Spring 2012

Alternative Therapy and Treatment of Type 2 Diabetes

Carmine R. Grieco
Old Dominion University

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By

Carmine R. Grieco, MS
Bachelor of Science in Exercise Science, 1996, University of Wyoming
Master of Science in Education, Exercise Science, 2008, Old Dominion University

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

DOCTOR OF PHILOSOPHY

HUMAN MOVEMENT SCIENCE

OLD DOMINION UNIVERSITY

May, 2012

Approved by:

Sheri R. Colberg-Ochs (Director)

Aaron I. Vinik (Director)

C. Thomas Somma (Member)

ABSTRACT

ALTERNATIVE THERAPY AND TREATMENT OF TYPE 2 DIABETES

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Carmine R. Grieco, MS
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The rise in prevalence and incidence of type 2 diabetes mellitus (T2D) in the developing world continues unabated. Current treatment strategies, however, fall short of achieving optimal glycemic control. The aim of project I was to investigate the effect of an acute bout of a yogic breathing exercise on heart rate variability (HRV) in individuals with T2D. Project II was designed to assess the effectiveness of the neurohormone melatonin in lowering short- and long-term glucose levels, lipids and oxidative stress in T2D. Project III investigated the differential effects of two different styles of aerobic exercise on postprandial glycemia, mood and HRV in T2D.

Project I investigated the effect of short-term breathing exercises and demonstrated significant differences between the T2D group and an age-matched normoglycemic group (CON) in resting measures of HRV. Standard deviation of consecutive heart beats (SDNN), the square root of the mean squared differences (RMSSD) and total spectral power (TP) were almost uniformly lower in the T2D group than the CON group. A within-group analysis revealed no significant effect of breathing exercise upon HRV in the CON group. However, a 10-minute breathing protocol involving selective breathing through only the left nostril demonstrated a significant reduction in resting heart rate in the T2D group (-1.2 beats per minute, or bpm) compared to the heart rate average during the entire breathing protocol, indicating a possible acute improvement in vagal tone.

Project II, which investigated the effect of six weeks of melatonin supplementation on short- and long-term glycemic control, lipids, and oxidative stress in T2D, yielded impressive results. There was a significant reduction in malondialdehyde, a marker of oxidative stress (-6.3 vs. 0.7nmol/ml), as well as a significant drop in glycated hemoglobin (-0.24%±0.23) in the melatonin group vs. the placebo group.

Project III examined the impact of a more recreational style of exercise (table tennis) following a dinner meal vs. a more traditional exercise (walking). Our results indicated that self-paced walking generated a significantly higher heart rate than table tennis, which translated into a significant drop in blood glucose levels following a 30-minute bout of exercise. We did not, however, note any difference in mood between the two groups.

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CHAPTER 1

INTRODUCTION

Currently it is estimated that 11.3% of adults over the age of 20 years and nearly 27% of adults over the age of 60 years have diabetes, with type 2 diabetes mellitus (T2D) accounting for 90–95% of all cases (CDC, 2011). Furthermore, the ranks of the diabetic population are swelling rapidly and it is predicted that the number of people with diabetes will nearly double within the next three decades (Chaturvedi, 2007). Additionally, it is estimated that 35% of the U.S. adult population is prediabetic (CDC, 2011). Complications arising from T2D represent a significant burden upon the national healthcare system, accounting for approximately 18.5% of all health care expenditures in 2002 (ADA, 2003).

The primary goal for the treatment and prevention of T2D is optimal glycemic control. T2D is characterized by a hyperglycemia-driven increase in oxidative stress (Giugliano and Ceriello, 1996), as well as a reduction in antioxidative capacity (Rysz et al., 2007). This dual attack contributes to autonomic nervous system damage (Singh et al., 2000), which can subsequently affect any organ of the body (Vinik and Erbas, 2001). It also results in micro- and macrovascular disease (Fowler, 2008). Consequently, individuals with T2D are 2–4 times more likely to die from heart disease and twice as likely to die of all-cause mortality (CDC, 2011).

There are a plethora of pharmacological agents designed to treat hyperglycemia. However, pharmacotherapeutic tactics still fall short of maintaining optimal glycemic control (Cefalu et al., 2009). In fact, only 37% of patients with T2D achieve and maintain optimal glycemic control (Saydah et al., 2004). A potential complicating factor is the economic cost of anti-diabetic drugs.

For example, it has been shown that oral antidiabetic drug use is inversely associated with adherence and a significant predictor of treatment failure (Barron et al., 2008).

It has been proposed that future directions of therapy should target the fundamental defects associated with T2D (Cefalu, Richards and Melendez-Ramirez, 2009). These fundamental properties are hyperglycemia and the resultant increase in oxidative stress, which, according to Brownlee's (2000) "unifying hypothesis," is the single most critical factor in complications of T2D. While exercise is a cornerstone treatment and has been previously validated for both treatment and prevention of T2D (Colberg et al., 2010), it has been demonstrated that individuals with T2D are less likely to engage in adequate amounts of physical activity (Nelson et al., 2002). Furthermore, adherence rates among individuals with T2D are distressingly low as well (Serour et al., 2007).

Current research into alternative and/or adjunctive therapy for T2D is sparse, but promising. Yoga training, for example, is associated with a higher adherence rate than traditional training (Flegal et al., 2007). The timing of exercise interventions around meal consumption is likewise a promising avenue of study as postprandial hyperglycemic spikes have demonstrated a greater impact on overall glycemic control than fasting glycemia (Woerle et al., 2007). Melatonin, a neurohormone and recently recognized antioxidant, has established a significant track record of reducing markers of oxidative stress in animal models and in in vitro studies (Korkmaz et al., 2009).

Therefore, further research into cost-effective alternative therapies, used in conjunction with standard pharmacotherapy, is warranted. Through the selective targeting of the fundamental

mechanisms which are responsible for the majority of complications arising from T2D it may be possible to more effectively treat and/or prevent this disease.

Project I

The Acute Effect of Short-Term Breathing Exercises on Sympathovagal Balance in Type 2 Diabetes

Statement of Purpose

The purpose of this study was to determine if a commonly practiced style of yoga breathing, alternate nostril breathing (ANB), significantly alters indices of heart rate variability (HRV) in individuals with T2D and an age-matched, normoglycemic control group.

Research Hypotheses

1. Baseline measures of all HRV measurements (SDNN, RMSSD and TP) will be reduced in the T2D group in comparison to the control group (CON).
2. ANB will induce significantly greater increases in SDNN, RMSSD and TP in the T2D group in comparison to the CON group.
3. The T2D group will have significantly greater heart rate response during the stand challenges (First and Last Stand) than the CON group.

Assumptions

1. All participants will adhere to the recommendations of abstaining from food for a minimum of three hours prior to testing.

2. All participants will adhere to the recommendations of abstaining from caffeine-containing foods and beverages on the day of testing.

Limitations

1. HRV is influenced by psychological as well as physical phenomena. It is not possible, however, to standardize mood or affective state.

Delimitations

1. Participants in the T2D group have diagnosed type 2 diabetes.

Operational Definitions

1. Heart rate variability (HRV) – Fundamentally, HRV represents the difference, in milliseconds, between consecutive heart beats. This inter-beat interval reflects dynamic cardiovascular control.

2. SDNN – The standard deviation of the normal-to-normal consecutive heart beats represents a global measure of the variability within the cardiac cycle.

3. RMSSD – The square root of the mean squared differences of successive normal heart beats. This is a measurement of HRV that is reflective of parasympathetic or vagal input.

4. TP – Total spectral power is a frequency domain method of quantifying all cyclic components of heart rate variability and is expressed in ms^2.

Project II

The Effect of N-Acetyl-5-Methoxytryptamine (Melatonin) Supplementation on Glycemic Control, Lipid Metabolism, and Oxidative Stress in Type 2 Diabetes Mellitus

Statement of Purpose

The purpose of this investigation is to evaluate the effect of a commercially available preparation of melatonin on glycemic control, lipid metabolism, and oxidative stress in uncomplicated T2D.

Research Hypotheses

1. There will be significantly greater reductions in fasting glucose levels in the melatonin supplemented group than the placebo group.
2. There will be significantly greater reductions in glycated hemoglobin (A1C) in the melatonin supplemented group than the placebo group.
3. There will be significantly greater reductions in malondialdehyde in the melatonin supplemented group than the placebo group.
4. There will be significantly greater reductions in total cholesterol in the melatonin supplemented group than the placebo group.
5. There will be significantly greater reductions in low density lipoprotein in the melatonin supplemented group than the placebo group.
6. There will be a significantly greater increase in high density lipoprotein in the melatonin supplemented group than the placebo group.

7. There will be significantly greater reductions in triglycerides in the melatonin supplemented group than the placebo group.

Assumptions

1. The melatonin capsules, a commercially prepared product, have been correctly verified for potency.
2. Participants will faithfully ingest one pill every evening, 30 minutes prior to sleep every night of the study.

Limitations

1. This investigation did not perform blood analyses to quantify markers of antioxidant activity.
2. The commercially produced melatonin capsules were verified via high performance chromatography by the manufacturer.

Delimitations

1. All participants must have a prior diagnosis of type 2 diabetes.
2. All participants must follow a standard diurnal cycle (i.e., no overnight work pattern).

Project III

The Effects of Walking vs. Table Tennis on Postprandial Glycemia, Heart Rate Variability, and Mood in Type 2 Diabetes

Statement of Purpose

The purpose of this study is to compare a traditionally prescribed exercise activity (i.e., walking) to a more recreational form of exercise (table tennis) on postprandial glycemic control, heart rate variability and mood in individuals with uncomplicated T2D.

Research Hypotheses

1. There will be significantly greater reductions in postprandial glucose levels in the table tennis treatment than the rest or walking treatments.
2. There will be significantly greater improvement in sympathovagal balance in the table tennis treatment than the rest or walking treatments.
3. A bout of table tennis will increase mood state to a significantly greater degree than an equal amount of time walking or resting.

Assumptions

1. Participants are not under unusual amounts of psychological stress as this can negatively impact HRV measurements.

Limitations

1. The self-selected pace of treadmill walking is not relative to the fitness level of the individual.

Delimitations

1. Participants are capable of undertaking 30 minutes of continuous exercise.
2. Participants have previously diagnosed type 2 diabetes

Operational Definitions

1. Mean RR – The interbeat interval, measured in milliseconds, from one QRS complex to the next
2. SDNN – the standard deviation of normal RR intervals
3. RMSSD – the square root of mean squared differences among consecutive normal RR intervals
4. Sympathovagal balance – A ratio-based representation of the independent influence of the sympathetic (low frequency power) and parasympathetic (high frequency power) inputs as reflected in the cardiac cycle
5. Valsalva ratio – The ratio between the slowest and fastest heart rate following a valsalva maneuver (a sustained exhale against pressure)
6. E/I ratio – The ratio of the longest RR interval during exhalation by the shortest RR interval during inspiration

7. 30:15 ratio – A ratio based on the slowest heart rate after standing (typically at the 30^{th} beat) by the fastest heart rate (typically at the 15^{th} beat)

REFERENCES

American Diabetes Association. Economic costs of diabetes in the U.S. in 2002. *Diabetes Care* 26: 917-932, 2003.

Barron J, Wahl P, Fisher M and Plauschinat C. Effect of prescription copayments on adherence and treatment failure with oral antidiabetic medications. *P & T: A Peer-Reviewed Journal of Formulary Management* 33(9): 532-553, 2008.

Cefalu WT, Richards RJ and Melendez-Ramirez LY. Redefining treatment success in type 2 diabetes mellitus: Comprehensive targeting of core defects. *Cleveland Clinical Journal of Medicine* 76(Suppl 5): S39-S47, 2009.

Center for Disease Control and Prevention. National diabetes fact sheet, 2011. http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Accessed on April 1, 2012.

Chaturvedi N. The burden of diabetes and its complications: trends and implications for intervention. *Diabetes Research and Clinical Practice* 76 Suppl 1: S3-S12, 2007.

Colberg SR, Albright AL, Blissmer BJ, Braun B, Chasan-Taber L, Fernhall B, Regensteiner JG, Rubin RR and Sigal RJ. Exercise and type 2 diabetes: American College of Sports Medicine and the American Diabetes Association: joint position statement. *Medicine and Science in Sports and Exercise* 42: 2282-2303, 2010.

Flegal KE, Kishiyama S, Zajdel D, Haas M and Oken BS. Adherence to yoga and exercise interventions in a 6-month clinical trial. *BMC Complementary and Alternative Medicine.* 2007; 7(37).

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

Giugliano D and Ceriello A. Oxidative stress and diabetic vascular complications. *Diabetes Care* 19: 257-267, 1996.

Kedziora-Kornatowska K, Szewczyk-Golec K, Kozakiewicz M, Pawluk H, Czuczejko J, Kornatowski T, Bartosz G and Kedziora J. Melatonin improves oxidative stress parameters measured in the blood of elderly type 2 diabetic patients. *Journal of Pineal Research* 46: 333-337, 2009.

Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S and Tan DX. Melatonin: An established antioxidant worthy of use in clinical trials. *Molecular Medicine* 15(1-2): 43-50, 2009.

Nelson KM, Reiber G, Boyko EJ; NHANES III. Diet and exercise among adults with type 2 diabetes: findings from the third national health and nutrition examination survey (NHANES III). *Diabetes Care* (25)10: 1722-1728, 2002.

Rysz J, Blaszczak R, Banach M, Kedziora-Kornatowska K, Kornatowski T, Tanski W, Kedziora J. Evaluation of selected parameters of the antioxidative system in patients with type 2 diabetes in different periods of metabolic compensation. *Archivum Immunologiae et Therapiae Experimentalis* 55(5): 335-340, 2007.

Serour M, Alqhenaei H, Al-Saqabi S, Mustafa AR and Ben-Nakhi A. Cultural factors and patients' adherence to lifestyle measures. *British Journal of General Practice* 57(537): 291-295, 2007.

Singh JP, Larson MG, O'Donnell CJ, Wilson PF, Tsuji H, Lloyd-Jones DM and Levy D. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). *American Journal of Cardiology* 86: 309-312, 2000.

CHAPTER II

REVIEW OF LITERATURE

The following review of literature will explore evidence as it relates to several specific aspects of alternative therapy for the treatment and prevention of T2D. There is significant literature established concerning the complications arising from T2D as well as mechanisms of action relating to the pathology of T2D, but there is still a significant need for research into alternative therapies for the treatment and prevention of T2D. This chapter will discuss the role of several individual alternative treatment interventions, provide a review of literature, and establish the need for further research in alternative treatments for T2D.

PROJECT I

The Acute Effect of Short-Term Breathing Exercises on Sympathovagal Balance in Type 2 Diabetes

Heart Rate Variability

HRV represents the changes that occur in the inter-beat intervals between successive cardiac cycles. Intrinsic heart rate is governed by the sino-atrial (SA) node, located in the right atrium of the heart. The instantaneous heart rate, however, represents the neurohumoral balancing of both sympathetic and vagal inputs. In the absence of autonomic input the SA node creates a spontaneous depolarization of the myocardium, which results in a heart rate in excess of 100 beats per minute (Jose et al., 1970). Autonomic inputs modify this base rate of cardiac cycles, with sympathetic and vagal inputs acting in a coordinated manner to adjust cardiac output

according to bodily needs. Therefore, in a resting state instantaneous heart rate represents either an increase in vagal dominance, sympathetic withdrawal, or a combination of the two.

HRV measurements of time and frequency domain are most commonly used in the literature (Task Force, 1996). Time domain measurements are the simplest form of calculating variability within the cardiac signal and revolve around statistical methods of measuring variance within time periods between successive QRS complexes (ventricular depolarization). Common time domain measurements include standard deviation of the normal-to-normal spontaneous cardiac cycles (SDNN) and the square root of the mean squared differences among successive cardiac cycles (RMSSD). The SDNN is a global measure of variability, while the RMSSD is thought to reflect only the vagal influence. Frequency domain measurements are statistical descriptions of power spectral density, a metric of how variance is distributed as a function of the frequency within the signal. Commonly cited measurements of frequency are low frequency power ($LFms^2$; 0.04-0.15Hz), high frequency power ($HFms^2$; 0.15-0.40Hz) and total spectral power ($TPms^2$; $\geq$0.40Hz). The interpretation of LF power is somewhat controversial, with some parties considering it a marker of sympathetic power and others a combination of both sympathetic and vagal power. HF power, however, is seen as a marker of vagal activity, while TP is regarded as a marker of total variance.

Heart Rate Variability and Relationship to Diabetes

A loss of HRV is associated with T2D and prediabetes and is inversely associated with plasma glucose levels (Singh et al., 2000). In fact, in a recent study of 1,987 older individuals (55-74 years), Ziegler et al. (2006) determined that the primary factor predicting decreased HRV

was presence of diabetes. Both loss of HRV and resting tachycardia are common signs of autonomic neuropathy of the cardiovascular system (Vinik and Erbas, 2001) and may be an early predictor of macrovascular disease in T2D (Gottsäter et al., 2006). Decreasing HRV is also associated with increasing carotid artery atherosclerosis in T2D (Gottsäter et al., 2006). More specifically, decrements of vagal activity appear early in T2D, manifest as diastolic dysfunction and may contribute to the high incidence of cardiovascular disorders in T2D (Poirier et al., 2003). Therefore, measurement of HRV represents a simple and non-invasive tool which is useful for understanding the status of the autonomic nervous system in T2D (Acharya et al., 2006).

Training Effect of Breathing Exercises on Cardiovascular Control

Several investigations support the concept that yogic breathing exercises (pranayama) may have an ability to influence cardiovascular control via the autonomic nervous system. In an early study by Telles et al. (1994), 48 young (34.1±5.9 years), healthy male subjects who were yoga teachers in-training were randomly assigned to one of three groups for a four-week intervention that consisted of the following breathing exercises conducted four times daily: right nostril breathing (RNB), left nostril breathing (LNB) or alternate nostril breathing (ANB). Subjects performed 27 respiratory cycles of the assigned breathing exercise four times per day, for 30 consecutive days. Following training the RNB group showed a significant increase in resting oxygen consumption (37%, $p<0.05$). In contrast, the LNB group demonstrated a significant increase in galvanic skin resistance, indicating a reduction in sympathetic outflow to the sweat glands. Interestingly, both the ANB and RNB groups displayed a significantly higher resting

heart rate of 1.9 and 2.8 beats per minute (bpm), respectively, at the conclusion of the four-week intervention ($p<0.001$).

In a study of similar duration, Dhungel et al. (2008) enrolled 36 young (24.7±2.4 years), predominantly male subjects (32 male, 4 female) in an investigation of ANB, conducted daily in 15 minutes sessions, on cardiovascular function. At the conclusion of the four-week intervention there were significant decreases in respiratory rate (-2.1 bpm, $p<0.001$) and diastolic blood pressure (DBP) (-5.1mm/Hg, $p<0.001$) and an increase in peak expiratory flow rate (12.2L/min, $p<0.001$). However, in contrast to Telles et al. (1994), the present investigation realized a significant decrease in resting heart rate (-3 bpm, $p<0.001$).

In a more elegant study, Srivastava et al. (2005) investigated both the acute and chronic effect of ANB between equal groups of young males and females ($n=20$ each). The acute effect was assessed following a single 15-minute bout of ANB. This was followed by eight weeks of 15 minutes of daily practice. Acutely the single bout of ANB decreased heart rate in females (-10 bpm, $p<0.0001$), but not males. Contrarily, the males realized a significant decrease in systolic blood pressure (SBP) (-2.3 mmHg, $p<0.05$), but the female subjects did not. Following eight weeks of daily training both male and females demonstrated significant drops in resting heart rates (-12.6 and -11.7, respectively, $p<0.001$). Similarly, both genders significantly decreased SBP. However, contrary to the acute effect, females demonstrated a significant drop in DBP, while the males DBP (which was acutely lowered) rose to non-significance following training. In contrast to Telles et al. (1994), neither gender demonstrated an acute or training effect upon galvanic skin resistance.

In a study that more directly assessed autonomic function, Pal et al. (2004) randomly assigned 60 healthy, young (17.7±0.2 years) subjects to either a fast (FB) or slow (SB) breathing group for

a three-month intervention. Each group was further divided into experimental or control groups (n=15 each). The FB group practiced 60 minutes daily by performing one minute of deep and fast inspiration and expiration, followed by a three-minute rest period. This process was repeated eight to ten times throughout the two daily 30-minute sessions. The SB group performed two daily sessions of 30 minutes each in which they practiced slow (6 breaths per minute) ANB. The control groups (CON) participated in no breath training. There were no significant changes in any variable in the FB group or the two CON groups. In contrast, the SB group had a significant decrease in resting HR (-8 bpm, $p<0.05$), maximum heart rate response to standing (-10.1 bpm, $p<0.05$), minimum heart rate response to standing (-8.1 bpm, $p<0.01$), and mean heart rate after standing (-8.0 bpm, $p<0.01$). Collectively these decreases would suggest an improvement in vagal tone.

Recently, Veerabhadrappa et al. (2011) investigated the effect of Mukh Bhastrika (a fast-paced pranayama) on resting heart rate, blood pressure response to standing, and heart rate response to deep breathing in 50 young (19.5±1.2 years), healthy male participants following 12 weeks of training (5 days per week). The Mukh Bhastrika consisted of a rapid inhalation and exhalation "like the bellows of a blacksmith" for 10 respiratory cycles. This was followed by a final exhalation in which the breathing cycle was delayed as long as possible, and followed by a maximal inhale, after which the participant retained the breath for as long as possible. This process was repeated three times per session. At the conclusion of the study, there was a significant decrease in resting heart rate (-13.4 bpm, $p<0.001$), an increase in both the maximum (12.5 bpm) and minimum (7.4 bpm) heart rate response to standing ($p<0.001$, respectively) and a decrease in systolic blood pressure response to standing (-3.6 mmHg, $p<0.001$). Since resting heart rate is a product of vagal drive, a significant decrease in resting heart rate is indicative of an

increase in parasympathetic activity. Concomitantly, there was a decrease in systolic blood pressure response to standing. Taken together, these findings would suggest an increase in vagal tone.

In another recent investigation, Ankad et al. (2011) enrolled 50 subjects covering a substantially larger age range and gender distribution than previous studies (24 male, 26 female, 20-60 years, mean of 38.6±8.9 years). Significantly, participants with prior yoga history were excluded from the investigation. Subjects participated in a daily two-hour practice involving 45 minutes of eight different pranayama exercises for 15 days. Each session also involved yoga-based lectures (30 minutes) and meditation (20 minutes). Baseline and post-training measurements of resting heart rate, systolic and diastolic blood pressure and mean arterial pressure were taken three times during a 30 minute period of rest in a supine position. For statistical purposes scores were stratified according to gender (male, female), age (>40 and >40 years) and BMI (<25 and >26 kg/m^2). There were significant reductions in resting heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure, across all gender, age, and BMI delineations. While this is indicative of a training response, the results are clouded by a multifactorial intervention.

