creager MA, Beckman JA, Cosentino F. Diabetes and vascular disease: Pathophysiology, clinical consequences and medical therapy: Part II. *Circulation.* 2003;108(13):1655-1661.

45. Bate KL, Jerums G. Preventing complications of diabetes. *The Medical Journal of Australia.* 2003;179(9):498-503.

46. Krzymien J, Karnafel W. Lactic acidosis in patients with diabetes. *Pol Arch Med Wewn.* 2013;123(3):91-97.

47. Seaquist ER, Anderson J, Childs B, et al. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. *Diabetes Care.* 2013;36(5):1384-1395.

48. Jacobson AM, Musen G, Ryan CM, et al. Long term effect of diabetes and its treatment on cognitive function. *New England Journal of Medicine.* 2007;356:1842-1852.
49. Brands AMA, Kessels RPC, de Haan EHF, Kappelle LJ, Biessels GJ. Cerebral dysfunction in type 1 diabetes: Effects of insulin, vascular risk factors and blood-glucose levels. *European Journal of Pharmacology.* 2004;490(1–3):159-168.
50. Rosano C, Aizenstein HJ, Studenski S, Newman AB. A regions-of-interest volumetric analysis of mobility limitations in community-dwelling older adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences.* 2007;62(9):1048-1055.
51. Maillet D, Rajah MN. Association between prefrontal activity and volume change in prefrontal and medial temporal lobes in aging and dementia: A review. *Ageing Research Reviews.* 2013;12(2):479-489.
52. Rosano C, Becker J, Lopez O, et al. Morphometric analysis of gray matter volume in demented older adults: Exploratory analysis of the Cardiovascular Health Study brain MRI database. *Neuroepidemiology.* 2005;24(4):221-229.
53. Ersche KD, Williams GB, Robbins TW, Bullmore ET. Meta-analysis of structural brain abnormalities associated with stimulant drug dependence and neuroimaging of addiction vulnerability and resilience. *Current Opinion in Neurobiology.* 2013;23(4):615-624.
54. Lopez-Garcia P, Aizenstein HJ, Snitz BE, Walter RP, Carter CS. Automated ROI-based brain parcellation analysis of frontal and temporal brain volumes in schizophrenia. *Psychiatry Research: Neuroimaging.* 2006;147(2–3):153-161.
55. Schutzer S, Angel T, Liu T, Schempoes A, Xie F, al. e. Gray matter is targeted in first-attack multiple sclerosis. *PloS one.* 2013;8(9):e66117.
56. Hägg S, Thorn LM, Putaala J, et al. Incidence of stroke according to presence of diabetic nephropathy and severe diabetic retinopathy in patients with type 1 diabetes. *Diabetes Care.* 2013;published ahead of print.
57. Brown WR, Thore CR. Review: Cerebral microvascular patholoy in aging and neurodegeneration. *Neuropathol Appl Neurobiol.* 2011;37(1):56-74.
58. Pantoni L. Cerebral small vessel disease: From pathogenesis and clnical characteristics to therapeutic challenges. *Lancet Neurol.* 2010;9:689-701.
59. Bakker W, Eringa EC, Sipkema P, van Hinsbergh VW. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. *Cell Tissue Res.* 2009;335(1):165-189.
60. Bennett L, Steffany A, Tenniswood M, et al. Chronic cerebral hypoperfusion elicits neuronal apoptosis and behavioral impairment. *Neuroreport.* 1998;9(1):161-166.
61. Gouw AA, Seewann A, van der Flier WM, et al. Heterogeneity of small vessel disease: A systematic review of MRI and histopathology correlations. *Journal of Neurology, Neurosurgery & Psychiatry.* 2011;82(2):126-135.
62. Pantoni L, Garcia JH. Pathogenesis of leukoaraisosi, A review. *Stroke.* 1997;28:652-659.
63. Perantie DC, Koller JM, Weaver PM, et al. Prospectively determined impact of type 1 diabetes on brain volume during development. *Diabetes.* 2011;60(11):3006-3014.
64. Wessels AM, Simsek S, Remijnse PL, et al. Voxel-based morphometry demonstrates reduced grey matter density on brain MRI in patients with diabetic retinopathy. *Diabetologia.* 2006;49(10):2474-2480.

65. Musen G, Lyoo KL, Sparks CR, al. E. Evidence for reduced gray matter density in patients with type 1 diabetes as measured by magnetic resonance imaging. *Diabetes.* 2004;2004(52 (Suppl. 2)):A57-A58.

66. Northam EA, Rankins D, Lin A, et al. Central nervous system function in youth with type 1 diabetes 12 years after disease onset. *Diabetes Care.* 2009;32(3):445-450.

