6-1-2013

Sleep-wake Cycle Assessment in Type 2 Diabetes and Salivary Melatonin Correlates

Paula Regina Aguiar Cavalcanti
Loma Linda University

LOMA LINDA UNIVERSITY
School of Allied Health Professions
in conjunction with the
Faculty of Graduate Studies

_____

Sleep-wake Cycle Assessment in Type 2 Diabetes and Salivary Melatonin Correlates

by

Paula Regina Aguiar Cavalcanti

_____

A Dissertation submitted in partial satisfaction of
the requirements for the degree
Doctor of Science in Physical Therapy

_____

June 2013

Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Science.

_____, Chairperson

Lee S. Berk, Associate Professor of Allied Health Professions, Allied Health Studies, Director Clinical Molecular Research Lab., School of Allied Health Professions and Associate Research Professor of Pathology and Humans Anatomy, School of Medicine

_____

Noha S. Daher, Associate Professor of Epidemiology and Biostatistics

_____

Everett Lohman, Assistant Dean of Graduate Academic Affairs, Professor of Physical Therapy

_____

Jerrold S. Petrofsky, Professor of Physical Therapy, Director of Physical Fitness Laboratory

ACKNOWLEDGEMENTS

I would like to first and foremost, express my greatest gratitude to my best friend, Savior and Lord, Jesus Christ who was always by my side giving me strength to face all the challenges and difficulties that arose along the way.

To Dr. Lee Berk, my chairperson, my mentor and my supervisor. Thank you for all the support, encouragement and help throughout this journey. Thank you for the laboratory experience and for providing me with the tools to learn and develop our research project. This journey would not have been the same without all your support.

I also would like to thank my committee members for their advice and direction. To Dr. Noha Daher, thank you for your constant help, encouragement and for the incredible support in developing our data analysis. To Dr. Jerrold Petrofsky, for all the attention and brilliant insights in this research journey. To Dr. Everett Lohman for providing me with the opportunity to be in this program and for your support from day one through completion.

To my family, your love and support through this long endeavor has been treasured and I could not have finished without all your continuous encouragement. Thank you for believing in me.

To my husband, Nelson, I am deeply grateful for your love, patience and support during this long journey. Thank you for the support and motivation in every single step of the way. You are an instrument of God in my life and your support gave me the strength to complete this journey.

CONTENTS

FIGURES

Figures Page

TABLES

Tables Page

ABBREVIATIONS

T2DM	type 2 diabetes mellitus
BDI	Beck’s Depression Inventory
ARES	Apnea Risk Evaluation System
HbA1c	Hemoglobin A1c
OSA	Obstructive sleep apnea
NREM	Non-rapid eye movement
S	Homeostatic process
C	Circadian process
EEG	Electroencephalogram
SCN	Suprachiasmatic nucleus
IV	Interdaily stability
IS	Intradaily variability
AMP	Amplitude
BMI	Body mass index
L5	Least active 5 h
M10	Most active 10 h
WASO	Wake after sleep onset

ABSTRACT OF THE DISSERTATION

Sleep-wake Cycle Assessment in Type 2 Diabetes and Salivary Melatonin Correlates

by

Paula Regina Aguiar Cavalcanti

Doctor of Science, Graduate Program in Physical Therapy
Loma Linda University, June 2013
Dr. Lee Berk, Chairperson

The aim of this study was to analyze the sleep-wake cycle of T2DM subjects and compare it to healthy controls using the nonparametric approach and to assess the changes in the circadian and homeostatic control of the sleep-wake cycle in type 2 diabetic (T2DM) and correlate it with melatonin concentration. The sample consisted of 21 subjects with diagnosis of T2DM for more than a year and 21 healthy controls matched for gender and age. Subjects were assessed using the Beck's Depression Inventory (BDI), the Apnea Risk Evaluation System (ARES), hemoglobin A1c (HbA1c), actigraphy and melatonin levels. The findings revealed that T2DM subjects demonstrate lower IS ($p=.03$), higher IV ($p=.046$) and lower rhythm amplitude ($p=.02$) when compared to healthy controls. Mean melatonin concentrations collected at bed time were significantly lower in the diabetic subjects than that of controls (11.7±7.27 pg/ml vs. 24.13±10.80pg/ml; $p<.01$). Actigraphic analysis during the wake phase demonstrated that diabetic subjects showed lower levels of activity ($p=.02$). Additionally, there was a significant difference decrease in sleep duration ($p=.03$), efficiency ($p=.02$); and higher activity counts during the sleep phase ($p=.02$) in the diabetic group. Sleep efficiency was significantly correlated with melatonin collected two hours before bed time ($\rho=.61$;

p=.047). Additionally, there were significant inverse relationships between melatonin collected at two hours before bed time and latency (ρ=-.87; p=.001), wake after sleep onset (ρ =-.69; p=.02) and nocturnal activity (ρ =-.67; p=.03). Latency was inversely correlated with melatonin collected at bed time (ρ =-.69; p=.02). These findings suggest that T2DM presents disturbances in the homeostatic and circadian drives, mainly characterized by less consistency across days of the daily circadian signal, higher rhythm fragmentation and lower rhythm amplitude. In addition to the lower melatonin levels, the decrease in the amplitude of the activity rhythm may also be involved in circadian alterations of the sleep-wake cycle.

CHAPTER ONE

INTRODUCTION

Diabetes and Sleep Dysfunction

There are approximately 25.8 million individuals with diabetes in the United States; of whom 90% to 95% are classified as type 2 diabetes mellitus (T2DM) (Department of Health and Human Services, 2011). Moreover, prediabetes has been predicted in around 79 million Americans (Department of Health and Human Services, 2011). T2DM is characterized by insulin resistance accompanied by impaired insulin secretion (Kahn, 2003). Insulin resistance and T2DM are both associated with an increased risk of cardiovascular disease (Kashyap & Defronzo, 2007).

According to Wild et al., 2004, the number of people diagnosed with T2DM will increase to 366 million by 2030, and countries such as China, India, the Middle East and sub-Saharan Africa will probably have the highest increase (Wild et al., 2004). Patients with diabetes have an augmented chance to develop coronary artery and peripheral vascular disease, stroke, blindness and renal failure; which lead to a significant increase in costs to the public health system (Yach et al., 2006). Among the most common studies, risk factors for T2DM include: unhealthy diet, smoking, physical inactivity, obesity, and alcohol abuse (American Diabetes Association, 2003). The Centers for Disease Control and Prevention have reported that sleep complaints have a high incidence rate in the United State's diabetic population, and individuals with diabetes are more likely to have sleep impairments when compared to healthy controls (Plantinga et al., 2012).