Acute Effect of Breathing Exercises on Cardiovascular Control

Perhaps the best evidence linking pranayama to alterations in autonomic function was conducted by Pramanik et al. (2009). In this investigation 39 subjects of indeterminate gender underwent pre/post readings of resting heart rate and systolic and diastolic blood pressure following five minutes of *Bhastrika* yogic breathing at a rate of 6 bpm (4s inhale/6s exhale). Another group of 10 subjects ingested 20 mg of hyoscine-*N*-butylbromide (Buscopan®), an anti-

cholinergic that is known to selectively inhibit the effects of parasympathetic activation, 30 minutes prior to performing the same yogic breathing exercise for five minutes. The group which ingested hyoscine-*N*-butylbromide demonstrated no significant change in any variable. However, the non-supplemented yogic breathing group significantly decreased systolic blood pressure (-7.6 mmHg, $p<0.05$) and diastolic blood pressure (-4 mmHg, $p<0.05$). While the heart rate decreased it did not reach the level of significance. This study demonstrates perhaps the most compelling evidence linking pranayama to cardiovascular function.

In one of the few published studies measuring parameters of HRV, Raghuraj and Telles (2008) recruited 21 young (27.5±6.3 years), healthy male volunteers. All subjects had previous pranayama training (mean 14.6±10.7 months). Subjects participated in five randomly assigned sessions of 40 minutes on different days. Each 40-minute session was broken down into 4 epochs of 7.5 minutes, equaling 30 minutes. Each session also began and ended with a five-minute transition period. During each epoch measurements of HRV were taken during the final five minutes and during each transition period (equaling six measurements during each session). Each session included four of the following five possible breathing exercises in random order: (1) RNB, (2) LNB, (3) ANB, (4) breath awareness, and (5) normal breathing. RNB and LNB had no significant effect upon measures of HRV. ANB, however, significantly increased low frequency power (a metric of sympathetic drive) while simultaneously decreasing high frequency power (a metric of vagal drive).

In another study Raghuraj et al. (1998) examined the acute effect two different styles of yogic breathing exercises on measures of HRV in young (25.6±3.1 years) and experienced (19.7±12.8 months) yogic practitioners. The two interventions, conducted on separate days, were

kapalabhati and ANB. Kapalabhati breathing was a rapid and forceful inhalation/exhalation conducted at a rate of 120 breaths per minute for only one minute. ANB involved inhaling through a single nostril and exhaling through the opposite nostril at a slow pace (pace not stated). This sequence was alternated and repeated for 15 minutes. Results indicate the kapalabhati breathing induced a significant reduction in high frequency power (vagal drive) and a significant increase in low frequency drive (sympathetic drive). Contrary to Raghuraj and Telles (2008), ANB did not significantly affect any metric of HRV.

Telles et al. (1996) investigated the differential effects of 45 minutes of RNB compared to an equal period of normal breathing (NB) in 12 young (27.2±3.3 years) experienced (~ three months) yoga practitioners (8 female, 4 male). Measurements of heart rate, blood pressure, galvanic skin resistance, and oxygen consumption were taken before and after each 45 minute session. RNB resulted in significant increases in systolic blood pressure (9.4mm/Hg, $p<0.05$) and resting oxygen consumption (43.7ml/min). Interestingly, both NB and RNB demonstrated a significant decrease in skin resistance, indicative of an increase in sympathetic tone.

In the most recent study, Ghiya and Lee (2012) investigated the effect of an acute bout of 30 minutes of ANB in comparison to an equal period of paced breathing (PB) on HRV (low frequency, high frequency and total power) in a young (22.3±2.9 years) mixed gender (8 male, 12 female) population. Both breathing protocols were conducted at a pace of 5 breaths per minute. Subjects, who were not regular practitioners of yoga, participated in one familiarization session. On a separate day participants were randomly assigned to begin with either ANB or PB. This was followed by a five-minute washout period and the protocols were crossed over. While there were statistically significant increases in low frequency, high frequency and total power following the protocols, there was no significant difference between the PB and ANB groups.

This would suggest that the pacing of the breathing exercise, independent of the type of exercise, was responsible for the change in HRV.

Conclusion

Several methodological concerns obscure the impact of pranayama on cardiovascular autonomic function. Firstly, the practice of pranayama is frequently used concurrently with other aspects of yoga training (i.e. the physical training of hatha yoga and/or the mental training or meditation commonly called dharana and/or dhyana); therefore, it is difficult to separate the specific effects of pranayama from the larger "yogic" intervention. Secondly, virtually studies have used young, healthy populations. Thirdly, the use of participants with knowledge of pranayama and the expected training outcomes creates a potential skewing effect. Also, to the authors knowledge there are no studies which have investigated either the chronic or acute effect of pranayamic training on older populations (>60 years) or individuals with T2D.

Nevertheless, studies involving the chronic effect of pranayamic training have generally provided consistent results. Of the four training studies that measured blood pressure, all had significant decreases in at least one aspect of blood pressure (Ankad et al., 2011; Dhungel et al., 2008; Srivastava et al., 2005; Veerabhadrappa et al., 2011). Similarly, five of six cited studies found a significant reduction in resting heart rate attributable to the pranayamic intervention (Ankad et al., 2011; Dhungel et al., 2008; Pal et al., 2004; Srivastava et al., 2005; Veerabhadrappa et al., 2011), while one study found a significant increase (Telles et al., 1994). In contrast, three of the acute studies found no effect on resting heart rate (Pramanik et al., 2009; Raghuraj et al., 1998; Telles et al., 1996) and one study found a drop in resting heart rate, but it

was not significantly different from the control group (Ghiya and Lee, 2012). Interestingly, of the studies that assessed some direct metric of autonomic function, three found that pranayama increased sympathetic output (Raghuraj et al., 1998; Raghuraj et al., 2008; Telles et al., 1996). A fourth study found a significant increase in HRV following the acute pranayamic intervention, but it was not significantly different from the control group (Ghiya and Lee, 2012). The best evidence of an acute effect remains Pramanik et al. (2009), which demonstrated reductions in diastolic and systolic blood pressure with pranayama which were absent with PNS blockade.

Therefore, the evidence supporting a training effect of pranayama on cardiovascular parameters is better established than the acute effect. Nevertheless, given that there is an absence of studies examining either the acute or chronic effect of pranayama on cardiovascular function in T2D it is worthwhile investigating this potentially beneficial aspect of alternative therapy.

REFERENCES

Acharya UR, Joseph KP, Kannathal N, Lim CM and Suri JS. Heart rate variability: a review. Medical and Biological Engineering and Computing 44:1030-1051, 2006.

Ankad RB, Herur A, Patil S, Shashikala GV and Chinagudi S. Effect of short-term pranayama and meditation on cardiovascular functions in healthy individuals. *Heart Views* 12(2): 58-62, 2011.

Dhungel KU, Malhotra V, Sarkar D and Prajapati R. Effect of alternate nostril breathing exercise on cardiorespiratory functions. Nepal Medical College Journal 10(1): 25-27, 2008.

Ghiya S and Lee CM. Influence of alternate nostril breathing on heart rate variability in non-practitioners of yogic breathing. *International Journal of Yoga* 5(1): 66-69, 2012.

Gottsäter A, Ahlgren AR, Taimour S and Sundkvist G. Decreased heart rate variability may predict the progression of carotid atherosclerosis in type 2 diabetes. *Clinical Autonomic Research* 16: 228-234, 2006.

Jose AD and Collison D. The normal range and determinants of the intrinsic heart rate in man. *Cardiovascular Research* 4: 160-167, 1970.

Pal GK, Velkumary S and Madanmohan. Effect of short-term practice of breathing exercises on autonomic functions in normal human volunteers. *Indian Journal of Medical Research* 120: 115-121, 2004.

Pramanik T, Sharma HO, Mishra S, Mishra A, Prajapati R and Singh S. Immediate effect of slow pace bhastrika pranayama on blood pressure and heart rate. *Journal of Alternative and Complementary Medicine* 15(3): 293-295, 2009.

Poirier P, Bogaty P, Philippon F, Garneau C, Fortin C and Dumesnil JG. Preclinical diabetic cardiomyopathy: relation of left ventricular diastolic dysfunction to cardiac autonomic neuropathy in men with uncomplicated well-controlled type 2 diabetes. *Metabolism* 52(8): 1056-1061, 2003.

Raghuraj P, Ramakrishnan AG, Nagendra HR and Telles S. Effect of two selected yogic breathing techniques on heart rate variability. *Indian Journal of Physiology and Pharmacology* 42(4): 467-472, 1998.

Raghuraj P and Telles S. Immediate effect of specific nostril manipulating yoga breathing practices on autonomic and respiratory variables. *Applied Psychophysiology and Biofeedback* 33: 65-75, 2008.

Singh JP, Larson MG, O'Donnell CJ, Wilson PF, Tsuji H, Lloyd-Jones DM and Levy D. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). *American Journal of Cardiology* 86: 309-312, 2000.

Srivastava RD, Jain N and Singhal A. Influence of alternate nostril breathing on cardiorespiratory and autonomic functions in healthy young adults. *Indian Journal of Physiology and Pharmacology* 49(4): 475-483, 2005.

Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *European Heart Journal* 17: 354-381, 1996.

Telles S, Nagarathna R and Nagendra HR. Breathing through a particular nostril can alter metabolism and autonomic activities. *Indian Journal of Physiology and Pharmacology* 38(2): 133-137, 1994.

Telles S, Nagarathna R and Nagendra HR. Physiological measures of right nostril breathing. *Journal of Alternative and Complementary Medicine* 2(4): 479-484, 1996.

Veerabhadrappa SG, Baljoshi VS, Khanapure S, Herur A, Patil S, Ankad RB and Chinagudi S. Effect of yogic bellows on cardiovascular autonomic reactivity. *Journal of Cardiovascular Disease Research* 2(4): 223-227, 2011.

Vinik AI and Erbas T. Recognizing and treating diabetic autonomic neuropathy. *Cleveland Clinical Journal of Medicine* 68(11): 928-944, 2001.

Ziegler D, Zentai C, Perz S, Rathmann W, Haastert B, Meisinger C and Löwel H. Selective contribution of diabetes and other cardiovascular risk factors to cardiac autonomic dysfunction in the general population. *Experimental and Clinical Endocrinology and Diabetes* 114: 153-159, 2006.

PROJECT II

The Effect of N-Acetyl-5-Methoxytryptamine (Melatonin) Supplementation on Glycemic Control, Lipid Metabolism, and Oxidative Stress in Type 2 Diabetes Mellitus

Substantial circumstantial evidence exists suggesting a functional relationship between melatonin and glucose homeostasis, as well as overwhelming evidence indicating that melatonin is a potent antioxidant. In this regard melatonin may have a significant role to play in the treatment of T2D.

Melatonin and Glucose Homeostasis

There is an abundance of research in animal models demonstrating melatonin plays a significant role in glucose homeostasis. However, there is a paucity of research examining this potentially useful aspect of melatonin in human subjects. Nevertheless, the limited human research has yielded intriguing results. For example, a recent article by Hussain et al. (2006) examined the effect of melatonin and zinc supplementation on glucose homeostasis in subjects with T2D (n = 46) and age-matched controls (n = 17), for a period of 90 days. The T2D subjects were assigned to one of three groups: metformin (2550 mg/day) and placebo (group A), metformin (2550 mg/day) and melatonin/zinc combination (10 mg/50 mg/day) (group B), or melatonin/zinc combination (10 mg/50 mg/day) (group C). All T2D subjects were on a dietary control program for the length of the intervention. Fasting blood samples were collected at 0, 30 and 90 days of treatment to assess fasting glucose, A1C and C-peptide. Additionally, 6 subjects

from group A, 7 from group B and 12 from Group C underwent a post-prandial glucose excursion (PPGE) test prior to and following 60 days of treatment, which consisted of a fasting blood draw followed by draws at 30, 60, 120, 180 and 240 minutes following a solid meal of 530 kcal. Results indicated that group B (metformin and melatonin/zinc) had a significant reduction in FPG and A1C at 30 days (-25%, p <0.01, -17%, p < 0.01), with a further reduction in A1Cat 90 days (-26%, p < 0.05). Group C (melatonin/zinc only) saw a reduction in FPG at 90 days (-23%, p < 0.01) and reductions in A1C at 30 and 90 days (-15% and -29%, p < 0.05). In comparison, group A (metformin and placebo) saw a more modest reduction in FPG at 90 days (-9%, p < 0.01) and a reduction in A1C at 30 days of 14% (p <0.01), which was maintained at 90 days. Results of the PPGE indicate group B (metformin and melatonin/zinc) had a significant reduction in area under the curve (AUC) from 0 to 60 days (-27.7%, p < 0.01). Overall results indicate that the combination of melatonin and zinc, when used singly or in combination with metformin, improved glycemic control in T2D subjects.

A study by Ha et al. (2006) investigated the effect of signaling pathways of glucose transport in mouse skeletal muscle. Monolayers of C_2C_{12} myoblasts were cultured for 3–7 days in order to obtain myotubes. Cell lines were pre-treated with melatonin (1 nM and 10 nM), insulin or luzindole (a receptor antagonist of melatonin) prior to exposure to 2-[^{3}H]-deoxy-$_D$-glucose for 20 minutes at room temperature. The prepared C_2C_{12} myotube cells were processed by SDS-polyacrylamide gel electrophoresis. Phosphoinositide 3-kinase was assayed and measured. Results demonstrated that melatonin treatment increased glucose uptake in myotubes at both 1 nM and 10 nM, increasing glucose uptake 1.9 and 1.8-fold (p < 0.01), respectively, over controls. More importantly, myotubes that were transfected with MTNR1B-containing pcDNA 3.0 plasmid and exposed to melatonin had a 13-fold increase in glucose uptake (p < 0.01),

compared to controls, while similarly treated myotubes exposed to insulin had only a 2-fold increase in glucose uptake (2.1, $p < 0.01$). The significance of this investigation indicates mouse skeletal muscle myotubes had a significantly greater glucose uptake that appeared to be mediated by melatonin receptor MTNR1B.

An intriguing study by Radziuk and Pye (2006) investigated the 24-hour glucose production and metabolic clearance rate (MCR) of 7 subjects with T2D and 6 healthy age and BMI-matched control subjects (CON). Subjects taking hypoglycemic agents discontinued use of medication 72 hours prior to the beginning of the investigation. Following an ad libitum breakfast at 8 am, subjects reported to the lab for 24 hours of fasted monitoring. At 2 pm subjects began an infusion of carbon-labeled glucose and lactate ([U-^{13}C]glucose & [3-^{14}C]lactate), which continued for 24 hours. Plasma insulin, glucose, glucagon, leptin, melatonin and cortisol were sampled according to periods of anticipated change throughout the 24 hour period. The MCR was calculated by analysis of non-steady state compartmental analysis. An index of gluconeogenesis was calculated by analyzing the incorporation of carbon-labeled lactate into glucose. The concentration of plasma glucose remained steady in CON subjects (4.9 mmol/l), with a slight decrease in the final hours (4.4 mmol/l). The T2D subjects, while beginning at a higher glucose concentration (7.2 mmol/l) than controls, exhibited an initial decrease in plasma concentration followed by a significant nocturnal increase (8.0 mmol/l) and a gradual decrease in the late-morning/early afternoon hours (6.3 mmol/l). Glycemic concentrations were significantly different between T2D and CON ($p = 0.0003$). The MCR curves were similar between T2D and CON; however, the CON group exhibited a significantly greater clearance rate (1.73 ml/kg/min vs. 1.4 ml/kg/min, $p = 0.04$). Endogenous glucose production (EGP) remained near constant in the CON group (8.2 μmol/kg/min), but dipped slightly after approximately 24 hours of fasting (7.2 μmol/kg/min).

The T2D group, however, exhibited a steady increase from 8.2 to 11.3 μmol/kg/min throughout the night, peaking in the early morning hours (6:30 am). Glucagon levels, while constant in both groups, were significantly higher in the T2D ($p = 0.04$). The gluconeogenic index demonstrated similar curves between groups; however, the T2D group exhibited a significant increase in gluconeogenic activity during the late-night/early morning hours ($p < 0.05$). Melatonin secretion displayed a phase-delay and attenuation in T2D subjects ($p = 0.02$) compared to CON and appeared to mirror changes in EGP. Overall, results indicate a cyclicity of EGP as well as a significant difference in glycemia between T2D and CON that appears to be driven by EGP.

An investigation by Zanquetta et al. (2003) examined the effect of calorie restriction, pinealectomy and melatonin supplementation on glucose homeostasis and glucose transporter-4 (GLUT-4) content in male Wistar rats. Subjects underwent a pinealectomy (PO) or sham operation (SO) 30 days prior to testing. PO subjects were further divided by daily placebo injection (PO) or melatonin injection (50 μg/100 g body weight) (POM). Testing procedures commenced 30 days later and consisted of an intravenous insulin tolerance test (ivITT), pinealectomy on SO rats and quantification of GLUT-4 gene expression (mRNA) in white adipose tissue (WAT). Results indicated that there was a significant reduction in GLUT-4 mRNA in PO rats (~ 70%, $p < 0.05$). Furthermore, there was a significant increase in plasma insulin ($p < 0.05$) and a significant decrease in glucose clearance in the PO rats ($p < 0.05$) indicating an overall suppressive effect on glucose homeostasis in pinealectomized rats in comparison to sham-operated controls or melatonin-supplemented pinealectomized rats.

Lima et al. (1998) examined the effect of either pinealectomy (PINX) or sham operation (SHAM) on glucose homeostasis and GLUT-4 content and effectiveness in adipocytes at two

different temporal locations (8 am and 4 pm) in male Wistar rats ($n = 22$). Five weeks following surgery all subjects underwent an intravenous glucose tolerance test (IVGTT) and assessment of fasting glucose and insulin. One week post-surgery all subjects were euthanized at either 8 am or 4 pm. The epididymal fat pad was removed and processed for adipocyte isolation. The adipocytes were assessed for insulin-stimulated 2-[^{3}H]-deoxy-$_D$-glucose uptake and insulin binding. Results indicated that plasma glucose and insulin did not differ during the IVGTT when performed at 8 am. However, when the IVGTT was administered at 4 pm, statistically significant differences in glucose and insulin levels were present, with the PINX rats having a decreased insulin secretion, accompanied by increased plasma glucose ($p < 0.05$). This difference was most apparent in the first 20 minutes of the IVGTT, indicating a reduced β-cell response in the PINX rats. An analysis of insulin receptor binding revealed no significant differences between PINX and SHAM at either time point. However, one commonality between both groups was an increase in receptor expression from 8 am to 4 pm, suggesting that differences in glucose disposal were not mediated by insulin receptor activity and also indicating a diurnal rhythm present in insulin receptor expression. Insulin-stimulated glucose uptake in isolated adipocytes demonstrated a 33% decrease in responsiveness in PINX rats at both time points ($p < 0.05$). Furthermore, GLUT-4 content of subjects was significantly reduced (-40%) in adipocytes of PINX rats ($p < 0.05$).

A study by Mazepa et al. (2000) investigated the effect of melatonin supplementation on muscle and liver glycogen in Wistar rats. Subjects were randomly assigned to either an exercise or rest group. Exercised rats ran on a motorized treadmill to exhaustion. Half of the rats in each group received intraperitoneal injections of melatonin at 0.5 or 2 mg/kg body weight. Immediately following exercise all rats were anesthetized and liver and muscle samples were

taken. Results indicated that melatonin supplemented exercise groups and rest groups had significantly reduced lactate elevations when compared to the non-melatonin exercise group ($p < 0.05$) and non-melatonin rest group ($p < 0.05$). Muscle glycogen content of soleus and gastrocnemius was significantly greater in the melatonin-supplemented exercise groups than the exercise control group ($p < 0.05$). Similarly, the melatonin-supplemented rest groups both had significantly higher muscle glycogen concentrations in comparison to the rest control group ($p < 0.05$). Plasma and liver β-hydroxybutyrate were significantly higher in both melatonin-supplemented exercise groups in comparison to the non-supplemented exercise group ($p < 0.05$), indicating that there was an enhancement of lipid utilization in melatonin-supplemented rats during exercise.

A recent study by Robeva et al. (2008) examined the interaction of melatonin and insulin secretion in a group of 40 young (32.6 ± 3.17 yr) subjects with metabolic syndrome (MS) and 19 clinically healthy controls (29.3 ± 2.45 yr). Fasting blood samples were drawn to measure glucose, insulin, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG). Furthermore, melatonin and insulin were sampled at 11 am, 7 pm and 3 am. Results demonstrated that there was an inverse correlation between 24 hour melatonin production and fasting glucose ($r = -0.316, p = 0.047$). In subjects with MS the night/day melatonin percentage difference correlated negatively with fasting glucose ($r = -0.494, p = 0.023$). There was also a weak negative correlation between percentage of day/night melatonin difference and LDL ($r = -0.367, p = 0.025$).

Melatonin and Lipid Metabolism

An intriguing article by Nishida et al. (2002) examined the effect of 30 weeks of melatonin supplementation on markers of lipid metabolism in male rats. Subjects were divided into three groups: control (CON), type 2 diabetes (T2D) or diabetes and implanted melatonin (T2DM). The melatonin implanted group received a dose of 1.1 mg/day. Non-fasting levels of plasma glucose, insulin, leptin, TG, total cholesterol (TC), phospholipids and glutamic oxalacetic transaminase (GOT) were measured at 0, 16 and 30 weeks. An assay was performed pre and post to determine the fatty-acid Δ-5 desaturase levels. Results indicated a significant decrease in plasma TG (-39%, $p < 0.05$) and TC (-27%, $p < 0.001$) in T2DM as compared to T2D. Melatonin decreased the level of plasma leptin at 16 (-33%, $p < 0.01$) and 30 (-43%, $p < 0.01$) weeks in T2DM in comparison to T2D. Melatonin had no significant effect on hyperinsulinemia at 16 weeks, but significantly reduced insulin levels at 30 weeks (-33%, $p < 0.01$) in T2DM as compared to T2D. The Δ-5 desaturase activity was significantly increased in the T2DM group in comparison to the T2D group (148%, $p < 0.005$).

In another investigation by Nishida et al. (2003) investigators examined the effect of reduced melatonin secretion (pinealectomy) on plasma and hepatic lipid levels in rats with (OLETF) and without (LETO) T2D. Subjects were divided into three groups: pinealectomized rats (PO), sham-operated OLETF rats (SO) and sham-operated LETO rats (SL). Plasma leptin, insulin, glucose, triglyceride (TG), free-fatty acid (FFA), total cholesterol (TC) and free cholesterol were measured at 0, 16 and 30 weeks. Hepatic acetyl-Co-A synthetase activity was measured by assay. Plasma TG levels were significantly higher in SO and PO at 0 and 30 weeks ($p < 0.05$, $p < 0.001$, respectively). Plasma free cholesterol was significantly higher in PO than SL at 30 weeks

($p < 0.05$). Hepatic TG levels were significantly higher in PO than SO ($p < 0.005$) and SL ($p < 0.001$). Plasma leptin levels were significantly higher in SO and PO at 16 ($p < 0.001$) and 30 ($p < 0.005$) weeks, in comparison to SL.