67. van Elderen SGC, Brandts A, van der Grond J, et al. Cerebral perfusion and aortic stiffness are independent predictors of white matter brain atrophy in type 1 diabetic patients assessed with magnetic resonance imaging. *Diabetes Care.* 2011;34(2):459-463.

68. Wessels AM, Rombouts SARB, Remijnse PL, et al. Cognitive performance in type 1 diabetes patients is associated with cerebral white matter volume. *Diabetologia.* 2007;50(8):1763-1769.

69. Wu M, Rosano C, Butters M, et al. A fully automated method for quantifying and localizing white matter hyperintensities on MR images. *Psychiatry Research: Neuroimaging.* 2006;148(2-3):133-142.

70. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: Systematic review and meta-analysis. *BMJ.* 2010;341.

71. Firbank MJ, Wiseman RM, Burton EJ, Saxby BK, O'Brien JT, Ford GA. Brain atrophy and white matter hyperintensity change in older adults and relationship to blood pressure. Brain atrophy, WMH change and blood pressure. *J Neurol.* 2007;254(6):713-721.

72. Ovbiagele B, Saver JL. Cerebral white matter hyperintensities on MRI: Current concepts and therapeutic implications. *Cerebrovascular Diseases.* 2006;22(2-3):83-90.

73. Gunning-Dixon FM, Brickman AM, Cheng JC, Alexopoulos GS. Aging of cerebral white matter: a review of MRI findings. *International Journal of Geriatric Psychiatry.* 2009;24(2):109-117.

74. Baloh RW, Vinters HV. White matter lesions and disequilibrium in older people: II. Clinicopathologic correlation. *Archives of Neurology.* 1995;52(10):975-981.

75. de Leeuw FE, de Groot JC, Achten E, et al. Prevalence of cerebral white matter lesions in elderly people: A population based magnetic resonance imaging study. The Rotterdam Scan Study. *Journal of Neurology, Neurosurgery & Psychiatry.* 2001;70(1):9-14.

76. Awad IA, Johnson PC, Spetzler RF, Hodak JA. Incidental subcortical lesions identified on magnetic resonance imaging in the elderly. II. Postmortem pathological correlations. *Stroke.* 1986;17(6):1090-1097.

77. Rostrup E, Gouw AA, Vrenken H, et al. The spatial distribution of age-related white matter changes as a funciton of vascular risk factors - Results from the LADIS study. *NeuroImage.* 2012;60(3):1597-1607.

78. Smith EE, Salat DH, Jeng J, et al. Correlations between MRI white matter lesion location and executive function and episodic memory. *Neurology.* 2011;76:1492-1499.

79. Rosano C, Chang Y-F, Kuller LH, et al. Long-term survival in adults 65 years and older with white matter hperintensity: Association with performance on the Digit Symbol Substitution Test. *Psychosomatic Medicine.* 2013;75(7):624-631.

80. DeCarli C, Murphy DG, Tranh M, et al. The effect of white matter hyperintensity volume on brain structure, cognitive performance, and cerebral metabolism of glucose in 51 healthy adults. *Neurology.* 1995;45(11):2077-2084.

