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# The Effect of a Square-Stepping Exercise Intervention on Heart Rate Variability in Older Individuals with Type 2 Diabetes and Subjective Cognitive Complaints

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Graduate Program in Kinesiology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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## **Abstract**

Aging is associated with increased onset of diseases such as type 2 diabetes (T2D) and cognitive impairment. Heart rate variability (HRV), a measure of autonomic function, is reduced in T2D and may have the potential to indicate cognitive decline in this population. Square-stepping exercise (SSE) is a novel cognitive exercise recently implemented in cognitive research, which may have the potential to improve global cognitive function in T2D individuals. Participants with T2D (N=25, aged  $\geq 50$ y) and self-reported cognitive complaints were randomized into either an SSE intervention or a wait-list (WL) control group for 24-weeks. HRV parameters (time and frequency domain) and GCF domains were assessed at baseline and 24-weeks. No significant differences were found in HRV parameters between groups at baseline and 24-weeks. However, heart rate was significantly reduced from baseline to 24-weeks in the SSE group,  $p = 0.046$ . Additionally, low and high frequency power were significantly decreased from baseline to 24-weeks in the WL group,  $p = 0.05$  and  $p = 0.043$  respectively. This study elucidates the impact of cognitive exercise training on HRV, however, it is inconclusive as to why a shift towards vagal modulation was observed in both the SSE and WL groups.

## **Keywords**

Heart rate variability; type 2 diabetes mellitus; autonomic dysfunction; global cognitive function; square-stepping exercise

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## List of Term and Abbreviations

- $\alpha_1$** —Short-term scaling component
- $\alpha_2$** —Long-term scaling component
- A $\beta_{42}$** —Alzheimer biomarker
- ACSM**—American college of sports medicine
- ANS**—Autonomic nervous system
- AR**—Autoregression
- BMI**—Body mass index
- BP**—Blood pressure
- BRS**—Baroreflex sensitivity
- CAN**—Cardiac autonomic neuropathy
- CBS**—Cambridge brain sciences
- CES-D**—Center of epidemiologic studies depression scale
- CGSA**—Coarse graining spectral analysis
- CI**—Confidence interval
- CV**—Cardiovascular
- CVD**—Cardiovascular disease
- DFA**—Detrended fluctuation analysis
- ECG**—Electrocardiogram
- FFT**—Fast Fourier transform
- GCF**—Global cognitive function
- HbA<sub>1c</sub>**—Glycated hemoglobin
- HF**—High frequency
- HR**—Heart rate
- HRV**—Heart rate variability
- IQR**—Interquartile range
- LF**—Low frequency
- LOI**—Letter of information

**MCI**—Mild cognitive impairment  
**Md**—Median  
**MI**—Myocardial infarction  
**MMSE**—Mini-mental state examination  
**MOCA**—Montreal cognitive assessment  
**PC**—Personal computer  
**pNN50**—Percentage of R-R intervals with difference >50ms  
**PNS**—Parasympathetic nervous system  
**PSD**—Power spectral density  
**RCT**—Randomized controlled trial  
**rho**—Spearman correlation  
**RMSSD**—Root mean square of successive R-R interval differences  
**RRI**—R-R intervals  
**SD**—Standard deviation  
**SD1**—Poincaré plot width  
**SD2**—Poincaré plot length  
**SDNN**—Normal-to-normal R-R intervals  
**SNS**—Sympathetic nervous system  
**SSE**—Square-stepping exercise  
**T2D**—Type 2 diabetes mellitus  
**TP**—Total power  
**ULF**—Ultra low frequency  
**VLF**—Very low frequency  
**VO<sub>2</sub>max**—Maximal oxygen intake  
**WC**—Waist circumference  
**WL**—Wait-list control group  
**YMCA**—Young men’s Christian association

## Chapter 1

### 1.1 Literature Review

#### 1.2 Epidemiology of Diabetes Mellitus

In 2011, an estimated 366 million people around the world were diagnosed with Type 2 diabetes (T2D), and this number is projected to rise by 51%, reaching 552 million by 2030 (Whiting et al., 2011). The prevalence of T2D diagnosis of Canadians aged 12 years or older in 2014 was 6.7% overall (approximately 2.0 million people) with 5.8% reported in women and 7.5% in men (Diabetes, 2015). In 2006, persons aged 75 to 79 years had the highest prevalence of T2D for both men and women at an average of 23.4%. Recently however, this trend has shifted to a younger age bracket (60 to 64 years) as the “baby boomer” generation ages and the prevalence of obesity rises (Report from the National Diabetes Surveillance System, 2009). Not only is T2D a burden on quality of life and independence for the individual, but it is also a burden on families, caregivers and the healthcare systems with regards to costs and liabilities associated with aging and disease (Hayes et al., 2014).

##### 1.2.1 Types 2 Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disease that affects the way the body metabolizes glucose (Canadian Diabetes Association, 2008). In healthy individuals following a meal, carbohydrates are broken down into glucose (a form of simple sugar) and enter the blood stream. Insulin, a hormone produced by beta cells in the pancreas, is released into the blood stream to absorb glucose (Harlan, 2014) and stores it as glycogen in the liver and skeletal muscles. Diabetics are either unable to produce insulin, unable to produce enough insulin or unable to effectively use the insulin that is produced to control blood glucose levels (Mayo Clinic, 2014). Due to insulin resistance, diabetics experience high levels of blood glucose (hyperglycemia).

There are three main types of diabetes: gestational, Type 1 and Type 2. Gestational diabetes is a form of diabetes that develops during pregnancy that may complicate pregnancy and affect the health of the baby (Harlan, 2014). Type 1 diabetes, formerly

known as juvenile diabetes, occurs when the body's immune system attacks pancreatic beta cells resulting in the inability to produce insulin which causes hyperglycemia following a meal (Canadian Diabetes Association, 2008). And T2D, formally known as adult-onset diabetes, is the most common form, at 90-95% of cases diagnosed (Harlan, 2014). Diagnosis of hyperglycemia and T2D is confirmed and monitored via blood work—i.e. glycated hemoglobin (HbA<sub>1c</sub>), fasting plasma glucose and oral glucose tolerance tests. T2D was originally seen in older adults, however, incidences are beginning to appear at younger ages due to physical inactivity and increased childhood obesity (Hannon et al., 2005). Over time hyperglycemia causes nerve and vasculature damage, leading to severe health problems such as cardiovascular disease (CVD)—i.e. hypertension, atherosclerosis, heart attack and stroke, kidney disease, retinopathy/blindness, peripheral neuropathy, autonomic dysfunction, cognitive impairment, reduced mobility and depression (Harlan, 2014; Matei et al., 2013). The risk factors associated with the development of T2D are either modifiable or non-modifiable. Age (> 40 years), gender (male), family history, and race (Aboriginal, Asian, African and Hispanic) are all non-modifiable risk factors, whereas, weight (overweight and obese), physical inactivity, dyslipidemia and hypertension are all modifiable risk factors (Mayo Clinic, 2014; Harlan, 2014). If left untreated, T2D complications can lead to death. Treatment prevents some complications but it does not restore normoglycemia or completely eliminate all adverse consequences associated with T2D (Knowler et al., 2002).

### **1.3 Aging and Cognitive Impairment in Diabetes**

Global cognitive function embodies overall cognitive function of the brain including executive function (planning, inhibition, task switching, maintenance and manipulation of information), episodic memory, reasoning, thinking, attention, language and processing speed (Hayes et al., 2014). Successful aging is defined by Rowe & Kahn (1997) as multidimensional, consisting of disease and disability avoidance, maintenance of high physical and cognitive function, and sustained engagement in social and productive activities. Poor social integration is associated with higher mortality rates in aging populations (Rowe & Kahn, 1997). In individuals aged 50 years and older, there is also a

higher prevalence of cognitive decline regardless of successful aging (Acee, 2012). Nonetheless, many older adults do not meet this definition of successful aging and instead are at risk for various diseases such as CVD, T2D and dementia (Rowe & Kahn, 1997). These chronic diseases are correlated with increased age and contribute to the loss of mobility and decrease in overall well-being. T2D is not the only chronic disorder expected to rise in the oncoming years. Of the other disorders related to chronological aging that are expected to increase significantly, dementia is the foremost (Acee, 2012). In Canada, it was estimated that nearly 15% (747,000) of older adults were diagnosed with dementia or Alzheimer's disease in 2011. It was also predicted that unless treatments were implemented, this figure is expected to reach 1.4 million by 2031 (Alzheimer Society, 2012). The Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MOCA) are both simple cognitive tests with a battery of questions aimed at screening for cognitive impairment. The MMSE is both reliable and a valid test for estimating the severity of cognitive impairment in individuals with dementia, delirium, schizophrenia or affective disorder (Folstein et al., 1975). The MOCA is highly sensitive in identifying individuals with subtle cognitive decline, however, it may not be significant enough to warrant a diagnosis of dementia (Gill et al., 2016).

Cognitive impairment can be classified as subjective cognitive complaints, mild cognitive impairment (MCI), and dementia. A subjective cognitive complaint is the belief that one's thinking and/or memory has gotten worse recently, and occurs frequently in the elderly. Longitudinal studies have identified subjective cognitive complaints as a future predictor of MCI and dementia (Jessen et al., 2007; Xu et al., 2010). MCI represents the transitional phase between normal cognitive function and dementia, although not all people with MCI will develop dementia (Ettorre et al., 2012; Xu et al., 2010). In a study by Xu et al (2010), pre-diabetes and T2D were found to accelerate the progression from MCI to dementia, as well as T2D increased the incidence of dementia by 50-100% when compared to people without diabetes (Xu et al., 2010). Dementia is a broad term encompassing many forms of cognitive impairment. Vascular dementia (also known as vascular cognitive impairment), was found to be two to three times more likely in T2D individuals than normal age-matched individuals, and is the most prevalent of the dementias seen in this population (Acee, 2012). Vascular dementia is thought to be caused by small artery disease linked

with CVD risk factors (age, hypertension, dyslipidemia, smoking and alcoholism) and poor glycemic control. It is characterized by chronic reduced blood flow in the brain, ultimately leading to brain tissue death and dementia (Acee, 2012). Cerebral damage such as reduced brain volume and increased white matter hyperintensity volume lead to reduced cognitive performance (Gauthier et al., 2015). Decline in cognitive function is particularly found in executive function, processing speed and episodic memory and is due to structural and functional alterations in the fronto-parietal and medial temporal brain regions (Hayes et al., 2014).

#### **1.4 Cardiovascular Autonomic Function**

The autonomic nervous system (ANS) is composed of two major branches, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), which work together to control involuntary functions such as heart rate (HR), blood pressure (BP), respiratory rate, digestion, urination and sexual arousal (Barrett et al., 2010). The SNS is associated with energy mobilization whereas the PNS is associated with restorative functions. The dynamic interaction between the two systems is called autonomic balance and allows for environmental adaptation (Thayer et al., 2010). The SNS and PNS are important regulators of the cardiovascular (CV) system. Activation of sympathetic nerves going to the heart and blood vessels are excitatory and mediated by the release of epinephrine and norepinephrine. They increase myocardial contractility, resulting in elevated cardiac output, HR and BP. Parasympathetic activation, provided primarily by the vagus nerve, is mediated by acetylcholine. It provides an inhibitory response and opposes SNS activity resulting in decreased myocardial activity, cardiac output, HR and BP (Stuckey & Petrella, 2013; Task Force of the European Society of Cardiology, 1996).

##### **1.4.1 Autonomic Responses to Postural Changes**

Under resting conditions, such as a supine position, vagal activity prevails due to the sinus node being rich in acetylcholinesterase, resulting in rapid vagal transmission thus reducing HR (Task Force of the European Society of Cardiology, 1996). There is no influence of gravity and activation of baroreceptors is only determined by changes in BP mediated by respiratory movements (Michel-Chávez et al., 2015). Baroreceptors are

stretch receptors located in the carotid arteries and aortic arch. They respond to changes in BP via a control loop known as the baroreflex. The baroreflex is the most important short-term mechanism for BP regulation (Stuckey & Petrella, 2013). Raised BP results in a baroreflex-mediated increase in PNS and/or reduction in SNS activity, and vice versa for reduced BP. Baroreflex sensitivity (BRS) refers to the capacity of the baroreflex to adjust HR and vascular resistance in response to changes in BP (Stuckey & Petrella, 2013).

Transitional changes, such as sitting and standing invoke physical challenges imposed by gravity, and cause a temporary drop in BP and increase in HR, as a consequence of BRS activation (Michel-Chávez et al., 2015). These changes from supine to sitting and subsequently to standing, result in stronger SNS stimulation with corresponding vagal withdrawal (Hnatkova et al., 2013). In disease populations, in which sympathetic denervation of the blood vessels is occurring, autonomic dysfunction correlates clinically with postural hypotension and impaired BRS function (Michel-Chávez et al., 2015). In T2D, arterial stiffness prevents stretch receptors in the carotid arteries and the aorta from sensing subtle changes in BP, thus increasing the time it takes to activate the baroreflex. This delay is indicative of reduced BRS and leads to postural hypotension (Michel-Chávez et al., 2015).

Chemoreflexes are also important modulators of SNS activation. Peripheral chemoreceptors are located in the carotid arteries and respond primarily to hypoxaemia (low oxygen), while central chemoreceptors are located in the brainstem and respond to hypercapnia (high carbon dioxide). Activation of either the hypoxic or hypercapnic chemoreflex results in both hyperventilation and SNS activation (Kara et al., 2003). In healthy individuals, SNS and PNS are closely regulated to maintain autonomic balance. In aging and clinical populations, sympathetic and parasympathetic nerves innervating the heart and blood vessels become damaged leading to autonomic imbalance and dysfunction (Thayer et al., 2010).

