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Donepezil's Effect on Cardiac Function in Patients with Alzheimer's Disease through an In Vivo, Non Invasive Measure of Cardiac Autonomic Function

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DONEPEZIL'S EFFECT ON CARDIAC FUNCTION IN PATIENTS WITH

ALZHEIMER'S DISEASE THROUGH AN IN VIVO, NON INVASIVE

MEASURE OF CARDIAC AUTONOMIC FUNCTION

by

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ABSTRACT

DONEPEZIL'S EFFECT ON CARDIAC FUNCTION IN PATIENTS WITH
ALZHEIMER'S DISEASE THROUGH AN IN VIVO, NON INVASIVE MEASURE
OF CARDIAC AUTONOMIC FUNCTION

Lewis Patrick Hackett
Old Dominion University, 2015
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The aim of the current study was to explore the effects of the medication Donepezil (Aricept®) on cardiac autonomic function for individuals diagnosed with mild to moderate (probable) Alzheimer's disease (AD). Using age (± 3 years) and gender matched controls, differences in heart rate variability (HRV) among individuals with Alzheimer's disease were compared to healthy controls over a period of six months (session 1 [baseline], session 2 [3 months], and session 3 [6 months]) in both supine and standing positions. HRV was obtained through frequency-domain (Low Frequency Power, High Frequency Power, RMSSD, LF/HF Ratio) and time-domain (six minutes) measures using ECG technology. Data were analyzed using 2x2x2 (AD group $n= 12$; HC group $n= 12$) and 2x2x3 (AD group $n= 8$; HC group $n= 8$) repeated measures ANCOVA's while controlling for age, gender, body mass index (BMI), blood pressure, medications, dose of Donepezil (Aricept®), alcohol use, caffeine use, and physical activity. Results from the ANCOVA did not reveal statistically significant between group differences for the HRV frequency-domain measures. However, data indicated a trend towards greater reductions in normalized high frequency power (parasympathetic depression) and greater increases in normalized low frequency power (sympathetic exacerbation) among the AD group versus healthy controls at all three time points despite the use of Donepezil (Aricept®).

This dissertation is dedicated to the geropsychology community and all persons who are committed to improving the life and well-being of the aging population.

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CHAPTER I

INTRODUCTION

The aim of the current study was to explore the effects of the medication Donepezil (Aricept®) on cardiac autonomic function for individuals diagnosed with Alzheimer's disease (AD) and taking the medication Donepezil (Aricept®). Donepezil is a widely used cholinesterase inhibitor (ChEls) that is designed to increase cholinergic function among persons with mild to moderate AD. Cholinergic function regulates cardiac autonomic activity via the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). Cholinergic function also plays an integral role in neurocognitive function. Depletion of cholinergic resources often results in cardiac autonomic dysfunction and neurocognitive impairment in persons with AD (Allan et al., 2006; Bartus, 1999; Giubilei, et al., 1998; Toledo & Junqueira, 2008, 2009; Zulli et al., 2005). Cardiac autonomic dysfunction generally refers to the imbalance of the sympathetic and parasympathetic nervous system. This imbalance is a recognized risk factor for cardiovascular mortality, characterized by higher risk of arrhythmias, parasympathetic depression, syncope, and sudden death (Allan et al., 2007; Kleiger, Miller, Bigger, & Moss, 1986; Schliebs & Arendt, 2006). Previous research has indicated some potential cardio-protective benefits of taking the medication Donepezil, and this is of particular interest in the present study.

As research has demonstrated that individuals with AD often present with cardiac autonomic dysfunction, a growing body of evidence has supported the use of heart rate variability (HRV) measures in identifying cardiac autonomic dysfunction in persons with AD (Allan et al., 2007; Balocchi et al., 2006; Da Costa Dias Dias, Da Silva, De Moraes,

& Caramelli, 2013; Giubilei et al., 1998; Masuda & Kawamura, 2003; McLaren, Allen, Murray, Ballard, & Kenny, 2003; Siepmann, Mück, Engel, Rupprecht, & Mück-Weymann, 2006; Task Force on Heart Rate Variability, 1996; Zulli et al., 2005). Heart rate variability (HRV) is frequently measured using non-invasive electrocardiogram (ECG) technology. This typically involves the patient wearing a heart rate sensor positioned close to the heart and a receiver is then positioned in close proximity to the sensor. In its most basic form, HRV refers to the fluctuation of intervals between consecutive heart beats as well as the fluctuation between consecutive instantaneous heart rates (Task Force on Heart Rate Variability, 1996). The HRV algorithm is not necessarily a measure of heart beats, but is rather a measure of the time and variation between beats (Task Force on Heart Rate Variability, 1996). This variation between beats is referred to as inter beat interval and is measured in milliseconds (ms). HRV more generally describes the variations in both heart beat intervals (RR intervals) and heart rate cycles (instantaneous heart rate) over a designated time frame. It is the variations in HRV intervals that are of particular interest here, as they represent changes in cardiac autonomic activity (Bernardi et al., 2011).

Using HRV technology, frequency-domain and time-domain (e.g., six minutes) measures can be used to provide an algorithm that represents cardiac autonomic output. ECG technology provides frequencies that reflect different levels of cardiac outflow and these include; very low frequency (0–0.04 Hz), low frequency (0.04–0.15 Hz), and high frequency (0.15–0.50 Hz) spectral bands. Low frequency (LF) output provides a measure of sympathetic and parasympathetic nervous activity, while high frequency (HF) output provides an exclusive measure of parasympathetic (cholinergic) nervous activity (Allan et

al., 2007; Task Force on Heart Rate Variability, 1996). In addition to this, the LF/HF ratio is computed and can be used as a measure of sympathovagal balance to reflect sympathetic modulation (Task Force on Heart Rate Variability, 1996). Frequency-domain measures have yielded significant results in a number of studies looking at cardiac autonomic function in persons with AD (Allan et al., 2007; Da Costa Dias et al., 2013; Giubilei et al., 1998; Masuda & Kawamura, 2003; Umegaki & Khookhor, 2013; Zulli et al., 2005). The most commonly used time-domain measure includes the mean squared differences of successive R-R intervals (RMSSD). This is used as a measurement to estimate the high frequency variations in heart rate (Toledo & Junqueira, 2008). It is commonly perceived that a regular heartbeat is a sign of strong cardiovascular health. However, the rhythm of a healthy heart or the successive cardiac cycle length is characterized by significant variability in healthy individuals. Therefore, normal and healthy heart rate is characterized by more variability and reductions in variability are often indicative of disease conditions (Sharma, Paudel, Singh, & Limbua, 2009). More specifically, greater high frequency (HF) HRV is an indicator of more healthful cardiac autonomic functioning, and those with AD tend to present with lower high frequency (HF) output (or parasympathetic depression; Sharma, Paudel, Singh, & Limbua, 2009; Toledo & Junqueira, 2008).

The aim of the present study is to not only explore the differences in cardiac autonomic function in persons with AD compared to healthy controls, but to also explore the effects of the cholinesterase inhibitor (ChEl) Donepezil on cardiac autonomic function. Research has indicated potential cardiac autonomic benefits when using the popular cholinesterase inhibitor Donepezil (Nordstrom, Religa, Wimo, Winblad, &

Eriksdotter, 2013; Umegaki & Khookhor, 2013). The medication Donepezil hydrochloride (Aricept®) is one of the most popular ChEls used to treat cognitive impairment among persons with AD. ChEls such as Donepezil operate by preventing acetylcholine from being hydrolyzed thus increasing cholinergic resources. As cholinergic transmission is a mechanism involved in the regulation of the autonomic nervous system, it is hypothesized that Donepezil may provide some additional cardio-protective benefits. These cardio-protective benefits can be assessed through changes in HRV as measured by ECG technology.

Previous research has identified differences in cardiac autonomic function between individuals with AD versus healthy controls. Toledo & Junqueira (2008) and Zulli et al (2005) monitored cardiac autonomic function via ECG technology among those with AD, but in the absence of ChEl treatment. Both authors found significant differences in HRV between AD participants and healthy controls such that those with AD demonstrated parasympathetic depression (suggesting lower cholinergic function) in comparison to healthy controls. The authors of both studies assessed HRV at a single, fixed, time frame. The present study however, will extend the research from a single, fixed, time frame to look at changes in HRV over a period of six months. HRV data will be obtained at three different time points; baseline (session 1), and at three (session 2) and six month (session 3) follow-up intervals. Despite the single time-frame (cross sectional) design in the previous work, studies support the need for further investigation into cardiac autonomic dysfunction in persons with AD and support the use of ECG technology in obtaining HRV data.

Previous research has also shown that Donepezil stabilizes HRV among participants with AD by increasing parasympathetic nervous activity and decreasing sympathetic nervous activity (Giubilei, 1998; Umegaki & Khookhor, 2013). Giubilei et al (1998) explored the effects of a now discontinued and no longer available cholinesterase inhibitor (eptastigmine) on cardiac autonomic function via ECG technology over a period of one month. Participants included 12 individuals who were diagnosed with probable AD and 10 healthy controls. The AD group participants were prescribed the cholinesterase inhibitor (eptastigmine) for one month. HRV recordings were obtained before and after ChEl treatment in both resting and tilting positions. The tilting position is often used to study heart rate abnormalities by positioning the participant at different angles (e.g., 90 degrees; Kochiadakis, et al., 1998). This change in positioning often causes a decrease in high frequency (HF) HRV and subsequent vasovagal syncope (light-headedness; Efremov, Brisinda, Venuti, & Iantorno, 2015). Results from the study indicated that both low and high frequency power generated by the AD group in the tilting position were similar to that of healthy controls following one month of ChEl treatment. However, major limitations to this study include the use of a now discontinued ChEls. According to the Alzheimer's Association (2013), eptastigmine was discontinued by the Food and Drug Administration (FDA) due to its potentially harmful hematologic (blood related) adverse effects. This included reductions in red and white blood cells (pancytopenia) and the development of granulocytopenia (abnormally low concentration of granulocytes in the blood; Braida & Sala, 2001). Therefore, this medication is no longer available for use. The lack of FDA approval and negative side-effects associated with the medication certainly undermine its treatment efficacy and this is a limitation of

the study. Nonetheless, this study demonstrates some of the potential cardiac benefits associated with ChEl treatment.

Additional limitations of the Giubilei et al (1998) study include the limited time frame in which HRV was monitored (one month). Previous studies have indicated that the benefits of Donepezil tend to be more evident after six weeks of ingestion. For example, a study by Rogers et al (1998) looked at changes in cognitive function over a period of 24 weeks (six months) among those with mild to moderate AD who were treated with Donepezil versus placebo-controls. At six weeks of using the medication, the authors reported no significant differences between those taking Donepezil versus healthy controls. However, after six weeks, cognitive performance for the Donepezil-treated participants improved with time whereas the placebo group showed significant deterioration (Rogers, 1998). According to the author, the beneficial effects of Donepezil were most pronounced at the 12 week visit and these effects persisted with no decrease in magnitude at weeks 18 and 24 (Roger, 1998). Other researchers have also demonstrated greater gains in cognitive function with Donepezil use after 24 weeks (six months; Mohs et al., 2001) while other researchers have identified more immediate gains in cognitive function (Feldman, 2001; Umegaki & Khookhor, 2013). The present study will explore changes in HRV among persons with AD who are taking Donepezil versus healthy controls over a period of six months. Participants' HRV will be recorded at baseline and at three month and six month time points, for a total of three sessions. Each session must be within one to two weeks of the three month mark. Therefore, each session occurs every three months (± two weeks). Based on the research by Feldman, 2001; Mohs et al.,

2001; Rogers, 1998), this will provide more than adequate time for Donepezil to reach a therapeutic dose.

Results from these aforementioned studies suggest positive gains in cognitive function as a result of taking Donepezil which may also reflect an increase in cholinergic function. As cholinergic function influences both cognitive function and cardiac autonomic function, it is probable that HRV may also improve more significantly at six months of taking the medication. Thirdly, although the authors used the tilting position to measure HRV as recommended by the Task Force on Heart Rate Variability (1996), LF and HF indices have been shown to change in different positions and under different conditions such as sitting to standing (Risk, Broadbridge, & Cohen, 2001; Task Force on Heart Rate Variability, 1996). Obtaining HRV readings from a still resting position (supine) to a standing position (orthostatic challenge) can help to identify any significant changes in SNS and PNS activity (Aysin & Aysin, 2007; Risk, Broadbridge, & Cohen, 2001). The present study will employ two commonly used and recommended resting positions when measuring HRV (supine and standing), and will utilize the participants respiration when calculating HF HRV (Aysin & Aysin, 2007; Risk, Broadbridge, & Cohen, 2001; Task Force on Heart Rate Variability, 1996). The adjustment using respiration in a HRV algorithm is known as respiratory sinus arrhythmia (RSA). The RSA integrates changes in heart rate during inhalation (heart rate increases) and exhalation (heart rate decreases). Respiratory linked variations in heart rate usually occur in the high frequency (HF) range (Lehrer, Vaschillo, and Vaschillo, 2000). RSA has been shown to provide a more accurate index of cardiac vagal tone and parasympathetic activity (Grossman & Taylor, 2006).

In contrast to the Giubilei et al (1998) study, Masuda & Kawamura (2003) explored changes in HRV using a 24-hour ECG among individuals diagnosed with AD and who were taking the medication Donepezil for six weeks. The participants were initially taking 3mg of Donepezil which was later titrated to 5mg (after one week) and remained at this dose for six weeks. Results from the study indicated reductions in LF and HF power as a result of Donepezil treatment six weeks after beginning the medication. The reduction in the HF component was also greater than the reduction in the LF component, indicating a decrease in PNS activity (and reduced cholinergic activity) over the period of six weeks despite taking Donepezil. These findings suggest that taking Donepezil for six weeks did not improve HRV among persons taking the medication. There are however several methodological limitations to this study. Firstly, the time frame in which HRV was monitored (six weeks) is fairly short. As mentioned earlier, studies have indicated that cognitive improvement appears to take place after six weeks and continues to show therapeutic efficacy at six months (Rogers, 1998; Mohs, 2001). This suggests Donepezil requires at least six weeks in order to fully metabolize (Rogers, 1998; Mohs et al., 2001). Secondly, participants were provided with a 3mg dose of Donepezil followed by a 5mg dose for six weeks. It is recommended that persons taking Donepezil start at a 5mg dose and titrate to a 10 mg dose (the therapeutic dose) after four to six weeks. Thirdly, participants HRV data was recorded using a 24-hour ECG while engaging in daily activities. This is problematic, as monitoring HRV while engaging in activities has been shown to cause variable oscillations in blood pressure creating interference or "noise" which may reduce accuracy (Gianfrance, Saul, Di Rienzo, & Mancia, 1994).

The present study will include participants who are taking both 5mg and 10mg doses of Donepezil as recommended by pharmacological studies (Feldman et al., 2001). Fourthly, respiration count was not factored into the HRV algorithm in this previous work. Obtaining a respiration count and incorporating this into the HRV algorithm is important for purposes of accuracy, particularly when measuring HF HRV (parasympathetic activity; Grossman & Taylor, 2006). Lastly, Masuda & Kawamura (2003) did not use normalized units (n.u.) when analyzing and reporting their HRV frequency data (LF, HF, and LF/HF). According to the Task Force on Heart Rate Variability (1996), measurement of LF and HF Power components are typically measured in absolute values of power (ms^2) but LF and HF can also be measured in normalized units (n.u.). Using normalized units (n.u.) emphasizes changes in sympathetic and parasympathetic regulation by minimizing the effects of Very Low Frequency power. Normalized units display sympathetic and parasympathetic activity as a percentage, allowing the clinician to more easily gauge sympathetic versus parasympathetic dominance. The advantage of using normalized units surrounds the emphasis on the controlled and balanced behavior of the sympathetic and parasympathetic nervous system. Normalized units are also advantageous for tracking changes over time (e.g., when using a repeated measures design). Additionally, the normalization process expresses the quantities more easily by using percentage values (0%-100%). Normalization also reduces most of the large within and across-subject variability in the total raw HRV spectral power (Burr, 2007).

The present study proposes the following three hypotheses: 1) participants that are diagnosed with mild to moderate AD and who are taking a stable dose of Donepezil

(Aricept®) will show increased parasympathetic nervous activity over a period of six months. This will be indicated by decreases in LF Power (n.u.) and LF/HF Ratio in the standing position at session 3 (six months) compared to session 1 (baseline). Thus, there will be a decrease in LF Power (n.u.) and LF/HF Ratio from session 1 (baseline) to session 3 (six months) when engaging in the orthostatic challenge (supine to standing). In contrast, HF Power (n.u.) and RMSSD will increase in the standing position at session 3 (six months) compared to session 1 (baseline). Therefore, there will be an increase in HF Power (n.u.) and RMSSD from session 1 (baseline) to session 3 (six months) when engaging in the orthostatic challenge (supine to standing); 2) Healthy controls will show significant increases in HF Power (n.u.) from a supine to standing position at all three HRV recorded sessions (baseline, 3 months, and 6 months). The AD group will demonstrate minimal change in HF Power (n.u.) from a supine to a standing position at the baseline session, but will show increased HF Power (n.u.) from supine to standing at sessions 2 and 3. Changes in HF Power (n.u.) from a supine to a standing position at session 3 will be similar among both AD participants and healthy controls; and 3) Donepezil (Aricept®) will improve parasympathetic nervous activity through increased acetylcholine transmission, thus supporting the medication's use as a potential protective agent against cardiac autonomic dysfunction.

Currently, research examining cardiac cholinergic function among individuals with AD using Donepezil is limited. Implications of the current study include the potential utilization of Donepezil as a protective agent for those experiencing cardiac autonomic dysfunction. Early detection of parasympathetic depression among persons with AD and the prescription of Donepezil may help safeguard against a future cardiac

event such as bradycardia, arrhythmias, transient ischemic attack (TIA), myocardial infarct, or sudden death.

CHAPTER II

LITERATURE REVIEW

This chapter will outline research surrounding AD pathology, AD risk factors, cholinergic and cardiac autonomic function, the treatment efficacy of Donepezil (Aricept®), heart rate variability (HRV) and the significance of the presenting topic.

Alzheimer's Disease (AD)

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is characterized by neuropsychological, neuropsychiatric and neurological symptoms. AD is the most common cause of dementia and currently affects over five million US Americans aged 65 and older (Alzheimer's Association, 2013; Center for Disease Control, 2013). By 2025, it is estimated that the number of people aged 65 and older with AD will reach a staggering 7.1 million. In 2012, caregivers provided approximately 17 billion hours of unpaid care to persons with AD, and caregiver stress related to AD care is becoming increasingly problematic (Alzheimer's Association, 2013). Typically, AD is diagnosed when the individual meets a set diagnostic criteria as provided by the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5).

According to the DSM-5, in order to make a diagnosis of AD, the individual must meet criteria for either a "minor" or "major" neurocognitive disorder (5th ed., DSM–5; American Psychiatric Association, 2013). A person who meets criteria for a major neurocognitive disorder must present with evidence of significant cognitive decline from a previous level of functioning in one or more of the following cognitive domains:

complex attention, executive functioning, learning and memory, language, perceptual-motor, or social cognition (5th ed., DSM–5; American Psychiatric Association, 2013). This evidence must be based on the concern or knowledge of an informant, or there must be strong evidence of cognitive impairment as revealed by neuropsychological testing. The cognitive impairment must also interfere with the person's ability to carry out activities of daily living (e.g., paying bills or managing medications), and cannot be the direct result of delirium or a severe mental illness (e.g., major depressive disorder). A diagnosis of mild neurocognitive disorder is assigned if the individual only exhibits mild to moderate cognitive decline in the same categories (5th ed., DSM-5; American Psychiatric Association, 2013).

Once the criteria for a minor or major neurocognitive disorder has been satisfied, the individual must meet additional diagnostic criteria in order to be diagnosed with AD. The likelihood of this diagnosis as being "possible" or "probable" is also specified in accordance with accumulated evidence. The diagnostic criteria required to determine major and probable AD should include one or more of the following: an insidious or progressive onset, evidence of causative AD based on genetic evidence, clear evidence of memory decline, learning and decline in at least one other cognitive domain (as determined by neuropsychological testing), and a steady progressive or gradual decline in cognition. Finally, the change in cognition should not be the direct result of another condition such as cerebrovascular disease. A "possible" specifier is allocated if the evidence of AD is limited e.g., neuropsychological assessment data is absent but the patient displays AD features. A "probable" specifier is allocated if there is causative evidence of AD (e.g., as revealed by genetic testing). Lastly, mild AD is determined

based on similar criteria but the individual must display only mild to moderate symptoms (5th ed., DSM-5; American Psychiatric Association, 2013). A "possible" or "probable" specifier is assigned in a similar fashion as with major AD.

AD is an insidious disease that presents with both positive and negative neuropathological features. These neuropathological features include both amyloid plaques and neurofibrillary tangles. These amyloid plaques (Aβ) and neurofibrillary tangles (NFT's) were first described by Alois Alzheimer at the turn of the century following autopsies of a 51-year-old woman and 50-year-old man both of whom had dementia (Muir, 1996). The classic positive symptoms of AD include the presence of extracellular amyloid plaque deposits, neurofibrillary tangles (NFT's), and neuropil threads (Nitsch, Slack, Wurtman, & Growdon, 1992; Serrano-Pozo, Frosch, Masliah & Hyman, 2011). Amyloid plaques are characterized by an accumulation of the amyloid-β peptide (Aβ) which is generated from the amyloid precursor protein (APP; Bertram & Tanzi, 2008). Neurofibrillary tangles in contrast, contain ribbon-like collections of tau proteins. Neuropil threads often accompany neurofibrillary tangles, and are thought to be the result of the breakdown of cell dendrites and axons that are constricted by the NFT's.

The topographic distribution of amyloid plaques originates in the basal areas of the frontal, temporal, and occipital lobe, but as the disease progresses, amyloid deposits can be found in the thalamus and hypothalamus (Braak & Braak, 1991). The distribution of NFT's are more widespread than amyloid plaques, with the degeneration beginning in the allocortex of the medial temporal lobe, and later spreading to the isocortical region (Serrano-Pozo, Frosch, Masliah & Hyman, 2011). Neurodegenerative studies attribute the build-up of these plaques and tangles to the process of protein oligomarization. Protein

oligomarization occurs when certain neuronal proteins which are typically soluble (e.g., tau and α-synuclein), pathologically fold and oligomerize resulting in a type of protein meld (Selkoe, Mandelkow & Holtzman, 2012). This process of abnormal protein folding (or oligomarization) underscores the disease-like nature of AD.

