Spring 2016

Neuropathy Detection, Quality of Life Tools & Treatment for Type 2 Diabetes

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NEUROPATHY DETECTION, QUALITY OF LIFE TOOLS & TREATMENT FOR TYPE 2 DIABETES

by

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A Dissertation Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

HUMAN MOVEMENT SCIENCE

OLD DOMINION UNIVERSITY
May 2016

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ABSTRACT

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FOR TYPE 2 DIABETES

Jennifer J. Brown
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Type 2 diabetes (T2D) mellitus has become the epidemic of the new millennium, with an estimated 382 million people affected worldwide as of 2013, and statistics projected towards 592 million by the year 2035. With the development of diabetes, complications have risen, with diabetic neuropathy becoming one of the most prevalent, affecting between 10–90% of those with the disease. Diabetic peripheral neuropathy (DPN) is difficult to detect in early stages of pathology, yet devastating once significant damage has taken place. Cardiac autonomic neuropathy (CAN), which is often silent, is associated with autonomic nervous system (ANS) dysfunction and increased risk for sudden death. Therefore, the purposes of this dissertation were early detection, assessment of quality of life (QOL) and disease intervention. Study I explored the effectiveness of the 128-Hz tuning fork, the 1-g and 10-g monofilaments, and the QOL-DN as tools for the early detection of DPN in overweight, obese and inactive (OOI), prediabetes (PD), and type 2 diabetes (T2D) individuals. Study II compared three QOL assessments: the QOL-DN, the PN-QOL-97 and the NeuroQOL-28, in OOI, PD and T2D individuals. Study III involved the execution of a double blinded, placebo controlled exploration of melatonin as a potential intervention for the improvement of ANS and sleep dysfunction in T2D.

The results of Study I suggest that the 1-g monofilament and QOL-DN measures correlate to NC-Stat DPN Check portable nerve conduction study (NCS) findings, that these

measures function well for early detection purposes, and that the 128-Hz tuning fork is a useful screening tool in OOI, PD and T2D populations, despite lack of correlation to NCS measures. The results of Study II suggest that the QOL-DN and the NeuroQOl-28 QOL instruments significantly predict NCS results, indicating that these measures are useful for screening and accurately assessing neuropathy within our populations of interest. Study III results indicate that a 10 mg dose of melatonin taken 30 minutes prior to bedtime for four weeks has a positive effect on PSQI Subjective Sleep Quality, systolic blood pressure (SBP) in deep breathing and Valsalva maneuvers, and HRV SDNN measures in individuals with T2D.

This dissertation is dedicated to the academic heroes of the

Human Movement Sciences Department of Old Dominion University.

Thank you for investing in me through all my years of training.

Dr. Sheri Colberg, Dr. Kim Baskette, Dr. Shana Pribesh and Dr. Aaron Vinik: thanks for your help through the challenges life has brought. I'll be sure to pay it forward.

To my father, Dr. A. Andrew Robertson (1939-2009);

you were with me every late night of writing on this project.

You will always be my hero, closest friend, and greatest inspiration.

ACKNOWLEDGMENTS

First, I must acknowledge that these projects have been accomplished with the financial support of Old Dominion University's Darden College of Education (DCOE) and the Human Movement Sciences (HMS) Department. Working to achieve a doctorate is a lofty goal, and to have the support of one's university is both an honor and a necessity to achieve success. The DCOE and HMS Department provided support through GTA teaching positions, stipends, tuition scholarships, and a Doctoral Dissertation Fellowship. These valuable gifts allowed me to complete my training and doctoral projects with greater focus.

My committee has gone above and beyond to assist me in my studies, offering insight throughout my doctoral career and guidance through the projects on the following pages. My mentor, Dr. Sheri Colberg-Ochs, has invested countless hours into my training, and willingly made herself available to assist me in any way possible to help me achieve success, and I am forever indebted to her for her investment. Dr. Colberg, I am grateful beyond words for the example you have set for me as a professor, investigator, mentor, collaborator, role model and approachable human being. Thank you, from the bottom of my heart, for your patience and encouragement through all of the moments leading to this success. I wish you well in all of your new endeavors, and know that Old Dominion University will miss your motivating influence. You've made an incredible impact in my life and in the lives of many others while you have been here. Dr. Baskette, thank you for the investment of your time, ideas, and support, all of which have been provided at key moments during this journey. I would also like to thank Dr. Aaron I. Vinik, who has both challenged and supported me in countless ways during my training. Dr. Vinik, your keen insight into the world of diabetes and neuropathy has provided inspiration as I've spent countless hours reading articles and preparing this manuscript. Thank you for

investing your facility resources and staff to support the Melatonin project. Lastly, I thank Dr. Pribesh for her support, for pushing me at key moments and challenging me on many levels during my training as a doctoral student.

At this level, it takes more than a committee to accomplish this many projects in such a short time period. Many thanks to Dr. Carolina Casellini, and in her absence, Dr. Aaron Vinik, for performing the physicals on the melatonin subjects, and to Dr. Henri Parson for negotiating the joint venture project between ODU and EVMS. Special thanks to the EVMS Strelitz Diabetes Center research and clinic staff, for all of their support. Much appreciation to Benjamin Liu, Morgan Huskey and Anthony Ponce for their commitment to show up and help throughout data collection at Old Dominion University.

To my husband, Gil and two children, Natalie and Keagan: your support as I have walked through this degree has meant the world. Thank you for the countless loving ways you have arrived to help make this happen during the moments that I have needed it the most. In your efforts to show care for me as your wife, mother and friend, you have given me a great gift by supporting me as I have pursued this training. I, in turn, hope that the use of this degree will become a great gift to others. To my mother, Doris Robertson, thank you for your unwavering support for me in all that I set out to do. Your love of education, and encouragement as I have pursued it, has been a blessing for all three of my degrees. To my father, Dr. A. Andrew Robertson, who was not here to see this degree started or completed; you were and still are, an incredible example of tenacity, love and faith. Thank you for instilling in me the values of faith, family, hard work and persistence. Without these qualities, I would have surely not have reached the finish line of this great academic event.

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CHAPTER I

INTRODUCTION

Diabetes is a metabolic disorder, and when present, indicates that an individual has high blood glucose (BG) levels relating to insulin production or usage, or potentially both of these metabolic processes (Center for Disease Control and Prevention, 2014). This elevated BG status, or hyperglycemia, affects various cells throughout the body that perform both vital and secondary functions, including key brain, organ and muscle tissue processes. Debilitating by nature, diabetes often goes undetected, with one out of four unaware that they have developed the disease until symptoms elevate to the point where significant damage may be present or a major event may occur (Center for Disease Control and Prevention, 2014).

The debilitating effects of diabetes stretches worldwide, affecting 382-387 million people, creating an impact on 29.1 million, or 9.3% of the United States population (Center for Disease Control and Prevention, 2014; Guariguata et al., 2013; International Diabetes Federation, 2014). With an estimated 21 million who have been diagnosed and another 8.1 million who remain undiagnosed, the International Diabetes Federation and research estimates the impact of diabetes to climb into the staggering 590-592 million range across the globe by the year 2035 (Guariguata et al., 2013; International Diabetes Federation, 2014). The largest number of new diabetes cases are in the 45 to 64 age range, and interventions aiming to stop diabetes processes at earlier stages in younger populations seem prudent, as the disease is gaining a tighter grip on the United States and the population worldwide (Center for Disease Control and Prevention, 2014).

Great emphasis has been placed on developing management strategies for commonly associated complications such as neuropathy (Shah & Mueller, 2012) and weight gain (Courcoulas, 2015; Golomb, Ben David, Glass, Kolitz, & Keidar, 2015); yet long term resolution remains elusive once disease pathways have set their course. Extensive research has been performed determining the linkage between diabetes and other diseases, including the effects of insulin on the brain, metabolic syndrome, depression, and various forms of neuropathy (Alberti et al., 2009; Chen, Wang, Zhu, Li, & Teng, 2014; Mezuk, Eaton, Albrecht, & Golden, 2008; Smith, Gerardi, Lessard, Reyna, & Singleton, 2013; Vinik, Nevoret, Casellini, & Parson, 2013). Existing research provides foundational opportunities for positive impact on the health of millions who currently suffer with diabetes, as a major focus is on the modification of management of the disease, in hopes to halt its progression. Yet, there is still much to learn regarding how to prevent the initial onset in the earliest stages, and how to ignite changes before long standing hyperglycemia sets in, negatively affecting quality of life (QOL) (Alexander, Landsman, & Grundy, 2006; Nichols, Alexander, Girman, Kamal-Bahl, & Brown, 2006; Phillips, Ratner, Buse, & Kahn, 2014).

Hyperglycemia at any stage is an unhealthy process that lays the groundwork for significant pathophysiological processes (Mustafa, Alemam, & Hamid, 2012; Papanas & Ziegler, 2012). The development of earlier intervention practices is logical for numerous reasons. The cutoff points dictating glycemic levels that define diabetes relate to the associations found between particular glucose levels and significant increases in microvascular complications such as retinopathy and nephropathy (Buysschaert & Bergman, 2011). It is vital to encourage individuals whose glucose levels reside within cautionary ranges, such as prediabetes (PD), to take heed and implement aggressive measures to avoid the common progression to type 2

diabetes (T2D). Research indicates that individuals with PD in the HbA1C range of 6.1–6.4% are at significant risk for the development of T2D and typically progress to T2D within three to five years (ADA, 2016). Developing new tools and programs that assess risk prior to this time of pathophysiological dysfunction is a practical course of action.

Early detection and intervention is key if society desires to attenuate the impending impact that T2D and prediabetes is expected to have on the modern world in the upcoming years of 2030 to 2035. Diabetes in its latter forms has already launched its assault on the body, often in irreparable ways and, thus, early detection of complications and treatment of them is key to achieve the best scenarios for health and positive outcomes. It is with these concepts in mind that I built a framework for my research.

CHAPTER I

PART A: NEUROPATHY SCREENING TOOLS

The Problem

T2D and PD are worldwide health problems, with rapidly increasing numbers (International Diabetes Federation, 2014; Ruterbusch, 2014). DPN is a significant complication associated with acute and chronic hyperglycemic conditions such as T2D and PD, yet little research exists evaluating the ability of low-cost screening tools to effectively detect the earliest stages of the disease. Given that DPN is frequently debilitating (Vinik et al., 2013), early detection of the disease would best allow for treatment and management, hopefully deterring long-term deficits in ambulation (Eikenberg & Davy, 2013; Papanas & Ziegler, 2012). Research scientists agree that catching the pathology in the earliest stages is important to prevent major complications and loss of quality of life, thus targeted efforts must be made to detect DPN during the earliest stages of hyperglycemia (Ferrannini, Gastaldelli, & Iozzo, 2011; Papanas & Ziegler, 2012; *Prevention of Type 2 Diabetes: From Science to Therapy*, 2012). This involves evaluating PD and individuals with elevated, but not clinically diagnosable hyperglycemia. Multiple tools exist to screen T2D populations, yet these tools have not been used extensively in PD or subclinical populations.

Purpose and Significance of the Study

Evaluating simple screening tools and their ability to effectively detect earlier stages of DPN in new research populations, such as in overweight, obese and inactive (OOI) subjects,

alongside PD and T2D subjects allowed a fresh look at how these particular tools could be used. The hope of this research was that we might uncover simple ways to disclose early DPN or subclinical neuropathy. PD and T2D populations served as control subjects, allowing us to compare our data to previous research, while seeking new answers for early DPN and subclinical neuropathy detection. We aimed to evaluate the effectiveness of three neuropathy screening tools: the 128-Hz tuning fork, the 1-g and 10-g monofilaments, and the QOL–DN for the purposes of early DPN detection, utilizing predefined definitions from literature (Tesfaye, 2010). We also hoped to determine which screening tool was the most effective, while comparing our results back to a standardized criterion measure of portable nerve conduction in the form of the NC-Stat DPN Check (Neurometrix Inc., Waltham, MA).

Research Design

Study Design Approach. The proposed study took an observational and correlational approach paired with a quantitative data collection. Individuals were screened and categorized by HbA1C values and prior diagnoses, creating specific groups to study (see Appendix A) (OOI, PD, T2D).

Variables. A criterion variable approach was implemented, with the NC-Stat DPN Check device providing a nerve conduction testing evaluation of the sural nerve, bilaterally. Our other variables, the 128-Hz tuning fork, 1-g and 10-g monofilaments, and the QOL–DN (see Appendix B), were compared to this standard of testing. HbA1C test data combined with previous PD or T2D diagnosis identified OOI, PD and T2D populations with continuous numerical data, into 1 of 3 categories: OOI, 4.0–5.6%; PD, 5.7–6.4%, T2D, 6.5% and above.

Dependent variables (DV) included the tuning fork data, monofilament testing, and QOL–DN results, which consisted of continuous, interval level data.

Research Questions

RQ1: To what extent will:

1a: the 128-Hz tuning fork detect early DPN in an OOI, PD and T2D population?

1b: the 1-g and 10-g monofilaments detect early DPN in an OOI, PD and T2D population?

1c: the QOL–DN detect early DPN in an OOI, PD and T2D population?

RQ2: Which tool will be the most sensitive for detecting early DPN?

Research Hypotheses

H1: The 128-Hz tuning fork and QOL–DN tools will provide excellent mechanisms for detecting early DPN in OOI, PD and T2D populations.

H2: The QOL-DN will be the most sensitive measure to detect early or undisclosed DPN in an OOI, PD and T2D population.

Assumptions. Assumptions included accurate reporting on the part of our participants. This includes believing that they would be invested in accurately participating in tests that involved their voluntary response, such as tuning fork, and monofilament testing and that participating individuals would answer questions honestly on the provided patient reported outcome measure (PROM) questionnaires. We justified that individuals choosing to engage in

our research were likely to report truthfully and be invested in the task at hand, as they were not paid for their time, and willingly participated of their own free will in the study. The psychometric properties of the QOL-DN have been previously evaluated for populations with diabetes and also, for the purposes of revealing undisclosed DPN in varied populations; therefore, in this research study we assumed that the psychometric properties of this instrument would be effective in our OOI individuals.

Limitations. Old Dominion University has limited clinical equipment for related to diabetes testing. The HbA1C testing machine that was used within the study is a validated machine (Lenters-Westra & Slingerland, 2010), yet oral glucose tolerance testing is preferred by some research scientists, particularly for individuals with cardiac autonomic neuropathy (CAN) (Farhan et al., 2012). DPN and CAN often coexist, yet we did not test for CAN and, therefore, cannot account for unknown discrepancies. Temperature and humidity have been found to affect monofilament results, by affecting the potential validity of the instrument in extremely high temperatures as well as high testing volumes in short periods of time (Booth & Young, 2000; Haloua, Sierevelt, & Theuvenet, 2011). Temperature was accounted for by limiting monofilament storage and use to normal climate controlled room temperatures and monitored these values. Humidity was monitored, but not controlled beyond what the Old Dominion University air-conditioning and heating systems accounted for. Preparation for monofilament usage followed previously stated guidelines and recommendations, with testing amounting to far less than 100 compressions per day per instrument (Booth & Young, 2000). The NC-Stat DPN Check device was used solely to test the sural nerve; therefore, deficits in nerve function relating to other nerves of the lower leg were not confirmed through this device. Previous research has not investigated the validity of the QOL-DN specifically within an overweight, obese and

inactive population and, therefore, we should take this into account in the interpretation of our findings.

Delimitations. Individuals were screened through the provided screening questionnaire located in the appendix (see appendix A). None of the research staff were trained physicians. We did not ask for medical records to confirm individual reporting. We did not evaluate medical conditions or attempt to diagnose neuropathy, but instead referred individuals to appropriate medical staff if research findings indicated potential deficits related to DPN**.**

Operational Definitions

Confirmed DSPN: "The *presence of an abnormality of nerve conduction* and symptom or symptoms or a sign or signs of neuropathy confirms DSPN. If nerve conduction is normal, a validated measure of small fiber neuropathy (SFN) (with class one evidence) may be used. To assess for the severity of DSPN, several approaches can be recommended: the graded approach; various continuous measures of sum scores of neurologic signs, symptoms, or nerve test scores; scores of function of activities of daily living (ADLS); or scores of predetermined tasks or of disability" (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Additional resources to support this definition include Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, and Vinik (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Diabetes: The American Diabetes Association clearly classifies diabetes into four categories (ADA, 2014, 2016; Zhou & Zhou, 2014):

1. Type 1 diabetes (T1D): caused by beta cell destruction, and most often leads to

complete insulin deficiency.

2. Type 2 diabetes (T2D): caused by a progressive insulin secretory defect combined with insulin resistance).

3. Gestational diabetes mellitus (GDM): diabetes which is diagnosed during pregnancy, usually in the second or third trimester, yet is not considered overt diabetes.

4. Diabetes due to other causes, such as neonatal diabetes, maturity-onset diabetes, pancreatic disease causes (cystic fibrosis), drug or chemical causes (HIV/AIDS treatment, organ transplants).

Diabetes Diagnostic Criteria: Diabetes may be diagnosed with a hemoglobin A1C (HbA1C) value of $\geq$ 6.5% or a fasting glucose of $\geq$ 126 mg/dL (7.0 mmol/L) (ADA, 2014, 2016; Zhou & Zhou, 2014) with clear diagnostic criteria. Without clear diagnostic criteria, an immediate retest should be done to confirm the results, or two diagnostic tests with clear confirmation confirms diagnosis.

Diabetic neuropathy (DN): "DN is represented by clinical syndromes affecting distinct regions of the nervous system, singly or combined. It may be silent and go undetected while exercising its ravages; or it may present with clinical symptoms and signs that, although nonspecific and insidious, with slow progression, also mimic those seen in many other diseases" (Vinik et al., 2013, pg. 747).

Diabetic peripheral neuropathy (DPN): DPN is commonly experienced by individuals who have been diagnosed with diabetes. It is frequently reported as a late complication resulting in multiple syndromes, with no universal viewpoint for classification (Vinik, Mitchell, et al., 1995; Vinik et al., 2013). Generally, DPN is viewed in subdivisions, such as: focal/multifocal neuropathies, diabetic amyotrophy, symmetric polyneuropathies and

sensorimotor polyneuropathy (DSPN), which is a common type of neuropathy experienced by diabetes patients (Sadosky, 2008; Vinik, Ullal, Parson, & Casellini, 2006; Vinik et al., 2013).

Fasting plasma glucose: "a check of a person's blood glucose level after the person has not eaten for 8 to 12 hours (usually overnight) (ADA, 2014, 2016). This test is used to diagnose prediabetes and diabetes. It is also used to monitor people with diabetes."

Glycosylated hemoglobin testing: (HbA1C testing) is a test measuring an individual's average blood glucose levels for the past 2 to 3 months (ADA, 2014, 2016). The hemoglobin (HEE-mo-glo-bin) is the part of a red blood cell that carries oxygen to the cells and sometimes joins with the glucose in the bloodstream. This test is called hemoglobin A1C or glycosylated (gly-KOH-sih-lay-ted) hemoglobin, and represents the percentage of red blood cells with glucose attached to the A1c component, which is proportional to the amount of glucose in the blood.

Oral glucose tolerance testing: This is a test used to diagnose prediabetes and diabetes. The oral glucose tolerance test is given by a health care professional after an overnight fast. A blood sample is taken, then the patient drinks a high-glucose beverage provided by the health care professional. Blood samples are taken at intervals for 2 to 3 hours and the results are compared with a standard and show how the body uses glucose over time (ADA, 2014, 2016).

Possible DSPN: The presence of *symptoms or signs* of DSPN including any of the following: symptoms—decreased sensation; positive neuropathic sensory symptoms (e.g. "asleep numbness," prickling or stabbing, burning or aching pain) predominantly in the toes, feet or legs; or signs—symmetric decrease of distal sensation, or unequivocally decreased or

absent ankle reflexes (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Prediabetes: Prediabetes is considered to be a condition of elevated but not yet clinically diagnosable diabetes blood glucose or HbA1C levels (ADA, 2014, 2016).

Prediabetes Identification Criteria: Prediabetes may be defined with an HbA1C value of 5.7–6.4% or a fasting glucose of > 100–125 mg/dL (7.0 mmol/L) (ADA, 2014, 2016; Zhou & Zhou, 2014). These individuals should be considered to be in a place of increased risk for both cardiovascular disease (CVD) and diabetes development. HbA1C values > 6.0 should be considered very high risk for the development of diabetes and aggressive interventions are advised.

Probable DSPN: "The presence of a *combination of symptoms and signs* of neuropathy including two or more of any of the following: neuropathic symptoms, decreased distal sensation, or unequivocally decreased or absent ankle reflexes" (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Small fiber neuropathy (SFN): "SFN should be graded as follows: (1) possible: the presence of length-dependent symptoms and/or clinical signs of small fiber damage; (2) probable: the presence of length dependent symptoms, clinical signs of small fiber damage, and normal sural nerve conduction; and (3) definite: the presence of length dependent symptoms, clinical signs of small fiber damage, normal sural nerve conduction, and altered IENFD

at the ankle and/or abnormal thermal thresholds at the foot (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Subclinical: Subclinical refers to a condition in terms of being elevated but not yet clinically diagnostic, symptomology; may apply to blood glucose, HbA1C levels, DPN, or other diabetes symptomology (Mustafa et al., 2012).

Subclinical DSPN: "The presence of *no signs or symptoms of* neuropathy is confirmed with *abnormal nerve conduction or a validated measure of SFN* (with class 1 evidence)" (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Definitions relating to possible, probable and confirmed DSPN can be used for clinical practice and confirmed DSPN and subclinical definitions can be used for research studies. Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Expected Outcomes

This study examined the diagnostic utility of the 128-Hz tuning fork, 1-g and 10-g monofilaments, and QOL-DN for the purposes of subclinical neuropathy screening and to determine which was the most effective. Previous studies indicated that all three of these measures have been both sensitive and reliable in T2D populations; therefore, we expected the same outcome within the study utilizing OOI, PD and T2D volunteer participants. Each tool has

had limited usage with PD populations, and their utility needs further validation, yet we expected high reliability and specificity from all three measures in detecting DPN in a PD population. The unique outcome of the study was determined to be ascertaining which measure is the most sensitive and specific for determining subclinical DPN detection, and we predicted that the QOL-DN would provide a mechanism for detecting sensation loss that is able to be noticed by the participant. We also expected that the 128-Hz tuning fork would be a useful mechanism for detecting subclinical DPN in individuals without signs or symptoms.

CHAPTER I

PART B: NEUROPATHY QUALITY OF LIFE TOOLS

The Problem

DN is often experienced in T2D, and research has also confirmed DN in PD populations, raising questions as to when DN develops (Marrero et al., 2014; Papanas & Ziegler, 2012) and how soon it affects QOL. QOL is a significant issue in diabetes management, and quick assessment seems prudent for medical screenings in research, yet little attention has been given to the completion burden related to QOL instruments, as the time to complete certain measures are reported as unknown or somewhat lengthy (Smith, Lamping, & Maclaine, 2012). Accurate and effective assessment of QOL measures can be a benefit for healthcare providers and patients alike in a multitude of settings, yet some debate exists regarding which instruments are the most effective for determining QOL.

Purpose & Significance of the Study

The purpose of the study was to compare the measures of the QOL–DN, the PN-QOL-97, and the NeuroQOL-28 in a mixed population that included OOI, PD and T2D individuals to determine which instrument was the most effective at detecting DPN at various stages while comparing the findings back to a criterion standard of NC–Stat DPN Check (NeuroMetrix, Waltham, MA). Completion times were tracked for each instrument, allowing a comparison of the time investment needed to utilize each chosen method.

By determining the effectiveness of these tools to detect DPN within this predefined

population, it may open up new opportunities to evaluate the effectiveness of these instruments in early DPN and subclinical populations. Further examination of these instruments could provide opportunity to strengthen the measures and reveal unknown caveats.

Research Design

Study Design Approach. The proposed study took an observational and correlational approach paired with a quantitative data collection. Individuals were screened and categorized by HbA1C values and prior diagnoses, creating specific groups to study (see Appendix A) (OOI, PD, T2D). A criterion variable was implemented, with NC-Stat DPN Check nerve conduction testing being presented as the criterion that determining normal or abnormal values for the sural nerve. The effectiveness of the content of the other variables, the QOL-DN, the NeuroQOL-28 and PN-QOL-97, was compared to this standard of testing (see Appendices B, C and D).

Research Questions

RQ 1: To what extent will the three instruments differ in their ability to detect DN in OOI, PD and T2D populations?

RQ 2: To what extent will the results of the three instruments correlate with the NC-Stat DPN Check?

RQ 3: To what extent will the surveys differ in the amount of time they take to complete?

Research Hypotheses

H 1: We hypothesized that the QOL–DN and PN-QOL-97 would more clearly identify signs of early DN when compared to the NC-Stat DPN Check results.

H 2: We hypothesized that all three instruments would correlate with the NC-Stat DPN Check results at 60 or higher, with the QOL-DN yielding the strongest relationship.

H 3: We hypothesized that the NeuroQOL-28 would be quickest to complete, followed by the QOL-DN and the PN-QOL-97.

Assumptions. Assumptions included accurate reporting on the part of our participants, that they would answer questions honestly on the provided questionnaires, and be invested in accurate participation. We justified that individuals choosing to engage in our research were likely to report truthfully and be invested in the task at hand, as they were not paid for their time and were involved in the study by their own choice. The psychometric properties of the QOL-DN have been previously evaluated for populations with diabetes and also, for the purposes of revealing undisclosed DPN in varied populations; therefore, in this research study we assumed that the psychometric properties of this instrument would be effective in our OOI individuals.

Limitations. Old Dominion University has limited clinical equipment for related to diabetes testing. The HbA1C testing machine that was used within the study is a validated machine (Lenters-Westra & Slingerland, 2010), yet oral glucose tolerance testing is preferred by some research scientists, particularly for individuals with cardiac autonomic neuropathy (CAN) (Farhan et al., 2012). We did not test for CAN and, therefore, cannot account for unknown discrepancies. Lack of random assignment and use of volunteers for subjects created potential selection bias, with clinical population research targeting and low available funding heavily

influencing this method. The NC-Stat DPN Check device was used solely to test the sural nerve; therefore, deficits in nerve function relating to other nerves of the lower leg were not confirmed through this device. Previous research has not investigated the validity of the QOL-DN, the PN-QOL-97 and the NeuroQOL-28 specifically within an overweight, obese and inactive population and, therefore, we should take this into account in the interpretation of our findings.

Delimitations. Individuals were screened to help determine eligibility for participation (see appendix A). The researchers were not trained physicians. We did not ask for medical records to confirm individual reporting. We did not evaluate medical conditions or attempt to diagnose neuropathy, but instead referred individuals to medical staff if research findings indicated potential deficits related to PN or other medical conditions that presented themselves.

Operational Definitions

Confirmed DSPN: "The *presence of an abnormality of nerve conduction* and symptom or symptoms or a sign or signs of neuropathy confirms DSPN. If nerve conduction is normal, a validated measure of small fiber neuropathy (SFN) (with class one evidence) may be used. To assess for the severity of DSPN, several approaches can be recommended: the graded approach; various continuous measures of sum scores of neurologic signs, symptoms, or nerve test scores; scores of function of activities of daily living (ADLS); or scores of predetermined tasks or of disability" (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

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Diabetes Diagnostic Criteria: Diabetes may be diagnosed with a hemoglobin A1C (HbA1C) value of $\geq$ 6.5% or a fasting glucose of $\geq$ 126 mg/dL (7.0 mmol/L) (ADA, 2014, 2016; Zhou & Zhou, 2014) with clear diagnostic criteria. Without clear diagnostic criteria, an immediate retest should be done to confirm the results, or two diagnostic tests with clear confirmation confirms diagnosis.

Diabetic neuropathy (DN): "DN is represented by clinical syndromes affecting distinct regions of the nervous system, singly or combined. It may be silent and go undetected while exercising its ravages; or it may present with clinical symptoms and signs that, although nonspecific and insidious with slow progression, also mimic those seen in many other diseases" (Vinik et al., 2013, pg. 747).

Diabetic peripheral neuropathy (DPN): DPN is commonly experienced by individuals who have been diagnosed with diabetes. It is frequently reported as a late complication resulting in

multiple syndromes, with no universal viewpoint for classification (Vinik, Mitchell, et al., 1995; Vinik et al., 2013). Generally, DPN is viewed in subdivisions, such as: focal/multifocal neuropathies, diabetic amyotrophy, symmetric polyneuropathies and sensorimotor polyneuropathy (DSPN), which is a common type of neuropathy experienced by diabetes patients (Sadosky, 2008; Vinik et al., 2006; Vinik et al., 2013).

Fasting plasma glucose: "a check of a person's blood glucose level after the person has not eaten for 8 to 12 hours (usually overnight) (ADA, 2014, 2016). This test is used to diagnose prediabetes and diabetes. It is also used to monitor people with diabetes".

Glycosylated hemoglobin testing: (HbA1C testing) is a test measuring an individual's average blood glucose levels for the past 2 to 3 months (ADA, 2014, 2016). The hemoglobin (HEE-mo-glo-bin) is the part of a red blood cell that carries oxygen to the cells and sometimes joins with the glucose in the bloodstream. This test is called hemoglobin A1C or glycosylated (gly-KOH-sih-lay-ted) hemoglobin, and represents the percentage of red blood cells with glucose attached to the A1c component, which is proportional to the amount of glucose in the blood.

Oral glucose tolerance testing: This is a test used to diagnose prediabetes and diabetes. The oral glucose tolerance test is given by a health care professional after an overnight fast. A blood sample is taken, then the patient drinks a high-glucose beverage provided by the health care professional. Blood samples are taken at intervals for 2 to 3 hours and the results are compared with a standard and show how the body uses glucose over time (ADA, 2014, 2016).

Possible DSPN: The presence of *symptoms or signs* of DSPN including any of the following: symptoms—decreased sensation; positive neuropathic sensory symptoms (e.g. "asleep

numbness," prickling or stabbing, burning or aching pain) predominantly in the toes, feet or legs; or signs—symmetric decrease of distal sensation, or unequivocally decreased or absent ankle reflexes (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Prediabetes: Prediabetes is considered to be a condition of elevated but not yet clinically diagnosable diabetes blood glucose or HbA1C levels (ADA, 2014, 2016).

Prediabetes Identification Criteria: Prediabetes may be defined with an HbA1C value of 5.7–6.4% or a fasting glucose of > 100–125 mg/dL (7.0 mmol/L) (ADA, 2014, 2016; Zhou & Zhou, 2014). These individuals should be considered to be in a place of increased risk for both cardiovascular disease (CVD) and diabetes development. HbA1C values > 6.0 should be considered very high risk for the development of diabetes and aggressive interventions are advised.

Probable DSPN: "The presence of a *combination of symptoms and signs* of neuropathy including two or more of any of the following: neuropathic symptoms, decreased distal sensation, or unequivocally decreased or absent ankle reflexes" (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Small fiber neuropathy (SFN): "SFN should be graded as follows: (1) possible: the presence of length-dependent symptoms and/or clinical signs of small fiber damage; (2) probable: the presence of length dependent symptoms, clinical signs of small fiber damage, and normal

sural nerve conduction; and (3) definite: the presence of length dependent symptoms, clinical signs of small fiber damage, normal sural nerve conduction, and altered IENFD at the ankle and/or abnormal thermal thresholds at the foot (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Subclinical: Subclinical refers to a condition in terms of being elevated but not yet clinically diagnostic, symptomology; may apply to blood glucose, HbA1C levels, DPN, or other diabetes symptomology (Mustafa et al., 2012).

Subclinical DSPN: "The presence of *no signs or symptoms of* neuropathy is confirmed with *abnormal nerve conduction or a validated measure of SFN* (with class 1 evidence)" (Prevention of Type 2 Diabetes: From Science to Therapy, 2012, p. 120). Definitions relating to possible, probable and confirmed DSPN can be used for clinical practice and confirmed DSPN and subclinical definitions can be used for research studies. Additional resources to support this definition include Tesfaye et al. (2010) and Vinik et al. (2013) (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, Vinik, et al., 2010; Vinik et al., 2013).

Expected Outcomes

This research attempted to evaluate the QOL-DN, the PN-QOL-97 and the NeuroQOL-28 in a mixed population in order to determine which instrument was the most effect at detecting DN at various stages, while further comparing the results back to a standardized measurement of

the sural nerve provided by the NC-Stat DPN Check (NeuroMetrix, Waltham, MA). Average completion times were determined, allowing an estimated time investment for each instrument.

CHAPTER I

PART C: MELATONIN AND AUTONOMIC NERVOUS SYSTEM FUNCTION

The Problem

CAN is a serious complication of diabetes, presenting with various degrees of severity throughout research. While the concept of neuropathy research is well represented for the diabetic foot, CAN dysfunction and appropriate treatment for this disorder has not been well investigated. Circadian patterns are often disrupted in T2D and associated dysfunction and pathology aligns with this disruption.