#### **1.4.2 Autonomic Dysfunction in Type 2 Diabetes**

Autonomic imbalance occurs when one branch of the ANS dominates over the other. It is associated with a lack of dynamic flexibility and reduced health (Thayer et al., 2010), and

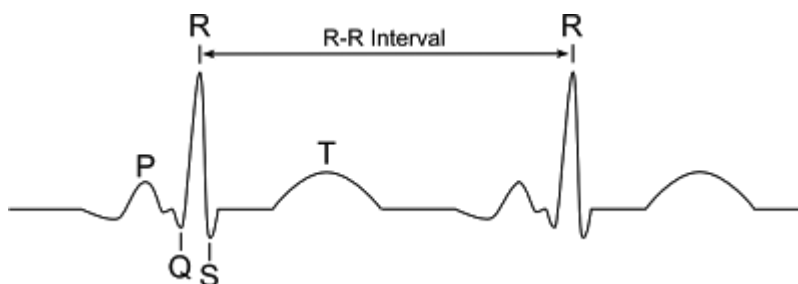
progresses to autonomic dysfunction. Autonomic imbalance typically occurs when the SNS is hyperactive and the PNS is hypoactive, causing a predominance of sympathetic activity (Michel-Chávez et al., 2015). Hypoactivity may be a result of denervation to the vagus nerve caused by chronic hyperglycemia (Brownlee, 2005) which is responsible for nearly 75% of PNS activity (Balcioglu & Müderrisoğlu, 2015). PNS nerve fibers may become damaged earlier with autonomic dysfunction, however, over time SNS nerve fibers are also affected (Vinik & Ziegler, 2007). When the SNS dominates for long periods of time, energy demands on the system become excessive, and ultimately cannot be met, eventually leading to neuronal death (Thayer et al., 2010). In T2D, cardiac autonomic neuropathy (CAN) is a form of autonomic dysfunction and causes prevalent, chronic CV complications with life-threatening outcomes. CAN is characterized by damage to the autonomic nerve fibers regulating HR, cardiac output, myocardial contractility and blood vessel constriction/dilation, which causes a wide range of cardiac disorders (Balcioglu & Müderrisoğlu, 2015). Diabetic risk factors such as hyperglycemia, older age, obesity, and physical inactivity all contribute to CAN (Vinik & Ziegler, 2007). CAN is often overlooked due to clinical symptoms appearing late in the progression of the disease. Clinical manifestations include resting tachycardia, orthostatic hypotension, exercise intolerance, hypertension and silent myocardial infarctions (MI) (Vinik & Ziegler, 2007). Autonomic denervation is partly reversible and can be slowed down in the early stages of disease, therefore early detection and screening for autonomic dysfunction in patients with T2D is crucial. Reduced heart rate variability (HRV) may be a potential early indicator of autonomic dysfunction and can be seen at subclinical stages of disease (Matei et al., 2013; Thayer et al., 2010; Vinik & Ziegler, 2007)).

### **1.5 Heart Rate Variability**

HRV is a non-invasive measure of cardiac autonomic regulation (Britton et al., 2008), which may be a promising tool for early diagnosis. Measurement of HRV involves the collection of consecutive R-R intervals (RRI) (Figure 1) from either an electrocardiogram (ECG) or HR monitor. Long-term recordings of 24-hour duration and short-term recordings of five minutes duration are commonly analysed (Figure 2). A high HRV reflects cardiac adaptability and implies good health, as seen in healthy populations, while



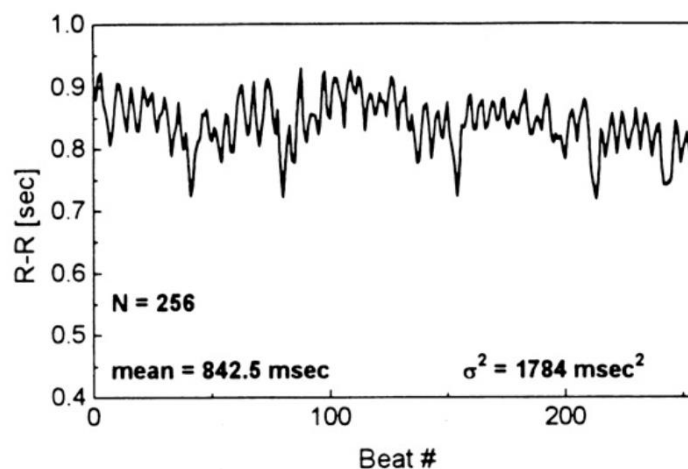
low HRV reflects autonomic dysfunction, as seen in clinical populations (Grieco et al., 2014; Balcioglu & Muderrisoğlu, 2015). It is important that HRV only be compared within or between individuals if the same length of data was analyzed, due to variability being length-dependent (Task Force of the European Society of Cardiology, 1996). Non-sinus beats such as ectopic beats, noise, and premature ventricular contractions should be removed prior to HRV analysis (Kamath et al., 2012). HRV analysis methods are divided into linear (time and frequency domains) and non-linear methods.



**Figure 1: ECG signal**—Depicting the RRI used to assess HRV (Thompson, 2010).

### 1.5.1 Time Domain Analysis

Time domain parameters are easily calculated. The most common time domain parameter of HRV is the standard deviation of normal-to-normal RRI (SDNN). Other parameters include root mean square of successive RRI differences (RMSSD) and percentage of RRI with difference  $>50\text{ms}$  (pNN50) (Kamath et al., 2012). RMSSD and pNN50 represent rapid vagal responses in RRI, while SDNN has both SNS and PNS activity and represents overall autonomic activity (Matei et al., 2013). The main limitation of this method is the lack of discrimination between the effects of sympathetic and parasympathetic autonomic branches (Kamath et al., 2012).



**Figure 2: Consecutive R-R intervals**—Depicting consecutive RRI (Task Force of the European Society of Cardiology, 1996)

### 1.5.2 Frequency Domain Analysis

Power spectral density (PSD) is a more advanced analysis of HRV than the time domain analysis and has been used extensively in research. In the frequency domain, HRV is described as the sum of oscillatory components and defined by frequency and amplitude (power) (Chemla et al., 2005). Total power (TP) of the RRI is represented by the total area under the PSD curve. The power spectrum consists of four power frequency bands: ultra low frequency (ULF; 0-0.003Hz), very low frequency (VLF; 0.003-0.04Hz), low frequency (LF; 0.04-0.15Hz) and high frequency (HF; 0.15-0.4Hz) (Kamath et al., 2012). LF and HF are generally conveyed as short-term spectral components whereas ULF and VLF require long-term recordings to perform valid analysis. LF and HF can be expressed as a ratio LF/HF to emphasize the sympathovagal balance between PNS and SNS activity (Task Force of the European Society of Cardiology, 1996). HF represent vagal activity and LF includes both PNS and SNS effects. Increases in LF/HF are assumed to reflect a shift to sympathetic dominance, while decreases correspond to parasympathetic dominance (Billman, 2013). However, despite wide acceptance of the LF/HF ratio, it remains a controversial measure of sympathovagal balance in healthy and disease populations (Billman, 2013). Billman (2013) proposed that the LF/HF representing sympathovagal balance rested upon four assumptions: 1) SNS activity is exclusively responsible for the LF peak in the HR power spectrum; 2) PNS activity is exclusively

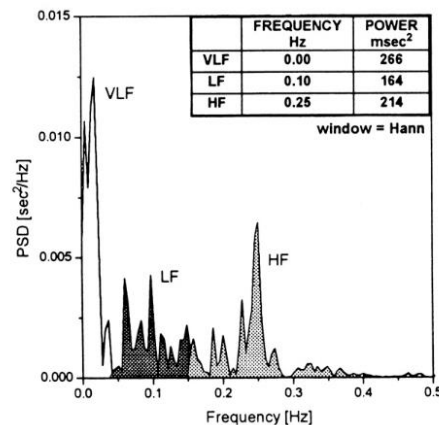
responsible for the HF peak in the HR power spectrum; 3) disease or physiological challenges provoke reciprocal changes in SNS and PNS activity; and 4) there is a simple linear interaction between the effects of SNS and PNS activity on HRV. Billman concludes that the LF/HF ratio cannot accurately quantify cardiac sympathovagal balance in healthy or disease populations (Billman, 2013).

Frequency bands are thought to represent physiological processes and are used to estimate the contributions of PNS and SNS contributions in HR. The physiological background of ULF and VLF bands are not well known and attributing physiological explanation may be called into question (Task Force of the European Society of Cardiology, 1996). LF and HF bands are more commonly used in HRV analysis. LF bands are mediated by both the sympathetic and parasympathetic branches of the ANS, whereas HF bands are primarily mediated by parasympathetic branches (Tarvainen et al., 2013; Kamath et al., 2012). HF corresponds to HR variations related to respiratory sinus arrhythmia and fluctuations in PNS activity. Breathing volume and frequency influence HRV measures; as breathing volume decreases, so does HF power (Kamath et al., 2012). Roughly speaking, SNS activity increases HR and decreases HRV, whereas PNS activity decreases HR and increases HRV (Tarvainen et al., 2013).

In a normal population, the ability of the CV system to maintain a high level of variability is a sign of health. Conditions such as chronic heart failure are characterized by high SNS drive to both the heart and peripheral blood vessels and would be expected to manifest as predominantly LF oscillations, which however, is not the case (Van De Borne et al., 1997). In severe cardiac disease conditions, such as chronic heart failure, LF and thus LF/HF, are unable to measure SNS drive due to impaired BRS regulation and tonic SNS activation without LF oscillatory patterns (Van De Borne et al., 1997).

Methods for calculating PSD provide information on the power distribution across frequencies and are classified as either nonparametric or parametric (Task Force of the European Society of Cardiology, 1996). The fast Fourier transform (FFT) is the most commonly used nonparametric method. FFT is a simple algorithm used to convert the time domain data into frequency domain data, and is not filtered prior to processing (Kamath et al., 2012). FFT allows the signal to be easily interpreted using a graphical

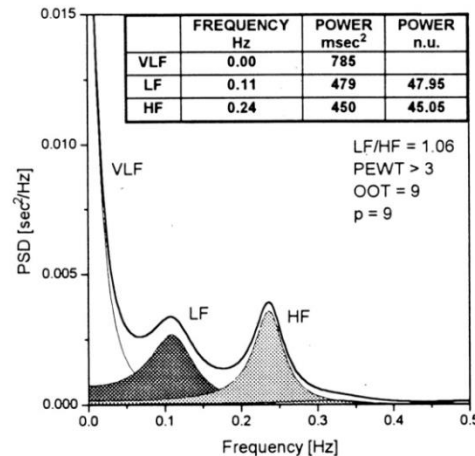
representation of the data (Figure 3). The power spectrum is divided into VLF, LF and HF bands (Task Force of the European Society of Cardiology, 1996). The resolution of the frequency scale depends on the number of data samples and the sampling interval. A low sampling rate may produce a jitter and may not be a good representation of the original signal. Alternatively if the sampling rate is too high, the signal is accompanied by spectral noise and inaccuracies. Applying a linear regression analysis to the HRV signal decreases the error (Kamath et al., 2012). Between each adjacent signal there is overlap causing leakage (loss of data) between frequencies in the power spectral estimate. Leakage from one frequency into the other can be corrected for using data windowing (i.e. Hanning) to smooth the spectral signal (Kamath et al., 2012).



**Figure 3: FFT spectral analysis**—Depicting the VLF, LF and HF peaks of the signal (Task Force of the European Society of Cardiology, 1996)

Autoregression (AR) is a parametric model that is used to analyze RRI and determine the central frequency (frequency peaks) and powers of the oscillatory components. Each time series (RRI) depends linearly on the previous time series values (Kamath et al., 2012). The advantages of parametric methods are simplicity of the algorithm, high processing speed (Task Force of the European Society of Cardiology, 1996) and smoother spectral components that can be distinguished independently of the frequency bands (Pichon et al., 2006). Similar to FFT, the AR model requires a set of parameters (model order) to establish the correct model order of the frequency spectrum. The Akaike information criteria is employed as a measure of model quality and Burg's algorithm is used to select the specific model order (Kamath et al., 2012). Excessively high model orders capture

noise and produces a spectrum with false or split peaks, while too low a model order smooths spectral peaks, causes peak shifting or removes peaks altogether (Kamath et al., 2012). The AR model is also divided into VLF, LF and HF bands (Figure 4).

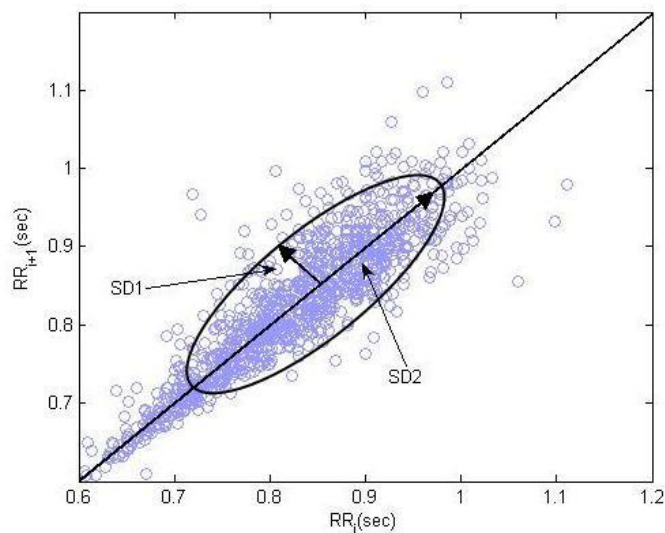


**Figure 4: AR spectral analysis**—Depicting the VLF, LF and HF bands of the spectrum (Task Force of the European Society of Cardiology, 1996)

### 1.5.3 Non-Linear Analysis

Non-linear analysis methods of analyzing HRV also elicit valuable information for physiological interpretation and the risk of sudden death (Task Force of the European Society of Cardiology, 1996). Commonly used non-linear HRV methods are: Poincaré plot, sample entropy, detrended fluctuation analysis (DFA) and Coarse Graining Spectral Analysis (CGSA). The Poincaré plot is a scatterplot between successive RRI (Figure 5). They are used to extract indexed such as short-term variability (width; SD1) and long-term variability (length; SD2) which are non-linearly connected to time-domain parameters (Goit et al., 2014; Tarvainen et al., 2014; Kamath et al., 2012). Sample entropy is a commonly used measure of signal complexity of short time series and is computed using an embedding dimension of length ( $m=2$ ) and tolerance of 0.2 times the standard deviation of the time series (Tarvainen et al., 2014). Sample entropy is not sensitive to changes in single data values, however it is sensitive to factors that affect tolerance parameters (i.e. ectopic beats) (Kamath et al., 2012). DFA is a measure of self-affinity of a signal, separated into short-term ( $\alpha_1$ ) and long-term ( $\alpha_2$ ) fluctuations within the RRI time series (Tarvainen et al., 2014). And finally, CGSA samples every other data

point of the time series to eliminate noise, which allows for more accurate quantitative analysis by acquiring clearer peaks in the LF and HF bands (Yamamoto & Hughson, 1991).