In contrast to the positive features of the disease, negative features primarily include neuronal and synaptic loss (Serrano-Pozo, Frosch, Masliah & Hyman, 2011). Neuronal loss is the main cause of cortical atrophy, and there are two main factors that result in neuronal death. Firstly, NFT's occur within the neuron and displace the cell nucleus resulting in cell death. These tangle-bearing neurons (also known as "ghost tangles") are identified by the appearance of a vacant nucleus. Secondly, neuronal death may occur as a result of apoptosis or programmed cell death (PCD) as a result of DNA fragmentation (Cotman & Su, 1996). Neuronal loss largely outnumbers NFT's, thus neuronal loss has a much greater correlation with cognitive impairment. The cognitive impairment associated with AD is generally caused by neurodegeneration in the limbic system, neocortical regions, and the basal forebrain as well as synaptic and dendritic degeneration (Scheff, Price, Schmitt, & Mufson, 2005; Serrano-Pozo, Frosch, Masliah & Hyman, 2011). Research indicates a significant correlation between the number of synapses and cognitive function, with lower numbers of synapses resulting in poorer cognitive performance (Scheff, Price, Schmitt, & Mufson, 2005). Synaptic loss is a precursor to neuronal loss and is a main contributor to cortical atrophy. Synaptic loss disrupts the connections both within and between neurons, which is why synaptic density has the highest correlation with cognitive decline.

Aside from cognitive decline, individuals with AD present with deficiencies in numerous bodily functions. Cardiovascular disease (CVD) has been shown to correlate highly among persons with non-genetic AD. Non-genetic AD includes persons who present with AD pathology but lack the predisposing genetic traits commonly found in persons with the disease (e.g., Apolopoprotein E gene). A high percentage of individuals with AD present with cardiovascular conditions such as atherosclerosis, high blood pressure, hypertension, and coronary artery disease (De La Torre, 2002; Stewart, 1998). Epidemiological research suggests that CVD may directly contribute to AD pathology and more specifically, CVD may actually have a direct impact on AD pathology (De La Torre, 2002; Newman et al., 2005; Stewart, 1998). Conditions such as atherosclerosis have been shown to reduce cerebral blood flow to the brain (known as cerebral perfusion) resulting in impairment and white matter hyperintensity (De La Torre, 2002). Medications that improve cognitive function such as cholinesterase inhibitors (ChEls), also increase cerebral perfusion reinforcing the link between the cardiovascular system and neurocognitive functioning (De La Torre, 2002).

Despite some of the distinct and unique differences in the symptomology of vascular dementia (VaD) and AD, there is also overlap with both clinical and pathological presentations. For example, individuals with VaD and AD both present with white matter changes, cerebral hypoprefusion (reduced cerebral blood flow), and genetic predispositions (De La Torre, 2002). The overlap in AD and VaD pathologies suggests that both diseases are influenced by similar processes. In addition to CVD, another bodily function which demonstrates decline as a result of AD pathology includes respiratory rate (Hrouova et al., 2012). Respiratory rate is controlled by the autonomic nervous system,

which is a subsystem of the peripheral nervous system. Autonomic nervous function is influenced and regulated by the neurotransmitter acetylcholine (ACh; Wessler, et al., 2007). Therefore, decreases in respiratory rate may be the direct result of changes in cholinergic function and autonomic nervous function as a result of AD pathology. As indicated in this research, AD pathology appears to extend beyond the neurodegenerative symptoms commonly associated with the disease and cardiac autonomic function appears to play an important role in the disease.

AD Risk Factors

A large number of demonstrable risk factors have been shown to increase the etiology and likelihood of developing AD. Well-supported and correlated risk factors include: genetic vulnerability, cardiovascular disease (CVD), aging, ethnicity, physical inactivity, cognitive inactivity or low educational attainment, diabetes, midlife obesity, smoking, mild cognitive impairment, and ethnic background (Alzheimer's Association, 2013; Barnes & Yaffe, 2011; Kalaria et al., 2008).

Genetics. The role of genetics in AD is heterogeneous and complex. Those who have a family member with AD are at greater risk for developing the disease (Alzheimer's Association, 2013). AD that occurs before the age of 60 to 65 years of age is quite rare, and is referred to as autosomal dominant Alzheimer's disease (ADAD) or early-onset AD. Early-onset AD is caused by mutations of three primary genes, all of which alter the production of amyloid-β (Aβ) peptide (Bertram & Tanzi, 2008). As previously mentioned, the production of Aβ is generated from the amyloid precursor protein (APP). The mutations associated with early-onset AD are located on the APP

gene or in the genes that are responsible for encoding proteins; these are presenilin 1 (PSEN1) and presenilin 2 (PSEN2). The three primary genes involved in early-onset AD are referred to as deterministic genes, and almost guarantee that anyone with these specific gene mutations will develop the disease. Unlike early-onset AD, late-onset AD appears to develop from a multitude of risk alleles across different genes. These risk alleles are associated with the production, aggregation, and removal of Aβ peptide (Bertram & Tanzi, 2008). The gene most consistently linked to late-onset AD, is the Apolopoprotein E (apoE) gene (Blacker et al., 1997). The apoE gene contains four chromosomes, the fourth chromosome (apoE-4) of which is overrepresented in persons with late-onset AD. Although apoE-4 allele increases the risk of developing AD, only 20-25% with inherent apoE-4 actually develop the disease suggesting that other factors are contributing to the etiology of the disease (Alzheimer's Association, 2013).

Cardiovascular Function. Research into the relationship between cardiovascular disease (CVD), AD, and cognitive decline is well established (Barnes & Yaffe, 2011; Newman et al., 2005; Stamper, 2006). Results from a cohort study of 4,971 older adults indicated that mean scores were lower on a test of general cognitive ability (Mini Mental State Examination) among individuals with a previous vascular event. Other factors that correlate with lower general cognitive ability scores include the presence of plaques in the carotid arteries and peripheral arterial atherosclerotic disease (PAD; Breteler, Claus, Diederick, Grobbee, & Hofman, 1994; Stampfer, 2006). Furthermore, research has indicated a higher prevalence of AD among those with clinical and subclinical coronary artery disease, raised systolic blood pressure, high serum cholesterol in mid-life, and hypertension (Barnes & Yaffe, 2011; Kivipelto et al., 2001; Newman, Ftizpatrick, &

Lopez, 2005). Autopsy studies have demonstrated a close relationship between the number of microvascular ischemic lesions as a result of cardiovascular incidents and Alzheimer lesions, indicating a probable interaction between the two (Stampfer, 2006). In corroboration with the above cardiovascular risk factors, Snowdon (2001) identified a 34% prevalence rate of stroke among 118 nuns diagnosed with AD. This study was unique, because the nuns used in the study did not present with a history of negative lifestyle behaviors (such as alcohol use, drug use, or poor diet) therefore reducing the likelihood of confounding variables. Snowdon (2001) also demonstrated that older women required an eight fold increase of NFT's to produce the same dementia severity as a person who has experienced a stroke, suggesting that dementia severity is mediated by cardiovascular incidents. It is also well established that certain ethnic groups present with a higher prevalence of both AD and CVD. For example, Latinos and African-Americans present with higher prevalence rates of AD compared to Caucasians, which coincides with a greater prevalence of CVD, metabolic syndromes (namely diabetes), high blood pressure, and high cholesterol (Alzheimer's Association, 2013). Therefore there is a probable link between these two factors.

Aging. Increased age is one of the most highly correlated risk factors associated with AD. The risk of developing AD doubles every five years after the age of 65 and after the age of 85 the risk increases to nearly 50% (Alzheimer's Association, 2013). Exposure to deleterious conditions throughout life such as poverty, infectious diseases, malnutrition, and prenatal stress, may compromise healthy aging (Kalaria et al., 2008).

Ethnicity. Research has indicated that HRV indices vary among different ethnic populations (e.g., African-American's compared to European American's). In particular,

African-American's tend to generate higher levels of high frequency power and RMSSD and lower levels of low frequency power as compared to European Americans (Wang, Thayer, Treiber, & Snieder, 2005). This suggests that African-Americans have a propensity towards greater parasympathetic modulation and greater sympathovagal balance in comparison to European Americans. Therefore, it is important to consider these factors when evaluating HRV among different ethnic groups.

Physical Inactivity. According to a meta-analysis of 16 prospective studies exploring physical inactivity and AD prevalence, approximately 1.1 million AD cases are potentially attributable to physical inactivity (Barnes & Yaffe, 2011). Individuals who engage in physical activity at least twice a week have 60% lower odds of developing AD and cognitive impairment compared to those who engage in frequent sedentary behavior (Barnes & Yaffe, 2011; Rolland, Kan & Vellas, 2008; Rovio et al., 2005). The effects of physical inactivity on cognitive decline are also more pronounced among individuals that carry the apoE-4 gene, suggesting that physical activity may somehow modify the development of apoE-4 gene mutations (Rovio et al., 2005). Reductions in cognitive decline among persons who exercise regularly are likely to be related to the cardiovascular benefits of physical activity. Physical activity mitigates conditions that increase the risk of developing dementia such as hypertension, hypercholesterolemia, diabetes mellitus, and obesity (Rovio et al., 2005).

Cognitive Inactivity & Low Educational Attainment. Both cognitive inactivity and shorter years of education pose as risk factors for AD pathology (Barnes & Yaffe, 2011; Hall, Sujuan, Frederick & Hendrie, 2000). Individuals with higher levels of education (more years in education) and higher occupational attainment show less

clinical signs of memory loss when compared to those with lower educational and occupational attainment (Attix & Welsh-Bohmer, 2006). However, research findings suggest that persons with higher educational attainment are better able to find ways to compensate for memory decline using compensatory strategies (also known as cognitive reserve). Those with higher cognitive reserve tend to show a slower decline in memory function at the early stage of the disease, but experience more rapid/stepwise progression when global functions become impaired (Reed, et al., 2010; Scarmeas, Albert, Manly & Stern, 2006).

Obesity. Longitudinal epidemiological meta-analyses demonstrate higher rates of AD among persons with a history of obesity (Barnes & Yaffe, 2011; Profenno, Porsteinsson, & Farone, 2010). In particular, high body mass index (BMI) is associated with an increased risk of all-cause dementia. According to the National Heart, Lung, and Blood Institute (2014), a normal BMI is considered to be between 18.5 (kg) and 24.9 (kg) while a BMI of 30 (kg) or greater is considered overweight. It is thought that obesity may exacerbate the causal mechanisms involved in the development of the disease via a genetic interaction. Those with a higher BMI present with a greater prevalence of the fat mass and obesity associated gene or the FTO polymorphism (Keller, Xu, Wang, Winbald, Fratiglioni, & Graff, 2010). An interaction seems to exist between the FTO gene and the apoE-4 gene, and those carrying the FTO gene appear to have a greater expression of the apoE-4 gene resulting in a greater risk of AD. The effects of obesity have been shown to coincide with the increased risk of developing CVD and metabolic syndromes such as diabetes, both of which significantly increase AD risk (Profenno, Porsteinsson, & Farone, 2010; Sharma, 2003). Individuals who present with both diabetes and an apoE-4 allele,

have approximately double the risk of developing the disease in comparison to those who possess neither (Messier, 2003). Overall, obesity appears to be a risk factor for AD due to its negative impact on the cardiovascular system.

Smoking. Smoking furthers the risk of developing AD particularly among those who carry the apoE-4 gene (Barnes & Yaffe, 2011; Ott et al., 1998; Rusanen et al., 2010). Smoking is a heavy detriment to the cardiovascular system, often resulting in a higher risk of a myocardial infarct (MI), coronary artery disease (CAD), carotid wall thickening, and internal carotid stenosis (Ambrose & Rajat, 2004; Tell et al., 1994).

Mild Cognitive Impairment. Mild cognitive impairment (MCI) is a prodromal state of cognitive function that falls between the changes seen in healthy aging and meeting criteria for dementia (Peterson, 2011; Kalaria et al., 2008). MCI is classified into two subtypes; amnestic and non-amnestic mild cognitive impairment. Amnestic MCI primarily affects memory function, and persons with amnestic MCI often present with increasing forgetfulness but other functions are spared (e.g., executive functioning). The capacities affected by non-amnestic MCI generally include executive and visuospatial function with memory largely remaining intact. The prevalence of MCI among those aged 65 years and older is approximately 10-20%. The rate in which those diagnosed with MCI who eventually go on to develop AD is approximately 5-10% in community samples and 10-15% in clinical samples (Peterson, 2011). Individuals who present with the apoE-4 gene, a hippocampal volume below the 25th percentile, and elevated levels of cerebral spinal fluid (CSF) tau protein, are at greater risk of developing AD after being diagnosed with MCI.

Overall, there are numerous factors that exacerbate the risk of developing AD. Although individual factors pose an increased risk, combinations of risk factors such as the apoE-4 genotype and high BMI seem to drastically increase the risk of developing the disease above and beyond individual factors. Two of the greatest risk factors for AD include a genetic predisposition and CVD. These risk factors are then mediated by negative lifestyle behaviors such as physical inactivity, and alcohol and tobacco use. The relationship between CVD and negative lifestyle behaviors may also be bi-directional, with negative lifestyle behaviors increasing the likelihood of developing CVD. Therefore, it is the interaction of multiple risk factors that may increase the overall risk of a person developing AD.

Cholinergic Function and Cardiac Autonomic Function

In addition to the positive and negative symptoms present in the AD brain, changes in the neurotransmitter system also occur in persons with the disease. The neurotransmitter acetylcholine (ACh) plays a primary role in neurocognitive function (mainly learning and memory) and cardiac autonomic function. Cholinergic resources become increasingly deficient in the AD brain resulting in neurocognitive impairment and cardiac autonomic dysfunction (Bartus, 1999; De Vilhena & Fernando, 2009; Feldman, 2001; Schliebs & Arendt, 2006). Decreases in choline acetyl transferase (ChAT), a biochemical marker for cholinergic neurons, are found in the brain of AD patients (Muir, 1996). Reductions in levels of ACh are caused by the loss of presynaptic neuronal structures (e.g., ChAT) in the basal forebrain. A landmark study by Perry, Tomlinson, Blessed, Bergman, & Gibson et al (1978) demonstrated a strong correlation between cholinergic neuronal loss identified via autopsy and decreased mental state

examination scores (Muir, 1996). These findings along with others were significant in the development of the "cholinergic hypothesis", which theorized a link between cholinergic resource depletion in the central nervous system and decreased cognitive function (Bartus, 1999). The cholinergic hypothesis in comparison to a number of other theories pertaining to the neurodegenerative pattern in AD, has produced strong evidence of efficacy in well controlled, multicenter trials (Muir, 1996). Early studies showed support for the theory when the symptoms of memory loss seen in the AD presentation were replicated by blocking cholinergic receptors in young adults (Schliebs & Arendt, 2006). More recent studies have provided supporting evidence for the hypothesis using in-vivo measurements of cholinergic receptor expression via positron emission tomography (PET) technology (Schliebs & Arendt, 2006). Additionally, researchers Toledo & Junqueira (2009) explored correlations between MMSE scores from AD participants and HRV indices. Results from this study indicated that cognitive deficiency was correlated with lower cardiac parasympathetic modulation and a trend for higher cardiac sympathetic modulation.

Cholinergic neurons can be subdivided into two categories: projection neurons and interneurons. Projection neurons are primarily concentrated in the forebrain and upper brain stem, whereas interneurons are located in the caudate-putamen (cerebral cortex), nucleus accumbens (basal forebrain), hippocampus, cerebral cortex, hypothalamus, and spinal cord (Schliebs & Arendt, 2006). Most importantly, cholinergic projection neurons also comprise of motor neurons in the spinal cord and cholinergic neurons in the sympathetic (SNS) and parasympathetic nervous system (PNS). Cholinergic function is involved in controlling cerebral blood flow, cortical activity, the

sleep-wake cycle, and modulating cognitive function (Schliebs & Arendt, 2006). Cholinergic function is a primary regulatory neurotransmitter of the autonomic nervous system (ANS). The autonomic nervous system regulates both the sympathetic and parasympathetic nervous system, and is involved in cardiac regulation as well as respiration (Task Force on Heart Rate Variability, 1996).

For more than 40 years, AD has been classified as a genetic neurodegenerative disorder but epidemiological studies indicate that AD may also be non-genetic and initiated by vascular factors that precede the neurodegenerative process (De La Torre, 2002). Cardiac autonomic dysfunction is prevalent among individuals with AD, and this generally reflects an increase in sympathetic activity and a decrease in parasympathetic activity (or parasympathetic depression; Toledo & Junqueira, 2009). This could be a direct result of cholinergic dysfunction, and it is well established that persons with AD generally present with depleted cholinergic resources in tandem with autonomic dysfunction indicating a probable link between the two. Examples of autonomic dysfunction among those with AD can include increased cardiovascular mortality, higher risk of arrhythmias, sudden death, and other cardiac events (Allan et al., 2007; Kleiger, Miller, Bigger, & Moss, 1986). A relationship between higher cognitive function and autonomic function has been demonstrated which could be mediated by cholinergic function (Critchley, 2005; Hugdahl, 1996; Royall et al., 2006). Therefore, it is possible that cardiac autonomic dysfunction may represent a decrease in cholinergic resources as well as increased cardiovascular risk.

In contrast to the evidence indicating a direct and causal link between cardiac autonomic dysfunction and AD (or vice-versa), other studies have indicated a parallel

process whereby AD and cardiac autonomic dysfunction co-occur. For example, the existence of cardiac autonomic dysfunction (e.g., cerebrovascular disease) may lead to deep white matter infarction and subsequent white matter disease (WMD; Andin, Passant, Gustafson, & Englund, 2006). White matter disease correlates highly with AD and cerebral amyloid angiopathy (CAA). CAA is the build-up of amyloid plaques within the walls of brain arteries, and these plaques are similar to the Aβ plaques found in the AD brain. As vascular complications progress (e.g., atherosclerosis and CAA), the severity of AD tends to progress in a similar fashion (Andin, Passant, Gustafson, & Englund, 2006; De La Torre, 2002).

In corroboration with the evidence surrounding a parallel process, cardiovascular conditions have been shown to cause reduced cerebral perfusion (cerebral blood flow) or hypoperfusion as well as cognitive impairment (Toledo Ferraz Alves, Ferreira, Wajngarten, & Busatto, 2010). This hypoperfusion pushes oxidative stress towards the brain resulting in cognitive decline (De La Torre, 2002). Hypoperfusion is likely a precursor to AD pathology but may also mediate AD symptomology. It is also well-known that Aβ and NFT's have been found in the brains of healthy older adults who show no signs of AD (De La Torre, 2002). As mentioned previously, Snowdon (2001) demonstrated that older women required an eight fold increase of NFT's to produce the same dementia severity as a person who has experienced a stroke, suggesting that dementia severity is mediated by cardiovascular health. This could indicate that a parallel process involving both cardiovascular and neurocognitive systems is necessary to induce AD pathology.

Donepezil

The medication Donepezil hydrochloride (Aricept®) is a popular ChEls used to treat cognitive impairment among persons with AD. Donepezil is a highly selective ChEls designed to prevent and slow the progression of cholinergic deficiency which greatly impacts neurocognitive function (Feldman et al., 2001; Bartus, 1999). ChEls operate by blocking the acetylcholine esterase (AChE) and butylcholinesterase (BuChE) enzymes which typically hydrolyze acetylcholine (Rogers, 1998). The recommended dose for persons taking Donepezil is 5mg over a period of four to six weeks which is then titrated to the therapeutic dose of 10mg if the medication is well tolerated (Feldman et al., 2001; Mohs et al., 2001). Results from a number of placebo-controlled studies looking at the effects of Donepezil on cognitive function have indicated significant reductions in the rate of cognitive decline (neurodegeneration) and mild cognitive gains in persons with mild to moderate AD who are taking the medication (Mohs et al., 2001; Rogers et al., 1998; Feldman et al., 2001). This reduction in cognitive decline and improvement in cognitive functioning during Donepezil treatment appears to be evident after six weeks of taking the medication (Mohs et al., 2001; Rogers, 1998) but greater gains can be seen at six months (Mohs et al., 2001). Additional findings suggest that Donepezil may also help reduce cognitive decline among persons with more progressive and severe stages of the disease (Feldman, 2001).

Although Donepezil may slow the rate of cognitive decline, the medication and other ChEls have been shown to cause uncommon side effects such as dizziness and syncope, bradycardia (abnormally slowed heart rate), atrial arrhythmias, myocardial infarction, angina, seizures, atrioventricular block, and orthostatic hypotension (Bordier,

2006; Calvo-Romero & Ramos-Salado, 1999; Newby, Kenny, & McKeith, 2006; Rowland, Rigby, Harper, & Rowland, 2007). These adverse effects are however mostly seen in cases where the amount of Donepezil metabolized has greatly exceeded the therapeutic dose, but some have reported mild side-effects from the recommended dose (Bordier et al., 2005). The prevalence of bradycardia as a result of taking Donepezil is rare with the base rate being around 1% (Bordier et al., 2005; Rowland, Rigby, Harper, & Rowland, 2007). It is important to mention that bradycardia is fairly common among the general population (all ages), with approximately 15% of males and 2% of females meeting criteria for clinically defined bradycardia (Ostchega, Porter, Hughes, & Dillon, 2011). Thus, the percentage of individuals developing bradycardia as a result of taking Donepezil is quite low in comparison to the general base rate. The frequency of adverse events such as dizziness and syncope are more common, and are reported in around 1-10% of persons taking the medication (Rowland, Rigby, Harper, & Rowland, 2007). Atrioventricular block is exceptionally rare, and occurs in 0.001-0.1% of those taking the medication.. It is hypothesized that side-effects such as bradycardia and arrhythmia are triggered by increased acetylcholine production or increased vagal neurotransmission which acts to slow heart rate. However, Donepezil has also been shown to improve cardiac autonomic dysfunction by increasing heart rate variability (HRV) among persons with AD as well as animal subjects (Giubilei et al., 1998; Umegaki & Khookhor, 2013). Limitations to these studies include measuring changes in HRV at a single (and varied) time frame (12 weeks ± 4 weeks), using a now discontinued ChEI, measuring changes in HRV over a short time frame (2 weeks), the absence of normalized units when identifying changes in HRV over time (Masuda & Kawamura, 2003; Umegaki &

Khookhor, 2013), using animal subjects (rats), and the lack of a healthy control group (Da Costa Dias et al., 2013; Giubilei et al., 1998; Umegaki & Khookhor, 2013).

Previous research has also explored the medium term effects of cholinesterase inhibitors on cardiac health. For example, researchers Nordstrom et al (2013) studied the prevalence of myocardial infarcts (MI) and rate of death among individuals with AD who were taking a cholinesterase inhibitor ($N=$ 7,073) over a period of five years. Results from the study found that participants who used ChEls had a 34% lower risk of experiencing an MI. Cholinesterase inhibitor use was also associated with a lower risk of death. Therefore, Donepezil may present with cardio-protective properties in addition to improving neurocognitive function. These positive side-effects of the medication Donepezil are likely the result of changes in cardiac autonomic function as a result of increased ACh production.