Sleep disturbances, such as obstructive sleep apnea (OSA) (Ip et al., 2002), inadequate sleep duration (Spiegel et al., 2005; Van Cauter et al., 2007b), insomnia, restless leg syndrome and poor sleep quality are associated with an increased risk of developing chronic systemic conditions (Gangwisch et al., 2005; Gangwisch et al., 2006). Metabolic syndrome (Jennings et al., 2007; Hall et al., 2008), including hypertension (Gangwisch et al., 2006), obesity (Hasler et al., 2004; Gangwisch et al., 2005), and cardiovascular disease (Nilsson et al., 2001), have been described as such conditions that are directly related to sleep disturbances. Furthermore, sleep disorders have been both identified as a risk factor for developing T2DM (Knutson et al., 2006; Tasali et al., 2009), as well as associated with aggravation of the diabetes condition (Nilsson et al., 2004; Hayashino et al., 2007).

The main connections between type 2 diabetes and poor sleep have been attributed to impairments in glucose metabolism and circadian variation of hormone levels (Gottlieb et al., 2005; Van Cauter et al., 2007a; Spiegel et al., 2009). Glucose tolerance and insulin secretion are adjusted according to the sleep-wake cycle (Van Cauter et al., 1991). The homeostasis of normal glucose metabolic process is directly influenced by inadequate sleep through disruption of circadian rhythms, clock gene function and variation in autonomic nervous system activity (Laposky et al., 2008; Gangwisch, 2009). Moreover, glucose consumption is greatest during wake and lowest during non-rapid eye movement (NREM) sleep; also glucose metabolism expresses a diurnal pattern with variations in glucose tolerance throughout the day (Van Cauter et al., 1991; Scheen et al., 1996).

Sleep deprivation is one of the most studied sleep disturbances in T2DM. Associations between reduced sleep duration and high glucose levels have been previously reported. This relationship has been mainly explained by the reduced glucose metabolism, increased insulin resistance and high cortisol concentrations (Vgontzas et al., 1999; Gottlieb et al., 2005; Nedeltcheva et al., 2009a; Spiegel et al., 2009; Buxton et al., 2010). Experimental studies in healthy individuals have reported that restricting sleep to four hours for a period of two or more nights, reduce glucose tolerance by 40%, and acute insulin response to glucose by 30% (Spiegel et al., 1999; Spiegel et al., 2005). Another study showed that even one night of sleep restriction has negative effects on glucose metabolism in healthy individuals (Donga et al., 2010).

Results from the Sleep Heart Health Study showed that sleeping six hours or less, and sleeping nine hours or more per night have a greater prevalence of type 2 diabetes and impaired glucose tolerance when compared to people sleeping seven to eight hours per night (Gottlieb et al., 2005). Data from cross-sectional studies suggest that short and long duration of sleep are associated with increased prevalence of metabolic dysfunction with greater occurrence of high fasting plasma glucose and high Hemoglobin A1c (HbA1c) level (Gottlieb et al., 2005; Nakajima et al., 2008). Furthermore, quality and amount of sleep were reported to be a significant predictor of HbA1c (Knutson et al., 2006). Indeed, sleep duration above eight hours per day or less than seven hours per day are at a moderately increased risk of all-cause of mortality, cardiovascular disease, and symptomatic diabetes (Phillips et al., 2006).

Leptin, a hormone secreted during sleep, has also been associated with metabolic consequences of sleep disturbances (Spiegel et al., 2009). Its action is linked with the

balance between satiety and the need for food and energy consumption. The levels of Ghrelin tend to decrease throughout the night and the level of leptin tend to elevate (Van Cauter et al., 1991; Simon et al., 1994). Hypersecretion of leptin is observed during sleep deprivation, which leads to increase food intake, and blood glucose levels (Nedeltcheva et al., 2009b). Sleep apnea has also been reported to be related to the increase of leptin concentration and the increase in resistance to leptin action (Patel et al., 2004). This condition is one of the most common disorders observed in T2DM (Skomro et al., 2001). Epidemiologic studies have reported that the prevalence of sleep apnea in diabetes patients is around 50% (Frye et al., 2007).

Circadian Control of Sleep-wake Cycle, Melatonin and Diabetes

According to the two-process model proposed by Borbély 1982, the homeostatic process (process S) is determined by the previous amount of sleep and wakefulness, in which the sleep pressure is greatest at the beginning of sleep and gradually decreases throughout this phase (Borbely, 1982). The circadian process (process C) organizes sleep and wakefulness alternations over the 24 hours of the day. During wakefulness, little or no homeostatic drive remains, but begins to accumulate and grows steadily during this phase. For this reason, its strength is dependent upon the amount of time elapsed since the last sleep period. When we are awake, the rising homeostatic sleep drive is opposed by a circadian drive that activates wakefulness and influences this component until just before sleep, when the circadian influence gradually declines (Beersma & Gordijn, 2007). In situations such as sleep deprivation, the drive to sleep increases due to the accumulated debt of sleep. Additionally, a decrease in sleep latency, and an enhancement of

electroencephalogram (EEG) synchrony in NREM sleep are also observed (Borbely & Achermann, 1999).

In addition to the homestatic drive influencing the circadian rhythmicity of the sleep-wake cycle, the circadian process controls the timing and organization of sleep through the Circadian System. This system aims to temporarily organize the physiological processes of the body by specialized neural structures such as the retinohypothalamic track, which is responsible for transmitting light signals from the retina to the circadian master clock (the suprachiasmatic nucleus -SCN). The SCN generates signals to the pineal gland to regulate melatonin (N-acetyl-5-methoxytryptamine) production (Lewy et al., 1980; Scheer & Czeisler, 2005). In response to light, the excitatory signal from the SCN and consequent suppression of melatonin production are believed to be related to wakefulness promotion during the day. In the dark phase, this inhibition is released and melatonin is produced, leading to sleep promotion (Lewy et al., 1980; Scheer & Czeisler, 2005; Khullar, 2012). In this context, the endogenous circadian melatonin rhythm is of great importance for synchronization of the sleep-wake cycle (Nagtegaal, 2002).

Melatonin concentration in plasma begins to increase approximately 2 h before habitual bedtime, it peaks around the first hours of the morning, and after waking, decreases to levels close to those found during the day (Tzischinsky et al., 1993).