**Figure 5: Poincare plot**—Depicting SD1 and SD2 (Karmakar, 2009)

#### 1.5.4 Heart Rate Variability during Postural Changes

Changes in body position or posture can alter the activity of SNS or vagal influence on HR in a T2D population (Mahananto et al., 2015). Positions such as sitting and standing undergo physical challenges imposed by gravity, which cause a temporary drop in BP and increase in HR (Michel-Chávez et al., 2015). As the posture becomes more physically demanding, initial decreases in HF and increases in LF are observed (Malliani et al., 1991). After approximately one minute of sitting or standing, HRV parameters are regulated by the body’s negative feedback mechanism and stabilized back to a homeostatic “set point” (Finucane et al., 2014; Zhang, 2007). In T2D, this “set point” is influenced by greater SNS activation, and results in higher HR, LF and reduced HF.

#### 1.5.5 Heart Rate Variability as a Diagnostic Tool

HRV is used extensively in research and is considered a valuable clinical tool due to its simplicity and non-invasiveness. Clinical applications of HRV include assessment of risk of death after an MI, monitoring disease progression of congestive heart failure and early

diagnosis of autonomic dysfunction (Malik, 1998). The usefulness of HRV has expanded from cardiac applications to utilization during exercise and diverse pathological conditions, such as chronic renal failure, autonomic neuropathy, fibromyalgia and hypertension (Kamath et al., 2012). Several studies have investigated the usefulness of short-term HRV analysis by examining autonomic imbalances (exhibited as reduced HRV) that occur in the aforementioned pathological conditions. Power spectral analysis of HRV has also been considered a useful tool in assessing autonomic function in patients with T2D (Canani et al., 2013).

In a longitudinal study on aging, researchers investigated the association between HRV and cognitive performance (as measured by MOCA). A multivariate linear regression was used to model the association between HRV and cognition. Results indicated that lower quantiles of SDNN, LF and LF/HF ratio were significantly associated with poorer MOCA scores (Frewen et al., 2013). A possible explanation for the association between reduced HRV and cognitive decline could be the cholinergic anti-inflammatory pathway. Efferent vagal nerve activity inhibits pro-inflammatory cytokine release, which protects against systemic inflammation. Reductions in LF, HF and SDNN, along with an increase in HR are associated with higher levels of C-reactive proteins and interleukin-6, which are both associated with cognitive decline. Therefore, inflammation may mediate the relationship between reduced HRV and cognitive performance (Frewen et al., 2013). Thus, HRV may have potential predictive application in cognitive decline.

## **1.6 Type 2 Diabetes Management**

### **1.6.1 Pharmaceuticals**

Glycemic control in T2D is managed via pharmaceutical treatments and/or through diet and exercise. Lifestyle management, through diet and exercise are initially prescribed, however if there is poor adherence and glycemic targets are not met in the first 2 to 3 months, pharmaceuticals are administered (Canadian Diabetes Association, 2008). Most T2D patients will require insulin therapy to maintain long-term glycemic control, either as a monotherapy or combined with oral antidiabetic therapy (Krentz & Bailey, 2005). There are many oral antidiabetic medications available. The main classes include agents that stimulate insulin secretion (sulphonylureas and rapid-acting secretagogues), reduce

hepatic glucose production (biguanides), delay digestion and absorption of intestinal carbohydrate ( $\alpha$ -glucosidase inhibitors) or improve insulin action (thiazolidinediones) (Krentz & Bailey, 2005). Metformin, a biguanide agent, is widely regarded as the drug of choice for patients with T2D as it improves insulin sensitivity and decreases insulin levels (Kravitz et al., 2013). Initial monotherapy with metformin does not promote weight gain, unlike other antidiabetic medications, and has beneficial effects on CVD risks such as reduced MI and diabetes-related deaths (Krentz & Bailey, 2005).

In a study by Knowler et al (2002) pre-diabetic individuals were randomly assigned to either placebo, metformin (850mg twice daily), or a lifestyle modification program (150 minutes of moderate-intensity exercise per week; low-calorie, low-fat diet). After three years, researchers found the incidence of T2D was 11.0, 7.8 and 4.8 cases per 100 people in the placebo, metformin and lifestyle intervention groups respectively. The lifestyle intervention significantly reduced the risk of T2D by 58% while metformin reduced the risk of T2D by 31% (Knowler et al., 2002). Lifestyle interventions are better short- and long-term regulators of T2D and should therefore be an essential constituent in both the prevention and treatment of T2D.

### **1.6.2 Aerobic Exercise in Type 2 Diabetes**

Extensive literature exists on the effects of aerobic exercise in healthy and clinical populations. Aerobic exercise, also known as endurance exercise, utilizes the cardiorespiratory systems to perform exercise. In aerobic exercise, the circulatory and respiratory systems work together to supply oxygenated blood to the working skeletal muscles during prolonged exercise (Hayes et al., 2014). According to the American College of Sports Medicine (ACSM) and American Diabetes Association, people with T2D should accumulate at least 150 minutes of moderate to vigorous aerobic exercise a week, spread out over at least 3 days of the week, and with no more than 2 consecutive days of rest in between (Colberg et al., 2010). CV protective results are not immediately observed with acute aerobic exercise, and due to the exercise intolerance commonly seen in individuals with T2D (caused by excessive weight and low respiratory fitness), adherence to exercise is low, outweighing the associated positive benefits (Grieco et al., 2014). Special exercise considerations for T2D individuals should be implemented to



increase compliance. Exercise can be modified to better accommodate overweight individuals by dividing exercise time into smaller intervals and reducing intensity. Blood glucose levels should also be monitored before and after exercise to prevent hypoglycemia during exercise (American College of Sports Medicine, 2013). Strong dose-response relationships are documented between regular exercise and reduced risk of conditions such as CVD, T2D, hypertension, and cognitive decline (American College of Sports Medicine, 2013).

### **Aerobic Exercise and Cognition**

Overall, evidence suggests that exercise has cognitive benefits that may offset declines associated with aging and disease (Hayes et al., 2014). Cognitive gains achieved with aerobic exercise training are most prominent in executive functions, processing speed and episodic memory, which are processes required for daily living (Hayes et al., 2014).

Reduced physical fitness is associated with brain alterations, reduced cerebral blood flow, increased white matter lesions, a thinner cortex and smaller total and regional (i.e. hippocampus, cingulate gyrus) grey matter volume in CVD populations (Hayes et al., 2014). Research suggests exercise may attenuate adverse brain changes in CVD through improvements in physical fitness and subsequent increased cerebral perfusion (Hayes et al., 2014).

Baker et al (2010) extended these findings by observing improvements in executive function, cardiorespiratory fitness and insulin sensitivity following 6-months of aerobic exercise intervention (45-60 minute sessions, 75-85% HR reserve, 4 days per week) when compared to the control group (<50% HR reserve; balance and stretching exercises) in older glucose intolerant adults. In addition, circulating levels of the Alzheimer biomarker A $\beta$ <sub>42</sub> tended to decrease for subjects in the aerobic group relative to the control (Baker et al., 2010).

In contrast, a study by Fiocco et al (2013) examined the effects of a 24-week intervention program (60 minutes combined aerobic and resistance exercise; 30 minute lecture) on cognitive performance, and CV and metabolic factors. Results showed an increase in CV fitness and peak HR, and a decrease in body mass index and depressive symptoms.

Surprisingly however, analysis showed that cognitive performance on immediate recall, short-delay recall and category fluency (as measured by the California Verbal Learning Test, Digit Symbol Substitution Task and fluency test respectively) declined following the intervention (Fiocco et al., 2013). It was concluded that due to small sample size and lack of control group, the findings of this study should be interpreted with caution. Furthermore, it was suggested that the addition of hypertension exacerbated the deleterious effects of T2D on cognitive function.

A systematic review by Angevaren et al (2008) concluded that aerobic exercise is beneficial for cognitive function in healthy older adults. Effects were observed for motor function, cognitive speed, auditory and visual attention, however, the majority of comparisons yielded no significant results and larger studies were required. Alternatively, the National Institutes of Health State of Science Conference Statement on Preventing Alzheimer's Disease and Cognitive Decline concluded that data were insufficient to state that aerobic exercise improved or maintained cognitive function (Daviglius et al., 2010). A meta-analysis of randomized control trials (RCT) found a small, higher quality study that showed modest benefit in reducing cognitive decline following exercise in people with confirmed memory problems; however, the data is considered preliminary and further investigation is required (Daviglius et al., 2010).

### **Aerobic Exercise and Heart Rate Variability**

The effects of exercise on HRV have been examined thoroughly among patients with T2D, however, findings have been inconclusive. In healthy individuals, aerobic exercise training typically increases vagally mediated HRV (Tulppo et al., 2003), the increased stroke volume of the heart being the most potent mechanism in this response (Cornelissen & Fagard, 2005). Zoppini et al (2007) examined long-term CV autonomic adaptation to moderate aerobic exercise in individuals with T2D. Following 6-months of aerobic training (twice weekly, 1 hour sessions, 50-70% HR reserve), there were no exercise-related changes in power spectral analyses or LF/HF ratio in the prone position. In a standing position, however, HF was significantly increased and LF and LF/HF ratio were significant decreased. Researchers infer that moderate aerobic exercise significantly

improves CV autonomic function in T2D individuals, however the study did not comply with ACSM exercise recommendations in T2D.

These findings were enforced by a study that did meet exercise guidelines by Goit et al (2014) on the effects of 6-months of aerobic exercise intervention (3 days per week, moderate-intensity exercise) in T2D individuals. The results showed that the time domain parameters reflecting PNS activity (RMSSD and pNN50) were significantly increased, whereas SDNN, representing both SNS and PNS activity, was unaffected after exercise. Findings were also significant in the frequency domain; an increase in HF and decrease in both LF and LF/HF ratio were observed. The non-linear findings showed a significant increase in Poincaré plot SD1, whereas SD2 was unaffected following exercise (Goit et al., 2014).

Contrarily, Loimaala et al (2003) concluded that 12-months of exercise training (aerobic exercise 2 times per week at 65-75% maximal oxygen intake ( $VO_2$ max); strength training 2 times per week at 70-80% maximal voluntary contraction, 3 sets of 10-12 repetitions) is not long enough to improve HRV in T2D patients. However, a decrease in HR suggested vagal tone was likely increased following training. There was also a nonsignificant trend towards improved HRV parameters in the exercise group, suggesting a longer training period could improve HRV in T2D.

In clinical populations, HRV parameters reflecting PNS activity have been shown to increase whereas parameters reflecting SNS activity have been observed to decrease following aerobic exercise training. These findings suggest that individuals with initial low HRV are responsive to changes in HRV following aerobic exercise (Brown & Brown, 2006). However, improved autonomic function is not consistently observed with exercise training, for instance, in CAD patients regardless of comorbid T2D (Karjalainen et al., 2015).

### **1.6.3 Resistance Exercise in Type 2 Diabetes**

As opposed to the extensive literature on aerobic exercise, fewer studies have examined the effects of resistance training. Resistance training, also known as strength training or weight training, is any exercise that causes the muscles to contract against an external

resistance with the intent to increase muscle strength and mass (American College of Sports Medicine, 2013). Resistance exercise is recommended at least 2-3 days/week for people with T2D as part of a well-rounded exercise program by ACSM (Colberg et al., 2010). It maintains or increases skeletal muscular strength, prevents osteoporosis and lowers the risk of all-cause mortality and CVD events (American College of Sports Medicine, 2013).

### **Resistance Exercise and Cognition**

Resistance exercise plays a role in cognitive function. In a study by Cassilhas et al (2007), improvements were found in global cognitive function following both moderate- and high-intensity resistance training when compared to control, with no difference between the two intensities. Since the beneficial effects did not depend on the intensity of exercise, moderate-intensity resistance exercise may be more appropriate for an elderly population. Following resistance exercise, participants experienced better brain blood flow, and transportation of nutrients and oxygen, which is a likely mechanism for the improved cognitive performance (Cassilhas et al., 2007).

In a comparative study by Ozkaya et al (2005), researchers investigated the effects of 9-weeks of either moderate strength or endurance exercise training on cognition, evaluated by event-related potentials in older individuals. Participants were divided into either aerobic training (50 minutes/session, 3 times per week, at 70% heart rate reserve), strength training (7 exercises, 12 repetitions, 60-80% of 1 repetition maximum, 3 times per week) or control. Ozkaya et al (2005) found no differences in functional fitness between the strength and endurance training groups, however strength training may have facilitated effects on early information processing and cognition. They concluded that changes in cerebral blood flow, neurotransmitter functioning or increased cell complexity might occur in different brain regions and contribute to central nervous system integrity, however, the mechanisms behind the event-related potentials remain unknown and their preliminary findings await replication in a larger sample size (Ozkaya et al., 2005). Positive advancements have been made on the effects of resistance exercise on cognition, however research is limited and results have been modest.

## **Resistance Exercise and Heart Rate Variability**

There is currently no research on the sole effects of resistance training on HRV in T2D population, however, effects have been investigated in other diseases affecting cardiac autonomic function. In a study examining the effects of a 3-month moderate-intensity resistance exercise training program (circuit training, 3 times per week) in chronic heart failure participants, significant increases in HRV (increase in HF power and decrease in LF power and LF/HF ratio) were observed (Selig et al., 2004). More recently, Figueroa et al (2008) conducted a study on 16-weeks of moderate-intensity resistance training (30 minutes circuit training, 2 times per week, 8-12 repetitions, 50-80% of 1 repetition maximum) in women with fibromyalgia who had pre-training reductions in HRV. Findings showed increased HRV (increase in TP and HF power) following the training program (Figueroa et al., 2008). The mechanisms involved in these responses are believed to be a result of increased peripheral SNS activity stimulating the BRS which in turn promotes a decrease in SNS and an increase in cardiac PNS activity (Lima et al., 2011). Likewise, the improvements in cardiovagal control following resistance exercise in those with autonomic dysfunction may be due to a greater physical deconditioning and adiposity (Kingsley and Figueroa, 2016).

### **1.6.4 Cognitive Exercise**

Interventions that rely on complex mental activities present a new approach to combating age-related decline and dementia (Gates & Valenzuela, 2010). Cognitive exercise or training is defined by Gates & Valenzuela (2010) as a standardized set of mental exercises that are repeated and focus on specific cognitive functions that progressively become more difficult. Benefits from cognitive training in older cognitively intact adults remains controversial, however longitudinal RCTs implementing cognitive training in older adults without cognitive impairment have been shown to slow the rate of age-related cognitive decline (Gates & Valenzuela, 2010).

In a large scale study by Ball et al (2002), older adults without cognitive impairment were randomized into either training for memory (verbal episodic memory), reasoning (problem solving serial patterns), speed of processing (visual search and identification) or a control group. Findings showed that cognitive interventions improved normal elderly

individuals' performance on the cognitive tasks for which they were trained. Although the impact of training had decreased after 2 years, participants in the cognitive intervention groups had significantly better cognitive skills than individuals in the control group.