Wolden-Hanson et al. (2000) investigated the effect of 12 weeks of melatonin supplementation on body weight, intra-abdominal adiposity, food intake and hormone levels in three groups of Sprague Dawley rats (young, middle-aged melatonin-supplemented and middle-aged control). Physical activity was assessed by infrared beam and body composition by DEXA. Plasma glucose, insulin, leptin, melatonin and corticosterone levels were measured by assay. Melatonin was administered via water bottle and was approximately 1.3-1.7 μg/h throughout the night (10 hours). Results indicated that the melatonin-supplemented group experienced a 3% loss of body weight while the middle-aged control group had a 3.6% increase ($p < 0.0001$). Core body temperature and physical activity were both significantly higher in the melatonin-supplemented group than the age-matched control ($p < 0.01, p < 0.05$, respectively). The melatonin group had a significant reduction in intra-abdominal fat in comparison to age-matched controls ($p < 0.05$). Plasma leptin and insulin levels were significantly reduced in melatonin-supplemented rats in comparison to age-matched controls (-33% and -25%, respectively, $p < 0.05$).

Kadhim et al. (2006) examined the effect of melatonin and zinc supplementation on lipid profile using the same population and methodology as Hussain et al. (2006). Briefly, participants with T2D ($n = 46$) and age-matched controls ($n = 17$) were randomly assigned to one of three groups for a period of 90 days: metformin (2550 mg/day) and placebo (group A), metformin (2550 mg/day) and melatonin/zinc combination (10 mg/50 mg/day, group B) or melatonin/zinc

combination (10 mg/50 mg/day, group C). Fasting blood samples were collected to measure total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Urine samples were collected to measure microalbuminuria (MAU). Results indicated a significant decline in TC for groups B and C in comparison to group A after 90 days of treatment ($p < 0.05$), indicating that melatonin/zinc and melatonin/zinc in combination with metformin lower TC more than metformin alone. Similarly, there was a significant reduction in TG in groups B and C in comparison to group A ($p < 0.05$), indicating that melatonin/zinc and melatonin/zinc in combination with metformin lower TG more than metformin alone. Groups B and C also significantly increased HDL levels in comparison to group A ($p < 0.01$). LDL levels were significantly reduced in groups B and C at 30 and 90 days in comparison to group A ($p < 0.05$) and significantly reduced in comparison to baseline values. Likewise, groups B and C also experienced a significant decline in MAU in comparison to baseline values and group A ($p < 0.05$).

Melatonin and Antioxidant Capability

Oxidative stress can be broadly described as an imbalance between reactive oxygen species (ROS) production and the cellular ability to reduce these potentially harmful substances through antioxidant defenses. At physiological levels ROS are important cellular signaling molecules and a normal byproduct of cellular metabolism. Under optimal conditions ROS molecules are rigidly controlled by endogenous antioxidant defenses, effectively nullifying any deleterious effect. An oxidative imbalance, which is a direct result of hyperglycemia, will result in cellular damage and

is a significant contributing factor to the pathophysiology of T2D (Brownlee, 2001; Ceriello and Testa, 2009).

While there is a strong theoretical background advocating the use of conventional antioxidant therapy to ameliorate oxidative stress, studies using supplemental vitamins C, E and beta-carotene have yielded disappointing results (Beckman et al., 2003). In fact, two meta-analyses that have investigated the effect of antioxidant status on the prevention of cardiovascular disease and all-cause mortality found no relationship with the cardiovascular death rate (Vivekananthan et al., 2003). Taken in entirety the evidence supporting conventional antioxidants translating into improved health outcomes is equivocal.

Conventional antioxidants, however, represent only a small portion of a biologically active class of compounds that inhibit oxidation. Given the lackluster performance of conventional antioxidants in addressing health-related outcomes induced by oxidative stress it has been suggested that a "new antioxidant" approach to oxidative stress should focus on increasing intracellular antioxidant defenses and controlling free radical formation at its source (Ceriello and Testa, 2009).

Under optimal conditions conventional antioxidants quench oxidants as they are formed. However, T2D represents a disease state in which there is an imbalance between antioxidative resources and oxidant production that results in a state of oxidative stress. The "holy grail" of antioxidants would be capable of directly scavenging free radicals, but more importantly, it would also be capable of: a) stimulating antioxidative enzymes, b) directly promoting glutathione synthesis, and c) reducing production of free radicals in the mitochondria. Evidence

from animal and human models suggests that melatonin is capable of achieving these results (Reiter et al., 2007).

An investigation by Paskaloglu, Sener and Ayanoglu-Dulger (2004) examined the effect of exogenous melatonin supplementation on glutathione synthesis and diabetes-induced changes in rat aorta and corpus cavernosum. Twenty four male Wistar rats were divided into one of three groups: control group (CON), diabetes-induced group (DM) or melatonin treated (MEL). The DM group was further divided into four subgroups: melatonin treated (10 mg/kg/day), insulin treated (6 U/kg/day), insulin and melatonin treated, or no treatment. At eight weeks the rats were euthanized. The corpus cavernosum and aorta were removed and analyzed. Excised tissues were analyzed for isometric contraction using a force-displacement transducer. Glutathione was assessed by spectrophotometry and malondialdehyde was assessed by assay. Smooth muscle contractility of the aorta was significantly reduced in the DM group compared to CON ($p < 0.001$). Treatment of insulin, melatonin or both in the diabetes-induced group significantly improved contractility. Levels of malondialdehyde, a by-product of lipid peroxidation, were significantly higher in diabetes-induced rats than in CON ($p < 0.001$). Melatonin or the melatonin/insulin combination significantly reduced malondialdehyde levels in diabetes-induced rats in comparison to the diabetes control group ($p < 0.001$). Glutathione levels were decreased in the DM group when compared to the CON group ($p < 0.001$). Melatonin or melatonin/insulin significantly increased glutathione synthesis in comparison to the diabetes control group ($p < 0.001$).

An article by Chang et al. (2000) investigated the influence of melatonin supplementation on oxidative damage. Sixty five male Wistar rats were divided into four groups: untreated control

(group 1), injected control (group 2), melatonin supplemented (5 mg/kg) (group 3) and melatonin supplemented (100 mg/kg) (group 4). Sixty of the rats underwent a hypoglossal nerve (HN) transection, while five of the untreated controls had a sham operation in which the HN was exposed, but not transected. Subjects were processed at 3, 7, 14, 21 or 30 days. NADPH-d and nitric oxide synthase (NOS) were quantified from tissue sections. In group 1 NADPH-d and NOS reactivity were increased at 3 (1.71%), 7 (37.6%) and 14 (65.7%) days, before beginning a gradual decline. Group 3 was significantly decreased in comparison to group 1 at days 7 (30.5% vs. 37.6%, $p < 0.05$), 14 (48.6% vs. 65.7%, $p < 0.05$), 21 (43.1% vs. 36.9%, $p < 0.05$) and 30 (19.9% vs. 15.7%) days. Group 4 experienced even greater reductions in comparison to group 1 at days 7 (37.6% vs. 21.2%, $p < 0.05$), 14 (65.7% vs. 31.8%, $p < 0.05$), 21 (43.1% vs. 23.6%, $p < 0.05$) and 30 days (19.9% vs. 12.3%), indicating an inverse dose-response relationship between level of oxidative stress and melatonin dose.

Toxicology of Exogenous Melatonin

A study by Seabra et al. (2000) investigated the toxicological effects of melatonin supplementation over a period of five weeks. Forty healthy male volunteers (25–55 years) were assigned to either control ($n = 10$) or melatonin group ($n = 30$). An initial baseline visit included polysomnography testing (PSG), the Epworth Somnolence Scale (ESS), plasma melatonin levels and clinical lab exams, including blood cell count, albumin, glucose, triglycerides, total cholesterol, HDL, LDL, very low-density lipoprotein (VLDL), urea, creatinine thyroid stimulating hormone (TSH), T_3, T_4, cortisol and melatonin levels. All subjects were further required to keep a sleep diary. Melatonin (10 mg) and/or placebo pills were provided with

instructions to ingest pill one hour before bedtime. Three site visits, each spaced seven days apart, followed, in which subjects filled out side effects (SE) questionnaires and underwent blood sampling, PSG and ESS testing. The intervention period was 28 days. A fifth round of testing occurred seven days post-intervention. There were no significant differences between groups for any parameter in the clinical exam or biochemical analysis. The most common side effects were somnolence (n = 17) and headache (n = 14). The placebo had a rate of SE of 83.3% and the melatonin group 60%, with no significant difference between groups. There were no significant adverse events reported.

Jan et al. (2000) investigated the effective dose of controlled release (CR) and fast release (FR) melatonin necessary to treat chronic sleep-wake cycle disorders in 42 multi-disabled children (4-21 yr). An initial randomized, double-blind crossover design involving 16 children currently taking melatonin for three months was implemented. The subjects received either FR or CR at varying doses (2-20 mg) for a period 11 days, after which the type of melatonin was crossed over for an additional 11 days. Following the initial 22 day period all children received the CR melatonin for several weeks; the parents were encouraged to alter the dose in order to find the minimal most effective dosage. A follow-up study was conducted with 42 children that were currently taking melatonin (avg. duration of treatment 2.2 years). The subjects received CR, FR or a combination thereof for an average duration of 2.8 years. No significant adverse effects were reported.

Melatonin has been extensively studied in humans at dosages ranging from 5 mg up to 20 grams per day (Kedziora-Kornatowska et al., 2007; Lissoni et al., 1997) with no serious adverse events reported and is widely regarded as a nontoxic and benign substance (Reiter et al., 2009).

Two recent meta-analyses', which examined 27 studies with a cumulative population of 873 subjects, concluded that melatonin was safe within the investigated dosages (0.5 – 10mg) and timeframes (1 – 90 days) and there were no significant differences in reported side effects between melatonin and placebo. The most commonly reported side-effects of melatonin supplementation are nausea, headache, dizziness and drowsiness (Buscemi et al., 2005; Buscemi et al., 2006).

Conclusion

Recent articles suggest that glycemic control may be related to melatonin secretion (Radziuk and Pye, 2006; Robeva et al., 2008). Interestingly, melatonin secretion is diminished in T2D (Peschke et al., 2007), which is characterized by hyperglycemia, as well as being negatively correlated with aging (Claustrat et al., 2005; Lunenfeld et al., 2001). Similarly, there also exists a relationship between aging and insulin resistance, with advancing age being positively correlated with increasing insulin resistance (Fujita et al., 2007). It has been demonstrated in animal models that the suppression of melatonin through pinealectomy has a significant negative impact upon glucose homeostasis by decreasing insulin sensitivity, as well as reducing GLUT-4 content and insulin-stimulated glucose uptake in rats (Zanquetta et al., 2003; Lima et al., 1998). Furthermore, melatonin suppression through pinealectomy also appears to have a deleterious effect upon plasma and hepatic triglycerides and plasma cholesterol (Nishida et al., 2003).

Melatonin supplementation, however, has demonstrated significant improvements in glycemic control in subjects with T2D (Hussain et al., 2006), has increased glucose uptake in in vitro skeletal muscle (Ha et al., 2006), and has shown a glycogen sparing effect in skeletal

muscle (Mazepa et al., 2000). Its supplementation is also associated with up-regulation of GLUT-4 content (Zanquetta et al., 2003; Mazepa et al., 2000).

Perhaps as importantly, there also appears to be a relationship between melatonin and lipid metabolism. Melatonin supplementation has demonstrated increased lipid metabolism during exercise (Mazepa et al., 2000) as well as decreasing abdominal adiposity (Wolden-Hanson et al., 2000) in rats. Likewise, melatonin has shown significant improvements for total cholesterol, triglycerides and LDL cholesterol (Kadhim et al., 2006), while reducing long-term hyperinsulinemia and increasing hepatic lipid metabolism (Nishida et al., 2002).

T2D contributes to a state of hyperglycemia-induced oxidative stress that increases endothelial dysfunction (Paskaloglu, Sener and Ayanoglu-Dulger, 2004) and microvascular and macrovascular damage (CDC, 2011; ADA, 2007). Melatonin, however, is a potent antioxidant (Korkmaz et al., 2009; Tan et al., 1993) and appears to have the capability to ameliorate many of the associated deleterious processes by increasing antioxidant defense (Paskaloglu, Sener and Ayanoglu-Dulger, 2004; Reiter et al., 1995). Furthermore, melatonin supplementation has been found to be nontoxic at physiologic and pharmacologic levels in both humans and animals (Hussain et al., 2006; Jan et al., 2000; Seabra et al., 2000; Nishida et al., 2002).

There is, however, a relative dearth of research investigating the effect of exogenous melatonin on glucose homeostasis, lipid metabolism and oxidative stress in humans. This gap in the literature provides a compelling reason to undertake an investigation into the role of supplemental melatonin.

REFERENCES

American Diabetes Association. Diagnosis and classification of diabetes mellitus: Position statement. *Diabetes Care* 30: S42-47, 2007.

Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF and Creager MA. Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. *American Journal of Physiology: Heart and Circulatory Physiology* 285(6): H2392-H2398, 2003.

Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813-820, 2001.

Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Baker G, Klassen TP and Vohra S. The Efficacy and Safety of Exogenous Melatonin for Primary Sleep Disorders. *Journal of General Internal Medicine* 2(12): 1151-1158, 2005.

Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold R, Hartling L, Vohra S, Klassen TP and Baker G. Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. *British Medical Journal* 332(7538): 385-393, 2006.

Ceriello A and Testa R. Antioxidant anti-inflammatory treatment in type 2 diabetes. *Diabetes Care* 32(Suppl 2): S232-S236, 2009.

Chang HM, Ling EA, Lue JH, Wen CY and Shieh JY. Melatonin attenuates neuronal NADPH-d/NOS expression in the hypoglossal nucleus of adult rats following peripheral nerve injury. *Brain Research* 873: 243-251, 2000.

Claustrat B, Brun J and Chazot G. The basic physiology and pathophysiology of melatonin. *Sleep Medicine Reviews* 9 (1): 11-24, 2005.

Center for Disease Control and Prevention. National diabetes fact sheet, 2011. http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Accessed on April 3, 2012.

Fujita S., Rasmussen B. B., Cadenas J. G., Drummond M. J., Glynn E. L., Sattler F. R. and Volpi E. Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and kat/mammalian target of rapamycin signaling. *Diabetes* 56: 1615-1622, 2007.

Ha E, Yim SV, Chung JH, Yoon KS, Kang I, Cho YH and Baik HH. Melatonin stimulates glucose transport via insulin receptor substrate-1/phosphatidylinositol 3-kinase pathway in C_2C_{12} Murine skeletal muscle cells. *Journal of Pineal Research* 41: 67-72, 2006.

Hussain AS, Khadim HM, Khalaf BH, Ismail SH, Hussein KI and Sahib AS. Effects of melatonin and zinc on glycemic control in type 2 diabetic patients poorly controlled with metformin. *Saudi Medical Journal* 27 (10): 1483-1488, 2006.

Jan JE, Hamilton D, Seward N, Fast DK, Freeman RD and Laudon M. Clinical trials of controlled-release melatonin in children with sleep-wake cycle disorders. *Journal of Pineal Research* 29: 34-39, 2000.

Kadhim HM, Ismail SH, Hussein KI, Bakir IH, Sahib AS, Khalaf BH and Hussain SAR. Effects of melatonin and zinc on lipid profile and renal function in type 2 diabetic patients poorly controlled with metformin. *Journal of Pineal Research* 41: 189-193, 2006.

Kedziora-Kornatowska K, Szewczyk-Golec K, Czuczejko J, van Marke de Lumen K, Pawluk H, Motyl J, Karasek M and Kedziora J. Effect of melatonin on the oxidative stress in erythrocytes of healthy young and elderly subjects. *Journal of Pineal Research* 42: 153-158, 2007.

Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S and Tan DX. Melatonin: An established antioxidant worthy of use in clinical trials. *Molecular Medicine* 15 (1-2): 43-50, 2009.

Lima FB, Machado UF, Bartol I, Seraphim PM, Sumida DH, Moraes SMF, Hell NS, Okamoto MM, Saad MJA, Carvalho CR and Cipolla-Neto J. *American Journal of Physiology, Endocrinology and Metabolism* 38: E934-941.

Lissoni P Paolorossi F, Adrizzoia A, Barni S, Chilelli M, Mancuso M, Tancini G, Conti A and Maestroni GJM. A randomized study of chemotherapy with cisplatin plus etospside versus chemoendocrine therapy with cisplatin, etoposide and the pineal hormone melatonin as a first-line treatment of advanced non-small cell lung cancer patients in a poor clinical state. *Journal of Pineal Research* 23: 15-19, 1997.

Lunenfeld B. Aging men-challenges ahead. *Asian Journal of Andrology* 3 (3): 161-168, 2001.

Mazepa RC, Cuevas MJ, Collado PS and Gonzalez-Gallego J. Melatonin increases muscle and liver glycogen content in nonexercised and exercised rats. *Life Sciences* 66 (2): 153-160, 2000.

Nishida S, Segawa T, Murai I and Nakagawa S. Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Δ-5 desaturase activity. *Journal of Pineal Research* 32: 26-33, 2002.

Nishida S, Sato R, Murai I and Nakagawa S. Effect of pinealectomy on plasma levels of insulin and leptin and on hepatic lipids in type 2 diabetic rats. *Journal of Pineal Research* 35: 251-256, 2003.

Paskaloglu K, Sener G and Ayanoglu-Dulger G. Melatonin treatment protects against diabetes-induced functional and biochemical changes in rat aorta and corpus cavernosum. *European Journal of Pharmacology* 499: 345-354, 2004.

Peschke E, Stumpf I, Bazwinsky I, Litvak L, Dralle H and Muhlbauer. Melatonin and type 2 diabetes – a possible link? *Journal of Pineal Research* 42: 350-358, 2007.

Radziuk J and Pye S. Diurnal rhythm in endogenous glucose production is a major contributor to fasting hyperglycemia in type 2 diabetes. Suprachiasmatic deficit of limit cycle behaviour? *Diabetologia* 49: 1619-1628, 2006.

Reiter RJ. The role of the neurohormone melatonin as a buffer against macromolecular oxidative damage. *Neurochemistry International* 27 (6): 453-460, 1995.

Reiter RJ, Tan DX, Manchester LC, Pilar Terron M, Flores LJ and Koppisepi S. Medical implications of melatonin: Receptor-mediated and receptor-independent actions. *Advances in Medical Sciences* 52: 11-28, 2007.

Reiter RJ, Paredes SD, Manchester LC and Tax DX. Reducing oxidative/nitrosative stress: A newly-discovered genre for melatonin. *Clinical Reviews in Biochemistry and Molecular Biology* 44(4): 175-200, 2009.

Robeva R, Kirilov G, Tomova A and Kumanov P. Melatonin-insulin interactions in patients with metabolic syndrome. *Journal of Pineal Research* 44: 52-56, 2008.

Seabra MLV, Bignotto M, Pinto LR Jr. and Tufik S. Randomized, double-blind clinical trial controlled with placebo, of the toxicology of chronic melatonin treatment. *Journal of Pineal Research* 29: 193-200, 2000.

Tan DX, Chen LD, Poeggler B, Manchester LC and Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. *Journal of Endocrinology* 1: 57-60, 1993.

Vivekananthan DP, Penn MS, Sapp SK, Hsu A and Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: Meta-analysis of randomized trials. *Lancet* 361: 2017-2023, 2003.

Wolden-Hanson T, Mitton DR, McCants RL, Yellon SM, Wilkinson CW, Matsumoto AM and Rasmussen DD. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. *Endocrinology* 141 (2): 487-497, 2000.

Zanquetta MM, Seraphim PM, Sumida DH, Cipolla-Neto J and Machado FM. Calorie restriction reduces pinealectomy-induced insulin resistance by improving GLUT-4 gene expression and its translocation to the plasma membrane. *Journal of Pineal Research* 35: 141-148, 2003.

PROJECT III

The Effects of Walking vs. Table Tennis on Postprandial Glycemia, Heart Rate Variability, and Mood in Type 2 Diabetes

Postprandial Glycemia

The primary goal for the treatment of T2D is the strict control of blood glucose levels. Several clinical trials have found that reductions in A1C, a long-term marker of glycemic control, are associated with decreased risk of vascular complications (Shichiri et al., 2000; UKPDS, 1998). This is significant as micro- and macrovascular complications represent the primary source of mortality and morbidity in diabetes (Fowler, 2008). The American Diabetes Association recognizes an A1C level of 6.5% as a threshold of diagnosis, as well as being associated with an increased risk of retinopathy (ADA, 2012). However, several recent investigations have indicated that control of postprandial glycemia may play a more prominent role in A1C maintenance than simply controlling fasting blood glucose (Shichiri et al., 2000; Woerle et al., 2007).

Woerle et al. (2007) investigated the effect of a forced, titrated glycemic treatment program on 164 T2D individuals with poor glycemic control (A1C>7.5%). Subjects participated in 10.5 hours of diabetes training and optimal therapy modification was overseen by a physician. Participants self-monitored blood glucose levels seven times per day; three preprandial measurements were taken at 7 am, 1 pm and 7 pm, three postprandial measurements were taken 90 minutes after each of three meals, and a final measurement was taken at 11 pm. A1C levels were measured at baseline and following the three month intensive glycemic control

intervention. Goals for the intervention included achieving a fasting glucose level <100mg/dL and a 90 minute postprandial glucose level <140mg/dL. At the conclusion of the intervention subjects were stratified into two groups, those that achieved an A1C < 7% and those that remained > 7%. There was a significant overall reduction in fasting plasma glucose levels in both groups (~174 mg/dL to ~117 mg/dL). However, the <7% group had significantly lower average postprandial (-27 mg/dL, $p<0.001$) and daylong glucose levels (-24 mg/dL, $p<0.001$) than the >7% group. Within the <7% group only 64% achieved a fasting glucose level <100 mg/dL; however, 94% of those that achieved a postprandial glucose level <140 mg/dL had an A1C level <7%. The results of this investigation demonstrate that postprandial glycemia contributes more than fasting glucose levels to A1C.

The role of postprandial glycemic spikes in complications of T2D is multifactorial. For example, a recent study by Tobin et al. (2011) investigated the effect of a 75 gram dose of glucose on postprandial microcirculation in eight male subjects with T2D and eight age- and BMI-matched normoglycemic subjects. Ultrasound was used to measure blood flow of the subcutaneous abdominal adipose tissue and the musculature of the forearm (brachioradialis). Ultrasound measurements were taken at 60, 120 and 180 minutes following the glucose load. Results demonstrated that the T2D group had no change in blood flow to adipose tissue at any time point. In contrast, the age- and BMI-matched control group realized a significant increase in blood flow to adipose tissue at 60 and 120 minutes following glucose ingestion. The T2D group demonstrated significantly higher plasma glucose levels than the control group at all time points. In the aftermath of meal ingestion, an increase in blood flow to adipose tissue aids in regulating plasma macronutrient levels as excess glucose and lipids may be stored and removed from circulation. However, in this investigation, individuals with T2D demonstrated a blunted

vascular response following glucose ingestion. In the short-term this reduction in circulation could be a contributing factor to postprandial glycemic spikes. The long-term consequences, however, could be much more significant and could potentially include increased incidence of micro- and macrovascular disease.