Heart Rate Variability (HRV)

Over the past 25 years, there has been an increased recognition in the relationship between the autonomic nervous system (ANS) and cardiovascular mortality (Task Force on Heart Rate Variability, 1996). Following the recognition of ANS markers and the consequences of autonomic dysfunction, interest in obtaining heart rate variability (HRV) measures via electrocardiogram (ECG) have grown significantly (Balocchi et al., 2006). Epidemiological and clinical data using ECG technology indicate autonomic and neuroanatomic dysfunction in persons with AD, and more specifically parasympathetic depression (Bartus, 1999; Giubilei et al., 1998; Toledo & Junqueira, 2008, 2009; Zullii et al., 2005). Heart rate and heart rhythm are primarily controlled by the autonomic nervous

system (ANS), which comprises of two distinct subsystems; the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The SNS and PNS work in complementary and opposing ways. The PNS influences heart rate by the release of ACh neurotransmission via the vagus nerve. The release of ACh results in diastolic depolarization which causes heart rate activity to slow (Task Force on Heart Rate Variability, 1996). Reductions in PNS activity is often referred to as vagal withdrawal, and is indicative of impaired autonomic function. Neurotransmitters including the release of epinephrine and norepinephrine, activate adrenergic (adrenaline) receptors causing an increased SNS response and slowed diastolic depolarization.

Heart rate variability is monitored using ECG technology which provides an algorithm of SNS and PNS activity. Assessment of SNS and PNS activity can be obtained via spectral analysis of HRV which provides both low frequency (LF) and high frequency (HF) cardiovascular output. Increased vagal afferent excitation results in vagal efferent activity (PNS) which (in turn) inhibits the sympathetic nervous response. Efferent vagal activity is of high interest here, because it can be measured by HF HRV output from an electrocardiogram (ECG) thus providing a quantitative measure of PNS activity. The simplest measurement of HRV is the time domain method, which utilizes the individual's heart rate at a certain point in time and the intervals between successive normal complexes (also known as RR interval variability; Task Force on Heart Rate Variability, 1996). The time domain method can be used to capture both short-term (e.g., 5-7 minutes) or long-term recordings (e.g., 24 hour period). Due to the nature of this study, short-term recordings will be obtained over a period of six minutes in two different positions (supine and standing). Short-term recordings (5 minute intervals) have been

used in previous studies to accurately capture HRV data in persons with AD (Allan et al., 2006; Giubilei et al., 1998; Siepmann et al., 2006; Toledo & Junqueira, 2008). The most commonly used time-domain measures include the mean squared differences of successive R-R intervals (RMSSD). This is used as a measurement to estimate the high frequency variations in heart rate.

Using short-term recordings, the following spectral components can be derived from the HRV algorithm; low frequency (LF), high frequency (HF) and LF/HF ratio. The proportional variations of LF and HF output are represented by the LF/HF ratio. The spectral components are available in both absolute values (ms^2) or normalized units (n.u.) and represent changes in autonomic heart function. The advantage of using normalized units surrounds the emphasis on the controlled and balanced behavior of the sympathetic and parasympathetic nervous system (Task Force on Heart Rate Variability, 1996). Using normalized units (n.u.) emphasizes changes in sympathetic and parasympathetic regulation by minimizing the effects of Very Low Frequency power. The Task Force on Heart Rate Variability (1996) state that power in normalized units (n.u.) should always be reported with absolute values of LF and HF Power in order to describe in total the distribution of power in the spectral components. Normalized units are also advantageous for tracking changes over time (e.g., when using a repeated measures design) and are similar to a standard score. In addition to this, the normalization process expresses the quantities more easily by using percentage values (0%-100%). Normalization also reduces most of the large within and across-subject variability in the total raw HRV spectral power as well (Burr, 2007).

With regards to standardized HRV data using normalized units, a meta-analysis of 44 publications exploring short-term HRV among healthy adults (≥ 18 years) suggests that mean normal values of low frequency power (expressed in normalized units) should be approximately 52% (*SD*= 10), while mean normal values of high frequency power (expressed in normalized units) should be approximately 40% (*SD*= 10; Nunan, Sandercock, & Brodie, 2010). Additionally, mean normal values of RMSSD should be approximately 42 (ms; *SD*= 15) while the mean normal value of the LF/HF Ratio should be approximately 2.8 (*SD*= 2.6; Nunan, Sandercock, & Brodie, 2010). It is important to mention that the mean values generated from this study were based on HRV data obtained in a range of positions (e.g., supine, standing, resting etc.) and from individuals of varying ages (≥18).

Previous studies have explored frequency domain analysis using LF, HF, and LF/HF ratio indices to determine cardiac autonomic change among those with AD versus healthy controls (Allan et al., 2007; Da Costa Dias, et al., 2013; Giubilei et al., 1998; Masuda & Kawamura, 2003; Toledo & Junqueira, 2009). Using a cross-sectional design, researchers Toledo & Junqueira (2008) studied cardiac autonomic function and cognitive function in 22 participants with probable AD (*Mean age* = 79.6) and compared them to 24 healthy participants (*Mean age*= 68.6). Cardiac autonomic function was measured during five minute HRV intervals and frequency domain indexes in both supine and standing positions. The HRV analysis was performed according to the methodological standards recommended by the Task Force on Heart Rate Variability (1996). Each individual completed a five minute series of R-R intervals in both supine and standing positions using digital ECG technology and compatible software. Both time-domain and

frequency-domain analyses were conducted (Toledo & Junqueira, 2008). Cardiac sympathetic and parasympathetic modulation was measured using a time-domain analysis, a mean R-R interval series and two variability indices. This included the standard deviation of intervals (SDNN) and the coefficient of variation (SDNN/mean). Two separate indices were also used and included the percentage of successive adjacent R-R intervals greater than 50 ms (pNN50%), and the square root of the mean successive differences between adjacent R-R intervals (RMSSD; Task Force on Heart Rate Variability, 1996). These reflect the rapid beat-to-beat shifts in intervals that are derived from the parasympathetic modulation associated with respiration (Toledo & Junqueira, 2008).

Frequency domain analyses were also conducted by the authors, which provided a functional measure of HRV frequency distribution. These frequencies included very low frequency (0–0.04 Hz), low frequency (0.04–0.15 Hz), and high frequency (0.15–0.50 Hz) spectral bands. The frequency domain measures include total power spectral area, which demonstrates the level of the overall autonomic modulation. Low frequency bands are a measure of SNS activity while high frequency bands are an indication of PNS activity (Task Force on Heart Rate Variability, 1996). The ratio of the low to high frequency indices (LF/HF ratio) provides an estimative of the sympathovagal balance. If the LF/HF ratio is less than 1, then this indicates a dominance of parasympathetic modulation whereas if the ratio is greater than 1, this is indicative of sympathetic dominance (Toledo & Junqueira, 2008). The mean and frequency domain indices in each group were compared using Student t-tests. Multiple logistic regression analyses were conducted to verify if the HRV indices were affected by independent confounding

variables (e.g., caffeine use, blood pressure, and alcohol use). Logistic regression analyses revealed changes in sympathovagal balance, which was altered towards parasympathetic depression and sympathetic exacerbation in both the supine and standing posture among persons with AD (Toledo & Junqueira, 2008).

Supporting evidence for reductions in HRV were identified in similar studies by Zulli et al (2008) and Allan et al (2007). Zulli et al (2008) compared HRV markers of 33 participants with AD and 29 participants with MCI to healthy controls using 24-hour ECG monitoring. The researchers also monitored blood pressure changes over a 24-hour period, and controlled for various lifestyle behaviors including smoking habits and hypertension. High and low blood pressure have been linked to changes in cardiac autonomic function, which is why this factor was a potential confound (Acharya, et al., 2006; Pavithran, Prakash, Dutta, & Madanmohan, 2010). Persons with a history of heart failure, coronary artery disease, diabetes mellitus, or severe clinical conditions (e.g., cerebrovascular disorders) were excluded from the study. Student *t*-tests were conducted to detect changes in continuous variables, and categorical variables were analyzed using a chi-square analysis. The authors then used a Pearson linear correlation analysis to evaluate the correlation between HRV and cognitive impairment. Results indicated that HRV time and frequency domain parameters were lower in patients with AD in comparison to patients with MCI and healthy controls (Zulli et al., 2008). More specifically, the AD group showed much lower high frequency output compared to healthy controls. Allan et al (2007) compared HRV among persons with AD, VaD, and Lewy Body Dementia (LBD) using ECG technology. Results further indicated decreased

PNS activity among individuals with all types of dementia suggesting that decreased autonomic dysfunction is not unique to dementia of the AD type.

Currently, there are only a few studies exploring the cardio-protective benefits of ChEl treatment among individuals diagnosed with AD. One of these studies was conducted by researchers Giubilei et al (1998). In this study, authors Giubilei et al (1998) explored cardiac autonomic function in persons with probable AD ($n=$ 12) versus healthy controls ($n=$ 12). The AD group were receiving ChEl treatment for one month using a now discontinued medication named eptastigmine. Before the participants received eptastigmine, a recording of the HRV was performed during a head-up tilt test. This was then repeated on the last day of treatment. The same frequency bands used in the Toledo & Junqueira (2008) study were used in this study (HF, LF, and LF/HF ratio). Results indicated that before treatment, HF Power (parasympathetic function) was lower in AD participants compared to healthy controls while in the resting position. Following one month of ChEl treatment, AD participants showed a similar magnitude of HF Power in the resting position compared to healthy controls. Additionally, AD participants showed similar patterns of HRV output following ChEl treatment when placed in the tilting position compared to healthy controls. Overall, both groups showed similar increases in the magnitude of LF Power and decreased HF Power when placed in the tilting position. The authors concluded that cardiac autonomic dysfunction may be present in persons with AD as a result of a cholinergic deficit in the peripheral autonomic nervous system. However, this cardiac autonomic dysfunction can be normalized by ChEl treatment (Giubilei et al.,1998). A major limitation to this study includes the use of a now

discontinued ChEls and the short time frame in which pre/post measures were obtained (one month).

Another study conducted by Masuda & Kawamura (2003) explored the effects of the more modern and widely used cholinesterase inhibitor Donepezil hydrochloride (Aricept®). The authors examined the effects of Donepezil on autonomic nervous activity among 17 participants with a diagnosis of probable AD. The severity of AD was controlled for in the analysis by quantifying dementia severity using a dementia screening tool. Using 24-hour ECG technology, Masuda & Kawamura (2003) obtained HRV before the participants began taking Donepezil and six weeks after the participants started Donepezil. Participants were however initially administered a 3mg dose of Donepezil in the morning for 2 weeks as a running-in period and after these 2 weeks, the dosage was increased to 5mg per day where it remained. HRV was measured using frequency domain analyses over a 24-hour period. Results indicated that low frequency and high frequency components were significantly reduced with treatment and ultralow and very low frequency components were not affected. The reduction in the high frequency (cholinergic) component was also greater than the reduction in the low frequency component, indicating a greater decrease in parasympathetic (cholinergic) activity in comparison to sympathetic activity over the period of six weeks. Results from this study indicate that Donepezil reduced parasympathetic activity and cholinergic function among those taking Donepezil, which contradicts the findings from Giubilei et al (1998) and Umegaki & Khookhor (2013).

Similar findings to the Masuda & Kawamura (2003) study were also obtained by Siepmann et al (2006) and McLaren et al (2003). Siepmann et al (2006) explored the

effects of Donepezil treatment on RMSSD among individuals diagnosed with AD while in the resting position. RMSSD was captured using HRV technology at a single time frame. The duration in which ChEl treatment was administered was not specified. The authors also used an AD control group who were not being treated with Donepezil. Results indicated a lower magnitude of RMSSD (parasympathetic nervous activity) among the AD group during Donepezil treatment versus the AD (non-ChEl treatment) control group. Major limitations to this study include the use of a single time frame to capture HRV, the absence of normalized units, as well as the unspecified length of time in which individuals with AD were taking the medication Donepezil. Additionally, researchers McLaren et al (2003) explored the effects of Donepezil on HRV among individuals diagnosed with AD and Lewy Body Dementia before and after ChEl treatment. HRV was obtained through the use of a five minute HRV analysis. Results indicated that HRV was significantly reduced following treatment with Donepezil as indicated by reductions in high frequency power. Limitations to this study include the use of a single time frame, absence of a control group, and absence of normalized units. The researchers of both studies did not account for various confounding factors (e.g., physical activity, caffeine use, gender) which have been shown to influence HRV.

A more recent study conducted by researchers Umegaki & Khookhor (2013), explored the effects of Donepezil (3mg) on HRV using animal (rats) subjects. The authors administered Donepezil to 32 rats and monitored their low frequency (LF), high frequency (HF) and the LF/HF Ratio indices over a period of two weeks. The rats HRV was obtained on the day the initial dose was ingested and at one and two weeks after the initial dose was ingested. On day one, authors found an increase in sympathetic nervous

activity as evidenced by LF dominance (LF/HF Ratio). However, at weeks one and two, the magnitude of the LF output had decreased significantly and HF dominance was apparent. The authors concluded that initially ChEl treatment induced sympathetic nervous activation acutely, but longer-term ingestion (two weeks) induced parasympathetic activation. Additionally, another recent study exploring the effects of ChEl treatment on HRV and blood pressure, identified improvements in cardiac autonomic balance and diastolic and systolic blood pressure following ChEl treatment (Da Costa Dias, et al., 2013). In older adults, increasing blood pressure produces cardiac slowing as a result of reductions in baroreceptor reflex sensitivity (Simpson & Wicks, 1988). The baroreceptor reflex is a strong reflex of the brainstem and baroreceptors are located in the auricles of the heart and vena cavaeis (Berntson, 1997). The baroreceptor reflex is involved in maintaining blood pressure and homeostasis. More specifically, if blood pressure decreases the baroreceptor reflexes act to help restore blood pressure by increasing heart rate. Baroreceptor reflexes exert powerful inhibitory influences on sympathetic outflow and also stimulate vagal (ANS) activity (Berntson, 1997). As such, older adults have the tendency to demonstrate poorer HRV with age but disease pathology (e.g., dementia) causes larger decreases in HRV (Toledo & Junqueira, 2008). The study by Da Costa Dias et al (2013) may suggest that decreases in high blood pressure as a result of improved baroreceptor reflex sensitivity may in turn, improve heart rate variability. Previous research has also indicated a strong relation between high blood pressure and greater LF Power (sympathetic exacerbation) among hypertensive participants versus healthy controls (Piccirillo, Munizzi, Fimognari, & Marigliano.,

1996). Therefore, LF Power is thought to reflect baroreceptor-mediated regulation of blood pressure (Bernston, et al., 1997; Friedman & Thayer, 1998).

Respiratory Sinus Arrhythmia (RSA)

In order to improve the accuracy of measuring parasympathetic activity via high frequency HRV, respiration count is often incorporated into the HRV algorithm. This measure is known as the respiratory sinus arrhythmia or RSA (Grossman & Taylor, 2006). The respiratory sinus arrhythmia is attributed to parasympathetic activity, and is frequently utilized as an index of cardiac vagal tone (Grossman & Taylor, 2006). Cardiac vagal tone is a process by which the vagus nerve controls and regulates the parasympathetic nervous system and is responsible for homeostatic regulation. Heart rate has been shown to synchronize with respiratory rhythm, and the R-R interval on an ECG is shortened during inspiration and prolonged during expiration (Yasuma & Hayano, 2004). Therefore, obtaining respiratory parameters can be advantageous in providing a more accurate measure of parasympathetic activity.

HRV Positioning

Research has demonstrated that HRV, diastolic and systolic blood pressure (BP), high and low frequency band power of HRV all show significant changes when positioning is altered from a supine to a standing posture (Acharya, et al., 2006; American Heart Association, 1996; Aysin & Aysin, 2007). The orthostatic challenge (supine to standing) is also one of the most common tests to detect cardiac autonomic dysfunction (Aysin & Aysin, 2007). Physical activity has also been shown to cause oscillations in blood pressure creating interference or "noise" with HRV measures

(Gianfrance, Saul, Di Rienzo, & Mancia, 1994). Obtaining HRV readings from a still resting position to a standing position will help identify any significant changes in SNS and PNS activity. Toledo & Jaunqueira (2008) demonstrated large differences in high frequency (HF) bands among persons in both supine ($M=$ 28.0 ms^2) and standing ($M=$ 13.8 ms^2) positions, which is consistent with previous research (Fagard, 2001). The heart rate response to standing evaluates the cardiovascular response initiated by a change from horizontal to a vertical position. The typical heart rate response to standing (increased sympathetic activity) is largely mitigated by parasympathetic activity (Risk, Broadbridge, & Cohen, 2001). In healthy individuals, there is a characteristic and rapid response to standing which is followed by a relaxation bradycardia (Risk, Broadbridge, & Cohen, 2001). Research has shown that shifts in positioning (e.g., a head tilt or sit to stand) among persons with AD and healthy older adults can alter HRV (Toledo & Junqueira, 2008). The present study will use both supine and standing positions to measure HRV. Using a still resting position will also decrease the likelihood of additional frequency interference (Gianfrance, Saul, Di Rienzo, & Mancia, 1994). It is recommended that the participant should be in a resting position prior to commencing the standing measurement. So far, the results of the few studies examining cardiac autonomic function in AD present with varying results in frequency-domains. This is likely the result of varying methods of measuring heart rate variability such as using a stand versus a supine position or a 24-hour ECG (Giubilei et al., 1998; Masuda & Kawamura, 2003; Siepmann et al., 2006; Toledo & Junqueira, 2008).

HRV Confounds

When assessing HRV in the present study, it will be important to identify factors that may influence cardiac autonomic function as these factors could undermine the validity of the HRV analysis. It is well known that persons who present with severe coronary artery disease, congestive heart failure, atherosclerosis, high blood pressure, or stroke present with decreased heart rate variability (Kleiger, Miller, Bigger, & Moss, 1986; Kwon et al., 2008; Naver, Bloomstrand, & Wallin, 1996). More specifically, measures of high frequency HRV appear to be significantly lower among persons with cardiovascular disease indicating decreased parasympathetic nervous response (or vagal withdrawal; Evrengul et al., 2006). Therefore, it will be important to exclude individuals with severe cardiac conditions such as a history of stroke from the study, and control for more mild cardiac conditions (e.g., high blood pressure) in the analyses.

Frequent exercise (two to three times per week) has also been shown to increase heart rate variability as demonstrated by increases in high frequency HRV among young and older adults (Sandercock, Bromley, & Brodie, 2005; Schuit et al., 1999). Therefore, it will be necessary to document the amount of hourly exercise each participant engages in per week as this could potentially inflate their levels of high frequency HRV. In addition to this, body mass index, long-term smoking behaviors, and moderate and long-term alcohol consumption have also been shown to decrease HRV and decrease parasympathetic nervous response as measured by high frequency HRV (Harte & Meston, 2013; Hayano et al., 1990; Koskinen, Virolainen, & Kupari, 1994; Molifino et al., 2009). Individuals with alcohol dependence (based on DSM-5 criteria) will not be eligible for the study as this can cause long-term vagal neuropathy (Malpas, Whiteside, &

Maling, 1991). Older age is also associated with a global reduction in HRV and research has indicated that gender may impact HRV as well (Fagard, 2001; Stein, Kleiger, & Rottman 1997; Umetani, Singer, & McCraty, 1998). Interestingly, the level of decreased HRV as a result of aging appears to be more pronounced in males in comparison to females (Acharya, et al., 2006; Stein, Kleiger, & Rottman 1997; Umetani, Singer, & McCraty, 1998). As a result of this, AD participants in the current study will be matched with a healthy control based on age and gender.

The influence of medication should also be considered when interpreting HRV as certain classes of medications can cause changes HRV. For example, beta-blockers that are used to reduce hypertension and high blood pressure have been shown to improve PNS activity and decrease SNS activity (Acharya, et al., 2006). Other medications such as selective serotonin reuptake inhibitors (SSRI's) and benzodiazepines can cause adverse changes in blood pressure resulting in orthostatic hypotension (Tinetti & Kumar, 2010). These medications may interfere with HRV accuracy due to the resultant changes in blood pressure. Therefore, medications will be controlled for in the analysis. Lastly, acute caffeine ingestion has been shown to cause increased blood pressure, increased SNS activity, and decreased HRV (Sondermeijer, Van Marle, Kamen, & Krum, 2002). Therefore, participants will be required to refrain from caffeine ingestion 12 hours prior to completing the ECG as this may interfere with the HRV reading.

Alzheimer's Disease and Heart Rate Variability

To date, there are no studies exploring changes in autonomic function among those diagnosed with AD who are taking Donepezil compared to an age and gender

matched healthy control group over a period of six months using supine and standing positions. This study will obtain time and frequency-domain HRV data using both supine and standing positions at three separate time frames (session 1 [baseline]; session 2 [3 months]; and session 3 [6 months]). Special attention will be paid to the low and high frequency indices as well as the LF/HF modulation ratio and the square root of the mean successive differences between adjacent R-R intervals (RMSSD). Data pertaining to the covariates described in the HRV confounds section will be obtained each session and controlled for in the analyses.

The results of the few studies examining cardiac autonomic function in AD are conflicting, which is likely due to the use of varying methods of measuring heart rate variability (e.g., standing vs. supine vs. tilting positions vs. 24-hour). Limitations in the methodology of previous studies include the lack of a healthy control group, the use of a single time frame (cross sectional design) for data collection, the short period of time in which participants were taking Donepezil, the absence of controlling for multiple covariates, the failure to report HRV data in normalized (standardized) units (n.u.), the absence of a respiration count and a respiratory sinus arrhythmia (RSA) measure, the use of animal subjects, and the use of a single position to capture HRV (Allan et al., 2005; Da Costa Dias Dias, et al., 2013; Giubilei et al., 1998; Masuda & Kawamura, 2003; McLaren, et al., 2003; Siepmann, et al., 2006; Umegaki & Khookhor, 2013; Zullii et al., 2005). Despite the methodological issues with these studies, the majority of the researchers have indicated the presence of parasympathetic depression and sympathetic exacerbation in persons with AD.