Likewise, Klusmann et al (2010) found that 6-months of cognitive training (cognitive computer course) had similar effects to that of the exercise intervention (aerobic, resistance and flexibility training) on episodic memory in older women with MCI. They conclude that different activity types (mental activity or exercise) are equally suitable for maintaining cognitive performance in older women with MCI. Multimodal cognitive training has also been suggested to provide greater benefit than unimodal (Gates & Valenzuela, 2010) which demonstrates the importance of researching interventions that combine exercise with cognitive training.

### **Square-Stepping Exercise**

The square-stepping exercise (SSE) program was developed by Shigematsu & Okura in Japan as a fall prevention strategy to aid participant balance and reduce the incidence of falls in the elderly (Shigematsu & Okura, 2006). SSE was found to be as effective, if not more so, than strength and balance training when preventing falls in older adults (Shigematsu et al., 2008) and is now starting to show the possibility of improving cognitive status among older adults. SSE is a form of mind-motor exercise, which combines both cognitive and mild exercise tasks. SSE is low cost, easily administered and social group-based exercise intervention that can be described as a visuo-spatial working memory task with a stepping response. One recent study by Teixeira et al (2013) revealed positive influences on global cognitive function (MMSE score), concentrated attention (Toulouse-Pierón Attention Test) and mental flexibility (Modified Card Sorting Test) following 16-weeks of SSE (40 minute sessions, 3 days per week) among older adults.

These findings were supported by a study by Gill et al (2016), who found that following a 26-week intervention (exercise and dual task), there were improvements in global cognitive function when compared to control (exercise only). Both exercise groups performed either a minimum of 50 minutes (classes 2 times per week) to a maximum of 75 minutes (classes 3 times per week) of aerobic exercise. In combination with the aerobic exercise, both groups took part in beginner-level SSE (45 minutes per week, 2 to

3 days per week). In addition to the beginner-level SSE, the dual-task group were required to respond to cognitively challenging questions while participating in SSE. These results indicate that there are further improvements in cognitive function when combining a dual task with exercise. Improvements however were not seen at 12-weeks nor 26-weeks following the completion of the intervention. SSE as a form of cognitive training is a recent development, and few studies have investigated its effect on cognition. Currently there have been no reports in the literature observing the effects of SSE on functional fitness, such as HR and BP (Teixeira et al., 2013) nor have there been any studies on the effects of SSE on HRV. More high-quality studies are needed to assess the effects of SSE on HRV in older adults experiencing cognitive decline.

### **1.7 Summary**

The current literature review highlights areas of research that are lacking and require further investigation. As previously stated, the effect of exercise on alleviating cognitive decline in T2D remains limited and incongruous. The presence of T2D has been previously linked to autonomic dysfunction, however to date, there is a lack of research and mixed findings to show whether or not low HRV, as seen in T2D, is associated with cognitive impairments and dementia. An improvement in HRV has been observed in traditional aerobic and resistance exercises in clinical populations, however little research has been done on novel cognitive exercises such as the SSE. The SSE is a relatively new group-based exercise program that has only recently been implemented in cognitive research. The SSE is beneficial through its multifaceted impact on cognition, not only combining visuo-spatial memory tasks with mild exercise, but ascertaining social benefits through a group environment. It is believed that such a novel cognitive exercise, suitable for older and disease populations, can be seen to develop new steps in addressing the prominent issues related to mitigating autonomic dysfunction and cognitive decline.

## Chapter 2

### **The effect of a square-stepping exercise intervention on heart rate variability in older individuals with type 2 diabetes and subjective cognitive complaints**

#### **2.1 Introduction**

The findings from the aforementioned literature demonstrate the necessity for an intervention-based study in older diabetic adults. Therefore, the primary objective of this RCT was to investigate HRV parameters (time and frequency domains) in community-dwelling older adults with T2D and subjective cognitive complaints following a 24-week SSE intervention. A secondary objective was explored to determine if there was a relationship between HRV parameters (HR and RMSSD) and global cognitive function following 24-weeks of SSE training. Lastly, a tertiary objective was used to examine the effects of this novel intervention on other CVD risk factors associated with T2D, such as resting BP. It was hypothesized that HRV parameters representative of predominant SNS activity would be high and HRV parameters representative of PNS activity would be low in all participants at baseline. Following 24-weeks of SSE training, it was further hypothesized that the intervention group would shift towards predominant vagal modulation (increased RMSSD and HF and decreased HR, SDNN, LF and LF/HF), with no change in the control group (Task Force of the European Society of Cardiology, 1996). These shifts towards vagal modulation were speculated to only be observed in the sitting and standing postures due to the physical challenges imposed by gravity (Michel-Chávez et al., 2015). It was hypothesized that RMSSD (a measure chosen to represent predominant PNS activity) would exhibit a positive correlation with global cognitive function following 24-weeks of SSE training, with no change in the WL group. Contrarily, HR (a measure chosen to represent predominant SNS activity) would exhibit a negative correlation with global cognitive function following 24-weeks in the SSE group with no change in the WL group. Finally, resting SBP and DBP were predicted to decrease following 24-weeks in the SSE group, with no change in the WL group (Cornelissen & Fagard, 2005).



## **2.2 Methods**

The current study was a pilot-study of a larger 2-site RCT conducted out of London and Woodstock, Ontario, Canada. The larger study was designed to investigate the effects of a square-stepping exercise on global cognitive function and was cleared by the Health Sciences Research Ethics Board at Western University (REB# 105883; Appendix A; Appendix B; Appendix C). All participants provided written informed consent prior to enrolling in the study. Outcome measures were recorded at baseline, following 12-weeks (interim) and 24-weeks (intervention end point) of the exercise intervention.

### **Participants**

Twenty-five older adults over the age of 50 years (range: 54 to 82 years; 8 women) with stable T2D and self-reported cognitive complaints were recruited from both sites (London and Woodstock). Participants were recruited using a variety of methods including posters, newspapers, electronic medical records, local stress test database, previous study lists (i.e., approved by ethics to go back to individuals from former studies) and a community-base, hospital supported Primary Care Diabetes Program. Once informed consent was completed and the participants were deemed eligible through a screening process, participants were randomized. A randomization stratification of 1:1 was used and balanced at every 4 participants. The person who generated the sequence was independent of those doing the allocation. Allocation involved concealed envelopes, to keep the randomization unknown to the assessors until after the screening process.

### **Study Design**

This study was designed as a 24-week RCT, whereby participants were randomized into either a SSE intervention or a WL control group. Participants randomized into the intervention group took part in 24-weeks of SSE. The WL group did not receive supervised exercise sessions during the initial 24-week period. Participants were instructed to continue their daily routines, and keep their eating and exercise habits the same. Following the initial 24-week period, the WL group began the 24-week SSE program.

The study was broken down into three waves to enroll more participants into the RCT. Waves 1 and 2, containing twelve and six participants respectively, were held in London. Wave 3, containing seven participants, was located in Woodstock. All participants from both London and Woodstock were included in the same analyses.

### **Location**

All assessments for Waves 1 and 2 were held in London and all assessments for Wave 3 were held in Woodstock. Screening and baseline assessments were conducted at St. Joseph's Primary Care Diabetes Clinic (London), the University of Western Ontario (London) and the Salvation Army Community Church (Woodstock). Final assessments were conducted at the University of Western Ontario and the Salvation Army Community Church. The exercise classes were held at the Stoney Creek Young Men's Christian Association (YMCA) in London and the Woodstock YMCA.

### **Screening**

Screening was implemented to ensure subjects met the correct inclusion criteria. Screening comprised of reading the study's letter of information (LOI) and consenting documentation. Tests and questionnaires including the MMSE (as an assessment of cognitive function) and the CES-D (to determine the presence of severe depression) were administered. Participants were also required to agree to attend a minimum of 75% of sessions.

### **Inclusion/Exclusion Criteria**

Participants were deemed eligible to participate in the study if they were 50 years of age or older, had stable T2D, and self-reported a cognitive complaint. Stable diabetes was defined as having an HbA<sub>1c</sub> of <9.0mmol/L (at the advice of Dr. Sonja Reichert and Dr. Stewart Harris) for the past 6 months (last 2 readings). These values were verified using electronic medical records. A self-reported cognitive complaint was defined as believing that one's thinking and/or memory had gotten worse recently (recently was subjective). Participants were excluded if they displayed any of the following criteria: clinical depression (CES-D score  $\geq 16$  needed confirmation from a physician), diagnosis of dementia, diagnosis of other neurological disorders (i.e. Parkinson's disease), severe

history of CV event (within the past 12 months), severe orthopedic limitations that could impair ability to exercise, uncontrolled diabetes ( $HbA_{1c} >9.0\text{mmol/L}$ ), unstable angina, untreated retinopathy, foot ulcers with or without severe peripheral neuropathy, severe sensory impairment and the inability to commit to 75% of sessions. Those eligible for the study, continued on to baseline assessments.

### **Assessments**

In addition to the following assessments, participants were taken through a battery of measures that pertained to the larger study outcomes, and will not be discussed in the current paper. A flowchart of assessments pertaining to the current study can be found in Figure 6. Participants were termed “non-compliant” if they did not attend assessments at 24-weeks.

#### *Baseline Assessment*

Demographic information including age, sex, ethnicity, marital status, general health (smoking status, drinks consumed in a typical week), duration of T2D, duration of subjective cognitive complaint, years of education, number of medications and medical history/conditions were obtained. The Montreal Cognitive Assessment (MOCA) and the clock drawing test were administered as part of the cognitive assessments.

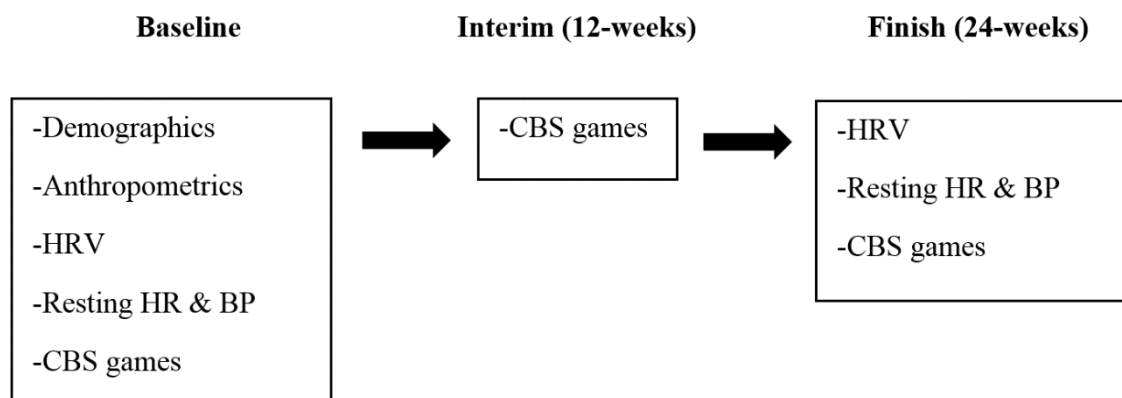
Anthropometric measures including weight, height, body mass index (BMI), and waist circumference were recorded. Participants then underwent the HRV protocol and resting HR and BP measures. Finally, participants were introduced to twelve Cambridge Brain Sciences (CBS) computer games. Further explanations for anthropometric measures, HRV and CBS games are located below in the *Instruments and Measures* section. The baseline assessments lasted approximately 1.5 hours.

#### *Interim Assessment*

CBS was the only assessment conducted at 12-weeks for the current study.

#### *Final Assessment*

A list of each participant’s medication was updated at final assessments. The CBS games, HRV and resting HR and BP were collected.



**Figure 6: Assessment flowchart**—Assessments collected at baseline, interim and completion of study.

## Instruments and Measures

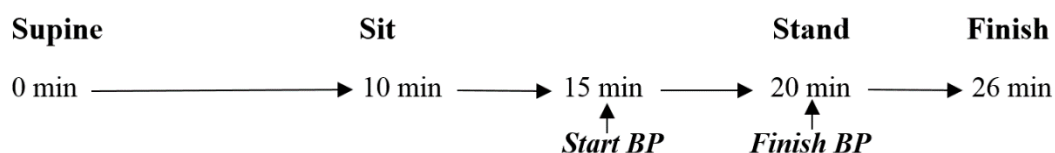
### *Anthropometrics*

Anthropometric measurements included the participant's height (m), weight (kg), waist circumference (cm) and body mass index ( $\text{kg}/\text{m}^2$ ). Height was measured using a stadiometer and weight was measured using a standard scale. Measurements were taken with clothing (large sweaters and coats were removed) and without shoes. According to the Canadian Diabetes Association Clinical Practice Guidelines, waist circumference was measured by placing a measuring tape just above the lateral border of the iliac crest, and instructing the participant to take two normal breaths. The measuring tape was tightened until snug upon the second exhale (Managing Weight & Diabetes, 2013). If the difference between the first two measures was greater than 0.5cm, a third measure was taken, and the three recordings were averaged. BMI was calculated using height and weight measurements.

### *Heart Rate Variability and Blood Pressure*

Participants were instructed to stand and lift their shirt while their chest was scrubbed with an alcohol swab along the ribs at the level of the xiphoid process. The Suunto Memory Belt, containing 2 electrodes on the posterior surface, was wet with water and placed snugly around the chest directly on the skin (Suunto User's Guide, 2014). The participant was instructed to sit on the examining table while an automated sphygmomanometer (BpTRU<sup>TM</sup>) was placed over the brachial artery on either the left or

right arm before lying down to begin the supine-sit-stand protocol. The protocol consisted of 10 minutes of supine, 10 minutes of sitting and 6 minutes of standing (Figure 7). Protocol times were established to provide accurate HRV recordings in the final 5 minutes of each position while excluding transitional influences. Standing time was set to 6 minutes because the orthostatic HR response is stabilized after approximately one minute of standing (Finucane et al., 2014) and for some participants it would have been difficult to remain standing for 10 minutes.



**Figure 7: Timeline of HRV protocol**—10 minutes of supine, 10 minutes of sitting, 6 minutes of standing.

Participants were instructed to stay as still as possible throughout the protocol and to breathe normally. Three resting BP and HR measures were taken halfway through the sitting position (at 15 minutes); readings were taken 2 minutes apart (Quinn et al., 2010). Recorded resting BP was measured as the average of the final two SBP readings over the average of the final two DBP readings. Resting HR was recorded as the average of the final two readings.