The acute effect of a bout of aerobic exercise has been shown to lower blood sugar levels in T2D for 24 to 72 hours (ACSM/ADA, 2010). The timing of an exercise intervention, however, may have an important impact of postprandial glycemic spikes in individuals with T2D. Recently, Colberg et al. (2009) compared the effect of 20 minutes of low-to-moderate intensity exercise (~40% of heart rate reserve) undertaken before and after a standardized meal of approximately 400 kilocalories. Two exercise trials, before and after dinner (PRE and POST, respectively), and one non-exercise control trial (CON) were undertaken in random order by 12 older (61.4±2.7years) individuals with uncomplicated T2D. Blood glucose was measured via an intravenous catheter at 30 minutes intervals during the four-hour intervention. The change in plasma glucose at 90 minutes, immediately following the cessation of exercise, was significantly lower in the POST group (-0.41±0.34 mmol/L) compared to the Pre (1.83±0.66) and CON (0.73±0.56) groups. These results indicated low-to-moderate intensity postprandial exercise may be more effective than preprandial exercise at controlling a postprandial glycemic spike.

Similarly, Poirier et al. (2000) investigated the effect of a bout of moderate-to-vigorous aerobic exercise on plasma glucose levels in 10 sedentary males with T2D (54±5years). Subjects participated in two randomly assigned trials during which they exercised for 60 minutes at 60% of VO_{2peak}. The trials consisted of exercise in the fasted state (CON) or following a 395-calorie standardized breakfast meal (BF). Plasma glucose levels were measured at 15 minute intervals

throughout the exercise bout and up to 30 minutes post-exercise. Plasma glucose levels were significantly reduced at the end of 60 minutes of exercise in CON, however, plasma glucose returned to baseline levels within 15 minutes. In contrast, in the BF trial 60 minutes of exercise following a breakfast meal resulted in a significant plasma glucose reduction immediately following exercise (60 minutes) and during recovery (at 75 and 90 minutes, respectively). These results indicate that 60 minutes of moderate-to-vigorous exercise had a significantly greater impact on plasma glucose levels when in a postprandial state than in a fasted state.

Heart Rate Variability

Heart rate variability (HRV) is measured by the time oscillation between successive heart beats and represents the inputs of autonomic control over the cardiac cycle. In the absence of external stimuli the intrinsic heart rate is $\geq$ 100 bpm (Jose et al., 1970). This baseline value, however, is in a constant flux created by a balance between inputs from the sympathetic and parasympathetic branches of the autonomic nervous system. These two autonomic inputs, collectively termed sympathovagal balance, act in a coordinated manner and represent the hearts ability to adapt to changing stimuli (Acharya et al., 2006).

HRV measurements are categorized as time, frequency or geometric domain, with time and frequency domain the most commonly cited measurements in the literature (Task Force, 1996). This measurement reflects the variability within the cardiac cycle or, more succinctly, the balance between sympathetic and parasympathetic influence and has demonstrated an ability to provide important information about autonomic modulation (Task Force, 1996). T2D is associated with a reduction of heart rate variability (Singh et al., 2000). Reduced HRV has also

been associated with increasing carotid artery atherosclerosis and may be an early predictor of macrovascular disease in T2D (Gottsäter et al., 2006).

Autonomic nervous system damage, termed autonomic neuropathy (AN), is a common complication of T2D and can affect any organ of the body (Vinik and Erbas, 2001). AN is a serious neurological deficit and the mortality rate of those with symptomatic AN is 25-50% within 5–10 years (Ewing, Boland and Neilson, 1991; Rathman et al., 1993). Damage to the vagal component of the autonomic nervous system appear early in T2D, contribute to the imbalance between sympathetic and parasympathetic inputs (sympathovagal balance), and manifest as diastolic dysfunction (Poirier et al., 2003). Importantly, however, the onset of cardiovascular autonomic dysfunction may appear prior to diagnosis of T2D. In a study by Laitinen et al. (2011) researchers investigated the prevalence of cardiovascular autonomic dysfunction among 177 older (62±7years) overweight or obese (BMI 29.6±4.5kg/m^2) participants enrolled in the Finnish Diabetes Prevention Study. Parasympathetic dysfunction was assessed via the E/I ratio during three to four cycles of deep breathing and sympathetic dysfunction was measured by the decrease of systolic blood pressure $\geq$ 20 mm/Hg during standing. Parasympathetic dysfunction comprised 25% of the subject population, while sympathetic dysfunction was only 6%.

Exercise training, however, has demonstrated an important ability to improve HRV in T2D. A study by Zoppini et al. (2007) investigated the effect of a six-month aerobic training program on HRV in 12 older (65.7±5.6years) individuals with T2D. Seven males and five females participated in a twice weekly program of moderate-to-vigorous exercise at 50 to 70% of heart rate reserve. There were no significant changes in measurements of HRV during 10 minutes of supine rest. There was, however, a significant increase in high frequency power (vagal

modulation), a significant decrease in low frequency power, and a significant improvement in sympathovagal balance with measurements taken during a 10-minute period of standing. These results indicate a significant shift toward vagal improvement in individuals with T2D following an aerobic training program.

Furthermore, Figueroa et al. (2007) demonstrated improvements in HRV in obese women with and without T2D following a 16-week training program. Prior to the beginning of the training program, eight females with T2D (50±1year) and 12 obese females (48±2years) without T2D underwent a maximal oxygen consumption (VO_{2max}) test. On a separate day resting measurements of HRV were taken in the supine position for five minutes. Immediately thereafter each participant performed a treadmill exercise session at 65% of VO_{2max}. Approximately 20 minutes after the cessation of exercise another five minute recording was taken in the supine position. Measurements were repeated at the end of the 16–week, thrice-weekly training program. Similar to Zoppini et al. (2007), there were no significant changes in HRV taken at rest following the training intervention. However, measurements taken 20 minutes after an acute bout of exercise at 65% of VO_{2max}, following the training intervention, demonstrated increases in high frequency power, low frequency power and baroreflex sensitivity.

Affective Response

Exercise is a core recommendation for the treatment and prevention of T2D (ACSM/ADA, 2010). However, it has been reported that exercise levels among individuals with T2D fall far below the recommended levels (Nelson et al., 2002). For example, Morrato et al. (2007) reported that among a sample of 23,283 U.S. adults, nearly 60% of individuals without diabetes engaged

in 30 minutes or more of moderate-to-vigorous exercise at least three times per week. Among individuals with diabetes, however, only 38.5% reported regular exercise. Interestingly, there was an inverse relationship between the number of diabetes risk factors and likelihood of engaging in regular moderate-to-vigorous exercise.

The affective response to exercise is an important predictor of exercise adherence. An investigation by Williams et al. (2008) examined the association between affective response to a single bout of exercise and future exercise participation over an unsupervised 12-month follow-up period. At baseline 37 older (43.9±8.6 years), sedentary participants filled out a Physical Activity Recall (PAR) questionnaire, an interviewer-administered instrument that measures physical activity participation within the prior seven days. Along with the PAR, the Feeling Scale (FS), a single-item 11-point dimensional scale that measures affect, was also administered prior to undergoing a single bout of graded exercise, which terminated at 85% of age-predicted maximal heart rate. The exercise test, the Balke treadmill protocol, began at 3 mph and increased grade by 2.5% in two-minute intervals. The FS was administered at the end of each interval and the PAR and FS were administered again at six and 12 months. Results indicated there was a positive correlation between affective response on the FS during the single bout of exercise and subsequent minutes of physical activity participation at six and 12 months of follow-up.

Conclusion

Exercise is a proven and widely prescribed form of treatment for T2D (ACSM/ADA, 2010). However, individuals with T2D are less likely to participate in exercise (Morrato et al., 2007). Postprandial glycemia may play a disproportionately large role in glycemic control (Woerle et

al., 2007). Exercise is known to improve glycemia, however, and may be more impactful when undertake following a meal (Colberg et al., 2009). Affective response to exercise is predictive of exercise adherence (Williams et al., 2008). Damage to the autonomic nervous system occurs early and extensively in T2D (Vinik and Erbas, 2001). HRV is an accepted, non-invasive method of assessing autonomic modulation (Task force, 1996). Furthermore, exercise has demonstrated a significant ability to improve parameters of HRV in T2D (Figueroa et al., 2007; Zoppini et al., 2007). Therefore, a more recreational form of exercise, which improves affective response, may improve exercise adherence and positively impact postprandial glycemia and autonomic function.

REFERENCES

Acharya UR, Joseph KP, Kannathal N, Lim CM and Suri JS. Heart rate variability: a review. Medical and Biological Engineering and Computing 44:1030-1051, 2006.

American College of Sports Medicine, American Diabetes Association. Exercise and type 2 diabetes: joint position stand. *Medicine and Science in Sports and Exercise* 42(12): 2282-2303, 2010.

American Diabetes Association. Clinical practice recommendations. *Diabetes Care* 35 (Suppl. 1): S64-S71, 2012.

Colberg SR, Zarrabi L, Bennington L, Nakave A, Somma CT, Swain DP, Sechrist SR: Postprandial walking is better for lowering the glycemic effect of dinner than pre-dinner exercise in type 2 diabetic individuals. *Journal of the American Medical Directors Association* 10(6): 394-397, 2009.

Ewing DJ, Boland O, Neilson JM, Cho CK and Clarke BF. Autonomic neuropathy, QT interval lengthening, and unexpected deaths in male diabetic patients. *Diabetologia* 34: 182-185, 1991.

Figueroa A, Baynard T, Fernhall B, Carhart R and Kanaley JA. Endurance training improves post-exercise cardiac autonomic modulation in obese women with and without type 2 diabetes. *European Journal of Applied Physiology* 100: 437-444, 2007.

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

Gottsäter A, Ahlgren AR, Taimour S and Sundkvist G. Decreased heart rate variability may predict the progression of carotid atherosclerosis in type 2 diabetes. *Clinical Autonomic Research* 16: 228-234, 2006.

Jose AD and Collison D. The normal range and determinants of the intrinsic heart rate in man. *Cardiovascular Research* 4: 160-167, 1970.

Laitinen T, Lindstrom J, Eriksson J, Ilanne-Parikka P, Aunola S, Keinanen-Kiukaanniemi S, Tuomilehto J and Uusitupa M. Cardiovascular autonomic dysfunction is associated with central obesity in persons with impaired glucose tolerance. *Diabetic Medicine* 28: 699-704, 2011.

Morrato EH, Hill JO, Wyatt HR, Ghushchyan V, Sullivan PW. Physical activity in U.S. adults with diabetes and at risk for developing diabetes, 2003. *Diabetes Care* 30(2):203-9, 2008.

Nelson KM, Reiber G, Boyko EJ; NHANES III. Diet and exercise among adults with type 2 diabetes: findings from the third national health and nutrition examination survey (NHANES III). *Diabetes Care* (25)10: 1722-1728, 2002.

Poirier P, Bogaty P, Philippon FO, Garneau C, Fortin C, and Dumesnil JG. Preclinical Diabetic Cardiomyopathy: Relation of Left Ventricular Diastolic Dysfunction to Cardiac Autonomic Neuropathy in Men With Uncomplicated Well-Controlled Type 2 Diabetes. *Metabolism* 52(8): 1056-1061, 2003.

Poirier P, Mawhinney S, Grondin L, Tremblay A, Broderick T, Cleroux J, Catellier C, Tancrede G and Nadeau A. Prior meal enhances the plasma glucose lowering effect of exercise in type 2 diabetes. *Medicine and Science in Sports and Exercise* 33(8): 1259-1264, 2001.

Rathman W, Ziegler D, Jahnke M, Haastert B and Gries FA. Mortality in diabetic patients with cardiovascular autonomic neuropathy. *Quarterly Journal of Medicine* 10: 820-824, 1993.

Shichiri M, Kishikawa H, Ohkubo Y and Wake N. Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care* 23 (Suppl. 2), B21-B29, 2000.

Singh JP, Larson MG, O'Donnell CJ, Wilson PF, Tsuji H, Lloyd-Jones DM and Levy D. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). *American Journal of Cardiology* 86: 309-312, 2000.

Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *European Heart Journal* 17: 354-381, 1996.

Tobin L, Simonsen L and Bülow J. The dynamics of the microcirculation in the subcutaneous adipose tissue is impaired in the postprandial state in type 2 diabetes. *Clinical Physiology and Functional Imaging* 31: 458-463, 2011.

U.K. Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. *Lancet* 352: 837-853, 1998.

Vinik AI and Erbas T. Recognizing and treating diabetic autonomic neuropathy. *Cleveland Clinical Journal of Medicine* 68(11): 928-944, 2001.

Williams DM, Dunsiger S, Ciccolo JT, Lewis BA, Albrecht AE and Marcus BH. Acute affective response to a moderate-intensity exercise stimulus predicts physical activity participation 6 and 12 months later. *Psychology of Sport and Exercise* 9(3): 231-245, 2008.

Woerle HJ, Neumann C, Zschau S, Tenner S, Irsigler A, Schirra J, Gerich JE and Göke B. Impact of fasting and postprandial glycemia on overall glycemic control in type 2 diabetes. Importance of postprandial glycemia to achieve target HvA1c levels. *Diabetes Research and Clinical Practice* 77: 280-285, 2007.

Zoppini G. Cacciatori V, Gemma ML, Moghetti P, Targher G, Zamboni C, Thomaseth K, Bellavera F and Muggeo M. Effect of moderate aerobic exercise on sympatho-vagal balance in type 2 diabetes. *Diabetic Medicine* 24: 370-376, 2007.

CHAPTER III

PROJECT I

The Acute Effect of Short-Term Breathing Exercises on Sympathovagal Balance in Type 2 Diabetes

INTRODUCTION

The autonomic nervous system (ANS), via the sympathetic and parasympathetic branches, act in a coordinated manner to maintain cardiovascular homeostasis. Heart rate variability (HRV) reflects the beat-to-beat fluctuations, as measured in milliseconds, of the cardiac cycle that are reflective of the dynamic response of the cardiovascular control systems to physiological perturbations. Under resting conditions HRV is a measure of the underlying tone or balance between sympathetic and vagal inputs, with high variability representing an enhanced adaptive ability and low variability reflecting a diminished adaptive ability.

While type 2 diabetes (T2D) is associated with a total reduction of HRV decrements of parasympathetic activity are independently associated with diastolic dysfunction (Poirier et al., 2003) and contribute to the high incidence of cardiovascular disorders in T2D. Importantly, however, decrements in sympathetic activity are also present in T2D and may decrease the threshold for ventricular fibrillation and predispose an individual with T2D to ventricular tachyarrythmias (Acharya et al., 2006). Conversely, increased tone of the parasympathetic nervous system (PNS) may be protective against sudden cardiac death (Task Force, 1996). As such, HRV represents a simple and non-invasive tool which is useful for understanding the status of the autonomic nervous system in T2D (Acharya et al., 2006).

Damage to the autonomic nervous system is a common complication of T2D, which can result in autonomic neuropathy (AN) and have a deleterious effect on any organ of the body (Vinik and Erbas, 2001). AN is a serious neurological deficit and the mortality rate of those with symptomatic AN is 25–50% within 5–10 years (Ewing, Boland and Neilson, 1991; Rathman et al., 1993). Furthermore, the onset of AN occurs early in the pathology of T2D and is frequently identifiable within the first year after diagnosis (Pfeiffer et al., 1984).

A common sign of AN is a reduction in HRV, which may be an early predictor of macrovascular disease in T2D (Gottsater et al., 2006). In fact, there are detectable deficits in HRV prior to the onset of T2D in the presence of impaired fasting glucose (Singh et al., 2000). Reductions of HRV are also associated with duration of T2D and, significantly, there is a correlation between decreasing HRV and increasing carotid artery atherosclerosis in T2D (Gottsäter et al., 2006).

Yoga is an ancient vedic science composed of eight distinct disciplines. The modern practice of yoga, however, focuses on the three disciplines of hatha yoga (physical postures), pranayama (breathing exercises), and dhyana (meditation). Yogic breathing exercises have independently demonstrated improvements across a diverse variety of ailments and diseases, including hypertension (Dhungel et al., 2008), irritable bowel syndrome (Taneja et al., 2004) and pulmonary function (Malhotra et al., 2002). Pranayama is associated with improved autonomic tone, specifically increased PNS activity (Jerath et al., 2006). Many studies have demonstrated significant short-term improvements in cardiovascular variables with pranayama that may be attributable to improvements in autonomic tone and/or improvements in sympathovagal balance (Dhungel et al., 2008; Srivastava et al., 2005; Pal et al., 2004).

There is, however, only a limited amount of data investigating the effect of pranayama on HRV and sympathovagal balance. Furthermore, to the author's knowledge this is the first study specifically investigating the effect of pranayama on HRV in T2D. Pranayama represents a simple, inexpensive and non-invasive tool that may play a role in improving HRV and, more specifically, parasympathetic drive. Therefore, the purpose of this study was to determine the extent to which a commonly employed yogic breathing technique (alternate nostril breathing, or ANB) affects HRV in individuals with T2D.

METHODS

Subjects

Twelve subjects with uncomplicated T2D (7 female, 5 male; 54.9±7.4 years) and 14 normoglycemic subjects (12 female, 2 male; 54.7±6.8 years) participated in this investigation. The average length of diagnosis within the T2D group was 8.4±6.2 years. All individuals with T2D were treated with oral anti-hyperglycemic drugs and/or diet and exercise. All subjects were pre-screened via a health screening questionnaire to determine eligibility. Exclusionary criteria included congested nasal passages (i.e., common cold or allergies), a deviated septum, congestive heart failure, myocardial infarction, arrhythmia or any cardiovascular event in the previous year, liver disease, kidney disease or pulmonary disease. The study was approved by the Old Dominion University Institutional Review Board and all subjects provided signed informed consent.

Protocol

Subjects were asked to refrain from eating within three hours of testing or consuming any caffeine-containing products on the morning of the testing day. Subjects were fitted with a Bioharness (Zephyr Technology Corp., Annapolis, MD). After pre-moistening the electrodes on the Bioharness, which is similar to a standard heart rate monitor and consists of a single Velcro-adjusted strap, it was placed against the skin, under clothing, at the level of the sternum.

Subjects were positioned in a comfortable chair with the feet flat on the ground prior to receiving detailed verbal instructions regarding the breathing protocol. A laptop computer displaying a breath-pacing program was positioned at eye level to provide both auditory and visual cues for breath pacing (E-Z Air Plus, Thought Technology Ltd., West Chazy, NY). For the ANB portion of the protocol the subjects were instructed to use the contralateral hand to depress and seal off the assigned nostril by placing a single finger (the ring finger) over the nasal vestibule and depressing the tip of the finger until the cessation of air flow was confirmed (Saraswati, 2008). Briefly, the breathing protocol consisted of: 5 minutes of spontaneous breathing (non-paced) (Baseline 1), 2 minutes of spontaneous breathing standing in place (Stand 1), 5 minutes of paced breathing at a rate of 6 breaths per minutes (Baseline 2), 10 minutes of randomly assigned uni-nostril breathing (left nostril breathing (LNB), or right nostril breathing (RNB)), 5 minutes of paced breathing (Baseline3), 10 minutes of uni-nostril breathing (using the opposite nostril) (ANB2), and finally 2 minutes of paced breathing while standing in place. The paced breathing portion of the protocol was adjusted so each inhale was 4 seconds and each exhale was 6 seconds in duration. The total breathing protocol lasted 39 minutes. HRV measurements are presented as individual epochs of time, as well as an "overall" epoch, which encompasses the entire 39 minute protocol.

Heart Rate Variability

HRV data were recorded on a Bioharness collecting at a sampling rate of 250 Hz. The data were then exported as a text file to the HRV analysis software program Kubios HRV 2.0 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Finland). Data was visually inspected on a computer monitor to minimize presence of artifact. Detrending of raw data was performed according to Tarvainen et al. (2002) and power frequency analysis was performed using a fast Fourier transform with Welch's periodogram (256s window with 50% overlap). The following time and frequency domain indices were calculated from the R-to-R intervals: standard deviation of the normal-to-normal RR intervals (SDNN), the square root of the mean squared differences of successive RR intervals (RMSSD) and total spectral power (TP).

Statistics

Data analyses were performed with PASW 17.0 (SPSS, Chicago, IL). All data, except instantaneous heart rate (HR) were logarithmically transformed to induce normality. Within group comparisons were analyzed using ANOVA with repeated measures on 8 time points (Baseline 1, Stand 1, Baseline 2, ANB 1, Baseline 3, ANB 2, Stand 2 and Overall). Between group comparisons were analyzed using independent samples t-test.

RESULTS

Between Groups

Baseline RMSSD in the T2D group was significantly lower than CON ($p=0.03$). Baseline 2 SDNN and RMSSD were significantly lower in T2D compared to CON ($p=0.01$ and $p=0.02$, respectively). LNB SDNN, RMSSD and TP were significantly lower in T2D compared to CON ($p=0.001$, $p=0.002$ and $p=0.001$, respectively) (Figure III.1). Baseline 2 SDNN and RMSSD were significantly lower in T2D than CON ($p=0.003$ and $p=0.002$, respectively). RNB SDNN, RMSSD and TP were significantly lower in T2D compared to CON ($p=0.01$, $p=0.004$ and $p=0.01$, respectively). Baseline 3 measurements of SDNN and RMSSD were significantly lower in the T2D group ($p<0.05$) (Figure III.3). Last Stand SDNN, RMSSD and TP were significantly lower in T2D than in CON ($p=0.02$, $p=0.007$ and $p=0.02$, respectively). Mean HR during Last Stand was significantly higher in the T2D group (7.7bpm, $p<0.05$) (Figure III.2). Overall SDNN, RMSSD and TP were significantly lower in T2D than in CON ($p=0.009$, $p=0.005$ and $p=0.02$, respectively). See Figures III.4, III.5 and III.6 for full protocol comparison of SDNN, RMSSD and TP.

Within Groups

In the T2D group the LNB protocol resulted in a significantly lower mean heart rate (-1.2bpm, $p=.014$) in comparison to the overall protocol measures (Figure III.7). The LNB also resulted in a significant decrease in TP compared to Last Stand (-0.15, $p<0.05$). There were no other significant interactions within the CON or T2D groups.

DISCUSSION

Our findings indicate that two acute bouts of 10 minutes of ANB had no effect on HRV within the T2D group or an age-matched normoglycemic group. Comparisons between groups, however, demonstrated significant reductions in nearly all measures of HRV in the T2D individuals compared to the CON group.

The intrinsic control of heart rate is regulated by the sino-atrial (SA) node. In the absence of outside neurohumoral inputs the SA node maintains a rhythm of approximately 106 beats per minute (bpm) and displays an age-related decline (Jose et al., 1970). Actual resting heart rate, however, is controlled by the counter-balancing of sympathetic and parasympathetic (vagal) inputs to the SA node. While these autonomic inputs occur simultaneously, given that a normal resting heart rate is $\leq$ 70 bpm (Hoffman, 2006), the vagal influence predominates. Therefore, resting heart rate may be used as an index of autonomic tone (Lahiri et al., 2008). In fact, a higher resting heart rate in middle age is predictive of onset of T2D later in life (Carnethon et al., 2008). An increased resting heart rate is also associated with cardiac autonomic neuropathy (CAN) (Ewing and Clarke, 1986). Increased resting heart rate in individuals with T2D is also predictive of increased mortality and cardiovascular complications (Hillis et al., 2012). Significantly, HRV is also inversely associated with plasma glucose levels in T2D, as well as in prediabetes (Singh et al., 2000).