In summary, HRV measures can be used to assess cardiac autonomic function, and in some studies, ChEls have been shown to improve cardiac autonomic function in persons with AD and animal subjects (Da Costa et al., 2013; Giubilei et al., 1998; Nordstrom et al., 2013; Umegaki & Khookhor, 2013). The present study will address some of the methodological limitations present in previous research. Firstly, participants taking the medication Donepezil will be matched with a healthy control of the same age (± 3 years) and gender, as both age and gender have been shown to influence HRV (Stein, Kleiger, & Rottman 1997; Umetani, Singer, & McCraty, 1998). Secondly, the participants in the current study will be taking Donepezil for six months and data will be obtained at three different time points (baseline, three months, and six months). Few studies (including Giubilei et al., 1998; Masuda & Kawamura, 2003; McLaren, et al., 2003; Siepmann et al., 2006; Umegaki & Khookhor, 2013) have examined the cardiovascular benefits of the medication after six weeks. Thirdly, the present study will control for the following confounds as previous research has indicated that these factors can interfere with HRV measures: age, gender, body mass index (BMI), blood pressure (hypertension), medications (blood pressure, allergy, anxiolytic or antidepressant), length of time on Donepezil, alcohol use, caffeine use, and physical activity (Harte & Meston, 2013; Hayano et al., 1990; Koskinen, Virolainen, & Kupari, 1994; Molifino et al., 2009; Sandercock, Bromley, & Brodie, 2005; Sondermeijer, Van Marle, Kamen, & Krum, 2002; Stein, Kleiger, & Rottman 1997; Umetani, Singer, & McCraty, 1998). Participants will not be eligible for the study if they are taking certain medications that may interfere with HRV measures (e.g., anti-depressants), have a recent history of a cardiovascular event (e.g., stroke), a recent history of a severe medical condition (e.g., cancer), or suffer

from a severe psychological disorder (e.g., bi-polar disorder or schizophrenia). Fourthly, the results will be reported in normalized units (n.u.) to emphasize the controlled and balanced behavior of the sympathetic and parasympathetic nervous system (Task Force on Heart Rate Variability, 1996). The normalization process will also provide more meaningful quantities by using percentage values (0%-100%).

Fifthly, respiration count will be obtained as heart rate has been shown to synchronize with respiratory rhythm. Capturing respiration and adding this to the HRV algorithm will also serve to improve the accuracy of parasympathetic data via a measure of respiratory sinus arrhythmia (Grossman & Taylor, 2006). This is an important indices because heart rate increases during inhalation and decreases during exhalation. Respiratory linked variations in heart rate also usually occur in the high frequency (HF) range (Lehrer, Vaschillo, and Vaschillo, 2000). Finally, the present study will obtain HRV measures in both supine and standing positions as previous studies (Da Costa et al., 2013; Giubilei et al., 1998; Masuda & Kawamura, 2003; Siepmann et al., 2006; Toledo & Junqueira, 2008) have demonstrated differences in HRV as a result of varying methods of measuring heart rate (e.g., supine vs. standing. vs. tilting vs. 24-hour). Overall, the current study will provide additional and more robust evidence into the effects of Donepezil on cardiac autonomic function in persons with AD.

CHAPTER III

METHODS AND PROCEDURE

Participants

Recruitment for the present study was completed in February, 2015. There were two groups in which individuals were recruited; an Alzheimer's Disease (AD) group and a healthy control group. The AD group is comprised of individuals who were newly diagnosed with probable AD of mild to moderate severity as reflected by Mini Mental State Examination (MMSE) scores between 16 and 28. The AD group consists of individuals taking Donepezil. AD participants were not taking Donepezil for longer than 3 months at Session 1. Doses of Donepezil were flexible and typically ranged from 5mg to 10mg (after titration). The therapeutic dose may differ for each individual as dose is based on the physician's discretion. If participants discontinued Donepezil at any point during the study, they were no longer eligible to participate. The healthy control group consists of individuals who were not diagnosed with AD or any other type of cognitive disorder, and who were not taking Donepezil. The Healthy Control group self-reported to be free from cognitive impairment and obtained MMSE scores within the normal range (27-30; Crum, Anthony, Bassett, & Folstein, 1993). Both groups were matched for gender as well as age (± 3 years). This matching process is similar to that used by Stein et al (1997) who successfully matched male and female older adult participants (± 3 years) to study the differing effects of age on HRV between men and women. AD participants were matched with a healthy control on an ongoing basis.

All participants recruited for the study had at least a 6th grade education level, which was ascertained during the phone screen. Probable AD was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). All participants were free from the following conditions: severe and chronic diseases affecting general health (e.g., treatment for cancer [within the past year], diagnosed cardiovascular disease, chronic kidney or liver disease (end stage renal disease requiring dialysis), stroke or cerebrovascular events or disease, or brain tumors. Participants were all fluent in English, and did not have any current substance dependence (based on DSM-5 criteria). Participants were not taking other cholinesterase inhibitors, N-Methyl-D-aspartate (NMDA) receptor antagonists (e.g., Namenda), anti-psychotics, anticonvulsants or tricyclic antidepressants as these medications could interfere with the measures used in this study. Participants were not diagnosed with severe psychiatric disorders such as psychotic disorders, schizophrenia, or bipolar disorder. Lastly, participants did not have a history of multiple head injuries, traumatic brain injury or multiple losses of consciousness.

For sessions 1 and 2, the AD group ($n= 12$) and the healthy control group ($n= 12$) were larger than the AD group ($n= 8$) and the healthy control group ($n= 8$) in session 3. This was largely due to attrition rates and user error/unrecoverable data among the AD group. More specifically, one AD participant ($n= 1$) withdrew from the study due to the worsening of AD symptoms (cognitive decline) and a second AD participant ($n= 1$) withdrew from the study due to adverse medication reactions which resulted in the discontinuation of Donepezil (Aricept®). HRV data from two AD participants ($n= 2$) were lost due to technological difficulties with the receiver (transferring the data to a PC

failed) as well as user error (failure to capture the correct HRV time-interval data).

Therefore, two separate series of group analyses were conducted. In the first group of

HRV analyses, the AD group ($n= 12$) were compared to the healthy control group ($n= 12$) over a period of three months (session 1 and session 2; $N= 24$). In the second set of

HRV analyses, the AD group ($n= 8$) were compared to the healthy control group ($n= 8$)

over six months (session 1, session 2, and session 3; $N= 16$).

In the first set of HRV analyses ($N= 24$), the AD group consisted of seven males

(*Mean age* $= 75.71$, *SD*$= 6.99$) and five females (*Mean age* $= 73.80$, *SD*$= 6.14$) who were

matched with the healthy control group based on age and gender. Therefore, the healthy

control group also consisted of seven males (*Mean age* $= 75.14$, *SD*$= 7.43$) and five

females (*Mean age* $= 73.00$, *SD*$= 5.70$) of similar respective age. In the second set of

HRV analyses ($N= 16$), the AD group consisted of five males (*Mean age* $= 77.20$, *SD*$=

6.26$) and three females (*Mean age* $= 70.33$, *SD*$= 5.51$) who were again matched with the

healthy control group based on age and gender. Therefore, the healthy control group also

consisted of five males (*Mean age* $= 77.40$, *SD*$= 4.92$) and three females (*Mean age* $=

70.33$, *SD*$= 6.11$) of similar respective age. The total number of participants recruited for

the present study for sessions 1, 2, and 3 ($N= 16$) exceeded the total sample size

generated by the power analysis for the supine position. The total number of participants

recruited for the present study for sessions 1, 2, and 3 ($N= 16$) were equal to the total

sample size generated by the power analysis for the standing position (please refer to the

Power Analysis Section in Appendix G).

Recruitment. Participants diagnosed with probable AD were recruited from the

Glennan Center at Eastern Virginia Medical School (EVMS). The physicians were

provided the eligibility criteria for the present study. If a patient met eligibility criteria for the AD study, he/she was informed about the study and provided a verbal consent as to whether or not they wanted to be contacted by a research team member. No study data was obtained from the referring physicians. Flyers advertising for AD participants were placed in the Glennan Center, offices of Internal Medicine, the Neuropsychology Center, and public bulletin boards (PrimePlus senior center and the YMCA). All methods of advertising received Internal Review Board (IRB) approval.

The age-matched healthy control group were recruited by local advertisement as well as referrals from EVMS clinicians. Flyers advertising the need for volunteers were placed in the offices of the Glennan Center, Internal Medicine, the Neuropsychology Center, and public bulletin boards. Caregivers and local physicians were advised to give the flyers to healthy individuals and/or individuals with recently diagnosed Alzheimer's disease that might be interested in participating in the study.

Once a potential participant provided a verbal consent to be contacted by a member of the research team, a research assistant contacted the potential participant via telephone and conducted a brief eligibility screen (see Appendix A). This included questions surrounding Donepezil (Aricept®) use, the presence or history of cardiovascular disease(s) or incidents (e.g., stroke), pertinent psychiatric history (e.g., a history of schizophrenia or bipolar disorder), and medication use such as tricyclic anti-depressants. If the participant was eligible for the study, he/she was invited to attend three sessions at Eastern Virginia Medical School (EVMS), Department of Psychiatry and Behavioral Sciences, located in Andrews Hall, Suite 244, Norfolk, Virginia. If an AD participant was not able to attend the sessions at EVMS due to transportation issues (a

common barrier for older adults with AD), the sessions were conducted in the participant's home. In-home sessions only took place if the participant lived within the Hampton Roads area. All equipment was easily portable making this a very viable option. All three sessions were completed in the same location to reduce the likelihood of the environment impacting HRV recordings across sessions. Therefore, AD participants either completed all three sessions in the home or at Eastern Virginia Medical School (EVMS). A total of four AD participants completed all three HRV sessions in the home environment. Participants in the healthy control group completed all sessions at EVMS.

Proposed Research Design and Methods

The present study has three specific hypotheses: 1) participants that are diagnosed with mild to moderate AD and who are taking a stable dose of Donepezil (Aricept®) will show increased parasympathetic nervous activity over a period of six months. This will be indicated by decreases in LF Power (n.u.) and LF/HF Ratio in the standing position at session 3 (six months) compared to session 1 (baseline). Thus, there will be a decrease in LF Power (n.u.) and LF/HF Ratio from session 1 (baseline) to session 3 (six months) when engaging in the orthostatic challenge (supine to standing). In contrast, HF Power (n.u.) and RMSSD will increase in the standing position at session 3 (six months) compared to session 1 (baseline). Therefore, there will be an increase in HF Power (n.u.) and RMSSD from session 1 (baseline) to session 3 (six months) when engaging in the orthostatic challenge (supine to standing); 2) Healthy controls will show significant increases in HF Power (n.u.) from a supine to standing position at all three sessions. The AD group will demonstrate minimal change in HF Power (n.u.) from a supine to a standing position at the baseline session, but will show increased HF Power (n.u.) from

supine to stand at sessions 2 and 3. Changes in HF Power (n.u.) from a supine to a standing position at session 3 will be similar among both AD participants and healthy controls; and 3) Donepezil (Aricept®) will improve parasympathetic nervous activity through increased acetylcholine transmission, thus supporting the medications use as a potential protective agent against cardiac autonomic dysfunction. This study will assess heart rate variability (HRV) at baseline (initial session), three months after the first session (session 2), and six months after first session (session 3). Each session will last approximately 90 minutes in duration. Basic information such as height, weight, blood pressure, body mass index (BMI), and heart rate variability measurements will be obtained at each session. Again, the primary goal of the present research is to develop an understanding of the relationship between cardiac autonomic function and AD while identifying the cardiovascular effects of Donepezil on participants with AD.

Study Protocol

The present study obtained short-term time-domain (six minutes) and frequency-domain HRV data over the course of six months. HRV was obtained using a noninvasive and reliable method that is widely used to measure cardiac autonomic function. More specifically, Polar Performance Technology was utilized to evaluate overall cardiac autonomic modulation including sympathetic and parasympathetic nervous system function. HRV data were captured at baseline, three month, and six month intervals from both AD and healthy control participants in both supine and standing positions as recommended by the Task Force on Heart Rate Variability (1996). In order to obtain HRV in a standing position, it was recommended that the participant sit in a resting position prior to commencing the standing measurement (Risk, Broadbridge, & Cohen,

2001). Obtaining HRV readings from a still resting position (supine) to a standing position (orthostatic challenge) helped identify any significant changes in SNS and PNS activity (Aysin & Aysin, 2007). In the present study, participants were seated in the supine position for six minutes while an ECG was administered and HRV was obtained. The participants then stood for six minutes and the ECG was re-administered for a second time. Research suggests that the heart rate response to standing evaluates the cardiovascular response initiated by a change from horizontal to a vertical position (Risk, Broadbridge, & Cohen, 2001). The typical heart rate response to standing (increased sympathetic activity) is largely mitigated by parasympathetic activity (Risk, Broadbridge, & Cohen, 2001).

The potential impact of hypertension, obesity (BMI), exercise, caffeine use, alcohol use, Aricept® dose, and related medications were tracked and evaluated in tandem with the effects of Donepezil in the AD participants. This medical and lifestyle-behavior information was collected for each participant at every session over the six month period. All tests used in this protocol had been widely and safely used with diverse populations (Allan et al., 2006; Da Costa Dias Dias, et al., 2013; Task Force on Heart Rate Variability, 1996; Toledo & Junqueira, 2008). All necessary precautions were taken during the course of participation to ensure the safety and wellbeing of the participant. The protocol was carried out under the supervision of the principal investigator, Serina Neumann, Ph.D. Although the risks associated with participation were minimal, adverse events were reported in compliance with EVMS's Internal Review Board (IRB) policies. When a clinically significant physical or psychiatric disorder was identified during the

course of participation, an appropriate referral to a medical or psychological treatment facility was offered.

It is important to mention that the current study is part of a larger study exploring the effects of Donepezil (Aricept®) on cardiac autonomic function and neuro-cholinergic function among patients diagnosed with probable AD. In addition to the time- and frequency-domain HRV measures discussed in the present study, neuropsychological testing was also completed at the three month intervals. More specifically, neuropsychological measures were obtained at baseline, three months and six months in tandem with the respective HRV measures. Specific neuropsychological test measures at these intervals included the Symbol Digit Modalities Test, Trails Making Tests A and B, Controlled Oral Word Association Test, the Test of Premorbid Functioning (TOPF), and Hopkins Verbal Learning Test. The same battery of testing was administered during all sessions. However, for the purposes of this dissertation, the present study will primarily explore Donepezil's effect on cardiac function in patients with AD through an in vivo, non-invasive measure of cardiac autonomic function.

Measures

Demographics/Socioeconomic Status (SES) form. This form was used to collect demographic information such as age, gender, ethnicity, educational attainment, and job and work history (see Appendix B). Age, gender, and ethnicity have been shown to influence HRV (Stein, Kleiger, & Rottman 1997; Umetani, Singer, & McCraty, 1998) and the Demographics/Socioeconomic Status (SES) form was used to capture this information. These variables were then controlled for in the analyses.

Lifestyle Behaviors Questionnaire. The lifestyle behaviors questionnaire was used to collect information on lifestyle choices such as tobacco and alcohol use, daily caffeine consumption, and hours of physical activity. The questions on this form were compiled using selected questions from the following: SCID-NP (Structured Clinical Interview for DSM-IV: Non-patient edition), Paffenbarger Physical Activity Questionnaire, and Tobacco use interview (see Appendix C). Alcohol use, tobacco use, caffeine use, and physical activity can also interfere with HRV measures, and this information was captured using the lifestyle behaviors questionnaire and controlled for in the analyses (Harte & Meston, 2013; Hayano et al., 1990; Koskinen, Virolainen, & Kupari, 1994; Molifino et al., 2009; Sandercock, Bromley, & Brodie, 2005; Sondermeijer, Van Marle, Kamen, & Krum, 2002).

Medical History Checklist form. The medical history form was used to obtain detailed information concerning the participant's medical history. This included any medical conditions surrounding the heart or blood vessels, the brain including neurological conditions, and other diseases (see Appendix D). Participants were screened for severe medical conditions prior to enrolling in the study and those with severe medical conditions such as a recent history of stroke or a diagnosis of multiple sclerosis were not be eligible to participate.

Medication Update Form. The medication update form was used to record and follow changes in medication use and adherence to medical instructions during both sessions. This included a list of all medications prescribed to participants, the prescribed purpose, as well as the dosage and frequency. Information specific to the administration of Donepezil was also gathered (see Appendix E). Certain medications such as beta-

blockers and antihistamines can also effect blood pressure and HRV (Acharya et al., 2006; Tinetti & Kumar, 2010). Therefore, these medication types and their prescribed doses were controlled for in the analysis. If participants indicated that they had discontinued Donepezil, they were no longer eligible to participate in the study. Participants who reported adverse side-effects from their medications were referred to their physician immediately. These adverse events were then reported in compliance with EVMS's Internal Review Board (IRB) policies.

Physical and Biological Assessments. The physical and biological assessments were used to obtain the following: body mass index (BMI), blood pressure, and heart rate variability. These data points were collected at all three sessions. Blood pressure was obtained after the participant had been seated for five minutes (See appendix F). High body mass index and high blood pressure have been shown to interfere with HRV measures, and these were be captured by the physical and biological assessment and subsequently controlled for in the analyses (Kleiger, Miller, Bigger, & Moss, 1986; Kwon et al., 2008; Naver, Bloomstrand, & Wallin, 1996).

Measurement of HRV. HRV was measured from a continuous time series of beat-to-beat intervals with Polar Performance Technology. Participants were required to attach the Polar Performance sensor strap around their chest with the aid of a research assistant to ensure the sensor was correctly placed. Participants were also required to wear the Polar Performance receiver on their wrist. To estimate vagal activity precisely, respiratory rate was assessed for one minute intervals at minute one and minute three of the HRV recording. Respiration rate was captured by visual observation (rise and fall of chest). Time and frequency-domain measures of HRV were recorded by the Polar

Performance Technology. Time domain analyses provided the root mean of successive differences in R-R intervals (RMSSD) and heart rate. Based on the Task Force on Heart Rate Variability (1996), spectral analyses was performed on the beat-to-beat intervals derived from the electrocardiogram (ECG) data collection to obtain both low frequency (LF: 0.04-0.15 Hz) and high frequency (HF: 0.15-0.40 Hz) indices. As discussed in the previous chapter, LF and HF Power bands measure differential autonomic nervous system influences. HF Power primarily reflects respiratory-modulated parasympathetic outflow, whereas LF frequency reflects sympathetic control and varying amounts of parasympathetic influences (Toledo & Junqueira, 2008; Task Force on Heart Rate Variability, 1996). LF Power is also thought to reflect baroreceptor-mediated regulation of blood pressure (Bernston, et al., 1997; Friedman & Thayer, 1998). To interpret sympathetic influences on the heart, an LF/HF ratio was computed to measure of sympathovagal balance (Malliani, Lombard, Pagani, & Cerutii, 1990). This process was completed for both supine and standing positions as LF Power in particular shows significant changes when positioning is altered from a supine to a standing posture (Acharya, et al., 2006; American Heart Association, 1996). Additionally, HRV has been shown to vary as a function of gender, age, body mass index, high blood pressure, caffeine use, heavy alcohol consumption, tobacco use, and physical activity, and these factors were controlled for in the analyses if they correlated with HRV indices. More information regarding the use of the HRV data can be found in the HRV data reduction section below.

   Data storage. All data were disassociated from participant's names and other identifying information by randomly assigning a four digit ID number. The connection

relating the participant name with their ID number was held by the principal investigator under lock and key and was destroyed after participant recruitment and data collection was completed. Research staff training procedures also incorporated explicit instruction regarding procedures for protecting participant confidentiality.

HRV Data Reduction

HRV data obtained from the Polar Performance Technology equipment during the three sessions were screened for outliers and technological errors and these were modified as needed. Once the data were checked for errors, average respiration rate adjustments were made to the HF Power bands. The average number of respirations from minute one and minute three were then divided by 60, and parameters were created by adding and subtracting .03 (Berntson, et al., 1997). The HF Power band represented Respiratory Sinus Arrhythmia (RSA). This data was then analyzed using the Kubios HRV Analysis Software − Version 2.0 (Biosignal Analysis and Medical Imaging Group, 2008) which provided reports of HRV frequency-domains. Once these reports were generated, data from the Autoregressive approach (AR) were used over the fast Fourier transformation (FFT), as the AR method provides better frequency resolution than the fast Fourier transformation (Malliani, Pagani, & Lombardi, 1994). The AR technique concentrates on the more significant heart rate peaks which ultimately excludes additional noise or interference (Berntson et al., 1997).

As recommended by the Task Force on Heart Rate Variability (1996), both LF and HF Power bands were represented in normalized units (n.u.) which emphasizes the controlled and balanced behavior of the sympathetic and parasympathetic nervous

system. Normalized units are also advantageous as they minimize the effect of the change in total power on the values of the LF and HF indices (Task Force on Heart Rate Variability, 1996). The two normalized HRV power bands (LF Power n.u.) and (HF Power n.u.) were then used to calculate the LF/HF ratio. The LF/HF ratio is a widely used HRV index of sympathovagal balance between the two branches of the autonomic nervous system (Burr, 2007). The LF/HF ratio is calculated by dividing the LF Power by the LF plus the HF Power (Burr, 2007). Additionally, the square root of the mean of the squared successive differences between adjacent R-R intervals (RMSSD) was analyzed, which reflects the rapid beat-to-beat changes in intervals that are dependent on the parasympathetic modulation associated with the respiratory sinus arrhythmia (RSA). For each session (session 1 [baseline], session 2 [3 months], and session 3 [6 months]) averages from the HF Power n.u., LF Power n.u., RMSSD, and the LF/HF ratio were computed for both the Alzheimer's group and the healthy control group while in the supine position and while standing (Toledo & Junqueira, 2008). These four HRV indices in both standing and supine positions were compared across three different time frames in separate analyses for both groups (between and within). Therefore, there were eight different HRV dependent measures for each time point.

Statistical Analyses

Power analysis. *A priori* power analyses were conducted for both supine and standing positions over three different time points. The power analysis was used to determine an appropriate sample size to achieve adequate power to conduct the ANOVA for the three hypotheses. Cohen's *d* effect sizes were estimated for both supine and standing positions from the Toledo & Junqueira (2008) cross sectional study evaluating

HRV in AD patients. The Cohen's d's were considered medium (d's= .65) for the supine AD high frequency power (n.u.) group mean (M= 0.28; SD= 0.03) and the control group mean (M= 0.38; SD= 0.04; Cohen, 1988). The Cohen's d's were considered large (d's = .82) for the standing AD high frequency power (n.u.) group mean (M= 0.28 n.u.; SD= 0.03) and the control group mean (M= 0.34; SD= 0.04; Cohen, 1988). Given these effect sizes and using a two-tailed alpha of .05, a power analysis was conducted with g*power software (version 3.1.7). The results from the power analysis indicated that the total number of participants required to obtain a similar effect size in the supine position for a repeated measures, between-subjects, ANOVA would be 12. The total number of participants required to obtain a similar effect size in the standing position for a repeated measures, between-subjects, ANOVA would be 16 (see Appendix G).