Autonomic nervous system function (ANS) is frequently affected in T2D, leaving an altered state of dysfunction that negatively affects circadian rhythms. This altered state affects sleep quality, ANS function, and creates a state of CAN, which ultimately places individuals at significant risk for early mortality.

The Purpose & Significance of the Study

Melatonin supplementation provides an opportunity for rebalancing the ANS in T2D. Melatonin has been found to be effective in resetting the circadian clock of the ANS, and we postulated that it may help improve ANS function in T2D patients, providing relief from sleep dysfunction, ANS pathology, and ultimately attenuate the pathophysiology of CAN.

The overall purpose of this research was to investigate whether the underlying central, cardiac, and peripheral defects that were observed in T2D could be improved or reversed by a known chronotropic hormone, melatonin, given as a daily supplement. The physiological impact

was evaluated through the effects of a high dose supplemental melatonin on autonomic balance in baroreflex sensitivity (BRS).

Research Design

This study investigated the potential positive effects of a single, high dose of nightly melatonin supplements on autonomic balance and baroreflex sensitivity in adults with T2D. Autonomic balance in T2D subjects was studied at baseline and following 4 weeks of 10 mg dose of melatonin or placebo in a double-blinded, randomized design, with no washout period between the melatonin and placebo trials. Variables of interest included autonomic balance and baroreflex sensitivity.

Research Questions

RQ1: To what extent is the ANS altered in individuals with T2D at baseline testing?

RQ2: To what extent will melatonin improve baseline study measures of ANS function?

Research Hypotheses

H1: We hypothesized that the ANS misconducts, causing a neuroinflammatory response, leading to impairment and that the proposed study measures would evaluate this phenomenon.

H2: We hypothesized that ANS function would improve in participants when engaging in melatonin supplementation.

Assumptions. Assumptions included accurate reporting on the part of our participants. This includes the basic assumption that individuals would answer questions honestly on the provided questionnaires, be invested in accurately participating in tests that involve their voluntary response, and accurately report consumption of the provided pills for each leg of the study. The Eastern Virginia Medical School Strelitz Diabetes Center provided the ANSAR and Sudoscan testing for patient evaluation on location at their outpatient facility. We assumed that all equipment beyond our control was well maintained and in proper working order for the use of this study.

Limitations. Old Dominion University has excellent movement exercise laboratory testing equipment, still, limited clinical testing equipment for clinical diabetes testing. The hemoglobin A1C (HbA1C) testing machine that was transported to Eastern Virginia Medical School is valid and reliable, yet oral glucose tolerance testing is preferred for working with CAN patients with diabetes (Farhan et al., 2012). Our sample was quite small for this pilot study, and all inferences within the written literature should take this into account.

Delimitations. We did not ask for medical records to confirm individual reporting. Oral glucose tolerance testing was not performed with the CAN patients due to financial limitations. Bloodwork was not drawn to confirm fasted states of participants due to financial limitations.

Operational Definitions

Diabetes: The American Diabetes Association clearly classifies diabetes into four categories (ADA, 2014, 2016; Zhou & Zhou, 2014):

1. Type 1 diabetes (T1D): caused by beta cell destruction, and most often leads to complete insulin deficiency.

2. Type 2 diabetes (T2D): caused by a progressive insulin secretory defect combined with insulin resistance).

3. Gestational diabetes mellitus (GDM): diabetes which is diagnosed during pregnancy, usually in the second or third trimester, yet is not considered overt diabetes.

4. Diabetes due to other causes, such as neonatal diabetes, maturity-onset diabetes, pancreatic disease causes (cystic fibrosis), drug or chemical causes (HIV/AIDS treatment, organ transplants).

Diabetes Diagnostic Criteria: Diabetes may be diagnosed with a hemoglobin A1C (HbA1C) value of $\geq$ 6.5% or a fasting glucose of $\geq$126 mg/dL (7.0 mmol/L) (ADA, 2014, 2016; Zhou & Zhou, 2014) with clear diagnostic criteria. Without clear diagnostic criteria, an immediate retest should be done to confirm the results, or two diagnostic tests with clear confirmation confirms diagnosis.

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Diabetic peripheral neuropathy (DPN): DPN is commonly experienced by individuals who have been diagnosed with diabetes. It is frequently reported as a late complication resulting in multiple syndromes, with no universal viewpoint for classification (Vinik, Mitchell, et al., 1995; Vinik et al., 2013). Generally, DPN is viewed in subdivisions, such as: focal/multifocal neuropathies, diabetic amyotrophy, symmetric polyneuropathies and sensorimotor polyneuropathy (DSPN), which is a common type of neuropathy

experienced by diabetes patients (Sadosky, 2008; Vinik et al., 2006; Vinik et al., 2013).

E/I Ratio: Represents a ratio of expiration to inspiration; standardized cardiac reflex test based on deep breathing recommended by American Diabetes Association for the evaluation of CAN; primarily tests cardiac parasympathetic functions (Gulichsen, Fleischer, Ejskjaer, Eldrup, & Tarnow, 2012; Vinik et al., 2013).

Fasting plasma glucose: "a check of a person's blood glucose level after the person has not eaten for 8 to 12 hours (usually overnight) (ADA, 2014, 2016). This test is used to diagnose prediabetes and diabetes. It is also used to monitor people with diabetes".

Glycosylated hemoglobin testing: (HbA1C testing) is a test measuring an individual's average blood glucose levels for the past 2 to 3 months (ADA, 2014, 2016). The hemoglobin (HEE-mo-glo-bin) is the part of a red blood cell that carries oxygen to the cells and sometimes joins with the glucose in the bloodstream. This test is called hemoglobin A1C or glycosylated (gly-KOH-sih-lay-ted) hemoglobin, and represents the percentage of red blood cells with glucose attached to the A1c component, which is proportional to the amount of glucose in the blood.

Heart rate variability: Heart rate variability (HRV) is a measureable, physiological phenomenon involving the variation of intervals of time between successive heart beats and is measured in beat to beat intervals.

High frequency (HF) component: Refers to a primarily parasympathetic dominant pathway in the autonomic nervous system (Heathers, 2014; Lieb, Parson, Mamikunian, & Vinik, 2012).

Low frequency (LF) component: Refers to low frequency component of HRV; often reflected in literature as a sympathetically dominant component; however, debate exists on the interpretation of this measure (Heathers, 2014; Lieb et al., 2012).

Oral glucose tolerance testing: This is a test used to diagnose prediabetes and diabetes. The oral glucose tolerance test is given by a health care professional after an overnight fast. A blood sample is taken, then the patient drinks a high-glucose beverage provided by the health care professional. Blood samples are taken at intervals for 2 to 3 hours and the results are compared with a standard and show how the body uses glucose over time (ADA, 2014, 2016).

Prediabetes: Prediabetes is considered to be a condition of elevated but not yet clinically diagnosable diabetes blood glucose or HbA1C levels (ADA, 2014, 2016).

Prediabetes Identification Criteria: Prediabetes may be defined with an HbA1C value of 5.7–6.4% or a fasting glucose of > 100–125 mg/dL (7.0 mmol/L) (ADA, 2014, 2016; Zhou & Zhou, 2014). These individuals should be considered to be in a place of increased risk for both CVD and diabetes development. HbA1C values > 6.0 should be considered very high risk for the development of diabetes and aggressive interventions are advised.

RMSSD: The square root of the mean of the sum of the squares of differences between successive NN intervals; a measure primarily of parasympathetic activity (American Heart Association Inc.; European Society of Cardiology, 1996; Vinik & Ziegler, 2007).

SDNN: Refers to the standard deviation of NN intervals (normal R to R intervals); a measure that reflects both sympathetic and parasympathetic pathways within the autonomic nervous system; is a reflection of cyclic components that are responsible variability during the period of recording (American Heart Association Inc.; European Society of Cardiology, 1996; Lieb et al., 2012).

Subclinical: Subclinical refers to a condition in terms of being elevated but not yet clinically diagnostic, symptomology; may apply to blood glucose, HbA1C levels, DPN, or other

diabetes symptomology (Mustafa et al., 2012).

Valsalva Ratio: Evaluates sympathetic adrenergic pathways by utilizing HR and BP responses; evaluates parasympathetic pathways in the form of HR responses; involves timed, dynamic breathing patterns which create a dynamic, active exhalation against pressure (Gulichsen et al., 2012).

30:15 Ratio: A ratio derived from the duration of inspiration to the duration of exhalation; taken from the lowest heart rate after position change (standing) in relationship to the fastest heart rate. Typically, the ratio is derived from the 30^{th} and 15^{th} heart beats.

Expected Outcomes

We expect that melatonin will have a positive effect on the ANS, attenuating some of the effects of T2D pathophysiology demonstrated in altered baseline HRV measures and disrupted sleep patterns. We expect that individuals will likely experience better sleep during the melatonin part of the trial and that sleep will likely remain the same during the four-week period placebo part of the study.

CHAPTER II

REVIEW OF THE LITERATURE

This purpose of this chapter is to review the pertinent literature relating to neuropathy detection, screening, and treatment. Chapter II Part A, Neuropathy Screening Tools, discusses diabetic peripheral neuropathy (DPN), its pathophysiological basis, and examines the diagnostic accuracy of specific tools for the assessment of DPN, including the 128-Hz tuning fork, 1-g and 10-g monofilaments, the QOL-DN, the NC-Stat DPN Check and HbA1C testing. Chapter II Part B, Neuropathy Quality of Life Tools, discusses health-related quality of life (HQOL) within the context of T2D and DPN, and examines neuropathy specific QOL instruments of interest, including the QOL-DN, the PN-QOL-97, and the NeuroQOL-28 in conjunction with the NC-Stat DPN Check and HbA1C testing. Each of these modules of the study (Part A and B) will present research findings and synthesize the current literature relating to reliability and validity for the aforementioned diagnostic tools, questionnaires, and testing methods within the context of screening for and identifying neuropathy in healthy, PD and T2D populations.

Chapter II Part C, Melatonin and the Autonomic Nervous System, discusses melatonin and its effect on autonomic nervous system function in T2D, symptoms of cardiovascular autonomic neuropathy (CAN) in diabetes, symptoms and measurement of cardiac dysfunction. This section presents research findings and synthesizes the current literature relating to these concepts and instruments, including their reliability and validity. The focus of the literature reviewed is on T2D populations.

CHAPTER II

PART A: NEUROPATHY SCREENING TOOLS

Diabetic Peripheral Neuropathy

The International Diabetes Federation (IDF) estimates the global problem of diabetes to have reached 387 million people as of 2014, and estimate the reach of diabetes to increase to 592 million by the year 2035 (International Diabetes Federation, 2014). With such significant impact, it is easy to understand why the complications of T2D draws significant attention. DPN is one of the most common and troublesome complications that T2D patients may encounter as it is both a silent and damaging opponent, even in early stages (Divisova, 2012; Mustafa et al., 2012; Papanas & Ziegler, 2012). Chronic or acute hyperglycemia, microvascular insufficiency, oxidative and nitrosative stress, defective neurotropism or autoimmune-related nerve destruction all may contribute to the destruction of nerve cells or structures, and produce damage to key organs and systems, such as the kidneys, retina or neurons (Marcovecchio, Lucantoni, & Chiarelli, 2011; Vinik et al., 2013). Such damage may lead to diabetic cardiac autonomic neuropathy (CAN), retinopathy, diabetic nephropathy, damage to various organs, or our focus, DPN.

Evaluating DPN is not a simple task, and physicians generally rule out other possible causes for any presenting symptoms before assigning a DPN diagnosis (Tesfaye, 2010; Tesfaye, 2015; Vinik et al., 2013). Diagnosis is difficult, and misdiagnosis is common as many specialists are not trained to recognize the signs and symptoms of the disease, and this ultimately aids in the progression of DPN without treatment (Herman & Kennedy, 2005). DPN is also viewed in subdivisions, or branches, including focal/multi focal neuropathies, diabetic amyotrophy,

symmetric polyneuropathies and sensorimotor polyneuropathy (DSPN), further elaborating the scenario for proper diagnosis. DSPN presents as the most commonly occurring neuropathy to date, manifesting with length-dependent, symmetrical sensorimotor polyneuropathy, often developing in patients with a history of extended hyperglycemia (Marcovecchio et al., 2011; Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, & Vinik, 2010). Glycemic control, therefore, is an important prevention implementation or risk covariate, and is dependent on the history of the patient and whether stabilization is achievable. The damage caused is microvascular by nature, linking this manifestation to similar diabetes complications such as retinopathy and nephropathy (Tesfaye, 2010).

Literature is divided relating to the true prevalence of DPN, with variability in reporting ranging from 30–90% (Dixit & Maiya, 2014; Duby, Campbell, Setter, & Rasmussen, 2004; Vinik, 1999; Vinik et al., 2013). Reporting rates vary based on the testing methods used, with the highest rates of reporting associated with sophisticated testing equipment such as nerve conduction studies (NCS) or in research reporting, neuropathic pain syndromes (Rota, 2005, 2007). Both type 1 diabetes (T1D) and T2D patients are affected by the disease, and may or may not present with neurologic signs or symptoms (Boulton, 2015; Singh, Armstrong, & Lipsky, 2005; Vinik et al., 2013). Strong community reporting indicates rates of DPN prevalence at 25%, with DPN contributing to 30% of diabetes related hospitalizations, and some research reports that as many as one out of four diabetes patients will develop foot ulcers within the course of their lifetime (Lipsky et al., 2006; Vinik et al., 2013; Zgonis, Stapleton, Girard-Powell, & Hagino, 2008).

DPN has been commonly reported as a late complication of diabetes, and described as a combination or variety of syndromes, rather than a particular clinical presentation (Vinik et al.,

2013). A lack of universal classification makes DPN especially difficult to diagnose, even for seasoned professionals (McKinlay, Piccolo, & Marceau, 2013). This is even more true for the untrained eye, as physicians will offer competing diagnoses with great levels of certainty (McKinlay et al., 2013). DN affects distinct areas of the nervous system in a singular or combined fashion, and may do so silently for quite some time (Vinik et al., 2013). Conversely, DPN may present with very distinct symptoms that are diagnosable or, nonspecific, difficult to diagnose symptoms and, therefore, these symptoms may be confused with other illnesses. While symptoms vary, common complaints may include burning, tingling, or numbness in the lower extremities (Dixit & Maiya, 2014), see Table IIA.1.

When classifying sensation loss, there are several fiber types, including small, large, and sensory fiber involvement. Small fiber sensation loss generally affects thermal and pain perception while large fiber dysfunction will result in dysfunction related to touch and vibration sensations (Vinik et al., 2013). Sensory fiber involvement causes variations of pain or abnormal sensations such as tingling, pins and needles or prickly sensations.

Neuropathy Screening & Assessment

The clinical assessment of DPN is recommended when receiving a T2D diagnosis or within five years of receiving a T1D diagnosis, and a detailed examination is key in order to reveal the presence of DPN (ADA, 2016; Baraz, Zarea, Shahbazian, & Latifi, 2014; Dixit & Maiya, 2014; Katon, Reiber, & Nelson, 2013). Screening should include an examination of ankle reflexes and sensory tests associated with DPN, along with a full examination of feet. Sensory function may be evaluated in a number of ways, including the Wartenberg Pinwheel (pinprick sensation), temperature, 128-Hz tuning fork (vibration), or the 1-g or 10-g

monofilament applied at the distal halluces. It is recommended for examinations to utilize more than one test during screenings, as previous research indicates an 87% sensitivity for the detection of DPN when two tests are combined (Boulton et al., 2005; Vinik et al., 2013; Vinik, Suwanwalaikorn, et al., 1995).

Small fiber neuropathy may be examined with Neurotips, which evaluates nociceptors for pain and warmth, or a cold tuning fork, which evaluates cold thermoreceptors (see Table IIA.2; Vinik et al., 2013). Large fiber neuropathy may be evaluated via vibration distributed through a 128-Hz tuning fork, which tests the mechanoreceptors and Ruffini corpuscle, or alternatively one may choose to implement a wisp of cotton, which evaluates Meissner corpuscles through light touch (see Table IIA.2, Vinik at al., 2013). Pacinian corpuscle may be tested via 1-g or 10-g monofilament, which ultimately tests pressure and large fiber sensitivity.

The staff of the Strelitz Diabetes Center, which is part of the Eastern Virginia Medical School in Norfolk, VA has compiled the following definitions for the diagnostic assessment of DPN:

1. "Possible DSPN. The presence of *symptoms or signs* of DSPN and may include the following: *symptoms*—decreased sensation, positive neuropathic sensory symptoms (e.g., "asleep numbness", prickling or stabbing, burning or aching pain) predominantly in the toes, feet, or legs; *or signs*—symmetric decrease of distal sensation or unequivocally decreased or absent ankle reflexes.

2. Probable DSPN. The presence of a combination of symptoms and signs of neuropathy including any two or more of the following: neuropathic symptoms, decreased distal sensation, or unequivocally decreased or absent ankle reflexes.

3. Confirmed DSPN. The presence of an abnormality or nerve conduction and a symptom

or symptoms, or a sign or signs, of neuropathy confirm DSPN. If nerve conduction is normal, a validated measure of small fiber neuropathy (SFN) (with class one evidence) may be used. To assess for the severity of DSPN, several approaches can be recommended: for example, the graded approach outlined (see Table IIA.3); various continuous measures of sum scores of neurologic signs, symptoms, or nerve test scores; scores of function of activities of daily living; or scores of predetermined tasks of disability.

4. Subclinical DSPN. The presence of no signs or symptoms of neuropathy are confirmed with abnormal nerve conduction or a validated measure of DSPN (with class 1 evidence). Definitions 1, 2, or 3 can be used for clinical practice, and definitions 3 or 4 can be used for research studies.

5. Small fiber neuropathy (SFN). SFN should be graded as follows: (1) possible: the presence of length-dependent symptoms and/or clinical signs of small-fiber damage; (2) probable: the presence of length-dependent symptoms, clinical signs of small-fiber damage, and normal sural nerve conduction; and (3) definite: the presence of length-dependent symptoms, clinical signs of small-fiber damage, normal sural nerve conduction, and altered intra-epidermal nerve fiber (IENF) density at the ankle and/or abnormal thermal thresholds at the foot" (Vinik at al., 2013, p.755–756).

As previously mentioned, presentation of neuropathy differs based on the type of neuropathy present. Vinik & Mehrabyan (2004) prepared a simple, clear figure to represent this (see Figure IIA.1). Neuropathy is a worldwide concern, with many organizations making great efforts to not only advance research, but also classify the disease. Accepted testing parameters for assessment are further elaborated in Table IIA.3. Mayo Clinic has efficiently presented

options for staging neuropathy as presented in Table IIA.4.

The Tuning Fork. The 128-Hz tuning fork has been effectively used in research to test for vibration sensation loss (Divisova et al., 2012; Perkins, Olaleye, Zinman, & Bril, 2001; Robinson, Balbinot, Silva, Achaval, & Zaro, 2013) by detecting the loss of sensation in the associated Ruffini mechanoreceptors of the large nerve fibers (Vinik et al., 2013). It is a low cost means of assessing vibration thresholds (VT), easily accessible and has been widely used in the assessment of the diabetic foot for diagnosing polyneuropathy (Meijer et al., 2005).

The Canadian Diabetes Association clearly outlines practices for the rapid screening of diabetic neuropathy and includes exact methodology for the tuning fork on/off test, including test scoring, site application and reproducible familiarization details ("Rapid Screening for Diabetic Neuropathy," 2013). This method, within research, has presented as one of the most clearly defined methodologies and has been recommended by clinical practitioners.

Jayaprakash et al. (2011) investigated the validation of bedside testing methods specifically for the evaluation of DPN (Jayaprakash et al., 2011). Vibration perception thresholds (VPT) were measured via a biothesiometer probe and compared to the 10–g monofilament, and the 128-Hz tuning fork. Detailed histories were taken from 1044 patients with DM, questionnaires were filled out relating to Diabetic Neuropathy Symptom scoring, with a score greater than 1 being considered as significant. Results indicated that tuning fork testing and 10-g monofilament testing resulted in lower sensitivity (62.5 and 62.8%); however, more desirable specificity (95.3 and 92.8%). Accuracy was relatively high for both instruments (78.9 and 77.9%) for tuning fork testing and monofilament screening, respectively. The final interpretations of the study indicate these tools as useful in the assessment of DPN (Jayaprakash et al., 2011).

Pourhamidi evaluated clinical tools and their diagnostic usefulness in detecting distal symmetric polyneuropathy and found sensitivity to be relatively low in the tuning fork compared to electronic measures such as the biothesiometer (Pourhamidi, Dahlin, Englund, & Rolandsson, 2014). In contrast to the study, other research has found the tuning fork to be quite useful for detecting large fiber related sensation loss, even when compared to a neurothesiometer (Kastenbauer, Sauseng, Brath, Abrahamian, & Irsigler, 2004).

The 1-g and 10-g Monofilaments. While monofilaments have been widely used for PN detection (Gregg et al., 2004; Katon et al., 2013), literature indicates a lack of continuity in testing across studies and debate regarding which monofilament methods (sites, grams, number of trials) are the most effective and reliable for detecting early sensation loss (Dros, Wewerinke, Bindels, & van Weert, 2009). Studies suggest numerous site testing possibilities ranging from one to ten, including four-site SWM testing (Dixit & Maiya, 2014), three-site (Katon et al., 2013), two-site (Lee et al., 2003), or one-site monofilament testing (Bourcier et al., 2006). Baraz et al. chose multiple tests to evaluate with their research, testing at three, four, eight and ten sites, and found that three and four sites were significantly accurate and that adding additional points did not increase accuracy (Baraz et al., 2014). Varying approaches have been established, with some studies utilizing full kits and multiple sites for testing, and others relying solely on one site, while utilizing the 1-g and 10-g for testing.

The 10-g monofilament has been widely used in neuropathy screening both as a simple bedside screening tool and during general diabetes exams, and in the United States is considered a target tool for the evaluation of “loss of protective sensation” (Lavery & Gazewood, 2000; "Rapid Screening for Diabetic Neuropathy," 2013). The Canadian Diabetes Association supports the “Rapid Screening for Diabetic Neuropathy”, and provides simple, easy to use

screening methods that physicians have utilized and approved ("Rapid Screening for Diabetic Neuropathy," 2013). This standardized screening method highlights protocols for the 10-g monofilament and the 128-Hz tuning fork on/off method.

As research literature points out, monofilaments are highly desirable for their portability, ease of use, and non-invasiveness in detecting developing problems within the insensate foot, and more recently have been used in subclinical neuropathy screening efforts (Divisova, 2012; Lavery & Gazewood, 2000). Monofilaments have been used to help define DPN previously in literature: for example, Katon, Reiber, and Nelson (2013) utilized the 10-g monofilament data from the NHANES study (1999–2004), which used this screening tool to detect insensate sites on the foot in a massive study that examined 7818 individuals (Gregg et al., 2004; Katon et al., 2013). DPN was defined as one or more insensate sites on the foot. Relative risks were calculated relating to diabetes and DPN, and results indicated modest increases in risk and DPN for those with PD and undiagnosed diabetes and a 74% higher risk of DPN for those with diagnosed diabetes. Such ventures in literature demonstrate the usefulness of the 10-g for simple screening in large populations.

The precision and accuracy of the 10-g monofilament may come into question when used in a repeated loading fashion, without rest (Lavery et al., 2012). This factor should be taken into consideration in how testing is administered by limiting testing loads per day. If high volume testing is to take place, monofilament loading force should be regularly checked during research testing and clinical exams, as high repeated (200 cycles per day, 15 consecutive days) use will shorten the service life of this instrument. Lavery et al. (2012) endorses the use of this unique instrument, but recommends seeking a quality manufacturer and combining this tool with other neuropathy screening tools for the best identification of sensory dysfunction (Lavery et al.,

2012).

Other issues which must be addressed are age, temperature and humidity, as monofilaments are affected by all three (Haloua, Sierevelt, & Theuvenet, 2011). Age had a relatively minor effect within this research; however, temperature and humidity both significantly contributed to buckling force changes, as much as 39%. When unaccounted for, this has the potential to mislead the examiner regarding the levels of sensation detected. Haloua et al. (2011) recommends awareness regarding the environmental effects of temperature and humidity (Haloua et al., 2011). Controlling for environmental factors and screening with multiple bedside tests to confirm lack of sensation seems prudent.

The Norfolk Quality of Life Diabetic Neuropathy Tool. The Norfolk Quality of Life Diabetic Neuropathy Tool (QOL-DN) was developed as a neuropathy screening tool, aiming to differentiate between typical features that present in DPN (Vinik, Hayes, Oglesby, & Vinik, 2004; Vinik et al., 2005), particularly issues related to changes in sensation in small and large fibers, as well as typical alterations to autonomic nervous system functioning (see Appendix B) (Vinik et al., 2005). Twenty-eight questionnaire items were developed through 1000 structured patient interviews and combined with activities of daily living (ADLs), general health and status items of interest and tested on DPN patients. Initial testing revealed that the QOL-DN successfully identified domains of a fiber–specific nature relating to DPN with reliability, and discriminates between individuals who do and do not have DPN in an English-speaking population.

The QOL-DN instrument is comprised of a Total QOL score, and five subscale items (symptoms, ADLS, small fiber, large fiber, autonomic), each targeting to measure a specific area of interest. Furthering its impact and validation, it was translated into German and tested as a

fiber-specific QOL tool in this new population in 2014 (Vinik, Paulson, Ford-Molvik, & Vinik, 2008). This research demonstrated the tool's ability to cross language barriers, and confirmed prior findings relating to the same factors as previously identified in the English version. Important findings include the Norfolk QOL-DN's ability to detect levels of neuropathy within and across different populations (Vinik, Vinik, et al., 2014). This fiber specific, self-report questionnaire was translated into Romanian and effectively revealed a high prevalence of undisclosed neuropathy in 25,000 Romanian patients (Veresiu et al., 2015). The QOL–DN effectively and accurately determined the QOL of the participants, while also establishing its ability to operate within a new language and population to ferret out undisclosed neuropathy (Veresiu et al., 2015; Vinik et al., 2008).

The QOL-DN has also been used to assess baseline and improved QOL in randomized, double-masked, placebo-controlled, clinical studies and was sensitive enough to differentiate where improvement developed within fiber types (Boyd, Casselini, Vinik, & Vinik, 2011; Casellini, 2007). The questionnaire has been successfully used in research to detect both the severity and impact of neuropathy on the QOL experiences in 61 patients diagnosed with transthyretin familial amyloid polyneuropathy (TTR-FAP) (Coelho et al., 2012; Coelho et al., 2013; Vinik, Vinik, et al., 2014) and in patients with neuroendocrine tumors (Vinik, Silva, & Vinik, 2010). This questionnaire has been effectively utilized to perform cost analyses of the financial impact of DPN within research and used within postal surveys within research efforts (Currie et al., 2006; Happich, John, Stamenitis, Clouth, & Polnau, 2008).

Smith et al. analyzed the QOL-DN in relationship to other available measures in 2012 and recommended it for DN screening (Smith et al., 2012). Furthermore, this research points out the strengths of the QOL-DN as being able to serve effectively in multiple languages, being

fiber-specific, demonstrating test/re-test reliability and emphasizes the strong clinical background that serves as the foundation for the instrument. The time to complete the instrument is unknown and criterion validity had not yet been assessed when the article was written.

NC-Stat DPN Check. The NC-Stat DPN Check (Neurometrix Inc., Waltham, MA) is a point-of-care nerve conduction device that has been developed with the intent to serve as a substitute for more advanced nerve conduction study (NCS) devices (Lee et al., 2014). It is a simple, portable device that was made to be both user and patient friendly. This method of evaluation of the sural nerve allows for a quick assessment (less than 10 minutes), with results that are easily uploaded to a laptop computer for evaluation. The NC-Stat DPN Check has been proposed as being able to serve as a potential substitute for the more expensive and less accessible clinic driven NCS (Lee et al., 2014) and provides an opportunity for accessibility in community health care that has not been previously available. This point of care device (POCD) requires limited training and supplies to operate it and flexible options for testing, thus enabling a low-cost and short time investment to screen for potential nerve damage in the lower leg in order to determine if further evaluation is warranted at a more complex facility.

The NC-Stat DPN Check was tested as a POCD across multiple sites with 72 patients in order to determine its potential as an alternative to traditional NCS (Perkins et al., 2008). Patients underwent extensive testing that included neurological examination and NCS. Spearman correlation coefficients indicated a relationship between the POCD and other measures ranging from .76 to .91, confirming reasonable accuracy for the device to serve as an alternative. Lee et al. (2014) continued the validation of this device when his research team evaluated 44 T1D and T2D subjects with the NC-Stat DPN Check POCD and standardized NCS

(Lee et al., 2014). The sural nerve conduction velocity (SNCV) and sural nerve action potential (SNAP) were recorded. Reliability and validity were evaluated via intraclass correlation coefficients, Bland-Altman analysis, and receiver operating characteristic curves and results indicated. Two trained testers were utilized and interrater reproducibility ICC values were .97 for SNAP (interrater value, .83) and .94 for SNCV (interrater value, .79), with 88% sensitivity and 94% specificity for SNAP reference values and 94% sensitivity and 71% specificity. Excellent reliability and acceptable accuracy was demonstrated by the device, and POCD normative threshold values were validated.

This validated POCD has been shown to be a reliable and accurate alternative to traditional NCS through successful evaluation of the sensory nerve action potential (SNAP) and sensory nerve conduction velocity (SNCV) of the sural nerve in multiple research studies (Lee et al., 2014; Perkins, Grewal, Ng, Ngo, & Bril, 2006; Perkins et al., 2008). Multiple methodologies have been employed in its validation within these studies, including unilateral and bilateral applications, with one to two trials.

Glycohemoglobin Testing. Glycohemoglobin testing, or HbA1C, testing has been reliably used to categorize BG values within research and has been proven as a simple, portable method of screening for diabetes that is accurate, relatively quick to perform on-site (10 minutes), and results are easily relayed (Feng, Schlosser, & Sumpio, 2009; Sumpio et al., 2013). This method of screening also provides the unique caveat of accessible health care upon demand to individuals that might not otherwise engage in or be able to afford more expensive tests such as oral glucose tolerance testing (OGTT). When considering the validity of HbA1C screening for diabetes, it should be noted that the Canadian Task Force on Preventive Health Care, American Task Force and WHO recommendations all support HbA1C testing of $\geq$ 6.5% for the

screening and diagnosis of diabetes (American Diabetes Association, 2012; Canadian Task Force, 2012; World Health Organization, 2011). In a clinical setting, if positive results are obtained, results should be confirmed with repeated testing.

HbA1C testing has been shown in research to be strongly associated as an accurate predictor of glycemic control, especially when compared to fasting plasma glucose (FPG) (ADA, 2016; Bernal-Lopez et al., 2011; Mannarino, Tonelli, & Allan, 2013). While agreement is strong between HbA1C testing and FBG, issues arise with nonparallel findings between HbA1C and oral glucose tolerance testing (OGTT) (Farhan et al., 2012; Mannarino et al., 2013). Studies independently performed by Mannarino et al. and Farhan et al. point out that there is discordance between OGTT and HbA1C regarding T2D diagnosis outcomes, with Farhan's findings indicating discordance when cardiac autonomic neuropathy (CAN) is present. Such issues may lower the incidence of diagnosis when using HbA1C as a determining test (Farhan et al., 2012). Additionally, HbA1C testing is not appropriate in situations with individuals who have hemoglobinopathies, as it may not be reliable (Hare, Shaw, & Zimmet, 2012). Current recommendations by the American Diabetes Association, however, align HbA1C as a valid test in equal measure to other diagnostic tests that may be used and that one test is not preferred over the other (ADA, 2016).

The DCA Vantage from Siemens Corporation has been evaluated within research as a POCD for HbA1C testing and found to have good correlation with laboratory methods and acceptable precision (Lenters-Westra & Slingerland, 2010; Sanchez-Mora et al., 2011). Sanchez-Mora et al. examined 53 blood samples from diabetic patients over a wide range of HbA1C values (4–14%), with results examined by both a DCA Vantage Analyzer and a POCT Analyzer. These results were compared to on-site lab testing, and both were found to be highly

correlated (r = 0.973) with clinical lab testing results.

HbA1C testing remains as a recommended test for the screening and diagnosis of diabetes. Increased accuracy over FPG, high portability, and financial accessibility in comparison to OGTT make it an excellent screening test, despite limitations with certain diabetic populations. The DCA Vantage Analyzer provides quality, on-site testing for HbA1C values, with a proven wide range of testing capabilities that are clinically acceptable.