81. Rossi R, Boccardi M, Sabattoli F, et al. Topographic correspondence between white matter hyperintensities and brain atrophy. *J Neurol.* 2006;253(7):919-927.
82. Wen W, Sachdev PS, Li JJ, Chen X, Anstey KJ. White matter hyperintensities in the forties: Their prevalence and topography in an epidemiological sample aged 44–48. *Human Brain Mapping.* 2009;30(4):1155-1167.
83. Le Bihan D, Mangin J-F, Poupon C, et al. Diffusion tensor imaging: Concepts and applications. *Journal of Magnetic Resonance Imaging.* 2001;13(4):534-546.
84. Alexander A, Lee J, Lazar M, Field A. Diffusion tensor imaging of the brain. *Neurotherapeutics.* 2007;4(3):316-329.
85. Wedeen VJ, Wang RP, Schmahmann JD, et al. Diffusion spectrum magnetic resonance imaging (DSI) tractography of crossing fibers. *NeuroImage.* 2008;41(4):1267-1277.
86. Chung H-W, Chou M-C, Chen C-Y. Principles and limitations of computational algorithms in clinical Diffusion Tensor MR Tractography. *American Journal of Neuroradiology.* 2011;32(1):3-13.
87. Roine T, Jeurissen B, Perrone D, et al. Isotropic non-white matter partial volume effects in constrained spherical deconvolution. *Frontiers in neuroinformatics.* 2014;8:28.
88. Le Bihan D, Poupon C, Amadon A, Lethimonnier F. Artifacts and pitfalls in diffusion MRI. *Journal of Magnetic Resonance Imaging.* 2006;24(3):478-488.
89. Baron CA, Beaulieu C. Acquisition strategy to reduce cerebrospinal fluid partial volume effects for improved DTI tractography. *Magnetic Resonance in Medicine.* 2014:e-pub ahead of print.
90. Dejgaard A, Gade A, Larsson H, Balle V, Parving A, Parving HH. Evidence for diabetic encephalopathy. *Diabetes Medicine.* 1991;8(2):162-167.
91. Perros P, Deary I, Sellar R, Best J, Frier BM. Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia. *Diabetes Care.* 1997;20(6):1013-1018.
92. Brands AMA, Kessels RPC, Hoogma RPLM, et al. Cognitive performance, psychological well-being, and brain magnetic resonance imaging in older patients with type 1 diabetes. *Diabetes.* 2006;55(6):1800-1806.
93. Ferguson SC, Blane A, Perros P, et al. Cognitive ability and brain structure in type 1 diabetes: Relation to microangiopathy and preceding severe hypoglycemia. *Diabetes.* 2003;52(1):149-156.
94. Ferguson SC, Blane A, Wardlaw J, et al. Influence of an early-onset age of type 1 diabetes on cerebral structure and cognitive function. *Diabetes Care.* 2005;28(6):1431-1437.
95. Weinger K, Jacobson AM, Musen G, et al. The effects of type 1 diabetes on cerebral white matter. *Diabetologia.* 2007;51(3):417-425.
96. Lobnig BM, Kromeke O, Optenhostert-Porst C, Wolf OT. Hippocampal volume and cognitive performance in long-standing type 1 diabetic patients without macrovascular complications. *Diabetic Medicine.* 2006;23(1):32-39.
97. Yousem D, Tasman W, Grossman RI. Proliferative retinopathy: Absence of white matter lesions at MR imaging. *Radiology.* 1991;179:229-230.

98. Brands AMA, Biessels GJ, Kappelle LJ, et al. Cognitive functioning and brain MRI in patients with type 1 and type 2 diabetes mellitus: A comparative study. *Dementia and Geriatric Cognitive Disorders.* 2007;23(5):343-350.
99. Kodl CT, Franc DT, Rao JP, et al. Diffusion tensor imaging identifies deficits in white matter microstructure in subjects with type 1 diabetes that correlate with reduced neurocognitive function. *Diabetes.* 2008;57(11):3083-3089.
100. Franc DT, Kodl CT, Mueller BA, Muetzel RL, Lim KO, Seaquist ER. High connectivity between reduced cortical thickness and disrupted white matter tracts in long-standing type 1 diabetes. *Diabetes.* 2011;60(1):315-319.
101. Antenor-Dorsey JAV, Meyer E, Rutlin J, et al. White matter microstructural integrity in youth with type 1 diabetes. *Diabetes.* 2013;62(2):581-589.
102. Ge Y, Grossman RI, Babb JS, Rabin ML, Mannon LJ, Kolson DL. Age-related total gray matter and white matter changes in normal adult brain. Part I: Volumetric MR imaging analysis. *American Journal of Neuroradiology.* 2002;23(8):1327-1333.
103. Inglese M, Oesingmann N, Casaccia P, Fleysher L. Progressive multiple sclerosis and gray matter pathology: An MRI perspective. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine.* 2011;78(2):258-267.
104. Salerno JA, Murphy DG, Horwitz B, et al. Brain atrophy in hypertension. A volumetric magnetic resonance imaging study. *Hypertension.* 1992;20(3):340-348.
105. Schmidt-Wilcke T, Gänßbauer S, Neuner T, Bogdahn U, May A. Subtle grey matter changes between migraine patients and healthy controls. *Cephalalgia.* 2008;28(1):1-4.
106. Ho MS, Weller NJ, Ives FJ, et al. Prevalence of structural central nervous system abnormalities in early-onset type 1 diabetes mellitus. *The Journal of Pediatrics.* 2008;153(3):385-390.
107. Aye T, Reiss AL, Kesler S, et al. The feasibility of detecting neuropsychologic and neuroanatomic effects of T1D in young children. *Diabetes Care.* 2011;34:1458-1462.
108. Hughes TM, Ryan CM, Aizenstein HJ, et al. Frontal gray matter atrophy in middle aged adults with type 1 diabetes is independent of cardiovascular risk factors and diabetes complications. *Journal of Diabetes and its Complications.* 2013;27(6):558-564.
109. Hershey T, Perantie DC, Wu J, Weaver PM, Black KJ, White NH. Hippocampal volumes in youth with type 1 diabetes. *Diabetes.* 2010;59(1):236-241.
110. Perantie DC, Wu J, Koller JM, et al. Regional brain volume differences associated with hyperglycemia and severe hypoglycemia in youth with type 1 diabetes. *Diabetes Care.* 2007;30(9):2331-2337.
111. Deibler AR, Pollock JM, Kraft RA, Tan H, Burdette JH, Maldjian JA. Arterial spin-labeling in routine clinical practice, part 1: Technique and artifacts. *American Journal of Neuroradiology.* August 1, 2008 2008;29(7):1228-1234.
112. Zaharchuk G. Arterial spin–labeled perfusion imaging in acute ischemic stroke. *Stroke.* 2014;45(4):1202-1207.
113. Mangia S, Tesfaye N, De Martino F, et al. Hypoglycemia-induced increases in thalamic cerebral blood flow are blunted in subjects with type 1 diabetes and hypoglycemia unawareness. *J Cereb Blood Flow Metab.* 2012;32(11):2084-2090.
114. de la Monte SM, J.R. W. Alzheimer's disease is type 3 diabetes - Evidence reviewed. *Journal of Diabetes Science and Technology.* 2008;2(6):1101-1113.