T2D and prediabetes are both associated with a reduction in HRV (Singh et al., 2000), as was confirmed in the present investigation. Parasympathetic damage occurs earlier in the pathology of T2D than sympathetic damage, results in sympathovagal imbalance, which manifests as diastolic dysfunction, and contributes to the high incidence of cardiovascular disorders in T2D

(Poirier et al., 2003). This occurs, in part, because sympathetic activity decreases the fibrillation threshold and may predispose an individual to ventricular fibrillation, while vagal activity increases the threshold and has a protective effect against malignant ventricular tachyarrythmias (Acharya et al., 2006).

Numerous studies have found a significant training effect of yogic breathing exercises upon vagal drive and sympathovagal balance in healthy populations. For example, Udupa et al. (2003) demonstrated that three months of pranayama training (20 minutes per day, five days per week) significantly lowered resting heart rate among adolescent boys in comparison to a control group. However, the pranayama training involved a combination of four different yogic breathing exercises and did not involve any direct measures of HRV. More recently, Dhungel et al. (2008) demonstrated that four weeks of ANB conducted daily in 15-minute sessions significantly reduced resting heart rate, as well as respiratory rate and diastolic blood pressure. Similar to other studies with significant effects, however, Dhungel et al. used healthy, young (24.7 years) predominantly male subjects. In a more recent investigation, Ankad et al. (2011) showed that 15 days of pranayama training significantly lowered resting heart, systolic and diastolic blood pressure and mean arterial pressure in a group of 50 individuals with a diverse age range (20-60 years, mean of 38.6±8.9) and evenly matched gender (24 male, 26 female). Complicating the interpretation of this study, however, was the fact that the daily intervention also included several other components such as meditation.

While several studies have demonstrated a significant training effect of pranayama on autonomic function, fewer have demonstrated an acute effect. Additionally, results have been equivocal and inconsistent. For example, an investigation of the acute effect of a single bout of 15 minutes of ANB in young, healthy male subjects found no significant effect upon HRV

(Raghuraj et al., 1998). In contrast Shannahoff-Khalsa and Kennedy (1993) found that a single bout of ANB conducted for 15 minutes significantly altered heart rate, with LNB decreasing and RNB increasing resting heart rate. The subjects in this investigation, while older (45.1 years), were also previously experienced in yogic breathing exercises. Raghuraj and Telles (2008) found mixed results in a study investigating the acute effect of 30 minutes of ANB breathing on HRV in young, healthy male subjects. However, inconsistencies within the design of that study make interpretation of this investigation problematic. Perhaps the best evidence linking the acute effect of yogic breathing exercises to autonomic function was a study by Pramanik et al. (2009). Using a pharmaceutical blockade of parasympathetic stimulation researchers compared the effect of five minutes of slow pranayama on resting heart rate and blood pressure in 49 participants (10 subjects received the PNS blockade 30 minutes prior to pranayama). Results indicated significant reductions in diastolic and systolic blood pressure in the group without PNS blockade, and no change within the PNS blockade group.

The inconsistency within acute studies, while intriguing, may be based upon the potential mechanism through which pranayama exerts it autonomic-modifying effect. While there is no clear consensus on how pranayama works it has been hypothesized that the autonomic nervous system is "functionally reset" through stimulation of slowly adapting pulmonary stretch receptors (SAR) and hyperpolarization of fibroblasts located within connective tissue found within the lungs (Jerath et al., 2006). Located within smooth muscle of the airways, the SARs, a class of pulmonary vagal receptor (Wang and Zhang, 2003), continuously gauge tension within the elastic components of the tracheobronchial tree. The structure and function of SARs remains controversial; however, they have been found to play a role in systemic vascular tone and heart rate (Jerath et al., 2001), potentially explaining the greater effect noted in training studies as

opposed to acute studies. Further clarification of the interplay between the autonomic nervous system and afferent lung receptors will be needed before the potential mechanism of action underlying pranayama may be elucidated.

The present investigation diverges from previous ones in several important ways. Firstly, the population within this investigation was substantially older. Secondly, no studies have previously investigated the effect of pranayama in T2D. The present investigation found no significant effect of ANB on measurements of HRV in an older (54.7 years), but normoglycemic, population or an age-matched population of individuals with T2D. It is worth noting, however, that this investigation adds to the abundance of literature demonstrating significant reductions in markers of HRV in individuals with T2D.

Conclusion

In summary, two acute bouts of ANB had no impact upon HRV in an older, but healthy, population. There was, however, a significant effect of LNB on resting heart in individuals with T2D. Given the rapid proliferation of T2D, the established association between vagal modulation and cardiovascular complications and the relative ease of yogic breathing techniques further research into the autonomic-modifying effects of pranayama is warranted.

REFERENCES

Acharya UR, Joseph KP, Kannathal N, Lim CM and Suri JS. Heart rate variability: a review. Medical and Biological Engineering and Computing 44:1030-1051, 2006.

Carnethon MR, Yan LY, Greenland P, Garside DB, Dyer AR, Metzger B and Daviglus ML. Resting heart rate in middle age and diabetes development in older age. *Diabetes Care* 31(2): 335-339, 2008.

Dhungel KU, Malhotra V, Sarkar D and Prajapati R. Effect of alternate nostril breathing exercise on cardiorespiratory functions. *Nepal Medical College Journal* 10(1): 25-27, 2008.

Ewing DJ and Clarke BF. Diabetic autonomic neuropathy: present insights and future prospects. *Diabetes Care* 9: 648-665, 1986.

Hillis GS, Woodward M, Rodgers A, Chow CK, Li Q, Zoungas S, Patel A, Webster R, Batty GD, Ninomiya T, Mancia G, Poulter NR and Chalmers J. Resting heart rate and the risk of death and cardiovascular complications in patients with type 2 diabetes mellitus. *Diabetologia* (epub ahead of print).

Hoffman J. Norms for Fitness, Performance and Health (1st edition). Champaign, IL. Human Kinetics, 2006, p. 121.

Jose AD and Collison D. The normal range and determinants of the intrinsic heart rate in man. *Cardiovascular Research* 4: 160-167, 1970.

Jerath R, Edry JW, Barnes VA and Jerath V. Physiology of long pranayamic breathing: Neural respiratory elements may provide a mechanism that explains how slow deep breathing shifts the autonomic nervous system. *Medical Hypotheses* 67: 566-571, 2006.

Lahiri MK, Kannankeril PJ, Goldberger JJ. Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. Journal of the American College of Cardiology 51(18): 1725-1733, 2008.

Malhotra V, Singh S, Singh KP, Gupta P, Sharma SB, Madhu SV and Tandon OP. Study of yoga asanas in assessment of pulmonary function in NIDDM patients. *Indian Journal of Physiology and Pharmacology* 46(3): 313-320, 2002.

Pal GK, Velkumary S and Madonmohan. Effect of short-term practice of breathing exercises on autonomic functions in normal human volunteers. *The Indian Journal of Medical Research* 120: 115-121, 2004.

Poirier P, Bogaty P, Philippon F, Garneau C, Fortin C and Dumesnil JG. Preclinical diabetic cardiomyopathy: relation of left ventricular diastolic dysfunction to cardiac autonomic

neuropathy in men with uncomplicated well-controlled type 2 diabetes. *Metabolism* 52(8): 1056-1061, 2003.

Raghuraj P, Ramakrishnan AG, Nagendra HR and Telles S. Effect of two selected yogic breathing techniques on heart rate variability. *Indian Journal of Physiology and Pharmacology* 42(4): 467-472, 1998.

Raghuraj P and Telles S. Immediate effect of specific nostril manipulating yoga breathing practices on autonomic and respiratory variables. *Applied Psychophysiology and Biofeedback* 33: 65-75, 2008.

Saraswati SS. Asana Pranayama Mudra Bandha (4th edition). Munger, India. Bihar School of Yoga, 2008, p. 96-112.

Shannahoff-Khalsa DS and Kennedy B. The effects of unilateral forced nostril breathing on the heart. *International Journal of Neuroscience* 73: 47-60, 1993.

Singh JP, Larson MG, O'Donnell CJ, Wilson PF, Tsuji H, Lloyd-Jones DM and Levy D. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). *American Journal of Cardiology* 86: 309-312, 2000.

Srivastava RD, Jain N and Singhal A. Influence of alternate nostril breathing on cardiorespiratory and autonomic functions in healthy young adults. *Indian Journal of Physiology and Pharmacology* 49(4): 475-483, 2005.

Taneja I, Deepak KK, Poojary G, Acharya IN, Pandey RM and Sharma MP. Yogic versus conventional treatment in diarrhea-predominant irritable bowel syndrome: a randomized control study. *Applied Psychophysiology and Biofeedback* 29(1), 19-33, 2004.

Tarvainen MP, Ranta-Aho PO, Karjalainen PA. An advanced detrending method with application to HRV analysis. *IEEE Transaction on Bio-medical Engineering* 49(2): 172-175, 2002.

Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation and clinical use. *European Heart Journal* 17(3): 354-381, 1996.

Udupa K, Madonmohan, Bhavanani B, Vijayalakshmi P and Krishnamurthy K. Effect of pranayama training on cardiac function in normal young volunteers. *Indian Journal of Physiology and Pharmacology* 47(1): 27-33, 2003.

Wang JYYF and Zhang JW. Structure of slowly adapting pulmonary stretch receptors in the lung periphery. *Journal of Applied Physiology* 95: 385-393, 2003.

Table III.1 Log transformed time and frequency domain measurements of heart rate variability (mean ±SEM)

	Mean HR (bpm)		**SDNN (ms)**		**RMSSD (ms)**		**Total Power (ms^2)**	
	CON	*T2D*	*CON*	*T2D*	*CON*	*T2D*	*CON*	*T2D*
Baseline	69.7±3.43	73.7±4.01	1.91±0.10	1.64±0.33	1.92±0.12	1.53±0.42*	3.67±0.26	3.07±0.21
First Stand	75.3±2.5	80.1±4.1	2.00±0.13	1.80±0.10	1.87±0.15	1.58±0.15	3.66±0.32	3.08±0.24
Baseline 2	67.0±2.4	72.0±3.5	2.06±0.08	1.72±0.10 *	2.01±0.11	1.62±0.12*	3.92±0.18	3.30±0.22
LNB	67.4±2.2	70.1±2.8‡	1.96±0.08	1.60±0.20 *	1.89±0.11	1.42±0.07*	3.86±0.17	3.07±0.12 *†
Baseline 3	67.5±1.8	70.1±2.9	2.00±0.08	1.63±0.07 *	1.93±0.10	1.47±0.08*	3.95±0.18	3.21±0.13
RNB	67.7±2.0	70.9±3.1	1.94±0.08	1.63±0.08 *	1.88±0.10	1.45±0.09*	3.87±0.21	3.17±0.17 *
Last Stand	71.4±1.9	79.1±3.4*	2.08±0.10	1.73±0.10 *	2.04±0.11	1.53±0.14*	3.95±0.23	3.22±0.20 *
Overall	68.5±2.4	71.3±2.9	2.03±0.07	1.76±0.06 *	1.97±0.09	1.58±0.08*	3.99±0.19	3.41±0.13 *

Mean HR is expressed as beats per minute (bpm), SDNN and RMSSD are expressed as milliseconds (MS) and total power is expressed as milliseconds squared (MS^2). Control subjects, CON; type 2 diabetic subjects, T2D.

*Significant difference vs. CON ($p<0.05$)
†Significant difference vs. Last Stand ($p<0.05$)
‡Significant difference vs. Overall Mean HR within T2D ($p<0.05$)

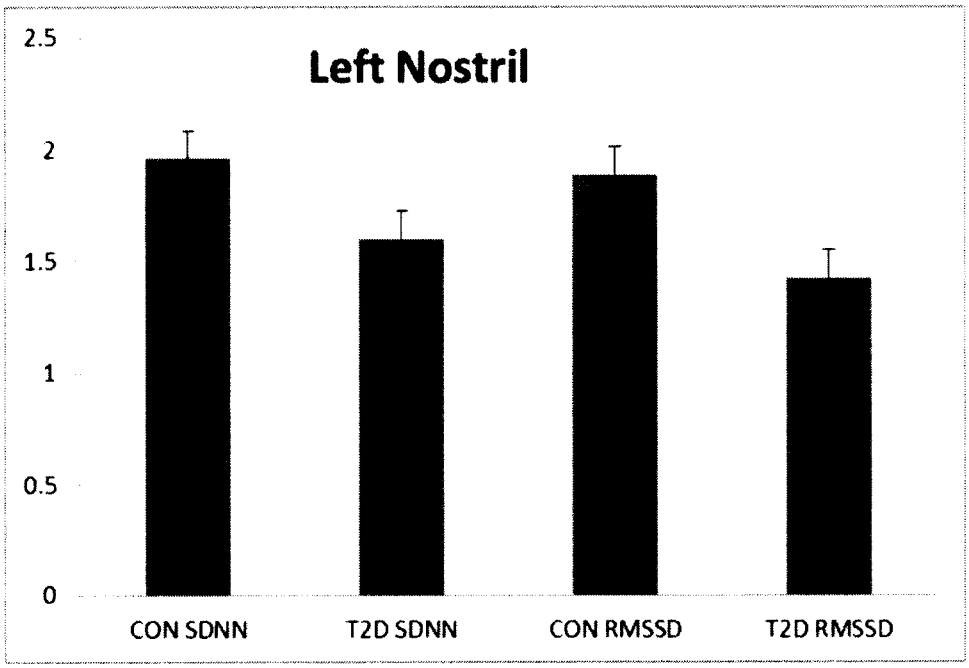

Figure III.1. Log transformed SDNN and RMSSD values during left nostril breathing in T2D compared to CON subjects.

*Significantly different from CON ($p<0.05$).

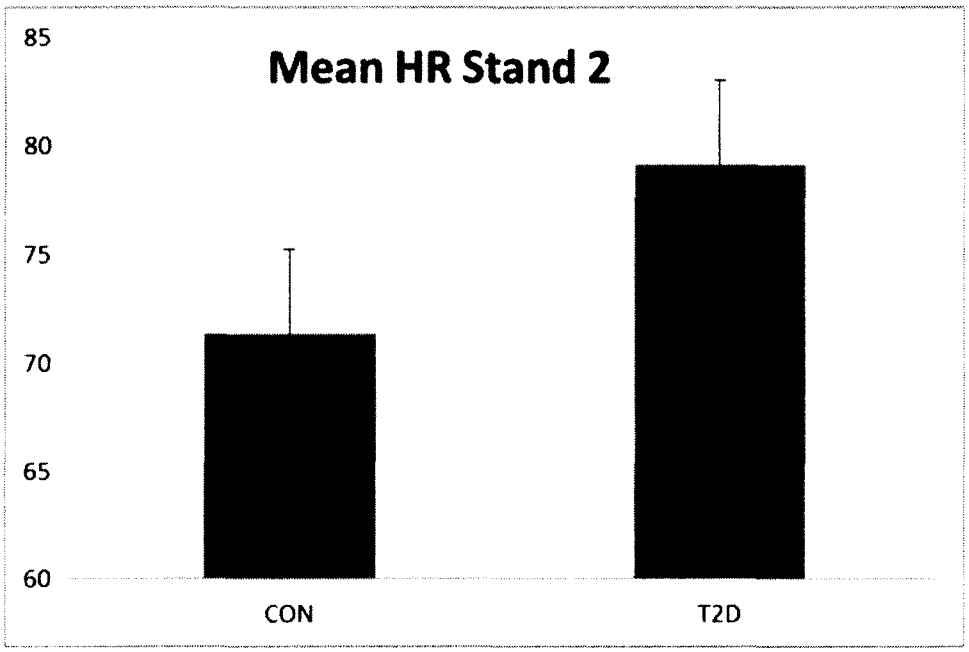

Figure III.2. Mean HR During Stand 2 comparing T2D to CON.

*Significantly different from CON ($p<0.05$).

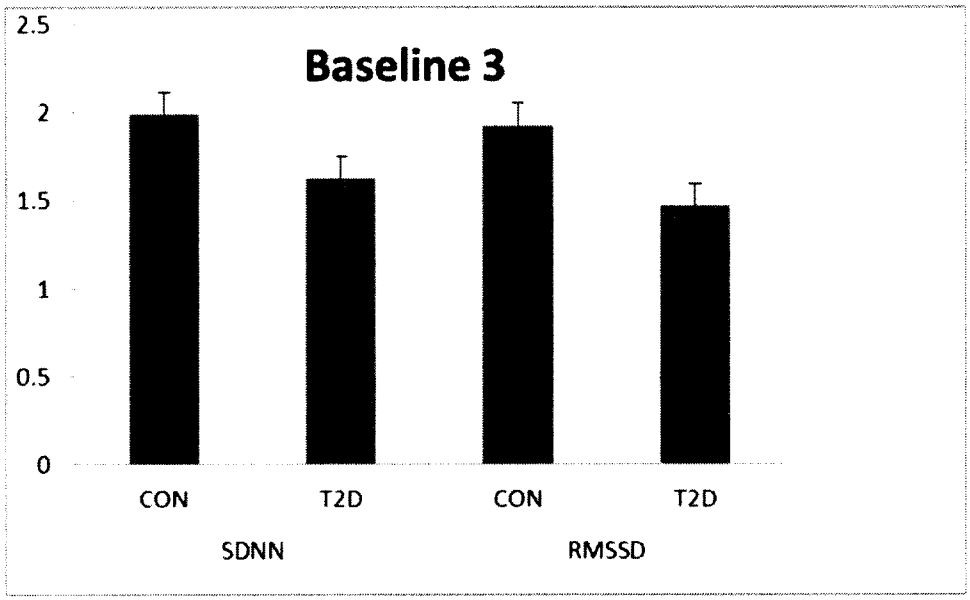

Figure III.3. Log transformed SDNN and RMSSD during Baseline 3 comparing T2D to CON.

*Significantly different from CON ($p<0.05$).

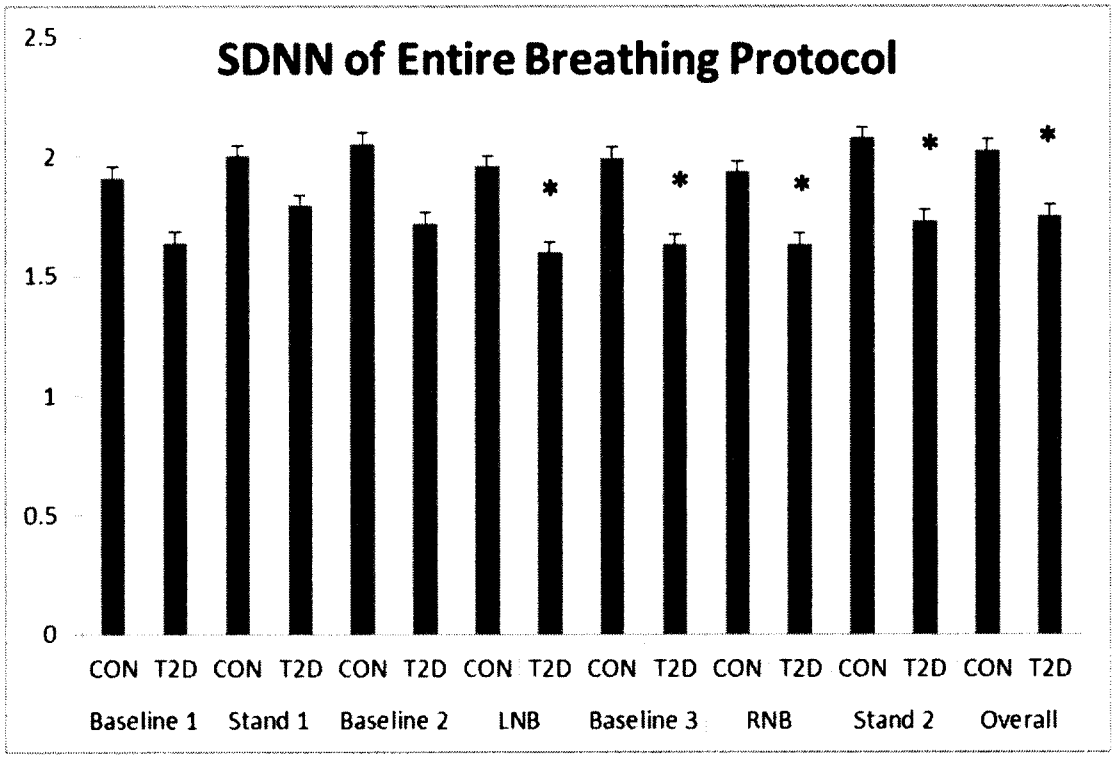

Figure III.4 Log transformed SDNN during entire 39 minute protocol in T2D compared to CON subjects.

*Significantly different from CON ($p<0.05$)

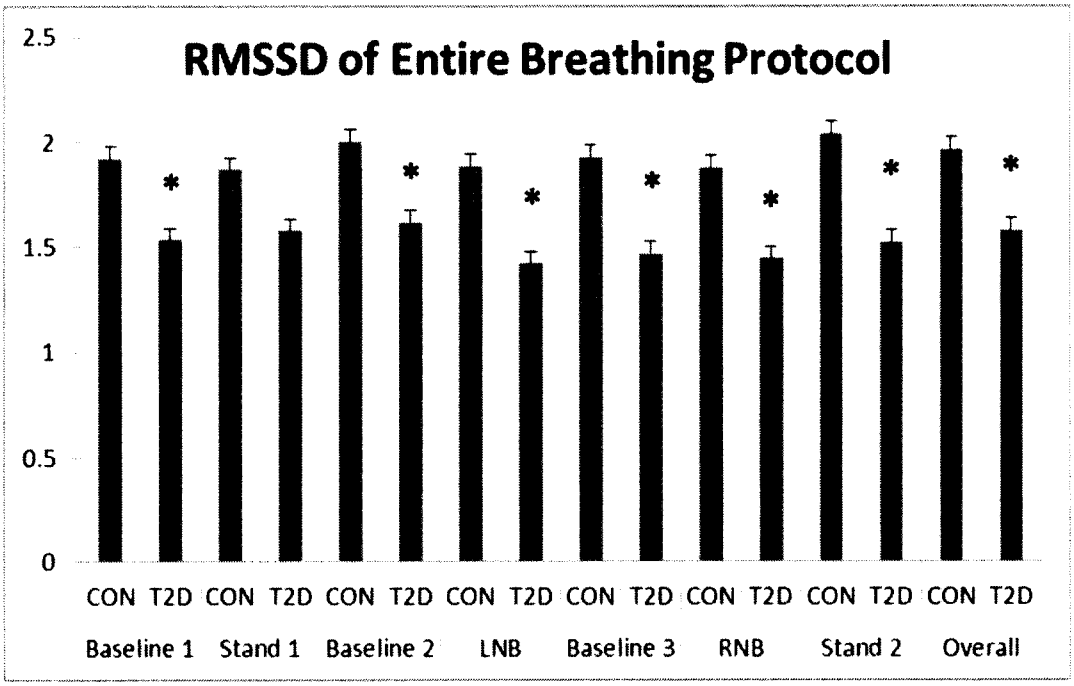

Figure III.5 Log Transformed RMSSD During Entire 39 Minute Protocol in T2D Compared to CON Subjects.