Summary

In summary, symptoms and testing results are reviewed in combination to present a background for which a determination may be made regarding the presence of neuropathy. Defining what types of sensory receptor deficiency is present aids in the determination of which fibers may be affected and ultimately, contributes to the determination of the type of neuropathy that the individual is experiencing. The usefulness of these three tests have been well established in addition to their clinical diagnostic assessment applications in the literature. Both large and small fiber deficiencies may be evaluated with simple bedside tests that have been repeatedly validated within research literature. It would be beneficial to evaluate the effectiveness of three neuropathy screening tools with specific aims to determine early DPN detection. Therefore, the purpose of the study was to evaluate the 128-Hz tuning fork, 1-g and 10-g monofilaments, and the QOL-DN for the purposes of DPN screening to determine which of the tools or combinations thereof would be the most effective for early DPN detection.

The potential for the study lies in investigating to what extent will the 128-Hz tuning fork, 1-g and 10-g monofilaments, and the QOL–DN detect DPN in an OOI, PD, and T2D population. Ascertaining which screening tools are the most effective for early or subclinical

DPN detection, specifically by fiber type, is a lofty, but worthy goal and an achievable aim for these tools. Establishing which screening tools provide the greatest reliability and accuracy for detecting early or subclinical DPN is a promising hope for the study. We hypothesized that the tuning fork will detect early sensation loss, indicating large fiber neuropathy, in OOI and PD. Furthermore, we hypothesized that the QOL–DN and the 128-Hz tuning fork would both provide excellent mechanisms for detecting early or subclinical DPN in HN and PD populations. This being said, we believed the QOL–DN has the potential to detect DPN in a HN and PD populations with the greatest reliability and accuracy. It is with these things in mind that we moved forward in discussion of our methods and how we executed the study (Alam, Ezhova, Kotovskaya, Dogotar, & Kobalava, 2015).

TABLES

Table IIA.1
Descriptors of different kinds of neuropathic pain

Dysesthesia	Paresthesia	Muscular Pain
Burning sensation	Pins and needles	Dull ache
Skin tingles	Electric like	Night cramps
	Numb but achy	Band like sensation
Painful sensation when bed sheet and stockings touch me	Knife like shooting	Deep aches, spasms
	Pain, lancinating pain	

(Dixit & Maiya, 2014; Journal of Postgraduate Medicine)

Table IIA.2

Diagnostic Assessment of DPN Using Bedside Tests

Sensory Modality	Nerve Fiber	Instrument	Associated Sensory Receptors
Vibration	Aβ(large)	128-Hz tuning fork	Ruffini corpuscle mechanoreceptors
Pain (pinprick)	C (small)	Neurotips	Nociceptors for pain and warmth
Pressure	Aβ, Aα (large)	1-g and 10-g monofilaments	Pacinian corpuscle
Light touch	Aβ, Aα (large)	Wisp of cotton	Meissner corpuscle
Cold	Aδ(small)	Cold tuning fork	Cold thermoreceptors

(Vinik et al., 2013, p.755)

Table IIA.3

Advanced objective testing for diabetic neuropathy

Neurologic Test	Type of Neuropathy	Measurement	Advantages
Quantitative sensory testing	Small and large fiber neuropathies	Assessment of sensory deficits	Uses controlled quantifiable stimuli with standard procedures
Skin biopsy and intro epidural nerve fiber (IENF) density	Small fiber neuropathy	Small-caliber sensory nerves including somatic unmyelinated IENFS, dermal myelinated nerve fibers, and autonomic nerve fibers	Quantitates small epidermal nerve fibers through various antibody staining
Corneal confocal microscopy	Small fiber neuropathy	Detects small nerve fiber loss in the cornea	Noninvasive technique that correlates with neuropathy severity
Contact heat evoked potentials	Small fiber neuropathy	Uses nociceptive heat as a stimulus that is recorded through electroencephalographic readings	Detects small fiber neuropathy in the absence of other indices
Sudomotor function	Distal small fiber neuropathy	Assesses the sweat response by analyzing sweat production or sweat chloride concentrations	Detects early neurophysiological abnormalities in peripheral autonomic function
Nerve conduction studies	Small and large fiber neuropathy	Measure the ability of nerves to conduct an electrical stimulus	Standardized universal technique that is well documented and recommended

(Vinik et al., 2013, p. 756)

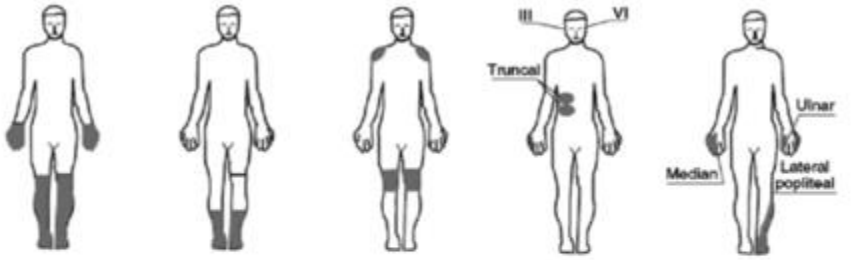
Table IIA.4
Stages in Diabetic Neuropathy

Stage	Description	Signs or Symptoms	Abnormal Quantitative Sensory Tests
0	No neuropathy	No	No
1	Subclinical neuropathy	No	Yes
2	Clinically evident neuropathy	Yes	Yes
3	Debilitating neuropathy	Yes	Yes

(Dixit & Maiya, 2014; Mayo Clinic)

FIGURES

Figure IIA.1 Clinical Manifestations of Neuropathy



Large fibre neuropathy	Small fibre neuropathy	Proximal motor neuropathy	Acute mono neuropthies	Pressure palsies
Sensory loss: 0 → +++ (touch, vibration) Pain: + → +++ Tendon reflex: N → ↓↓↓ Motor deficit 0 → +++	Sensory loss: 0 → + (thermal, allodynia) Pain: + → +++ Tendon reflex: N → ↓ Motor deficit: 0	Sensory loss: 0 → + Pain: + → +++ Tendon reflex: ↓↓ Proximal motor deficit: + → +++	Sensory loss: 0 → + Pain: + → +++ Tendon reflex: N Motor deficit + → +++	Sensory loss in nerve distribution: + → +++ Pain: + → ++ Tendon reflex: N Motor deficit + → +++

CHAPTER II

PART B: NEUROPATHY QUALITY OF LIFE TOOLS

Health Related Quality of Life (HRQOL)

HRQOL may be defined as subjective perceptions of how an illness and treatment for the illness is experienced, particularly in how it is perceived to affect physical, mental and social aspects, thereby providing an indication of the individuals perception of their overall well-being (Rajabally & Cavanna, 2015). T2D, as an illness, represents a collection of challenges that may affect several different facts of an individual's functioning, including physical, emotional, social, sexual, cognitive and self-perceptions surrounding health changes. The health issues surrounding T2D are formidable, and a variety of HRQOL instruments have been used throughout the last two decades to gain insight relating to the health perceptions experienced by T2D patients (Luscombe, 2000). Research conducted to determine associations between particular complications such as depression, HRQOL measures and ABC (HbA1C, BP, cholesterol) goal attainment in 808 adult T2D patients using the National Health and Nutrition Examination Survey (NHANES) (Shah, Mezzio, Ho, & Ip, 2015) found that longer disease duration and severe depression highly impacted individuals abilities to achieve health related goals. Likewise, research by Ji et al. relates findings regarding the effect of elevated BMI in an examination of 2052 Chinese T2D patients, who were examined for the HRQOL relationships between BMI, complications, glycemic control and comorbidities (Ji et al., 2015). Increased complications such as hypertension, dyslipidemia, and poorer HRQOL were associated with increasing BMI status, despite no significant differences between the groups for HbA1C. Such

examples highlight the gravity and impact of disease duration, underlying psychological challenges and BMI on the exacerbation of T2D.

HRQOL instruments have evolved over the last two decades, encompassing more domains, crossing language barriers and aiming to specialize in particular disease facets, such as in DPN (Hogg, Peach, Price, Thompson, & Hinchliffe, 2012; Vickrey, Hays, & Beckstrand, 2000; Vinik et al., 2004). Hogg et al. reviewed HRQOL diabetes-related foot disease measures through a meta-analysis of scientific literature available from 1996 to 2011, ultimately examining 53 studies that used a structured manner to directly assess HRQOL relating to foot problems (Hogg et al., 2012). Measures utilizing patient reported outcome measures (PROMs) to self-evaluate general diabetes topics were excluded. The NeuroQOL-28 was assessed as a disease specific instrument, providing proficiency in the assessment of advancing neuropathy and impact on HRQOL, but poor sensitivity to diabetic foot ulcers (DFU). The QOL-DN was discussed as an assessment of diabetes oriented neuropathy, with intentions for aiding in diagnosis and monitoring; however, Hogg et al. reports a lack of specificity for PN, thereby limiting the instrument to health impacts of a diabetes foot disease related nature (Hogg et al., 2012). This research further advises that the utilization of two tools would be more promising in detailing outcomes, but impractical in a clinical setting (Jeffcoate et al., 2009). After careful evaluation, Hogg et al. advises that each diabetes foot-specific PROM has some validity for measuring HRQOL, but also has limitations that are unique and specific to each tool and that these limitations should be considered when choosing an instrument. According to this research, no one specific PROM could be viewed as a gold standard measure.

Smith, Lamping and MacLaine reviewed HRQOL related to DPN in a systematic review in 2012 and ultimately compared the PN-QOL-97, NeuroQOL-28 and QOL-DN (Smith et al.,

2012). After a review of reliability, validity, content, language, development and prior use in research, this team concluded that all three instruments warranted support for their use in a DPN-specific manner based on evidence, to some degree; however, limitations exist. The NeuroQOL-28 lacks test-retest reliability, while the QOL-DN possesses this component as a strength. This being said the QOL-DN lacks content to assess the emotional and psychological impact of DPN, such as anxiety or depression components (Smith et al., 2012). Language variability is available with the QOL-DN, but the PN-QOL-97 is a valid option for assessing PN if language diversity is not a necessity. The authors suggest future research to compare the psychometric properties of all three instruments.

What once was only thought to be able to be accomplished by a specialist during an exam has now become a target for specific screening development in the form of self-reported QOL measures (Vinik et al., 2005). Efforts have been rewarded, as QOL measures relating to DN have successfully targeted and differentiated between small and large fiber deficits and levels of neuropathy severity (Vinik et al., 2004; Vinik et al., 2008). Such efforts allow more individuals to be screened and in a variety of locations, with or without clinical personnel, providing the ability to detect advancing disease at earlier stages with the hopes of earlier disease assessment and intervention. Earlier assessment of the debilitating effects of DN allows for the potential to grasp the impact and scope of accumulating pathophysiological processes in an individual, the formulation of targeted medical support plans and pre and post evaluations for T2D interventions (Wong et al., 2015). Such evaluations of physical, mental, emotional, social, and sexual functioning in additional to health perceptions, helps care providers in their efforts to effectively treat individuals experiencing DN.

Research indicates that obese individuals experience reduced HRQOL, with findings indicating a more significant impact on physical functions relating to health rather than mental capacity (Kolotkin, Crosby, & Williams, 2002). Kolotkin et al. examined HRQOL in 3353 patients of varying ethnicities in a geographically diverse study over a wide age range, 18 to 90 years of age, and found that higher BMIs, Caucasian status and women experienced obesity related reductions in HRQOL. Such results are not uncommon, as a meta-analysis of research examining 43,086 study participants indicated that increased BMI status relates to significant reductions in physical QOL, with the highest impact relating to individuals who were class III obese (Ul-Haq, Mackay, Fenwick, & Pell, 2013). At-risk individuals for T2D include those who are inactive. Hakkinen et al. (2009) evaluated 132 individuals for physical activity/inactivity patterns in relationship to HRQOL using the SF-36, and found that inactive individuals had a lower HRQOL in the pain and general health domains (Hakkinen et al., 2009). HRQOL decreased in a linear fashion in relationship to physical inactivity and increased in individuals who had higher physical activity levels. Individuals who were more active reported better subjective health and weight control, while researchers reported that high activity levels reduced the risk of T2D onset and associated complications.

Norfolk Quality of Life Diabetic Neuropathy Instrument (QOL–DN). The Norfolk Quality of Life Diabetic Neuropathy Tool (QOL-DN) was developed as a neuropathy-screening tool, aiming to differentiate between typical features that present in DN (see Appendix B) (Vinik et al., 2004; Vinik et al., 2005). Twenty-eight questionnaire items were developed through 1000 structured patient interviews, combined with ADLs, general health and status items of interest and tested on DN patients. Initial testing revealed that the QOL-DN successfully identified domains of a fiber-specific nature relating to DPN with reliability, and discriminates between

individuals who do and do not have DPN in an English-speaking population. The QOL-DN is unique due to its ability to detect issues that arise related to changes in sensation in small and large fibers, typical alterations to autonomic nervous system functioning and neuropathy severity (Vinik et al., 2008; Vinik, Stansberry, Ruck, & Vinik, 2003).

The QOL-DN instrument is comprised of a Total QOL score, and five subscale items (symptoms, ADLS, small fiber, large fiber, autonomic), each measuring a specific area of interest. Furthering its impact and validation, it was translated into German and was successfully tested as a fiber-specific QOL tool in this new population (Vinik et al., 2008). This research demonstrated the QOL-DN's ability to cross language barriers, and confirmed prior findings of the same five factors previously identified in the English version. Important findings include the Norfolk QOL-N's ability to detect levels of neuropathy within and across different populations (Vinik, Vinik, et al., 2014). This fiber specific, self-report questionnaire was utilized in a 25,000 person Romanian population, effectively revealing a high prevalence of undisclosed neuropathy in 25,000 Romanian patients (Veresiu et al., 2015). The QOL–DN effectively and accurately determined the QOL of the participants, while also establishing its ability to operate within a new language and population to ferret out undisclosed neuropathy (Veresiu et al., 2015; Vinik et al., 2008).

The QOL-DN has also been used to assess baseline and improved QOL in randomized, double-masked, placebo-controlled, clinical studies and was sensitive enough to differentiate where improvement developed within fiber types (Boyd et al., 2011; Casellini, 2007). The questionnaire has been successfully used in research to detect both the severity and impact of neuropathy on the QOL experiences in 61 patients diagnosed with transthyretin familial amyloid polyneuropathy (TTR-FAP) (Coelho et al., 2012; Coelho et al., 2013; Vinik, Vinik, et al., 2014)

and in patients with neuroendocrine tumors (Vinik et al., 2010). This questionnaire has been effectively utilized to perform cost analyses of the financial impact of DPN within research and used within postal surveys within research efforts (Currie et al., 2006; Happich et al., 2008).

Smith et al. (2012) analyzed the QOL-DN in relationship to other available measures in 2012 and recommended it for DPN screening (Smith et al., 2012). Furthermore, this research points out the strengths of the QOL-DN as being able to serve effectively in multiple languages as a fiber-specific tool, demonstrating test/re-test reliability and emphasizing the strong clinical background that serves as the foundation for the instrument. The time to complete the instrument is unknown at the time of the writing of the article.

The Peripheral Neuropathy Quality of Life Instrument (PN-QOL-97). Vickrey, Hays and Beckstrand (2000) also developed a questionnaire to evaluate peripheral neuropathy (see Appendix C) (Vickrey et al., 2000). The instrument was formed from items from the Rand-36, a widely used HRQOL PROM measure (Hays & Morales, 2001), and responses from focus group material, and evaluated in 80 patients at 3 and 6 month follow up evaluations in a clinical setting. The instrument was re-evaluated, and in the process, pared down from 162 items to 97 items during the study through examination of construct validity, reliability, and comparisons to HRQOL measures. Findings resulted in strong associations between the instrument's results and reported DN symptoms and support for reliability and validity for use in adults with DN. The revisions ultimately arrived at an instrument that is made of two base components, a physical component and a mental component, both of which are scored through a complex set of calculations provided by the author.

The PN-QOL-97 was utilized in a study aiming to determine the effectiveness of intraepidermal nerve fiber density (IENFD) in 72 sarcoidosis patients and 188 healthy

participants who had skin biopsies performed on the lower leg (Bakkers et al., 2009). The PN-QOL-97, along with a Symptoms Inventory Questionnaire (SIQ), were administered to participants and results were compared to IENFD values. The resulting comparisons of the PN-QOL-97 and SIQ helped to establish a validated realm of normative values for IENFD. A study examining the effect of social support on QOL was examined in 154 patients with polyneuropathy (Maxwell et al., 2013). The PN-QOL-97 and the Medical Outcome Study-Social Support Survey (MOSS-SSS) were utilized to determine QOL and social support. Results indicated that pain and autonomic symptoms strongly related to physical and mental components of QOL while social support only weakly correlated with the emotional/mental health components.

Research examining the characteristics of muscle cramps in individuals over the age of 18 with polyneuropathy utilized the PN-QOL-97 as a validated measure of QOL (Maxwell et al., 2014). Nerve conduction studies, the Toronto Clinical Neuropathy Score, the PN-QOL-97 and a demographic questionnaire assessing qualities of the symptoms and cramps were administered to 225 patients. Of the participating patients, 63% experienced muscle cramps, and nearly 44% described cramps of a disabling nature. This study confirmed that patients who experienced a disabling level of pain reported a lower QOL on the PN-QOL-97 than patients without disabling cramps and that muscle cramps were a common factor in individuals with polyneuropathy.
The PN-QOL-97 was reviewed by a research team in comparison to other QOL measures (Smith et al., 2012). Researchers pointed out that there are several strengths of the PN-QOL-97, such as expert review of focus group material, multi-dimensional design, psychological and emotional domains and comprehensiveness. Test-retest reliability had been established, yet it does take at

least 20 minutes to complete according to previously published materials and this QOL instrument represents one of the longest instruments available for use to assess DN today.

Neuropathy-and Foot Ulcer-Specific Quality of Life Instrument (NeuroQOL-28). The NeuroQOL-28 was developed to measure patient related QOL perceptions as they are impacted by DN and foot ulcers (see Appendix D) (Vileikyte et al., 2003). This research related to the development of an instrument designed to assess symptomology of DN, QOL and psychometric properties by working with 418 patients across U.K. and U.S. diabetes centers. Researchers found that the NeuroQOL-28 was able to reliably determine three measures relating to physical symptoms and two measures relating to psychosocial aspects of functioning (Vileikyte et al., 2003). When compared to the SF-12, results indicated stronger associations with neuropathic severity, DN's relationship to QOL, and was able to better explain variances in QOL when compared to the SF-12.

Vileikyte and fellow collaborators utilized the NeuroQOL-28 in a slightly differently research effort in 2005, as their team examined the associations between DN and symptoms of depression, in an attempt to develop associations that would lead to potential targets for interventions in future research (Vileikyte et al., 2005). The research successfully utilized the NeuroQOL-28 to evaluate this relationship while also using the Hospital Anxiety and Depression Scale (HADS) and demonstrated a link between neuropathy symptoms and depressive symptoms. Related research was performed in 2011, by Bergis, Hulman and Kulzer when they evaluated the effectiveness of the NeuroQOL-28 to detect psychological and neuropathic symptoms in 211 diabetic patients (Bergis, Hermanns, & Kulzer, 2011). Individuals were examined and tested by a physician and subsequently administered the NeuroQOL-28 instrument. Comparisons were made and the NeuroQOL-28 was found to be both valid and

reliable to assess neuropathy symptoms and to relay emotional problems arising from neuropathy-related symptoms.

With DPN rising as both a common and painful complication of diabetes, Davies et al. employed a study which examined the severity of symptoms in individuals with PN (Davies, Brophy, Williams, & Taylor, 2006). Surveys were sent out through the mail to known T2D patients in order to screen and determine the presence or absence of symptomology, followed by a more detailed neurological history collection through the Toronto Clinical Scoring System. PN or painful PN individuals were also administered a Neuropathic Pain Scale measure and the NeuroQOL-28. This research utilized the NeuroQOL-28 to establish that individuals with PN experience negative effects from the disease and are more likely to develop painful PN at a later point in time.

Further support for the validation of the NeuroQOL-28 developed with a study performed by Vileikyte and fellow researchers in 2007, when they examined NeuroQOL-28 scores in 295 DN patients in the U.S. and U.K. over a 9-month time period in comparison to the Neuropathy Disability Score (NDS) (Vileikyte et al., 2007). Results revealed continued validity for the NeuroQOL-28 through detecting changes in the severity of DN.

The use of the NeuroQOL-28 in DN related research is diverse, with efforts to measure improvements in sensation through at home light therapy among them (Lavery, Murdoch, Williams, & Lavery, 2008). Sixty-nine individuals participated in a study where they received sham or active treatments, with the active treatment consisting of 40 minutes of anodyne light therapy. The MNSI was utilized in conjunction with the NeuroQOL-28, SWM and nerve conduction studies in a repeated-measures evaluation and determined that there were no significant changes in the sham or treatment groups.

Finally, the translation of the NeuroQOL-28 into Brazilian Portuguese and evaluation of this translation in a 50 person Brazilian neuropathic population validated the NeuroQOL-28 as a reliable foot and ulcer specific instrument across different languages (Xavier et al., 2011). The NeuroQOL-28 was evaluated in conjunction with the SF-36, and found to be reliable and valid as a tool that could be utilized in DM populations by Brazilian medical staff.

A review of HRQOL instruments examined the NeuroQOL-28 in comparison to several other measures, detailing its strengths and weaknesses (Smith et al., 2012). This research determined that the NeuroQOL-28's target population was adults with DN through self-report measures and that at the time of the research, it was available in the US and UK, and in 10 different languages. The validation of the additional language versions was unclear despite personal communication from the author. Upon evaluation, Smith et al. determined that the DN version of the NeuroQOL-28 has 28 items that represent three physical domains, two psychosocial domains and one overall measure of QOL. Researchers commented that the time required to complete the NeuroQOL-28 was unknown. Test-retest reliability and criterion validity was not assessed in the main validation study.

NC-Stat DPN Check. The NC-Stat DPN Check (Neurometrix Inc., Waltham, MA) is a point-of-care nerve conduction device that has been developed with the intent to serve as a substitute for more advanced nerve conduction study (NCS) devices (Lee et al., 2014). It is a simple, portable device that was made to be both user- and patient-friendly. This method of evaluation of the sural nerve allows for a quick, easy assessment (less than 10 minutes), with results that are easily uploaded to a laptop computer for evaluation. The NC-Stat DPN Check has been proposed as being able to serve as a potential substitute for the more expensive and less accessible counterparts (Lee et al., 2014) and provides an opportunity for accessibility in

community health care that has not previously been available. This POCD requires limited training and supplies to operate it and flexible options for testing, thus enabling a low-cost and short time investment to screen for potential nerve damage in the lower leg in order to determine if further evaluation is warranted at a more complex facility.

The NC-Stat DPN Check was tested as a POCD across multiple sites with 72 patients in order to determine its potential as an alternative to traditional NCS (Perkins et al., 2008). Patients underwent extensive testing, which included neurological examination and NCS. Spearman correlation coefficients indicated a relationship between the POCD and other measures ranging from .76 to .91, confirming reasonable accuracy for the device to serve as an alternative.

Lee et al. (2014) evaluated 44 T1D and T2D subjects with the NC-Stat DPN Check POCD and standardized NCS (Lee et al., 2014). The sural nerve conduction velocity (SNCV) and action potential (SNAP) were recorded. Reliability and validity were evaluated via intraclass correlation coefficients, Bland-Altman analysis, and receiver operating characteristic curves and results indicated. Two trained testers were utilized and interrater reproducibility ICC values were .97 for SNAP (interrater value, .83) and .94 for SNCV (interrater value, .79), with 88% sensitivity and 94% specificity for SNAP reference values and 94% sensitivity and 71% specificity. Excellent reliability and acceptable accuracy was demonstrated by the device, and POCD normative threshold values were validated. This validated POCD has been shown to be a reliable and accurate alternative to traditional NCS through successful evaluation of the sensory nerve action potential (SNAP) and sensory nerve conduction velocity (SNCV) of the sural nerve in multiple research studies (Lee et al., 2014; Perkins et al., 2006; Perkins et al., 2008). Multiple

methodologies have been employed in its validation within these studies, including unilateral and bilateral applications, with one to two trials.

Glycohemoglobin Testing. Glycohemoglobin testing or HbA1C testing has been reliably used to categorize BG values within research and has been proven as a simple, portable method of screening for diabetes that is accurate, relatively quick to perform on-site (10 minutes), and results are easily relayed (Feng et al., 2009; Sumpio et al., 2013). This method of screening also provides the unique caveat of accessible health care upon demand to individuals that might not otherwise engage in or be able to afford more expensive tests such as oral glucose tolerance testing (OGTT). When considering the validity of HbA1C screening for diabetes, it should be noted that the Canadian Task Force on Preventive Health Care, American Task Force and WHO recommendations all support HbA1C testing of $\geq$ 6.5 for the screening and diagnosis of diabetes (ADA, 2016; Siu, 2015; World Health Organization, 2012). In a clinical setting, if positive results are obtained, the results should be confirmed with repeated testing.

HbA1C testing has been strongly associated as an accurate predictor of glycemic control, especially when compared to fasting plasma glucose (FPG) (ADA, 2016; Bernal-Lopez et al., 2011; Mannarino et al., 2013). While agreement is strong between HbA1C testing and FBG, issues arise with nonparallel findings between HbA1C and oral glucose tolerance testing (OGTT) (Farhan et al., 2012; Mannarino et al., 2013). Studies independently performed by Mannarino et al. and Farhan et al. point out that there is discordance between OGTT and HbA1C regarding T2D diagnosis outcomes, with Farhan's findings indicating particularly discordant results when cardiac autonomic neuropathy (CAN) is present. Such issues may lower the incidence of diagnosis when using HbA1C as a determining test (Farhan et al., 2012). Additionally, HbA1C testing is not appropriate in situations with individuals who have hemoglobinopathies, as it may

not be reliable (Hare et al., 2012). Current recommendations by the American Diabetes Association, however, align HbA1C as a valid test in equal measure to other diagnostic tests that may be used and that one test is not preferred over the other (ADA, 2016).

The DCA Vantage from Siemens Corporation has been evaluated within research as a point of care analyzer for HbA1C testing and found to have good correlation with laboratory methods and acceptable precision (Lenters-Westra & Slingerland, 2010; Sanchez-Mora et al., 2011). Within this research effort, 53 blood samples from diabetic patients over a wide range of HbA1C values (4–14%), were examined with both a DCA Vantage Analyzer and a POCT Analyzer and compared to on-site lab testing, and both were found to be clinically acceptable.

HbA1C testing remains as a recommended test for the screening and diagnosis of diabetes. Increased accuracy over FPG, high portability, and financial accessibility in comparison to OGTT make it an excellent screening test, despite limitations with certain diabetic populations. The DCA Vantage Analyzer provides quality, on-site testing for HbA1C values, with a proven wide range of testing capabilities that are clinically acceptable.

Summary

HRQOL relating to DPN is an important field of study, allowing for the assessment of disease impact for the potential early intervention and offers unique caveats to physicians to aid them in the diagnosis and treatment of DPN. HRQOL instruments vary greatly, ranging from general to disease specific, and researchers and clinicians both must be aware of the individual strengths and weaknesses of each instrument before utilizing them. Three HRQOL DN-specific instruments in particular stand out as points of interest: the QOL-DN, the PN-QOL-97 and the NeuroQOL-28. Each of these has been discussed within research literature and found to be

valuable in relationship to diabetes foot related disease or neuropathy. Researchers who have examined these instruments have recommended further evaluation, to compare certain aspects, such as the psychometric properties of these tools. Overweight and obese individuals experience reduced HRQOL, and are at risk for developing T2D, which makes them a unique population of interest. Activity status has been shown to be linked to risk for the development of T2D. Therefore, an overweight, obese, inactive population (OOI) is likely to be at increased risk for PD, T2D and potentially, early complications such as DPN. While limited studies have evaluated individuals with metabolic syndrome and obese populations, an OOI population has not been well investigated. The primary focus in literature has been on the utilization of HRQOL measures to assess individuals who have been diagnosed with disease, such as T2D.

We examined these three instruments to determine how to best detect early sensation loss and signs of neuropathy in an HbA1C categorized OOI, PD and T2D population, allowing for a beneficial appraisal of the instruments, an evaluation of potential early onset of disease and the timing of the instruments being employed. Furthermore, we compared the three instruments with the NC-Stat DPN Check POCD device, as a means of employing a criterion standard measure of determining the accuracy of the instruments themselves.

We hypothesized that the QOL–DN and PN-QOL-97 would more clearly identify signs of early or subclinical DPN when compared to the criterion standard of the NC-Stat DP Check, that all three instruments would correlate with our criterion standard at .60 or higher, with the QOL–DN yielding the strongest relationship and that the NeuroQOL-28 would be quickest to complete, followed by the QOL–DN and PN-QOL-97. These investigational concepts laid the framework for which we developed the methods for the execution of our research.

CHAPTER II

PART C: MELATONIN & AUTONOMIC NERVOUS SYSTEM FUNCTION

Melatonin

When attempting to develop treatments for the dysfunction that arises in T2D, the potential benefits of melatonin have just begun to be evaluated. Melatonin is a hormone that is made by the pineal gland in the human body (Claustrat, Brun, & Chazot, 2005). Produced in circadian patterns, this particular hormone has been found to regulate sleep and wake cycles and the circadian rhythms within healthy humans by means of hypothalamus receptors located in the suprachiasmatic nucleus (SCN) (Spadoni, Bedini, Rivara, & Mor, 2011). The hypothalamus acts as a dominant brain region, taking responsibility for sensing and responding to the levels of blood glucose within the body, and managing control of blood glucose during circadian rhythms (Cailotto et al., 2005; Page et al., 2009; Reiter et al., 2007; Vriend & Reiter, 2015).

Impairment of melatonin synthesis may have serious consequences related to hyperglycemia, as documented recently by Amaral et al., 2014. Rat studies involving sustained hyperglycemia-induced detrimental effects in melatonin synthesis, in vivo and in vitro, which suggest that given melatonin's antioxidant effects and roles in energy homeostasis, deficiencies in its release likely contribute toT2D progression (Amaral et al., 2014). Individuals with T2D often suffer the consequences of disrupted sleep processes, with reduced hypothalamic activity, and when tested, have been found to have decreased melatonin levels with phase delays (Kreier et al., 2007). Such process interruptions contribute to increased nocturnal liver glucose production (Radziuk & Pye, 2006), further indicating defects relating to glucose homeostasis.

The favorable effects of melatonin have been numerous, with positive outcomes in

metabolic research (Goyal et al., 2014) and improved glycemic control (Greico, Colberg, Somma, Thompson, & Vinik, 2013). Recent research documented protective effects on the cardiovascular system in older populations (Paredes, Forman, Vara, Escames, & Tresguerres, 2014) and reduced electrical instability after epinephrine application, suggesting positive roles for this easily accessible supplement (Vazan & Ravingerova, 2015). A similar study evaluated two major neurotransmitters of the sympathetic nervous system (SNS), plasma norepinephrine and dopamine levels, in the supine position, and found that after 60 minutes of melatonin administration, measured norepinephrine and dopamine levels were lower (Nishiyama et al., 2001), suggesting that melatonin administration influences cardiac vagal tone, potentially exerting suppressive effects on sympathetic influences from the ANS.

Dysfunction within the hypothalamus, particularly related to SCN output, may be particularly troublesome for T2D sufferers, creating irregular sleep and wake cycles, and making it difficult for them to avoid the exacerbation of the disease (Kreier et al., 2007). These deficits related to hypothalamic activity may contribute to the further development of T2D and cause individuals to progress further into the complications of diabetes. Melatonin has been researched as a synchronizer of the body's biological clock and has demonstrated a restorative ability within the SCN output context (Scheer, Kalsbeek, & Buijs, 2003). It is within this context that melatonin has the potential to improve ANS balance, inflammation, oxidative stress, and glycemic control within the T2D patient (Hussain et al., 2006; Kedziora-Kornatowska et al., 2009; Paskaloglu, Sener, & Ayangolu-Dulger, 2004; Reiter, 1995; Scheer et al., 2003; Tutuncu et al., 2005). It also lowers production of free radicals within the mitochondria (Okatani, Wakatsuki, Reiter, & Miyahara, 2002) and attenuates inflammation by inhibiting NF-κB (Jung et al., 2010). Thus, its primary effect appears to relate to the reduction of sympathetic influences,

while potentially increasing parasympathetic function, culminating in reduction in activation of the neuroinflammatory reflex arc.