The Circadian System organization occurs in a hierarchical manner. The SCN functions as a master oscillator and remains coupled with peripheral oscillators that are present in nearly all cells, through nerve connections of the autonomic nervous system and humoral signs (Brandstaetter, 2004). The major clock regulates the circadian rhythms

in glucose, corticosteroids, leptin and cardiovascular systems through these connections to the pancreas, liver, adrenal glands, adipose tissues and heart (Buijs et al., 2006). This interaction allows synchronizations between the central and peripheral tissue clocks. Moreover, various clock genes participate in the regulation of metabolic homeostasis (Bass & Takahashi, 2010). For instance, it has been shown that when a clock disorder occurs, it leads to disorders in glucose metabolism (Bass & Takahashi, 2010). Also, feeding time can entrain intrinsic oscillation of clocks in liver cells, similar to what occurs in central oscillators in the brain when entrained by light (Stokkan et al., 2001). Additionally, experimental studies have suggested that gene expression rhythm deregulation can cause metabolic dysfunction (Gatfield et al., 2009); and chronic circadian misalignment might be correlated with metabolic and cardiovascular dysfunction (Kohsaka & Bass, 2007; Ruger & Scheer, 2009; Scheer et al., 2009). These findings support the understanding that circadian and metabolic systems are reciprocally regulated (Young & Bray, 2007; Carneiro & Araujo, 2009; Bass & Takahashi, 2010; Carneiro, 2012).

Studies have also found that the decreased melatonin levels in T2DM might be related to the increased risk of developing T2DM (Peschke et al., 2006; Mantele et al., 2012; McMullan et al., 2013). Associations between melatonin alterations and insulin, glucose, lipid metabolism and antioxidant capacity have been reported (Nishida, 2005; Robeva et al., 2008) and an experimental analysis has shown a relationship between impaired melatonin levels and disturbances in insulin sensitivity that can be associated with development of T2DM (Peschke et al., 2006). In addition, the use of exogenous

melatonin has been suggested as an adjunct to improve glucose control and diabetic complications in T2DM (Garfinkel et al., 2011).

Physical Activity and the Sleep-wake Cycle

The benefits of physical activity for prevention and treatment of T2DM and cardiovascular diseases are well known and are mainly related to the potential to reduce insulin resistance in an inverse fashion (Waxman, 2004; Gill & Malkova, 2006; Ross & Despres, 2009; Zuo et al., 2012). However, T2DM has been intensively associated with sleep disorders (Knutson et al., 2006; Tasali et al., 2009; Plantinga et al., 2012), which has been reported to be related to the decrease in physical activity (Nedeltcheva et al., 2009a; Paparrigopoulos et al., 2010). Furthermore, a combination of low physical activity and insufficient amount of sleep is also associated with insulin resistance (Zuo et al., 2012). A study by Zuo et al., 2012, reported that adequate sleep duration together with different types of physical activity may assist the decrease of insulin resistance and its consequences (Zuo et al., 2012). Bromley et al. 2012, suggested that the decrease in physical activity is one of the factors mediating the association between chronic inadequate sleep with metabolic morbidity (Booth et al., 2012; Bromley et al., 2012).

Associations between sleep, physical activity, and insulin resistance include reports showing inadequate sleep being related to excessive daytime sleepiness and decreased physical functioning (Weaver et al., 1997). Also, it has been found that physical activity can be decreased by sleep restriction, and improvement in sleep quality can be seen after increasing physical activity (Nedeltcheva et al., 2009a; Paparrigopoulos et al., 2010). Therefore, it has been proposed that one of the factors mediating the

association of inadequate sleep and the increased incidence of metabolic morbidity is the decrease in physical activity. However, many studies had based the sleep analysis on subjective questionnaires that might be limiting the precision of the outcomes (Shephard, 2003; Lauderdale et al., 2008). Inconsistent results have been found on the association between self recalled sleep duration and physical activity. Liu et al. 2000 (Liu et al., 2000) and other authors (Ohida et al., 2001; Patel et al., 2008), found a positive relationship between self-reported sleep and physical activity; while other researchers reported a negative association (Chaput et al., 2008) or even no association between these two variables (Imaki et al., 2002; Youngstedt et al., 2003; Patel et al., 2006).

Although studies have shown a relationship between physical activity and sleep-wake cycle organization (Miyazaki et al., 2001; Buxton et al., 2003), we found no studies on diabetes addressing the consequences of physical inactivity on this circadian control. The influence of exercise on the circadian system is found when exercise is conducted between the middle and end of the nocturnal phase, in which a phase delay is observed (Miyazaki et al., 2001; Buxton et al., 2003). Conversely, phase advance is found when exercise is performed six hours after awakening (Buxton et al., 2003). Van Reeth et al., 1994, observed that physical exercise during night caused a clear phase delay of one to two hours a day after starting an exercise protocol (Van Reeth et al., 1994). Changes were also observed in melatonin and thyrotropin levels. The largest delays were found when exercise was performed three to five hours before the minimum temperature (Van Reeth et al., 1994). O'Connor and Youngstedt 1995, based on the hypothesis that a better quality of sleep causes less tiredness during the day and more provision for physical activity, supported that the quality of sleep of active people is better than inactive people

(O'Connor & Youngstedt, 1995). Vuori et al., 1988, reported that sleep is improved when exercise is introduced, and sedentary individuals are the ones that benefit the most (Vuori et al., 1988).

Due to the growing source of evidence demonstrating a relationship between physical activity and sleep, the American Society of Sleep Disorders reports physical activity as a nonpharmacological therapeutic modality for the treatment of sleep disorders (Shapiro & Flanigan, 1993; Youngstedt et al., 1997; Chasens et al., 2007).

Although exercise is cited as one of the major synchronizers in humans; it is not known for certain which mechanisms are responsible for this synchronization. Some researchers suggest, through experiments with rodents, that there is a participation of secondary neural pathways sending information to the SCN. The thalamic intergeniculate leaflet and raphe nuclei are part of the main pathways proposed, transmitting information about non-photic stimuli to the SCN (Vrang et al., 2003; Yannielli & Harrington, 2004). However, additional studies are needed to further describe specific physical activity parameters, such as intensity, duration, if accompanied by light or not, and what population would benefit from the relationship between the increase in physical activity level and the organization of the sleep-wake cycle.

Purpose of the Study

The risk of microvascular complications in patients with type 2 diabetes may be reduced by approaches that focus on maintaining good glycemic control (Chiasson et al., 2003; Gaede et al., 2003). The American Diabetes Association recommends maintaining HbA1c levels less than 7% as studies have shown that this approach reduces

microvascular complications due to diabetes (American Diabetes Association, 2010), and also reduces myocardial infarction in cases when metformin is used in overweight patients with diabetes (UK Prospective Diabetes Study Group, 1998a). Therefore, it is of great importance to address any action that reduces glycemic control such as inadequate sleep. Studies that address sleep-wake cycle are imperative to identify what parameters of this cycle may be influencing sleep in patients with diabetes, while also helping to recognize which approaches can be administered for sleep improvement in this population. Therefore, the aim of this study was to analyze the sleep-wake cycle in subjects with T2DM and compare it to healthy controls using the nonparametric approach.

Additionally, since there seems to be a complex interaction involving alterations in melatonin production, circadian rhythm disturbances, impaired insulin and glucose metabolism and T2DM; it is important to further investigate alterations in the sleep-wake cycle and melatonin to identify the steps that can be taken to minimize the consequences of these disturbances, and thus assist in improving the health of this population. Therefore, the aim of this study was also to assess the changes in the circadian and homeostatic control of the sleep-wake cycle in T2DM and correlate it with melatonin concentration.