*Significantly different from CON ($p<0.05$)

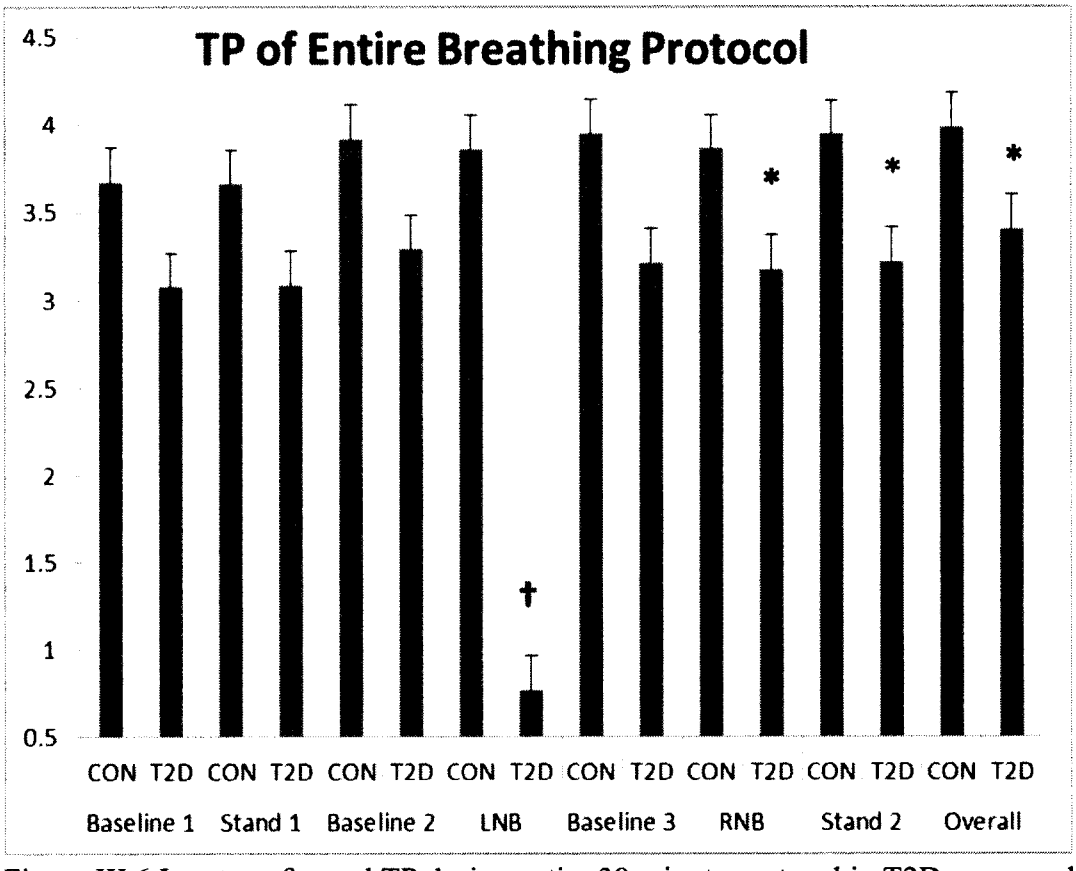

Figure III.6 Log transformed TP during entire 39 minute protocol in T2D compared to CON subjects.

*Significantly different from CON ($p<0.05$)
†Significantly different from Last Stand ($p<0.05$)

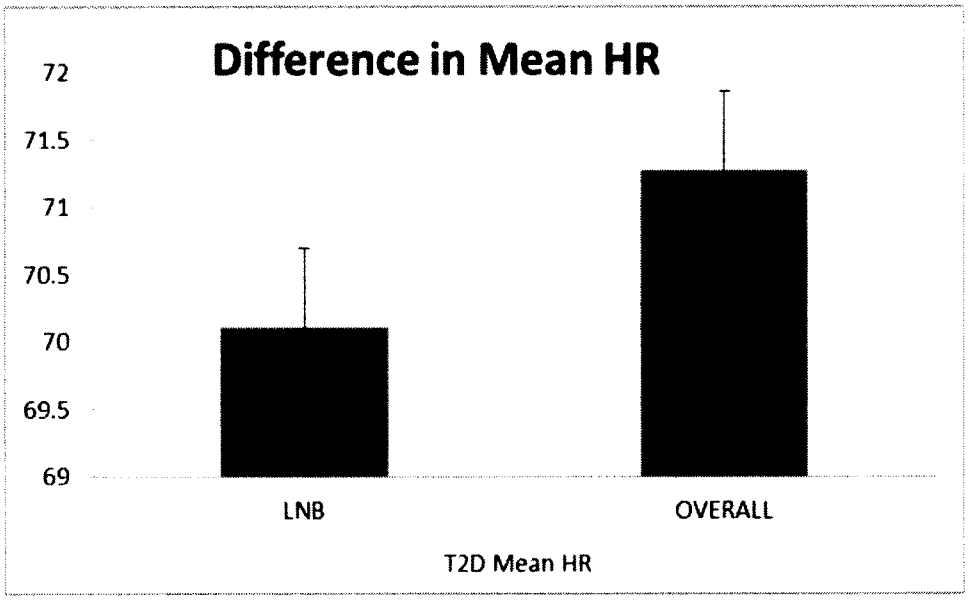

Figure III.7. Mean HR in T2D subjects during the left nostril breathing (LNB) compared to the overall mean HR through the entire breathing protocol.

*Significantly different from Overall mean HR ($p<0.05$).

CHAPTER IV

PROJECT II

The Effect of N-Acetyl-5-Methoxytryptamine (Melatonin) on Glucose Homeostasis, Lipid Metabolism, and Oxidative Stress in Type 2 Diabetes

INTRODUCTION

Complications of type 2 diabetes mellitus (T2D) are believed to result from hyperglycemia-driven increases in oxidative stress. A limited amount of in-vivo evidence indicates a potential role for melatonin in improving glucose regulation, lipid metabolism and oxidative stress in T2D. Melatonin, a hormone primarily secreted by the pineal gland, is most often associated with circadian rhythms and the sleep cycle. It is, however, a potent and unique antioxidant (Korkmaz et al., 2009) that has demonstrated an ameliorative effect on oxidative stress in human and animal models of T2D (Kedziora-Kornatowska et al. 2009; Rosales-Corral et al., 2003). Thus, the purpose of this investigation was to evaluate the effect of a commercially available preparation of melatonin on glycemic control, lipid metabolism, and oxidative stress in T2D.

METHODS

Fourteen sedentary subjects with uncomplicated T2D (10 female, 4 male; 52.5 ± 5.0 years) were randomly assigned to a melatonin group (MEL) or placebo group (PLA) for 42 days, followed by 42 days in the opposite group in a crossover design. Exclusionary criteria included sleep apnea or other sleep disorders, congestive heart failure, myocardial infarction, arrhythmia

or any cardiovascular event in the previous year, liver disease, kidney disease, orthostatic hypotension, or diagnosis of previous or current psychiatric disorder, according to DSM-IV (American Psychiatric Association, 1994). The study was approved by the Old Dominion University Institutional Review Board, and subjects provided signed informed consent prior to their participation.

During each supplementation period, subjects ingested either 10 mg of melatonin in capsule form (Quality Supplement and Vitamins, Inc., Ft. Lauderdale, FL) 30–60 minutes prior to sleep or an identical capsule (Capsuline, In., Pompano Beach, FL) containing white flour. Fasting blood draws occurred on three mornings: prior to supplementation, after 42 days, and after 84 days.

Blood Chemistry Analyses

Blood samples were collected in heparinized tubes using standard venipuncture technique. Lipids, fasting glucose and A1C were immediately analyzed using enzymatic assays (Cholestech Corp., Hayward, CA and Siemens Healthcare Diagnostics, Tarrytown, NY, respectively). Thereafter, samples were frozen and stored prior to spectrophotometric analysis of malondialdehyde (Zeptometrix Corp., Buffalo, NY)

Statistics

Mean differences were analyzed using a 2-way ANOVA with repeated measures on one factor (time), whereas one-tailed, paired t-tests were used to assess change from baseline

between groups. Results are presented as mean ±SEM. Data analyses were performed with PASW 17.0 (SPSS, Chicago, IL). Results were considered to be statistically significant at $P < 0.05$.

RESULTS

The change in A1C resulted in a total improvement of 0.33% after 42 days of melatonin supplementation compared to placebo (-0.24±0.23 % for MEL vs. 0.09±0.21 % for PLA, p=0.01) (Figure IV.1). After supplementation, levels of plasma malondialdehyde (MDA), a marker of oxidative stress, were significantly lower for MEL (-6.25±2.10 nmol/ml) compared to PLA (0.72±3.30, p=0.028) (Figure IV.2). This equates to a 55% reduction in MDA following melatonin supplementation. No significant changes were noted for total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, or fasting plasma glucose.

DISCUSSION

The results of this investigation add to the limited amount of in-vivo evidence demonstrating a potential role of melatonin in improving glycemic control and decreasing oxidative stress in T2D. Similar to animal and in-vitro data, the present investigation realized an ameliorative effect of melatonin supplementation on a marker of oxidative stress, as well as a reduction in long-term glycemia.

It is widely accepted that oxidative stress plays a significant role in the pathogenesis of T2D (Ceriello and Testa, 2009). While there is a strong theoretical background advocating the use of conventional antioxidant therapy to ameliorate oxidative stress, studies using supplemental vitamins C, E and beta-carotene have yielded disappointing results (Beckman et al., 2003). Given the lackluster performance of conventional antioxidants in addressing health-related outcomes induced by oxidative stress it has been suggested that a "new antioxidant" approach to oxidative stress should focus on increasing intracellular antioxidant defenses and controlling free radical formation at its source (Ceriello and Testa, 2009).

The "holy grail" of antioxidants would be capable of directly scavenging free radicals, but more importantly, it would also be capable of: a) stimulating antioxidative enzymes, b) directly promoting glutathione synthesis, and c) reducing production of free radicals in the mitochondria. Evidence from animal models and human in-vitro studies suggests that melatonin is capable of achieving these results (Reiter et al., 2007). Melatonin differs from conventional antioxidants by serving both as a direct scavenger of reactive oxygen species (ROS) (Korkmaz et al., 2009) as well as a potent stimulator of endogenous antioxidant enzymes (Urata et al., 1999).

Interestingly, reductions in plasma melatonin are independently associated with T2D (Radziuk and Pye, 2006). Consequently, this reduction in circulating melatonin, which is associated with significantly higher levels of oxidative stress and reduced antioxidant activity in diabetes (Kedziora-Kornatowska et al., 2009), is likely a direct result of an increased consumption of melatonin due to hyperglycemia precipitating increased levels of oxidative stress (Tan et al., 2007).

Prior to the present investigation, the only study to date that has investigated the effect of melatonin on oxidative stress in vivo in T2D was conducted in elderly patients (Kedziora-Kornatowska et al., 2009). Similar to the present investigation, a 5 mg/day dose of melatonin for 30 days resulted in a substantial reduction in MDA (-20%), as well as increasing superoxide dismutase (+16%), an important intracellular antioxidant defense, and serum melatonin (+70%).

While it is firmly established that oxidative stress is a significant contributing factor to the pathogenesis of T2D, current therapy remains primarily aimed at pharmacological interventions that decrease endogenous glucose production, enhance insulin secretion, or decrease insulin resistance. The current treatment strategy, however, is suboptimal. Diabetic complications are a direct result of hyperglycemia and hyperglycemia-induced oxidative stress, but it has been documented that only 37% of patients with T2D maintain an optimal glucose level (Saydah et al., 2004). Melatonin may hold the potential to be a low-cost adjunctive therapy in the treatment of complications arising from T2D.

Conclusion

In conclusion, this investigation demonstrated that a single daily dose of 10 mg of melatonin significantly decreased glycated hemoglobin and oxidative stress in individuals with T2D. Considering the prevalence and rapid growth of diabetes and the totality of evidence linking melatonin to reduced oxidative stress and increased antioxidant defenses, it would seem prudent to further investigate this unique antioxidant.

REFERENCES

Ceriello A and Testa R. Antioxidant anti-inflammatory treatment in type 2 diabetes. *Diabetes Care* 32(Suppl 2): S232-S236, 2009.

Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF and Creager MA. Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. *American Journal of Physiology: Heart and Circulatory Physiology* 285(6): H2392-H2398, 2003.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (4th edition). Washington, D.C. American Psychological Association, 2000.

Kedziora-Kornatowska K, Szewczyk-Golec K, Kozakiewicz M, Pawluk H, Czuczejko J, Kornatowski T, Bartosz G and Kedziora J. Melatonin improves oxidative stress parameters measured in the blood of elderly type 2 diabetic patients. *Journal of Pineal Research* 46: 333-337, 2009.

Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S and Tan DX. Melatonin: An established antioxidant worthy of use in clinical trials. *Molecular Medicine* 15(1-2): 43-50, 2009.

Radziuk J and Pye S. Diurnal rhythm in endogenous glucose production is a major contributor to fasting hyperglycemia in type 2 diabetes. Suprachiasmatic deficit or limit cycle behavior. *Diabetologia* 49: 1619-1628, 2006.

Reiter RJ, Tan DX, Manchester LC, Pilar Terron M, Flores LJ and Koppisepi S. Medical implications of melatonin: Receptor-mediated and receptor-independent actions. *Advances in Medical Sciences* 52: 11-28, 2007.

Rosales-Corral S, Tan DX, Reiter RJ, Valdivia-Velazquez M, Martinez-Barboza G, Acosta-Martinez P and Ortiz GG. Orally administered melatonin reduces oxidative stress and proinflammatory cytokines induced by amyloid-β peptide in rat brain: A comparative, in vivo study versus vitamin C and E. *Journal of Pineal Research* 35: 80-84, 2003.

Saydah SH, Fradkin J and Cowie CC. Poor control of risk factors for vascular disease among adults with previously diagnosed diabetes. *Journal of the American Medical Association* 291(3): 335-342, 2004.

Tan DX, Manchester LC, Terron MP, Flores LJ and Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? *Journal of Pineal Research* 42: 28-42, 2007.

Urata Y, Honma S, Goto S, Todoroki S, Iida T, Cho S, Honma K and Kondo T. Melatonin induces γ-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Radical Biology and Medicine* 27(7/8): 838-847, 1999.

Table IV.1 Effects of daily treatment with 10mg melatonin for 6 weeks vs. placebo (mean ± SEM)

	Baseline	**Post MEL**	**Post PLA**
A1C (%)	7.26 ± 0.30	7.02 ± 0.33*	7.35 ± 0.37
Fasting Plasma Glucose (mmol/L)	7.88 ± 0.62	7.36 ± 0.53	7.84 ± 0.99
Total Cholesterol (mmol/L)	4.43 ± 0.31	4.43 ± 0.33	4.43 ± 0.35
LDL Cholesterol (mmol/L)	2.54 ± 0.27	2.38 ± 0.24	2.58 ± 0.29
HDL Cholesterol (mmol/L)	1.11 ± 0.09	1.15 ± 0.09	1.07 ± 0.09
Triglycerides (mmol/L)	1.56 ± 0.18	2.06 ± 0.42	1.78 ± 0.30
MDA (nmol/ml)	11.5 ± 1.64	5.2 ± 1.22*	12.2 ± 3.30†

Melatonin supplementation, MEL; placebo supplementation, PLA; plasma malondialdehyde, MDA.

*Post MEL is significantly different from baseline ($p<0.05$)

†Non-significant trend between Post PLA and Post MEL ($p=.055$)

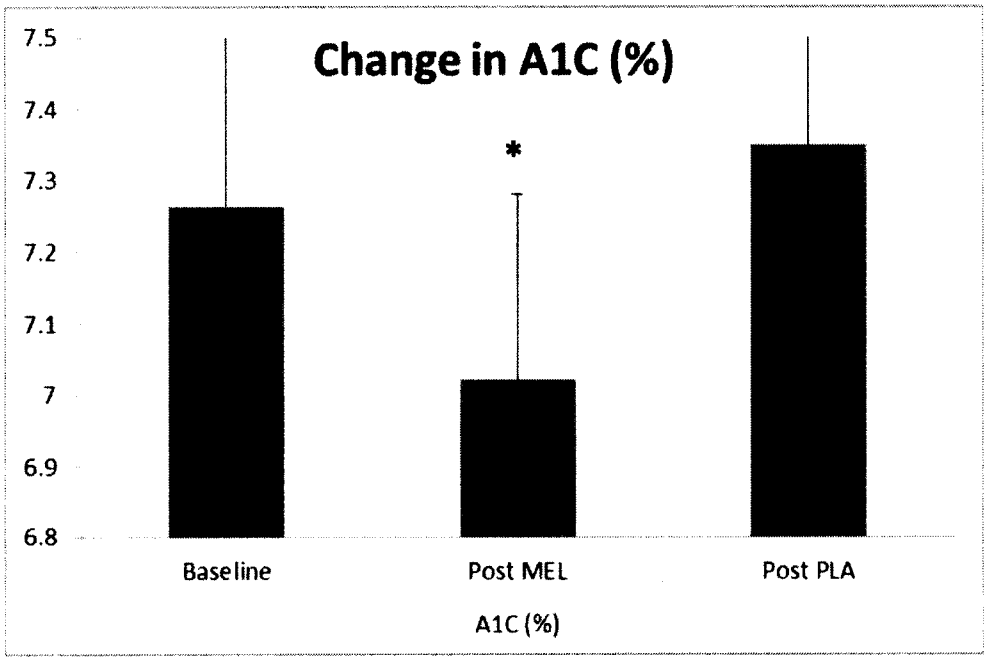

Figure IV.1. Change in A1C % following 6 weeks of melatonin supplementation (MEL) and placebo (PLA).

*Significantly different from baseline ($p<0.05$)

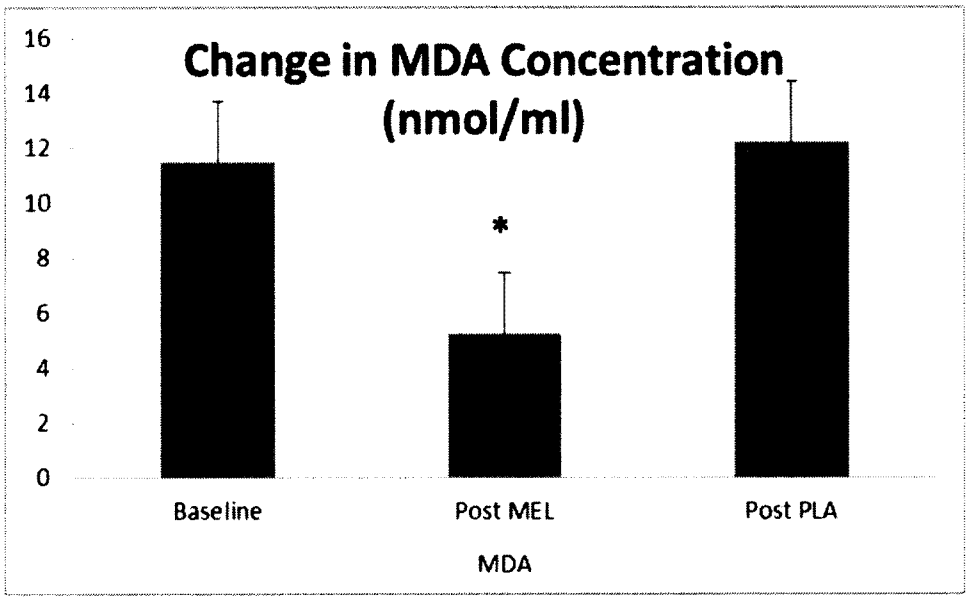

Figure IV.2. Change in plasma malondialdehyde (MDA) concentration between 6 weeks of melatonin supplementation and 6 weeks of placebo.

*Significantly different from placebo ($p<0.05$)

CHAPTER V

PROJECT III

The Effects of Walking vs. Table Tennis on Postprandial Glycemia, Heart Rate Variability, and Mood in Type 2 Diabetes

INTRODUCTION

Type 2 diabetes mellitus (T2D) is a widespread epidemic that now affects nearly one out of every ten Americans (CDC, 2011). Complications arising from T2D represent a significant strain upon the national healthcare system, accounting for approximately 18.5% of all health care expenditures (ADA, 2003). Distressingly, the ranks of the diabetic population are swelling rapidly and it is predicted that the number of people with diabetes will nearly double within the next three decades (Chaturvedi, 2007). Potentially more troubling, however, is an estimate by the Centers for Disease Control and Prevention suggesting that 35% of American adults over the age of 20 years and 50% of adults over the age of 65 years have prediabetes (CDC, 2011).

The treatment of T2D involves optimal regulation of glycemic levels, blood pressure and lipids, and physical activity is considered a cornerstone treatment and prevention strategy (Colberg and Grieco, 2009). Regular physical activity (PA) has been shown to improve glycemia and blood lipid profile, decrease blood pressure, and reduce both cardiovascular events and overall mortality in T2D (Colberg et al., 2010). Nevertheless, the majority of individuals with T2D are not regularly active (Morrato et al., 2007). In fact, almost one-third of U.S. adults with T2D report being completely sedentary, while an additional 38% receive less than the recommended levels of PA (Nelson et al., 2002). Interestingly, regular physical activity levels

among individuals with T2D mirror the maintenance of optimal glycemic levels, which remains distressingly low at only 37% (Saydah et al., 2004).

The timing of exercise can dramatically impact the glycemic response in T2D. For example, one hour of aerobic exercise significantly decreased plasma glucose in men with T2D following a breakfast meal, but had a minimal impact on glycemia when in a fasted state (Poirier et al., 2001). More recently, Colberg et al. (2009) demonstrated that 20 minutes of mild-to-moderate intensity walking significantly lowered postprandial glycemia to a greater extent in individuals with T2D when undertaken after the evening as compared to exercise before the evening meal (or no exercise).

Autonomic nervous system deterioration is a common complication of T2D that frequently results in autonomic neuropathy (AN) (Vinik and Erbas, 2001) and prevalence rates have been reported as high as 100% (Ziegler et al., 1992). A common complication resulting from AN is reduced heart rate variability (HRV). The HRV represents the time fluctuation in the beat-to-beat intervals between successive cardiac cycles and is reflective of the dynamic response of the sympathetic and parasympathetic branches of the autonomic nervous system. This reduction in HRV has been shown to be an early predictor of macrovascular disease as well as correlating with carotid artery atherosclerosis in T2D (Gottsater et al., 2006). Alarmingly, there are detectable deficits in HRV prior to the onset of T2D in the presence of impaired fasting glucose (Singh et al., 2000).

Decrements of parasympathetic nervous system (PNS) activity appear early in T2D, contribute to the imbalance between sympathetic and parasympathetic inputs (sympathovagal balance), and manifest as diastolic dysfunction (Poirier et al., 2003). Vagal activity, however, has

been shown to increase the cardiac fibrillation threshold, thereby imparting a protective effect against ventricular tachyarrythmias (Acharya et al., 2006). Importantly, exercise training has demonstrated significant improvements in HRV and sympathovagal balance in individuals with T2D (Figueroa et al., 2007; Zoppini et al., 2007; Sridhar et al., 2010).