The SNS is a powerful force, enabling the body to essentially push the gas pedal when it needs to gain momentum for a task; however, the system is often out of balance in patients with T2D, leaving the body in gear, so to speak. Parasympathetic nervous system (PNS) influences bring a more calming, and balance to the SNS, effectively representing the opposite end of the spectrum within the ANS. T2D patients experiencing cardiac autonomic neuropathy (CAN) in a progressive state, appear to be left in variations of full throttle or vacillating dysfunction, when every engine needs a period of rest in order to maintain reasonable performance levels. Melatonin appears to potentially have the ability to positively influence the ANS in such a way that it brings balance to the functions of the SNS and PNS by attenuating excessive SNS dominance dysfunction that is frequently found in T2D individuals.

Cardiovascular Autonomic Neuropathy & Diabetes

Neuropathy comes in many forms, including those that more specifically affect the cardiovascular system termed as cardiovascular autonomic neuropathy (CAN) (Vinik & Erbas, 2001). Incidence of the disease is incredibly high, with reported rates as high as 100% in some research findings (Ziegler, Gries, Spuler, & Lessmann, 1992). Microvascular damage occurring within the ANS exhibit itself in the form of CAN dysfunction, placing great risk to the individual being affected, as ANS dysfunction is a strong predictor of sudden death with intensive glycemic control (Vinik, Maser, & Ziegler, 2011). Mortality rates are significantly higher for individuals experiencing CAN compared to patients without this particular pathology (Ewing & Clarke, 1986; Vinik, Maser, Mitchell, & Freeman, 2003; Vinik & Erbas, 2006).

A unique T1D case proves the point, as Pop-Busui (2010) discussed the sudden death of a 26-year-old woman with severe CAN (Pop-Busui, 2010). Poor glycemic control over a 16-year period, with hyperglycemic unawareness is believed to have contributed to persistent orthostatic hypotension with BPs ranging in the 30 to 60 mmHg range. This case study revealed classic signs of the disease upon her last clinical visit, yet sadly this case is not isolated. T2D is often characterized by early damage to the ANS as well, which likely occurs prior to its onset (Laitinen et al., 2011).

CAN dysfunction and its relationship to diabetes is not clearly defined, although hyperglycemia appears to play a role, with glycation end products playing a significant role in creating inflammation in microvascular processes (Lieb et al., 2012). A proinflammatory state has been associated with ANS damage in diabetes (Lieb et al., 2012), and sympathovagal imbalance may either result from or be the cause of an increased state of inflammation (Lieb et al., 2012), which plays a key role in the development of both T2D and atherosclerosis.

This inflammatory response is controlled by the neural circuitry of the ANS. The afferent arc consists of nerves that sense injury and infection and, in turn, activate a cholinergic anti-inflammatory pathway that modulates the response (Vinik, 2012). The lymphoid organs of the immune system are innervated by cholinergic, catecholaminergic, dopaminergic, and peptidergic neurons, and neurotransmitters can alter the level of function of immune cells. In addition, sensory neurons detect inflammation and can lead to the release of dopamine and norepinephrine, causing depolarization of the vagal sensory fibers and initiation of a motor efferent arc in the brainstem (i.e. the cholinergic anti-inflammatory pathway) (Vinik, 2012). It is the loss of autonomic control with reduction of parasympathetic activity (a hallmark of T2D) that appears to initiate this cascade of inflammatory responses.

Symptoms. Symptoms associated with the disease include reduced resting heart rate variability (HRV), elevated HR at rest (tachycardia), exercise intolerance, orthostatic hypotension, abnormal circadian BPs, painless myocardial ischemia and intraoperative cardiovascular lability, leading to a two to three-fold increase in mortality in diabetic patients (Maser & Lenhard, 2005; Purewal & Watkins, 1995; Vinik & Ziegler, 2007). Others link CAN to lower survival rates post myocardial infarction (Vinik et al., 2013).

Resting tachycardia and fixed HR or blunted HR response tend to be late symptoms of CAN, likely due to vagal impairments that have developed over time (Vinik et al., 2013). Abnormal HR response is a simple, yet powerful marker of CAN, identifying individuals at higher risk. Hage et al. (2013) successfully identified blunted HR response to adenosine in otherwise asymptomatic diabetics (Hage et al., 2013). Results indicated that individuals with both abnormal MPI and HR were associated with the highest increased risk for cardiovascular events, further substantiating the stealth of CAN, and necessity of screening and treatment. Unique cases of dysfunction exist in the literature, as in the case of a 19 year old T1D experiencing palpitations, elevated HR, and postural orthostatic hypotension (POTS) (Meyer et al., 2015). Further examination resulted in hypotheses that related to PTSD in combination with T1D contributed to altering autonomic balance, thus inferring that altered mental states due to extreme stress may contribute to HR related autonomic dysregulation. Patients with POTS may also experience fatigue and sleeping disturbances, warranting intervention. Mallien et al. examined 38 POTS patients and 31 controls utilizing the Pittsburg Sleep Quality Questionnaire and the Epworth Sleepiness Scale (Mallien et al., 2014). Participants were examined at a sleep laboratory, where HRV analysis and other autonomic activity were recorded. POTS participants experienced lower sleep quality and diminished HRV parameters. De Wandele and fellow

researchers found dysregulation of autonomic function relating to increased sympathetic activity during rest and lowered sympathetic response to stimuli in 39 age matched females that underwent autonomic testing (De Wandele et al., 2014). Orthostatic intolerance, postural tachycardia and lowered sympathetic responses to stimuli suggested dysautonomia relating to CAN in these participants. A host of other symptoms are associated with CAN, including gastrointestinal, genitourinary, metabolic, Sudomotor, and pupillary dysfunction, yet these are not the focus of this research and are listed elsewhere (Vinik, Maser, et al., 2003).

Measuring Cardiac Autonomic Dysfunction. Several means exist to evaluate CAN, and most have been described in clinical and research literature (Vinik, Maser, et al., 2003; Vinik et al., 2013). Clinical and research evaluation and confirmation of CAN differ, however (see Table IIC.1). Research literature lists the following as acceptable means to evaluate CAN: heart rate response to deep breathing (an indication of beat-to-beat variations within the heart), heart rate response to standing, Valsalva maneuver, power spectral analysis (HRV analysis), 24 hour electrocardiogram (ECG) monitoring, systolic blood pressure (SBP) response to standing, diastolic blood pressure (DBP) responses sustained handgrip, and hemodynamic responses to tilt table tests (Poanta, Cerghizan, & Pop, 2010; Tarvainen, Laitinen, Lipponen, Cornforth, & Jelinek, 2014; Vinik, Maser, et al., 2003; Vinik et al., 2013).

HR, cardiovascular testing, and orthostatic hypotension testing have been evaluated and found to be acceptable measures for both research and clinical diagnosis of CAN (Vinik et al., 2013), and heart rate variability (HRV) has been used as a measure of CAN dysfunction. Tarvainen et al. (2014) performed work with these measures with 92 T2D patients, investigating time-domain, frequency-domain, and non-linear methods (Tarvainen et al., 2014). The investigation revealed significant decreases in HRV and mean increases in HR in T2D patients

when compared to healthy controls, providing relative evidence of CAN pathology within the first 5-10 years of T2D. Research is broad concerning the use of HRV, with power spectral analysis being endorsed as a primary means of evaluating and diagnosing CAN (Spallone, 2011; Vinik et al., 2013).

Baroreflex sensitivity measures have been used to evaluate coronary artery involvement related to impaired endothelial function, relating to patients with IGT (Wykretowicz, 2005), with vasodilator responses significantly being impaired in patients with IGT or diabetes. Rolim, de Souza, & Dib (2013) and Spallone (2011) endorse baroreflex sensitivity testing as a primary means of evaluating CAN and in response to the Toronto Consensus panel on Diabetic Neuropathy guidelines (Rolim, de Souza, & Atala Dib, 2013; Spallone, 2011).

More recently, Sudoscan has been introduced into research to evaluate the microvascular complications associated with neuropathy via electrochemical skin conduction (Eranki et al., 2013; Freedman, Bowden, Smith, Xu, & Divers, 2014; Smith, Lessard, Reyna, Doudova, & Singleton, 2014). This noninvasive skin conductive measurement evaluates sweat that is stimulated by a gentle electrical current (undetectable) that is passed quite gently through the soles of the feet and palms of the hands. Sudoscan is a measure of microvascular complications included in the study. Ease-of-use makes this tool a target for screening. Yajnik et al., 2012 discusses Sudomotor dysfunction testing as a simple means of alerting clinicians to both peripheral and cardiac dysfunction (Yajnik, Kantikar, Pande, & Deslypere, 2012), but advises additional research and clinical outcomes.

The presence of one abnormal cardiac vagal result indicates possible early CAN, while two abnormal cardiac vagal results confirm it (Spallone et al., 2011; Vinik et al., 2013).

Orthostatic hypotension combined with an abnormal HR should be considered severe or advanced CAN disease.

Glycohemoglobin Testing. Glycohemoglobin, or HbA1C, testing has been reliably used to categorize BG values within research (Feng et al., 2009; Sumpio et al., 2013) and has been proven as a simple, portable method of screening for diabetes that is accurate, relatively quick to perform on-site (10 minutes), with results that are easily relayed. This method of screening also provides the unique caveat of accessible health care upon demand to individuals that might not otherwise engage in or be able to afford more expensive tests such as oral glucose tolerance testing (OGTT). When considering the validity of HbA1C screening for diabetes, it should be noted that the Canadian Task Force on Preventive Health Care, American Task Force and WHO recommendations all support HbA1C testing of $\geq$ 6.5% for the screening and diagnosis of diabetes (American Diabetes Association, 2012; Canadian Task Force, 2012; World Health Organization, 2011). In a clinical setting, if positive results are obtained, results should be confirmed with repeated testing.

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Additionally, HbA1C testing is not appropriate in situations with individuals who have hemoglobinopathies, as it may not be reliable (Hare et al., 2012). Current recommendations by the American Diabetes Association, however, align HbA1C as a valid test in equal measure to other diagnostic tests that may be used and that one test is not preferred over the other (ADA, 2016).

The DCA Vantage from Siemens Corporation has been evaluated within research as a point of care analyzer for HbA1C testing and found to have good correlation with laboratory methods and acceptable precision (Lenters-Westra & Slingerland, 2010; Sanchez-Mora et al., 2011). Within this research effort, 53 blood samples from diabetic patients over a wide range of HbA1C values (4–14%), and results were examined with both a DCA Vantage Analyzer and a POCT Analyzer to be compared to on-site lab testing, and both were found to be clinically acceptable.

HbA1C testing remains as a recommended test for the screening and diagnosis of diabetes. Increased accuracy over FPG, high portability, and financial accessibility in comparison to OGTT make it an excellent screening test, despite limitations with certain diabetic populations. The DCA Vantage Analyzer provides quality, on-site testing for HbA1C values, with a proven wide range of testing capabilities that are clinically acceptable.

Summary

Melatonin has been shown in research to have a positive effect on ANS function through a variety of studies, many of which have addressed epinephrine, norepinephrine and glycemic control. It is postulated that melatonin may have a significant effect on circadian rhythms in T2D, yet this is not well researched to date, and remains to be elucidated through further testing.

The potential for the study lies in investigating to what extent the ANS is altered in T2D individuals at baseline testing, followed by to what extent melatonin would improve baseline study measures of ANS function. We hypothesized that the ANS is misconducting, causing a neuroinflammatory response, leading to impairment and that the proposed study would evaluate this phenomenon. We further postulated that ANS function would improve in participants with melatonin supplementation.

The overall purpose of this research was to investigate whether the underlying central, cardiac, and peripheral defects that are observed in T2D could be improved or reversed by a known chronotropic hormone, melatonin, given as a supplement. The physiological impact was evaluated through the effects of a high dose supplemental melatonin on autonomic balance in baroreflex sensitivity (BRS).

TABLES

Table IIC.1

Cardiovascular autonomic tests and suggested indications for their use

Test	Clinical Diagnosis	Research	End Point in Clinical Trials
Heart rate cardio vascular tests	Yes	Yes	Yes
Orthostatic hypotension test	Yes	Yes	No (low sensitivity)
QT interval	Yes (additional information and risk stratification)	Yes	No (low sensitivity)
Ambulatory blood pressure monitoring for dipping status (ABPM)	Yes (risk ratification)	Yes	No (low sensitivity)
HRV time and frequency domain indices	Yes (additional information and risk stratification)	Yes	Yes
Baroreflex sensitivity measures	No (early additional information and risk stratification but low availability)	Yes	Yes
Scintigraphy studies	No (low availability, limited standardization)	Yes	Yes
Muscle sympathetic nerve activity	No (low ability, limited data and cardiovascular autonomic neuropathy)	Yes	Possible (used in lifestyle intervention trials and obesity)
Catecholamine assessment	No (low availability)	Yes	Possible (using lifestyle intervention trials and obesity)

(Reproduced from Spallone et al., 2011; Vinik et al., 2013).

CHAPTER III

PROJECT I: NEUROPATHY SCREENING TOOLS

INTRODUCTION

Diabetes is known for its complications, with one of the most common being microvascular damage that leads to diabetic neuropathy (DN), an insidious pathology which comes in many forms, affecting various systems within the body, increasing a person's risk for amputation (Veresiu et al., 2015; Vinik et al., 2013). A common form of DN is diabetic peripheral neuropathy (DPN), which is a primary cause for balance issues (Schwartz et al., 2008; Vinik, Vinik, Colberg, & Morrison, 2015) and loss of sensation in the feet (Lamparter et al., 2014); it is also a major contributor to non-traumatic lower limb amputations (Vinik et al., 2013). DPN is a particularly significant problem for individuals with diabetes as it is relatively common, and often leads to disability, but is difficult to diagnose due to frequent asymptomatic onset or unusual presentation (Dixit & Maiya, 2014; Herman & Kennedy, 2005; McKinlay et al., 2013; Veresiu et al., 2015; Vinik et al., 2013). This complication affects the nerve endings in the feet, hands, and other regions of the body after an individual has experienced extended or acute hyperglycemia or other pathologies that lead to the destruction of various forms of sensation (Goh & Cooper, 2008; Goodarzi, 2014; Marcovecchio et al., 2011).

Earlier detection of such complications in at-risk individuals and in those with type 2 diabetes (T2D) or prediabetes (PD) allows for the best-case health and cost reduction scenarios for all concerned, including optimal intervention and lifestyle changes (Papanas & Ziegler, 2012; Phillips et al., 2014; Tabák, Herder, Rathmann, Brunner, & Kivimäki, 2012). Limited research has sought to detect subclinical changes utilizing expensive and non-portable nerve conduction

units, but research aiming to discover early changes in sensation using readily accessible, portable tools has not been a primary focus (Mustafa et al., 2012; Papanas & Ziegler, 2012; Smith & Singleton, 2006). DPN often develops silently, during early hyperglycemic processes, yet many find out far too late in the process to effectively intervene (Monnier, Hanefeld, Schnell, Colette, & Owens, 2013; Nichols et al., 2006; Phillips et al., 2014; Ruterbusch, 2014). Earlier intervention in the DPN disease process would allow individuals time to respond with appropriate choices to better direct their health, and low-cost tools to detect symptomology before T2D or PD has been diagnosed may be useful in this effort. Several tools, such as the 1-g and 10-g monofilaments and the 128-Hz tuning fork, have been successfully used within research, effectively serving PD and T2D populations for screening and disease assessment (Baraz et al., 2014; Bourcier et al., 2006; Divisova, 2012; Dros et al., 2009; Feng et al., 2009; Robinson et al., 2013; Tracey, Greene, & Doty, 2012). The Norfolk Quality of Life Diabetic Neuropathy Screening Tool (QOL-DN), the NC-Stat DPN Check, and hemoglobin A1C testing (HbA1C) have been validated within T2D and limited PD populations as well, making them likely candidates for success in early screening efforts (Boyd et al., 2011; Casellini, 2007; Lee et al., 2014; Perkins et al., 2006; Veresiu et al., 2015; Vinik, Vinik, et al., 2014).

While each measure has been shown to be reliable and valid in T2D and PD populations, overweight, obese and inactive (OOI) populations have not been a primary focus of studies using these measures; however, they are at high risk for the development of T2D and associated complications. Evaluation and screening for early signs of dysfunction in an OOI, PD and T2D population allows for the development of the appropriate refinement of methods for earlier detection. Thus, the purpose of this study was to evaluate the effectiveness of three neuropathy

screening tools, the 128-Hz tuning fork, 1-g and 10-g monofilaments, and QOL–DN, in these populations with the intent to identify early signs of DPN.

METHODS

Participants

Sampling. This study included a total of 34 adults of both sexes and varying ethnicities, divided into three groups: 10 overweight, obese and inactive normoglycemic adults (OOI) (6 females, 4 males; 59.6 ± 13.0 years), 13 with prediabetes (11 females, 2 males; 56.4± 12.2 years), and 11 with T2D (7 females, 4 males; 59.6 ± 12.1 years). Individuals with T1D, active tobacco use, presence of hepatitis B, hepatitis C, HIV, pregnancy, damage to the lower extremities, history of nerve disease (other than neuropathy), history of peripheral arterial disease, lower limb amputations, or foot ulcers were excluded from participation. Any individual possessing a serious medical condition that would compromise the subject's safety or the integrity of the study was also excluded.

Selection and Assignment. Volunteer subjects were recruited by flyers, email, word of mouth and university announcements. Subjects were screened by phone for exclusionary factors prior to reporting for testing. Assignment to groups was based on current HbA1C testing values obtained onsite during study procedures. This research was approved by the Old Dominion University Institutional Research Board and subjects participated in informed, signed consent procedures before participating (ODU IRB ID: 15-197).

Procedures

Subjects reported to the Old Dominion University Wellness Institute to be screened and participate in informed consent procedures prior to participation. Once they completed the screening measures, individuals participated in all of the following testing measures.

QOL-DN Questionnaires. The QOL-DN, a validated method instrument of assessing neuropathy, and differentiating between autonomic, large and small fiber impairment (Boyd et al., 2011; Veresiu et al., 2015; Vinik, Vinik, et al., 2014) and was utilized with each participant. Individuals were given the questionnaire in a quiet area of the testing facility where they could work undisturbed at their own pace (see Appendix B). Incomplete questionnaires were completed before proceeding further with the study (Boyd et al., 2011; Casellini, 2007; Veresiu et al., 2015; Vinik, Vinik, et al., 2014).

HbA1C Testing. Individuals were instructed prior to their appointment to drink several glasses of water within 2–3 hours prior to the study to avoid POCD errors, such as high total hemoglobin errors. Hydration instructions were assigned for the 24-hour period beforehand. Finger-stick testing was performed with a Siemens DCA Vantage 2000 Analyzer (Lenters-Westra & Slingerland, 2010) and DCA Vantage HbA1C test kits utilizing sterile techniques. HbA1C values and prior diagnoses were utilized to screen and categorize subjects as follows: OOI 4.0–5.6%; PD, 5.7–6.4%, T2D, 6.5% and above (Mannarino et al., 2013; Mustafa et al., 2012; Selvin, Steffes, Gregg, Brancati, & Coresh, 2011).

NC-Stat DPN Check. Nerve conduction study procedures utilized the POCD NC-Stat DPN Check (DPN-Check, NeuroMetrix Inc., Waltham, MA) and followed previously outlined methods as performed in Lee et al., 2014. The POCD test method involved a bilateral examination of the lower extremity with the focus of obtaining sural nerve amplitude potential (SNAP) and conduction velocity (SNCV) to assess large myelinated nerve fibers (Lee et al., 2014; Perkins et al., 2006; Perkins et al., 2008). The device allows for evaluation of the SNCV and SNAP by nonclinical personnel, assisting in DPN detection at a significantly earlier stage when compared to bedside tests (Pambianco, Costacou, Strotmeyer, & Orchard, 2011; Sharma,

Vas, & Rayman, 2015). The unit utilized biosensor technology paired with 2 probes coated in conductive gel and was applied directly to the skin posterior to the lateral malleolus. The single press of a button distributed 100 mA of current, which was detected by a single patient use disposable biosensor. A built-in thermometer accounted for variances in temperature between 23ºC and 30 ºC and notified the operator of skin temperatures too cold for testing, preventing testing until appropriate temperatures were present. Up to five attempts were utilized to collect three sets of SNCV and SNAP values, per leg. Device errors were not recorded; however, zero readings were recorded by hand and reattempts were made up to the 5-trial limit, as individuals permitted. When individuals could not tolerate the acquisition of 3 data points per leg, last observation carried forward (LOCF) methods were employed to complete the trial set (Vinik, Shapiro, et al., 2014). The validity and effectiveness of the NC-Stat DPN Check system has been confirmed in prior research (Perkins et al., 2006; Sharma et al., 2015). This test served as a criterion standard for the study and all other testing was compared to this measure.

Tuning Fork Testing. A 128-Hz tuning fork was used to assess vibration perception (Abbott et al., 2002; Shin, Seong, Lee, Kim, & Park, 2000) (See Figure III.1). Familiarization, the site and method of testing, and all procedures for the "On/Off" method followed standardized protocols as outlined by the Rapid Screening for Diabetic Neuropathy using the 128-Hz turning fork (Abbott et al., 2002; Divišová et al., 2012; Meijer et al., 2005; Perkins et al., 2001; "Rapid Screening for Diabetic Neuropathy," 2013; Shin et al., 2000). The timed tuning fork method was employed in the same manner as Perkins et al., 2001, bilaterally (Perkins et al., 2001). The procedural execution of both sets of tuning fork tests for peripheral neuropathy were performed with the subjects lying in the supine position, with eyes closed during testing (Perkins et al., 2001; "Rapid Screening for Diabetic Neuropathy," 2013).

Monofilament Testing. Commercially produced 1-g and 10-g monofilaments were used (North Coast Medical, San Jose, CA) with a standard lab testing table to evaluate sensation perception. Monofilament storage and testing took place in a temperature controlled environment, within the published parameters established by previous research (Haloua et al., 2011; Lavery et al., 2012). Testing loads were limited to appropriate testing and rest periods. Scheduling was spaced out over a period of six weeks, less than 10 subjects per day, followed by a 1-day rest period before subsequent use. Monofilaments were utilized to assess sensation according to previously published standardized guidelines (Baraz et al., 2014; Kafa et al., 2015; "Rapid Screening for Diabetic Neuropathy," 2013). Procedures for familiarization and testing followed the Canadian Diabetes Association for the Rapid Screening of Diabetic Neuropathy as laid out for 10-g monofilament testing at the dorsum of the great toe, just proximal to the nail bed (see Figure III.2). These procedures were applied to testing for the 4.17/1-g and 5.07/10-g monofilaments and included standardized procedures for familiarization procedures, subject response patterns, sites tested, number of stimuli and score assignment based off of prior literature (Perkins et al., 2001; "Rapid Screening for Diabetic Neuropathy," 2013; Shin et al., 2000). Monofilament testing was performed with the subject lying supine, eyes closed on a laboratory testing table. The 4.17 (1-g) and 5.07 (10-g) monofilaments from a full kit of North Coast Medical (North Coast Medical, San Jose, CA) monofilaments were utilized to assess sensation according to previously published standardized guidelines (Baraz et al., 2014; Kafa et al., 2015; "Rapid Screening for Diabetic Neuropathy," 2013). Individuals were allowed to keep shoes and socks on until the time of testing in order to maintain normal body temperature, but these items were removed just prior to commencing with screening.

Data Analyses

Statistical analyses were performed using SPSS version 22.0 for Windows (SPSS, Chicago, IL). Participant, group characteristics, SNAP and SNCV are presented in raw form. Criterion and dependent variable data were logarithmically transformed to best achieve normality for statistical analysis. Correlations were analyzed using Spearman's coefficients for the tuning fork, 1-g and 10-g monofilaments, QOL-DN and NC-Stat DPN Check results, and accounted for age, HbA1c and waist measurement (in cm). Kruskal-Wallis H tests were used to determine if there were differences between the three groups with pairwise comparisons using Dunn's (1964) procedure. Alpha was set at 0.05 for all analyses.

RESULTS

Population Characteristics

Our population included 10 males and 24 females of Caucasian and African American ethnicity, with HbA1C ranges varying from 4.4–14.0% (Tables III.1 and III.2). Fifteen of the 34 individuals reported no prior diagnosis or knowledge of hyperglycemia. Five out of 15 had PD HbA1C values and were grouped accordingly. Without specific recruitment for OOI, 33 of the 34 subjects were overweight or obese. Twenty-eight individuals reported having no prior neuropathy diagnosis or knowledge. Medication usage varied, with 10 of 34 participants reporting T2D specific medication usage as part of their personal medical plan. Two individuals with T2D reported a combination of T2D and neuropathy medication.

Sural Nerve Conduction Amplitude and Velocity Results

Overall group means for SNAP and SNCV characteristics did not significantly vary by HbA1C level (Table III.3). Kruskal-Wallis H testing revealed no significant differences among OOI, PD and T2D groups for SNAP and SNCV values (SNAP: R, $H(2) = 1.460$, $p = .482$; L, $H(2) = 2.369$, $p = .306$; SNCV: R, $H(2) = 1.874$, $p = .392$, L, $H(2) = 1.880$, $p = .391$). Raw data means and standard deviations are presented (Table III.3). Twenty-seven individuals obtained confirmed, individualized, abnormal NCS results, of which 25 were bilateral and symmetrical (Table III.4). Twenty-four participants presented with a combination of abnormal distal signs bilaterally, of which two also reported altered ADLs and four reported autonomic symptoms. Only two of the twenty-four reported changes in both ADLs and autonomic features. One individuals presented with no signs or symptoms. Seven cases presented with normal NCS findings, but in the presence of reported symptoms and reduced bilateral distal sensation.

Tuning Fork Testing, Monofilaments & QOL-DN Results

The tuning fork on/off test did not correlate with our criterion variables (see Table III.5); however, the tuning fork achieved a sensitivity of 53.8% and specificity of 75.0%. (Table III.5), Timed tuning fork testing yielded no significant correlations or relationships within the study, bilaterally. The 1-g total scores moderately correlated with both SNAPs [R, $r_s(34) = .364$, $p = .024$; L, $r_s(34) = .312$, $p = .047$], and left 1-g scores demonstrated a moderate relationship to both SNAPs [R, $r_s(34) = .393$, $p = .016$; L, $r_s(34) = .301$, $p = .053$] of the NC-Stat DPN Check. Sensitivity for the 1-g monofilament was 73.1% and specificity was low, at 25.0%.

The 10-g monofilament did not significantly correlate to our criterion variables. Sensitivity for the 10-g was 46.2% and specificity was 62.5%. Total QOL-DN scores negatively correlated with both SNAPs [R, $r_s(34) = -.317$, $p = .044$; L, $r_s(34) = -.311$, $p = .047$], as did the Symptoms subscale (both SNAPs) [R, $r_s(34) = -.332$, $p = .036$; L, $r_s(34) = -.375$, $p = .021$]. The small fiber subscale of the QOL-DN correlated with the RSCV [R, $r_s(34) = -.311$, $p = .047$] and the ADLS subscale correlated with the RSNAP both SNAPs [R, $r_s(34) = -.354$, $p = .028$]. QOL-DN components spanned a wide range in sensitivity (0–65.4%) and specificity (12.5–87.50%).

DISCUSSION

The integration of these testing methods provided an excellent framework to develop a better understanding of the onset of dysfunctional physiological processes within PD and OOI individuals during the beginning of disease onset and examination of relationships between symptoms and disease. This study compared the effectiveness of the 128-Hz tuning fork, 1-g and 10-g monofilaments, and the QOL-DN as screening measures for early DPN detection to established NCS criterion values as measured by the NC-Stat DPN Check. Our evaluation utilized the NC-Stat DPN Check and associated NC-Stat software to account for the age, height and weight of the subjects in conjunction with 3 bilateral sural NCS readings to assess the function of large myelinated nerve fibers, and thus we did not directly assess small fiber neuropathy associated deficits. This study offers a nonclinical analysis based off of the criteria required by Tesfaye et al. (2010) aiming to achieve minimal definition requirements for confirmed and subclinical DSPN classification, with the intent of developing early screening measures for DPN prone populations (Tesfaye, Boulton, Dyck, Freeman, Horowitz, Kempler, Lauria, Malik, Spallone, & Vinik, 2010).

Sural nerve conduction and amplitude values are validated quantitative physiological markers that assist in the assessment and confirmation of DPN status with, or without the presence of signs or symptoms. Twenty-six of 34 individuals had abnormal NCS, 24 of whom reported symptoms and bilateral symmetrical signs upon examination (1-g, 10-g monofilaments, 128-Hz tuning fork), meeting the requirements for confirmed DSPN according to some literature (Tesfaye et al., 2010); however, we find that this is a significant percentage of study participants in comparison to other research conducted with this device (Perkins et al., 2006). In addition, three individuals with abnormal NCS reported symptoms and unilateral presentation of signs,

potentially indicating pathology that is not the focus of this study, while one individual with abnormal NCS reported no symptoms or signs, confirming the likelihood of subclinical neuropathy. Six individuals obtained normal NCS studies, but had the presence of signs and reported symptoms, while one individual had normal NCS, but the presence of signs and no reported symptoms.

Perkins et al. 2006 experienced significant findings, yet their study only evaluated individuals with diagnosed diabetes (T1D and T2D), whereas our study examined a wide range of individuals, including "healthy" individuals that were recruited for our OOI population that we believed might be prone to DPN, as well as PD and T2D individuals. The fact that we report bilateral, abnormal findings in 71% of the individuals we tested, leaves room for questions. We applied rigorous testing preparation and methods, and while it is possible that there is an error we are unaware of, our findings may be questioned as valid. It is also possible that the NC-Stat DPN Check's current software components and algorithms are too sensitive for the subject population. For clarification, we compared our SNAPs to Perkins et al. and found that, overall, our SNAP values for our groups (see Table III.3) contained values ranging from 2–25μV, with means ranging from 6.6 to 10.5μV, compared to Perkins et al., who contained means of 5.6μV. Many of their participants (16) had undetectable levels, whereas we were able to achieve three readings on all but 4 individuals to whom LOCF was applied. At present, we interpret our readings as valid given that we acquired three readings on each leg, across a diverse collection of individuals, all of whom were likely to develop DPN. In support of our findings, the individuals with abnormal findings self-reported symptoms via QOL-DN and had documented distal sensation loss via 128-Hz tuning fork, and 1-g or 10-g monofilaments. It is, however, possible that our readings are altered in some way that we are unaware of at present.

In an attempt to offer specific recommendations of normal or abnormal findings based on applied individual characteristics, our assessment differed from previous research by evaluating each individual participant according to age, height and weight and determining appropriate cutoffs for normal and abnormal findings, thereby individualizing results to each participant with the built-in NC-Stat software. This method of analyses seemed particularly appropriate given the nature of the potential impact of overweight, obese status within our population. Having noted discrepancies between the two in the study he performed that analyzed both measures, Lee et al. 2014 notes that the SNCV values tend to be lower with a traditional NCS when compared to the NC-Stat DPN Check (Lee et al., 2014). This would prove to an interesting point to consider, if the same type of error were true, as it would likely boost the number of individuals who had abnormalities even higher.

To detect early DPN in normoglycemic OOI individuals, we had postulated that the 128-Hz tuning fork and QOL-DN would provide the best mechanisms for detection; however, our results only indicated partial support for this theory. The tuning fork on/off test did not correlate well with our NC-Stat DPN Check SNAP criterion variables, however, the QOL-DN, on several measures, did. This finding is different than some prior research, as the QOL-DN has not always been found to correlate with electrophysiological measures (Vinik et al., 2005; Vinik et al., 2008).

The QOL-DN ranged in sensitivity (0–65.4%) and specificity (12.5–87.5%), differing from previous research that resulting in high specificity and sensitivity. While there is no definitive answer for this, plausible considerations for this finding include the unusual distribution of our population, and our small pilot size across three groups in our attempts to discover DPN at its earliest point possible. Previous research expressed concern relating to the

QOL-DN: Hogg et al., 2012 reported the QOL-DN as a means to aid in diagnosis and monitoring, but expressed a lack of specificity for PN, stating that it may be limited its use to health impacts of a diabetes foot disease related nature (Hogg et al., 2012). We did not find this to be true in our study as QOL-DN measures not only correlated, but also provided vital standardized data relating to self-reported symptoms, ultimately contributing to our goal of the early identification of DPN.

Detecting such diabetes complications is an unfolding evolution that involves multiple dynamics. DPN may present in a completely silent manner, without pain, burning or symptoms of annoyance. In such cases, individuals will not disclose physical symptoms that they aren't currently experiencing. Individuals with early DPN may experience the disease in a varied manner, with some individuals experiencing asymptomatic disease patterns, ultimately requiring hands on screening to identify the silent progression of the disease. Future research should likely continue to examine the QOL-DN for early DPN detection, as several subscales indicate correlations.