Preliminary analyses. Preliminary analyses were conducted for two separate samples. The first sample included participants from session 1 (baseline) and session 2 (3 months; N= 24) while the second analyses included participants from session 1 (baseline), session 2 (3 months) and session 3 (6 months; N= 16). Using SPSS software, the preliminary analyses examined potential differences between the Alzheimer's Disease (AD) group and healthy controls on sample characteristics at session 1 (baseline), session 2 (3 months) and session 3 (6 months). This included age, gender, ethnicity, blood pressure, body mass index, average hours of weekly exercise, medications (blood pressure, allergy, anxiolytic or antidepressant), dose of Donepezil (Aricept®), average weekly alcohol use, caffeine use (12 hours prior to HRV recording), exercise (12 hours prior to HRV recording), and allergy medication (12 hours prior to HRV recording). Though it was initially proposed that tobacco use would be examined, there were no

current smokers in any group, and all previous smokers had been tobacco free for more than 25 years. Therefore, this variable was removed from the analyses. Data were checked for outliers, missing data points, skewness, and kurtosis. Normal distribution was determined by calculating z-scores and dividing the skewness values by their respective standard errors. If the absolute z-scores for skewness or kurtosis were larger than 1.96 corresponding with a significant alpha level of < 0.05, these were considered not-normal and subsequently transformed (Field, 2009). Both RMSSD and LF/HF Ratio demonstrated numerous indices that were not normally distributed (i.e. skewed). As such, all indices of RMSSD and LF/HF Ratio (supine and standing, sessions 1, 2, and 3) were uniformly normalized by base 10 logarithm. Following these transformations, RMSSD (log10) and LF/HF Ratio (log10) were re-checked for skewness and kurtosis and all were considered to be normally distributed (skewness and kurtosis ratio $= < 1.96$; Field, 2009).

Once normal distribution among HRV indices was established, the HRV indices were correlated with the following sample characteristics: age, gender, ethnicity, blood pressure (systolic and diastolic), body mass index, average hours of weekly exercise, medications (blood pressure, allergy, anxiolytic or antidepressant), dose of Donepezil, average weekly alcohol use, caffeine use (12 hours prior to HRV recording), exercise (12 hours prior to HRV recording), and allergy medication (12 hours prior to HRV recording). Pearson correlation coefficient non-parametric statistics were used for these continuous/interval sample characteristics (e.g., HF Power and age). The sample characteristics which were significantly correlated ($p <.05$) to any of the HRV indices were controlled for in the ANCOVA analyses. To determine whether or not the categorical (binomial) sample characteristics (gender, ethnicity, medication type [blood

pressure, allergy, anxiolytic or antidepressant], physical activity 12 hours before HRV session, alcohol or caffeine use 12 hours before HRV session, and cold and allergy medication 12 hours before HRV session) were correlated with the HRV indices, a Kendell's tau-b statistic was used. This was also recommended given the small sample size used in this study (Field, 2009). Sample characteristics that were significantly correlated with HRV indices ($p= <.05$) were controlled for in the ANCOVA model.

In order to identify between and within group differences among demographic variables and sample characteristics, continuous variables were added to a repeated measures ANOVA to test for independence. Continuous variables (e.g., average weekly alcohol use) that showed significant between and within group differences were added to the final ANCOVA model as covariates. Group differences among binomial variables were identified using a chi-square test of independence. Those variables which were not independent were added to the ANCOVA model as covariates. Lastly, using regression analyses, the standardized residuals (variance) from the Aricept® dose were saved as separate variables. The differences between the groups were considered statistically significant when a p-value was equal or less than 5% ($p < .05$). The standardized residuals were then correlated with the HRV indices using a Pearson correlation coefficient non-parametric statistic. The standardized residuals from the Aricept® dose that were significantly correlated with HRV indices were added to the final ANCOVA model as covariates.

Primary analyses. Firstly, for the three hypothesis, a 2 (group [AD group versus healthy controls]) x2 (task [supine to standing]) x2 (time [baseline and 3 month intervals]) repeated measures analysis of covariance (ANCOVA) was computed. This

analyses (2x2x2) tested the difference between the AD group versus the healthy control group on the time- and frequency-domain HRV indices while engaging in the supine and standing tasks over the course of three months. Secondly, a 2 (group [AD group versus healthy controls]) x2 (task [supine to standing]) x3 (time [baseline, 3 month and 6 month intervals]) repeated measures ANCOVA was computed. This analyses (2x2x3) tested the difference between the AD group versus the healthy control group on the time- and frequency-domain HRV indices while engaging in the supine and standing tasks over the course of six months.

Each AD participant was successfully matched with a healthy control based on age (± 3 years) and gender. The ANCOVA was used to control for the existing covariates that were identified in the preliminary analysis. The LF Power (n.u.), HF Power (n.u.), LF/HF Ratio (log10) and RMSSD (log10) obtained from the Alzheimer's and healthy control groups were compared across two and three different time points in both supine and standing positions. Levene's tests were checked for homogeneity of variance. For those indices where the Levene's tests were significant, the ratio of highest to lowest variance was calculated. It is recommended that if the critical values of the variance ratios are less than five, then the variance equality is considered acceptable (Field, 2009). Variances for each HRV indices were calculated by squaring the standard deviations of the HRV values. All HRV indices which showed Levene's tests violations ($p=$ <.05) were considered acceptable based on the variance ratios.

Secondary analysis. Tukey's HSD post-hoc tests for pairwise comparisons were used to identify between and within group differences in the 2 (group [AD group versus healthy controls]) x2 (task [supine to standing]) x2 (time [baseline and 3 month

intervals]) repeated measures ANCOVA and the 2 (group [AD group versus healthy controls]) x2 (task [supine to standing]) x3 (time [baseline, 3 month and 6 month intervals]) repeated measures ANCOVA. Tukey's HSD post-hoc tests were computed using Tukey's HSD post-hoc statistics software (Hall, 1998). The data from the ANCOVA tables generated in SPSS for LF Power (n.u.), HF Power (n.u.), RMSSD (log10) and LF/HF Ratio (log10) were transferred to this software. Separate Tukey's HSD post-hoc tests using the same processes were also conducted to identify any significant within group differences from supine to standing.

Expected study findings. The expected findings of the present study include the following: 1) participants that are diagnosed with mild to moderate AD and who are taking a stable dose of Donepezil (Aricept®) will show increased parasympathetic nervous activity over a period of six months. This will be indicated by decreases in LF Power (n.u.) and LF/HF Ratio (log10) in the standing position at session 3 (six months) compared to session 1 (baseline). Thus, there will be a decrease in LF Power (n.u.) and LF/HF Ratio (log10) from session 1 (baseline) to session 3 (six months) when engaging in the orthostatic challenge (supine to standing). In contrast, HF Power (n.u.) and RMSSD (log10) will increase in the standing position at session 3 (six months) compared to session 1 (baseline). Therefore, there will be an increase in HF Power (n.u.) and RMSSD (log10) from session 1 (baseline) to session 3 (six months) when engaging in the orthostatic challenge (supine to standing); 2) Healthy controls will show significant increases in HF Power (n.u.) from a supine to standing position at all three sessions. The AD group will demonstrate minimal change in HF Power (n.u.) from a supine to a standing position at the baseline session, but will show increased changes in HF Power

(n.u.) from supine to standing at sessions 2 and 3. Changes in HF Power (n.u.) from a supine to a standing position at session 3 will be similar among both AD participants and healthy controls; and 3) Donepezil (Aricept®) will improve parasympathetic nervous activity through increased acetylcholine transmission, thus supporting the medications use as a potential protective agent against cardiac autonomic dysfunction. If these hypotheses are not met, this suggests that Donepezil may not possess the cardio-protective qualities that were initially expected, and the medication may not improve cholinergic resources to the extent of enhancing cardiac autonomic function. These results would be consistent with the results obtained by Masuda & Kawamura (2003), McLaren et al (2003), and Siepmann et al (2006) who indicated a decrease in HF Power as result of taking the medication Donepezil. Additionally, if there are no changes in HF Power (n.u.) from a supine to a standing position at sessions 2 and 3, this will provide further evidence that Donepezil does not possess adequate cardio-protective properties. Furthermore, increases in LF Power (n.u.) and a higher LF to HF ratio may indicate the continued dominance of the sympathetic nervous system and the presence of parasympathetic depression despite the use of Donepezil. More specifically, this will indicate a decrease in sympathovagal balance to reflect greater sympathetic modulation.

CHAPTER IV

RESULTS

Table 1 summarizes demographic information for the 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x2 (time [baseline and 3 month intervals]) repeated measures ANCOVA. Demographic variables which were correlated with HRV indices are denoted by p–values.

Table 1. Descriptive Statistics and HRV Index Correlations of the AD and HC Sample for the 2x2x2 ANCOVA (N= 24).

Demographic	AD	HC	p
Total Number of Participants	n= 12	n= 12	
Gender			*
Male (n)	7	7	
Female (n)	5	5	
Age (years)			
Male M (SD)	75.71 (6.99)	75.14 (7.43)	
Female M (SD)	73.80 (6.14)	73.00 (5.70)	
Ethnic Background			*
Caucasian (n)	8	10	
Male (n)	6	7	
Female (n)	2	3	
African-American (n)	4	2	
Male (n)	1	0	
Female (n)	3	2	

Table 1. Continued

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p values were derived from the Pearson correlation coefficients and Kendall's tau-b statistic. Demographics with significant *p* values are correlated with one or more of the HRV indices. Note: *p <.05

Tables 2 and 3 display sample characteristics for the 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x2 (time [baseline and 3 month intervals]) analyses. Sample characteristics that were correlated with HRV indices or showed significant group differences are denoted by *p*-values. Results from the Pearson correlation coefficient and Kendall's tau-b statistic indicated that gender and ethnicity were correlated with LF Power (n.u.), HF Power (n.u.), and LF/HF Ratio (log10; *p=* <.05). Body mass index was correlated with all four HRV indices (*p=* <.05) and allergy medication was correlated with RMSSD (log10) only (*p=* <.05). Therefore, these variables were included as covariates in the preliminary ANCOVA. Between group differences in weekly alcohol consumption were also identified using a repeated measures ANOVA $F(1,22) = 6.95$, $p = .015$, 95% CI [2.38, 7.31], $\eta^2 = .240$. This variable was also included in the ANCOVA model as a covariate.

Table 2. Sample Characteristics and HRV Index Correlations for the AD Group (n= 12).

Characteristic	Baseline (S1)	3 Months (S2)	*p*
Body Mass Index (kg) *M* (*SD*)	26.60 (5.59)	27.73 (6.46)	*
Blood Pressure Systolic (mmHg) *M* (*SD*)	139.92 (16.72)	130.83 (15.68)	
Diastolic (mmHg) *M* (*SD*)	75.92 (6.68)	74.00 (8.64)	
Dose of Aricept (mg/day)	5.00	10.00	
Blood Pressure Medication Taken (*n*)	9	9	
Allergy Medication Taken (*n*)	0	2	**
Anxiolytic or Antidepressant Taken (*n*)	4	3	
Number Alcoholic Drinks Per Week (Avg) *M* (*SD*)	2.08 (6.01)	1.33 (4.03)	**
Hours Exercise Per Week (Avg) *M* (*SD*)	3.85 (3.36)	4.54 (4.04)	
Alcohol or Caffeine last 12 hrs (*n*)	0	3	
Exercise last 12 hrs (*n*)	0	0	
Allergy Meds last 12 hrs (*n*)	0	1	

p values were derived from the Pearson correlation coefficients, Kendall's tau-b correlation coefficients, and Analysis of Variance. Sample characteristics with significant *p* values are correlated with one or more HRV indices. Note: $*p < .05$ $**p < .01$

Table 3. Sample Characteristics and HRV Index Correlations for the HC Group (n= 12).

Characteristic	Baseline (S1)	3 Months (S2)	*p*
Body Mass Index (kg) *M* (*SD*)	26.25 (5.15)	26.49 (5.49)	*
Blood Pressure Systolic (mmHg) *M* (*SD*)	134.75 (22.54)	127.75 (16.30)	
Diastolic (mmHg) *M* (*SD*)	76.58 (12.13)	75.33 (8.09)	
Blood Pressure Medication Taken (*n*)	6	7	
Allergy Medication Taken (*n*)	1	1	**
Anxiolytic or Antidepressant Taken (*n*)	1	1	
Number Alcoholic Drinks Per Week (Avg) *M* (*SD*)	6.92 (5.39)	7.04 (6.00)	**
Hours Exercise Per Week (Avg) *M* (*SD*)	8.58 (5.10)	5.29 (3.58)	
Alcohol or Caffeine last 12 hrs (*n*)	4	1	
Exercise last 12 hrs (*n*)	1	2	
Allergy Meds last 12 hrs (*n*)	1	0	

p values were derived from the Pearson correlation coefficients, Kendall's tau-b correlation coefficients, and Analysis of Variance. Sample characteristics with significant *p* values are correlated with one or more HRV indices. Note: *p <.05 ** *p*

Table 4. *Mean and Standard Error of Heart Rate Variability Indices in Healthy Control and Alzheimer's Disease Groups in Supine and Standing Positions at Baseline (Session 1) and Three Month (Session 2) Intervals.*

| | Baseline (S1) | | | | 3 Months (S2) | | | | | |
| | AD | | HC | | AD | | HC | | | |
HRV Measure	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*	*p*	η^2
Supine:										
Low Frequency (n.u.)	58.03 ± 6.66		60.16 ± 8.27		65.61 ± 7.36		68.98 ± 5.32		.330	.056
High Frequency (n.u.)	41.97 ± 6.66		32.91 ± 7.15		34.39 ± 7.36		31.02 ± 5.32		.617	.015
RMSSD (log 10)	1.73 ± 0.14		1.43 ± 0.14		1.53 ± 0.19		1.52 ± 0.12		.864	.002
LF/HF Ratio (log10)	0.19 ± 0.15		0.19 ± 0.15		0.45 ± 0.21		0.43 ± 0.13		.254	.072
Standing:										
Low Frequency (n.u.)	72.10 ± 6.63		63.19 ± 5.77		72.25 ± 6.55		68.58 ± 6.36		.330	.056
High Frequency (n.u.)	27.90 ± 6.63		36.81 ± 5.77		27.75 ± 6.55		31.42 ± 6.36		.617	.015
RMSSD (log 10)	1.46 ± 0.18		1.54 ± 0.11		1.49 ± 0.23		1.38 ± 0.11		.864	.002
LF/HF Ratio (log10)	0.61 ± 0.19		0.32 ± 0.14		0.62 ± 0.20		0.45 ± 0.15		.254	.072

The data for the heart rate variability 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x2 (time [baseline and 3 month intervals]) ANCOVA analyses are presented in Table 4. Based on the ANCOVA, no indices showed statistically significant between group differences from supine to standing (see Table 4). However, the AD group displayed a trend towards relative sympathetic exacerbation from supine to standing at both time points. This was indicated by increases in LF Power (n.u.) from supine to standing at baseline and three months. The healthy control group also demonstrated an increase in LF Power (n.u.) from supine to standing at baseline and three months but to a smaller magnitude (see figure 1). The AD group demonstrated lower levels of LF Power (n.u.) in the supine position at both time points in comparison to the healthy controls. As expected, the sympathetic exacerbation from supine to standing among the AD group was accompanied by relative parasympathetic depression in comparison to the healthy control group. Among the AD group, this was indicated by decreases in HF Power (n.u.) from supine to standing at baseline and supine to standing at three months. In comparison, the healthy control group demonstrated parasympathetic enhancement as evidenced by increased HF Power (n.u.) from supine to standing at baseline and stable HF Power (n.u.) from supine to standing at three months (see figure 2). Consistent with lower levels of LF Power (n.u.), the AD group demonstrated higher levels of HF Power (n.u.) in the supine position in comparison to healthy controls at both time points.
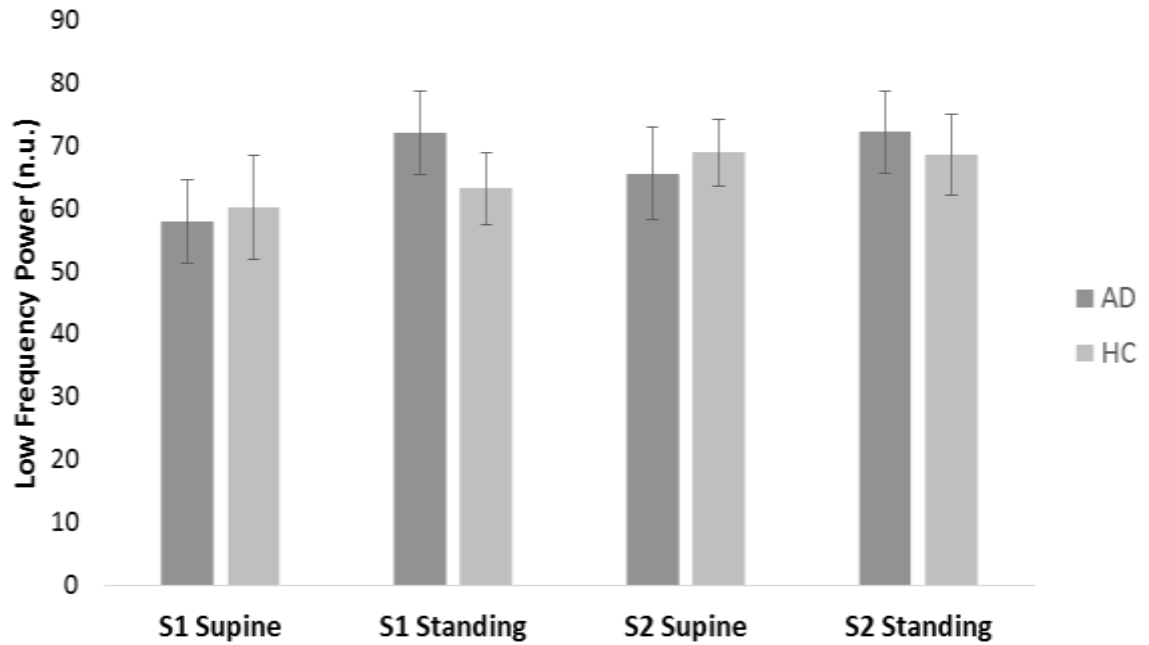
Figure 1. *Supine to standing mean low frequency power (n.u.) for the AD and HC group at baseline (S1) and 3 months (S2).*
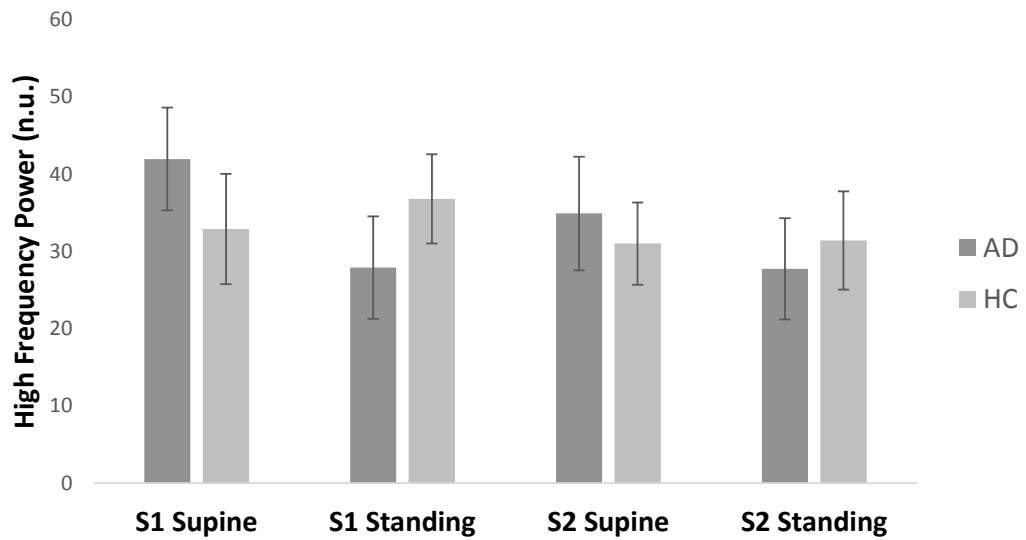
Figure 2. *Supine to standing mean high frequency power (n.u.) for the AD and HC group at baseline (S1) and 3 months (S2).*