Following the supine-sit-stand protocol, the following five questions were asked and answers were recorded on a datasheet:

- i. Have you consumed a large meal within the past 4 hours?
- ii. Have you had caffeine within the past 12 hours?
- iii. Have you smoked within the past 12 hours?
- iv. Have you performed vigorous exercise within the past 24 hours?
- v. Have you consumed alcohol within the past 24 hours?

The Suunto Memory Belt was then removed and the data was transferred to a personal computer (PC) using the Suunto Memory Belt docking station. The data was then retrieved from movescount.com as an Excel file and imported to the HEARTS software (Heart Signal Co., Oulu, Finland) for further HRV analysis.

All RRI time series were visually scanned for ectopic beats and artefacts before being subjected to HRV analysis. During editing, segments consisting of 10 or more consecutive beats ectopic or non-sinus beats were deleted, while segments less than 10 consecutive beats were interpolated (replaced with the average). Following editing, if the number of qualified beats (beats that were not subjected to editing) was less than 90%, the participant was excluded from analysis. HRV recordings were separated into 5 minute segments, including the last 5 minutes from each position. Spectral analysis was performed on each segment.

Six HRV parameters were obtained for all three postural positions and used in analyses: heart rate (beats/min), standard deviation of normal-to-normal R-R intervals (ms), root mean square of successive R-R interval differences (ms), low frequency ( $\text{ms}^2$ ), high frequency ( $\text{ms}^2$ ) and the low frequency/high frequency ratio. For the correlation between HRV parameters and GCF, only HR and RMSSD were used in analyses.

#### *Cambridge Brain Sciences*

Global cognitive function, as well as domain-specific cognitive function was calculated using the CBS games. The CBS games were developed by Dr. Adam Hampshire and Dr. Adrian Owen and designed to effectively evaluate cognitive function in large-scale, population-based studies (Hampshire et al., 2012). The CBS consists of 12 games that test memory (Monkey Ladder, Spatial Span Blocks, Digit Span, Paired Associates Task), concentration (Rotation Task, Feature Match Task, Polygon Task), reasoning (Grammatical Reasoning Task, Double Trouble, Odd One Out) and planning (Hampshire Tree Task, Token Search) (Hampshire et al., 2012).

Participants went through CBS assessments which took approximately one hour to complete. At baseline, participants went through familiarization with supervision from one of the research assistants. The purpose of familiarization was to get participants

acquainted with the games but also to prevent any learning effects that might occur due to the equipment. The games were randomly generated and constantly changed, so test-related practice effects were eliminated (Hampshire et al., 2012). The CBS familiarization was done using a mouse on a Toshiba PC laptop with the volume turned up so that participants could hear the game cues. Subsequent CBS familiarization was unsupervised and completed at the home of the participant. In the event of a participant experiencing technical difficulties or not owning a computer with access to the internet, arrangements were made to complete the games on the study laptop.

For CBS assessments, an email was sent to each participant with a link to the CBSTrials website. Participants logged in using their email address and clicked “Play now”. Participants played all twelve games two times. The first round was for familiarization of the games and the second round was scored. Instructions appeared before each game commenced and participants were instructed to complete all 12 tasks in one sitting. The CBS tasks were expected to be completed within three days of the assessment, if not, a follow-up email was sent as a reminder.

Composite scores for each cognitive domain was derived as follows: i) converting scores from each game into standardized z-scores (subtracting baseline group mean from the raw score and dividing by the baseline group standard deviation); ii) within each domain (memory, concentration, reasoning and planning), averaging game standardized scores to create domain-specific standardized scores; iii) the four domain-specific standardized scores were then averaged to create a global cognitive function (GCF) score (Monsell et al., 2012).

### **Stepping Exercise Intervention**

The SSE interventions took place between June 2015 and May 2016. Wave 1 was from June to December 2015; Wave 2 was from October 2015 to April 2016; and Wave 3 was from December 2015 to May 2016. The intervention group attended 60 minute square-stepping exercise sessions, twice a week on Tuesdays and Thursdays, for 24-weeks. Each session was broken up into 5-10 minutes of gentle warm-up suitable for diabetic patients, 45 minutes of SSE, and 5 minutes of cool-down. SSE is a form of mind-motor exercise performed on a gridded floor mat (250cm by 100cm; partitioned in to 10 rows of 4 equal-

sized squares), which combines cognitive tasks and mild exercise (Figure 8). A step pattern is demonstrated to the participants, who are then required to memorize and reproduce the pattern. Step patterns progressively become more difficult.

Each pattern was required to be completed 4 times error-free by each participant, or to be completed 4 times error-free by 80% of the group, before moving on to the next pattern. If the participants were unable to recreate the pattern after the first demonstration, the pattern was repeated. If after a second demonstration the pattern was still not understood, pauses would be added between each step sequence. If the pattern was not understood following the pause, the steps were counted out loud for the participants. The sequences could consist of forward, backward, lateral and diagonal steps, which became more complicated as the exercise progressed (Figure 9). SSE participants were not allowed to view the clipboard that had the stepping patterns on it, nor were they allowed to review the patterns while they were not on the mat. While performing a pattern, support from other participants was encouraged. The idea was to keep the participants motivated and aid in adherence to the program. The program sequences were organized into Beginner, Intermediate and Advanced, each containing multiple sublevels (Appendix D). If all SSE patterns were performed before study completion at 24-weeks, existing patterns were recycled and randomized in Microsoft Excel to generate a pattern new order. All Advanced sublevels were included in the randomization i.e. Advanced 1-17, Advanced 2-3, Advanced 3-21.



**Figure 8: Square-stepping exercise**—Gridded floor mats used for the square-stepping exercise.



i) Beginner Pattern—focused on walking-like movements and simple steps									
Start Walk	2		4		2		4		Finish Walk
		3		6		3		6	
	1		5		1		5		
ii) Intermediate Pattern—incorporates forward, lateral and diagonal movements									
Start Walk	3	5	3	5	3	5	3	5	Finish Walk
	1	4	1	4	1	4	1	4	
	2	6	2	6	2	6	2	6	
iii) Advanced Pattern—a large number of steps per pattern with multiple movement types									
Start Walk									Finish Walk
	1	3	7	5	1	3	7	5	
	4	2	6	8	4	2	6	8	

**Figure 9: Pattern examples**—Beginner, intermediate and advanced square-stepping patterns.

### Statistical Analysis

Statistical analysis was completed using IBM SPSS software version 23 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at  $p \leq 0.05$  and reported if it was met or was trending towards significance. Trending towards significance was characterized as ( $p=0.05-<0.10$ ). Baseline characteristics were compared between groups with independent sample t-tests for outcome measures with normal distribution or Mann-Whitney U tests for outcome measures that were not normally distributed. Non-parametric tests were used for outcomes measures with gross violations of normality and homogeneity of variance (HRV parameters and GCF domains). Mann-Whitney U tests were selected to determine if there were differences in parameters between groups at baseline and at 24-weeks. Wilcoxon Signed Ranks tests were used to examine changes over time and were employed separately for the intervention and control groups. Outcome measures that did not violate assumptions (SBP and DBP), used independent sample t-tests to examine differences between groups at each time point, and dependent sample t-tests to examine the changes from baseline to 24-weeks for each group.

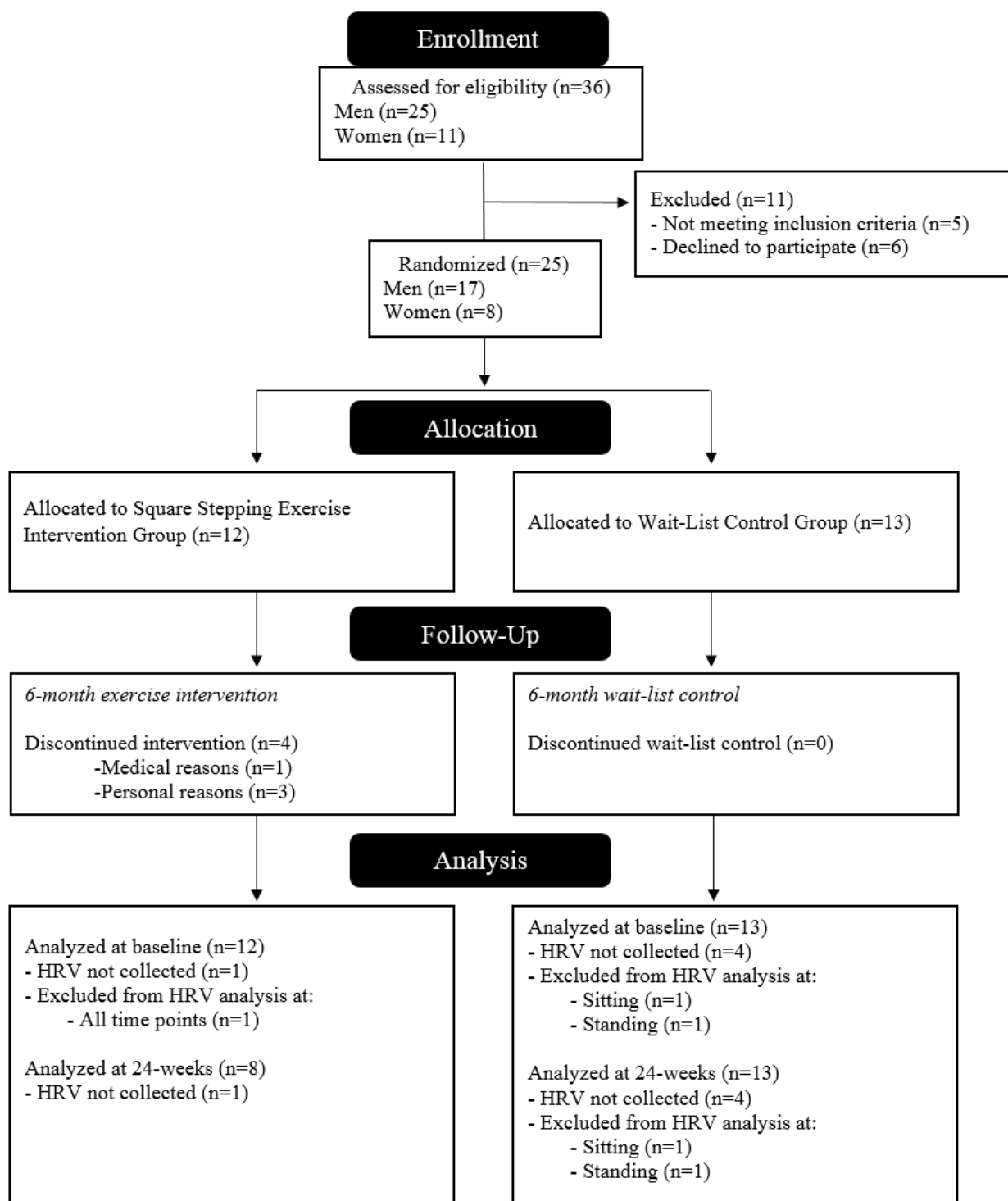
The correlation of HRV and GCF was performed using the Spearman ( $\rho$ ). Differences between groups were also examined in mean change from baseline to 24-weeks. Outliers were assessed using studentized residuals greater than  $\pm 3$  standard deviations and were removed prior to analysis. Estimates of effect sizes were calculated for t-tests ( $\eta^2$ ), and

Mann-Whitney U and Wilcoxon Signed Ranks tests,  $r = Z/\sqrt{N}$ . All results are shown as mean  $\pm$  (standard deviation; SD) for normally distributed data, and median (interquartile range; IQR) for non-parametric data.

## **2.3 Results**

### **Participant Flow**

Recruitment took place between February 2015 and December 2015. Figure 10 shows the flow of participants through the study. A total of 36 potential participants were assessed for eligibility. 11 individuals were excluded from the study due to not meeting inclusion criteria [clinical depression (n=3); orthopedic limitation (n=1); absence of a cognitive complaint (n=1)] or declining to participate (n=6). A total of 25 participants were randomized into the current study and allocated into either the square-stepping intervention (n=12) or the wait-list control group (n=13). Throughout the 24-weeks, 4 participants allocated to the square-stepping intervention group discontinued due to medical reasons (n=1) or personal reasons (n=3). There were no dropouts from the control group. HRV was not collected for 5 participants (HR monitors were unable to detect sinus rhythm). After removal of incomplete data, 19 participants were included in the final HRV analysis; 10 in the intervention and 9 in the control.



**Figure 10: Participant Flowchart**

### **Baseline Characteristics**

Participant baseline characteristics are located in Table 1 and presented as follows.

Overall, 68.0% of participants were men, with a mean age of 68.7 years. 92.0% of participants were White and had an average education of 13.5 years. Average HR was 72.2beats/min; average SBP was 134.0mmHg; and average DBP was 78.2mmHg.

Participant global cognitive function averaged a mean score of 28.7 on the MMSE and 25.6 on the MOCA. The average of total medications taken per person was 8.0. Out of all the participants who were taking medication, 88.0% were on diabetes medication, 80.0% were on hypertension medication and 84.0% were on cholesterol medication. There was a significant age difference between the intervention and control groups at baseline,  $t(23) = 2.152$ ,  $p = 0.042$ .

### **Intervention Characteristics**

Attendance was recorded at all sessions, which was used to calculate compliance to the SSE intervention. Attendance was calculated as the percentage of classes attended (2 classes per week) over the 24-week period. Session attendance for all waves of the SSE intervention group, excluding dropouts, was 52.2%.

Follow-up of the study at 24-weeks was 66.7% for the participants in the SSE intervention group (4 out of 12 participants dropped out before final assessments), while follow-up in the wait-list control group was 100% (all 13 participants completed final assessments).

In total, 7 participants (28.0%) experienced adverse events during the initial 24-week period of the square-stepping intervention. In the intervention group, a total of 10 adverse events were reported from 6 participants while the wait-list control group had one adverse event reported. Adverse events included back injuries ( $n=3$ ), neck strain/headaches ( $n=1$ ), knee injury ( $n=1$ ), dizziness ( $n=1$ ), bowel obstruction ( $n=1$ ) and general pain/illness ( $n=4$ ). Aside from the neck strain/headaches adverse event, all other adverse events were not caused by involvement in the current study. All participants recovered during the intervention period without further issues.