The 1-g monofilament proved to be useful within our study, with (30) individuals experiencing abnormal findings. This measure indicated high sensitivity (73.1%) and poor specificity (25.0%), yielding concerns. However, validation of 1-g physical findings was seen through moderate correlations back to our criterion SNAP variables. Our results relate to previous research efforts that reported high sensitivity and low specificity, as is the case of Takasande et al. 2011 and reviews performed by Feng et al. (Feng et al., 2009; Taksande, Ansari, Jaikishan, & Karwasara, 2011).

The 10-g monofilament testing lacked significant correlational relationships, yet the usefulness of this tool has been well established in T2D and limited PD populations in other

research. Our correlational findings did not add support for its use in normoglycemic obese populations, but insensate feet relate to neuropathy in later stages and this research focused instead on early detection. In contrast, Ylitalo et al. examined cardiometabolic and neuropathy factors in obese individuals and found that the 10-g monofilament was a useful tool for such research (Ylitalo, Sowers, & Heeringa, 2011).

We had hypothesized that the QOL-DN would be the most sensitive measure to detect undisclosed DPN in our population, and sensitivity results did not support this. The most sensitive tools for early DPN detection was the 1-g monofilament, which was reasonably sensitive at 73.1% but poorly specific at 25.0%, and the tuning fork on/off test, which was less sensitive (53.8%) and more specific (75.0%) in nature. Despite low sensitivity and specificity, the Total QOL-DN, Symptoms, ADLS and Small Fiber component aspects of the QOL-DN measure, should be considered, as this questionnaire proved to be invaluable to the study. The QOL-DN and its subscales are likely to be more successful in a more balanced study that is seeking both small and large fiber deficits related to early DPN detection, as this measure has been previously validated to detect both. Our criterion measure, the NC-Stat DPN Check was targeted towards screening for large fiber, and thus may not correlate as well with a well-rounded screening measure that targets multiple areas of neuropathy, such as the QOL-DN.

Finally, our results reflect a strong indication of neuropathy in this population, suggesting that careful screening of individuals at earlier stages may be quite beneficial in the early detection of DPN, even prior to hyperglycemia diagnosis. Smith and Singleton found elevated HbA1C status in such populations to be a concern for the development of large fiber-related neuropathy complications, as was found in our cohort (Smith & Singleton, 2013). Diabetes-related complications, such as decreased motor and sensory nerve conduction

velocities, may arise out of acute bouts of hyperglycemia experienced though postprandial excursions, which may be best reflected by HbA1C values (Marcovecchio et al., 2011).

Our study certainly has some limitations. As it is a pilot study, generalizations of findings may not be made to large populations. Lack of random assignment and use of volunteers for subjects created potential selection bias, with clinical population research targeting and low available funding heavily influencing this method. The HbA1C testing machine that was used within the study is a validated machine, yet oral glucose tolerance testing is preferred by some researchers, particularly for individuals with cardiac autonomic neuropathy (CAN) (Farhan et al., 2012). We did not test for CAN and, therefore, cannot account for unknown discrepancies. Temperature and humidity have been found to affect monofilament results, by affecting the potential validity of the instrument in extremely high temperatures as well as high testing volumes in short periods of time (Booth & Young, 2000; Haloua et al., 2011). Temperature was accounted for by limiting monofilament storage and use to normal climate controlled room temperatures and monitored these values. Humidity was monitored, but not controlled beyond what the laboratory air-conditioning and heating systems accounted for. Preparation for monofilament usage followed previously stated guidelines and recommendations, with testing amounting to far less than 100 compressions per day per instrument (Booth & Young, 2000). The NC-Stat DPN Check device was used solely to test the sural nerve; therefore, deficits in nerve function relating to other nerves of the lower leg were not confirmed through this device and two nerves were not evaluated, as some literature advises. The QOL-DN has been previously validated for individuals with diabetes and neuropathy, yet its specific validation to effectively target OOI individuals has not been performed and, therefore, this should be taken this into account when interpreting our findings.

CONCLUSION

This study aimed to detect DPN signs and symptomology prior to PD diagnosis in overweight, obese, and inactive adults using low-cost, established tools and compared these tools to a validated measure of nerve conduction. The 1-g monofilament was more useful for detection in this population than the 10-g monofilament. The tuning fork on and off test demonstrated reasonable use for this population, although it did not correlate with our criterion standard. The QOL-DN correlated on Total QOL and several subscales, providing valuable, standardized symptom information which may be incorporated into community screening models. Future research should continue to aim to refine and develop low-cost screening methods aimed at disclosing asymptomatic DPN earlier.

TABLES

Table III.1
Participant Characteristics

Variable	Frequency	Percent
Gender		
Male	10	29.4
Female	24	70.6
Ethnicity		
Caucasian	22	64.7
African American	12	35.3
Diabetes Diagnosis		
None	15	44.1
Prediabetes	8	23.5
T2D	11	32.4
Neuropathy Diagnosis		
No Prior Diagnosis	28	82.4
Prior Diagnosis	6	17.6
Medication		
No Medication	8	23.5
Not T2D Specific	14	41.2
T2D Specific	10	29.4
T2D and Neuropathy	2	5.9
HbA1C Category		
OOI	10	29.4
PD	13	38.2
T2D	11	32.4
BMI Category		
Normal	1	2.9
Overweight	9	26.5
Obese	24	70.6

Table III.2
Gender and Group Characteristics

	N	Min	Max	Mean	Std. Err	Std. Dev
Age						
Males	10	37.00	79.00	61.00	4.279	13.532
Females	24	35.00	74.00	57.20	2.364	11.581
Height						
Males	10	1.58	1.85	1.745	0.024	0.078
Females	24	1.48	1.74	1.66	0.013	0.064
Wt.						
Males	10	83.18	133.10	105.86	6.520	20.618
Females	24	65.36	122.73	89.40	3.083	15.103
Wt. By Group						
OOI	10	76.60	106.60	87.93	3.460	10.940
PD	13	65.90	133.10	98.03	7.350	23.260
T2D	11	78.40	127.70	101.29	5.590	17.680
BMI						
Males	10	28.20	41.50	34.85	1.570	4.966
Females	24	24.70	43.90	32.99	1.156	5.664
BMI by Group						
OOI	10	27.2	35.6	30.9	1.003	3.170
PD	13	24.7	43.9	34.2	1.860	6.707
T2D	11	27.0	41.5	35.1	1.516	5.029
HbA1C by Gender						
Males	9	4.4	7.1	6.0	0.289	0.915
Females	21	5.2	14.0	6.5	0.365	1.790
HbA1C by Group						
OOI	10	4.4	5.6	5.3	0.114	0.362
PD	13	5.6	6.4	5.9	0.06	0.218
T2D	11	6.5	14.0	7.8	0.632	2.095

Wt. = Weight in kg; OOI = Overweight, obese, inactive; T2D = Type 2 diabetes; BMI = Body mass index; HbA1C = hemoglobin A1C

Table III.3
NCS Results By Group

	NC-Stat DPN Check - Sural Nerve					
	N	Min	Max	Mean	Std. Error	Std. Dev.
SNAP-R (μV)						
OOI	10	2.0	14.3	6.631	1.444	4.567
PD	13	2.0	24.7	7.691	1.674	6.037
T2D	11	2.0	25.0	9.875	2.133	7.076
SNAP-L (μV)						
OOI	10	2.3	21.7	7.129	1.834	5.798
PD	13	3.0	21.7	7.277	1.186	4.277
T2D	11	3.0	21.7	10.572	2.064	6.847
SNCV-R (μV)						
OOI	10	35.3	55.7	46.2	1.902	6.016
PD	13	30.0	57.0	48.2	1.871	6.747
T2D	11	35.3	57.0	45.5	1.816	6.022
SNCV-L (μV)						
OOI	10	41.3	55.0	47.265	1.519	4.803
PD	13	43.0	55.0	49.637	1.072	3.865
T2D	11	37.3	57.0	46.876	1.946	6.455

*Displayed in untransformed form, as raw data
SNAP = sural nerve amplitude potential; SNCV = sural nerve conduction velocity

Table III.4
Sural NCS, Signs and Symptoms

	Variable	Total	Group		
			OOI	PD	T2D
Sural NCS	Normal	7	1	4	3
N = 34	Abnormal*	27	10	9	8
Signs *N* = 34	Tuning Fork				
	Normal	13	3	5	5
	Abnormal*	21	7	7	6
	Monofilaments				
	1-g				
	Normal	3	1	0	2
	Abnormal*	31	9	12	9
	10-g				
	Normal	3	1	0	2
	Abnormal*	31	9	13	9
Symptoms	None Reported	11	7	1	4
N = 34	Reported**	23	3	12	7
Autonomic	None Reported	21	7	8	6
N =34	Reported**	13	3	5	5
ADLS	None Reported	26	8	10	8
N = 34	Reported**	8	2	3	3
NCS, Sign & Symptom Combinations	AbNCS, Signs & Symptoms	17	3	9	5
	AbNCS, Signs or Symptoms	9	5	1	3
	AbNCS, No Signs or Symptoms	1	1	0	0
	NNCS, Signs & Symptoms	7	1	3	3

*Bilateral testing; abnormal findings on at least one limb;
**Self-reported on QOL-DN
AbNCS = Abnormal nerve conduction study; NNCS = Normal nerve conduction study

Table III.5
Spearman's Partial Correlations (Log Transformed)

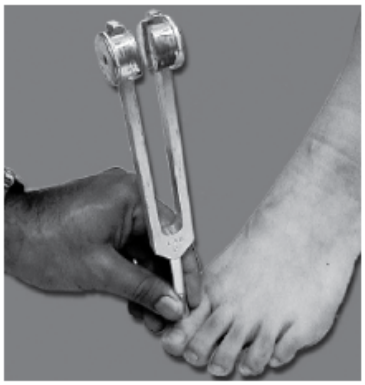
	NC-Stat DPN Check - Sural Nerve			
	SNAP-R	SNAP-L	SNCV-R	SNCV-L
Tuning Fork	*N = 34*	*N = 34*	*N = 34*	*N = 34*
On/Off	0.221	0.137	0.235	-0.089
Sig	0.121	0.235	0.106	0.319
Timed–R	-0.066	-0.019	-0.019	-0.099
Sig	0.365	0.461	0.459	0.302
Timed–L	-0.063	-0.052	-0.018	-0.081
Sig	0.371	0.392	0.463	0.355
Monofilaments	*N = 34*	*N = 34*	*N = 34*	*N = 34*
Total 1-g	0.364*	0.312*	-0.060	-0.141
Sig	0.024	0.047	0.377	0.229
1-g R	0.229	0.206	0.024	0.077
Sig	0.112	0.138	0.451	0.342
1-g L	0.393*	0.301*	-0.191	-0.313
Sig	0.016	0.053	0.155	0.046
Total 10-g	0.098	0.088	0.032	0.030
Sig	0.304	0.321	0.432	0.438
10-g R	0.096	0.160	0.005	-0.066
Sig	0.306	0.200	0.489	0.364
10-g L	0.137	0.070	0.031	0.054
Sig	0.235	0.356	0.436	0.388
QOL-DN	*N = 34*	*N = 34*	*N = 34*	*N = 34*
Total	-0.317*	-0.311*	0.162	-0.117
Sig	0.044	0.047	0.197	0.269
Symptoms	-0.332*	-0.375*	0.213	-0.003
Sig	0.036	0.021	0.129	0.493
Large Fiber	-0.297	-0.284	0.107	-0.163
Sig	0.056	0.064	0.286	0.195
Small Fiber	-0.241	-0.187	-0.311*	-0.366
Sig	0.099	0.161	0.047	0.023
ADLS	-0.354*	-0.263	0.104	-0.065
Sig	0.028	0.080	0.293	0.366
Autonomic	-0.236	-0.245	0.149	-0.044
Sig	0.105	0.096	0.216	0.408

Accounts for HbA1C, Age and Waist in cm; *Significance at the .05 level.

FIGURES

Figure III.1 128-Hz Tuning Fork

Rapid Screening for Diabetic Neuropathy Using the 128-Hz Vibration Tuning Fork (The "On-Off" Method)



1. Strike the tuning fork against the palm of your hand hard enough that it will vibrate for approximately 40 seconds.
2. Apply the base of the tuning fork to the patient's forehead or sternum and ensure that the vibration sensation (not just the touch sensation) is understood.
3. With the patient's eyes closed, apply the tuning fork to the bony prominence situated at the dorsum of the first toe just proximal to the nail bed. Ask if the vibration sensation is perceived.
4. Ask the patient to tell you when the vibration stimulus is stopped, and then dampen the tuning fork with your other hand.
5. One point is assigned for each vibration sensation perceived (vibration "on"). Another point is assigned if the correct timing of dampening of the vibration is perceived (vibration "off ").
6. Repeat this procedure again on the same foot, then twice on the other foot in an arrhythmic manner so the patient does not anticipate when the stimulus is to be applied.
7. Though this test can be used to rule out the presence of neuropathy, unlike for the monofilament described above, threshold scores do not exist to indicate the risk of future onset of neuropathy.

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Figure III.2 Monofilament Application

Rapid Screening for Diabetic Neuropathy Using the 10-g Semmes-Weinstein Monofilament

1. Show the 10-g Semmes-Weinstein monofilament to the patient.
2. Touch it first to the patient's forehead or sternum so that the sensation is understood.
3. Instruct the patient to say "yes" every time the monofilament stimulus is perceived.
4. With the patient's eyes closed, apply the monofilament to the dorsum of the great toe proximal to the nail bed as shown in the illustration below. Use a smooth motion-touch the skin, bend the filament for a full second, then lift from the skin.

5. Perform this stimulus 4 times per foot in an arrhythmic manner so the patient does not anticipate when the stimulus is to be applied.
6. For each of the 8 stimuli, assign a score of 0 if it is not perceived, 0.5 if it is substantially less than that perceived on the forehead or sternum, and 1 if it is perceived normally. A score of 3 out of 8 correct responses means that the presence of neuropathy is likely. A score of 3.5 to 5 means that the risk of new onset neuropathy in the next four years is high. A score of 5.5 or greater indicates that there is a low risk of neuropathy onset in the next four years.

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CHAPTER IV

PROJECT II: NEUROPATHY QUALITY OF LIFE TOOLS

INTRODUCTION

Diabetes is an overwhelming disease that places significant demands on individuals, often leading to distress and ultimately degradation of consistent self-care behaviors (Guo et al., 2015; Karlsen, Oftedal, & Bru, 2012). Such stressors and inconsistent monitoring behaviors invite damage caused by extended or acute hyperglycemia (Vinik et al., 2013). Hyperglycemia promotes early microvascular complications related to diabetic neuropathy (DN), including altered eyesight, kidney and psychosocial functioning, all of which may bring significant impact on an individual. An individual's outlook on life, how he or she experiences it, interacts with others, and chooses activities may be affected by a DN diagnosis and individual symptomology. Such adverse outcomes on an individual body system, and the ability to perform tasks and psychosocial functioning is referred to as health-related quality of life (HRQOL) (Luscombe, 2000). HRQOL is an important concept within diabetes care management, particularly due to the rising impact of the disease itself, as projected estimations for 2035 indicate diabetes will impact over 592 million individuals worldwide across the globe (Guariguata et al., 2013).

Research over the past several decades has made great strides in developing several HRQOL assessments that specialize in assessing DN-related measures and address HRQOL as a significant factor (Bredfeldt, Altschuler, Adams, Portz, & Bayliss, 2015; Smith et al., 2012; Vickrey et al., 2000; Vileikyte et al., 2003; Vinik et al., 2004). Within the realm of HRQOL, DN has been of particular interest, with individuals dedicating significant research effort to the validation of neuropathy-specific measures. Individuals at risk for or who are experiencing DN

should be promptly screened in order to facilitate optimal health outcomes (Marrero et al., 2014; Sinclair, Dunning, & Rodriguez-Mañas, 2015).

DN is often experienced in T2D and PD, raising questions as to when DN develops (Marrero et al., 2014; Papanas & Ziegler, 2012) and how soon it affects QOL. Furthermore, overweight, obese, or inactive status (OOI) places an individual at increased risk for disease, including potential progression to PD and T2D and other forms of physiological dysfunction, yet sparse research is available relating to how these individuals may or may not experience DN (Miscio et al., 2005). Neuropathy screening is considered a standard care for individuals diagnosed with T2D, but not for OOI individuals. Therefore, the purpose of this study was to compare three measures of QOL, the NQOL–DN, the PN-QOL-97, and the NeuroQOL-28, in OOI, PD and T2D adults to determine which instrument is the most effective at detecting DN at various stages while comparing the findings back to a criterion standard, the NC–Stat DPN Check (NeuroMetrix, Waltham, MA).

METHODS

Participants

Sampling. This study included a total of 34 adults of both sexes and varying ethnicities, divided into three groups: 10 overweight, obese and inactive normoglycemic individuals (OOI) (6 females, 4 males; 59.6± 13.0 years), 13 individuals with prediabetes (11 females, 2 males; 56.4±12.2 years), and 11 individuals with T2D (7 females, 4 males; 59.6±12.1 years). Individuals with T1D, active tobacco use, presence of hepatitis B, hepatitis C, HIV, pregnancy, damage to the lower extremities, history of nerve disease (other than neuropathy), history of peripheral arterial disease, lower limb amputations, or foot ulcers were excluded from participation. Any individual possessing a serious medical condition that would compromise the subject's safety or the integrity of the study was also excluded.

Selection and Assignment. Volunteer subjects were recruited by flyers, email, word of mouth and university announcements. Subjects were screened by phone for exclusionary factors prior to reporting for testing (see Appendix A). Assignment to groups was based on current HbA1C testing values obtained onsite during study procedures.

Protection of Subjects. Participants were closely monitored during the study. Sterile techniques were used to collect blood samples and perform HbA1C testing. This research was approved by the Old Dominion University Institutional Research Board and subjects participated in informed, signed consent procedures before participating (ODU IRB ID: 15-197).

Procedure

Questionnaires were filled out after individuals were screened and consented into the study and prior to other data collection measures (see Appendices B, C and D). Completion

times were tracked for each instrument, allowing a comparison of the time investment needed to utilize each chosen method. Individuals were placed in a quiet room within the Wellness Institute with a volunteer research assistant who timed their completion of each instrument in minutes and seconds. Questionnaires were checked by volunteer research assistants and investigators for completeness before proceeding to HbA1C testing. Incomplete questionnaires were completed before proceeding with the study. Digital copies of all questionnaires were acquired directly from the authors (QOL–DN, PN-QOL-97 and NeuroQOL-28) via email correspondence, including scoring rubrics. Printed copies were used with each participant and are attached as appendices (see Appendices B, C and D).

Norfolk Quality of Life Diabetic Neuropathy Tool. The Norfolk Quality-of-Life Diabetic Neuropathy tool (QOL-DN) has been found to be reliable across different populations and sensitive to both small and large fiber impairment and improvements in neuropathy (see Appendix B) (Boyd et al., 2011; Casellini, 2007; Veresiu et al., 2015; Vinik, Vinik, et al., 2014).

PN-QOL-97. This instrument has been identified as a validated measure for identifying DPN and successfully used in research (Maxwell et al., 2013; Maxwell et al., 2014; Vickrey et al., 2000). It is a PN-specific HRQOL measure that offers multiple psychometric properties to be considered (see Appendix C) (Smith et al., 2012; Vickrey et al., 2000).

NeuroQOL-28. The NeuroQOL-28 questionnaire instrument has been validated as a neuropathy-and foot ulcer-specific QOL tool and subsequently utilized in myriad studies evaluating QOL identifying key factors involved in the DPN experience (see Appendix D) (Dixit & Maiya, 2014; Vileikyte et al., 2003).

HbA1C Testing. Hemoglobin A1C (HbA1C) finger-stick testing was performed with a Siemens DCA Vantage 2000 Analyzer (Lenters-Westra & Slingerland, 2010) and DCA Vantage

HbA1C test kits utilizing sterile techniques. HbA1C values were utilized to screen and categorize subjects as follows: OOI 4.0–5.6%; PD, 5.7–6.4%, T2D 6.5% and above (Mannarino et al., 2013; Mustafa et al., 2012; Selvin et al., 2011) HbA1C finger-stick testing followed a standardized protocol determined from Selvin et al. (2011) and Lenters-Westra and Slingerland, (2010) (Lenters-Westra & Slingerland, 2010; Selvin et al., 2011). HbA1C testing was performed after individuals have been screened, and consented into the study, and after all other paperwork has been filled out. Individuals were instructed prior to their appointment to drink several glasses of water within 2–3 hours prior to the study to avoid errors on the test, such as high total hemoglobin errors. Individuals were also instructed to stay well hydrated for the 24-hour period beforehand.

NC-Stat DPN Check. NC-Stat DPN Check (DPN-Check, NeuroMetrix Inc., Waltham, MA) procedures followed previously outlined methods as performed in Lee et al. (2014) (Lee et al., 2014). The POCD test method involved a bilateral examination of the lower extremity with the focus of obtaining sural nerve amplitude potential (SNAP) and conduction velocity (SNCV) (Lee et al., 2014; Perkins et al., 2006; Perkins et al., 2008). The device allows for evaluation of the SNCV and SNAP by nonclinical personnel, assisting in DPN detection at a significantly earlier stage when compared to bedside tests (Pambianco et al., 2011; Sharma et al., 2015). The unit utilized biosensor technology paired with 2 probes was applied directly to the skin posterior to the lateral malleolus. A single press of a button distributed 100 mA of current, which was detected by a one use disposable biosensor. A built-in thermometer accounted for variances in temperature between 23^{o}C and 30^{o}C and notified the operator if skin temperatures were too cold for testing. Thee SNCV and SNAP values were attempted for each leg with up to five attempts to collect the trials. Device errors were not recorded; however, zero readings were recorded by

hand and re-attempts we made up to the 5 trial limit, as individuals permitted. The validity and effectiveness of the NC-Stat DPN Check system has been confirmed in prior research (Perkins et al., 2006; Sharma et al., 2015). This test served as a criterion standard for the study and all other testing was compared to this measure.

Data Analyses

Statistical Evaluation. Questionnaires were considered valid if complete biographic information, including age and sex was provided (Veresiu et al., 2015). Summary statistics, in the form of continuous data is presented with means and standard deviations. Pertinent Spearman's partial correlations are presented. NC-Stat DPN Check, measuring (3 trials) the right sided sural nerve amplitude potential (RSNAP) served as the comparable criterion standard, determining confirmed DSPN or subclinical DSPN.

Multiple regressions were run to attempt to predict the right SNAP criterion through modeling that accounted for HbA1C, age, BMI and selected correlated predictor variables from each questionnaire. Comparisons involved running separate multiple regression analyses with limited covariate and predictor variables with the aim to predict DPN. Covariates and predictors were entered at once, including accounting for known factors such as HbA1C, age, and BMI as a substitute measure for weight and height, in order to best preserve the regression model DOF (Herrera-Rangel, Aranda-Moreno, Mantilla-Ochoa, Zainos-Saucedo, & Jáuregui-Renaud, 2014). Selected neuropathy-related components were entered into each regression model based on potential relationships presented in Spearman's partial correlations and appropriate choices that meet the assumptions of regression, avoiding multicollinearity within regression models.

Selections were first made from the highest correlations, with additional predictors options if multicollinearity issues could not be effectively resolved within the model.

Linearity was assessed by scatterplot analyses, partial regression plots and a plot of studentized residuals against the predicted values. Homoscedasticity, independence of observations (research design and Durbin-Watson), linear relationships, outliers ($\pm$3 std. dev.), influential leverage cases, and multi-collinearity components (correlations, tolerance, VIF values) were evaluated and addressed for each model independently.

Regression results were compared via confidence intervals, standard errors and regression coefficients in an effort to determine if one survey could be named as the optimal predictor survey with post hoc testing as necessary. All analyses were performed using SPSS Version 22.0 and significance was set at the $p < 0.05$ level.

RESULTS

Population Characteristics

Our population included 10 males and 24 females of Caucasian and African American ethnicity, with HbA1C ranges varying from 4.4–14.0% for all subjects (Tables IV.1 and IV.2). Fifteen of 34 individuals reported no prior diagnosis or knowledge of T2D or PD. Five of 15 individuals had PD HbA1C values and were grouped accordingly. A total of 33 out of 34 individuals were overweight or obese. Twenty-eight individuals reported having no prior neuropathy diagnosis or knowledge. Medication usage varied, with 10 of 34 participants reporting T2D specific medication usage as part of their personal medical plan. Two individuals with T2D reported a combination of T2D and neuropathy medication.

Sural Nerve Conduction Amplitude and Velocity Results

Overall group means for SNAP and SNCV characteristics did not significantly vary by HbA1C level (Table IV.3). Kruskal-Wallis-H testing revealed no significant differences among OOI, PD and T2D groups for SNAP and SNCV values (SNAP: R, $H(2) = 1.460$, $p = .482$; L, $H(2) = 2.369$, $p = .306$; SNCV: R, $H(2) = 1.874$, $p = .392$, L, $H(2) = 1.880$, $p = .391$). Raw data means and standard deviations are presented (Table IV.5). Twenty-seven individuals obtained confirmed, individualized, abnormal NCS results, of which 25 were bilateral and symmetrical (Table IV.4). Twenty-four participants presented with combinations of abnormal distal signs bilaterally, meeting criteria for confirmed DSPN, and one case presented with no signs or symptoms, indicating the presence of subclinical neuropathy. Seven cases presented with normal NCS findings, but in the presence reported symptoms and reduced bilateral distal sensation.

Correlations

Spearman's partial correlations were run between NC-Stat DPN Check criterion standard variables, which were the right and left SNAP and SNCV values and all questionnaire data components while accounting for age and HbA1C values. Significant correlations were identified and are presented (Table IV.5). The QOL-DN symptom component moderately correlated with the right SNAP criterion [R, $r_s(34)$ -.365, p = .044]. The PN-QOL-97 physical component score moderately correlated with both the right and left SNAP criterions [R, $r_s(34)$ =.375, p = .038; L, $r_s(34)$*:* .366, p = .043], as did the mental component scores; however, the relationship was considerably stronger [R, $r_s(34)$ = .522, p = .003; L, $r_s(34)$ = .451, p = .011]. NeuroQOL-28 neuropathy specific components moderately strongly correlated to the left SNAP [$r_s(34)$ = -.426, p = .017], and the NeuroQOL-28 overall QOL judgment score strongly [RSNAP, $r_s(34)$ =.541, p = .002] and moderately correlated [LSNAP, $r_s(34)$ =.396, p = .028] to our criterion SNAP values.

Completion Times

Completion times analyses revealed that the QOL-DN (M = 5.17; SD = 1.83) was the quickest, on average to complete, followed by the NeuroQOL-28 (M = 5.58; SD = 3.56) and QOL-97 (M = 13.23; SD = 3.606) (Table IV.6).

QOL-DN Questionnaires

A multiple regression was run to attempt to predict the right SNAP criterion with a regression model that accounted for HbA1C, age, BMI, QOL-DN Symptoms and Total QOL Scores as predictors. The multiple regression model significantly predicted the right SNAP

value, $F(5,28) = 6.118$, $p < .001$, adj. $R^2 = .52$. Age ($p = .000$) and Total QOL ($p = .019$) significantly added to the prediction. Regression coefficients and standard errors can be found in Table IV.7.

PN-QOL-97 Questionnaires

A multiple regression was run to attempt to predict the right SNAP criterion with a regression model that accounted for HbA1C, age, BMI, and PN-QOL-97 Physical and Mental Scores as predictors. This model significantly predicted the right SNAP value, $F(5,25) = 7.465$, $p < .0005$, adj. $R^2 = .52$. Age ($p = .000$) and HbA1C ($p = .025$) significantly added to the prediction. Regression coefficients and standard errors can be found in Table IV.8.

NeuroQOL-28 Questionnaires

A multiple regression was run to attempt to predict the right SNAP criterion with a regression model that accounted for HbA1C, age, BMI, and the NeuroQOL-28 Neuropathy Specific Component and Overall QOL Judgment as predictors. The multiple regression model significantly predicted the right SNAP value, $F(5,28) = 7.238$, $p < .0005$, adj. $R^2 = .49$. Age ($p = .000$) and Overall QOL Judgment ($p = .017$) significantly added to the prediction. Regression coefficients and standard errors can be found in Table IV.9.

DISCUSSION

Although the QOL-DN, PN-QOL-97 and NeuroQOL-28 have been validated for use in research as neuropathy instruments to detect DPN, further analysis of these instruments has been recommended (Bredfeldt et al., 2015; Smith et al., 2012). We sought to determine which of three instruments, the QOL-DN (Vinik et al., 2005), the PN-QOL-97 (Vickrey et al., 2000) or the NeuroQOL-28 (Vileikyte et al., 2003) would be the best predictor of neuropathy when compared to our criterion standard measurements in OOI, PD and T2D populations. The QOL-DN Symptoms component correlated with our LSNAP, but not with the RSNAP, thus the fact that the regression results revealed a predictor relationship between the Total QOL and RSNAP was not surprising. Examination of bilateral results will be reported elsewhere.

We had anticipated that our first hypothesis would likely be supported, with the QOL-DN more clearly identifying early, or subclinical PN, and the Total QOL-DN (p = .019) component supports this hypothesis. Our results indicate that QOL-DN, but not the PN-QOL-97, predicted our criterion standard RSNAP value within our regression models, although the PN-QOL-97's Mental Score was relatively close to significance (p = .073). The NeuroQOL-28 Overall Judgment of QOL (p = .017) demonstrated significant predictive qualities for early detection, giving further validation to this short questionnaire, yet asymmetry existed in its correlational relationship across the RSNAP and LSNAP variables. Normal variants within our target population could account for such asymmetries. Twenty-six of 34 individuals had abnormal NCS. Of these 26, 24 reported symptoms (recorded via QOL-DN) and the presence of bilateral symmetrical signs as evidenced by 1-g and 10-g monofilaments, 128-Hz tuning fork, and reported symptoms, meeting the requirements for confirmed DSPN (reported elsewhere).

In hypothesizing which instrument would be most effective to detect DPN in our OOI, PD and T2D population, we had predicted correlation of .60 or higher between these tools and the NC-Stat DPN Check criterion SNAP and SNCV values. However, our results indicate that all three measures failed to meet this level; although they correlated to our criterion, the association was not as strong as clinically desirable. Correlations revealed significant relationships between the RSNAP and the Neuropathy Overall Judgment QOL (r_s = .523), and PN-QOL-97 Mental Scores (r_s = 505) for the RSNAP, but not for the QOL-DN.

Early detection is considered critical, yet is difficult to accomplish with currently available methods. Papanas and Ziegler (2012) emphasize the importance of early DPN detection in their research, advising small fiber evaluation as a means to catch the pathophysiological process in the earliest stages (Herrera-Rangel et al., 2014; Papanas, Vinik, & Ziegler, 2011; Papanas & Ziegler, 2012). Clinical exams readily identify small fiber pathology, often using Neurotips (pain and warmth detection), or a cold 128-Hz tuning fork (thermoreceptor evaluation) (Vinik et al., 2013). Large fiber neuropathy, which is the primary focus of the NC-Stat DPN Check tool, may also be evaluated through hands-on measures (NC-Stat DPN-Check, 1-g, 10-g monofilaments, 128-Hz tuning fork) in clinical or on site applications to test pressure and large fiber sensitivity changes. Small fiber dysfunction, however, is difficult to detect, often requiring skin biopsy for confirmed status, paired with abnormal QST and clinical exams, requiring clinical appointments. Such clinical tests are useful, if one can get individuals to report for testing at a clinical site or participate in on-site screening that utilizes them. However, we emphasize the necessity of research to develop easy-to-use screening tools that may be utilized in short time commitment community screening efforts. The often silent beginnings of small fiber dysfunction do not readily lead individuals to seek the clinical assessment necessary to catch the

pathology. Unless experiencing symptoms, such as brief pins-and-needles, pricks, or shock sensations, they have little to move them towards clinical evaluation.

While we knew that each instrument had its strengths and weaknesses as we entered this study, one thing we did not fully consider was that each may pick up on different components of fiber loss, meaning one may be more adept at detecting small fiber, another identifies with large fiber loss and another may do well with both of these and autonomic as well. Our criterion focused on large fiber measurement, and future study designs may want to incorporate multiple means to assess the effectiveness of these QOL tools, ones that address small, large and autonomic neuropathy components, to better detect the abilities of each individual instrument for screening. This might include simple bedside tests, such as a cold tuning fork and Neurotips, in order to evaluate small fiber components.