The relative sympathetic dominance in the standing position among the AD group was also reflected by larger LF/HF Ratio (log10). More specifically, the degree in which the LF/HF Ratio (log10) for the AD group increased from supine to standing at baseline and supine to standing at three months was incrementally larger than the healthy control group. In comparison, the healthy control group demonstrated increases in sympathetic nervous activity from supine to standing at baseline and supine to standing at three months, but to a lower magnitude (see figure 3). As expected, the AD group showed reductions of a larger magnitude in the mean squared differences of successive R-R intervals (RMSSD [log10]) from supine to standing at baseline in comparison to the healthy control group who showed an increase in RMSSD (log10) from supine to standing. Interestingly, this was not the case at the three month time period. At this time interval, the healthy control group demonstrated smaller magnitudes of RMSSD (log10) from supine to standing in comparison to the AD group (see figure 4). Larger magnitudes of RMSSD (log10) among the AD group in the supine position at sessions 1 and 2 in comparison to healthy controls were also reflective of greater HF Power (n.u.) at these time points. Consistent with greater HF Power (n.u.) in the supine position, the AD group showed a greater magnitude of RMSSD (log10) at both points and a slightly smaller magnitude of LF/HF Ratio (log10) at session 2. Secondary analyses using Tukey's post-hoc tests did not reveal any significant between or within group differences for the 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x2 (time [baseline and 3 month intervals]) ANCOVA's
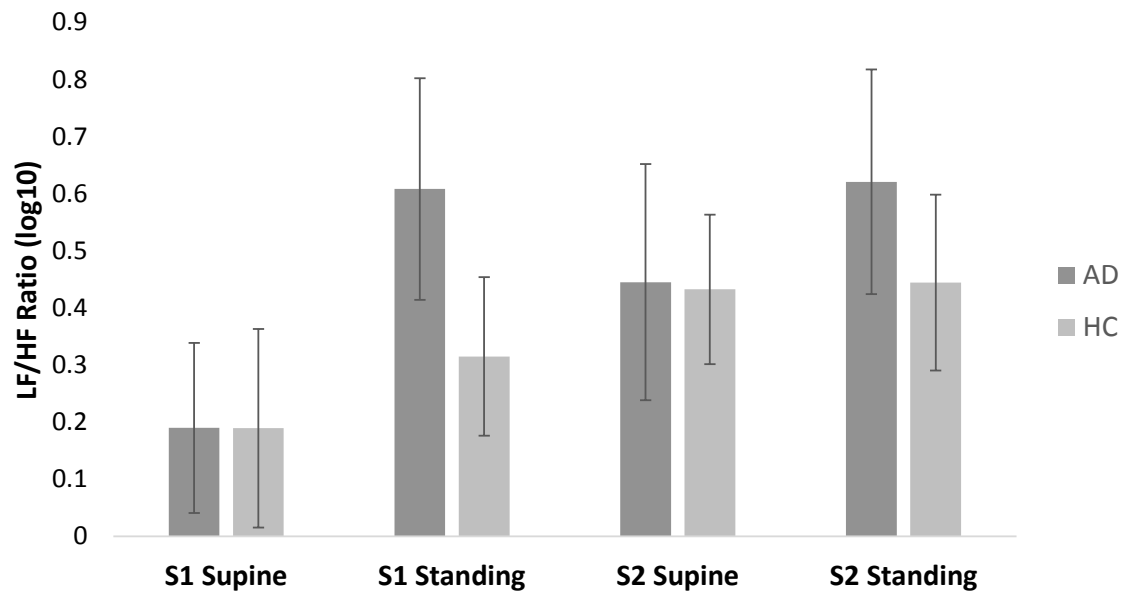
Figure 3. *Supine to standing mean LF/HF Ratio (log10) for the AD and HC group at baseline (S1) and 3 months (S2).*
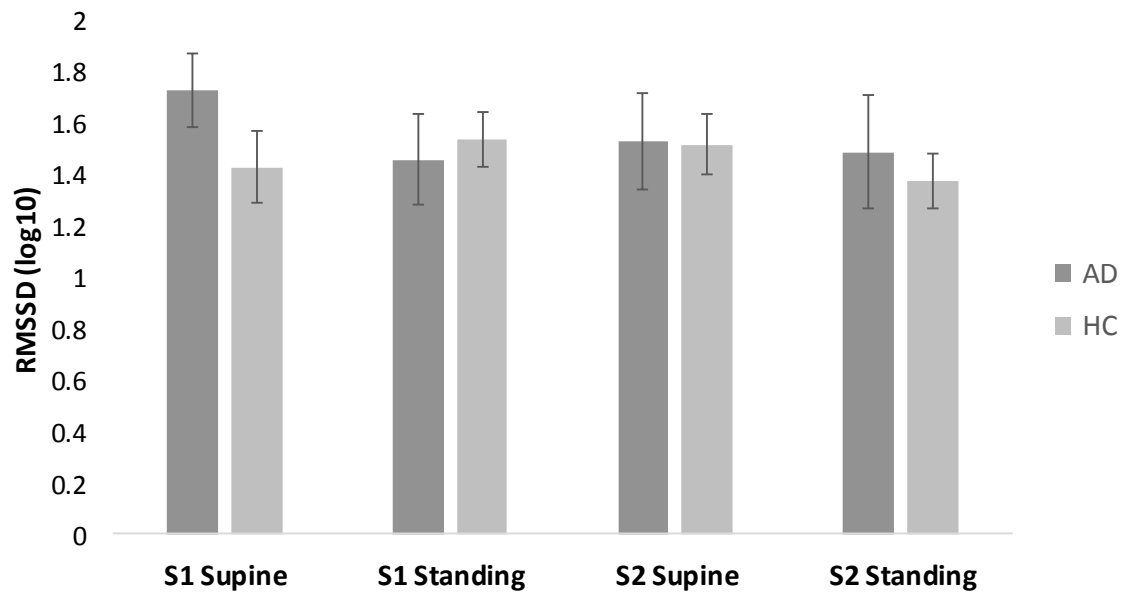
Figure 4. *Supine to standing mean RMSSD (log10) for the AD and HC group at baseline (S1) and 3 months (S2).*

Table 5 summarizes demographic information for the 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x3 (time [baseline, 3 month and 6 month intervals]) ANCOVA analyses. Demographic variables which were correlated with HRV indices are denoted by *p*–values.

Table 5. Descriptive Statistics and HRV Index Correlations of the AD and HC Sample for the 2x2x3 ANCOVA (N= 16).

Demographic	AD	HC	*p*
Total Number of Participants	*n*= 8	*n*= 8	
Gender			*
Male (*n*)	5	5	
Female (*n*)	3	3	
Age (years)			**
Male *M* (*SD*)	77.20 (6.26)	77.40 (4.93)	
Female *M* (*SD*)	70.33 (5.51)	70.33 (6.11)	
Ethnic Background			*
Caucasian (*n*)	6	7	
Male (*n*)	5	5	
Female (*n*)	1	2	
African-American (*n*)	2	1	
Male (*n*)	0	0	
Female (*n*)	2	1	

p values were derived from the Pearson correlation coefficients and Kendall's tau-b statistic. Demographics with significant *p* values are correlated with one or more of the HRV indices. Note: *p <.05 ** p <.01

Tables 6 and 7 display sample characteristics for the 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x3 (time [baseline, 3 month and 6 month intervals]) analyses. Sample characteristics that were correlated with HRV indices or showed significant group differences are also denoted by *p*-values. Results from the Pearson correlation coefficient and Kendall's tau-b statistic indicated that gender was correlated with all four HRV indices ($p= <.05$) while age was correlated with LF Power (n.u.), HF Power (n.u.) and LF/HF Ratio (log10; $p= <.01$). Ethnicity was only correlated with LF Power (n.u.) while body mass index was correlated with LF Power and LF/HF Ratio (log10; $p= <.05$). Systolic blood pressure was correlated with RMSSD (log10) while Aricept® dose was correlated with RMSSD (log10) and LF/HF Ratio (log10; $p= <.05$). Anxiolytic and antidepressant medications were correlated with HF Power (n.u.), LF Power (n.u.) and LF/HF Ratio (log10; $p= <.05$). Lastly, average number of hours of exercise per week was correlated with LF Power (n.u.), HF Power (n.u.), and LF/HF Ratio (log10; $p= <.05$). Therefore, these variables were included as covariates in the ANCOVA's. Between group differences in weekly alcohol consumption were also identified using a repeated measures ANOVA $F(1,14) = 7.62$, $p = .015$, 95% CI [2.52, 7.28], $\eta^2 = .352$. This variable was also included in the ANCOVA model as a covariate.

Table 6. Sample Characteristics and HRV Index Correlations for the AD Group (n= 8).

Characteristic	Baseline (S1)	3 Months (S2)	6 Months (S3)	*p*
Body Mass Index (kg) *M* (*SD*)	25.86 (5.99)	26.56 (5.58)	25.49 (5.25)	*
Blood Pressure Systolic (mmHg) *M* (*SD*)	139.88 (19.50)	132.63 (15.70)	127.38 (17.86)	*
Diastolic (mmHg) *M* (*SD*)	75.00 (7.46)	75.63 (7.96)	72.00 (8.30)	
Dose of Aricept (mg/day)	5.00	10.00	10.00	*
Blood Pressure Medication Taken (*n*)	6	6	5	
Allergy Medication Taken (*n*)	0	2	2	
Anxiolytic or Antidepressant Taken (*n*)	2	1	1	*
Number Alcoholic Drinks Per Week (Avg) *M* (*SD*)	3.13 (7.28)	2.00 (4.90)	0.38 (0.74)	*
Hours Exercise Per Week (Avg) *M* (*SD*)	4.96 (3.53)	5.81 (4.21)	5.47 (2.63)	*
Alcohol or Caffeine last 12 hrs (*n*)	0	3	0	
Exercise last 12 hrs (*n*)	0	0	0	
Allergy Meds last 12 hrs (*n*)	0	0	1	

p values were derived from the Pearson correlation coefficients, Kendall's tau-b statistic, and Analysis of Variance. Sample characteristics with significant *p* values are correlated with one or more of the HRV indices. Note: *p <.05

Table 7. Sample Characteristics and HRV Index Correlations for the HC Group (n= 8).

Characteristic	Baseline (S1)	3 Months (S2)	6 Months (S3)	*p*
Body Mass Index (kg) *M* (*SD*)	25.03 (5.20)	25.18 (5.50)	24.79 (5.06)	*
Blood Pressure Systolic (mmHg) *M* (*SD*)	135.50 (24.58)	126.75 (15.49)	129.13 (16.66)	*
Diastolic (mmHg) *M* (*SD*)	75.50 (11.61)	74.88 (6.33)	75.50 (8.38)	
Blood Pressure Medication Taken (*n*)	4	5	3	
Allergy Medication Taken (*n*)	1	1	0	
Anxiolytic or Antidepressant Taken (*n*)	1	1	1	*
Number Alcoholic Drinks Per Week (Avg) *M* (*SD*)	8.50 (5.15)	8.00 (4.96)	7.31 (5.38)	*
Hours Exercise Per Week (Avg) *M* (*SD*)	9.00 (4.17)	5.81 (4.09)	6.38 (3.29)	*
Alcohol or Caffeine last 12 hrs (*n*)	3	1	1	
Exercise last 12 hrs (*n*)	1	1	1	
Allergy Meds last 12 hrs (*n*)	1	0	0	

p values were derived from the Pearson correlation coefficients, Kendall's tau-b statistic, and Analysis of Variance. Sample characteristics with significant *p* values are correlated with one or more of the HRV indices. Note: *p <.05

Table 8. *Mean and Standard Error of HRV Indices in Healthy Control and Alzheimer's Disease Groups in Supine and Standing Positions at Baseline, Three Month, and Six Month Intervals.*

| HRV Measure | Baseline (S1) | | | | 3 Months (S2) | | | | 6 Months (S3) | | | | | |
| | AD | | HC | | AD | | HC | | AD | | HC | | | |
	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE	p	η^2
Supine:														
Low Frequency (n.u.)	52.43 ± 8.26		69.04 ± 6.07		63.49 ± 10.15		60.35 ± 5.59		60.76 ± 7.76		61.08 ± 5.79		.415	.097
High Frequency (n.u.)	47.58 ± 8.27		30.96 ± 6.07		36.51 ± 10.15		39.65 ± 5.59		39.24 ± 7.76		38.91 ± 5.78		.339	.102
RMSSD (log 10)	1.82 ± 0.19		1.29 ± 0.16		1.59 ± 0.23		1.66 ± 0.12		1.83 ± 0.19		1.53 ± 0.12		.442	.060
LF/HF Ratio (log10)	0.43 ± 0.19		0.19 ± 0.14		0.37 ± 0.25		0.23 ± 0.14		0.23 ± 0.17		0.22 ± 0.12		.051	.440
Standing:														
Low Frequency (n.u.)	67.90 ± 7.56		58.63 ± 6.27		66.44 ± 8.76		65.70 ± 7.18		74.00 ± 7.41		65.04 ± 7.76		.415	.097
High Frequency (n.u.)	32.10 ± 7.56		41.38 ± 6.27		33.56 ± 8.76		34.30 ± 7.18		26.00 ± 7.40		34.93 ± 7.66		.339	.102
RMSSD (log 10)	1.63 ± 0.23		1.54 ± 0.14		1.63 ± 0.26		1.40 ± 0.15		1.59 ± 0.20		1.47 ± 0.17		.442	.060
LF/HF Ratio (log10)	0.43 ± 0.19		0.19 ± 0.14		0.49 ± 0.26		0.34 ± 0.16		0.64 ± 0.24		0.40 ± 0.21		.051	.440

The data for the heart rate variability 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x3 (time [baseline, 3 month and 6 month intervals]) analyses are presented in Table 8. Results from the 2 (group [AD group versus healthy controls]) x2 (task [supine to standing]) x3 (time [baseline, 3 month and 6 month intervals]) ANCOVA's, did not demonstrate statistically significant between group differences from supine to standing. As in the 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x2 (time [baseline and 3 month intervals]) analyses, between group differences among LF Power (n.u.), HF Power (n.u.), LF/HF Ratio (log10) and the mean squared differences of successive R-R intervals (RMSSD [log10]) were not statistically significant. There were no significant within group differences among the HRV indices from supine to standing at the three time points. Despite the lack of statistical significance, the AD group demonstrated a trend towards larger increases in LF Power (n.u.) from supine to standing in comparison to the healthy control group at all three sessions. The healthy control group demonstrated lower levels of sympathetic exacerbation from supine to standing at session 1, supine to standing at session 2, and supine to standing at session 3 in comparison to the AD group. Additionally, the level of LF Power (n.u.)/sympathetic nervous activity exacerbated over time for the AD group in comparison to the healthy control group which remained relatively stable. Results from the 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x3 (time [baseline, 3 month and 6 month intervals]) ANCOVA also indicated that the healthy control group demonstrated higher levels of LF Power (n.u.) and lower levels of HF Power (n.u.) in the supine position at sessions 1 and 3 compared to the AD group, but once the AD group initiated the standing task, their LF Power (n.u.) exacerbated at a

much larger rate than the healthy control group (see figure 5). As expected, the increase in LF Power (n.u.) resulted in a decrease in HF Power (n.u.) among both groups with the exception of the healthy control group at baseline who demonstrated an increase in HF Power (n.u.) from supine to stand. Overall, the AD group demonstrated a higher magnitude of parasympathetic depression in comparison to the healthy control group at all three time points when engaging in the orthostatic challenge (supine to standing; see figure 6).

Figure 5. *Supine to standing mean low frequency power (n.u.) for the AD and HC group at baseline (S1), 3 months (S2), and 6 months (S3).*
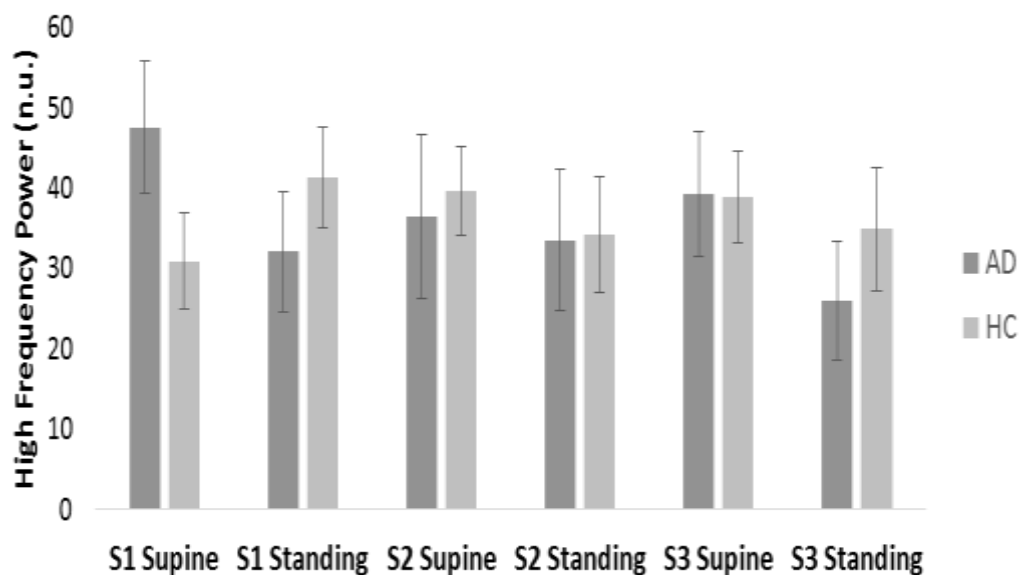
Figure 6. *Supine to standing mean high frequency power (n.u.) for the AD and HC group at baseline (S1), 3 months (S2), and 6 months (S3).*

Results from the 2 (group [AD group versus healthy control group]) x2 (task [supine to standing]) x3 (time [baseline, 3 month and 6 month intervals]) ANCOVA did not indicate significant between group differences for the LF/HF Ratio (log10) but were very close $F(1, 8) = 5.50$, $p = .051$, 95% CI [0.19, 0.50], $\eta^2 = .440$. Results from the ANCOVA also indicated a medium effect for the LF/HF Ratio (log10) between group differences. The mean LF/HF Ratio (log10) reflected greater sympathetic dominance (larger LF/HF ratios) in both supine and standing positions at sessions 1, 2 and 3 among the AD group in comparison to the healthy control group. When transitioning from supine to standing position, the AD group demonstrated larger increases in LF/HF Ratio (log10) as well (see figure 7). This is consistent with the relative sympathetic

exacerbation and parasympathetic depression demonstrated by the AD group as evidenced by increased LF Power (n.u.) from supine to standing and decreased HF Power (n.u.) from supine to standing. Lastly, the AD group showed a larger magnitude of mean squared differences of successive intervals (RMSSD [log10]) from supine to standing at all three time points in comparison to the healthy control group. The greater magnitude of RMSSD (log10) among the AD group is reflective of the larger variations in LF Power (n.u.) and HF Power (n.u.) from supine to standing (see figure 8). Secondary analyses using Tukey's post-hoc tests revealed significant between group differences at baseline for RMSSD (log10) between the AD group (*M*=1.82, *SD*= 0.54) and the healthy control group (*M*=1.29, *SD*= 0.46) while in the supine position (*p* <.05).
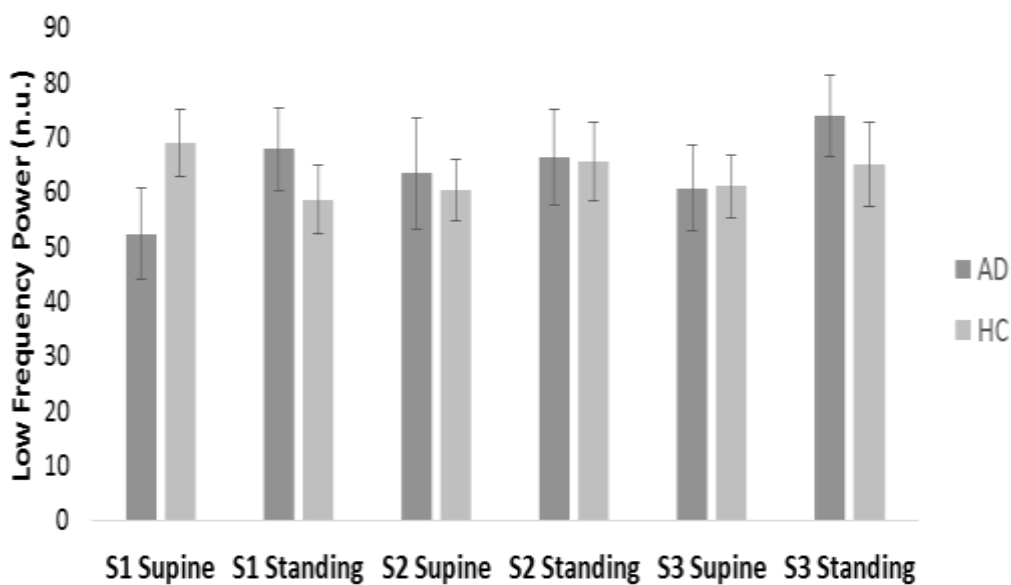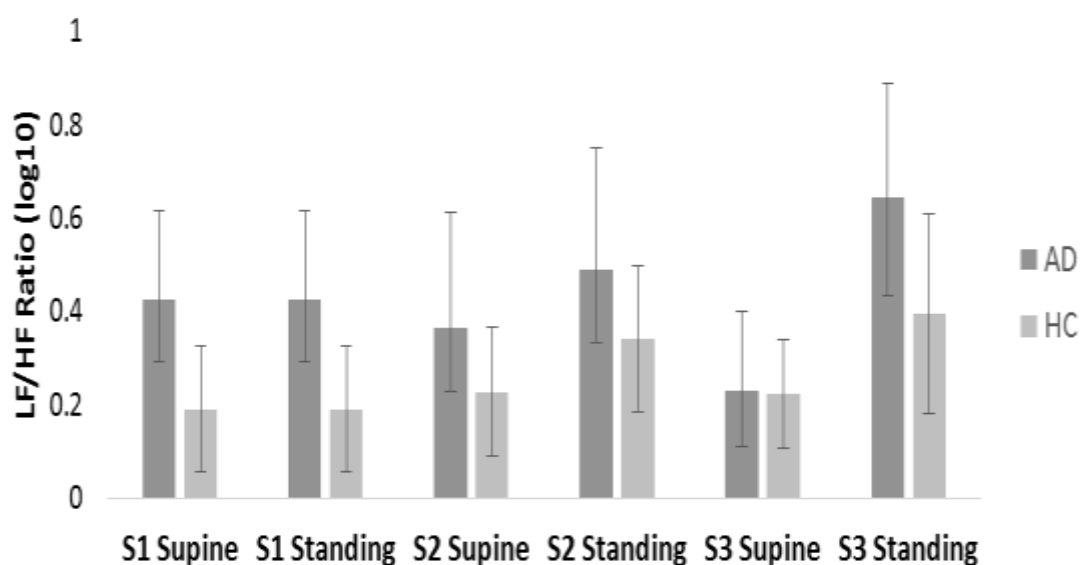

Figure 7. *Supine to standing mean LF/ HF Ratio (log10) for the AD and HC group at baseline (S1), 3 months (S2), and 6 months (S3).*
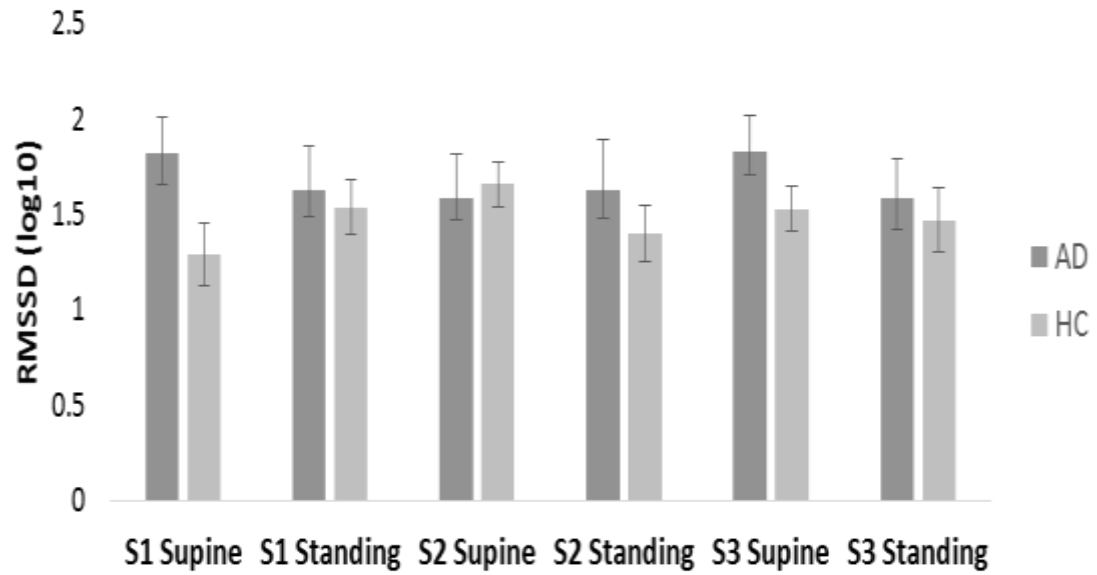
Figure 8. *Supine to standing mean RMSSD (log10) for the AD and HC group at baseline (S1), 3 months (S2), and 6 months (S3).*

CHAPTER V

SUMMARY AND DISCUSSION

The goal of the present study was to explore the effects of Alzheimer's disease (AD) pathology on cardiac autonomic function for individuals diagnosed with AD and taking the medication Donepezil (Aricept®). Previous research and epidemiological data using HRV technology have indicated variable disturbances of autonomic function in persons with AD (Allan, et al., 2007; Giubilei et al., 1998; Masuda & Kawamura, 2003; Siepmann et al., 2006; Toledo & Junqueira, 2008; Zulli et al., 2005). More specifically, cardiac autonomic dysfunction is prevalent among individuals with AD as reflected by exacerbated sympathetic nervous activity and decreased parasympathetic nervous activity (Allan et al., 2007; Giubilei et al., 1998; Masuda & Kawamura, 2003; Siepmann et al., 2006; Toledo & Junqueira, 2008; Zulli et al., 2005). This sympathovagal imbalance (sympathetic dominance) is a recognized risk factor for cardiovascular mortality, characterized by higher risk of arrhythmias, parasympathetic depression, syncope, and sudden death (Allan et al., 2007; Kleiger, Miller, Bigger, & Moss, 1986).