Approaches of the Study

The analysis of the sleep-wake cycle on T2DM needs to be explored. To our knowledge, this is the first study to further discuss the sleep-wake cycle in T2DM applying the nonparametric methodological approach, and base the analysis on the Two-process

Model Theory to assess the circadian and homeostatic controls; furthermore it is the first study to correlate these variables with melatonin concentration in diabetic subjects. The rest-activity rhythm was assessed through actigraphy, using the Actiwatch 2® from Philips. The raw activity counts were extracted from this device and used for analysis of the nonparametric approach. Additionally, sleep-wake variables were obtained from Respironics Actiware 5.70.1 software. The nonparametric methodological approach of the rest-activity rhythm assessment, previously reported by Van Someren et al., 1996, is composed of three main variables: interdaily stability (IS), intradaily variability (IV) and amplitude (AMP). This approach was performed in our study and its discussion is in Chapter 2 of this dissertation. The analysis on the Two-Process Model Theory, to further analyze the rest-activity rhythm, and the correlation with salivary melatonin levels is in Chapter 3 of this dissertation.

Significance of the Study

According to the Centers for Disease Control and Prevention, sleep complaints have a high incidence rate in the United States diabetic population, and individuals with diabetes are more likely to have sleep impairments when compared to controls. Additionally, sleep disorders have been both identified as a risk factor for developing T2DM, as well as associated with worsening the diabetes condition. Authors have suggested that irregular sleep-wake cycle patters and short and long sleep may enhance dysfunction in the glucose metabolism. However, little is known about the circadian and homeostatic control of the sleep-wake cycle in this population. The results of this study contribute to the understanding of these parameters and identify specific disturbances of the circadian

and homeostatic control of the sleep-wake cycle. We believe that with further analysis on the sleep-wake cycle in type 2 diabetics, it will be possible to better identify therapeutic procedures that might help to achieve a better sleep-wake cycle organization, better sleep parameters and ultimately, a better glycemic control in type 2 diabetics.

CHAPTER TWO

A NONPARAMETRIC METHODOLOGICAL ANALYSIS OF SLEEP-WAKE CYCLE IN TYPE 2 DIABETES

By [1]Paula Regina Aguiar Cavalcanti, DSc, PT; [1,2]Lee Berk, DrPH, MPH, CLS; [3]Noha Daher, DrPH, MSPH; [4]Tania Campus, Phd, PT; [4]Araujo, John, Phd, MD; [1]Jerrold S. Petrofsky, Phd, J.D.; [1]Everett Lohman, DSc, PT, OCS

[1]Dept. of Physical Therapy, School of Allied Health Professions, Loma Linda University

[2]Dept. Pathology and Humans Anatomy, School of Medicine, Loma Linda University

[3]Dept. of Computing, Research and Statistics, School of Allied Health Professions, Loma Linda University

[4] Neurobiology and Biological Rhythm Laboratory, School of Psychobiology, Federal University of Rio Grande do Norte

Abstract

Type 2 diabetics have a high incidence of sleep impairments. Since sleep deficiency can be associated with impaired glycemic control, studies of the sleep-wake cycle in diabetics are important in identifying which parameters of the cycle may influence sleep. The purpose of this study was to analyze the sleep-wake cycle of type 2 diabetics and compare them to healthy controls using the nonparametric analytic approach. Twenty-one diabetics and 21 healthy subjects matched for gender and age were recruited to participate in the study. Subjects were assessed by the Beck's Depression Inventory (BDI), the Apnea Risk Evaluation System (ARES) questionnaire, hemoglobin A1c (HbA1c) and actigraphy. The nonparametric methodological approach of rest-activity rhythm assessment is composed of three main variables: interdaily stability (IS), intradaily variability (IV) and amplitude (AMP). Data were analyzed using the Independent t-test, Mann-Whitney U test, and Spearman's correlation. Type 2 diabetics subjects demonstrate lower IS (p=.03), higher IV (p=.046) and lower rhythm amplitude (p=.02) when compared to healthy controls. Also, there was a positive correlation between IS and most active 10 hr (M10)) in the average of 24 hours pattern (r =.44; p=.046) in the diabetes group and a negative correlation between IV and M10 in the control group (r =-.57; p=.007). These data together suggest that type 2 diabetes mellitus (T2DM) exhibits a dysfunction in the sleep-wake cycle due to alterations in the circadian function as well as in the homeostatic capacity to maintain sleep; mainly characterized by less consistency across days of the daily circadian signal, higher rhythm fragmentation and lower rhythm amplitude. Future approaches may be developed considering the

influence of circadian glucose variations throughout the day and meal times on the coupling of the rest-activity rhythm to zeitgeber and rhythm fragmentation.

Key words: actigraphy, interdaily stability, intradaily variability, amplitude, most active 10 h, least active 5h

Introduction

There are approximately 25.8 million individuals with diabetes in the United States; of whom 90% to 95% are classified as type 2 diabetes mellitus (T2DM) (Department of Health and Human Services, 2011). Among the common studied risk factors for T2DM include: unhealthy diet, smoking, physical inactivity, obesity, and alcohol abuse (American Diabetes Association, 2003; van Dam, 2003). Furthermore, the Centers for Disease Control and Prevention have reported that sleep complaints have a high incidence rate in the United States diabetic population, and individuals with diabetes are more likely to have sleep impairments when compared to controls (Plantinga et al., 2012).

The main connections between type 2 diabetes and poor sleep have been attributed to impairments in glucose metabolism and circadian variation of hormone levels (Gottlieb et al., 2005; Van Cauter et al., 2007a; Spiegel et al., 2009). Glucose tolerance and insulin secretion are adjusted according to the sleep-wake cycle (Van Cauter et al., 1991). The homeostasis of normal glucose metabolic process is directly influenced by inadequate sleep through disruption of circadian rhythms, clock gene function and variation in autonomic nervous system activity (Laposky et al., 2008; Gangwisch, 2009). Moreover, glucose consumption is greatest during wake and lowest during non-rapid eye movement (NREM) sleep; also glucose metabolism expresses a diurnal pattern with variations in glucose tolerance throughout the day (Van Cauter et al., 1991; Scheen et al., 1996).