The further development of paper questionnaires to effectively screen for small fiber component dysfunction should be a priority, as much of the general public does not seek medical attention until symptoms have become obvious. Ultimately, the focus of patient reported outcomes such as the QOL-DN, PN-QOL-97 and the NeuroQOL-28 is DPN screening and detection, thus evaluating these instruments for different facets of the targeted disease population and determining each tool's viability in that subset was a useful objective of the current study. Both the QOL-DN and NeuroQOL-28 likely identified key subjective measures that align well with objective screening measure in early hyperglycemic processes within our small pilot population. The QOL-DN was effectively employed to identify key symptomology necessary for the diagnosis of DPN, aiding and assisting in a patient centered, cumulative approach. Both of these instruments are available in US and UK versions, with the NeuroQOL-28 reported to be available in 10 additional languages and the QOL-DN available in 8 (Smith et al., 2012),

indicating widespread availability for use in research and screening efforts. Additional strengths of the QOL-DN are highlighted in research efforts by Vinik et al. (2005), where the QOL-DN demonstrates a well-rounded approach, uncovering multiple neuropathy-related components, including complications, medication use, autonomic factors, fiber specific domains and validated use for revealing undisclosed neuropathy (Veresiu et al., 2015; Vinik et al., 2005). Our study showed similar results, disclosing DPN in individuals who were unaware of their deteriorating physiological state, revealing promise for the QOL-DN in revealing disease in diverse population settings. The NeuroQOL-28 focuses on painful symptoms, reduced sensation, ADLS, overall diffused sensory and motor changes, emotional changes and overall QOL, which likely explains its usefulness in our study (Vileikyte et al., 2003). These facets relate to our research, as the completion of results reflect a strong indication of neuropathy in this population, suggesting that careful screening of individuals at earlier stages may be quite beneficial in the DPN detection process, even prior to acute hyperglycemia diagnosis. Elevated HbA1C status in such populations is associated with the development of decreased motor and sensory nerve conduction velocities, which may arise out of acute bouts of hyperglycemia experienced though postprandial excursions (Marcovecchio et al., 2011; Smith & Singleton, 2013). Our participants were likely to report a variety of component changes, including psychometric properties that are evaluated and reported by this measure. Currently, each questionnaire has its strengths and should be applied accordingly.

Previous research has reported unknown completion times for the NeuroQOL-28 and QOL-DN (Smith et al., 2012). Our study, therefore, is the first to document time to completion for all three measures. Our finding that completion times were shorter for the QOL-DN and NeuroQOL-28 suggests that these two would ultimately make better choices for community

screening efforts, as evaluations will need to take these times into account for optimal participation. Both instruments can be employed within a short time, and the choice between which measure to use in future early DPN investigations is a difficult one, as these instruments are typically applied in populations that are likely further along in their disease process than the ones in this pilot work. On a practical note, quickly completed PROMs provide more leeway for integration into community screenings, but the measure also must be able to be quickly scored to be of immediate use to the individual. Of the 3 measures, the scoring is easiest for the NeuroQOL-28, which can be done by hand in a face-to-face setting as necessary within less than 5 minutes. The QOL-DN requires scoring, that although simple, requires additional time to provide feedback, likely needing contact information or a second reporting to disseminate results. The PN-QOL-97, while thorough, requires more elaborate scoring and calculations accomplished through programs such as Excel, and same day reported outcomes would not be realistic without the cooperation of multiple researchers with designated roles. Although not done in this study, examining these measures directly in the field, within whatever screening context they are being honed for, would have allowed for evaluation of these measures within the context of administering the instruments in a less controlled atmosphere and should be considered for future evaluations.

Our study has limitations that should be considered. We performed a pilot study, and generalizations may not be made to large populations. Lack of random assignment and use of volunteers for subjects created potential selection bias, with clinical population research targeting and low available funding heavily influencing this method. The HbA1C machine that was used within the study is a validated machine (Lenters-Westra & Slingerland, 2010), yet oral glucose tolerance testing is preferred by some research scientists, particularly for individuals with cardiac

autonomic neuropathy (CAN) (Farhan et al., 2012). We did not test for CAN and, therefore, cannot account for unknown discrepancies. The NC-Stat DPN Check device was used solely to test the sural nerve; therefore, deficits in nerve function relating to other nerves of the lower leg were not confirmed through this device. Previous research has not investigated the validity of the QOL-DN, PN-QOL-97 and the NeuroQOL-28 within an overweight, obese and inactive population and, therefore, this should be taken this into account when interpreting our findings. Furthermore, each of these instruments detects particular types of neuropathy, and we only assessed large fiber components with our NC-Stat DPN check device.

CONCLUSION

Both the QOL-DN and NeuroQOL-28 significantly predict neuropathy criterion standard components in OOI, PD and T2D subjects, adding validity to their use as screening measures as early DPN detection tools. The PN-QOL-97 effectively identified multiple DPN-related issues; however, its ability to predict our criterion standard was not statistically significant. Time completion studies revealed that the QOL-DN and NeuroQOL-28 may be posed as excellent short screening measures, completed in approximately 6 minutes or less, with reasonable scoring for both; however, if immediate feedback is needed, the NeuroQOL-28 is likely to be a better fit with time constraints and limited staff. Consideration should be given to adding fiber specific domains to the NeuroQOL-28 and psychological measures assessing the impact of depression to the QOL-DN, thus adding potential to both instruments to more closely align with different facets potentially experienced by the target population, hopefully increasing the power of their constructs. Asymmetry in NCS findings warrants proposing that future research consider how falls and injuries may contribute to the uneven pathogenesis of SNAP values in subacute and acute hyperglycemic populations and to further explore other options for effective screening for early DPN. Priority should be given to investigations seeking to evaluate the effectiveness of these tools to detect DN within early, DN prone, predefined populations, providing new opportunities to increase the effectiveness of these and other instruments in subclinical population screening efforts.

TABLES

Table IV.1
Participant Characteristics

Variable	Frequency	Percent
Gender		
Male	10	29.4
Female	24	70.6
Ethnicity		
Caucasian	22	64.7
African American	12	35.3
Diabetes Diagnosis		
None	15	44.1
Prediabetes	8	23.5
T2D	11	32.4
Neuropathy Diagnosis		
No Prior Diagnosis	28	82.4
Prior Diagnosis	6	17.6
Medication		
No Medication	8	23.5
Not T2D Specific	14	41.2
T2D Specific	10	29.4
T2D and Neuropathy	2	5.9
HbA1C Category		
OOI	10	29.4
PD	13	38.2
T2D	11	32.4
BMI Category		
Normal	1	2.9
Overweight	9	26.5
Obese	24	70.6

Table IV.2
Gender and Group Characteristics

	N	Min	Max	Mean	Std. Err	Std. Dev
Age						
Males	10	37.00	79.00	61.00	4.279	13.532
Females	24	35.00	74.00	57.20	2.364	11.581
Height						
Males	10	1.58	1.85	1.745	0.024	0.078
Females	24	1.48	1.74	1.66	0.013	0.064
Wt.						
Males	10	83.18	133.10	105.86	6.520	20.618
Females	24	65.36	122.73	89.40	3.083	15.103
Wt. By Group						
OOI	10	76.60	106.60	87.93	3.460	10.940
PD	13	65.90	133.10	98.03	7.350	23.260
T2D	11	78.40	127.70	101.29	5.590	17.680
BMI						
Males	10	28.20	41.50	34.85	1.570	4.966
Females	24	24.70	43.90	32.99	1.156	5.664
BMI by Group						
OOI	10	27.2	35.6	30.9	1.003	3.170
PD	13	24.7	43.9	34.2	1.860	6.707
T2D	11	27.0	41.5	35.1	1.516	5.029
HbA1C by Gender						
Males	9	4.4	7.1	6.0	0.289	0.915
Females	21	5.2	14.0	6.5	0.365	1.790
HbA1C by Group						
OOI	10	4.4	5.6	5.3	0.114	0.362
PD	13	5.6	6.4	5.9	0.06	0.218
T2D	11	6.5	14.0	7.8	0.632	2.095

Wt. = Weight in kg; OOI = Overweight, obese, inactive; T2D = Type 2 diabetes; BMI = Body mass index; HbA1C = hemoglobin A1C

Table IV.3
NCS Results By Group

	NC-Stat DPN Check - Sural Nerve					
	N	Min	Max	Mean	Std. Err	Std. Dev
SNAP-R (μV)						
OOI	10	2.0	14.3	6.631	1.444	4.567
PD	13	2.0	24.7	7.691	1.674	6.037
T2D	11	2.0	25.0	9.875	2.133	7.076
SNAP-L (μV)						
OOI	10	2.3	21.7	7.129	1.834	5.798
PD	13	3.0	21.7	7.277	1.186	4.277
T2D	11	3.0	21.7	10.572	2.064	6.847
SNCV-R (μV)						
OOI	10	35.3	55.7	46.2	1.902	6.016
PD	13	30.0	57.0	48.2	1.871	6.747
T2D	11	35.3	57.0	45.5	1.816	6.022
SNCV-L (μV)						
OOI	10	41.3	55.0	47.265	1.519	4.803
PD	13	43.0	55.0	49.637	1.072	3.865
T2D	11	37.3	57.0	46.876	1.946	6.455

*Displayed in untransformed form, as raw data
SNAP = sural nerve amplitude potential; SNCV = sural nerve conduction velocity

Table IV.4
Sural NCS, Signs and Symptoms

	Variable	Total	Group OOI	PD	T2D
Sural NCS *N* = 34	Normal	7	1	4	3
	Abnormal*	27	10	9	8
Signs *N* = 34	Tuning Fork				
	Normal	13	3	5	5
	Abnormal*	21	7	7	6
	Monofilaments 1-g				
	Normal	3	1	0	2
	Abnormal*	31	9	12	9
	10-g				
	Normal	3	1	0	2
	Abnormal*	31	9	13	9
Symptoms *N* = 34	None Reported	11	7	1	4
	Reported**	23	3	12	7
Autonomic *N* =34	None Reported	21	7	8	6
	Reported**	13	3	5	5
ADLS *N* = 34	None Reported	26	8	10	8
	Reported**	8	2	3	3
NCS, Sign & Symptom Combinations	AbNCS, Signs & Symptoms	17	3	9	5
	AbNCS, Signs or Symptoms	9	5	1	3
	AbNCS, No Signs or Symptoms	1	1	0	0
	NNCS, Signs & Symptoms	7	1	3	3

*Bilateral testing; abnormal findings on at least one limb; **Self-reported on QOL-DN
AbNCS = Abnormal nerve conduction study; NNCS = Normal nerve conduction study

Table IV.5
Spearman Partial Correlations

		RSNAP	LSNAP	RSNCV	LSNCV
QOL-DN		*N*=34	*N*=34	*N*=34	*N*=34
Total Score	Corr.	-0.289	-0.352	-0.004	-0.242
	Sig.	0.128	0.061	0.985	0.205
Large Fiber	Corr.	-0.275	-0.322	-0.058	-0.290
	Sig.	0.149	0.088	0.765	0.127
Small Fiber	Corr.	-0.251	-0.185	-0.340	-0.361
	Sig.	0.189	0.336	0.071	0.054
Symptoms	Corr.	-0.291	*-0.417	0.047	-0.102
	Sig.	0.126	0.024	0.808	0.597
ADLS	Corr.	-0.331	-0.260	0.066	-0.074
	Sig.	0.079	0.164	0.734	0.701
Autonomic	Corr.	-0.188	-0.297	-0.091	-0.188
	Sig.	0.328	0.117	0.638	0.329
PN-QOL-97		N=34	N=34	N=34	N=34
Physical	Corr.	0.350	*0.399	0.107	0.166
	Sig.	0.063	0.032	0.579	0.389
Mental	Corr.	*0.505	*0.479	0.052	-0.101
	Sig.	0.005	0.009	0.791	0.603
NeuroQOL-28		N=34	N=34	N=34	N=34
Total Score	Corr.	-0.194	-0.334	-0.288	-0.279
	Sig.	0.314	0.077	0.129	0.142
Neuropathy Specific	Corr.	-0.305	*-0.464	-0.177	-0.204
	Sig.	0.108	0.011	0.358	0.287
Overall QOL Judgement	Corr.	*0.523	*0.426	0.194	0.025
	Sig.	0.004	0.021	0.312	0.897

All correlations account for HbA1C, age, height and weight.
* significance at the .05 level

Table IV.6
Instrument Completion Times

	N	Mean	Std. Dev.	Std. Error
QOL-DN	34	5.17	1.834	0.315
NeuroQOL-28	34	5.58	3.566	0.612
QOL-97	34	13.23	3.606	0.618

Table IV.7
QOL-DN Regression Results

Model		Unstandardized Coeff.		Std. Coeff.	t	Sig.	95.0% Conf. Int. for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	28.084	7.291		3.852	.001	13.150	43.018
	Age	-.311	.066	-.629	-4.738	.000	-.446	-.177
	HbA1C	.262	.593	.069	.441	.663	-.954	1.477
	Body Mass Index	-.110	.149	-.101	-.741	.465	-.416	.195
	Symptoms	3.613	2.523	.347	1.432	.163	-1.555	8.780
	Total QOL	-2.719	1.096	-.550	-2.481	.019	-4.964	-.474

a. Dependent Variable: RLLOCF3Trials

Table IV.8
PN-QOL-97 Regression Results

Model		Unstandardized Coeff.		Std. Coeff.	t	Sig.	95.0% Conf. Int. for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-47.420	43.910		-1.080	.290	-137.853	43.014
	Age	-.348	.077	-.730	-4.525	.000	-.507	-.190
	HbA1C	2.608	1.090	.374	2.392	.025	.362	4.853
	Body Mass Index	-.207	.156	-.178	-1.326	.197	-.528	.114
	Physical Score	4.470	11.653	.076	.384	.705	-19.529	28.470
	Mental Score	12.102	6.456	.349	1.874	.073	-1.195	25.399

a. Dependent Variable: RLLOCF3Trials

Table IV.9
NeuroQOL-28 Regression Results

Model		Unstandardized Coeff.		Std. Coeff.	t	Sig.	95.0% Conf. Int. for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-.235	18.513		-.013	.990	-38.157	37.688
	Age	-.357	.067	-.721	-5.344	.000	-.494	-.220
	HbA1C	.918	.549	.243	1.671	.106	-.207	2.042
	Body Mass Index	.036	.151	.032	.236	.815	-.273	.345
	Neuropathy Specific	-5.358	6.438	-.129	-.832	.412	-18.545	7.829
	Overall QOL Judgement	15.748	6.180	.422	2.548	.017	3.089	28.406

a. Dependent Variable: RLLOCF3Trials

CHAPTER V

PROJECT III: MELATONIN AND THE AUTONOMIC NERVOUS SYSTEM

INTRODUCTION

The complications relating to diabetes are numerous, potentially relating to most regions of the body as microvascular pathology develops over an extended period, yet no complication of the disease may be as dangerous as cardiac autonomic neuropathy (CAN) (Vinik & Erbas, 2001; Ziegler et al., 1992). Mortality rates are significantly higher for individuals experiencing CAN when compared to patients without this particular pathology (Ewing & Clarke, 1986; Vinik, Maser, et al., 2003; Vinik & Erbas, 2006). CAN dysfunction and how it relates to diabetes is not clearly defined, although hyperglycemia appears to be related, with glycation end products playing a significant role in creating inflammation in microvascular processes (Hardeland, Cardinali, Brown, & Pandi-Perumal, 2015; Lieb et al., 2012; Tarvainen et al., 2014). A proinflammatory state has been associated with ANS damage in diabetes (Hardeland et al., 2011; Lieb et al., 2012) and sympathovagal imbalance may either result from or be the cause of an increased state of inflammation (Lieb et al., 2012), which plays a key role in the development of both T2D and atherosclerosis.

Interest has developed in melatonin as a substance that may provide answers for elements of dysfunction that arise in T2D, particularly those associated with circadian disorders (Ferrell & Chiang, 2015; Scheer et al., 2003; Spadoni et al., 2011). Melatonin regulates sleep patterns and wake cycles within healthy individuals, and is produced in circadian patterns (Claustrat et al., 2005; Spadoni et al., 2011). Sleep and wake cycles are often disturbed in T2D, and impaired

melatonin production is suspected, and thought to be related to the consequences of hyperglycemia (Reutrakul & Van Cauter, 2014).

Interruption in sleep and wake cycles may be particularly difficult for T2D patients, speeding along symptoms of the disease (Kreier et al., 2007). Melatonin synchronizes the biological clock (Reiter et al., 2007; Scheer et al., 2003), increasing restorative capabilities, lowering inflammation (Jung et al., 2010), and attenuates neurotransmitters of the sympathetic nervous system (Nishiyama et al., 2001; Vazan & Ravingerova, 2015). Thus, it has been hypothesized that melatonin may have promise to improve ANS balance, inflammation, oxidative stress, and glycemic control within the T2D patient (Hussain et al., 2006; Kedziora-Kornatowska et al., 2009; Paskaloglu et al., 2004; Reiter, 1995; Scheer et al., 2003; Tutuncu et al., 2005).

Given melatonin's effects on resetting the circadian clock of the ANS, we postulated that it might help improve ANS function in T2D patients. Thus, the purpose of this research was to investigate whether the underlying central, cardiac, and peripheral defects observed in T2D could be improved or reversed by this known chronotropic hormone given as a daily supplement.

METHODS

Participants

Sampling. A total of 10 adults of both sexes and varying ethnicities, 40–75 years of age, who had diagnosed T2D were recruited from a local population. Exclusionary criteria included: congestive heart failure, recent myocardial infarction, unstable arrhythmia, any cardiovascular event in the previous year, liver or kidney disease, severe orthostatic hypotension, active tobacco use, type 1 diabetes, hepatitis B or C, presence of HIV, active malignancy (diagnosed or treated with in the last year), nighttime shift work, current or recent use of supplemental melatonin, pregnancy and/or breast-feeding, or other serious medical conditions that investigators believed would compromise the subject's well-being or participation in the study.

Protection of Subjects. This research was approved by the Old Dominion University Institutional Research Board and Eastern Virginia Medical School's Institutional Review Board, and subjects participated in informed, signed consent procedures before participating (ODU IRB ID: 15-260). Documented, informed consent was obtained from each subject prior to participation. Throughout the study, patients were evaluated by a medical professional at each visit (baseline, 4 and 8 weeks) to the Eastern Virginia Medical School (EVMS) Strelitz Diabetes Center. Potential side effects were recorded for both the placebo and melatonin portions of the study.

Procedures

This study utilized a single over-the-counter daily dose of melatonin (10 mg) to determine its effect on both autonomic balance and baroreflex sensitivity. Up to 10 mg doses are safe for adults, (Burgess, Revell, & Eastman, 2008; Burgess, Revell, Molina, & Eastman, 2010)

and even as little as 0.5 mg can affect circadian rhythm entrainment (Hack, Lockley, Arendt, & Skene, 2003). Individuals were screened by phone as potential candidates for the study utilizing inclusion/exclusion criteria (see Appendix F) before scheduling volunteers to arrive in a fasted state for a 2-hour appointment at the EVMS Strelitz Diabetes Center, in Norfolk, VA. During visit one, the inclusion/exclusion form was completed (see Appendix F), and individuals meeting study requirements were consented into the study prior to receiving an exam that included height, weight, an electrocardiogram, blood pressure measurements (supine, standing, seated), medication recording, health condition disclosure, basic diabetes screening and a neurological physical to determine the health of the individual to participate in the research study (see Appendix E). Qualified candidates continued with testing, which included sleep questionnaires, HbA1C finger-stick testing, Sudoscan testing, HRV and baroreflex sensitivity testing (see Appendix G). Each of the 3 visits followed the same pattern, with HbA1C testing on visits 1 and 3, and 4-week melatonin or placebo assignment on visits 1 and 2.

HbA1C Testing. A Siemens DCA Vantage 2000 Analyzer (Lenters-Westra & Slingerland, 2010), DCA Vantage HbA1C test kits, alcohol prep pads, lancets, medical gloves, anti-bacterial wipes, and a Hazard Sharps container were utilized for finger-stick testing on visits 1 and 3.

Melatonin. After qualifying for the study and giving their informed consent, subjects were randomly assigned a tablet order. Subjects received a single 4-week quantity of 10 mg melatonin capsules or placebo capsules and were instructed to consume one capsule every evening 30 minutes before bedtime. The crossover dose (melatonin or placebo) was distributed to each subject after 4 weeks and compliance reassessed after the second 4 weeks until each subject had taken both melatonin and placebo. Commercially-produced pure melatonin capsules

(Life Extension, Ft. Lauderdale, FL) contained 10 mg each. Placebo capsules contained white flour.

Autonomic Nervous System Function Testing. Before and after each of the two 4-week trials (melatonin and placebo), the ANSAR device (ANSAR; ANX 3.0 software; ANSAR Group, Inc., Philadelphia, PA) was utilized to assess systemic (vagal) autonomic function and sympathetic balance (Vinik & Erbas, 2006). Subjects underwent three tests of autonomic function (R-R intervals): 1) deep breathing (expiratory/inspiratory ratio; E/I); 2) Valsalva maneuver (breath holding); and 3) postural change (standing from a seated position). All ANS testing was done at the same time of day both before and after supplementation to minimize individual diurnal variations of any residual melatonin following each overnight period.

Power Spectral Analysis. Power spectral analysis of HRV was performed with previously validated methods (La Rovere, Pinna, Maestri, & Sleight, 2012) under resting conditions with the ANSAR device for determination of low frequency (LF) and high-frequency (HF) components. The LF component of the power spectrum of HRV primarily is now considered to reflect baroreflex function. The HF component primarily reflects parasympathetic activity. LF/HF ratios were calculated to provide a measure of ANS balance. The total spectral power (TSP) was calculated, along with the standard deviation of all normal R-R intervals (SDNN), a measure of both sympathetic and parasympathetic action on HRV, and the root-mean square of the difference of successive R-R intervals (RMSSD), a measure primarily of parasympathetic activity (Vinik & Ziegler, 2007). Abnormalities in SDNN and RMSSD precede inflammation in adults with newly-diagnosed T2D (Lieb et al., 2012). All power spectral analyses were conducted at baseline and again after both 4-week intervals (10 mg melatonin and placebo).

Baroreflex Sensitivity Testing. IBSF was measured with the ANSAR device using validated methods (Dimitropoulos, Tahrani, & Stevens, 2014; La Rovere et al., 2012). Sudoscan (Aspire Medical Solutions, NY) testing was also used to quantify changes in sudomotor and small nerve fiber function (i.e., peripheral sympathetic tone) and, together with BSF, to determine parasympathetic balance. The physiological impact was evaluated through the effects of a high dose supplemental melatonin on autonomic balance in baroreflex sensitivity (BRS).

Sleep Quality. Although melatonin supplements may or may not improve sleep quality in older adults (Baskett et al., 2003; Baskett et al., 2001; Wade et al., 2007), to account for any possible effects of changes in sleep quality alone due to exogenous melatonin, subjects completed the Pittsburgh Sleep Quality Index (PSQI), (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989; Buysse et al., 1991; Lemoine, Wade, Katz, Nir, & Zisapel, 2012; Nunes et al., 2008; Yu et al., 2011), a validated, self-rated questionnaire assessing sleep quality and disturbances over a 1-month time interval, before and after each 4-week supplementation period. Nineteen individual items generated seven "component" scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction (see Appendix G).

Data Analyses

Statistical Evaluation. Repeated measures analysis of variance (ANOVA) was used to compare pre- and post-supplementation values for melatonin and placebo. Data were normalized. Relationships among autonomic function, baroreflex sensitivity, sleep quality measures, and melatonin dose were determined with Spearman's partial correlations. Friedman's ANOVA was employed for non-normally distributed data. Comparison of ANS

assessment variables were performed after age and HbA1C adjustment (Agelink et al., 2001; Rodrigues et al., 2010). The level of significance for all analyses was set at $p < 0.05$.

RESULTS

Ten individuals participated in a double blinded, randomized crossover study comparing 4 weeks of placebo to 4 weeks of 10 mg of melatonin supplementation. Participant characteristics are displayed in Table V.1. HbA1C values did not significantly differ from visit 1 to visit 3. Careful logging was kept of all potential side effects and events during the study. After medical examination, the only side effect reported possibly related to melatonin administration was sleepiness, as reported by one subject.

Autonomic Function & Power Spectral Analysis

Spearman's partial correlations accounted for age and HbA1C, and key correlations of interest are included in Table V.2 for review. Friedman's ANOVA tests were run to determine if there were significant differences between baseline, placebo and melatonin time points relating to autonomic ratio tests (E/I Ratio, Valsalva Ratio, 30:15 Ratio) and for all HRV variables. Ratio testing results indicate that there were increases from the baseline condition to placebo and from baseline to melatonin; however, none were statistically significant (see Table V.3). Assessment of individual ANSAR results indicated that that nine of ten individuals presented with initial ANS dysfunction, ranging from mild to advanced, with the ninth specifically demonstrated what was likely to be sympathetic withdrawal. Only one participant presented with no evidence of dysfunction at initial baseline evaluation. Four of these individuals also had abnormal Sudomotor function, further validating ANSAR findings.

Orthostatic hypotension (OH) testing revealed that none of our participants were experiencing it. This was tested twice each visit; once with ANSAR BP testing values (seated to standing) and once with clinic measures values (lying to standing). Likewise, HR was evaluated

twice each visit for tachycardia (90–130 bpm) (Dimitropoulos et al., 2014), and none experienced tachycardia, or even notable HR elevation/events throughout testing. All participants were within a normal or slightly bradycardic range at baseline, placebo and melatonin conditions. BP values did not vary significantly from baseline to placebo or baseline to melatonin within the conditions of deep breathing, Valsalva or postural change (30:15), with the exception of SBP response to deep breathing and SBP response to Valsalva (reported in Baroreflex Sensitivity).

Individual evaluation of frequency domain components, revealed that Valsalva LFnu was significantly different between the baseline, placebo and melatonin conditions (p = .045). Pairwise comparisons revealed significant differences between baseline and melatonin conditions (p = .042), but not between baseline and placebo (p =.353) or placebo and melatonin (p = .371) (see Table V.3). Valsalva HFnu was significantly different between the baseline, placebo and melatonin conditions (p = .045). Pairwise comparisons revealed significant differences between baseline and melatonin conditions (p = .042), but not between baseline and placebo (p = .353) or placebo and melatonin (p = .371). Analysis of other power spectral analysis components (LF, HF and LF/HF ratio) did not reveal any significant differences across the conditions, at any time point.

Standing SDNN, a time domain component, was significantly higher in the melatonin measurement (p = .032) than baseline or placebo time measurements. Pairwise comparisons revealed significant differences between placebo and melatonin (p = .042), but not between baseline and placebo (p = .371) or baseline and melatonin (p = .353). There were no other significant interactions relating to time domain variables. See Table V.3 for nonparametric Friedman ANOVA results.

Baroreflex Sensitivity

BP and HR were examined across all conditions (deep breathing, Valsalva, 30:15) via Friedman's ANOVA, and heart rates did not significantly differ across any of the tested conditions. SBP response, however, significantly differed across two tests. SBP changes related to deep breathing [X^2 (2, N = 10) = 6.821, p = .033] with pairwise comparisons indicate differences between baseline and the melatonin condition (p = .042). SBP changes during Valsalva [X^2 (2, N = 10) = 7.947, p = .019] were also present, with pairwise comparisons indicating significant differences between the placebo and melatonin conditions (p = .030).

A one-way repeated measures ANOVA examined differences in Sudoscan results at baseline, and after placebo and melatonin conditions. There were no outliers and the data were normally distributed at each time point, with the assumption of sphericity met by Mauchly's test of sphericity. There were no statistically different changes between the conditions over time, $F(2,18) = .055$, $p = .844$, $\eta^2 = .006$. There were no statistically significant differences between the means at the different time points ($p > .05$).

Sleep Quality

A Friedman's test was conducted to determine if there were significant differences between the baseline, placebo and melatonin Sleep Questionnaire scores. Median Sleep Quality scores were generated for all sleep components (subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, sleep medication, daytime dysfunction, and global PSQI (see Appendix G. Subjective sleep quality scores were significantly different between the baseline, placebo and melatonin scores, X^2 (3, N = 10) = 12.929, p = .002. Post hoc analysis revealed statistically differences in SSQ scores from placebo to melatonin ($p < .011$). An

evaluation of subjective sleep quality revealed significant differences between the groups ($p <$ .002) (Table V.5). Pairwise comparisons were performed with a Bonferroni correction for multiple comparisons and subjective sleep quality were significantly different between placebo (*Mdn* = .00) and melatonin (*Mdn* = 1.00) interventions ($p < .011$), not between the placebo and baseline or baseline and melatonin interventions (see Table V.6). Friedman's test was performed on sleep latency, sleep duration, sleep efficiency, sleep disturbances, sleep medication, daytime dysfunction, and global PSQI, to determine if there were differences between baseline, placebo and melatonin; however, differences were not statistically significant on these measures (see Table V.5).

DISCUSSION

This study aimed to determine baseline ANS function in individuals with T2D with the intent to investigate to what extent melatonin would improve individual measures. We hypothesized that the ANS misconducts in T2D, causing a neuroinflammatory response and leading to impairment, and that ANS function would improve in participants through melatonin supplementation. However, our results indicate unexpected significant changes from melatonin supplementation in the HRV variables of LFnu and HFnu. LFnu significantly increased from baseline after melatonin supplementation (p =.042), as did HFnu (p =.032) during the Valsalva portion of ANSAR testing. Heathers (2014) discusses the interpretation of HRV variables in great depth, explaining that the LF component's dominance is an ongoing debate, with multifaceted points to consider (Goldstein, Bentho, Park, & Sharabi, 2011; Heathers, 2014) and not to be confused with HRV components with normalized units, such as LFnu and HFnu. Theoretically, Heathers submits that one may view that LFnu and HFnu are extremely close in nature, more clearly representing a continuum of outcomes, representative of not just a deviation or change but potential changes that happen over a period or continuum and represent small deviations of measurement rather than two separate variables (Heathers, 2014). Such an explanation may be plausible over a dynamic activity, such as Valsalva conditions, where a continuum applies, yet only one specific reading is evaluated, suggesting that our results are worthy of additional exploration. An interesting finding in our study was a decrease in SBP during the Valsalva melatonin condition. When viewed collectively with HFnu and LFnu findings, it becomes of greater interest as we found a positive effect on lowering SBP during dynamic activity. Likewise, SBP dropped during the DB portion of the melatonin condition when compared to baseline, showing another adaptation that is likely due to our 4-week

intervention. Although not measured in the present study, research relating to melatonin supplementation in T2D should likely utilize 24-hr halter monitors that would capture a range of values and continuums to evaluate, during sleep, ADLS and activities, paired with clinically controlled ANSAR testing protocols.

We had hypothesized that ANS function would improve with melatonin supplementation, creating a positive effect and this was found to be partly true within our study. Power spectral measures, such as LF, which is generally thought to represent a balance of both the parasympathetic and sympathetic branches of the ANS and HF, a reflection of parasympathetic balance, did not significantly change across any of our testing conditions, despite changes in other measures of consideration. SDNN, however, which is a time domain component representing the standard deviation of normal RR intervals, rose in the standing condition in our population of T2D subjects. Subbalakshmi, Adhikari, Poornima, and KN (2015) investigated correlates of SDNN in T2D and found that higher heart rates, DBP and Q-T dysfunction were related to a reduction in SDNN in such individuals (Subbalakshmi et al., 2015). Our subjects were screened via ECG at each visit, and monitored for cardiac abnormalities throughout the study, and abnormal Q-T interval, DBP and elevated HR findings were not found in our cohort. Depressed or lowered values of SDNN have been associated with increases in risk of sudden death, particularly in patients with known heart issues (Farrell et al., 1991; Kleiger, Miller, Bigger, & Moss, 1987). Previous research indicates that E:I Ratio and SDNN are valid markers for monitoring CAN, yet our study revealed no significant differences in the evaluation of these ratios across baseline, placebo and the melatonin conditions (Subbalakshmi, Adhikari, Rao, & Jeganathan, 2012). Our findings suggest a potential positive effect on SDNN in a small pilot population of adults with T2D. Furthermore, our entire cohort was overweight or obese (BMI

Range: 28.7-47.0), with 9 individuals with a BMI over 30, with most participants on BP lowering medications. HRV parameters have been researched in similar cohorts with nonsignificant findings; however, this study indicates that individuals with overweight, obese status may benefit from melatonin supplementation to increase SDNN or impact key physiological processes, or potentially positively impact this HRV features before more extreme forms of dysfunction are diagnosed (Stuckey, 2013).