Prior research has indicated that the enhancement of sympathetic nervous activity and parasympathetic depression among persons with AD is even more profound following a shift from a supine to a standing position (Toledo & Junqueira, 2008). This shift in position (known as the orthostatic challenge), is an excellent way to measure cardiac autonomic dysfunction due to the increase in sympathetic nervous activity triggered from the supine to standing movement (Aysin & Aysin, 2007).

As poor cardiovascular health appears to be a mechanism of AD pathology, research looking into the potential cardiovascular benefits of cholinesterase inhibitors (ChEls) has gained much interest (Da Costa Dias et al., 2013; Giubilei et al., 1998; Masuda & Kawamura, 2003; McLaren et al., 2003; Nordstrom et al., 2013; Siepmann et al., 2006; Umegaki & Khookhor, 2013). However, results from studies exploring the cardio protective benefits of ChEls have been mixed (Da Costa Dias et al., 2013; Giubilei et al., 1998; Masuda & Kawamura, 2003; McLaren et al., 2003; Siepmann et al., 2006; Umegaki & Khookhor, 2013). These studies also contain various methodological limitations including the use of a now discontinued ChEls (Giubilei et al., 1998), the short duration of study (e.g., two weeks; McLaren, et al., 2003; Umegaki & Khookhor, 2013), measuring changes at a single, variable (± 4.8 weeks) time frame (Da Costa Dias Dias et al., 2013), the use of animal subjects as opposed to human subjects (Umegaki & Khookhor, 2013), the absence of normalized units when exploring changes in HRV over time (Masuda & Kawamura, 2003; Toledo & Junqueira, 2008; Umegaki & Khookhor, 2013), the lack of a healthy control group comparison (McLaren, et al., 2003), and controlling for few factors which have been shown to interfere with HRV measurement (Da Costa Dias Dias et al., 2013; Giubilei et al., 1998; Masuda & Kawamura, 2003; McLaren, et al., 2003; Siepmann et al., 2006; Umegaki & Khookhor, 2013).

In the present study, differences in HRV among individuals with Alzheimer's disease versus healthy controls were studied over a period of six months in both supine and standing positions. HRV was obtained through the utilization of non-invasive electrocardiogram (ECG) technology using frequency-domain (LF Power, HF Power, RMSSD, LF/HF Ratio) and time-domain (six minutes) measures. The results of the

current study indicated a trend towards greater reductions in parasympathetic function and greater increases in sympathetic function among the AD group in comparison to the healthy controls during the orthostatic challenge. This was the case across all three time points. More specifically, the AD group showed a lower magnitude of normalized high frequency power in comparison to healthy controls (session 1= 9.28% [lower]; session 2= 3.67% [lower]; session 3= 8.93% [lower]) while in the standing position. This trend was accompanied by a greater LF/HF ratio among the AD group (session 1= 0.24% [greater]; session 2= 0.15% [greater]; session 3= 0.24% [greater]) reflecting greater sympathovagal imbalance. Statistically, this trend towards greater LF/HF Ratio among the AD group versus healthy controls at all three sessions also corresponded with a medium effect size (95% CI [0.19, 0.50], η^2 =.440). Therefore, Cohen's effect size value suggested a moderate level of practical significance, which is reflected by the large trend toward sympathetic exacerbation among the AD group when engaging in the orthostatic challenge. Despite this trend toward sympathetic exacerbation among the AD group while engaging in the orthostatic challenge, it is important to note that the results from the ANCOVA's were not statistically significant. In addition, although there was a medium effect size for the LF/HF ratio, it is important to consider the potentially large amount of error variance that may be inherent with a small sample. Therefore, these results need to be interpreted with caution.

During the supine task, the AD group showed a larger magnitude of normalized high frequency power in comparison to the healthy control group (session 1= 16.62% [greater]; session 2= 3.37% [greater]; session 3= 0.33% [greater]). However, this was substantially reduced when engaging in the standing task (orthostatic challenge).

Therefore, the supine position may mask the presence of cardiac autonomic dysfunction, which is then revealed when the individual engages in the orthostatic challenge. While engaging in the orthostatic challenge, the healthy control group showed an increase in normalized high frequency power from supine to standing at session 1, indicating better sympathovagal balance when changing posture. Not surprisingly, the healthy control group demonstrated greater high frequency power at session 1 (9.28% [greater]) in comparison to the AD group while engaging in the orthostatic challenge. At sessions 2 and 3, the healthy control group demonstrated a reduction in high frequency power from supine to standing. However, the magnitude of the high frequency power generated by the healthy control group while in the standing position at sessions 2 (3.67% [greater]) and 3 (8.93% [greater]) was still comparatively larger than the AD group. Therefore, the healthy control group generated a greater magnitude of high frequency power at all three sessions when engaging in the orthostatic challenge.

Based on a meta-analysis of short-term heart rate variability recordings among healthy adults ($\geq$ 18 years; Nunan, Sandercock, & Brodie, 2010), the mean normal values of low frequency power (expressed in normalized units) should be approximately 52% (*SD*= 10), while mean normal values of high frequency power (expressed in normalized units) should be approximately 40% (*SD*= 10). While in the supine position, the AD group generated more similar magnitudes of low and high frequency power with respect to this normative sample. However, while engaging in the orthostatic challenge, the healthy control group generated more similar magnitudes of low and high frequency power with respect to this normative sample. This may provide further corroborating evidence for more healthy cardiac autonomic function among the healthy control group

while engaging in the orthostatic challenge. It is important to mention that the Nunan, Sandercock, & Brodie (2010) study combined all HRV positions (e.g., supine and standing) and did not provide separate mean scores for older adults. Therefore, the conclusions that can be drawn between the data obtained in the present study and this normative data are limited.

The results from the current study are similar to that of three previous studies that identified a significant reduction in high frequency power among AD participants undergoing ChEls treatment (McLaren et al., 2003; Siepmann et al., 2006; Masuda & Kawamura, 2003). Additionally, the current study supports general findings from authors Toledo & Junqueira (2008) and Zulli et al (2005) who identified relative parasympathetic depression and sympathetic exacerbation among individuals with AD versus healthy controls. However, AD participants in the Toledo & Junqueira (2008) study were not undertaking ChEls treatment and also demonstrated parasympathetic depression and sympathetic exacerbation in the supine position. Findings from the current study suggest that individuals with AD who were taking the medication Donepezil (Aricept®) showed better high frequency power in the supine position at sessions 1, 2 and 3 in comparison to healthy controls. These findings may indicate that cardiac autonomic function is improved among individuals with AD when in the supine position as a result of the cholinesterase inhibitor Donepezil (Aricept®). However, when the AD participants are standing, the potential beneficial effects of Aricept® appear to be minimized.

Previous research has demonstrated that changes in blood pressure, high and low frequency band power show significant changes when positioning is altered from a supine to a standing posture (Acharya, et al., 2006; American Heart Association, 1996;

Aysin & Aysin, 2007). This was particularly evident among the AD group who showed exacerbated low frequency power and reduced high frequency power from supine to standing positions at all three time points in comparison to healthy controls. This decrease in high frequency power among the AD group when engaging in the orthostatic challenge may be a more accurate reflection of cardiac autonomic dysfunction given that the orthostatic challenge is commonly used to measure ANS function (Aysin & Aysin, 2007). As there is a strong relation between the autonomic nervous system and blood pressure via the baroreceptor reflex (the baroreceptor reflex helps restore blood pressure by increasing heart rate), it is possible that the AD group experienced greater oscillations in blood pressure (e.g., orthostatic hypotension) while engaging in the orthostatic challenge (Berntson, 1997). Previous research has indicated a strong relation between high blood pressure and greater low frequency power (sympathetic exacerbation) among hypertensive individuals (Piccirillo, Munizzi, Fimognari, & Marigliano., 1996).

Although findings from the current study are similar to that of previous research (Masuda & Kawamura, 2003; McLaren et al., 2003; Siepmann et al., 2006; Toledo & Junqueira., 2008; Zulli et al., 2005), it is important to mention that these results also contradict previous findings as well (Giubilei, et al., 1998). For example, authors Giubilei et al (1998) identified improved cardiac autonomic function among individuals with AD who were undertaking ChEl treatment. More specifically, the AD group showed similar magnitudes of high frequency power and low frequency power compared to healthy controls while in the tilting position following ChEl treatment. However, there were various limitations to this study including the short time frame studied (one month) and the use of a now discontinued ChEls. Overall, results from the present study extend the

majority of the previous findings regarding autonomic dysfunction in persons with Alzheimer's disease and healthy older adults, reinforcing patterns of parasympathetic depression and sympathetic exacerbation (Masuda & Kawamura, 2003; McLaren, et al., 2003; Siepmann et al., 2006; Toledo & Junqueira, 2008, 2009; Zulli et al., 2005).

With regards to the expected findings outlined previously, the AD group showed greater high frequency power at session 1 (16.62% [greater]), and slightly better high frequency power at session 2 (3.37%[greater]) and session 3 (0.33% [greater]) in comparison to the healthy control group in the supine position. Although the magnitude of high frequency power at session 3 had decreased by 8.34% from session 1 for the AD group, the magnitude of high frequency power was still larger than that of the healthy control group at all three sessions while in the supine position. The larger proportion of high frequency power generated by the AD group at baseline may be related to the proximity to the first dose of Aricept® and the stabilization (therapeutic dose) of the medication at the six month time point. More specifically, two previous studies have indicated initial increases in high frequency power after short periods of ingestion of a ChEls (two weeks and one month respectively; Giubilei et al., 1998; Umegaki & Khookhor, 2013). This may be of particular significance because researchers Rogers et al (1998) found that the effects of Donepezil on cognitive functioning were most pronounced at the 12 week visit (three months). These neurocholinergic effects also persisted with no decrease in magnitude at weeks 18 and 24 (Rogers, 1998). During the baseline phase, AD participants were fairly early in their ChEl treatment (less than three months) and were exposed to lower doses (5mg) of Aricept®. By session 2 (three months) and session 3 (six months), AD participants were taking Aricept® for an

extended period of time and were titrated to larger doses (10mg). Therefore, the results from the current study may suggest that chronic administration of Aricept® could have positive effects of PNS activity at the six month interval in comparison to healthy controls through an increase in cholinergic resources. However, the AD group demonstrated large reductions in high frequency power from baseline to six months particularly in the standing position. Therefore, this suggests that participants diagnosed with mild to moderate AD continued to show cardiac autonomic dysfunction over a period of six months while taking Donepezil (Aricept®). Given that the orthostatic challenge has been widely used as a measure of cardiac autonomic function, the results demonstrating decreases in high frequency power among the AD group when in the standing position appear to be more compelling.

Secondly, it was hypothesized that healthy controls would show significant increases in high frequency power from a supine to standing position at all three sessions, while the AD group would demonstrate minimal change in high frequency power from a supine to standing position at the baseline session, but would gradually show increased high frequency power from supine to standing at sessions 2 and 3. Results from this study indicated that healthy controls demonstrated both increases in high frequency power from supine to standing at session 1 (10.42% [increase]) and decreases in high frequency power from supine to standing at sessions 2 (5.35% [decrease]) and 3 (3.98% [decrease]). In contrast, the AD group showed larger decreases in high frequency power from supine to standing at all three sessions (session 1= 15.48% [decrease]; session 2= 6.82% [decrease]; session 3= 13.24% [decrease]). However, these results were not statistically significant. The current HRV literature also indicates that healthy older adults have the

tendency to show reductions in high frequency power from supine to standing positions as well (Toledo & Junquiera, 2008). Although it was hypothesized that the healthy controls would display increases in high frequency power from supine to standing, the trend for reductions in high frequency power from supine to standing among the healthy older adults at sessions 2 and 3 found in the present study are consistent with previous findings (Giubilei, et al., 1998; Toledo & Junquiera, 2008).

Thirdly, it was hypothesized that Donepezil (Aricept®) would improve parasympathetic nervous activity through increased acetylcholine transmission, thus supporting the medications use as a potential protective agent against cardiac autonomic dysfunction. Results from this study indicate that high frequency power was greater for individuals with AD in comparison to healthy controls at sessions 1, 2 and 3 in the supine position only. Therefore, it is possible that Donepezil (Aricept®) may provide cardio-protective factors which are more evident in the supine position. However, as mentioned previously, the potential cardiac benefits of Donepezil (Aricept®) appear to be nullified during a standing task. These results are consistent with the results obtained by Masuda & Kawamura (2003) and McLaren et al (2003) who indicated a decrease in high frequency power as result of taking the medication Donepezil. Increases in low frequency power and a larger LF/HF ratio among the AD group also indicated the continued dominance of the sympathetic nervous system and the presence of parasympathetic depression despite the use of Donepezil. Thus, there is a tendency towards sympathovagal imbalance reflecting greater sympathetic modulation during a supine to standing task among the AD group.

One of the goals of the present study was to address the methodological limitations of studies exploring the effects of ChEls on HRV among individuals with AD. Firstly, participants taking the medication Donepezil were matched with a healthy control of the same age (± 3 years) and gender. The previous studies exploring the effects of ChEl treatment on individuals with AD did not match AD participants with healthy controls (Da Costa Dias et al., 2013; Masuda & Kawamura, 2003; McLaren, et al., 2003; Umegaki & Khookhor, 2013). Secondly, the participants in the current study were taking Donepezil for six months and data was obtained at three different time points (session 1 [baseline], session 2 [three months], and session 3 [six months]). Few studies have examined the cardiovascular benefits of the medication after six weeks (Da Costa Dias et al., 2013; Masuda & Kawamura, 2003; McLaren, et al., 2003; Siepmann et al., 2006; Umegaki & Khookhor, 2013). Thirdly, the present study controlled for the following confounds, which are known to influence HRV measures: age, gender, body mass index (BMI), blood pressure (systolic and diastolic), medications (blood pressure, allergy, anxiolytic or antidepressant), dose on Donepezil (Aricept®), alcohol use, caffeine use, and physical activity (Harte & Meston, 2013; Hayano et al., 1990; Koskinen, Virolainen, & Kupari, 1994; Molifino et al., 2009; Sandercock, Bromley, & Brodie, 2005; Sondermeijer, Van Marle, Kamen, & Krum, 2002; Stein, Kleiger, & Rottman 1997; Umetani, Singer, & McCraty, 1998). The results from the Pearson correlation coefficients and the Kendall's tau-b correlation coefficients demonstrated that age, body mass index, blood pressure, medications (blood pressure, allergy, anxiolytic or antidepressant), dose of Aricept, and physical activity all correlated with one or more of the HRV indices. Significant between group differences in alcohol use were also identified from the

between subjects ANOVA's. Thus, it is probable that these factors could influence variations in multiple HRV indices.

With regards to the statistical analyses, although there were fairly clear mean differences in HRV indices and a large effect in LF/HF Ratio between the AD group and the healthy control group, none of these mean comparisons were statistically significant. As HRV is very sensitive to various confounding conditions, it is possible that some of the variance in HRV in previous studies can be accounted for by these confounding factors. For example, the Toledo & Junquiera (2008) study indicated significant differences between individuals with AD and healthy controls from a supine to standing position. However, the mean age of the AD group (*Mean age*= 79.6) was higher than the age of the control group (*Mean age*= 68.6). Therefore, some of this between group variance might be explained by age differences between the AD group and the healthy control group.

Fourthly, some of the indices (low frequency power and high frequency power) used in this study were reported in normalized units to emphasize the controlled and balanced behavior of the sympathetic and parasympathetic nervous system (Task Force on Heart Rate Variability, 1996). Normalized units display sympathetic and parasympathetic activity as a percentage (0%-100%). This allows the clinician to easily ascertain changes in sympathetic versus parasympathetic output over time (e.g., when using a repeated measures design) but also provides the opportunity to determine sympathetic versus parasympathetic modulation (Task Force on Heart Rate Variability, 1996). Previous studies have not utilized normalized units to monitor changes in HRV among individuals with AD taking the medication Donepezil (Aricept®; Masuda &

Kawamura, 2003; McLaren, 2003; Umegaki & Khookhor, 2013). Using normalized units also provides the opportunity to compare HRV indices with normative data or previous studies that express HRV data in normalized units.

Fifthly, respiration count was captured and added to the HRV algorithm using the Kubios HRV Analysis Software. This was accomplished by observing the frequency of breaths (rise and fall of the chest) at two separate one minute intervals (minute one and minute three) during the supine and standing position at all three sessions. As discussed previously, the adjustment using respiration in a HRV algorithm is known as respiratory sinus arrhythmia (RSA). The RSA is generated by integrating changes in heart rate during inhalation (heart rate increases) and exhalation (heart rate decreases). Respiratory linked variations in heart rate usually occur in the high frequency range (Lehrer, Vaschillo, and Vaschillo, 2000) and thus, RSA has been shown to provide a more accurate index of cardiac vagal tone and parasympathetic activity (Grossman & Taylor, 2006). Finally, the present study obtained HRV measures in both supine and standing positions as previous studies have demonstrated differences in HRV as a result of varying methods of measuring heart rate (e.g., supine vs. standing. vs. tilting vs. 24-hour; (Giubilei et al., 1998; Masuda & Kawamura, 2003; Siepmann et al., 2006; Toledo & Junqueira, 2008; Zulli et al., 2005).

Clinical Implications for Health Psychology

Given the strong evidence for cardiac autonomic dysfunction among individuals with AD as evidenced in the present study, HRV technology could be utilized to capture sympathetic and parasympathetic nervous activity among patients who are at risk for

developing AD (e.g., genetically or recently diagnosed with mild cognitive impairment). Subsequent changes in HRV (e.g., low frequency dominance) in tandem with decreased cognitive status could provide corroborating evidence for reductions in acetylcholine production, transmission, and probable dementia. As results from this study demonstrated large decreases in high frequency power during the orthostatic challenge among the AD group, it is may be beneficial to obtain HRV recordings in two separate positions. More specifically, when obtaining HRV data from individuals with suspected AD, HRV recordings should be performed in both the supine and standing position as the orthostatic challenge revealed greater cardiac autonomic dysfunction among the AD group when changing position. If the clinician obtains HRV readings in the supine position only, then the presence of cardiac autonomic dysfunction may be missed. Once the clinician obtains HRV data, results could be communicated to the patient's primary care physician and the interdisciplinary care team who may use this information to guide treatment interventions. For example, if the individual shows impaired cardiac autonomic function via sympathetic dominance, referral to a health psychologist for HRV biofeedback, relaxation training, or progressive muscle relaxation may reduce the risk for potential cardiac events. Both relaxation training and progressive muscle relaxation exercises have been successfully implemented with older adults (Hirokawa, 2004; Morone & Greco, 2007). One particular study significantly reduced hypertension among older adults using guided relaxation training (Tang, Harms, Speck, Vezeau, & Jesurum, 2009).

Another clinical intervention that may reduce the likelihood of future cardiac events is slow (diaphragmatic) breathing. Research has indicated that people are capable of voluntarily producing substantial increases in respiratory sinus arrhythmia (RSA) or

high frequency power using biofeedback techniques (Lehrer, Vaschillo, & Vaschillo, 2000). It is well established that HRV is systematically influenced by breathing frequency, with higher frequency being generated by slower respiration (Lehrer & Gevirtz, 2014). As such, when breathing rate is reduced, R–R interval fluctuations and spectral power are increased (Eckberg, 2003). The recommended rate of breathing to achieve increased R-R fluctuations and higher frequency output is six breaths per minute (Lehrer & Gevirtz, 2014). In order to achieve a rate of six breaths per minute, a pacing stimulus can be presented on a computer display screen prompting patients when to slowly inhale and exhale using their diaphragm (diaphragmatic breathing; Lehrer, Vaschillo, and Vaschillo, 2000). The introduction of HRV biofeedback technology then teaches the patient to breathe approximately in phase with heart rate changes through a different visual display (Lehrer, Vaschillo, & Vaschillo, 2000). According to a summary of the HRV biofeedback literature by Wheat & Larkin (2010), HRV biofeedback has been shown to significantly reduce cardiovascular mortality and hypertension in adults. Progressive muscle relaxation and relaxation training in combination with HRV biofeedback has also demonstrated reductions in cardiovascular risk and hypertension among adults as well (Wheat & Larkin, 2010). This is highly pertinent given the relation between AD and cardiac autonomic dysfunction, the high rate of cardiac events among older adults, and the prevalence of hypertension among older adults.

Pharmacological interventions may also help to improve cardiac autonomic function as well. Certain blood pressure medications such as antihypertensives (beta blockers) have been shown to improve high frequency power and cardiac autonomic function after eight weeks among adults diagnosed with hypertension (Pavithran,

Prakash, Dutta, & Madanmohan, 2010). Therefore, individuals who present with parasympathetic depression following a HRV recording may be referred to a physician for a medication evaluation. The impact of the prescribed medication on the autonomic nervous system could be easily monitored via standardized pre/post HRV recordings using normalized units.

Other potential risk factors associated with cardiac autonomic dysfunction (e.g., parasympathetic depression) include an increased risk for falls (American Geriatrics Society, 2010; Melillo, Jovic, De Luca, Morgan, & Pecchia, 2015). This is a major problem for older adults as one in ten falls results in serious injury and up to one in five in a fracture (Tinetti, 2003). Individuals with autonomic dysfunction and hypertension are also at higher risk for experiencing postural (orthostatic) hypotension which is a well-known risk factor for falls among the elderly (American Geriatrics Society, 2011). The use of Donepezil has also been shown to cause orthostatic hypotension in some individuals as well (Bordier, 2006). Orthostatic hypotension is defined as a decrease in systolic blood pressure of 20 mmHg (or 20%) or more when the individual transitions from a supine to standing position (Tinetti, 2010). In the present study, the AD group showed greater increases in low frequency power compared to healthy controls when engaging in the orthostatic challenge (supine to standing). Previous research has indicated a relation between increased sympathetic nervous activity (low frequency power) and hypertension (Melillo, Jovic, De Luca, Morgan, & Pecchia, 2015). Although blood pressure was not measured in the standing position, it is possible that the AD group experienced greater fluctuations in blood pressure (e.g., hypertension) following the orthostatic challenge. Therefore, identifying cardiac autonomic dysfunction among older

adults via non-invasive HRV measures and providing interventions to improve cardiac autonomic function may reduce the likelihood of a fall. Interventions which have been shown to reduce the likelihood of falls among older adults include medication management, increased fluid intake, physical therapy (PT), increased exercise, and deep breathing (American Geriatrics Society, 2011; Jáuregui-Renaud, Márquez, Hermosillo, Sobrino, & Lara, et al., 2003).