115. Vagelatos NT, Eslick GD. Type 2 diabetes as a risk factor for Alzheimer's Disease: The confounders, interactions, and neuropathology associated with this relationship. *Epidemiologic Reviews.* 2013;35(1):152-160.
116. McIntyre R, Kenna H, Nguyen H, et al. Brain volume abnormalities and neurocognitive deficits in diabetes mellitus: Points of pathophysiological commonality with mood disorders? *Adv Therapy.* 2010;27(2):63-80.
117. Xu W, Qiu C, Gatz M, Pedersen NL, Johansson B, Fratiglioni L. Mid- and late-life diabetes in relation to the risk of dementia: A population-based twin study. *Diabetes.* 2009;58(1):71-77.
118. Northam EA, Lin A. Hypoglycaemia in childhood onset type 1 diabetes--part villain, but not the only one. *Pediatr Diabetes.* 2010;11(2):134-141.
119. Alvarez EO, Beauquia J, Revsin Y, et al. Cognitive dysfunction and hippocampal change in experimental type 1 diabetes. *Behavioural Brain Research.* 2009;198:224-230.
120. Beauquis J, Saravia F, Coulaud J, et al. Prominently decreased hippocampal neurogenesis in a spontaneous model of type 1 diabetes, the nonobese diabetic mouse. *Exp Neurol.* 2008;210(2):359-367.
121. Christman AL, Vannorsdall TD, Pearlson GD, Hill-Briggs F, Schretlen DJ. Cranial volume, mild cognitive deficits, and functional limitations associated with diabetes in a community sample. *Archives of Clinical Neuropsychology.* 2010;25(1):49-59.
122. Asvold BO, Sand T, Hestad K, Bjorgaas MR. Cognitive function in type 1 diabetic adults with early exposure to severe hypoglycemia; a 16-year follow-up study. *Diabetes Care.* 2010;33(9):1945-1947.
123. Ba-Tin L, Strike P, Tabet N. Diabetic peripheral microvascular complications: relationship to cognitive function. *Cardiovasc Psychiatry Neurol.* 2011;2011:723434.
124. van Duinkerken E, Klein M, Schoonenboom NSM, et al. Functional brain connectivity and neurocognitive functioning in patients with long-standing type 1 diabetes with and without microvascular complications: A magnetoencephalography study. *Diabetes.* 2009;58(10):2335-2343.
125. van Duinkerken E, Schoonheim MM, Ijzerman RG, et al. Diffusion tensor imaging in type 1 diabetes: decreased white matter integrity relates to cognitive functions. *Diabetologia.* Apr 2012;55(4):1218-1220.
126. Brismar T, Maurex L, Cooray G, et al. Predictors of cognitive impairment in type 1 diabetes. *Psychoneuroendocrinology.* 2007;32(8-10):1041-1051.
127. Brands AMA, Biessels GJ, de Haan EHF, Kappelle LJ, Kessels RPC. The effects of type 1 diabetes on cognitive performance: A meta-analysis. *Diabetes Care.* 2005;28(3):726-735.
128. Gaudieri PA, Chen R, Greer TF, Holmes CS. Cognitive function in children with type 1 diabetes: A meta-analysis. *Diabetes Care.* 2008;31(9):1892-1897.
129. Strachan MWJ, Frier BM, Deary IJ. Cognitive assessment in diabetes: the need for consensus. *Diabetic Medicine.* 1997;14(6):421-422.
130. Boyko EJ. Progress in the estimation of mortality due to diabetes. *Diabetes Care.* 2005;28(9):2320-2321.
131. Miller RG, Secrest AM, Sharma RK, Songer TJ, Orchard TJ. Improvements in the life expectancy of type 1 diabetes: The Pittsburgh Epidemiology of Diabetes Complications Study cohort. *Diabetes.* 2012;61(11):2987-2992.