Baroreflex sensitivity was evaluated in our study, yet HR responses to SBP did not reveal any significant relationships, indicating that vagal cardiac activity most likely did not significantly change in relationship to HR. Some participants experienced mild bradycardia (58/59 BPM) during resting conditions, indicating higher parasympathetic tone and possible sympathetic withdrawal. Of interest, SBP did change in relationship to DB when considering the baseline to placebo and baseline to melatonin conditions. When examining the data, it is apparent that the placebo SBP rose higher during the placebo condition in comparison to baseline, whereas the melatonin SBP dropped significantly, moving the opposite direction. We cannot explain the rise in SBP across our participants, but it appears that the melatonin had a positive effect on SBP during DB. In a similar manner, SBP for Valsalva relating to the placebo and melatonin condition moved the same directions, with the melatonin SBP dropping and the placebo elevating, although neither was significant in relationship to baseline, the resulting SBPs were significantly different when compared to each other. Our results are similar to Cavallo, Daniels, Dolan, Bean, and Khoury (2004) who performed BP research with T1D individuals who utilized ambulatory BP monitors and took 10 mg of melatonin daily (Cavallo et al., 2004). This research documented decreases in SBP during sleep; however, our research differs in that they documented declines in DBP as well while we did not.

Our evaluation of Sudoscan measures to assess small fiber and autonomic nerve activity (baroreflex) found no significant differences between baseline, placebo and melatonin conditions, yet Sudoscan has become a validated and useful tool for measuring sudomotor changes at the microvascular level (Calvet, Dupin, Winiecki, & Schwarz, 2013). Our results suggest that a 4-wk 10 mg melatonin intervention does not impact measurable small fiber and baroreflex changes, although the potential effects of longer supplementation or different melatonin doses were not evaluated.

Subjective sleep quality significantly improved between the baseline and melatonin conditions, with 80% of subjects reporting improvements in sleep quality that were likely related to taking melatonin. While other measures (sleep latency, daytime dysfunction, global PSQI) improved from baseline to melatonin condition, the effect was not statistically significant. Similar research has indicated positive results in various PSQI sleep scores while utilizing 4 mg of melatonin supplementation one hour before bedtime over a 21-day time period, which differs from our methodology of administering 10 mg 30 minutes prior to bedtime over a 4-week period (Nunes et al., 2008). As observed in our subjects, 10 mg of melatonin at bedtime likely induces a positive, measurable effect on sleep quality in individuals with T2D.

Given that melatonin is secreted in the brain in all mammals, with primary synthesis rising from the pineal gland in a circadian manner orchestrated by the superchiasmatic nucleus, our introduction of a 10 mg supplementation of melatonin aimed to assist in what would, naturally be processes that are regulated by light and dark; however, how melatonin is used and orchestrated within the body can also be affected by medication usage. Our subjects had numerous health comorbidities and took numerous prescribed and over-the-counter medications during the course of the study, along with the melatonin supplements. Use of SSRIs to treat

depression is common in T2D, for example, and have debatable brain interactions (Härtter et al., 2001). BP medications have varying interactions with melatonin, as melatonin has been found to lower BP nocturnally in beneficial ways and NSAIDS are known to reduce the efficacy of or suppress melatonin synthesis, which may have contributed to a type II error in our study (Aygün, Kaplan, Odaci, Onger, & Altunkaynak, 2012; Grossman et al., 2006; Murphy, Myers, & Badia, 1996; Reiter, Guerrero, Escames, Pappolla, & AcuÑA-Castroviejo, 1997). Melatonin may protect against neurotoxicity, and combats oxidative effects. We did not, however, account for potential effects of other medications in this pilot study, although individuals with major psychotic conditions were excluded from participation.

For the future, a larger cohort, with stricter medication parameters for admission to the study would be recommended. While our testing measures were rigorous, an additional A1C value would be advantageous, as would a longer trial period, including a minimum time period of 8 weeks on 10 mg melatonin in order to evaluate potential positive effects. There are a number of limitations to consider with this study. We did not control the medications of individuals participating in the study and do not know how their individual medications relate to our melatonin intervention. We used HbA1C to check diabetes status, despite literature that indicates that it is not ideal for working with individuals that might have CAN; however, our participants did have a prior medical history to confirm their diagnoses. Melatonin and placebo tablets were distributed to the participants, and although compliance was monitored, we also trusted that they would regularly participate in the study measures by being responsible to comply with instructions.

CONCLUSION

Some beneficial effects on autonomic nervous system function, such as improved SDNN HRV measures, decreased SBP during deep breathing and Valsalva maneuvers, may result from supplementation with 10 mg of melatonin at bedtime over a 4-week time period in adults with T2D who have signs of CAN dysfunction. Its effect on other HRV parameters (LFnu and HFnu) warrants additional investigation to evaluate the backdrop of dynamics activities, such as Valsalva breathing maneuvers. Its positive impact on sleep quality is promising, but sleep quality studies involving T2D individuals should measure the effects of different doses over longer periods of time and possibly include control groups without diabetes. Future research should focus on examining melatonin's potential impact on SDNN in T2D individuals who exhibit normal BP and HR status, in an effort to determine how protective mechanisms may be developed in this population.

TABLES

Table V.1
Participant Characteristics 10

Variable		N	Mean	Std. Dev.	Std. Error	Min	Max
Age	Cohort	10	62.8	6.030	1.907	54	70
	Baseline	10	218.340	31.134	9.845	172.600	277.800
Weight	Placebo	10	218.600	30.592	9.674	174.200	280.400
	Melatonin	10	217.620	31.156	9.852	171.600	276.400
BMI	Overweight	1	28.700	-	-	-	-
Category	Obese	9	36.433	5.486	1.829	30.500	47.000
HbA1C	Baseline, Visit 1	10	7.0%	0.865	0.274	-	-
	Final, Visit 3	10	6.9%	0.596	0.188	-	-

Table V.2
Spearman's Partial Correlations

Variable	Correlation/Significance Value
Melatonin Valsalva LF/HF	Melatonin Valsalva LFnu .976, *p* =.000 Melatonin Valsalva HFnu -.976, *p* =.000
Melatonin Valsalva TSP	Time Domain SDNN Baseline.693, *p* =.026 Time Domain SDNN Placebo .729, *p* =.017 Time Domain SDNN Melatonin .891, *p* =.001
Placebo Valsalva TSP	Time Domain RMSSD Baseline.697, *p* =.025 Time Domain RMSSD Placebo .867, *p* =.001 Time Domain RMSSD Melatonin .745, *p* =.013

Table V.3
Log10 Transformed Nonparametric Friedman's ANOVA Results

	Mean Rank				Test Statistics		
	Baseline	Placebo	Melatonin	N	Sig.	Test Statistic	DOF
ANSAR Testing							
E/I Ratio	1.600	2.300	2.100	10	0.273	2.600	2
Valsalva Ratio	1.850	2.150	2.000	10	0.794	0.462	2
30:15 Ratio	1.450	2.450	2.100	10	0.071	5.282	2
Deep Breathing							
LF/HF	1.800	1.800	2.400	10	0.301	2.400	2
LF nu	1.800	1.800	2.400	10	0.301	2.400	2
HF nu	2.200	2.200	1.600	10	0.301	2.400	2
TSP	1.700	2.100	2.200	10	0.497	1.400	2
SDNN	1.600	1.900	2.500	10	0.122	4.200	2
RMSSD	1.750	2.050	2.200	10	0.575	1.105	2
Valsalva Ratio							
LF/HF	1.500	2.000	2.500	10	0.082	5.000	2
LF nu	1.400	2.100	2.500	10	*0.045	6.200	2
HF nu	2.600	1.900	1.500	10	*0.045	6.200	2
TSP	2.100	1.900	2.000	10	0.905	0.200	2
SDNN	2.100	2.000	1.900	10	0.905	0.200	2
RMSSD	2.450	1.500	2.050	10	0.097	4.667	2
30:15							
LF/HF	2.500	1.800	1.700	10	0.150	3.800	2
LF nu	2.500	1.600	1.900	10	0.122	4.200	2
HF nu	1.850	2.150	2.000	10	0.794	0.462	2
TSP	2.200	1.900	1.900	10	0.741	0.600	2
SDNN	1.900	1.500	2.600	10	*0.032	6.889	2
RMSSD	2.100	1.900	2.000	10	0.889	0.235	2

*Significance is set at .05; see pairwise comparisons table for details.

Table V.4
Log10 Transformed Nonparametric Pairwise Comparisons

Variables & Comparisons	Rank			Test Statistics			
Valsalva	Baseline	Placebo	Melatonin	N	Sig.	Test Statistic	DOF
LF nu	1.400	2.100	2.500	10	*0.045	6.200	2
Pairwise Comparisons	Test Statistic	Std. Error	Std. Test Statistic	N	Sig.	Adj. Sig.	
Baseline/Placebo	-0.700	0.447	-1.565	10	0.118	0.353	
Baseline/Mel	-1.100	0.447	-2.46	10	0.014	*0.042	
Placebo/Melatonin	-0.400	0.447	-0.894	10	0.371	1.000	
Valsalva	Baseline	Placebo	Melatonin	N	Sig.	Test Statistic	DOF
HF nu	2.600	1.900	1.500	10	*0.045	6.200	2
Pairwise Comparisons	Test Statistic	Std. Error	Std. Test Statistic	N	Sig.	Adj. Sig.	
Melatonin/Placebo	0.400	0.447	0.894	10	0.371	1.000	
Baseline/Melatonin	1.100	0.447	2.46	10	0.014	*0.042	
Placebo/Baseline	0.700	0.447	1.565	10	0.118	0.353	
30:15	Baseline	Placebo	Melatonin	N	Sig.	Test Statistic	DOF
SDNN	1.900	1.500	2.600	10	*0.032	6.889	2
Pairwise Comparisons	Test Statistic	Std. Error	Std. Test Statistic	N	Sig.	Adj. Sig.	
Baseline/Placebo	0.400	0.447	0.894	10	0.371	1.000	
Placebo/Melatonin	-1.100	0.447	-2.46	10	0.014	*0.042	
Baseline/Melatonin	-0.700	0.447	-1.565	10	0.118	0.353	

*Significance level is set at .05

Table V.5
Friedman's ANOVA Sleep Questionnaire Results

	Mean Rank			Test Statistics			
	Baseline	Placebo	Melatonin	N	Sig.	Test Statistic	DOF
Sleep Questionnaire							
Subjective Sleep Quality	1.800	1.450	2.750	10	*0.002	12.929	2
Sleep Latency	2.200	2.100	1.790	10	0.393	1.867	2
Sleep Duration	1.850	1.850	2.300	10	0.368	2	2
Sleep Efficiency	1.750	2.250	2.000	10	0.210	3.125	2
Sleep Disturbances	2.000	2.000	2.000	10	1.000	0.000	2
Sleep Medication	1.700	2.000	2.300	10	0.135	4.000	2
Daytime Dysfunction	2,25	1.900	1.850	10	0.368	2.000	2
Global PSQI	2.400	1.850	1.750	10	0.130	4.083	2

*Significance is set at .05; see pairwise comparisons table for details.

Table V.6
Nonparametric Pairwise Comparisons

Variables & Comparisons	Rank			Test Statistics			
	Baseline	Placebo	Melatonin	N	Sig.	Test Statistic	DOF
Subjective Sleep Quality	1.800	1.450	2.750	10	*0.002	12.929	2
Pairwise Comparisons	Test Statistic	Std. Error	Std. Test Statistic	N	Sig.	Adj. Sig.	
Baseline/Placebo	0.350	0.447	0.783	10	0.434	1.000	2
Placebo/Melatonin	-1.300	0.447	-2.907	10	0.004	0.011	2
Baseline/Melatonin	-0.950	0.447	-2.124	10	0.034	0.101	2

*Significance level is set at .05

Table V.7
Sudoscan Results

	Baseline		Placebo		Melatonin		N
Lg10+1Transformed	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	
Feet Mean ESC	1.748	0.142	1.761	0.173	1.746	0.186	10
Feet Mean Asymmetry	0.679	0.142	0.825	0.325	0.679	0.436	10
Hands Mean ESC	1.704	0.166	1.631	0.233	1.682	0.121	10
Hands Mean Asymmetry	0.814	0.367	0.815	0.404	0.754	0.280	10

CHAPTER VI

CONCLUSIONS

The impact of T2D is significant, affecting increasing numbers of individuals within the United States and across the globe in the range of 390−392 million and beyond at the present time. Despite current efforts, diabetes diagnoses have dramatically increased over the past two decades, rising from 7.6 million globally in 2004 to 21.9 million in 2014. Estimated impact by the year 2035 is projected to be in the 590 million range globally. Despite medical and research efforts, the disease has continued to increase in prevalence. With such rising numbers and increasing health impact, the research community must continue to seek out new ways to prevent and effectively manage the disease.

The research efforts within this dissertation include three distinct investigations related to neuropathy screening and treatment. Project I investigated the effectivenss of the 128-Hz tuning fork, 1-g and 1-g monofilament and the QOL-DN as potential early screening tools while comparing each measure back to the portable NC-Stat DPN Check nerve conduction device. These screening tools have been reported to be effective for the screening of DPN in T2D populations when used together or in conjunction with other bedside tests. Each screening tool has unique functions as to what type of sensation they are reported to detect and this should be considered when choosing screening options. Previous research has focused on T2D and limited PD populations for their research; however, we utilized these screening options within a mixed population of OOI, PD and T2D subjects. Our results indicated that the 1-g monofilament and the QOL-DN show promise for development for the screening of early DPN and each correlates well with the portable NC-Stat DPN Check device. Additionally, the 128-Hz tuning fork performed well as a measure within the study, despite a lack of correlation back to our criterion.

HRQOL measurements have become an interest in research, with emphasis developing on unique subsets of health linked to particular diagnoses such as DN. Several DN-related QOL instruments have been developed and implemented within research in the past several decades in an effort to detect DN-related health deficits, in hopes of revealing and treating related complications and improving QOL. Research efforts, beyond meta-analyses, to compare which measures might be most effective in an active research population have not been prevalent thus far, nor have many instruments been compared to nerve conduction studies. Portable nerve conduction units, such as the NC-Stat DPN Check have more recently come available; thus, in keeping with advancing neuropathy screening efforts, we sought to compare three accepted DN specific QOL measures in an OOI, PD and T2D population, while comparing our results back to the validated NC-Stat DPN Check device. Our results indicate that the QOL-DN and NeuroQOL-28 effectively predicted our neuropathy derived criterion, making them effective tools to administer in an OOI, PD and T2D population for early screening and detection of DPN. Effectively administered in approximately 6 minutes or less, both instruments are research screening friendly. The NeuroQOL-28 is the easiest to administer and score on site, making it ideal for community screening efforts.

Research literature proposes that T2D disrupts circadian rythms, alters autonomic function and places individuals with diabetes at significant risk for cardiovascular events. Studies have evaluated the effect of melatonin and found it to be beneficial for resetting sleep patterns in healthy adults. We sought to determine the effectiveness of resetting the ANS in individuals with T2D, with the hope of improving sleep patterns and positively altering HRV measures by adminisering a 10 mg dose of over-the-counter melatonin. Our investigation revealed that there were significant differences between HRV variables relating to LFnu and

HFnu in the Valsalva condition when comparing the baseline to melatonin and placebo treatments. This indicates a unique phenomenon to be evaluated further in order to investigate the continuum of how dynamic movement HRV measures are affected by melatonin supplementation. SBP in deep breathing and SBP in the Valsalva maneuver were significantly lowered following melatonin supplementation, indicating a positive effect. SDNN was signficantly different from baseline to melatonin and between the placebo and melatonin condition, also indicating a beneficial effect from a 4-wk 10 mg melatonin supplementation. Sleep quality measures showed significant changes in subjective sleep quality measures, suggesting that melatonin had a positive impact on perceived sleep quality.

While these pilots studies show promising results, more research in all of these areas is needed. Future research should focus on the continued development of effective methods for early detection, disease assessment, management and reversal in DN prone populations to facilitate the best possible health outcomes.

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APPENDICES

A. SCREENING QUESTIONNAIRE

Screening Questionnaire

Name: _____ Date: _____

Please answer the following questions as completely and honestly as possible.

Current Age: _____ Gender: _____

Circle **One**

Yes No Have you ever been told you have high blood sugar, prediabetes or diabetes?

Yes No Do you have a first degree relative that has diabetes?

Yes No Have you been told that you have type I diabetes?

Yes No Have you been told that you have type 2 diabetes?

If yes, how long have you had diabetes? _____ years/diagnosis date

Yes No Have you been told you have hepatitis B or C?

Yes No Have you been told you have HIV?

Yes No Do you currently have a sore, ulcer, cut or other damage to either foot?

Yes No Have you ever had any part of either lower extremity amputated?

Yes No Do you have any numbness or pain in your feet?

Yes No Do you have any type of foot deformity?

Yes No Have you been diagnosed with peripheral vascular disease or nerve problems in your lower extremities?

Yes No Have you been diagnosed with kidney or liver problems, or are you on dialysis?

Yes No Are you currently on any medications? (Please list at the bottom)

Yes No Do you have any type of visual impairment?

Yes No Do you smoke?

If yes to any of the above, please explain:

_____

_____

To be filled out by research staff: Height: _____ Weight: _____

BMI: _____Waist circumference: _____ Seated BP: _____

Approved to be in the study? _____ HbA1C Value: _____

B. QOL-DN

Quality of Life Questionnaire (QOL-DN)

Diabetic Neuropathy Version

Name:_____**Date:**_____
Subject #:_____**Visit:** _____
Date of Birth:_____**Gender:** □Male □Female

Race: □White □Black □Hispanic
□Native American[1] □Pacific Area[2] □Other:
[1] Native American includes American Indian, Eskimo, and Aleut
[2] Pacific Area Embraces Polynesian (including Hawaiian and Samoan), Micronesian (including

Do you have diabetes? □ Yes □ No

Do you have neuropathy (nerve damage)? □ Yes □ No

Do you have any known medical condition that causes pain or weakness? □ Yes □ No
-- If yes, what condition: _____

How long have you had any symptoms of neuropathy?_____Years_____Months

Are the symptoms the same on the right as on the left? ..... □ Yes
□ No…which is worse? □ Left □ Right
…only one side? □ Left □ Right

Are the symptoms usually worse at night? ..... □ Yes □ No

How many medications or other treatments have you used for any of these symptoms (both in the past and presently)?

Please write the number on the line. _____

Have you ever been told that you have neuropathy? ..... □ Yes □ No
Have you ever had ulcer(s) on your feet?..... □ Yes □ No
Have you ever had gangrene? ..... □ Yes □ No
Have you had any toes (or fingers) amputated?..... □ Yes □ No

In the past 4 weeks, have you had a problem with involuntary urinating when laughing or coughing?
□ Yes □ No

(MALES ONLY) In the past 4 weeks, have you had a problem with obtaining or maintaining erections?
□ Yes □ No

(FEMALES ONLY) In the past 4 weeks, have you had a problem with vaginal dryness during intercourse?
□ Yes □ No

Part I: Symptoms

Have you had any of the following symptoms in the past 4 weeks? Please Check all that apply.

	Feet	Legs	Hands	Arms	None
1. Numbness	□		□	□	
2. Tingling, Pins and Needles	□		□	□	
3. Electric Shocks	□		□	□	
4. Other Unusual Sensations	□		□	□	
5. Superficial Pain	□		□	□	
6. Deep Pain	□		□	□	
7. Weakness	□		□	□	

Part II: Activities of Daily Life

Answer these questions according to the following scale:	Not a problem **0**	Very mild problem **1**	Mild problem **2**	Moderate problem **3**	Severe problem **4**
8. In the past 4 weeks, has pain kept you awake or woken you at night?	□	□	□	□	□
9. In the past 4 weeks, has the touch of bed sheets, clothes, or wearing shoes	□	□	□	□	□
10. In the past 4 weeks, have you burned or injured yourself and been unable to feel it?	D	D	D	D	D
11. In the past 4 weeks, have any symptoms kept you from doing your usual activities during the day?	D	D	D	D	D
12. In the past 4 weeks, have you had difficulty doing fine movements with your fingers, like buttoning your clothes, turning pages in a book, picking up coins from a table?	D	D	D	D	D
13. In the past 4 weeks, have you felt unsteady on your feet when you walk?	D	D	D	D	D
14. In the past 4 weeks, have you had any problem getting out of a chair without pushing with your hands?	D	D	D	D	D
15. In the past 4 weeks, have you had a problem walking down stairs?	D	D	D	D	D
16. In the past 4 weeks, have you been unable to feel your feet when walking?	D	D	D	D	D
17. In the past 4 weeks, have you been unable to tell hot from cold water **with your hands**?	D	D	D	D	D
18. In the past 4 weeks, have you been unable to tell hot from cold water **with your feet**?	D	D	D	D	D
19. In the past 4 weeks, have you had a problem with vomiting, particularly after meals (but not due to flu or other illness)?	D	D	D	D	D
20. In the past 4 weeks, have you had a problem with diarrhea and/or loss of bowel control?	D	D	D	D	D
21. In the past 4 weeks, have you had a problem with fainting or dizziness when you stand?	D	D	D	D	D

In the past 4 weeks, how much difficulty have you had performing the following activities:

22. Bathing/Showering?	D	D	D	D	D
23. Dressing?	D	D	D	D	D
24. Walking?	D	D	D	D	D
25. Getting on or off the toilet?	D	D	D	D	D
26. Using eating utensils?	D	D	D	D	D

Answer these questions according to the following scale:	Not at all **0**	A little **1**	Somewhat **2**	Moderately **3**	Severely **4**
In the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical or emotional health?					
27. Cut down on the amount of time you spent on work or other activities?……	D	D	D	D	D
28. Accomplished less than you would like?…….	D	D	D	D	D
29. Were limited in the kind of work or other activities you could perform?…….....	D	D	D	D	D
30. Had difficulty performing the work/other activities (it took extra effort)?……..	D	D	D	D	D

31. In general, would you say your health now is:

Excellent	Very Good	Good	Fair	Poor
D	D	D	D	D

32. Compared with 3 months ago, how would you rate your health in general now?

Much Better	Somewhat Better	About the Same	Somewhat Worse	Much Worse
D	D	D	D	D

Answer these questions according to the following scale:	Not at all **0**	A little **1**	Somewhat **2**	Moderately **3**	Severely **4**
33. In the past 4 weeks, to what extent has your physical health interfered with your normal social activities with family, friends, neighbors, or groups?	D	D	D	D	D
34. In the past 4 weeks, how much did **pain** interfere with your normal work (including work both outside the home and housework)?	D	D	D	D	D
35. In the past 4 weeks, how much did **weakness or shakiness** interfere with your normal work (including work both outside the home and housework)?	D	D	D	D	D

Manual and Scoring Algorithm for QOL-DN
1) Description:

The QOL-DN is a self-administered questionnaire, designed to capture and quantify the impact of diabetic neuropathy on the quality of life of individual patients with diabetic neuropathy. Fourteen of the items are of a health-related, biographical nature and are not scored. These are on the front page, and they are not numbered nor scored. The remaining 35 scored questions are numbered items that comprise the entire scale, and they are arranged thematically so that the wording of the questions and the type of response is grouped together. However, the content and topic of each individual question concerns particular functions or symptoms that are related to the following themes:

Total Quality of Life Score

Physical Functioning/Large Fiber Neuropathy

Activities of Daily Living (ADLs)

Symptoms

Small Fiber Neuropathy

Autonomic Neuropathy

These scales and the administration of the questionnaire are described in detail below. In general, items 1-7 (Part I) are a simple inventory of symptoms of neuropathy. The presence of the symptom is checked in whichever box applies, and an absence of a symptom is checked under "none." Positive responses are scored as 1; and negative responses, as 0. Items 8-35 (Part II) pertain to Activities of Daily Life, and most of these are scaled on a 5-point Likert scale ranging from 0 ("Not a problem") to 4 ("Severe problem"). However, Questions 31 and 32 are scored differently. In Question 31, "Good", the middle item, is scored as O. "Very Good" is scored as –1, Excellent" is scored as –2. "Fair is scored as 1, and "Poor" is scored as 2. In Question 32, "About the Same", the middle item, is scored as 0. "Somewhat better" is scored as –1, "Much better" is scored as -2. "Somewhat worse" is scored as 1, and "Much worse" is scored as 2.
A final important point of the overall instrument is that the patient/subject is instructed to rate most items **over the last 4 weeks**, so responses should be interpreted as cumulative over that time period - not merely an inventory of the patient's status at the moment of filling out the questionnaire.

2) Administering the questionnaire:

Administering the questionnaire to the patient or experimental subject is very straightforward: the patient simply fills out the paper form. It is important that the patient is in a quiet area, free of undue distractions, and patients are encouraged to answer the questions themselves (i.e. spouses and significant others should not fill out the questionnaire or influence the patient's responses). *These are subjective patient responses.* The responses are coded and scored when they are entered into the appropriate database, and the algorithm is supplied below. All questions should be answered. The gender-specific sexual functions questions located on the biographical page should obviously be answered according to gender. It is not recommended to compare responses on this questionnaire directly to the patient's medical history or any other sources of similar information such as other pain questionnaires, etc.

3) Data Accumulation:

De-identified data are accumulated in database format (e.g. MS Excel 2000) and entered by a HIPAA certified research assistant. The original hard copies of the responses are retained as source documents in the patient/subject's medical record. The database is secured by password access to authorized users only. The structure is that of a single table containing all fields for a single questionnaire.

4) Sub-scales and Scoring Algorithm:

The scales listed above were determined based on an exploratory factor analysis, so the questions have loaded into their respective domains. All symptoms (1-7) are scored as either a 1 or a 0, indicating a presence or absence of the symptom. With the exception of Questions 31, and 32, the other items are scored according to the 5-point Likert Scale (0-4, "No Problem" to "Severe Problem"). In Question 31, "Good", the middle item, is scored as O. "Very Good" is scored as –1, Excellent" is scored as –2. "Fair is scored as 1, and "Poor" is scored as 2. In Question 32, "About the Same", the middle item, is scored as 0. "Somewhat better" is scored as –1, "Much better" is scored as -2. "Somewhat worse" is scored as 1, and "Much worse" is scored as 2.
The Total QOL and five domains should be summed as follows:
Total QOL Σ(1-7, 8-35)
Physical Functioning/Large Fiber Σ(8, 11, 13-15, 24, 27-35)
Activities of Daily Living (ADLs) Σ(12, 22, 23, 25, 26)
Symptoms Σ(1-7, 9)
Small Fiber Σ(10, 16, 17, 18)
Autonomic Σ(19, 20, 21)

These scales and subscales are calculated without weighting of any kind, and reported as the integer sum of the listed questionnaire items.

C. PN-QOL-97

Peripheral Neuropathy

Quality-of-Life Instrument - 97

- Section I -

The questions in **Section I** ask for your views about your health in general.

As you answer these questions, please consider **ALL** your health problems.

1. In general, would you say your health is: *(Mark an ☒ in the one box that best describes your answer.)*

   Excellent ..... ☐ 1
   Very good ..... ☐ 2
   Good ..... ☐ 3
   Fair ..... ☐ 4
   Poor ..... ☐ 5

2. **Compared to six months ago**, how would you rate your health in general **now**?

   Much better now than six months ago ..... ☐ 1
   Somewhat better now than six months ago ..... ☐ 2
   About the same ..... ☐ 3
   Somewhat worse now than six months ago ..... ☐ 4
   Much worse now than six months ago ..... ☐ 5

3. The following questions are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

(Mark an ☒ in a box on each line.)

	Yes, limited a lot	**Yes, limited a little**	**No, not limited at all**
a. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c. Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d. Climbing **several** flights of stairs	☐ 1	☐ 2	☐ 3
e. Climbing **one** flight of stairs	☐ 1	☐ 2	☐ 3
f. Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g. Walking **more than a mile**	☐ 1	☐ 2	☐ 3
h. Walking **several hundred yards**	☐ 1	☐ 2	☐ 3
i. Walking **one hundred yards**	☐ 1	☐ 2	☐ 3
j. Bathing and dressing yourself	☐ 1	☐ 2	☐ 3

4. During the **past 4 weeks**, how much of the time have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**? *(Mark an ☒ in a box on each line.)*

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the **amount of time** you spent on work or other activities?	☐1	☐2	☐3	☐4	☐5
b. **Accomplished less** than you would have liked?	☐1	☐2	☐3	☐4	☐5
c. Were limited in the **kind** of work or other activities?	☐1	☐2	☐3	☐4	☐5
d. Had **difficulty** performing the work or other activities? (for example, it took extra effort)	☐1	☐2	☐3	☐4	☐5

5. During the **past 4 weeks**, how much of the time have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)? *(Mark an ☒ in a box on each line.)*

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spend on work or other activities?	☐1	☐2	☐3	☐4	☐5
b. Accomplished less than you would have liked?	☐1	☐2	☐3	☐4	☐5
c. Did work or other activities less carefully than usual?	☐1	☐2	☐3	☐4	☐5

6. During the **past 4 weeks**, to what extent have your **physical health or emotional problems** interfered with your normal social activities with family, friends, neighbors, or groups? *(Mark an ☒ in one box that best describes your answer.)*

- Not at all ☐1
- Slightly ☐2
- Moderately ☐3
- Quite a bit ☐4
- Extremely ☐5

7. How much **bodily** pain have you had during **the past 4 weeks**?

- None ☐1
- Very mild ☐2
- Mild ☐3
- Moderate ☐4
- Severe ☐5
- Very severe ☐6

8. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- Not at all ☐1
- A little bit ☐2
- Moderately ☐3
- Quite a bit ☐4
- Extremely ☐5

9. These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks** . . .

(Mark an ☒ in a box on each line.)

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	☐1	☐2	☐3	☐4	☐5
b. Have you been very nervous?	☐1	☐2	☐3	☐4	☐5
c. Have you felt so down in the dumps that nothing could cheer you up?.....	☐1	☐2	☐3	☐4	☐5
d. Have you felt calm and peaceful?	☐1	☐2	☐3	☐4	☐5
e. Did you have a lot of energy?	☐1	☐2	☐3	☐4	☐5
f. Have you felt downhearted and depressed?	☐1	☐2	☐3	☐4	☐5
g. Did you feel worn out?	☐1	☐2	☐3	☐4	☐5
h. Have you been happy?	☐1	☐2	☐3	☐4	☐5
i. Did you feel tired?	☐1	☐2	☐3	☐4	☐5
j. Have you felt depressed?	☐1	☐2	☐3	☐4	☐5
k. Have you enjoyed life?	☐1	☐2	☐3	☐4	☐5

10. During the **past 4 weeks**, how much of the time have your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

(Mark an ☒ in the one box that best describes your answer.)

- All of the time ☐ 1
- Most of the time ☐ 2
- Some of the time ☐ 3
- A little of the time ☐ 4
- None of the time ☐ 5

11. Please choose the answer that best describe how TRUE or FALSE each of the following statements is for you.

(Mark an ☒ in a box on each line.)

	Definitely True	Mostly True	Not Sure	Mostly False	Definitely False
a. I seem to get sick a little easier than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. I have been feeling bad lately	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f. I am somewhat ill	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

12. For how many of the **last 30 days** have you been unable to work or attend school **because of your health** (for **any** health reason)?

(Write in a number between 0 and 30)

_____ days out of the last 30 days.

13. How much of the time during the **past 4 weeks** . . .

(Mark an ☒ in a box on each line.)

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Were you frustrated about your health?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Was your health a worry in your life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Did you feel weighed down by your health problems?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Have you had difficulty concentrating and thinking? . ..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. Did you have trouble keeping your attention on an activity for long?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f. Have you had trouble with your memory?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

- Section II -

The following questions in **Section II** ask for your views about your symptoms and problems **due to your peripheral neuropathy.** As you answer these questions, please consider **ONLY** your **peripheral neuropathy.**

14. **Because of your peripheral neuropathy,** how much difficulty have you had performing the following activities during the **past 4 weeks**?

(Mark an ☒ in a box on each line.)

	No difficulty	A little difficulty	Moderate difficulty	A great deal of difficulty	Unable to do
a. Washing your hair?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Working buttons, zippers or laces?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Walking up a ramp?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Handwriting or printing? .	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. Doing work around the house such as cleaning, yard work, or home maintenance?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f. Going to social events outside your home?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g. Holding onto or using small objects such as keys, pens, or coins?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

15. **Because of your peripheral neuropathy**, how careful have you had to be to avoid falling when moving during the **past 4 weeks**?

(Mark an ☒ in the one box that best describes your answer.)

- Didn't have to be careful ☐ 1
- A little careful ☐ 2
- Moderately careful ☐ 3
- Very careful ☐ 4
- Extremely careful ☐ 5

16. **Because of your peripheral neuropathy**, how much pain in your hands have you had during the **past 4 weeks**?

- None ☐ 1
- Very mild ☐ 2
- Mild ☐ 3
- Moderate ☐ 4
- Severe ☐ 5
- Very severe ☐ 6

17. **Because of your peripheral neuropathy**, how much pain in your feet have you had during the **past 4 weeks**?