Limitations of the Current Study and Suggestions for Future Studies

There are various limitations to the current study, which warrant discussion. Firstly, although the sample size identified by the power analyses was met for both supine and standing positions, previous studies have used slightly larger samples to assess changes in HRV and obtained significant results (Da Costa Dias Dias et al., 2013; Masuda & Kawamura, 2003; Siepmann et al., 2006; Toledo & Junquiera, 2008; Zulli, et al., 2003). Results from the ANCOVA also indicated a medium effect for the LF/HF Ratio (log10) between group differences. The mean LF/HF Ratio (log10) reflected greater sympathetic dominance (larger LF/HF ratios) in both supine and standing positions at sessions 1, 2 and 3 among the AD group in comparison to the healthy control group.. However, based on the small sample used, this statistic was interpreted with caution given the potential for error variance within a small sample. The results from the present study do however indicate the need for larger controlled trials to further investigate the cardiovascular effects of Donepezil. One of the drawbacks of the small sample used in this study was the impact of variable scores on the mean of each HRV index. Using a larger sample would reduce the likelihood of more variable scores impacting the means of each index and increase the likelihood of detecting a statistical

significance. Secondly, it is difficult to determine the extent to which Donepezil may improve cardiac autonomic function compared to individuals with AD who are not taking Donepezil. Having a third comparison group (an AD group who are not taking Donepezil) or normative HRV data based on age, gender, and HRV positioning would provide more information of the extent to which Donepezil impacts the trajectory of cardiac autonomic dysfunction. However, recruiting individuals who are diagnosed with AD and who are not taking a ChEls would be challenging as the large majority of individuals diagnosed with probable AD are prescribed a cholinesterase inhibitor or a N-Methyl-D-aspartate (NMDA) receptor antagonists (e.g., Namenda).

Thirdly, given the increase in sympathetic nervous activity from the supine to standing position among individuals with AD, it is possible that baroreceptor reflex sensitivity is diminished among this population. Obtaining a blood pressure reading from AD participants in the standing position (e.g., measuring postural hyper/hypotension) may help identify any interference in the participants homeostatic mechanisms that help maintain blood pressure (Berntson, 1997). Fourthly, although transforming the RMSSD and LF/HF Ratio HRV indices using base 10 logarithm reduced the skewness and kurtosis of the distribution, interpretation of these indices became more difficult as the values were no longer expressed in normalized units and ratio values (LF/HF Ratio). However, Toledo & Junquiera (2008) used similar log transformations to correct non-normal distribution among their HRV indices and used similar interpretations. Fifthly, future studies may want to explore whether the combination of a ChEls and N-Methyl-D-aspartate (NMDA) receptor antagonist (e.g., Namenda) improves HRV above and beyond a ChEls alone. Sixthly, HRV was captured in the home environment for four of the AD

participants. Although obtaining HRV in the home was advantageous for recruitment purposes, this may have influenced HRV indices. Research has indicated that individuals with white coat hypertension (high blood pressure in the clinical setting) have a tendency towards higher levels of LF power and a propensity towards sympathovagal imbalance (Fagard, Stolarz, Kuznetsova, Seidlerova, & Tikhonoff, 2007). Based on this research, it is possible that individuals whose HRV was obtained in the clinical/laboratory setting were more likely to experience greater low frequency power compared to individuals whose HRV was captured in the home environment. Therefore this could be a potential confound, which was not accounted for in this study design. In order to reduce this confound in future studies, it is recommended that HRV be obtained in the same environment across and within groups. Lastly, the inclusion of neurocognitive measures, in addition to HRV measures, would provide a more accurate indication of the potential cholinergic benefits of Donepezil (Aricept®). As mentioned previously, cholinergic resources become increasingly deficient in individuals with AD resulting in neurocognitive impairment (Miur, 1996). Furthermore, the use of biomarkers such as volumetric magnetic resonance imaging (MRI) may help validate changes in HRV as a result of ChEl treatment. Overall, it appears that Donepezil's effect on cardiac function in patients with Alzheimer's disease is limited and requires further exploration.

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APPENDIX A

Phone Screen

Donepezil's Effect on Cardiac Function in Patients with Alzheimer's Disease Through an In Vivo, Non Invasive Measure of Peripheral Neuro-cholinergic Function: Relation to Therapeutic Efficacy

Name:_____

To be read to subject before screening questions: *We are calling from Eastern Virginia Medical School regarding the research study that you expressed interest in. We wanted to see if you would be interested in participating in* **a research study that is examining the relation between heart and brain function and potential effects of medications on these functions.** *Your involvement in the study would last for 6 months with a total commitment of about 5 hours over that time. We are collecting several measures at session 1 and following up with similar measures during session 2 and session 3. This information is being asked to determine your eligibility in our research study, this information will not be used other than for the purposes of this research study. If you decide not to participate or for some reason are ineligible to participate, this information will be destroyed. Does this sound like something you would like to participate in?* (If no, thank the person for their time. If yes, ask the following screening questions. If the potential subject is determined ineligible for any of the screening criteria below, please provide the explanation for their ineligibility presented in italics below each screening question.)

1. How old are you?_____

If not between the ages of 65-85

[I am sorry at this time we are looking for subjects between the ages of 65-85, but thank you for your time and interest]

2. How would you describe your ethnic background?_____

3. Do you have any assistive devices such as a pacemaker? Y   N

[I am sorry, we are unable to have people with any electrical assistive devices such as yours, because it can send a false signal when measuring heart rate, but we thank you for your time and interest]

4. Have you ever had a stroke or TIA (Transient ischemic attack, or "mini stroke"),
        Y   N   Date:_____

[I am sorry, we are unable to have people who have had a stroke previously because a stroke can affect measures that we are collecting, but we thank you for your time and interest]

5. Have you ever been diagnosed with arrhythmias (irregular heart rate) or vascular dementia (loss of mental function due to interruption of blood flow to the brain such as a stroke or heart attack)? Y   N

[Arrhythmia and vascular dementia can interfere with the measures that we are collecting.]

6. Are you currently taking any medication? Y   N

      Which ones?_____

not to be taking certain psychotropic medications (e.g., tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRI's), anticonvulsants, or antipsychotics). *[Some medications can interfere with the measures we are taking, but thank you for your interest and time]*

7. Have you ever been diagnosed with cancer?   Y   N

      Within the last year?   Y   N

Were you under any treatment for cancer?   Y   N

Within the last year?   Y   N

[Treatments for cancer can often interfere with the measures we are taking, so we are unable to have you participate in the study, but thank you for your time and interest]

8. Have you ever been diagnosed with Kidney or Liver Disease?   Y   N

[Certain illnesses and diseases may interfere with nerve conduction in some of the measures we are collecting]

9. Have you ever been diagnosed with a neurological illness? (ex. Multiple sclerosis or Parkinson's Disease)

                                              Y   N

[Certain illnesses and diseases may interfere with nerve conduction in some of the measures we are collecting]

10. Do you suffer from significant hearing, sight, or sensory impairment?   Y   N

Please describe:_____

_____

[Impairment of vision, hearing, and other sensory functions may interfere with your ability to complete the testing required for this study]

11. How far did you get in school? _____

12. Have you ever been hospitalized for a psychiatric problem?

Have you ever been diagnosed with a psychiatric illness?   Y   N

    Which one?_____

    When?_____

    Were you given any medications for treatment?   Y   N

    Which ones?_____

Must have no lifetime history of certain psychiatric symptoms or illnesses (including psychotic or delusional symptoms, bipolar disorder and schizophrenia).

[Unfortunately we are unable to accept people who suffer from these diseases due to the possible interference in the measures we collect during the study]

13. On average about how many alcoholic beverages do you consume in a week? _____

    (One beverage is equal to a 12oz. beer, 5 oz. Wine, or 1.5 oz of hard liquor)

14. Have you ever had a concussion or head injury?   Y   N

    How many? _____

    When? _____

Is subject eligible?   Y   N

Yes: *Well, Mr/Mrs. _____ it appears you would be a good candidate for this study, would you be able to come in for the first visit on_____? At_____. This first visit takes about 1 hour and 30 minutes to complete so please wear comfortable clothes. You will come into our office and be given informed consent which details the study and you can determine if the study is right for you. If you decided that you would like to participate, you will at that time have a medical history interview and EKG. We will then administer a series of neuropsychological tests. [*Be sure to gather home contact information*]*

No: *I am sorry but you are not eligible at this time to participate in this study. We appreciate your time and interest.*

Criteria:

a) Meets age criteria and reports no clinical history of severe and chronic diseases affecting general health (cancer or treatment for cancer [within the past year], neurological disorders [e.g., multiple sclerosis], chronic kidney or liver disease, stroke or cerebrovascular events or disease)

b) Is fluent in English (i.e., have commonly used English in everyday speaking and reading for at least 10 years)

c) Has no electronic assistive devices that may interfere with measuring HRV

d) Does not have current substance dependence

e) Is not taking certain psychotropic medications (e.g., tricyclic antidepressants, anticonvulsants, or antipsychotics)

f) Has no lifetime history of certain psychiatric symptoms or illnesses (including psychotic or delusional symptoms, bipolar disorder and schizophrenia)

g) Does not suffer from significant hearing, sight, or sensory impairment

h) Has no history of arrhythmias or vascular dementia

i) Has not sustained any significant head trauma that may impair cognitive function

APPENDIX B

Demographics/Socioeconomic Status Form

A. PERSONAL INFORMATION *(Please circle)*

1.   Sex:

      (1)   Male

      (2)   Female

2.   Age: _____

3.   Date of birth: _____/_____/_____

             mm   dd   yy

4.   Racial categories:

      (1)   Caucasian, white

      (2)   African American, black

      (3)   Asian

      (4)   Latino or Hispanic

      (5)   Native American

      (6)   Bi- or multiracial, *please specify:* _____

      (6)   Other, *please specify:* _____

5.   What is your current marital status?

      (1)   Married/living with partner

      (2)   Widowed

      (3)   Separated

      (4)   Divorced

      (5)   Single

6.   Total family income *(before taxes; including your income and your spouse or partner's income)*:

(1)	Less than $10,000/year	*or*	$0 - 833/month
(2)	$10,000 - 14,999/year	*or*	$834- 1249/month
(3)	$15,000 - 24,999/year	*or*	$1250 - 2083/month
(4)	$25,000 - 34,999/year	*or*	$2084 - 2916/month
(5)	$35,000 - 49,999/year	*or*	$2917 - 4166/month
(6)	$50,000 - 64,999/year	*or*	$4167 - 5416/month
(7)	$65,000 - 79,999/year	*or*	$5417 - 6666/month
(8)	$80,000 - 94,999/year	*or*	$6667 - 7916/month
(9)	$95,000 - 109,999/year	*or*	$7917 - 9167/month
(10)	$110,000 - 125,000/year	*or*	$9168 - 10,416/month
(11)	More than $125,000/year	*or*	more than 10,417/month

7.   Individual income *(before taxes)*:

(1)	Less than $10,000/year	*or*	$0 - 833/month
(2)	$10,000 - 14,999/year	*or*	$834- 1249/month
(3)	$15,000 - 24,999/year	*or*	$1250- 2083/month
(4)	$25,000 - 34,999/year	*or*	$2084-2916/month
(5)	$35,000 - 49,999/year	*or*	$2917- 4166/month
(6)	$50,000 - 64,999/year	*or*	$4167 - 5416/month
(7)	$65,000 - 79,999/year	*or*	$5417 - 6666/month
(8)	$80,000 - 94,999/year	*or*	$6667 - 7916/month
(9)	$95,000 - 109,999/year	*or*	$7917 - 9167/month
(10)	$110,000 - 125,000/year	*or*	$9168 - 10,416/month
(11)	More than $125,000/year	*or*	more than 10,417/month

B.   EDUCATION/OCCUPATION

8.   **How far did you get in school*?***

a. *Specify number of years attended school (PLEASE CIRCLE): This refers to the number of*

   full time (8-9 month) years of school.

GRADE SCHOOL   HIGH SCHOOL

1	2	3	4	5	6	7	8	9	10	11	12

COLLEGE   POST GRADUATE

13	14	15	16	17	18	19	20	21	22	23	24+

b. Education level *(please circle __highest__ level achieved/completed):*

(0)No High School diploma

(1)GED

(2)High School diploma

(3)Technical training

(4)Some college, no degree

(5)Associate degree

(6)Bachelors degree

(7)Masters degree

(8)MD/PhD/J.D./PharmD

10.   **What kind of work do you do?** *(i.e., What is/was your job? Do you work outside of your home)?*

_____

_____

_____

_____

_____

Main occupation - if not working due to retirement, disability, or unemployment but worked at least 6 months in past 12 months [at 1 or more jobs] ask job questions about most recent job.

11. **Do you own your own business (or are you self-employed)?**

     Yes   No

12.   **If currently employed, how many hours a week do/did you work?** _____ *hours*

         1 = FT (>= 35 hours/week)

         2 = PT (1-34 hours/week)

13.   **How long have you/did you work in that occupation or profession?**

     _____ *years*   _____ *months*

APPENDIX C

Lifestyle Behaviors Questionnaire Form

1. How many caffeinated beverages (coffee, tea, sodas, etc.) do you consume each day?

_____
(daily total)

2. Have you ever used any of the following tobacco products?

a. Cigarettes: No   Yes _____   Currently _____   Quit_____
                                    (# of years)   (# of years)   (year quit)

b. Pipe: No   Yes _____   Currently _____   Quit_____
                                    (# of years)   (# of years)   (year quit)

c. Cigar: No   Yes _____   Currently _____   Quit_____
                                    (# of years)   (# of years)   (year quit)

d. Chewing tobacco: No   Yes _____   Currently _____   Quit_____
                                    (# of years)   (# of years)   (year quit)

e. Snuff : Never   Occasionally or tried   Regular Use (# of yrs) _____

3. On average about how many alcoholic beverages do you consume in a week?

_____

 (One beverage is equal to a 12oz. beer, 5 oz. Wine, or 1.5 oz of hard liquor)

4. Did you drink any alcohol in the last week?   NO   YES

4a. We would like to know the number of alcoholic drinks you have

had in the last week. Today is _____. Let's begin with yesterday.

	BEER	WINE	LIQUOR	OTHER (SPECIFY)
M	_____	_____	_____	_____
T	_____	_____	_____	_____
W	_____	_____	_____	_____
R	_____	_____	_____	_____
F	_____	_____	_____	_____
SA	_____	_____	_____	_____
SU	_____	_____	_____	_____

_____
(7 day total)

5. At least once a week, do you engage in regular activity such as brisk walking, jogging, bicycling, swimming, etc., long enough to work up a sweat, get your heart thumping, or get out of breath?

_____ No.   Why not?

_____

_____ Yes.   How many times per week? _____

In what type of activities do you engage? _____

6. Approximately how many hours do you spend each week participating in physical activities such as walking, playing a sport, (golf, tennis, etc.), physical therapy, or group exercises such as water aerobics?

_____

(number of hours)

APPENDIX D

Medical History Checklist Form

Do you have or have you had any of the following? (PLEASE CHECK ONE)

	YES (1)	NO (0)	DON'T KNOW (-3)
Heart or Blood Vessels			
1. High blood pressure (hypertension)	( )	( )	( )
2. Heart attack (myocardial infarction, coronary occlusion or coronary thrombosis)	( )	( )	( )
3. Angina (chest pain brought on by exertion)	( )	( )	( )
4. Heart bypass surgery	( )	( )	( )
5. Heart balloon angioplasty, stent	( )	( )	( )
6. Congestive heart failure	( )	( )	( )
7. Valve problems (heart murmur or leaky valve)	( )	( )	( )
8. Heart pacemaker	( )	( )	( )
9. Rheumatic fever with involvement of heart	( )	( )	( )
10. Abnormal heart rhythm (e.g. atrial fibrillation)	( )	( )	( )
11. Other (Specify: _____)	( )	( )	( )
Brain			

12. Stroke (cerebrovascular accident)	( )	( )	( )
13. TIA or transient ischemic attack (brief stroke that completely resolved within 24 hours)	( )	( )	( )
14. Convulsions, fits or seizures, epilepsy	( )	( )	( )
15. Head injury or concussion or spinal cord injury, how many?	( )	( )	( )
16. Loss of consciousness, how long?	( )	( )	( )
17. Parkinson's disease, Multiple Sclerosis or Muscular Dystrophy	( )	( )	( )
18. Other (Specify:_____)	( )	( )	( )
Other Diseases	**YES** **(1)**	**NO** **(0)**	**DON'T KNOW** **(-3)**
19. Diabetes (high blood or urine sugar)	( )	( )	( )
20. Kidney problems (nephritis, kidney infection, kidney stones)	( )	( )	( )
21. Surgery (type: _____)	( )	( )	( )
22. Cancer (site:_____)	( )	( )	( )
23. Asthma	( )	( )	( )
24. Other lung problems (TB, emphysema, pleurisy, chronic bronchitis, or other problems)	( )	( )	( )
25. Thyroid condition	( )	( )	( )
26. Liver disease (hepatitis, cirrhosis or other problems)	( )	( )	( )

27. Arthritis	( )	( )	( )
28. Hives, hay fever, or other allergies	( )	( )	( )
29. Other major disease (specify: _____)	( )	( )	( )
30. Have you ever been hospitalized?	( )	( )	( )

Please indicate whether the following are true:	YES (1)	NO (0)
31. Full use of arms, hands, and fingers	( )	( )
32. Full use of legs and feet	( )	( )
33. Good eyesight (when wearing glasses or contact lens)	( )	( )

APPENDIX E

Medication Update Form

1. Are you currently taking Aricept (donepezil)?   Yes_____   No_____

   1a. If so how much do you take?   _____(mg)

   1b. How often do you take Aricept? (twice a day, once a day, etc.)

   _____

   _____

   _____

   1c. What time did you last take Aricept? (this morning, last night, etc.)

   _____

   _____

   _____

Note any change in dosage or instructions for administration here.

_____

_____

_____

_____

2. Are you currently taking any other medications that require a prescription from a doctor?

Yes _____   No_____

Medication Name	Purpose	Dosage (mg) and Frequency	Last taken

2. Do you take **any** supplements or over the counter medications?

 (For example, a multiple vitamin, vitamin C, calcium, iron, St. John's wort, glucosamine, ginkgo, allergy

 pill, cold medication, antacid or , laxative)

Yes_____   No_____

Supplement Name	Dosage (mg) and Frequency	Last taken

3.   Since your last session, have you taken aspirin or an over-the-counter pain medications

        (ibuprofen, Tylenol, etc)?

Yes_____   No_____

Medication Name	Purpose	Dosage (mg) and Frequency	Last taken

Notes:

_____
_____
_____

APPENDIX F

Physical and Biological Assessments

Height: _____

Weight: _____

Blood Pressure

    Cuff Size: _____

    1st: _____

    2nd: _____

HRV measurement:

    Time: _____

    Date: _____

Respiration:

    Minute 1:

Total Rate for minute: _____

    Minute 3:

Total Rate for minute: _____

Average of two readings: _____

    • Divide by 60:   _____

    • ADD (0.03)   _____

    • SUBTRACT (0.03)   _____

APPENDIX G

Power Analysis

<u>Supine:</u>

F tests - ANOVA: Repeated measures, between factors
Analysis: A priori: Compute required sample size
Input: Effect size f   = 0.82
                   α err prob   = 0.05
                   Power (1-β err prob)   = 0.80
                   Number of groups   = 2
                   Repetitions   = 3
                   Corr among rep measures   = 0.5
Output: Noncentrality parameter λ   = 12.103200
                   Critical F   = 4.964603
                   Numerator df   = 1.000000
                   Denominator df   = 10.000000
                   Total sample size   = 12
                   Actual power   = 0.879100

<u>Standing:</u>

F tests - ANOVA: Repeated measures, between factors
Analysis: A priori: Compute required sample size
Input: Effect size f   = 0.65
                   α err prob   = 0.05
                   Power (1-β err prob)   = 0.80
                   Number of groups   = 2
                   Repetitions   = 3
                   Corr among rep measures   = 0.5
Output: Noncentrality parameter λ   = 10.140000
                   Critical F   = 4.600110
                   Numerator df   = 1.000000
                   Denominator df   = 14.000000
                   Total sample size   = 16
                   Actual power   = 0.841313

VITA
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<u>Education</u>

2010 – present.	**Virginia Consortium Program in Clinical Psychology**
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	University-based, APA accredited program, jointly sponsored by: Eastern Virginia Medical School, Norfolk State University, and Old Dominion University.
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2008	**The Michigan School of Professional Psychology** M.A. in Clinical Psychology
2005	**The University of Derby** Bachelor of Arts

<u>Pre-Doctoral Internship:</u>

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<u>Research Experience</u>

Dissertation	Donepezil's Effect on Cardiac Function in Patients with Alzheimer's Disease Through an In Vivo, Non Invasive Measure of Cardiac Autonomic Function
Second Year Project	The Relationship of Trait Anxiety to Quality of Life Pre-Coronary Artery Bypass Graft (CABG) Surgery

<u>Recently Submitted Publications</u>

Hackett, L. P., Coogle, C. L., Owens, M. G., Ansello, E. F., & Mathews, J. H. (2015) Perceived Self-Efficacy Gains and Practice Change Following a Curriculum Development Training Program (Currently under review by the Journal of Interprofessional Care).

<u>Publications</u>

Hackett, L. P. (2013). Falls Risk and Falls Prevention among Older Adults. Virginia Psychogram, 38 (1), 17.

Simms, K., Bock, S., & Hackett, L. P., (2013). Does the Duration and Frequency of Physical Education Predict Academic Performance, Self-Concept, Social Skills, Food Consumption, and Body Mass Index? Health Education Journal, February 24, 2013.

<u>Grant Proposals</u>

Morrison, S., Hackett, L. P., Neumann, S., & Okhravi, H. (June, 2013). Balance, Mobility and Fear of Falling in High Risk Older Adults: The Effects of Cognitive Therapy and Balance Training, National Institute of Health (NIH) R21 Grant.