Walking is perhaps the most commonly prescribed form of PA in T2D. While walking as a form of exercise has many benefits, individuals with T2D are among the least likely to exercise on a regular basis; therefore, additional measures are necessary to encourage greater PA levels. A more recreational form of exercise (i.e., table tennis) may be perceived as being more enjoyable and, therefore, increase the volume of PA (Williams et al., 2008). No studies to date have examined the glycemic impact of a more recreational activity like table tennis compared to a more traditional exercise like walking. Similarly, the differential effects of an acute bout of standard vs. recreational PA on HRV in T2D are absent from the literature. Thus, the aim of this study was to compare the impact of a traditionally prescribed exercise (walking) with a more recreational activity (table tennis) on postprandial glycemia, mood and HRV following the dinner meal.

METHODS

Subjects

A total of 12 subjects (9 female, 3 male) with uncomplicated T2D treated with diet and/or oral anti-diabetic medications participated in this investigation. Subject characteristics are presented in Table V.1. Eleven of the subjects were currently taking oral glucose-lowering drugs and one

subject was diet only. The study was approved by the Old Dominion University Institutional Review Board, and subjects provided signed informed consent prior to their participation.

Protocol

Each subject performed one control and two research trials in a randomized order, on different days. Each trial was approximately 180 minutes in length (Table V.2) and were as follows: (1) non-exercise control period in which a standardized meal was consumed (CON); (2) 30 minutes of self-paced walking on a motorized treadmill, begun 30 minutes after eating a standardized meal (TM); (3) 30 minutes of continuous table tennis, played against an iPong automated table tennis robot (IPONG®, Rockville, MD) (TT), begun 30 minutes after eating a standardized meal. Each dinner was a microwavable meal of approximately 500 kcals with a macronutrient ratio representative of a typical American dinner. All participants were seated in a thermoneutral environment (25–28°C) for the duration of the trial, except during the 30 minutes of TM or TT. Exercising heart rates were recorded during the last few minutes of the intervention period. Exercise intensity was calculated as a percentage of age-predicted maximal heart rate (estimated using 220 minus age).

Baseline blood samples were collected at the beginning of trial one (regardless of which testing condition was scheduled) using standard venipuncture technique. A full lipid panel, including total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG), as well as glycated hemoglobin (A1C) were analyzed using enzymatic assays (Cholestech Corp., Hayward, CA and Siemens Healthcare Diagnostics, Tarrytown, NY, respectively). To measure changes in plasma glucose finger-stick measures of whole blood

glucose levels were obtained using a Lifescan OneTouch Ultra (Johnson & Johnson, Milpitas, CA). Measurements were taken at 30-minute intervals, beginning 30 minutes after arrival, for a total of five plasma glucose measurements.

To quantitatively evaluate changes in mood the Profile of Mood States-Brief (POMS) was completed before and immediately following each trial intervention (i.e., rest or either exercise). The POMS-Brief (McNair and Heuchert, 2007), a 30-item self-rating questionnaire, consists of six dimensions of mood, including: Tension/Anxiety, Depression, Anger/Hostility, Vigor, Fatigue, and Confusion (C). A value of total mood disturbance (TMD) is calculated by subtracting the V score from the sum of the remaining scores.

Heart Rate Variability

Measurements of HRV were taken at two time points during each trial, shortly after arrival to the lab and 30 minutes after the end of the intervention. Subjects were placed in a recumbent position, instructed to relax, to speak and move as little as possible, and allowed to breathe spontaneously for a 10-minute period. Heart rate data was recorded using the Polar WearLink system (Polar, Finland), with a sampling rate of 1000 Hz. HRV measurements were calculated from the final 5 minutes of the 10-minute rest period. Data was visually inspected on a computer monitor to minimize presence of artifact. Time and frequency domain measurements were analyzed using Kubios HRV analysis 2.0 software (University of Kuopio, Kuopio, Finland). Detrending of raw data was performed according to Tarvainen et al. (2002) and power frequency analysis was performed using a fast Fourier transform with Welch's periodogram (256s window with 50% overlap). Time domain HRV measurements included Mean RR interval, SDNN and

RMSSD. Frequency domain measurements included Low (LF) and high frequency (HF) power measurements and total power (TP), presented as absolute power (ms^2) and normalized units (n.u.). Normalized LF power is calculated as LF/(total power-VLF)x100 and HF power as HF/(total power-VLF)x100. LF and HF measurements represent the power of the heart period power spectrum between 0.04 – 0.15 Hz and 0.15 – 0.40 Hz, respectively. The LF/HF ratio is calculated as LF (ms^2)/HF (ms^2).

Autonomic Neuropathy

Measurement of AN was conducted on a separate day and included the following three tests: Valsalva ratio, E/I (expiration/inspiration) ratio and 30:15 ratio. These measures were obtained on the subjects during resting conditions using the Anscore System (Boston Medical Technologies, Wakefield, MA).

Accelerometry

A quantification of total motion was measured using the Bioharness BT (Zephyr Technology, Annapolis, MD). Each subject was securely fitted with a Bioharness, worn on the chest similar to a heart rate monitor, prior to the 30-minute collection period. The Bioharness incorporates a triaxial accelerometer and records in vector magnitude units (VMU) at a sampling rate of 125 Hz, as a 3 dimensional acceleration of g-forces averaged into 1 second epochs.

Statistics

Mean differences were analyzed using a 2-way ANOVA with repeated measures on one factor (time). In addition, correlational analyses were performed to examine the relationships among biometric data, AN values and measurements of HRV. Results are presented as mean ±SEM. Data analyses were performed with PASW 17.0 (SPSS, Chicago, IL). Given that the HRV data were not normally distributed, all data were logarithmically transformed prior to analysis to induce normality (except for instantaneous heart rate data). Results were considered to be statistically significant at $P < 0.05$.

RESULTS

Characteristics of subjects are presented in Table V.1. Mean heart rate during TM (122.8±5.4 beats per minute) was significantly higher than during TT (96.9±5.0, p=0.05) and CON (69.1±8.4, p=0.001). The percentage of age-predicted maximum heart rate (%MHR) during TM (76.4±0.04%) was also significantly higher than both CON (42.9±0.02%) and TT (60.5±0.04%) ($p<0.05$) (Figure V.4). TM demonstrated a significantly greater quantity of motion than CON (91%, p=0.01) and TT (72%, p=0.01) (Figure V.1). There was a significant decrease in postprandial glycemia in the TM group in comparison to CON (-25%, p=0.018) and TT (-23.5%, p=0.004) immediately following the bout of exercise, at 90 minutes (Figure V.2). Similarly, the change in plasma glucose was significantly lower in the TM group (-29.4±8.3 mg/dL) than in the CON (19.3±6.6 mg/dL, p=0.001) or TT group (12.6±10.4 mg/dL, 0.005) at 90 minutes. However, 30 minutes after the cessation of exercise, at 120 minutes, the TM group (21.3±5.5

mg/dL) was significantly higher than the CON (-5.2±5.3 mg/dL, 0.007) or TT (1.1±5.8 mg/dL, p=0.005) groups, respectively (Figures V.2 and V.3). There was no significant effect of time or intervention upon mood state.

Time Domain HRV

Mean resting HR during HRV measurements showed significant differences between trials with a decrease from pre to post in CON (-4 bpm, p=0.005) and a significant increase from pre to post in TM (6 bpm, p=0.011) (Table V.3). Mean resting HR was significantly lower in CON during post HRV measurement than either TM (-8.0 bpm, p=0.007) or TT (-5.8 bpm, p=0.007). The SDNN of the pre TM was significantly higher than the post TM (p=0.039) and post TT measurements (p=0.039). The RMSSD in CON post was significantly higher than all other trials (p=0.001) (Table V.3).

Frequency Domain HRV

The CON pre HF (n.u.) was significantly higher than TM pre (p=0.049) and TM pre LF (ms^2) was significantly higher than TT post (p=0.009). There were no significant differences for the log-transformed LF (n.u.) or the HF (ms^2) or for the sympathovagal balance (LF/HF ratio) (Table V.4).

Correlations with Autonomic Neuropathy

The 30:15 ratio was positively correlated with the TM pre values of LF (ms^2) (p=0.045, r=.587) as well as the TM post SDNN (p=0.046, r=.583). Neither the Valsalva ratio nor the E/I

ratio were significantly correlated with any time or frequency domain measure of HRV.

Correlations of Demographic and Biometric Data with Time and Frequency Domain HRV

No measurement of time domain frequency was significantly correlated with age, BMI, body fat percentage, A1C, or length of diagnosis. The HF (ms^2) of TM post was significantly negatively correlated with length of diagnosis ($p=0.032$, $r=-0.619$). Similarly, length of diagnosis was also negatively correlated with the HF (n.u.) of TT post ($p=0.04$, $r=-0.597$). Unadjusted correlation (non-log transformed) between time and frequency domain measurements were non-significant.

DISCUSSION

The present investigation examined the differential effects of two different styles of aerobic exercise following an identical dinner meal on mood, postprandial glycemia, and HRV. Postprandial walking resulted in a significant dip in plasma glucose immediately following 30 minutes of exercise at a self-selected pace. This decrease was, however, somewhat offset by a rebound spike in plasma glucose in the TM group 30 minutes after the cessation of exercise that was absent in the TT and CON groups. Exercise selection had no effect on mood. While the differential effects of exercise on HRV were minimal, there was a significant suppressive effect of recent exercise (independent of style) on vagal stimulation (RMSSD).

To our knowledge this was the first study to examine the differential effects of a recreational vs. standard aerobic exercise on postprandial glycemia. While there was a significant postprandial decrease in plasma glucose immediately following TM exercise, there was also a

rebound effect present at 120 minutes (30 minutes post-exercise), with the TM group demonstrating significantly higher plasma glucose than the CON or TT groups. This effect is likely due to the different self-selected intensities sustained during TM and TT (76.4 vs. 60.5% MHR). While the higher intensity achieved during the TM protocol was likely responsible for the post-exercise spike in plasma glucose at 120 minutes, it should be noted that glucose levels at 150 minutes were not significantly different among groups.

The timing of meals and exercise plays an important role in optimal glycemic control. The ability of postprandial exercise to attenuate blood glucose excursions has been well documented. For example, it was recently reported that 20 minutes of moderate-intensity self-paced walking (~40% of heart rate reserve) had a greater glycemic impact on individuals with T2D when performed following the dinner meal than when it occurred prior to the dinner meal (Colberg et al., 2009). Similarly, Larsen et al. (1997) found that a single 45 minute bout of cycling exercise conducted at ~50% VO_{2max} following the breakfast meal significantly reduced blood glucose area under the curve for a four hour period following the cessation of exercise in comparison to a non-exercise control period. This increase in glucose uptake, however, occurred in the presence of a significant decrease in insulin secretion. This apparent contradiction is explainable when considering two important pieces of evidence: 1) the onset of exercise increases sympathetic tone, resulting in an attenuation of insulin secretion (Borer, 2003), and 2) exercise is known to increase skeletal muscle glucose uptake independent of insulin action (Jessen and Goodyear, 2005).

The onset of exercise induces both a nervous and hormonal response, resulting in an increase in catecholamine secretion, primarily in the form of epinephrine (De Glisezinski et al., 2009),

which mobilizes glucose in an intensity-dependent manner (Kjaer, 1989), while simultaneously suppressing insulin action. The extra effort put forth during the TM protocol, as assessed through percentage of age-predicted maximum heart rate, was such that glucose disposal was likely temporarily increased while stimulating a latent increase in epinephrine secretion that became apparent with the over-shoot of plasma glucose levels at 120 minutes. Moreover, the higher exercise intensity in the TM group would have caused a shift in substrate utilization away from free fatty acids and toward greater glucose usage, which could explain the significant dip in plasma glucose at 90 minutes in the TM group, the subsequent rebound at 120 minutes, and the rapid attenuation of glucose levels at 150 minutes.

Glycemic control is the most important aspect in the treatment of T2D and the common goal of treatment is the overall reduction of plasma glucose to a level that is considered normal. Several large scale clinical trials have demonstrated that improvements in long-term glycemic control (A1C) are associated with decreases in micro- and macrovascular complications (UKPDS, 1998; Shichiri et al., 2000). However, the potentially deleterious role that postprandial excursions play in T2D is significant and an accumulating amount of evidence suggests that postprandial spikes may play a prominent role in the pathology of T2D. For example, postprandial glucose levels have been implicated as an important antecedent in the onset and progression of nephropathy in T2D (Shichiri et al., 2005). In another study, Woerle et al. (2007) investigated the relative contribution of fasting and postprandial glucose levels on overall glycemic control in 164 individuals with T2D. Results demonstrated that postprandial glycemia is a more significant contributor to A1C levels than fasting glucose. Therefore, strategies specifically targeting postprandial excursions may represent a more focused treatment

intervention, potentially increasing the importance of exercise in the amelioration of complications arising from T2D.

Previous studies on the chronic effect of aerobic exercise on HRV in T2D have demonstrated positive adaptations resulting in improved sympathovagal balance and increased vagal modulation. For example, Zoppini et al. (2007) found that a twice-weekly moderate-intensity aerobic exercise regimen conducted for six months increased vagal output while decreasing sympathetic output (measured as high and low frequency power, respectively) in individuals with uncomplicated T2D. These results, however, were only noted during a systemic challenge (response to standing); thus, there was no change in measures of HRV conducted at rest. Similarly, Figueroa et al. (2007) found that neither obese (but normoglycemic) nor T2D females improved resting measurements of HRV following 16 weeks of thrice weekly walking at ~ 65% of VO_2 peak. They did, however, demonstrate significant increases in both LF and HF power post-training when HRV measurements were taken following an acute bout of 20 minutes of treadmill walking at 65% of VO_2 peak. However, no previous studies have investigated the acute effect of a single bout of exercise on resting HRV values in T2D.

While it can be stated that chronic exercise training imparts a cardio-autonomic benefit in T2D, the measurement of those beneficial effects appear to be limited to periods in which there is (or has been) a cardiovascular challenge (i.e., response to standing or soon after a bout of exercise). Interestingly, there appears to be no improvement in cardio-autonomic function as a result of chronic exercise training that is measurable in a rested state in T2D. In the present study we investigated the acute effect of a bout of exercise on HRV, conducted approximately 30 minutes after the cessation of exercise. The primary finding was a residual suppression of

parasympathetic drive (RMSSD) in both exercise groups that was absent in the control group. Therefore, the findings of this investigation support no improvement of measures of HRV following an acute bout of exercise.

Conclusion

In summary, the present study found that 30 minutes of self-paced walking following the dinner meal may be more effective at lowering postprandial glycemia in individuals with T2D than a similar duration of table tennis. We did not, however, find that a more recreational form of exercise (i.e., table tennis) improved affective response, suggesting that traditionally prescribed exercise (i.e., walking) has no significant negative impact on mood and was superior to table tennis in postprandial glycemic control. Exercise also fails to produce an acute impact on measures of HRV. Despite the demonstrated improvement of HRV following chronic exercise training, it appears that a single bout of exercise does nothing more than contribute to a lingering suppression of vagal modulation following 30 minutes of exercise in T2D. Nevertheless, given the totality of available evidence, including the present investigation, exercise is strongly recommended as a method of postprandial glycemic control as well as eliciting improvements in cardio-autonomic modulation in T2D.

REFERENCES

Acharya UR, Joseph KP, Kannathal N, Lim CM and Suri JS. Heart rate variability: a review. *Medical and Biological Engineering and Computing* 44:1030-1051, 2006.

American Diabetes Association. Economic costs of diabetes in the U.S. in 2002. *Diabetes Care* 26: 917-932, 2003.

Borer KT. Exercise Endocrinology (1st edition). Champaign, IL. Human Kinetics, 2003, 36-38.

Center for Disease Control and Prevention. National diabetes fact sheet, 2011. http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Accessed on March 20, 2012.

Chaturvedi N. The burden of diabetes and its complications: trends and implications for intervention. *Diabetes Research and Clinical Practice* 76 Suppl 1: S3-S12, 2007.

Colberg SR, Albright AL, Blissmer BJ, Braun B, Chasan-Taber L, Fernhall B, Regensteiner JG, Rubin RR and Sigal RJ. Exercise and type 2 diabetes: American College of Sports Medicine and the American Diabetes Association: joint position statement. *Medicine and Science in Sports and Exercise* 42: 2282-2303, 2010.

Colberg SR, Grieco C: Exercise in the treatment and prevention of diabetes. *Current Sports Medicine Reports*, 8(4), July/August 2009 (Invited).

Colberg SR, Zarrabi L, Bennington L, Nakave A, Somma CT, Swain DP, Sechrist SR: Postprandial walking is better for lowering the glycemic effect of dinner than pre-dinner exercise in type 2 diabetic individuals. *Journal of the American Medical Directors Association* 10(6): 394-397, 2009.

De Glisezinski I, Larrouy D, Bajzova M, Koppo K, Polak J, Berlan M, Bulow J, Langin D, Marques MA, Lafontan M and Stich V. Adrenaline but noradrenaline is a determinant of exercise-induced lipid mobilization in human subcutaneous adipose tissue. *The Journal of Physiology* 13: 3393-3404, 2009.

Figueroa A, Baynard T, Fernhall B, Carhart R and Kanaley JA. Endurance training improves post-exercise cardiac autonomic modulation in obese women with and without type 2 diabetes. *European Journal of Applied Physiology* 100: 437-444, 2007.

Fujii H, Kono K, Nakai K, Goto S, Komaba H, Hamada Y, Shinohara M, Kitazawa S and Fukagawa M. Oxidative and nitrosative stress and progression of diabetic nephropathy in type 2 diabetes. *American Journal of Nephrology* 31(4): 342-352, 2010.

Jessen N and Goodyear LJ. Contraction signaling to glucose transport in skeletal muscle. *Journal of Applied Physiology* 99: 330-337, 2005.

Kjaer M. Epinephrine and some other hormonal responses to exercise in man: with special reference to physical training. *International Journal of Sports Medicine* 10: 2-15, 1989.

McNair DM, Heuchert JP. Profile of Mood States: Technical Update. North Tonawanda. NY, Multi-Health Systems, Inc., 2007.

Morrato EH, Hill JO, Wyatt HR, Ghushchyan V, Sullivan PW. Physical activity in U.S. adults with diabetes and at risk for developing diabetes, 2003. *Diabetes Care* 30(2):203-9, 2008.

Nelson KM, Reiber G, Boyko EJ; NHANES III. Diet and exercise among adults with type 2 diabetes: findings from the third national health and nutrition examination survey (NHANES III). *Diabetes Care* (25)10: 1722-1728, 2002.

Paskaloglu K, Sener G, Ayanoğlu-Dülger G. Melatonin treatment protects against diabetes-induced functional and biochemical changes in rat aorta and corpus cavernosum. *European Journal of Pharmacology* 499: 345-354, 2004.

Poirier P, Bogaty P, Philippon FO, Garneau C, Fortin C, and Dumesnil JG. Preclinical Diabetic Cardiomyopathy: Relation of Left Ventricular Diastolic Dysfunction to Cardiac Autonomic Neuropathy in Men With Uncomplicated Well-Controlled Type 2 Diabetes. *Metabolism* 52(8): 1056-1061, 2003.

Poirier P, Mawhinney S, Grondin L, Tremblay A, Broderick T, Cleroux J, Catellier C and Tancrede G. Prior meal enhances the plasma glucose lowering effect of exercise in type 2 diabetes. *Medicine and Science in Sports and Exercise* 33(8): 1259-1264, 2001.

Reiter RJ. The role of the neurohormone melatonin as a buffer against macromolecular oxidative damage. *Neurochemistry International* 27(6): 453-460, 1995.
Routledge FS, Campbell TS, McFetridge-Durdle JA and Bacon SL. Improvements in heart rate variability with exercise therapy. *Canadian Journal of Cardiology* 26(6): 303-312, 2010.

Shichiri M, Kishikawa H, Ohkubo Y, Wake N. Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care* 23 Suppl 2: B21-29, 2000.

Tiedge M, Lortz, S, Drinkgern J and Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* 46: 1733-1742, 1997.

U.K. Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. *Lancet* 352: 837-853, 1998.

Williams DM, Dunsiger S, Ciccolo JT, Lewis BA, Albrecht AE and Marcus BH. Acute affective response to a moderate-intensity exercise stimulus predicts physical activity participation 6 and 12 months later. *Psychology of Sport and Exercise* 9(3): 231-245, 2008.

Woerle HJ, Neumann C, Zschau S, Tenner S, Irsigler A, Schirra J, Gerich JE and Göke B. Impact of fasting and postprandial glycemia on overall glycemic control in type 2 diabetes.

Importance of postprandial glycemia to achieve target HvA1c levels. *Diabetes Research and Clinical Practice* 77: 280-285, 2007.

Ziegler D, Fies FA, Spuler M and Lessmann F. The epidemiology of diabetic autonomic neuropathy: diabetic Cardiovascular Autonomic Neuropathy Multicenter Study Group. *Journal of Diabetes Complications* 6: 49-57, 1992.

Table V.1 Subject baseline characteristics (mean ± SEM)

	All	Males	Females
Subjects (#)	12	3	9
Age (years)	58.7±2.4	64.0±4.6	55.7±2.9
Weight (lb)	211.4±13.0	191.1±15.7	218.2±16.4
Height (in)	65.7±0.7	69.2±1.0	64.4±0.4
Body mass index	34.8±2.4	28.1±2.4	37.1±2.8
Body fat (%)	39.5±2.8	27.7±3.5	43.5±2.3
Diabetes duration (yrs)	6.1±1.4	7.7±3.2	5.6±1.6
A1C (%)	6.6±0.2	6.4±0.3	6.7±0.2
Cholesterol (mg/dL)	149.5±14.2	112.0±6.7	162.0±16.9
HDL-Chol. (mg/dL)	40.0±4.9	23.7±0.9	45.4±5.4
Triglycerides (mg/dL)	143.8±21.3	190.7±44.3	128.2±23.4

Table V.2. Testing procedures timetable

	5:00 – 6:00 pm			**6:00 – 7:00 pm**		**7:00 – 8:00 pm**	
Time (min)	*0*	*30*	*60*	*90*	*120*	*150*	*180*
CON	HRV/POMS	Dinner	30 min Rest	POMS	HRV	Rest	Rest
TM	HRV/POMS	Dinner	30 min TM	POMS	HRV	Rest	Rest
TT	HRV/POMS	Dinner	30 min TT	POMS	HRV	Rest	Rest

Table V.3. Log transformed time domain measurements of heart rate variability

	CON		TM		TT	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*
Mean RR (ms)	869.1 ± 123.7	916.7 ± 120.5[1]	895.9 ± 131.6	822 ± 122.8[2]	867.8 ± 129.1	849.2 ± 128.4
Mean HR (bpm)	70.5 ± 10.0	66.5 ± 8.1[3]	68.5 ± 10.0	74.5 ± 10.7	70.8 ± 10.8	72.3 ± 10.6
SDNN	1.36+0.05	1.36±0.08	1.37+0.08[6,7]	1.26±0.07	1.36+0.08	1.26±0.07
RMSSD	1.38±0.07[5]	3.17±0.22[4]	1.36±0.11	1.26±0.09	1.38±0.09	1.26±0.09

Values are means ± SEM for rest (RE), treadmill (TM) and table tennis (TT) groups.