**Table 1: Baseline participant characteristics**— Values are reported as mean (SD) unless otherwise stated.

Demographics	All Participants (n=25)	SSE Intervention Group (n=12)	Wait-List Control Group (n=13)
Age (years)	68.7 (6.6)	65.9 (5.2)	71.2 (6.9) *
Male sex, no. (%)	17 (68.0)	8 (66.7)	9 (69.2)
White race, no. (%)	23 (92.0)	10 (83.3)	13 (100)
Education (years)	13.5 (2.3)	13.7 (2.5)	13.4 (2.1)
Height (m)	1.7 (0.1)	1.7 (1.0)	1.7 (0.1)
Weight (kg)	91.4 (15.8)	94.5 (19.6)	88.5 (11.4)
BMI (kg/m <sup>2</sup> )	32.6 (4.7)	33.3 (4.8)	32.0 (4.6)
WC (cm)	112.2 (12.2)	113.6 (15.4)	110.9 (8.9)
HR (beats/min)	72.2 (11.2)	75.9 (6.8)	68.8 (13.4)
SBP (mmHg)	134.0 (16.9)	131.7 (12.2)	136.5 (21.3)
DBP (mmHg)	78.2 (8.6)	79.9 (6.2)	76.5 (10.5)
CES-D (/60)	8.2 (7.8)	8.2 (8.4)	8.3 (7.5)
MMSE (/30)	28.7 (1.1)	28.9 (0.9)	28.5 (1.2)
MOCA (/30)	25.6 (2.7)	25.4 (2.7)	25.9 (2.7)
GCF domains (z-scores), Md (IQR)			
Memory	0.17 (1.34)	0.12 (1.47)	0.26 (1.38)
Concentration	-0.12 (1.05)	0.01 (1.16)	-0.21 (1.05)
Reasoning	0.03 (1.01)	-0.02 (1.21)	0.03 (1.09)
Planning	-0.02 (1.30)	-0.10 (1.17)	0.06 (1.35)
GCF	-0.02 (1.06)	-0.06 (1.18)	-0.02 (0.88)
Total medication, per person	8.0 (4.9)	7.0 (4.3)	9.0 (5.4)
Types of medication, no. (%)			
Diabetes	22 (88.0)	11 (91.7)	11 (84.6)
Hypertension	20 (80.0)	8 (66.7)	12 (92.3)
High cholesterol	21 (84.0)	9 (75.0)	12 (92.3)
Medical history, no. (%)			
Cardiac	9 (36.0)	5 (41.7)	4 (30.8)
Cancer	5 (20.0)	3 (25.0)	2 (15.4)

\*statistically significant difference between groups  $p \leq 0.05$

Outcome measures that were normally distributed used independent t-tests to determine if there were significant differences between groups at baseline.

Global cognitive function domains were not normally distributed, therefore Mann-Whitney U tests was used to determine if there were significant differences between groups at baseline (i.e. global cognitive function domains).

Key: BMI, body mass index; WC, waist circumference; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; CES-D, center of epidemiologic studies depression scale; MMSE, mini mental state examination; MOCA, Montreal cognitive assessment; GCF, global cognitive function; Md, median; IQR, interquartile range

### **Primary Outcome: Heart Rate Variability**

As previously reported in Table 1, there was a significant difference in age between the SSE intervention group and the WL control group. After removing the five participants who could not undergo HRV analysis, due to the HR monitor's inability to detect sinus rhythm, age was no longer found to be significantly different between groups,  $t(18) = 1.286$ ,  $p = 0.215$ .

No significant differences were found between groups at baseline and at 24-weeks, however HR in both the supine and standing positions were trending to be significantly higher in the SSE group compared to the WL group at baseline,  $U = 22.0$ ,  $Z = -1.885$ ,  $p = 0.059$ ,  $r = 0.43$  and  $U = 21$ ,  $Z = -1.729$ ,  $p = 0.084$ ,  $r = 0.41$ , respectively. No significant differences were found in mean change between groups.

Results for changes in HRV parameters from baseline to 24-weeks for the supine, sitting and standing positions are shown in Table 2, Table 3 and Table 4 respectively. A Wilcoxon Signed Ranks test revealed a statistically significant reduction in standing HR in the SSE group over 24-weeks,  $Z = -1.997$ ,  $p = 0.046$ ,  $r = 0.50$  (Figure 11). Significant reductions were also found in sitting LF (Figure 12) and HF (Figure 13) in the WL group over 24-weeks,  $Z = -1.960$ ,  $p = 0.05$ ,  $r = 0.48$  and  $Z = -2.028$ ,  $p = 0.043$ ,  $r = 0.49$ , respectively. Although not significant, the following were trending towards significance: i) a reduction in supine LF/HF in the SSE group over 24-weeks,  $Z = -1.859$ ,  $p = 0.063$ ,  $r = 0.45$ ; ii) a reduction in sitting SDNN in the WL group over 24-weeks,  $Z = -1.859$ ,  $p = 0.063$ ,  $r = 0.45$ ; iii) and reductions in supine and sitting RMSSD in the WL group over 24-weeks,  $Z = -1.690$ ,  $p = 0.091$ ,  $r = 0.44$  and  $Z = -1.820$ ,  $p = 0.069$ ,  $r = 0.44$ , respectively.

**Table 2: Changes in heart rate variability in the supine position from baseline to 24-weeks**— Values are reported as median (IQR).

<b>Variables</b>	<b>Baseline</b>	<b>24-weeks</b>	<b>Within Groups <math>\Delta P</math></b>	<b>Between Groups <math>\Delta P</math></b>
<b>HR (b/min)</b>				
SSE	76.0 (13.0)	72.0 (8.0)	0.734	
WL	66.0 (17.0)	68.0 (21.0)	0.326	
SSE vs. WL	0.059			0.709
<b>SDNN (ms)</b>				
SSE	23.0 (19.8)	20.0 (15.0)	0.249	
WL	22.5 (29.5)	18.0 (12.3)	0.123	
SSE vs. WL	0.929			0.524
<b>RMSSD (ms<sup>2</sup>)</b>				
SSE	147.5 (315.5)	133.0 (196.0)	0.310	
WL	108.0 (213.0)	100.0 (57.5)	0.091	
SSE vs. WL	0.495			0.949
<b>LF (ms<sup>2</sup>)</b>				
SSE	110.0 (251.8)	53.0 (249.0)	0.237	
WL	76.0 (273.0)	64.5 (62.5)	0.176	
SSE vs. WL	0.495			0.949
<b>HF (ms<sup>2</sup>)</b>				
SSE	50.0 (113.0)	56.0 (88.0)	0.310	
WL	30.0 (34.0)	29.0 (31.3)	0.398	
SSE vs. WL	0.464			0.789
<b>LF/HF</b>				
SSE	1.5 (4.2)	1.1 (2.1)	0.063	
WL	1.2 (4.3)	2.2 (1.1)	0.767	
SSE vs. WL	0.462			0.491

Statistically significant  $p \leq 0.05$

Within groups  $\Delta$  was calculated with the Wilcoxon Signed Ranks test.

Between groups  $\Delta$  was calculated with the Mann-Whitney U test.

The difference between SSE vs. WL at baseline was calculated with the Mann-Whitney U test.

Key: HR, heart rate; SDNN, normal-to-normal R-R intervals; RMSSD, root mean square of successive R-R interval differences; LF, low frequency; HF, high frequency; SSE, square-stepping exercise intervention; WL, wait-list control

**Table 3: Changes in heart rate variability in the sitting position from baseline to 24-weeks** — Values are reported as median (IQR).

Variables	Baseline	24-weeks	Within Groups $\Delta P$	Between Groups $\Delta P$
<b>HR (b/min)</b>				
SSE	76.0 (8.5)	74.0 (7.0)	0.670	
WL	67.0 (24.5)	67.0 (24.5)	0.767	
SSE vs. WL	0.122			0.974
<b>SDNN (ms)</b>				
SSE	22.0 (10.5)	21.0 (7.0)	0.399	
WL	23.5 (18.8)	17.0 (9.5)	0.063	
SSE vs. WL	0.846			0.352
<b>RMSSD (ms<sup>2</sup>)</b>				
SSE	109.0 (309.5)	111.0 (169.0)	1.000	
WL	126.5 (178.75)	86.0 (176.5)	0.069	
SSE vs. WL	1.000			0.203
<b>LF (ms<sup>2</sup>)</b>				
SSE	124.0 (71.0)	73.0 (102.0)	0.866	
WL	95.5 (116.8)	70.0 (46.5)	<b>0.05</b>	
SSE vs. WL	0.962			0.165
<b>HF (ms<sup>2</sup>)</b>				
SSE	49.0 (134.0)	34.0 (96.0)	1.000	
WL	41.5 (54.5)	27.0 (31.5)	<b>0.043</b>	
SSE vs. WL	0.773			0.203
<b>LF/HF</b>				
SSE	1.4 (2.5)	1.4 (1.9)	0.612	
WL	2.0 (3.2)	2.2 (2.5)	0.767	
SSE vs. WL	0.825			0.427

Statistically significant  $p \leq 0.05$

Within groups  $\Delta$  was calculated with the Wilcoxon Signed Ranks test.

Between groups  $\Delta$  was calculated with the Mann-Whitney U test.

The difference between SSE vs. WL at baseline was calculated with the Mann-Whitney U test.

Key: HR, heart rate; SDNN, normal-to-normal R-R intervals; RMSSD, root mean square of successive R-R interval differences; LF, low frequency; HF, high frequency; SSE, square-stepping exercise intervention; WL, wait-list control



**Table 4: Changes in heart rate variability in the standing position from baseline to 24-weeks** — Values are reported as median (IQR).

<b>Variables</b>	<b>Baseline</b>	<b>24-weeks</b>	<b>Within Groups <math>\Delta P</math></b>	<b>Between Groups <math>\Delta P</math></b>
<b>HR (b/min)</b>				
SSE	81.0 (10.0)	79.0 (7.0)	<b>0.046</b>	
WL	70.0 (25.0)	73.0 (28.5)	0.813	
SSE vs. WL	0.084			0.426
<b>SDNN (ms)</b>				
SSE	21.0 (6.5)	17.0 (7.0)	0.340	
WL	23.0 (29.0)	15.0 (24.5)	0.213	
SSE vs. WL	0.825			0.958
<b>RMSSD (ms<sup>2</sup>)</b>				
SSE	91.5 (94.8)	91.0 (130.0)	0.600	
WL	75.0 (1443.8)	62.5 (89.0)	0.176	
SSE vs. WL	0.674			0.153
<b>LF (ms<sup>2</sup>)</b>				
SSE	70.0 (72.0)	74.0 (48.0)	0.735	
WL	61.0 (138.25)	40.5 (35.75)	0.208	
SSE vs. WL	0.773			0.183
<b>HF (ms<sup>2</sup>)</b>				
SSE	47.0 (48.5)	31.0 (48.0)	0.917	
WL	25.0 (84.8)	17.0 (18.8)	0.128	
SSE vs. WL	0.736			0.277
<b>LF/HF</b>				
SSE	2.3 (4.9)	1.9 (1.6)	0.463	
WL	2.3 (3.2)	2.4 (1.9)	0.859	
SSE vs. WL	0.566			0.480

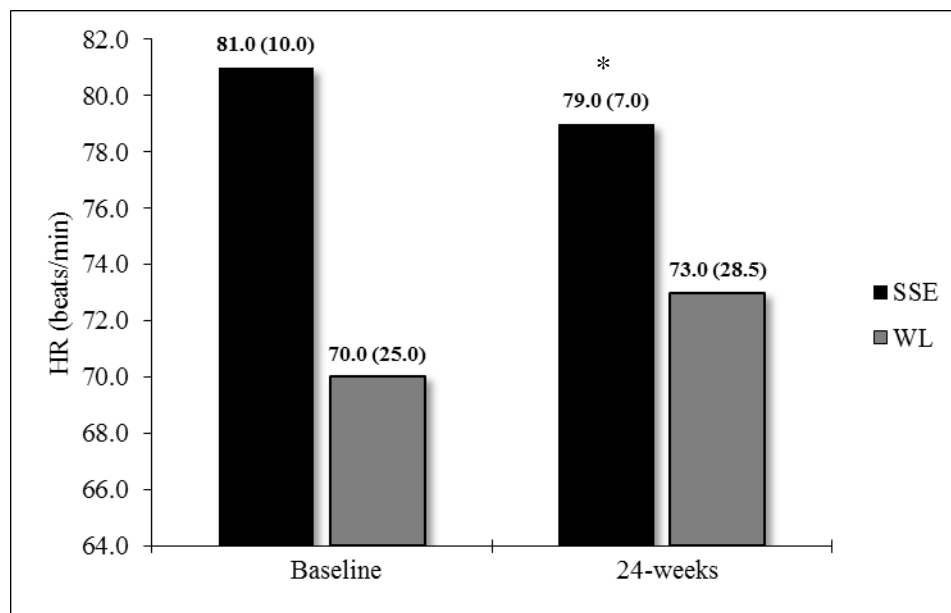
Statistically significant  $p \leq 0.05$

Within groups  $\Delta$  was calculated with the Wilcoxon Signed Ranks test.

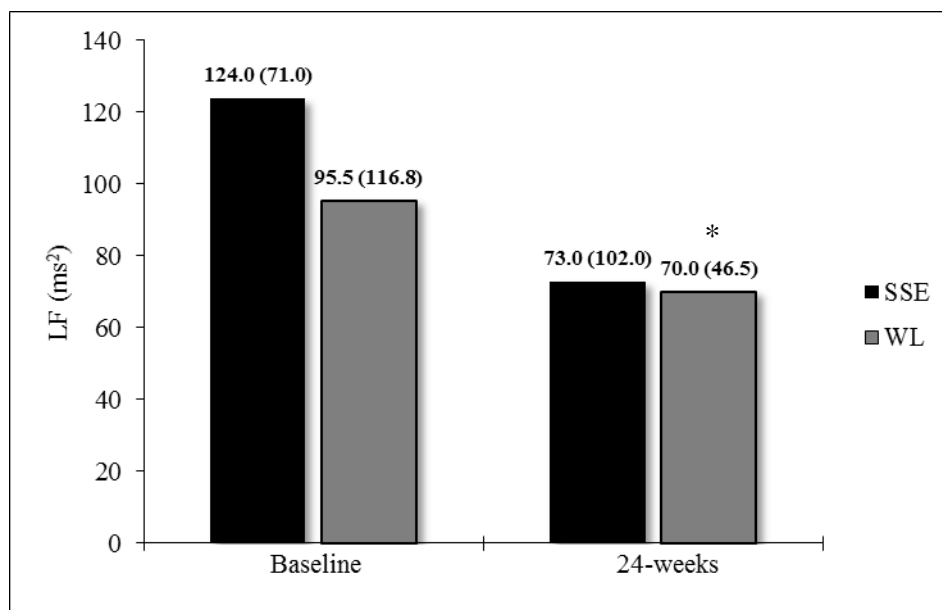
Between groups  $\Delta$  was calculated with the Mann-Whitney U test.