The Circadian System aims to temporally organize the physiological processes of the body by specialized neural structures such as input pathways, responsible for

transmitting signals such as light to the circadian master clock (the suprachiasmatic nucleus -SQN) (Dijk & von Schantz, 2005). This major clock regulates the circadian rhythms in glucose, corticosteroids, leptin and cardiovascular systems through the autonomic nervous system and humoral signs to the pancreas, liver, adrenal glands, adipose tissues and heart (Buijs et al., 2006). This interaction allows synchronizations between the central and peripheral tissue clocks. Moreover, various clock genes participate in the regulation of metabolic homeostasis (Bass & Takahashi, 2010). For instance, it has been shown that when a clock disorder occurs, it leads to disorders in glucose metabolism (Bass & Takahashi, 2010). Also, feeding time can entrain intrinsic oscillation of clocks in liver cells, similar to what occurs in central oscillators in the brain when entrained by light (Stokkan et al., 2001). These previous findings support the understanding that circadian and metabolic systems are reciprocally regulated (Young & Bray, 2007; Carneiro & Araujo, 2009; Bass & Takahashi, 2010; Carneiro, 2012).

The risk of microvascular complications in patients with T2DM may be reduced by approaches that focus on maintaining a good glycemic control (Chiasson et al., 2003; Gaede et al., 2003). The American Diabetes Association recommends maintaining HbA1c levels less than 7% as studies have shown that this approach reduces microvascular complications due to diabetes and also reduces myocardial infarction in cases when metformin is used in overweight patients with diabetes (UK Prospective Diabetes Study Group, 1998a, 1998b; American Diabetes Association, 2012). Therefore, it is of great importance to address any action that reduces glycemic control such as inadequate sleep. Studies that address sleep-wake cycle are imperative to identify what parameters of this cycle may be influencing sleep in patients with diabetes, while also

helping to recognize which approaches can be administered for sleep improvement in this population. Therefore, the aim of this study was to analyze the sleep-wake cycle in subjects with T2DM and compare it to healthy controls using the nonparametric approach.

Material and Methods

Sample

Twenty-five diabetic subjects (age range from 49 to 78 years old) were recruited for the study. The control group consisted of 21 healthy individuals matched for gender and age with the diabetes group. The study inclusion criteria consisted of diagnosis of diabetes for more than a year. Exclusion criteria were diagnosis of obstructive sleep apnea, neurological comorbidities such as Parkinson's and Alzheimer's disease, use of antidepressant or any other drug that may affect alertness or drowsiness (e.g. melatonin, antipsychotics, antidepressants, benzodiazepines, muscle relaxants), depression (a score of 17 and over was considered an indicator of the presence of the clinically significant depressive symptoms assessed by Beck's Depression Inventory), improper use of the actiwatch, incomplete sleep diary and important change in social routine during the week of data collection. A total of four diabetic subjects were excluded from the study due to incorrect use of the actiwatch, report of sleep apnea and severe depression (according to Beck's Depression Inventory).

Procedures

The study was approved by Loma Linda University Institutional Review Board

and all subjects were informed on the procedures of the investigation and signed an informed consent. The following demographic data were assessed: gender, age, weight and height, from which we calculated body mass index (BMI) (weight in kilograms divided by height in meters squared). The blood level of hemoglobin A1c (HbA1c) was measured using DCA Vantage^{TM} Analyzer (Siemens®, Malvern, PA, USA). HbA1c is a gold standard for monitoring glycemic control, and it reflects glucose levels over the past 2 to 3 months (International Expert Committee, 2009; American Diabetes Association, 2012). The study sample was classified as good (HbA1c < 7.5%) or poor glycemic control (HbA1c ≥7.5%) (Hartemann-Heurtier et al., 2001; Juarez et al., 2012). Duration of time since the diagnosis of diabetes and type of treatment for T2DM were also recorded.

Depression was assessed using the Beck's Depression Inventory (BDI), consisting of 21 questions that evaluate how severe is classified the depressive symptoms. The total score is a sum of all questions and range from 0-63. It is classified in four categories: "These ups and downs are considered normal" (1-10 points); "Mild mood disturbances" (11-16 points); "Borderline clinical depression" (17-20 points); "Moderate depression" (21-30); "Severe depression" (31-40 points); and "Extreme depression" (over 40 points) (Richter et al., 1998; Contreras S, 2004).

Subjects were screened for risk of obstructive sleep apnea (OSA) through Apnea Risk Evaluation System (ARES) questionnaire. This instrument considers demographic characteristics, diseases that are associated with risk for OSA (i.e., high blood pressure, heart disease, diabetes, or stroke), Epworth sleepiness scale, and a five-scale response to the frequency rating for snoring, waking up choking, and breathing difficulties during

sleep. Scoring between 4 or 5 is considered low risk for OSA, 6 to 10 high risk and 11 or more as very high risk (Levendowski D. J OR, 2007).

Rest-activity rhythm was measured in subjects' own homes using actimetry through Actiwatch 2 (Philips Respironics®, Andover, MA, USA). Actimetry consists of a non-invasive method for the indirect evaluation of the sleep-wake cycle and it is recognized by the American Academy of Sleep Medicine as helpful adjunct to clinically assess sleep disorders (American Sleep Disorders Association, 1995). The device was placed on subjects' non-dominant wrist for 7 days along with an explanatory pamphlet for the actiwatch and a sleep log. Subjects were also instructed to maintain their normal daily routine. Recordings per minute for six complete days and seven nights were used for the analysis. The Actiwatch 2 has a button to mark events and subjects were asked to press the button when they went to bed at night and when they got up in the morning; also, it has an ambient light monitor to assess the amount to Lux the participant has been exposed to. Subjects were asked not to remove the device from their wrist during the study week. Data on sleep and activity levels were primarily calculated using the Respironics Actiware 5.70.1 software (Philips Respironics®, Andover, MA, USA). The raw activity counts were extracted and analyzed by nonparametric approach (Van Someren et al., 1997a). This method of rest-activity circadian rhythm assessment is composed of three main variables: interdaily stability (IS)- shows how is the union between the rest-activity rhythm and the Zeitgeber (external time signaling stimuli) express the strength of coupling between the rest-activity rhythm and Zeitgebers; intradaily variability (IV) – representing the transitions periods between rest and activity representing fragmentation of periods of rest and activity; and amplitude (AMP)

(difference between least active 5 h (L5) and most active 10 h (M10)) in the average of 24 hours pattern, described previously by Van Someren et al., 1997 (Van Someren et al., 1997a). The calculations of each circadian variable were performed as follow:

IS is described as ratio between the variance of the average 24-hour pattern around the mean and the overall variance. The IS considers individual days to quantify how similar the activity patterns are. Higher values are indicative of rhythm stability, and it has been reported to be around 0.6 in healthy adults (Huang et al., 2002; Calogiuri et al., 2013).

The formula for IS is:

$$IS = \frac{N \sum_{h=1}^{p} (\bar{x}_h - \bar{x})^2}{p \sum_{i=1}^{N} (X_i - \bar{x})^2}$$

Where N is the total number of data, p is the number of data per day; $\bar{x}_h$ are the hourly means, $\bar{x}_i$ represent the individual data points, and $\bar{x}$ represents the mean of all days.