132. Jennings JR, Heim AF, Kuan DC-H, Gianaros PJ, Muldoon MF, Manuck SB. Use of total cerebral blood flow as an imaging biomarker of known cardiovascular risks. *Stroke.* 2013;44(9):2480-2485.
133. Jongen C, Biessels GJ. Structural brain imaging in diabetes: a methodological perspective. *European Journal of Pharmacology.* 2008;585(1):208-218.
134. Aizenstein HJ, Andreescu C, Edelman KL, et al. fMRI correlates of whiter matter hyperintensities in late-life depression. *American Journal of Psychiatry.* 2011;168(10):1075-1082.
135. Zheng JJJ, Delbaere K, Close JCT, et al. White matter hyperintensities are an independent predictor of physical decline in community-dwelling older people. *Gerontology.* 2012;58(5):398-406.
136. Dufouil C, Godin O, Chalmers J, et al. Severe cerebral white matter hyperintensities predict severe cognitive decline in patients with cerebrovascular disease history. *Stroke.* 2009;40(6):2219-2221.
137. Rosano C, Kuller LH, Chung H, Arnold AM, Longstreth WT, Jr., Newman AB. Subclinical brain magnetic resonance imaging abnormalities predict physical functional decline in high-functioning older adults. *J Am Geriatr Soc.* Apr 2005;53(4):649-654.
138. Jacobs HIL, Leritz EC, Williams VJ, et al. Association between white matter microstructure, executive functions, and processing speed in older adults: The impact of vascular health. *Human Brain Mapping.* 2013;34(1):77-95.
139. Jeerakathil T, Wolf PA, Beiser A, et al. Stroke risk profile predicts white matter hyperintensity volume: The Framingham Study. *Stroke.* 2004;35(8):1857-1861.
140. Tseng BY, Gundapuneedi T, Khan MA, et al. White matter integrity in physically fit older adults. *NeuroImage.* 2013;82(0):510-516.
141. Lipman TH, Levitt Katz LE, Ratcliffe SJ, et al. Increasing incidence of type 1 diabetes in youth: Twenty years of the Philadelphia Pediatric Diabetes Registry. *Diabetes Care.* 2013.
142. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes.* 2000;49(4):626-632.
143. Orchard TJ, Forrest KY, Ellis D, Becker DJ. Cumulative glycemic exposure and microvascular complications in insulin-dependent diabetes mellitus. The glycemic threshold revisited. *Archives of internal medicine.* Sep 8 1997;157(16):1851-1856.
144. Conway BN, Aroda VR, Maynard JD, et al. Skin intrinsic fluorescence is associated with coronary artery disease in individuals with long duration of type 1 diabetes. *Diabetes Care.* 2012;35(11):2331-2336.
145. Early treatment of diabetic retinopathy study coordinating center: Manual of operations. Chapters 5, 18. Baltimore: Univ. of Maryland Scool of Medicine; 1980.
146. Austin P, Escobar M, Kopec J. The use of the Tobit model for analyzing measures of health status. *Qual Life Res.* 2000;9(8):901-910.
147. Imperati D, Colcombe S, Kelly C, et al. Differential development of human brain white matter tracts. *PLoS One.* 2011;6(8):e23437.
148. Ladouceur CD, Peper JS, Crone EA, Dahl RE. White matter development in adolescence: the influence of puberty and implications for affective disorders. *Dev Cogn Neurosci.* 2012;2(1):36-54.