The difference between SSE vs. WL at baseline was calculated with the Mann-Whitney U test.

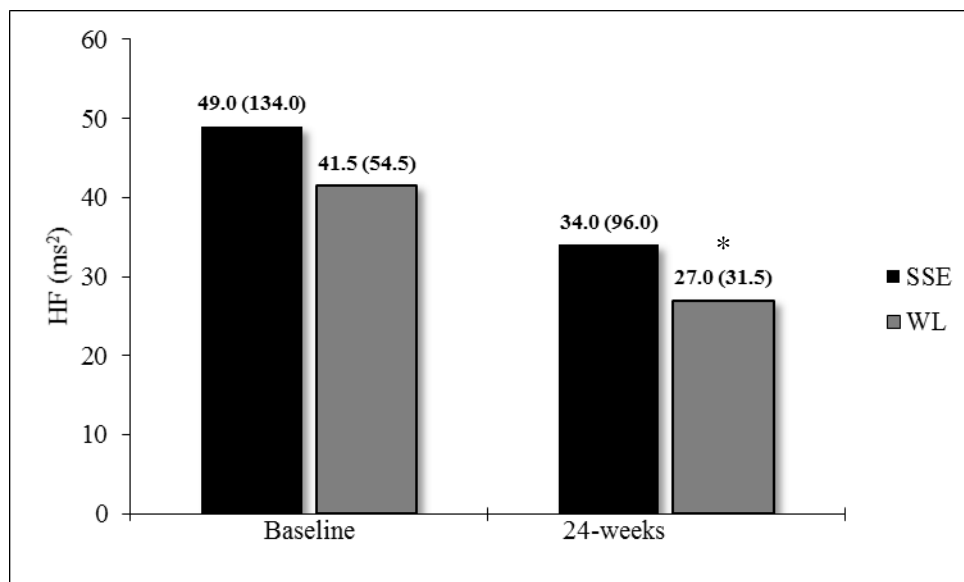
Key: HR, heart rate; SDNN, normal-to-normal R-R intervals; RMSSD, root mean square of successive R-R interval differences; LF, low frequency; HF, high frequency; SSE, square-stepping exercise intervention; WL, wait-list control



**Figure 11: Changes in heart rate in the standing position within groups at baseline and 24-weeks—** Values are reported as median (IQR); \*statistically significant differences to baseline  $p \leq 0.05$ ; HR, heart rate; WL, wait-list control; SSE, square-stepping exercise intervention



**Figure 12: Changes in low frequency in the sitting position within groups at baseline and 24-week—** Values are reported as median (IQR); \*statistically significant differences to baseline  $p \leq 0.05$ ; LF, low frequency; WL, wait-list control; SSE, square-stepping exercise intervention



**Figure 13: Changes in high frequency in the sitting position within groups at baseline and 24-week—** Values are reported as median (IQR); \*statistically significant differences to baseline  $p \leq 0.05$ ; HF, high frequency; WL, wait-list control; SSE, square-stepping exercise intervention

### **Secondary Outcome: Correlation between Heart Rate Variability and Global Cognitive Function**

A spearman correlation between HRV parameters (HR, RMSSD) and GCF showed no correlation for either group at baseline. At 24-weeks, there were large negative correlations in the WL group between RMSSD in both the sitting and standing positions versus GCF,  $\rho = -0.70$ ,  $p = 0.036$ ,  $n = 9$  and  $\rho = -0.76$ ,  $p = 0.031$ ,  $n = 8$ , respectively. Therefore, as RMSSD decreased, GCF increased. The coefficient of determination of RMSSD in the sitting position was 49%, which suggest that 49% of variability in one variable can be predicted by the other. Likewise for the coefficient of determination of RMSSD in the standing position was 58%, which suggest that 58% of variability in one variable can be predicted by the other. Also in the WL group at 24-weeks, a large positive correlation between HR in the sitting position versus GCF was trending towards significance,  $\rho = 0.61$ ,  $p = 0.081$ ,  $n = 9$ ; as HR increased, so did GCF. No significant correlations were found in the SSE group between HRV parameters versus GCF. No significant differences in mean change were found.

### Tertiary Outcome: Resting Blood Pressure

SBP and DBP did not differ statistically between groups at baseline or at 24-weeks. After running dependent sample t-tests for resting average BP, there was a significant decrease in the WL group for SBP from baseline (M = 138.1, SD = 21.7, n = 10) to 24-weeks (M = 122.0, SD = 20.4, n = 10),  $t(9) = 2.896$ ,  $p = 0.018$  (Table 5). The mean decrease in SBP (Figure 14) was 16.1mmHg (95% confidence interval, CI; 3.5 to 28.7), with an  $\eta^2$  (0.48) indicating a large effect size. Although not significant, DBP in the WL group was trending towards a significant decrease from baseline (M = 75.5, SD = 10.8, n = 10) to 24-weeks (M = 69.7, SD = 11.3, n = 10),  $t(9) = 2.251$ ,  $p = 0.051$ . The mean decrease in DBP was 5.8mmHg (95% CI -0.03 to 11.6), with an  $\eta^2$  (0.46) indicating a large effect size. There were no significant changes for resting average SBP and DBP from baseline to 24-weeks in the SSE group. Although no significant differences were found, the mean change in SBP between groups was trending towards significance,  $p = 0.091$ .

**Table 5: Changes in systolic and diastolic blood pressure from baseline to 24-weeks**— Values are reported as means (SD).

Variables	Baseline	24-weeks	Within Groups $\Delta P$	Between Groups $\Delta P$
<b>SBP (mmHg)</b>				
SSE	135.9 (14.3)	131.3 (13.4)	0.184	
WL	138.1(21.7)	122.0 (20.4)	<b>0.018</b>	
SSE vs. WL	0.523			0.091
<b>DBP (mmHg)</b>				
SSE	81.3 (7.8)	77.6 (9.9)	0.197	
WL	75.5 (10.8)	69.7 (11.3)	0.051	
SSE vs. WL	0.346			0.575

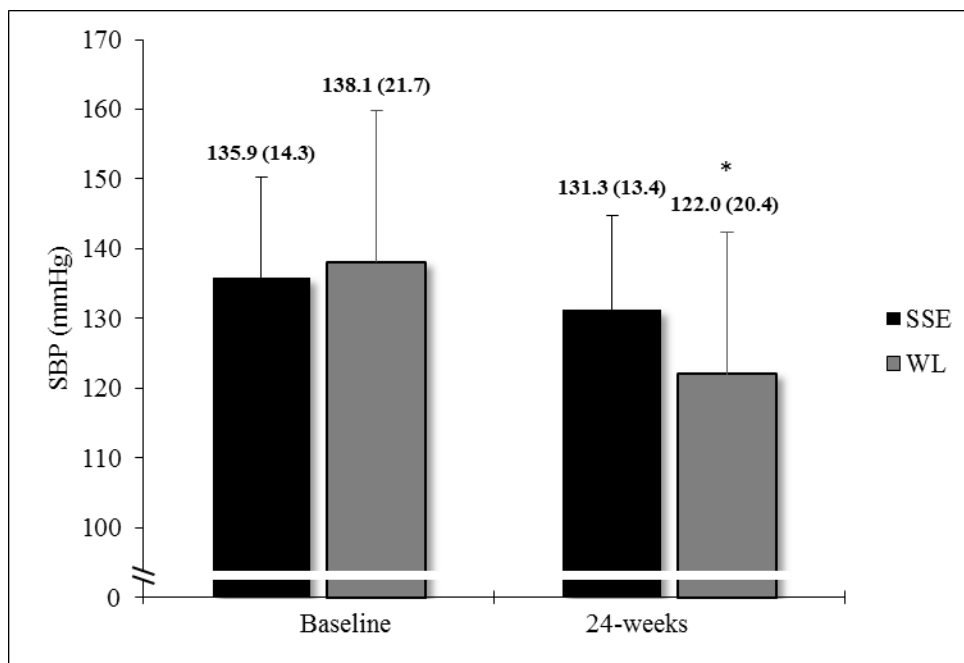
Statistically significant  $p \leq 0.05$

Within groups  $\Delta$  was calculated with dependent sample t-tests.

Between groups  $\Delta$  was calculated with independent sample t-tests.

The difference between SSE vs. WL at baseline was calculated with independent sample t-tests.

Key: SBP, systolic blood pressure; DBP, diastolic blood pressure; SSE, square-stepping exercise intervention; WL, wait-list control



**Figure 14: Changes in systolic blood pressure within groups at baseline and 24-week**—Values are reported as mean (SD); \*statistically significant differences to baseline  $p \leq 0.05$ ; SBP, systolic blood pressure; WL, wait-list control; SSE, square-stepping exercise intervention

## 2.4 Discussion

The primary research question was to elucidate whether a square-stepping exercise intervention could positively influence autonomic function, as measured by improved HRV, in older T2D adults. Five prominent and novel findings emerged from this study: 1) HR was significantly reduced in the standing position for the SSE group at 24-weeks compared to baseline; 2) LF was significantly reduced in the sitting position for the WL group at 24-weeks compared to baseline; 3) HF was significantly reduced in the sitting position for the WL group at 24-weeks compared to baseline; 4) there were large significant negative correlations between RMSSD in the sitting and standing positions versus GCF in the WL group at 24-weeks; and 5) resting SBP was significantly lower at 24-weeks compared to baseline in the WL group. Such findings are noteworthy because it is unclear as to why predominant vagal modulation was found in both the intervention and control.

Although studies examining the effects of exercise interventions on HRV in T2D are mixed, it was hypothesized that following SSE training there would be a shift towards vagal dominance (Sandercock et al., 2005); parameters representing predominant vagal modulation would increase (HF and RMSSD) and parameters representing sympathetic modulation (HR, SDNN, LF and LF/HF) would decrease (Tulppo et al., 2003). These shifts towards vagal dominance were predicted to only be observed in positions requiring greater BRS activation, such as sitting and standing (Michel-Chávez et al., 2015). Additionally, it was hypothesized that both SBP and DBP would be reduced following SSE training (Figueira et al., 2014). This investigation showed changes in HRV parameters and BP following SSE training that were unexpected.

### **Autonomic Responses during Postural Changes**

In the literature, exercise training lowers SNS activation, increases BRS and improves glycemic control in T2D (Loimaala et al., 2003). Following aerobic exercise, the autonomic balance is shifted towards vagal modulation and away from SNS modulation, thus improving HRV. Postural changes from supine to sit and stand still elicit SNS activation to compensate for the physical challenges imposed by gravity however, BP is stabilized faster and HRV parameters are stabilized back to a more vagally modulated homeostatic “set point” (Finucane et al., 2014; Zhang, 2007). In the current study, significant shifts towards vagal modulation were observed in the sitting and standing positions—resulting in improved HRV (increased HF and reduced LF and HR). Improvements in HRV may not have been observed in supine due to a lack of SNS modulation and baroreflex activation required for that position. These findings are consistent with the literature, as shifts towards vagal modulation have only been observed in positions that required greater SNS stimulation with corresponding vagal withdrawal (Hnatkova et al., 2013)

### **A Shift towards Vagal Modulation**

No statistical interaction was discovered between groups at 24-weeks for any HRV parameter, however, a significant decrease in HR was observed in the SSE intervention group following 24-weeks of SSE training. This observation is in line with both our hypothesis and the literature. HR has been considered a crude measure of autonomic

imbalance, and numerous studies have shown that a higher resting HR is associated with increased mortality and morbidity (Koenig et al., 2016). It has been suggested that aerobic exercise protects the heart against harmful cardiac events by increasing vagal tone and decreasing SNS activity (Hautala et al., 2004). Our findings suggest that the shift towards vagal dominance, as demonstrated by reduced HR, may also apply to cognitive exercises.

HRV parameters considered reflective of vagal activity under resting conditions (RMSSD and HF) showed no significant change from baseline to 24-weeks in the SSE group for the current study. A meta-analysis on the effects of exercise in HRV by Sandercock et al (2005) concluded that the change in HF power due to exercise training was homogeneously increased across all studies. There were no significant changes in HF for any of the postures in the SSE intervention group; however, a significant decrease in HF was found in the WL group. Therefore, one could argue that following 24-weeks of SSE training, the SSE group maintained HF power while the WL group showed significant reductions. The ability of the SSE group to maintain HF is indicative of a shift towards vagal modulation and improved HRV. Interestingly, Sandercock et al (2005) also demonstrated that old and middle-aged subjects showed small and moderate effect sizes of improved HF, respectively. This may suggest a reduced trainability of the heart and associated neural input with age. A reduced trainability in older adults may explain why HF power was maintained, rather than improved—especially given the older population recruited for the current study (54 to 82 years). Moreover, the meta-analysis excluded studies with disease populations, therefore omitting T2D populations in which trainability may further be reduced (Sandercock et al., 2005).

Furthermore, in a study by Zoppini et al (2007) examining HRV in the supine and standing positions following a 6 month supervised aerobic exercise in sedentary T2D adults, HF was found to be significantly increased and LF significantly decreased following exercise in the standing position. No significant results were found in the supine position. They concluded that the LF/HF ratio, which is reflective of sympathovagal balance, was markedly decreased after exercise (Zoppini et al., 2007). These results are in line with the current study, where LF was decreased and HF was increased in the sitting position after 24-weeks. However, contrary to the hypothesis

stating that the shift towards vagal modulation would be seen in the SSE intervention group, it was observed in the WL control group at 24-weeks.

### **Correlation between Heart Rate Variability and Global Cognitive Function**

Though the associations between HRV and GCF following cognitive exercise training in a diabetic population has never before been studied, a recent meta-analysis on perseverative cognition (the rumination about the past and worries about the future) in healthy subjects by Ottaviani et al (2015) may provide insight into the effects of HRV on cognitive health. To reiterate, a subjective cognitive complaint is the belief that one's thinking and/or memory has gotten worse recently. Worrying about worsening memory or cognition may further increase the risk for dementia in the T2D population (Xu et al., 2010). Analysis of eight studies showed an association between perseverative cognition and decreased HRV (Ottaviani et al., 2015). Although the meta-analysis did not divulge which HRV parameters were decreased, the negative correlation between perseverative cognition and HRV are in line with the findings of the current study, and may explain the significant correlation between reduced RMSSD and improved GCF. Further research is require to investigate the relationship between HRV parameters and cognition in disease populations.