The IV ranges from 0 to 2 and studies have shown that scores <1 are found in healthy adults. Rhythm fragmentation is characterized by higher values of IV. This variable is calculated as the ratio of the mean squares of the difference between successive hours and the mean squares around the grand mean, as shown below (Van Someren et al., 1997a):

$$IV = \frac{N \sum_{i=2}^{N} (X_i - X_{i-1})^2}{(N-1) \sum_{i=1}^{N} (\bar{x} - X_i)^2}$$

Data Analysis

Data analysis was performed using SPSS 21.0 software (Armonk, NY, USA). The Kolmogorov-Smirnov test was used to examine the distribution of the continuous variables. The independent *t*-test was used to compare groups in regards to mean of age, BMI, ARES score and BDI, as well as, the differences in circadian variables, which were followed by ANCOVA to control for risk of OSA, mean light exposition per minute and average of all light in Lux. The Pearson's correlation test was applied to examine the intra-group correlation among circadian variables and HbA1c. The chi-square Fisher's exact test was used to compare proportions of males and females by group. Comparisons between good control and poor control were assessed using Mann-Whitney U test. The level of significance was set at $p<.05$.

Results

The subjects' characteristics are shown in Table 1. There were no significant differences between the diabetic and control groups in terms of gender, weight, height and BMI. In the diabetic group, the mean time since diagnosed with diabetes was 7.8±9.6 years. The HbA1c level varied from 5.1 to 10.7; however, there was no significant difference in duration of diagnosis between good and poor controls. There was no significant difference in BDI score between groups (Diabetic 5.8±5.8 vs. Control 3.5±3.4; $p=.13$). Regarding the risk for sleep apnea, there was a significant difference between groups (5.8±2.7 vs. 3.4±2.7; $p=.009$). However, risk for OSA did not meet the modeling criterion for confounding when analyzing the differences between groups and the circadian variables.

Table 1 - Mean±SD of subjects'characteristics according to group.

Variables	Diabetic Group	Control Group	*p value*
n	21	21	
Gender [a] (%) Male Female	38.1 61.9	42.9 57.1	.50
Age[b] (years)	59.0 ± 8.6	59.5 ± 8.5	.68
Weight[b] (Kg)	77.0 ± 15.0	79.0± 15.3	.33
Height[b] (m)	1.7 ± .1	1.7 ± .1	.91
BMI[b] (kg/m^2)	27.6 ± 7.2	28.8± 4.3	.21
High blood pressure[a] (%)	85.7	14.3	<.01*
Duration of disease (years)	11.7 ± 9.6		
HbA1c[b] (%)	7.7 ± 1.4		
Diabetes treatment (%) Oral drug Insulin	 61.9 38.1		

n, number of subjects per group
[a]Fisher's test comparisons
[b]Independent test comparisons
*significant difference (p<.05)

Compared to controls, the diabetic group showed a significant decrease in IS (.37±.04 vs. .40±.07; p=.03) (Figure 1, A) and a significant increase in IV (.50±.05 vs. .45±.12; p=.046) (Figure 1, B). Additionally, diabetic subjects were significantly less active than healthy controls, as shown by the result of M10 (298.3±72.2 vs. 370.1±114.0; p=.02) (Figure 1, C) and during the least 5 hours of activity, diabetic subjects had higher active counts when compared to controls (17.6±8.3 vs. 12.2±5.7; p=.02) (Figure 1, D). Calculation from M10 and L5 demonstrated a significant lower amplitude of the rest-activity rhythm for the diabetic group (281.0±70.4 vs. 349.3±104.6; p=.02) (Figure 1, E).

There was a positive correlation between IS and M10 (ρ=.44; p=.046) in the diabetic group and a significant negative correlation between IV and M10 in the control group (ρ=-.57; p=.007). However, there was no significant correlation between IV and M10 in the diabetic group and IS and M10 in the control group.

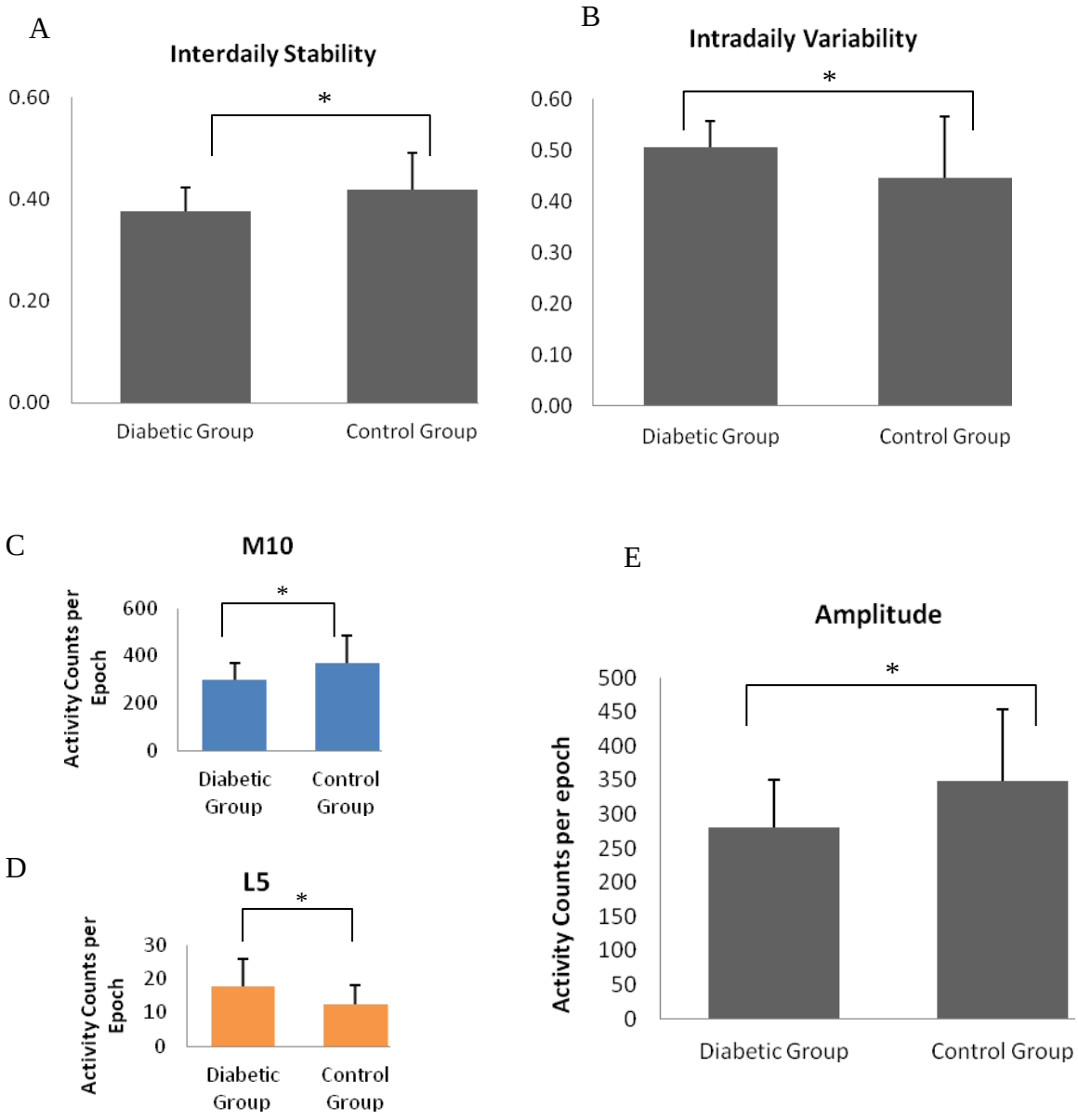
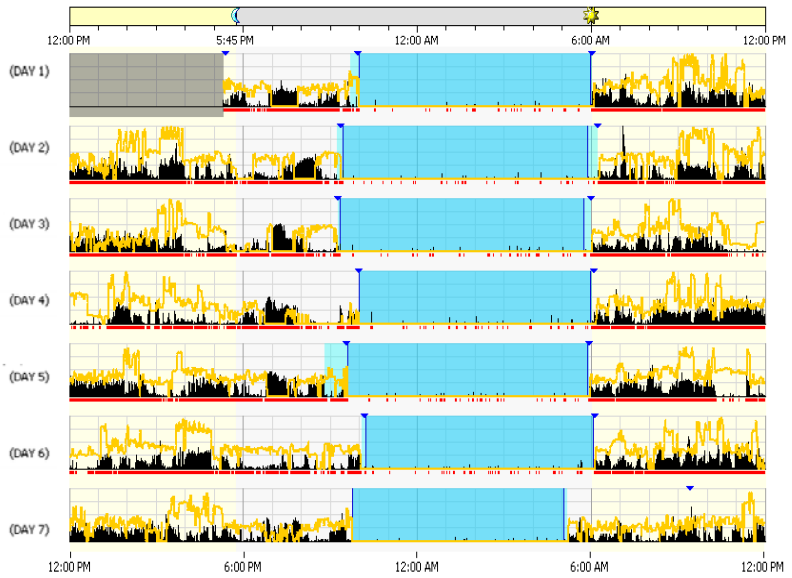