149. Lebel C, Gee M, Camicioli R, Wieler M, Martin W, Beaulieu C. Diffusion tensor imaging of white matter tract evolution over the lifespan. *NeuroImage.* 2012;60(1):340-352.
150. Gao FQ, Swartz RH, Scheltens P, et al. Complexity of MRI white matter hyperintensity assessments in relation to cognition in aging and dementia from the Sunnybrook Dementia Study. *J Alzheimers Dis.* 2011;26 Suppl 3:379-388.
151. Olsson E, Klasson N, Berge J, et al. White matter lesion assessment in patients with cognitive impairment and healthy controls: Reliability comparisons between visual rating, a manual, and an automatic volumetrical MRI method-The Gothenburg MCI Study. *J Aging Res.* 2013;2013:198471.
152. van Straaten EC, Fazekas F, Rostrup E, et al. Impact of white matter hyperintensities scoring method on correlations with clinical data: the LADIS study. *Stroke.* 2006;37(3):836-840.
153. Verzijl N, DeGroot J, Thorpe SR, et al. Effect ofcollagen turnover on the accumulation of advanced glycation end products. *Journal of Biological Chemistry.* 2000;275(50):39027-39031.
154. Hofmann B, Adam A-C, Jacobs K, et al. Advanced glycation end product associated skin autofluorescence: A mirror of vascular function? *Experimental Gerontology.* 2013;48(1):38-44.
155. Murray AD, Staff RT, Shenkin SD, Deary IJ, Starr JM, Whalley LJ. Brain white matter hyperintensities: relative importance of vascular risk factors in nondemented elderly people. *Radiology.* 2005;237(1):251-257.
156. Jorm A, Anstey K, Christensen H, et al. MRI hyperintensities and depressive symptoms in a community sample of individuals 60-64 years old. *Am J Psychiatry.* 2005;162:699-704.
157. Soderlund H, Nyberg L, Adolfsson R, Nilsson G, Launer LJ. High prevalence of white matter hyperintensities in normal aging: relation to blood pressure and cognition. *Cortex.* 2003;39(4-5):1093-1105.
158. Rosano C, Kuller LH, Chung H, Arnold AM, Longstreth WT, Newman AB. Subclinical brain magnetic resonance imaging abnormalities predict physical functional decline in high-functioning older adults. *Journal of the American Geriatrics Society.* 2005;53(4):649-654.
159. Kuller LH, Arnold AM, Longstreth WT, Jr., et al. White matter grade and ventricular volume on brain MRI as markers of longevity in the cardiovascular health study. *Neurobiol Aging.* Sep 2007;28(9):1307-1315.
160. Longstreth WT, Jr., Dulberg C, Manolio TA, et al. Incidence, manifestations, and predictors of brain infarcts defined by serial cranial magnetic resonance imaging in the elderly: the Cardiovascular Health Study. *Stroke.* Oct 2002;33(10):2376-2382.
161. Yang C, DeVisser A, Martinez JA, et al. Differential impact of diabetes and hypertension in the brain: adverse effects in white matter. *Neurobiol Dis.* Jun 2011;42(3):446-458.
162. Yan H, Rivkees SA. Hypoglycemia influences oligodendrocyte development and myelin formation. *NeuroReport.* 2006;17(1):55-59.
163. Brands AMA, Kessels RPC, Ryan CM. Cognition in Adults with Type 1 Diabetes. In: Biessels GJ, Luchsinger JA, eds. *Contemporary Diabetes: Diabetes and the Brain*: Humana Press of Springer Science+Business Media LLC; 2009:277-293.
164. Sima AA. Encephalopathies: the emerging diabetic complications. *Acta Diabetol.* 2010;47(4):279-293.

165. McCrimmon RJ, Ryan CM, Frier BM. Diabetes and cognitive dysfunction. *The Lancet.* 2012;379(9833):2291-2299.
166. Lopez OL, Jagust WJ, DeKosky ST, et al. Prevalence and classification of mild cognitive impairment in the Cardiovascular Health Study Cognition Study: Part 1. *Archives of Neurology.* 2003;60(10):1385-1389.
167. Deary IJ, Corley J, Gow AJ, et al. Age-associated cognitive decline. *British Medical Bulletin.* 2009;92(1):135-152.
168. Miller RG, Secrest AM, Sharma RK, Songer TJ, Orchard TJ. Improvements in the life expectancy of type 1 diabetes: The Pittsburgh Epidemiology of Diabetes Complications Study cohort. *Diabetes.* 2012.
169. Lopez OL, Jagust WJ, Dulberg C, et al. Risk factors for mild cognitive impairment in the Cardiovascular Health Study Cognition Study: Part 2. *Archives of Neurology.* 2003;60(10):1394-1399.
170. Teng E, Tassniyom K, Lu PH. Reduced quality-of-life ratings in mild cognitive impairment: Analyses of subject and informant responses. *Am J Geriatr Psychiatry.* 2012;20(12):1016-1025.
171. Stefanacci RG. The costs of Alzheimer's disease and the value of effective therapies. *Am J Manag Care.* 2011;17(Suppl 13):S356-362.
172. Wessels AM, Scheltens P, Barkhof F, Heine RJ. Hyperglycaemia as a determinant of cognitive decline in patients with type 1 diabetes. *European Journal of Pharmacology.* 2008;585(1):88-96.
173. Jacobson AM, Ryan CM, Cleary PA, et al. Biomedical risk factors for decreased cognitive functioning in type 1 diabetes: an 18 year follow-up of the Diabetes Control and Complications Trial (DCCT) cohort. *Diabetologia.* Feb 2011;54(2):245-255.
174. Frier BM. Cognitive functioning in type 1 diabetes: The Diabetes Control and Complications Trial (DCCT) revisited. *Diabetologia.* 2011;54:233-236.
175. Biessels GJ, Deary IJ, Ryan CM. Cognition and diabetes: a lifespan perspective. *The Lancet Neurology.* 2008;7(2):184-190.
176. Ryan CM, Geckle MO, Orchard TJ. Cognitive efficiency declines over time in adults with type 1 diabetes: effects of micro- and macrovascular complications. *Diabetologia.* 2003;46(7):940-948.
177. Northam EA, Rankins D, Cameron FJ. Therapy insight: the impact of type 1 diabetes on brain development and function. *Nat Clin Pract Neuro.* 2006;2(2):78-86.
178. Lee I-M, Paffenbarger RS. Associations of light, moderate and vigorous intensity physical activity with longevity, the Harvard Alumni Health Study. *Am J Epidemiology.* 2000;151(3):293-299.
179. Rosenthal R, Rosnow RL. *Esentials of behavioral research: Methods and data analysis (2nd ed.)*. New York: McGraw Hill; 1991.
180. Cohen J. *Statistical power analysis for the behavioral sciences (2nd ed.)*. Hillsdale NJ: Lawrence Earlbaum Associates; 1988.
181. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. *Annals of Internal Medicine.* 1999;130(6):461-470.