### **Resting Blood Pressure**

Historically, aerobic exercise training has been shown to reduce both SBP and DBP in T2D individuals (Figueira et al., 2014). Additionally, reductions in BP are known to be additive in reducing the chronic complications of T2D (Figueira et al., 2014). A meta-analysis by Cornelissen & Fagard (2005) examined resting BP and its regulating mechanisms in RCTs. In individual studies, the average net changes following aerobic exercise in resting BP ranged from -20.0 to +9.0mmHg for SBP and -11.0 to +11.3mmHg for DBP. The overall net effect on BP was -3.0/-2.4mmHg, with the greatest effect observed in hypertensive individuals (Cornelissen & Fagard, 2005; Figueira et al., 2014). Similarly, results from the current study showed significant reductions in SBP from baseline to 24-weeks, while DBP trended towards significance ( $p = 0.051$ ). The reductions in resting SBP and DBP for the current study, -16.1mmHg and -5.8mmHg respectively, fall within the range reported in the literature. Although these reductions in



BP were expected at 24-weeks, it was hypothesized that the SSE intervention group would experience this shift towards vagal modulation, not the WL control group. One possible explanation that may account for the aforementioned findings are medications blunting BP responses.

### **Medications Blunting Heart Rate Variability and Blood Pressure Responses**

Beta-blockers have been shown to improve HRV and are associated with a better prognosis following an MI (Malfatto et al., 1996). Airaksinen et al (1994) observed the effects of metoprolol and atenolol on stable CAD patients compared to a placebo group. They found that beta-blockers induced a shift towards vagal modulation by increasing PNS activation (higher HF) and reducing sympathetic activation (lower LF), thus improving HRV in CAD patients (Airaksinen et al., 1994). In the current study, 80.0% of all participants were on BP lowering medication at baseline— 92.3% of participants on hypertensive medication were in the WL group and 66.7% were in the SSE group. Upon completion of the study, medications for all participants remained the same. Considering that more participants in the WL were on hypertensive medication, the improvements in HRV, as demonstrated by reduced LF and BP, may be attributed to the medication responses.

T2D medications have also been shown to cause hypoglycemic events and alter HRV responses. Since 91.7% of participants in the SSE group and 84.6% of participants in the WL group were on at least one, or a combination of T2D medications, examining the effect of medications on HRV is appropriate. A study by Soydan et al (2013) demonstrated that the combination of glibenclamide (T2D medication) and exercise increased the number of hypoglycemic events by 33% when compared to glibenclamide alone, and 83% when compared to exercise alone. Results revealed that the mild hypoglycemia, caused by T2D medication, resulted in significant decreases of cardiac vagal outflow and HRV (SDNN, RMSSD, TP, HF and VLF). Researchers concluded that the HRV response to hypoglycemia is impaired in T2D patients, causing a higher than expected risk for cardiac arrhythmia following mild hypoglycemic episodes (Soyden et al., 2013).

Consequently, as medications may have caused reductions in LF and BP in the WL group, participants in the SSE group may also have reached their capacity for maximal autonomic improvements by way of medication. Therefore, the lack of improvement in RMSSD and HF may be due to medications blunting HRV responses, and not as the result of cognitive exercise training.

### **The Square-Stepping Exercise**

The SSE is a low-intensity novel exercise originally targeted as a fall prevention strategy in the elderly (Shigematsu & Okura, 2006). Recently it has been utilized as a cognitive exercise intervention to assist with GCF in older adults with subjective cognitive complaints (Gill et al., 2016; Teixeira et al., 2013). SSE has never been implemented in a diabetic population before. It was hypothesized that people with T2D, who are known to develop autonomic dysfunction and be at greater risk of cognitive decline, would improve both their HRV and GCF following a SSE program. However, results from the current study are peculiar and demonstrate improvement in HRV parameters for both the SSE and WL groups. Frequency and intensity of training may be important factors as to why there were no significant improvements in RMSSD and HF for the SSE group. In the literature, research studies with five to six exercise training sessions per week showed improvements in HRV, while less than four per week had mixed findings (Stuckey, 2013). Exercise interventions were also generally performed at a moderate- to high-intensity (Hautala et al., 2004; Tulppo et al., 2003; Cassilhas et al., 2007). Duration of exercise was not found to affect HRV modifications towards vagal dominance (Stuckey, 2013). Because SSE is such a novel exercise, there are currently no recommended exercise guidelines associated with it, therefore we created our own; 45 minute sessions, 2 times per week. It is likely that the low-intensity and low-frequency aspect of the SSE, which was suitable for our older diabetic population, was inadequate to invoke autonomic changes. It is possible that increasing the frequency and perhaps intensity of the SSE may improve HRV. However, if frequency and intensity are increased, the SSE may no longer be suitable for our target population.

## **Sample Size and Attendance**

Other reasons concerning the lack of effect could possibly be attributed to sample size (i.e. lack of statistical power). Regardless of the amount of time spent recruiting for this study, sample size remained low. This could be attributed to factors such as the duration of the intervention and our population of interest. The intervention period was 24-weeks, which is a substantial amount of time to commit to 75% of the session (exclusion from the study if the participant did not believe they could meet that requirement). The first wave of the intervention was to commence in the summer of 2015. Some potential participants were dissuaded from the study because of vacation plans or conflicting schedules on the days of the SSE classes.

The population under investigation was a very specific and relatively small population. Participants were required to have stable T2D, be 50 years or older and have a self-reported cognitive complaint. While recruiting, participants may have had stable T2D, however they may also have had other comorbidities which prevented inclusion (i.e. mobility issues) or prevented them from wanting to participate (i.e. constant pain). With regards to the cognitive criteria, some people either did not want to admit they had memory and/or thinking problem or they were not concerned about them, and therefore could not be included in the study. Another paradoxical issue when working with cognitively impaired subjects is that sometimes participants forgot about important assessments, regardless of reminders the day before. On occasion, this led to incomplete data.

As previously mentioned, the overall attendance for the SSE intervention group was 52.2%. Efforts to maintain adherence to the exercise program were made, however attendance to the SSE classes remained poor. Not only were all the dropouts for our study in the intervention group ( $n = 4$ ), but the remainder of the participants only attended on average 50% of the sessions. It is not surprising that only HR in the standing position was found to be significantly reduced in the SSE group at 24-weeks. The participants with the lowest levels of attendance often: 1) had more medical issues; 2) had work that conflicted with the classes; or 3) simply did not wish to attend. Seasonal effects may have also played a role in SSE class attendance. During the winter months, participants may have

been dissuaded to attend the classes due to unsafe driving conditions. Likewise in the summer, many participants had to care for grandchildren who were on summer break and therefore were unable to attend some classes.

### **Limitations and Future Research**

This novel study has developed knowledge on the effects of a cognitive exercise on HRV parameters. The current study was not without limitations; however it may provide future study directions that could help elucidate the nature of cognitive decline and develop preventative interventions for autonomic dysfunction. Firstly, it should be recognized that the current study was a pilot-study conducted to evaluate the feasibility of examining HRV in older T2D adults following the implementation of a mind-motor exercise program. Consequently, a major limitation is that the sample size remained small, regardless of multiple recruitment strategies. A larger sample size may have shown further increases in vagal modulation in the SSE group following the intervention. Due to our specific population, the HR monitors had a difficult time picking up sinus rhythm and participants were more likely to experience ectopic beats/irregularities in the ECG recordings. Thus rendering the data unanalyzable for five participants. HRV assessments were also done at different times of day. Therefore, there may be other factors apart from the SSE affecting HRV, such as lack of sleep, physiological stressors, caffeine, large meals, excessive exercise, and alcohol. Future studies may focus on keeping HRV assessments at a consistent time of day and controlling for food and caffeine intake before assessments. An older population was recruited for the current study. Some individuals were not familiar with computers and/or did not know how to properly work the mouse while playing the CBS games. Therefore, their scores may not necessarily be a reflection of their cognitive function, but their inability to use the equipment. Finally, future studies should also administer questionnaires regarding daily exercise, food and medication to both the SSE and WL groups to confirm that their daily habits do not change throughout the intervention period; as done in a study by Kitazawa et al (2015).

Despite these limitations, the current study holds promise in elucidating the effects of a mind-motor exercise in aging and disease populations. Therefore, larger studies examining HRV responses to recommended exercise guidelines in T2D populations are

warranted. Future studies could investigate the effects of increased SSE training frequency or the combination of aerobic and cognitive training on HRV parameters as well as focusing on maintaining breathing frequency during HRV assessments. Lastly, a follow-up assessment for the WL group after completion of the SSE training (at 48-weeks) may be used to determine if the changes in the WL group are consistent with the changes observed in the SSE group following the intervention.

## **2.5 Conclusion**

In conclusion, the implementation of a 24-week SSE program revealed a shift towards vagal modulation in both the intervention and control groups for T2D individuals with subjective cognitive complaints. It is unclear as to why this shift in vagal modulation was observed in the control group, however, medication and sample size may have played an influential role. Although there is promise, the overall finding of the current pilot-study remains inconclusive. It is crucial to understand the role of the ANS in HRV and how it may be accelerated with aging, disease and cognitive impairment; especially since these populations are on the rise. Examining the effects of cognitive exercises on HRV may provide invaluable insight into this relationship, therefore, additional research is required.

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## Appendices

### Appendix A: Western University Health Sciences Research Ethics Board HSREB Full Board Initial Approval Notice



Research Ethics

#### Western University Health Science Research Ethics Board HSREB Full Board Initial Approval Notice

**Principal Investigator:** Dr. Robert Petrella  
**Department & Institution:** Schulich School of Medicine and Dentistry\Geriatric Medicine, Parkwood Hospital

**HSREB File Number:** 105883  
**Study Title:** Mind-motor exercise in older adults with type 2 diabetes and self-reported cognitive complaints  
**Sponsor:**

**HSREB Initial Approval Date:** December 22, 2014  
**HSREB Expiry Date:** December 22, 2015

#### Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Instruments	MoCA - clean	2014/09/30
Instruments	Leisure activities- clean	2014/09/30
Instruments	MMSE- clean	2014/09/30
Instruments	CES-D- clean	2014/09/30
Other	NEW** Clinical trials.gov approval receipt- NCT #02292511	2014/11/14
Instruments	NEW** Clock Drawing Test collection assessment form	2014/11/18
Other	NEW** - Email script for Cambridge Brain Sciences outcome measure	2014/11/18
Data Collection Form/Case Report Form	Participant contact info - clean	2014/11/18
Data Collection Form/Case Report Form	CRF post intervention - clean	2014/11/18
Data Collection Form/Case Report Form	CRF interim - clean	2014/11/18
Data Collection Form/Case Report Form	CRF baseline - clean	2014/11/18
Data Collection Form/Case Report Form	CRF screen - clean	2014/11/18
Other	New** HealtheBrain screen shots	2014/12/16
Western University Protocol	Western protocol clean	2014/12/16
Letter of Information & Consent	LOI/Consent - clean with final recommendation #5 (email)	2014/12/19
Instruments	Activities-specific Balance Scale	2014/09/30
Instruments	Fullerton Balance Scale	2014/09/30
Other	Telephone Script - recruitment	2014/09/30

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing and submitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer to Contact for Further Information

*This is an official document. Please retain the original in your files.*

## Appendix B: Western University Health Sciences Research Ethics Board HSREB Amendment Approval Notice



Western  
Research

Research Ethics

### Western University Health Science Research Ethics Board HSREB Amendment Approval Notice

**Principal Investigator:** Dr. Robert Petrella  
**Department & Institution:** Schulich School of Medicine and Dentistry/Geriatric Medicine, Parkwood Institute

**Review Type:** Full Board  
**HSREB File Number:** 105883  
**Study Title:** Mind-motor exercise in older adults with type 2 diabetes and self-reported cognitive complaints  
**Sponsor:**

**HSREB Amendment Approval Date:** June 05, 2015  
**HSREB Expiry Date:** December 22, 2015

#### Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Recruitment Items	Poster	2015/04/28
Recruitment Items	Waiting room slide (received May 15/15)	
Revised Letter of Information & Consent	clean LOI	2015/05/05
Instruments	Clock Drawing Test	2015/04/28
Revised Western University Protocol		2015/05/14
Data Collection Form/Case Report Form	Post Intervention	2015/04/28
Data Collection Form/Case Report Form	Baseline	2015/05/05
Data Collection Form/Case Report Form	Screen	2015/04/28
Recruitment Items	handout (received May 15/15)	

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer to Contact for Further Information

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## Appendix C: Western University Health Sciences Research Ethics Board HSREB Annual Continuing Ethics Approval Notice



Western  
Research

Research Ethics

### Western University Health Science Research Ethics Board HSREB Annual Continuing Ethics Approval Notice

**Date:** November 30, 2015  
**Principal Investigator:** Dr. Robert Petrella  
**Department & Institution:** Schulich School of Medicine and Dentistry\Geriatric Medicine, Parkwood Institute

**Review Type:** Full Board  
**HSREB File Number:** 105883  
**Study Title:** Mind-motor exercise in older adults with type 2 diabetes and self-reported cognitive complaints  
**Sponsor:**

**HSREB Renewal Due Date & HSREB Expiry Date:**  
 Renewal Due -2016/11/30  
 Expiry Date -2016/12/22

The Western University Health Science Research Ethics Board (HSREB) has reviewed the Continuing Ethics Review (CER) Form and is re-issuing approval for the above noted study.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (ICH E6 R1), the Ontario Freedom of Information and Protection of Privacy Act (FIPPA, 1990), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

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[Redacted Signature]



**Appendix D: Breakdown of square-stepping patterns**

<b>Levels</b>	<b>Sublevels</b>	<b>Number of Patterns</b>
Beginner	1	1-8
	2	1-30
Intermediate	1	1-24
	2	1-30
	3	1-28
Advanced	1	1-36
	2	1-14
	3	1-26

## Curriculum Vitae

**Name:** Claire Riley

**Post-Secondary Education and Degrees:** B.Sc. (Hon) Kinesiology  
University of Waterloo  
Waterloo, ON, Canada  
2010 – 2014

M.Sc. Kinesiology Integrated Biosciences  
The University of Western Ontario  
London, ON, Canada  
2014 – present

**Related Work Experience:** Teaching Assistant  
The University of Western Ontario  
2014 – 2016

Stress Testing  
Parkwood Institute  
St. Joseph's Health Care London  
2014 – 2015

**Published Abstracts and Poster Presentations:** Shellington EM, **Riley CE**, Gill DP, Petrella RJ. Older adults with type 2 diabetes and self-reported cognitive complaints show sex-specific differences in global cognitive functioning: results from a proof-of-concept mind-motor exercise randomized controlled trial. Canadian Society for Exercise Physiology, Victoria, BC, Canada, October 12-15, 2016. (Poster Presentation)