Figure 1 –A, mean±SD interdaily stability; B, mean±SD intradaily variability; C, mean±SD amplitude of rest-activity rhythm; D, mean±SD of most active 10 hours; E, mean±SD of least active 5 hours of diabetes and control group. An asterisk (*) indicates that the group differs on this variables in the Student's *t*-test, p<.05.

Figure 2A illustrates the actogram of a healthy individual, in order to compare it with the actogram of a diabetic subject. The actogram in Figure 2B shows high instability between days. The subject in Figure 2C shows low amplitude of the activity rhythm and Figure 1D illustrates fragmentation in a subject with frequent records of periods of rest and activity. In regards to HbA1c levels in good and poor controls, there were no significant differences in the circadian variables.

A



B

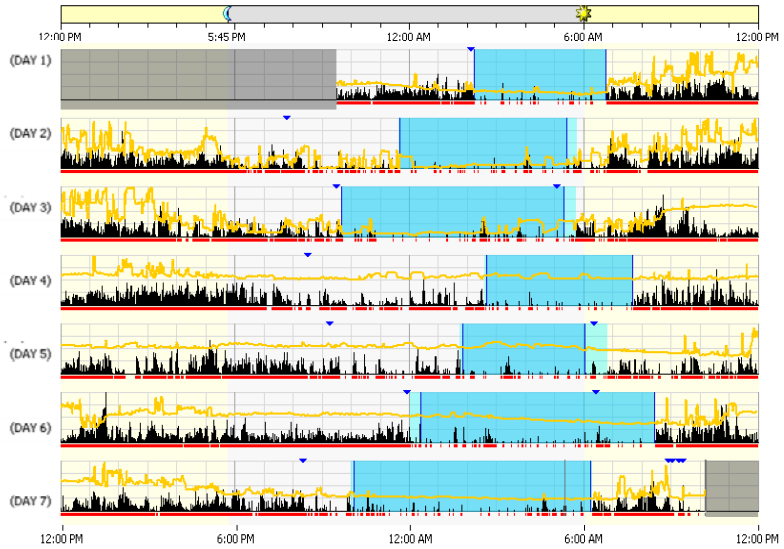
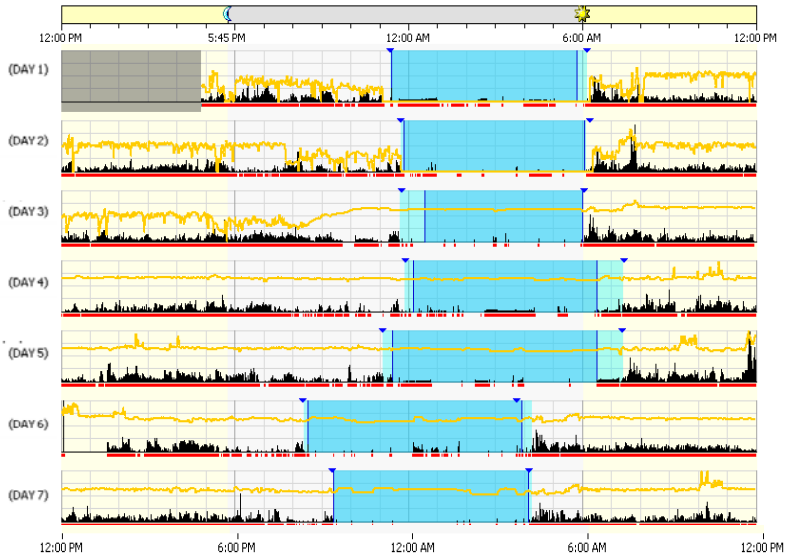


Figure 2: Actograms showing activity thresholds (vertical bar) divided into light phase (6:00 a.m. to 5:45 p.m.) and dark phase (5:45 p.m. to 6:00 a.m.), of (A) a control subject; (B) a diabetic subject showing high instability between days.