182. Lee H, Baniqued PL, Cosman J, et al. Examining cognitive function across the lifespan using a mobile application. *Computers in Human Behavior.* 2012;28(5):1934-1946.
183. Northam EA, Anderson PJ, Werther GA, Warne GL, Andrewes D. Predictors of change in the neuropsychological profiles of children with type 1 diabetes 2 years after disease onset. *Diabetes Care.* 1999;22(9):1438-1444.
184. Northam EA, Anderson PJ, Jacobs R, Hughes M, Warne GL, Werther GA. Neuropsychological profiles of children with tpe 1 diabetes 6 years after disease onset. *Diabetes Care.* September 1, 2001 2001;24(9):1541-1546.
185. Jacobson AM, Ryan CM, Cleary PA, et al. Biomedical risk factors for decreased cognitive functioning in type 1 diabetes: an 18 year follow-up of the Diabetes Control and Complications Trial (DCCT) cohort. *Diabetologia.* 2011;54(2):245-255.
186. Wrighten SA, Piroli GG, Grillo CA, Reagan LP. A look inside the diabetic brain: Contributors to diabetes-induced brain aging. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* 2009;1792(5):444-453.
187. Musen G. Cognition and brain imaging in type 1 diabetes. *Curr Diab Rep.* 2008;8(2):132-137.
188. Asvold BO, Sand T, Hestad K, Bjorgaas MR. Cognitive function in type 1 diabetic adults with early exposure to severe hypoglycemia: A 16-year follow-up study. *Diabetes Care.* 2010;33(9):1945-1947.
189. Frier B. Cognitive functioning in type 1 diabetes: The Diabetes Control and Complications Trial (DCCT) revisited. *Diabetologia.* 2011;54:233-236.
190. DeCarli C. Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. *The Lancet Neurology.* 2003;2(1):15-21.
191. Erten-Lyons D, Dodge HH, Woltjer R, et al. Neuropathologic basis of age-associated brain atrophy. *JAMA Neurology.* 2013;70(5):616-622.
192. Musen G, Lyoo IK, Sparks CR, et al. Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. *Diabetes.* 2006;55(2):326-333.
193. He J, Wong VSS, Fletcher E, et al. The contributions of MRI-based measures of gray matter, white matter hyperintensity, and white matter integrity to late-life cognition. *American Journal of Neuroradiology.* 2012;33(9):1797-1803.
194. Venkatraman VK, Aizenstein HJ, Newman AB, et al. Lower digit symbol substitution score in the oldest old is related to magnetization transfer and diffusion tensor imaging of the white matter. *Frontiers in Aging Neuroscience.* 2011;3.
195. Madden D, Bennett I, Song A. Cerebral white matter integrity and cognitive aging: Contributions from diffusion tensor imaging. *Neuropsychol Rev.* 2009;19(4):415-435.
196. Alosco ML, Gunstad J, Jerskey BA, et al. The adverse effects of reduced cerebral perfusion on cognition and brain structure in older adults with cardiovascular disease. *Brain and Behavior.* 2013;3(6):626-636.
197. Birdsill AC, Carlsson CM, Willette AA, et al. Low cerebral blood flow is associated with lower memory function in metabolic syndrome. *Obesity.* 2013;21(7):1313-1320.
198. Johnson NA, Jahng G-H, Weiner MW, et al. Pattern of cerebral hypoperfusion in Alzheimer Disease and mild cognitive impairment measured with arterial spin-labeling MR imaging: Initial experience. *Radiology.* 2005;234(3):851-859.