- None ☐ 1
- Very mild ☐ 2
- Mild ☐ 3
- Moderate ☐ 4
- Severe ☐ 5
- Very severe ☐ 6

18. Some people are bothered by the **effects of peripheral neuropathy** on their daily life, while others are not. How much do the **effects of peripheral neuropathy** bother you in each of the following areas?

(Mark an ☒ in a box on each line.)

	Not at all bothered	Some-what bothered	Moderately bothered	Very much bothered	Extremely bothered	Don't know
a. Your sleep?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
b. Your ability to work at a paying job?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
c. Your ability to travel?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
d. Your energy level?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
e. Your ability to walk on slick or slippery surfaces?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
f. Your ability to walk on ramps, driveways, or other surfaces that are not level?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
g. Your ability to walk on rough surfaces?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
h. Avoiding objects that could make you fall?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

19. During the **past 4 weeks**, to what extent have **symptoms of your peripheral neuropathy** interfered with your normal social activities with family, friends, neighbors, or groups?

(Mark an ☒ in the one box that best describes your answer.)

- Not at all ☐ 1
- A little bit ☐ 2
- Moderately ☐ 3
- Quite a bit ☐ 4
- Extremely ☐ 5

20. For how many of the **past 30 days** have you been unable to work or attend school **because of your peripheral neuropathy**?

(Write in a number between 0 and 30)

_____ days out of the last 30 days.

21. Overall, during **the past 6 months,** how would you rate the severity of your peripheral neuropathy symptoms?

(Mark an ☒ in the one box that best describes your answer.)

- No symptoms ☐ 1
- Mild symptoms ☐ 2
- Moderate symptoms ☐ 3
- Severe symptoms ☐ 4
- Extremely severe symptoms ☐ 5

22. How TRUE or FALSE is each of the following statements for you.

(Mark an ☒ in a box on each line.)

	Definitely True	Mostly True	Not Sure	Mostly False	Definitely False
a. I am embarrassed about how I look in public	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I am comfortable in social situations	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. I avoid doing some things in public because of my peripheral neuropathy	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. I worry about falling in front of other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. I feel well-coordinated	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f. I often have to explain how my peripheral neuropathy limits what I can do	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

23. How TRUE or FALSE is each of the following statements for you.

Because of your peripheral neuropathy, other people . . .

(Mark an ☒ in a box on each line.)

	Definitely True	Mostly True	Not Sure	Mostly False	Definitely False
a. Are uncomfortable around you	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Treat you as inferior	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Avoid you	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

- Section III -

These questions are about general symptoms or health problems you may or may not have.

24. For each symptom, please indicate **how much it bothered you during the past 4 weeks.** In answering each question, if you do not have that symptom at all, please indicate that you are **not at all bothered.**

(Mark an ☒ in a box on each line.)

	Not at all bothered	Some-what bothered	Moderately bothered	Very much bothered	Extremely bothered
a. Muscle or joint aches?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Pain in arms or legs?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. "Shaky" hands?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Unsteadiness on your feet?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. Trouble with your balance?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f. Difficulty feeling the shape of objects in your hand?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g. Stiffness or tightness of your hands or feet?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

The next set of questions is about your sexual function and your satisfaction with sexual function.

25. **During the past 4 weeks,** how much of a problem for you was lack of sexual interest? *(Mark an ☒ in the one box that best describes your answer.)*

- Not a problem ☐ 1
- A little of a problem ☐ 2
- Somewhat of a problem ☐ 3
- Very much of a problem ☐ 4

26. **During the past 4 weeks,** did your health interfere with your sexual relationships?

- No, not at all ☐ 1
- Yes, a little bit ☐ 2
- Yes, somewhat ☐ 3
- Yes, quite a bit ☐ 4
- Yes, a great deal ☐ 5

27. Overall, how satisfied were you with your sexual function **during the past 4 weeks?**

- Very satisfied ☐ 1
- Somewhat satisfied ☐ 2
- Neither satisfied nor dissatisfied ☐ 3
- Somewhat dissatisfied ☐ 4
- Very dissatisfied ☐ 5

28. Have you had any sexual activity **in the past 4 weeks?**

(Mark an ☒ in the one box that best describes you answer)

- Yes ☐ 1
- No ☐ 2

29. How often **during the past 4 weeks** did you . . .

(Mark an ☒ in a box on each line.)

	All of the time	Most of the time	Some of the time	Little of the time	None of the time
a. Get enough sleep to feel rested upon waking in the morning?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 6
b. Awaken short of breath or with a headache?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 6
c. Have trouble falling asleep?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 6
d. Have trouble staying awake during the day?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 6

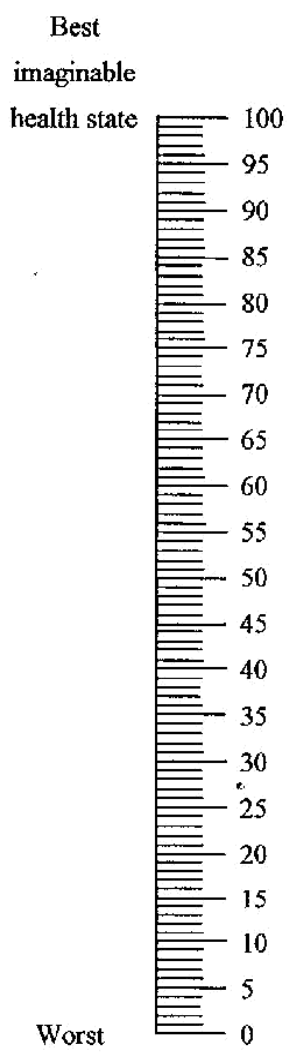
- Section IV -

This question asks about your current overall health.

30. To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked by 100 and the worst state you can imagine is marked by 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your current health is.

Your own health today



On the 0 to 100 thermometer above, my own health today is: _____.

- Section V -

The questions in **Section V** ask about your feelings in general, not just those related to your health.

31. How much do you agree or disagree with each statement below?

(Mark an ☒ in a box on each line)

	Strongly agree	Agree	Uncertain	Disagree	Strongly disagree
a. I feel that I have a number of good qualities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I feel I do not have much to be proud of	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. On the whole, I am satisfied with myself	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. I certainly feel useless at times	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. At times I think I am no good at all	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

SCORING ALGORITHM
PERIPHERAL NEUROPATHY QOL-97 SCORING FORM

Scale/Item Numbers	Response (raw score) 1	2	3	4	5	6	Subtotal Final Score 0-100 point scale
Physical Health Dimensions							
Physical Functioning-11							
3a	0	50	100	-	-	-	____
3b	0	50	100	-	-	-	____
3c	0	50	100	-	-	-	____
3d	0	50	100	-	-	-	____
3e	0	50	100	-	-	-	____
3f	0	50	100	-	-	-	____
3g	0	50	100	-	-	-	____
3h	0	50	100	-	-	-	____
3i	0	50	100	-	-	-	____
3j	0	50	100	-	-	-	____
14c	100	75	50	25	0	-	____
			Total	____	÷	11 =	____
Role Limitations Due to Physical Health-6							
4a	0	25	50	75	100	-	____
4b	0	25	50	75	100	-	____
4c	0	25	50	75	100	-	____
4d	0	25	50	75	100	-	____
18b [1]	100	75	50	25	0	-	____
14e	100	75	50	25	0	-	____
			Total	____	÷	6 =	____
Disease-Targeted Pain							
7	100	80	60	40	20	0	____
8	100	75	50	25	0	-	____
16	100	80	60	40	20	0	____
17	100	80	60	40	20	0	____
24a	100	75	50	25	0	-	____
24b	100	75	50	25	0	-	____
24g	100	75	50	25	0	-	____
			Total	____	÷	7 =	____

[1] For question 18a–18h recode to missing if the response is “Don’t know.”

SCORING ALGORITHM (cont.)
PERIPHERAL NEUROPATHY QOL-97 SCORING FORM

Scale/Item Numbers	Response (raw score) 1	2	3	4	5	6	Subtotal Final Score 0-100 point scale
Energy/Fatigue-5							
9a	100	75	50	25	0	-	____
9e	100	75	50	25	0	-	____
9g	0	25	50	75	100	-	____
9i	0	25	50	75	100	-	____
18d	100	75	50	25	0	-	____
			Total	____	÷	5 =	____
Upper Extremities							
14a	100	75	50	25	0	-	____
14b	100	75	50	25	0	-	____
14d	100	75	50	25	0	-	____
14g	100	75	50	25	0	-	____
24c	100	75	50	25	0	-	____
24f	100	75	50	25	0	-	____
			Total	____	÷	6 =	____
Balance							
15	100	75	50	25	0	-	____
18e	100	75	50	25	0	-	____
18f	100	75	50	25	0	-	____
18g	100	75	50	25	0	-	____
18h	100	75	50	25	0	-	____
22e	100	75	50	25	0	-	____
24d	100	75	50	25	0	-	____
24e	100	75	50	25	0	-	____
			Total	____	÷	8 =	____
<u>Mental Health Dimensions</u>							
Self Esteem							
22a	0	25	50	75	100	-	____
31a	100	75	50	25	0	-	____
31b	0	25	50	75	100	-	____
31c	100	75	50	25	0	-	____
31d	0	25	50	75	100	-	____
31e	0	25	50	75	100	-	____
			Total	____	÷	6 =	____

SCORING ALGORITHM (cont.)
PERIPHERAL NEUROPATHY QOL-97 SCORING FORM

Scale/Item Numbers	Response (raw score) 1	2	3	4	5	6	Subtotal	Final Score 0-100 point scale
Emotional Well Being-7								
9b	0	25	50	75	100	-		____
9c	0	25	50	75	100	-		____
9d	100	75	50	25	0	-		____
9f	0	25	50	75	100	-		____
9h	100	75	50	25	0	-		____
9j	0	25	50	75	100	-		____
9k	100	75	50	25	0	-		____
			Total	____	÷	7 =		____
Stigma								
23a	0	25	50	75	100	-		____
23b	0	25	50	75	100	-		____
23c	0	25	50	75	100	-		____
			Total	____	÷	3 =		____
Cognitive Function								
13d	0	25	50	75	100	-		____
13e	0	25	50	75	100	-		____
13f	0	25	50	75	100	-		____
			Total	____	÷	3 =		____
Role Limitations Due to Emotional Problems								
5a	0	25	50	75	100	-		____
5b	0	25	50	75	100	-		____
5c	0	25	50	75	100	-		____
			Total	____	÷	3 =		____
General Health Dimensions								
General Health Perceptions-7								
1	100	75	50	25	0	-		____
11a	0	25	50	75	100	-		____
11b	100	75	50	25	0	-		____
11c	0	25	50	75	100	-		____
11d	100	75	50	25	0	-		____
11e	0	25	50	75	100	-		____
11f	0	25	50	75	100	-		____
			Total	____	÷	7 =		____

SCORING ALGORITHM (cont.)
PERIPHERAL NEUROPATHY QOL-97 SCORING FORM

Scale/Item Numbers	Response (raw score) 1	2	3	4	5	6	Subtotal Final Score 0-100 point scale
Sleep							
18a	100	75	50	25	0	-	____
29a	100	75	50	25	0	-	____
29b	0	25	50	75	100	-	____
29c	0	25	50	75	100	-	____
29d	0	25	50	75	100	-	____
			Total	____	÷	5 =	____
Disease-Targeted Social Functioning							
6	100	75	50	25	0	-	____
10	0	25	50	75	100	-	____
14f	100	75	50	25	0	-	____
18c	100	75	50	25	0	-	____
19	100	75	50	25	0	-	____
22b	100	75	50	25	0	-	____
22c	0	25	50	75	100	-	____
22d	0	25	50	75	100	-	____
22f	0	25	50	75	100	-	____
			Total	____	÷	9 =	____
Sexual Function[2]							
25	100	66.7	33.3	0	-	-	____
26	100	75	50	25	0	-	____
			Total	____	÷	2 =	____
Health Distress							
13a	0	25	50	75	100	-	____
13b	0	25	50	75	100	-	____
13c	0	25	50	75	100	-	____
			Total	____	÷	3 =	____

[2] Recode questions 25 and 26 to missing if the response to question 28 is “no”.

SCORING ALGORITHM (cont.)
PERIPHERAL NEUROPATHY QOL-97 SCORING FORM

Scale/Item Numbers	Response (raw score) 1	2	3	4	5	6	Subtotal Final Score[3] 0-100 point scale
Single Items							
Severity							
21	100	75	50	25	0	-	_____ = ____
Disability days	(recoding not relevant)						
12							
20							
Health Change							
2	100	75	50	25	0	-	_____ = ____
Overall Health Rating							
30	(no recoding necessary)						
Satisfaction with Sexual Functioning[4]							
27	100	75	50	25	0	-	_____ = ____
Sexual Activity[5]							
28	(recoding not relevant)						

[3] Note: The total number of items in each scale is listed as the divisor for each subtotal. However, where all items in a scale are not answered, the divisor will be lower.

[4] Recode question 27 to missing if the response to question 28 is “no”.

[5] Item 28 on sexual activity is not counted as a quality-of-life item, but it is used for the scoring of the sexual function scale and the satisfaction with sexual functioning item.

Physical Health Summary Score:

(((**Pain Scale** - 65.48214286) / 21.91750078) * 0.19922) = _____

(((**Physical Functioning** - 72.96401515) / 25.74274538) * 0.20156) = _____

(((**Balance** - 79.18080357) / 19.11524675) * 0.19139) = _____

(((**Social Functioning** - 80.53993056) / 19.63613561) * 0.12088) = _____

(((**Role limitations—Physical Health** - 65.84375000) / 33.29916333) * 0.11810) = _____

(((**Energy/fatigue** - 56.80000000) / 23.41394154) * 0.12125) = _____

(((**Sleep Scale** -71.14375000) / 18.40712984) * 0.12537) = _____

(((**Upper Extremities** -91.89583333) / 11.21486152) * 0.12555) = _____

(((**General Health Perceptions** - 58.61904762) / 20.30108094) * 0.086309) = _____

(((**Emotional Well Being** - 71.40714286) / 17.15597900) *-0.08927) = _____

(((**Cognitive Functioning** - 76.75000000) / 20.07002509) *-0.03316) = _____

(((**Self-esteem** -79.91666667) / 16.93850049) *-0.05138) = _____

(((**Health Distress** - 68.25000000) / 25.46321911) * 0.02889) = _____

(((**Role limitations—Emotional** - 66.66666667) / 38.61162968) * 0.02849) = _____

(((**Stigma** - 93.54166667) / 14.76342984) *-0.00849) = _____

(((**Sexual Function** - 68.67094937) / 34.22217052) * 0.043288) = _____

Subtotal = **_____**

(((**Subtotal** - 0.0161271) / 0.9981705) * 10) + 50 = **Physical Health Summary Score**

Mental Health Summary Score:

(((**Pain Scale** - 65.48214286) / 21.91750078) * -0.05253) = _____

(((**Physical Functioning** - 72.96401515) / 25.74274538) * -0.07308) = _____

(((**Balance** - 79.18080357) / 19.11524675) * -0.07198) = _____

(((**Social Functioning** - 80.53993056) / 19.63613561) * 0.06578) = _____

(((**Energy/fatigue** - 56.80000000) / 23.41394154) * 0.05238) = _____

(((**Role limitations—Physical Health** - 65.84375000) / 33.29916333) * 0.06440) = _____

(((**Sleep Scale** - 71.14375000) / 18.40712984) * -0.01279) = _____

(((**Upper Extremities** - 91.89583333) / 11.21486152) * -0.03518) = _____

(((**General Health Perceptions** - 58.61904762) / 20.30108094) * 0.094966) = _____

(((**Emotional Well Being** - 71.40714286) / 17.15597900) * 0.29507) = _____

(((**Self-esteem** - 79.91666667) / 16.93850049) * 0.21516) = _____

(((**Cognitive Functioning** - 76.75000000) / 20.07002509) * 0.20817) = _____

(((**Role limitations—Emotional** - 66.66666667) / 38.61162968) * 0.15108) = _____

(((**Health Distress** - 68.25000000) / 25.46321911) * 0.15045) = _____

(((**Stigma** - 93.54166667) / 14.76342984) * 0.13053) = _____

(((**Sexual Function** - 68.67094937) / 34.22217052) * 0.076409) = _____

Subtotal = _____

(((**Subtotal** - 0.0137913) / 0.9993292) *10) + 50 = **Mental Health Summary Score**

SAS Code:

```
physic1=
(((DTPAIN-65.48214286)/21.91750078) * 0.19922 ) +
(((PHYFUN-72.96401515)/25.74274538) * 0.20156 ) +
(((BALANC-79.18080357)/19.11524675) * 0.19139 ) +
(((DTSFUN-80.53993056)/19.63613561) * 0.12088 ) +
(((PHROLE-65.84375000)/33.29916333) * 0.11810 ) +
(((ENERGY-56.80000000)/23.41394154) * 0.12125 ) +
(((SLEEP5-71.14375000)/18.40712984) * 0.12537 ) +
(((UPPERE-91.89583333)/11.21486152) * 0.12555 ) +
(((PERCEP-58.61904762)/20.30108094) * 0.086309) +
(((EMOTWB-71.40714286)/17.15597900) *-0.08927 ) +
(((COGFUN-76.75000000)/20.07002509) *-0.03316 ) +
(((SELFES-79.91666667)/16.93850049) *-0.05138 ) +
(((HDISTR-68.25000000)/25.46321911) * 0.02889 ) +
(((EMROLE-66.66666667)/38.61162968) * 0.02849 ) +
(((STIGMA-93.54166667)/14.76342984) *-0.00849 ) +
(((SEX2  -68.67094937)/34.22217052) * 0.043288 );

mental1=
(((DTPAIN-65.48214286)/21.91750078) *-0.05253 ) +
(((PHYFUN-72.96401515)/25.74274538) *-0.07308 ) +
(((BALANC-79.18080357)/19.11524675) *-0.07198 ) +
(((DTSFUN-80.53993056)/19.63613561) * 0.06578 ) +
(((ENERGY-56.80000000)/23.41394154) * 0.05238 ) +
(((PHROLE-65.84375000)/33.29916333) * 0.06440 ) +
(((SLEEP5-71.14375000)/18.40712984) *-0.01279 ) +
(((UPPERE-91.89583333)/11.21486152) *-0.03518 ) +
(((PERCEP-58.61904762)/20.30108094) * 0.094966) +
(((EMOTWB-71.40714286)/17.15597900) * 0.29507 ) +
(((SELFES-79.91666667)/16.93850049) * 0.21516 ) +
(((COGFUN-76.75000000)/20.07002509) * 0.20817 ) +
(((EMROLE-66.66666667)/38.61162968) * 0.15108 ) +
(((HDISTR-68.25000000)/25.46321911) * 0.15045 ) +
(((STIGMA-93.54166667)/14.76342984) * 0.13053 ) +
(((SEX2  -68.67094937)/34.22217052) * 0.076409 );

t_physic=(((physic1-0.0161271) /0.9981705) *10) +50;
t_mental=(((mental1-0.0137913) /0.9993292)
```

D. NEUROQOL-28

NEUROPATHY-SPECIFIC QUALITY OF LIFE Questionnaire Instructions UK Version	Patient Identification Visit Visit Date

- These questions ask about the effect your **FOOT PROBLEMS** may have on your daily life and well-being. By **foot problems** we mean **lost or reduced feeling** in your extremities**, pain, discomfort** and/or **ulcers (open sores) on your feet** and, in some cases **unsteadiness** while walking or standing.
- Please note that many **questions have two parts**. Answer every question by ticking one box for each part (tick two boxes per line). Please make sure you answer all questions.
- Please concentrate on how you have felt **IN THE PAST 4 WEEKS** for all of the questions.
- There are no right or wrong answers. If you are unsure about how to answer a question, you can ask the person who gave you the questionnaire. Please **DO NOT** ask a relative or friend to help you.
- **All of your responses will be held strictly confidential.**

In the past 4 weeks how often have you experienced the following symptoms?	All the time	Most of the time	Some of the time	Occasionally	Never	**How much bother did this cause you?** Very much	Some bother	None
1. Burning in your legs or feet								
2. Excessive heat or cold in your legs or feet								
3. Pins and needles in your legs or feet								
4. Shooting or stabbing pain in your legs or feet								
5. Throbbing in your legs or feet								
6. Sensations in your legs or feet that make them jump								
7. Irritation of the skin caused by something touching your feet, such as bedsheets or socks								

A. Have these **painful symptoms** reduced your quality of life?	Very much	Quite a lot	Somewhat	A little	Not at all

In the past 4 weeks how often have you experienced the following symptoms?	All the time	Most of the time	Some of the time	Occasionally	Never	**How much bother did this cause you?** Very much	Some bother	None
8. Numbness in your feet								

9. Inability to feel the difference between hot and cold with your feet								
10. Inability to feel objects with your feet								

B. Have these **last three symptoms** reduced your quality of life?	Very much	Quite a lot	Somewhat	A little	Not at all

In the past 4 weeks how often have you experienced the following symptoms?	All the time	Most of the time	Some of the time	Occasionally	Never	**How much bother did this cause you?**		
						Very much	Some bother	None
11. Weakness in your hands								
12. Problems with balance or unsteadiness while walking								
13. Problems with balance or unsteadiness while standing								

C. Have these **last three symptoms** reduced your quality of life?	Very much	Quite a lot	Somewhat	A little	Not at all

The following questions ask about how your FOOT PROBLEMS affect your daily activities, relationships and feelings.

Are you in <u>PAID WORK</u>?	Yes	No	If **YES** please go to Question 14. If **NO** please go to Question 15.

In the past 4 weeks, HOW MUCH have your foot problems interfered with your:	Very much	Quite a lot	Some what	A little	Not at all	**How important is this aspect of your life to you?** Very much	Some what	Not at all
14. Ability to perform your paid work?								
15. Ability to perform tasks around the house or garden?								
16. Ability to take part in leisure activities?								

D. Have these changes in **daily activities** as a result of your foot problems reduced your quality of life?	Very much	Quite a lot	Somewhat	A little	Not at all

In the past 4 weeks:	Very Much	Quite a lot	Some What	A little	Not at all	**How important is this aspect of your life to you?** Very much	Some what	Not at all
17. How much have your foot problems interfered with your relationships with people close to you?								

18.Have you felt more physically dependent than you would like to be on people close to you as a result of your foot problems?								
19.Have you felt more emotionally dependent than you would like to be on people close to you as a result of your foot problems?								
20. has your role in the family changed as a result of your foot problems?								

E. Have these changes in **relationships with other people** as a result of your foot problems reduced your quality of life?	Very much	Quite a lot	Somewhat	A little	Not at all

						How much bother did this cause you?		
How much do you agree with the following statements:	Completely agree	Partly agree	Neither agree or	Partly disagree	Completely disagree	Very much	Some bother	None
21. People treat me differently from other people as a result of my foot problems.								

22. I feel older than my years as a result of my foot problems.								
23. My self - confidence is affected as a result of my foot problems.								
24. My foot problems make my life a struggle.								
25. I generally feel frustrated because of my foot problems.								
26. My foot problems cause me embarrassment.								
27. I feel depressed because of my foot problems								

F. Have these **feelings about yourself** as a result of your foot problems reduced your quality of life?	Very much	Quite a lot	Some what	A little	Not at all

	Very much	Quite a lot	Some what	A little	Not at all
28. Overall, I would say problems with my feet reduced my Quality of Life:					
	Excellent	Very good	Good	Fair	Poor
29. Overall, I would rate my quality of life as:					

Assessment of Neuropathy- Specific Quality of Life (NeuroQoL)

The 35 item Neuropathy- Specific Quality of Life instrument (NeuroQoL) is an hierarchically organized scale *(Fries and Singh, 1996; Spilker and Revicki, 1996)* that assesses patients subjective reports *(Gill and Feinstein, 1994)* of functioning and quality of life in six specific domains. Following the hierarchical model, the base of each domain is assessed with items that measure specific somatic experiences, social and personal dysfunctions and emotional states, and end with an overall assessment of quality of life or satisfaction with experiences in that domain.

Thirteen items assess specific somatic experiences in three domains: i.e., Pain (items 1-7), Lost/reduced feeling (items 8-10); and Diffuse sensory-motor symptoms (items 11-13). Specific functional, social and emotional experiences are assessed in three domains with an additional 14 items: Restrictions in activities of daily living (items 14–16), and Disruptions in social relationships (items 17-20), and Emotional distress (items 21- 27). The frequency of these experiences, somatic, social and affective, are reported on 5 point scales (never, to all of the time). A participant's score for a domain is the mean of the items in that scale with higher scores representing more severe symptoms or greater disruption in functioning.

For each of these 27, specific items, patients are asked to judge the degree to which the somatic experience, restriction of activities, social function and emotional states have been a bother and/or important to them *(O'Boyle, McGee and Joyce,1994)*. The bother /importance items were scored as 1=none; 2= some; 3= very. Weighted scores were calculated by multiplying the scale score by the corresponding bother/importance score. Multiplying the frequency of experience by its bother and importance provides a more detailed picture of the degree to which the specific experience impacts satisfaction or quality of life.

In accord with the hierarchical model (*Fries and Singh, 1996; Spilker and Revicki, 1996)* and the accepted definition of quality of life as an overall judgment of satisfaction *(Spilker B (ed) (1996). Quality of life and pharmacoeconomics in clinical trials; Leventhal H & Colman S. Quality of life: a process view. Psychol Health 12:753-767)*, a single item assesses quality of life in each of the six domains (items A, B, C, D, E & F).

The two final items in the scale complete the hierarchical approach by assessing overall satisfaction or quality of life, one item requesting that the patient make a judgment specific to his or her experience with foot problems, and a final item asking for an overall judgment of quality of life (*Spilker B (ed) (1996). Quality of life and pharmacoeconomics in clinical trials).*

Satisfaction with quality of life in each domain and overall satisfaction are assessed using five point scales (not at all to very much). (NOTE: as the 6-item (lettered) QoL scale significantly correlates with a 1-item overall DN impact on QoL scale (item 28) at .88, item 28 could replace a 6-item scale if a shorter version of the NeuroQoL is needed. A short version of the NeuroQoL should, therefore, include items 1-13 (symptoms); 14-27 (psychosocial functioning); 28 (DN-specific impact on QoL) and 29 (overall QoL)).

E. NEUROLOGICAL FORM

Effect of Melatonin on ANS Study

PATIENT NAME:
SUBJECT ID:
DATE:

PHYSICAL EXAMINATION

SITE:	NORMAL	ABNORMAL	COMMENT
HEAD, NECK			
EYES			
EARS, NOSE, THROAT			
LYMPH NODES			
CHEST, LUNGS			
HEART			
ABDOMEN			
MUSCULOSKELETAL			
EXTREMITIES			
PERIPHERAL VASCULAR			
SKIN			
OTHER:			
ANEMIA			
CYANOSIS			
JAUNDICE			
CLUBBING			
JVP			
EDEMA			

SUPINE BP ____ **PULSE** _____

SITTING BP ____ **PULSE** _____

STANDING BP ____ **PULSE** _____

HEIGHT ___ **Inches**
BMI ____ **WEIGHT** ___ **Lbs.**

Exam performed by:_____

Transcribed by:_____

F. INCLUSION/EXCLUSION SCREENING FORM

Melatonin and Autonomic Nervous System (ANS) Function

INCLUSION/EXCLUSION SCREENING FORM

Subject Name _____Subject #_____Visit Date_____

INCLUSION CRITERIA
Must be "YES" for inclusion in the study.

☐ YES ☐ NO Subjects may be males or non-pregnant, non-lactating females age 40-75 years of age

☐ YES ☐ NO Subject must have been diagnosed with type 2 diabetes mellitus

EXCLUSION CRITERIA
Must be "NO" for Inclusion in the study.

☐ YES ☐ NO History of congestive heart failure

☐ YES ☐ NO Recent myocardial infarction or cardiovascular event within last year

☐ YES ☐ NO History of major macrovascular events such as myocardial infarction or stroke within the last 6 months

☐ YES ☐ NO History of unstable or irregular heartbeat

☐ YES ☐ NO Presence of end-stage renal disease (undergoing renal dialysis)

☐ YES ☐ NO Presence of moderate or severe hepatic insufficiency

☐ YES ☐ NO Presence of severe orthostatic hypotension

☐ YES ☐ NO Current tobacco use

☐ YES ☐ NO Presence of Type 1 diabetes

☐ YES ☐ NO Presence of hepatitis B or C

☐ YES ☐ NO Presence of HIV

☐ YES ☐ NO Presence of active malignancy (diagnosed or treated in last year)

☐ YES ☐ NO Night time shift work

☐ YES ☐ NO Current or recent use of supplemental melatonin (within 6 months)

☐ YES ☐ NO Amputation of any portion of a hand or a foot

☐ YES ☐ NO Other serious medical conditions which would compromise the subject's participation, in the opinion of the investigator

☐ YES ☐ NO Participation in another clinical trial concurrently or within the last month

FINAL DETERMINATION OF ELIGIBILITY (CHECK ONE)

☐ Based on medical history, physical exam, and lab tests patient has met all study criteria and can be enrolled in the study.

☐ Patient failed the screening process required for entry into the study. Please indicate reason for screen failure.

_____
_____
_____
_____

Transcribed By_____ Date_____

Reviewed By_____ Date _____

G. PSQI

PITTSBURGH SLEEP QUALITY INDEX (PSQI)

INSTRUCTIONS: The following questions relate to your usual sleep habits during the past month only.
Your answers should indicate the most accurate reply for the majority of days and nights in the past month.
Please answer all questions.

During the past month, when have you usually gone to bed at night?

USUAL BED TIME_____

During the past month, how long (in minutes) has it usually take you to fall asleep each night?

NUMBER OF MINUTES_____

During the past month, when have you usually gotten up in the morning?

USUAL GETTING UP TIME_____

During the past month, how many hours of actual sleep did you get at night? {This may be different than the number of hours you spend in bed.)

HOURS OF SLEEP PER NIGHT_____

INSTRUCTIONS: For each of the remaining questions, check the one best response.
Please answer all questions.

During the past month, how often have you had trouble sleeping because you...

		Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a)	...cannot get to sleep within 30 minutes	D	D	D	D
(b)	...wake up in the middle of the night or early morning	D	D	D	D
(c)	...have to get up to use the bathroom	D	D	D	D
(d)	...cannot breathe comfortably	D	D	D	D
(e)	...cough or snore loudly	D	D	D	D
(f)	...feel too cold	D	D	D	D
(g)	...feel too hot	D	D	D	D
(h)	...had bad dreams	D	D	D	D
(i)	...have pain	D	D	D	D
(j)	Other reason(s), please describe				

How often during the past month have you had trouble sleeping because of this?

No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
D	D	D	D

During the past month, how would you rate your sleep quality overall?

Very good	Fairly good	Fairly bad	very bad
D	D	D	D

During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
D	D	D	D

No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
D	D	D	D

During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
D	D	D	D

---

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
D	D	D	D

No bed Partner/ room, but not same bed	Partner in same roommate in same bed	Partner or other room	Partner in room
D	D	D	D

10. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
D	D	D	D

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
...loud snoring	D	D	D	D
...long pauses between breaths while asleep	D	D	D	D
...legs twitching or jerking while you sleep	D	D	D	D
...episodes of disorientation or confusion during sleep	D	D	D	D
Other restlessness while you sleep; please describe	D	D	D	D

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Licensed Content Publisher	Elsevier
Licensed Content Publication	Endocrinology and Metabolism Clinics of North America
Licensed Content Title	Diabetic Neuropathy
Licensed Content Author	Aaron I. Vinik, Marie-Laurie Nevoret, Carolina Casellini, Henri Parson
Licensed Content Date	December 2013
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Thesis: "Optimal Frequency of Slow Breathing Training to Cause a Therapeutic Effect on Autonomic Function in Healthy Adults

May 2010 Bachelors of Science, Exercise Science
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TEACHING

Bethel College, Fall 2014–Present
SCI 100: General Health & Wellness Lecture & Lab*, Online**

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EXSC 250: Strength & Conditioning Leadership Labs*
EXSC 415: Exercise Testing for Normal and Special Populations Labs*

Virginia Community College System, Fall 2005–Present, Various Schedules
PED 100*; PED 101*; PED 103*; PED 105*; PED 107*; PED 109*; PED 111*; PED 116*,**; PED 120*; PED 154; PED 206*,**
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SCHOLARSHIP

Brown, J. & Colberg, S. (2015). Pulse Wave Analysis of HbA1C Categorized Prediabetes, Type 2 Diabetes and Normoglycemic Populations: A Pilot Study. Manuscript in Progress.

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