[1]Significant difference vs. CON pre ($p<0.05$)
[2]Significant difference vs. CON post ($p< 0.05$)
[3]Significant difference vs. CON pre ($p< 0.05$)
[4]Significant difference vs. all other trials ($p<0.05$)
[5]Significant difference vs. CON post ($p<0.05$)
[6]Significant difference vs. TM post ($p<0.05$)
[7]Significant difference vs. TT post ($p<0.05$)

Table V.4. Log transformed frequency domain measurements of heart rate variability

	CON		**TM**		**TT**	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*
LF (ms^2)	2.20±0.10	2.26±0.17	2.30±0.17	2.03±0.17	2.24±0.14	2.02±0.15
LF (n.u.)	1.66±0.06	1.63±0.07	1.64±0.09	1.66±0.08	1.58±0.09	1.59±0.09
HF (ms^2)	2.18±0.17	2.27±0.17	2.17±0.24	1.96±0.18	2.29±0.19	2.05±0.24
HF (n.u.)	1.64±0.08	1.65±0.08	1.53±0.10	1.58±0.10	1.62±0.10	1.62±0.11
TP (ms^2)	2.560.11	2.64±0.15	2.68±0.18[1,2]	2.41±0.15	2.64±0.15	2.41±0.15
LF/HF	1.8 ± 2.1	1.7 ± 2.0	2.9 ± 3.3	2.6 ± 3.2	2.4 ± 3.5	2.8 ± 4.7

Values are means ± SEM for control (CON), treadmill (TM) and table tennis (TT) groups.

[1]Significant difference vs. TM post ($p<0.05$)
[2]Significant difference vs. TT post ($p<0.05$)

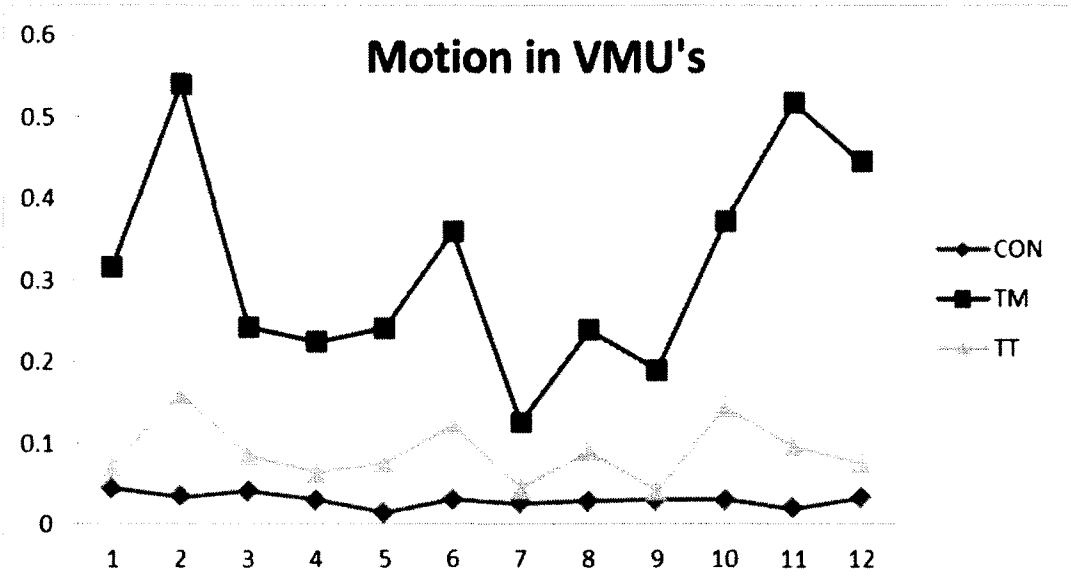

Figure V.1. Physical activity in vector magnitude units (VMU) of rest (CON), 30 minutes of self-paced treadmill walking (TM) and table tennis (TT) groups.

All 12 subjects are shown individually by number.

*Significant difference vs. CON ($p<0.05$)
†Significant difference vs. TT ($p<0.05$)

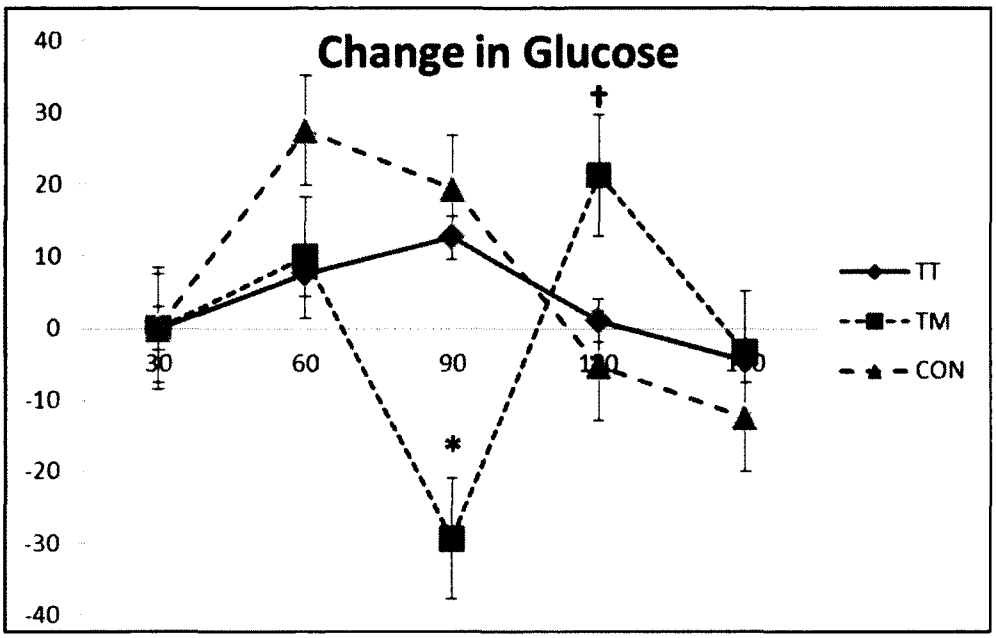

Figure V.2 Change in plasma glucose in response to standardized meal with no exercise (CON), 30 minutes of self-paced treadmill walking (TM), or continuous table tennis (TT).

*Significantly different vs. CON and TT ($p<0.05$)
†Significantly different vs. CON and TT ($p<0.05$)

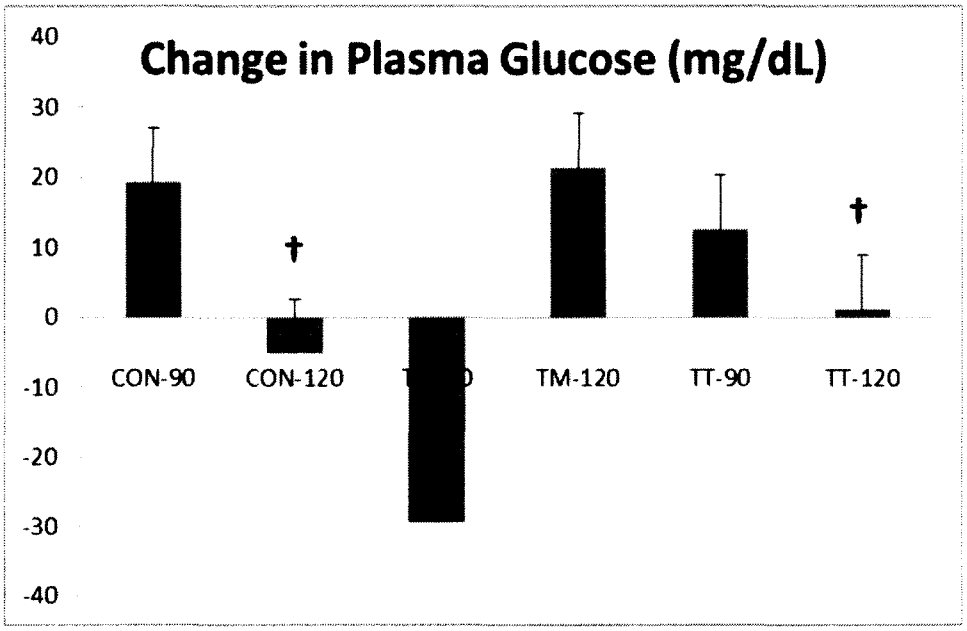

Figure V.3 Change in plasma glucose at 90 and 120 minutes with no exercise (CON), 30 minutes of self-paced walking on a treadmill (TM), or continuous table tennis (TT).

*Significantly higher than TM-90 ($p<0.05$)
†Significantly lower than TM-120 ($p<0.05$)

CHAPTER VI

CONCLUSIONS

The prevalence of T2D has reached epidemic proportions in the United States. Nearly 100 million Americans have, or are at increased risk of developing, T2D. Current treatment strategies fall far short of preventing or adequately treating this insidious disease. Alternative and adjunctive therapies are needed to more capably treat this chronic disease.

The projects included within this dissertation investigated three distinct alternative therapies for the treatment of T2D. The first project examined the efficacy of a little known aspect of yoga. Yoga is an ancient form of physical and mental discipline and has been growing rapidly in popularity within the U.S. Furthermore, yoga has been associated with a decreased exercise dropout rate. Pranayama, a collective term referring to yogic breathing exercises, have long been touted to have an ability to alter autonomic function. The previous research investigating the effect of pranayama has been equivocal and no studies have previously examined these effects in T2D. Our results indicated pranayama had no impact upon cardio-autonomic function. While yoga training is a viable and valuable form of exercise it appears that yogic breathing exercises do not positively impact autonomic function in individuals with T2D.

A widely accepted hypothesis suggests that oxidative stress may be the most important factor contributing to complications arising from diabetes. Following this logic antioxidant compounds should play a vital role in delaying or preventing diabetic complications. While there is a paucity of research investigating this possibility in T2D similar investigations examining the relationship of the most common vitamin-based antioxidants with cardiovascular disease prevention have yielded disappointing results. Melatonin, however, differs from the conventional class of

antioxidants by serving both as a direct scavenger as well as a potent stimulator of endogenous antioxidant enzymes. While there is a substantial amount of research that has investigated the efficacy of melatonin as a quencher of oxidative stress in animal models and in vitro lines, there is a near absence of in vivo research. Importantly, we found a significant impact of melatonin supplementation on markers of oxidative stress, as well improving the long-term glycemic profile of individuals with T2D. Melatonin, therefore, may be a viable adjunctive and/or alternative therapy for its treatment.

Individuals with T2D have been shown to have a very low exercise participation rate. Complications arising from diabetes result from hyperglycemia-driven processes. Therefore, reducing hyperglycemia is of paramount importance. Exercise has demonstrated an important ability to reduce both acute and chronic glycemic levels. However, it has been found recently that controlling postprandial glycemic spikes may be more important than reducing fasting blood glucose levels. Therefore, the timing of an exercise intervention may play a prominent role in modifying health outcomes in these individuals. Our project investigated the differential effects of a traditional exercise prescription and a more recreational form of exercise on postprandial glycemia. Our findings demonstrated that postprandial walking was more effective than table tennis in lowering plasma glucose levels following a standardized meal. We did not, however, find any improvement in affective response attributable to recreational exercise, indicating that walking may be a preferred method of exercise for most individuals with this disease. In conclusion, the results of these projects have demonstrated the efficacy of non-pharmaceutical forms of treatment as adjunctive and/or alternative therapy in T2D.

APPENDIX 1

Vita

Carmine R. Grieco

EDUCATION

Old Dominion University, Norfolk, VA

- **PhD** Education (Human Movement Sciences), *projected date of May, 2012*
  Dissertational Focus: "Alternative Therapy and Treatment of Type 2 Diabetes"

- **MS** Exercise Science, August, 2008
  Master's Thesis: "The Effect of Intensity of Aerobic Training on Insulin Sensitivity"

University of Wyoming, Laramie, WY

- **BS** Exercise Science, May 1996

TEACHING EXPERIENCE

Old Dominion University, Norfolk, VA

Lecturer, 2010 – 2012
Spring 2012
EXSC 409 Physiology of Exercise (61 students)
EXSC 431 Wellness Programming and Administration (60 students)
EXSC 368 Supervision of Exercise Science Internship (11 students)
SMGT 368 Supervision of Sports Management Internship/Practicum (4 students)

Fall 2011
EXSC 409 Physiology of Exercise (65 students)
EXSC 420 Research Methods in Exercise Science (26 students)
EXSC 431 Wellness Programming and Administration (56 students)
SMGT 368 Supervision of Sports Management Internship/Practicum (12 students)

Spring 2011
EXSC 403 Lifetime Fitness and Wellness (51 students)
EXSC 408 Nutrition for Fitness and Sport (74 students)
EXSC 409 Physiology of Exercise (41 students)
SMGT 368 Supervision of Sports Management Internship/Practicum (10 students)

Fall 2010
EXSC 250 Strength and Conditioning Leadership (Lab – 21 students)
EXSC 322 Anatomical Kinesiology/Human Anatomy (Lab – 14 students)
EXSC 403 Lifetime Fitness and Wellness (47 students)
EXSC 408 Nutrition for Fitness and Sport (62 students)
SMGT 368 Supervision of Sports Management Internship/Practicum (7 students)

Adjunct Instructor, 2008 - 2012
Summer 2012
EXSC 409 Physiology of Exercise

Spring 2010
EXSC 415 Exercise Testing for Normal and Special Populations (lab – 16 students)
EXSC 431 Wellness Programming and Administration (50 students)
EXSC 531 Wellness Programming and Administration (4 graduate students)

Summer 2008
EXSC 415 Exercise Testing for Normal and Special Populations (lab – 14 students)

Spring 2008
EXSC 415 Exercise Testing for Normal and Special Populations (lab – 16 students)
EXSC 431 Wellness Programming and Administration (23 students)

Teaching Assistantship, 2008 – 2009
Spring 2009
EXSC 415 Exercise Testing for Normal and Special Populations (lab, 2 sections – 31 students)
EXSC 431 Wellness Programming and Administration (36 students)
EXSC 531 Wellness Programming and Administration (3 graduate students)

Fall 2008
EXSC 250 Strength and Conditioning Leadership (26 students)
EXSC 403 Lifetime Fitness and Wellness (33 students)

REFEREED PUBLICATIONS

Grieco C, Cortes N, Greska E, Lucci S and Onate J. Effects of a Combined Resistance/Plyometric Training Program on Muscular Strength, Running Economy and VO_{2peak} in Division I Female Soccer Players. *Journal of Strength and Conditioning Research* (In Press)

Colberg SR, **Grieco C**: Exercise in the treatment and prevention of diabetes. *Current Sports Medicine Reports*, 8(4): 169-175, 2009 (Invited)

MANUSCRIPTS UNDER REVIEW

Grieco C, Swain D, Colberg S, Dowling E, Baskette K, Zarrabi L, Gandrakota R, Kotipalli U, Sechrist S and Somma T. Effect of Intensity of Aerobic Training on Insulin Sensitivity/Resistance in Recreationally Active Adults. *Journal of Strength and Conditioning Research.*

MANUSCRIPTS UNDER PREPARATION

Grieco C, Colberg SR and Somma CT. The Effect of Exercise Type on Postprandial Glycemia, Heart Rate Variability, and Mood in Individuals with Type 2 Diabetes.

REFEREED PUBLISHED ABSTRACTS

Grieco CR, Colberg SR, Somma CT, Vinik AI and Thompson AG. Effects of Walking vs. Table Tennis on Postprandial Glycemia, Heart Rate Variability, and Mood in Type 2 Diabetes. *Diabetes,* 61(Suppl. 2), 2012 (in press)

Grieco CR, Swain DP, Colberg SR, Dowling E, Baskette K, Zarrabi L, Gandrakota R, Kotipalli U, Sechrist SR, Somma CT. The effect of aerobic exercise intensity on insulin sensitivity. *Medicine & Science in Sports & Exercise*, 41 (5; Suppl.): S103, 2009.

ABSTRACTS

Thompson AG, **Grieco C**, Swain DP, Onate J, Cortes N. "Heart Rate Variability and VO_{2max} in Healthy College Students." Poster presentation at Annual meeting of the American College of Sports Medicine, San Francisco, CA, June 2012.

Alkatan MF, Dowling E, Branch JD, **Grieco C**, Kollock RO, Williams MH. "Effect of Caffeine on Maximum Strength and Rate of Force Development in Male Weight Lifters." Poster

presentation at the Annual meeting of the American College of Sports Medicine. Denver, CO, June 2011.

Thompson AG, **Grieco C**, Swain DP, Onate J, Cortes N. "Heart Rate Variability and VO_{2max} in Healthy College Students." PowerPoint presentation at South East regional meeting of American College of Sports Medicine, SC, February 2011.

Grieco C, Greska E, Lucci S, Cortes N, Colberg S, Onate J. "Effect of Neuromuscular Training on VO_{2peak} and Running Economy in Division I Female Soccer Players." Poster presentation at the annual meeting of the American College of Sports Medicine. Baltimore, MD, June 2010.

Cortes N, Lucci S, Quammen D, **Grieco C**, Onate J. "Aerobic Fatigue Changes Hip and Knee Sagittal Plane Kinematics During Unanticipated Athletic Tasks." Poster presentation at the annual meeting of the American College of Sports Medicine. Baltimore, MD, June 2010.

Grieco C, Swain DP, Colberg-Ochs S, Dowling E, Baskette K, Zarrabi L, Gandrakota R, Kotipalli U, Sechrist SR, Somma CT. "The Effect of Intensity of Aerobic Training on Insulin Sensitivity." PowerPoint presentation at the annual meeting of the American College of Sports Medicine. Seattle, WA, May 2009.

PROJECTS UNDER PREPARATION

Grieco C and Colberg SR. "The Effect of N-Acetyl-5-Methoxytryptamine (Melatonin) Supplementation on Glucose Homeostasis, Lipid Metabolism, and Oxidative Stress in Type 2 Diabetes Mellitus."

Grieco C and Colberg SR. "The Acute Effect of Short-Term Breathing Exercises on Sympathovagal Balance in Type 2 Diabetes Mellitus."

Grieco C, Greska E and Van Lunen B. "Effect of Heart Rate Training Program on VO_{2max} and Lactate Levels in Division I Male Tennis Players."

Swain DP, Simmons R, **Grieco C**, Kelleran K and Thompson A. "Effects of Stress on Heart Rate Variability Among Emergency Medical Service Providers."

GRANTS (Applied For)

External

The Effect of Exercise Type on Postprandial Glycemia, Heart Rate Variability, and Mood in Individuals with Type 2 Diabetes, Lifescan. Submitted: November, 2010

The Acute Effect of Yogic Breathing Exercises on Melatonin Secretion in Type 2 Diabetes Mellitus, American College of Sports Medicine. Submitted: January, 2010

Internal

Undergraduate Research Apprenticeship Program Site Grant (URAP), Honors College, Old Dominion University. November, 2011

Undergraduate Research Grant, Honors College, Old Dominion University. Effect of Music on Strength and Rate of Force Development. Submitted: November, 2011

Undergraduate Research Apprenticeship Program Site Grant (URAP), Honors College, Old Dominion University. Submitted: November, 2010

GRANTS (Awarded)

Undergraduate Research Grant, Honors College, Old Dominion University. Heart Rate Variability and VO2 Max in Healthy College Students. Accepted February, 2009, $1,500

Undergraduate Research Grant, Honors College, Old Dominion University. Effects of Stress on Heart Rate Variability Among Emergency Medical Service Providers. Accepted September, 2011, $1,500

HONORS and AWARDS

Graduate Doctoral Fellowship, Darden College of Education 2009-2010

CERTIFICATIONS

Personal Trainer 1996 – Present
American Council on Exercise

Yoga Teacher 2006 – Present
Bihar School of Yoga, India

Group Fitness Instructor 2001 – 2003
American Council on Exercise

Lifestyle and Weight Management Coach 2001 – 2003
American Council on Exercise

NIH Protection of Human Subjects 2007 - Present
National Institute of Health

CPR/AED Adult 1994 – Present
American Red Cross

SERVICE and MEMBERSHIPS

EDITORIAL EXPERIENCE

Editorial Board Member: *Frontiers in Endocrinology*, April 2012 2005-present

2010 - Present
Faculty Advisor, Human Movement Sciences Society, Old Dominion University

2011 - Present
Nutritional Consultant, Old Dominion University Men's Tennis Team

2009 – 2010
President, Human Movement Sciences Society, Old Dominion University

Presentations

National Strength and Conditioning Association Mid-Atlantic Conference, Old Dominion University, August, 2012
Pump You Up! Understanding the Anabolic Response to Exercise

Sigma Nu Ethical Leadership Series, Old Dominion University, April, 2012
Wellness and Leadership

Old Dominion University, New Student and Parent Program Presentation, November, 2011
Importance of Lifestyle Factors for Academic Performance

Old Dominion University Men's Tennis Team, September, 2011
Performance Nutrition in Tennis

First Colonial High School, Virginia Beach, VA, April, 2011
Nutrition and Optimal Performance

Sentara Obici Hospital, Suffolk, VA, July, 2010
Diabetes Support Group monthly meeting
You Don't Need a Prescription to Sweat

Kiwanis Club of Chesapeake, VA, February, 2009
Resistance Training and Your Health

City of Norfolk, VA, August, 2008
Calming the Monkey Mind: Yoga and Stress Management

Cape Henry Women's Club, March, 2008
You Don't Need a Prescription For Broccoli

Goddard School of Chesapeake, VA, March, 2008
Healthy Kids

Don Richards and Associates, March, 2008
Pain and Posture in the Workplace

Rotary Club of Chesapeake, VA, February, 2008
Where the Rubber Meets the Road: Health, Wellness and Your Business

Kiwanis Club of Chesapeake, VA, January, 2008
Nutrition and Your Health

Hampton Roads Chamber of Commerce, October, 2007
Fiscally Fit: Establishing a Health and Productivity Management Program

Don Richards and Associates, March, 2007
You Don't Need a Prescription For Broccoli

Memberships

National Speakers Association, Virginia Chapter	2011 – Present
National Strength and Conditioning Association	2011 - Present
American Diabetes Association	2008 – Present
Human Movement Sciences Society	2008 – Present
American College of Sports Medicine	2007 – Present
IDEA Health and Fitness Association	2000 – Present
American Council on Exercise	1996 – Present

Student Mentoring

Chris Futrell (Undergraduate Honors Thesis) 2011 – Present
Project: Under Development

Rachel Simmons (Undergraduate Honors College Project) 2010 – Present
Project: Heart Rate Variability in EMS Personnel in Response to a Standard Shift

Jennifer Brown (Master's Thesis) 2010 – Present
Project: Effect of Yogic Breathing Techniques on Pulmonary Function in Asthmatics

Andrew Thompson (Master's Thesis) 2010 – Present
Project: Effect of Environmental Stress on Heart Rate Variability and Accuracy During a Simulated Combat Shooting Task

Mohammed Alkatan (Master's Thesis) 2009–2010
Project: Effect of Caffeine on Maximum Strength and Rate of Force Development in Male Weight Lifters

Andrew Thompson (Undergraduate Honors College Project) 2008–2009
Project: Heart Rate Variability and VO_{2max} in Healthy College Students

Shawn Lucci (Master's Thesis) 2008–2009
Project: The Effects of Two Types of Fatigue on an Unanticipated Side-Step Cutting Task as Measured by Kinematic and Kinetic Variables

Dave Quammen (Master's Thesis) 2008–2009
Project: The Effects of Two Types of Fatigue on an Unanticipated Side-Step Cutting Task as Measured by Kinematic and Kinetic Variables