199. Griffiths PD, Hoggard N, Dannels WR, Wilkinson ID. In vivo measurement of cerebral blood flow: a review of methods and applications. *Vascular Medicine.* 2001;6(1):51-60.
200. Detre JA, Rao H, Wang DJJ, Chen YF, Wang Z. Applications of arterial spin labeled MRI in the brain. *Journal of Magnetic Resonance Imaging.* 2012;35(5):1026-1037.
201. American Psychiatric Assocation. *Diagnostic and statistical manual of mental disorders (4th ed., text rev.)*. Washington DC2000.
202. Five-year findings of the hypertension detection and follow-up program: I. reduction in mortality of persons with high blood pressure, including mild hypertension. *JAMA.* 1979;242(23):2562-2571.
203. Potier L, Roussel R, Labreuche J, et al. Interaction between diabetes and a high ankle–brachial index on mortality risk. *European Journal of Preventive Cardiology.* April 29, 2014 2014.
204. Ix JH, Miller RG, Criqui MH, Orchard TJ. Test characteristics of the ankle-brachial index and ankle-brachial difference for medial arterial calcification on X-ray in type 1 diabetes. *J Vasc Surg.* 2012;56(3):721-727.
205. Strauss E, Sherman EMS, Spreen O. *A compendium of neuropsychological tests: administrations, norms and commentary*. 3 ed. New York: Oxford University Press; 2006.
206. Ardila A. Normal aging increases cognitive heterogeneity: analysis of dispersion in WAIS-III scores across age. *Arch Clin Neuropsychol.* 2007;22(8):1003-1011.
207. Dore GA, Elias MF, Robbins MA, Elias PK, Brennan SL. Cognitive performance and age: norms from the Maine-Syracuse Study. *Exp Aging Res.* 2007;33(3):205-271.
208. Lee H-Y, Oh B-H. Aging and arterial stiffness. *Circulation Journal.* 2010;74(11):2257-2262.
209. Gorelick PB, Scuteri A, Black SE, et al. Vascular Contributions to Cognitive Impairment and Dementia: A Statement for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke.* 2011;42(9):2672-2713.
210. Rosano C, Watson N, Chang Y, et al. Aortic Pulse Wave Velocity Predicts Focal White Matter Hyperintensities in a Biracial Cohort of Older Adults. *Hypertension.* 2013;61(1):160-165.
211. Maser RE, Wolfson SK, Ellis D, et al. Cardiovascular disease and arterial calcification in insulin-dependent diabetes mellitus: interrelations and risk factor profiles. Pittsburgh Epidemiology of Diabetes Complications Study-V. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 1991;11(4):958-965.
212. Erickson KI, Glidengers AG, Butters MA. Physical activity and brain plasticity in late adulthood. *Dialogues Clin Neurosci.* 2013;15(1):99-108.
213. Tian Q, Erickson KI, Simonsick EM, et al. Physical Activity Predicts Microstructural Integrity in Memory-Related Networks in Very Old Adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences.* 2014.
214. Ryan JP, Fine DF, Rosano C. Type 2 Diabetes and Cognitive Impairment: Contributions From Neuroimaging. *Journal of Geriatric Psychiatry and Neurology.* 2014;27(1):47-55.
215. Koechlin E, Basso G, Pietrini P, Panzer S, Grafman J. The role of the anterior prefrontal cortex in human cognition. *Nature.* 1999;399(6732):148-151.
216. MacDonald AW, Cohen JD, Stenger VA, Carter CS. Dissociating the role of the dorsolateral orefrontal and anterior cingulate cortex in cognitive control. *Science.* 2000;288(5472):1835-1838.

217. Northam EA, Anderson PJ, Werther GA, Warne GL, Adler RG, Andrewes D. Neuropsychological complications of IDDM in children 2 years after disease onset. *Diabetes Care.* 1998;21(3):379-384.

218. Ward A, Arrighi HM, Michels S, Cedarbaum JM. Mild cognitive impairment: Disparity of incidence and prevalence estimates. *Alzheimer's & dementia : the journal of the Alzheimer's Association.* 2012;8(1):14-21.

219. Blair JR, Spreen O. Predicting pre-morbid IQ: A revision of the National Adult Reading Test. *Clin Neuropsychologist.* 1989;3:129-136.