C



D

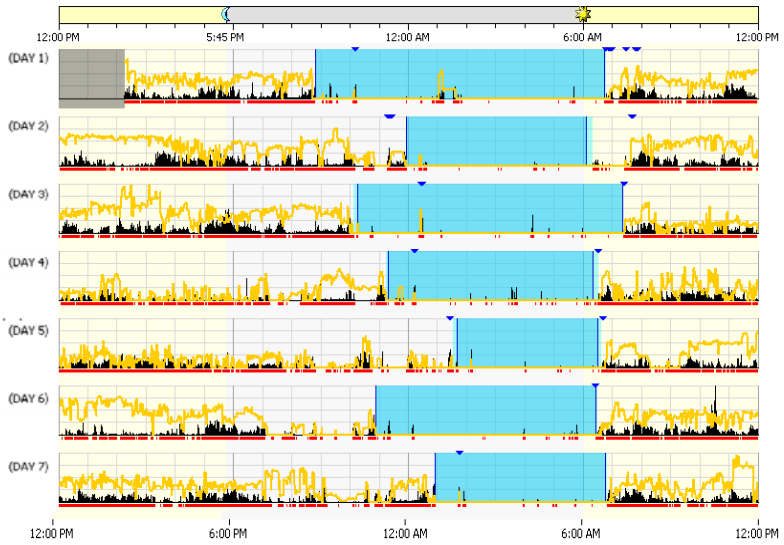


Figure 2: Actograms showing activity thresholds (vertical bar) divided into light phase (6:00 a.m. to 5:45 p.m.) and dark phase (5:45 p.m. to 6:00 a.m.), of (C) a diabetic subject showing low rhythm amplitude, and (D) a diabetic subject showing high fragmentation.

There were no significant differences in the sum of all light exposition (Lux multiplied by minute) and average of all valid light (Lux) from the start time to the end time of active interval between groups, however, the diabetic group was exposed to less amount of light (424654.1±263551.6 vs. 478923.9±414253.4; p=.61). Also, the amount of all valid light for the diabetic group was less than the control group (477.5±297.2 vs. 531.7±431.1; p=.64). Moreover, poor controls were significantly less exposed to light per minute than good controls (548322.2±257660.0 vs. 288619.2±203310.3; p=.02); and there was a significantly less amount of all valid light in Lux for the poor control, when compared to the good control (617.7±298.0 vs. 323.2±217.2; p=.02) (Figure 3).

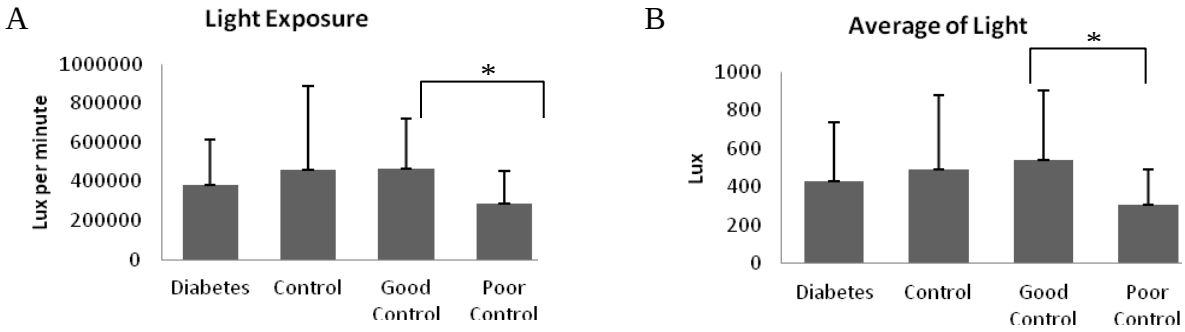


Figure 3: A, mean±SD light exposition (Lux multiplied by minute); and B, mean±SD average of all valid light (Lux) from the start time to the end time of active interval according to group. An asterisk (*) indicates that the group differs on this variables in the Mann-Whitney's test, p<.05.

Discussion

The present study examined the sleep-wake cycle variations on T2DM using a non-parametric approach. The findings revealed that T2DM individuals demonstrate lower IS, indicating a less consistency across days of the daily circadian signal; higher IV, showing greater variability within each 24-hour period; and lower peak activity

levels, indicating lower amplitude compared to healthy, age matched subjects. These data together suggest that T2DM exhibit a dysfunction in the sleep-wake cycle due to alterations in the circadian function as well as in the homeostatic capacity to maintain sleep. To our knowledge, this is the first study to assess the sleep-wake cycle on T2DM using the nonparametric approach.

Reports on the non-parametric approach on the analysis of the sleep-wake cycle have been previously published in healthy individuals, Alzheimer and Parkinson's disease but to date no published studies have used this approach in T2DM subjects (Van Someren et al., 1996; Van Someren et al., 1997b; Whitehead et al., 2008). For this reason, comparisons on circadian variables in this study were mainly examined indirectly.

Van Someren et al. (1996) found low IS in Alzheimer patients especially in those that were institutionalized. Our findings on T2DM also showed decrease in this circadian variable. The IS is indicative of how similar the activity pattern is expressed on different days. It can indicate how consistent the daily circadian signal is across days; also, revealing the strength of its coupling to stable zeitgebers. Typically, high values are indicative of a more stable rhythm, and it was found to be around 0.6 for healthy adults (Van Someren et al., 1997b; Calogiuri et al., 2013). Our finding is in agreement with this result as we observed lower values in IS for the diabetic group (.37±.04) compared to controls (.42±.07) (Van Someren et al., 1997b).

In regards to IV, the diabetic group showed significantly higher values when compared to controls and higher values of IV are indicative of more fragmented rhythm. Similar results were observed in Parkinson's disease. Whitehead et al. (2008) reported

that on the Parkinson's group, it was found a more fragmented rest-activity rhythm with constants transitions from high to low periods of activity. Authors reported that the rest-activity rhythm is less predictable in these patients. In regards to T2DM, studies have shown that nocturia is a significant variable that is positively correlated with difficulty to maintain sleep (Lamond et al., 2000) and it might as well contribute to increase IV in the diabetic population.

Whitehead et al. (2008) also found lower peak of physical activity and lower rhythm amplitude in subjects with Parkinson's disease. Authors suggested that this finding could be related to the motor impairment found in this population. Previous analysis on stroke patients also revealed alteration on the amplitude of the sleep-wake cycle that might as well impair the quality of life of these individuals (Cavalcanti, 2012; Cavalcanti, 2013). Motor alterations on stroke patients could be influencing the expression of this variable (Cavalcanti, 2012). Similarly, we observed lower levels of physical activity and low rhythm amplitude in the diabetic group. This finding could be related to the increased sedentary activity commonly found in these individuals.

Van Someren et al. (1996) reported a strong association between IS and IV with daytime activity. They explained that this association does not result from the mathematical model, but from the organized pattern of the high levels of daytime activity which lead to an organized circadian rhythm. Moreover, as activity level functions as an output and as an input of the circadian timing system, Van Someren et al. (1996) suggested that daytime activity level is of great importance in circadian rest-active disturbances and might as well be one of the causes for the rhythm disturbances in the Alzheimer population. These findings are in line with our results in which IS showed

positive association with daytime activity in the diabetic group. However, more studies are needed to test if increasing the amplitude of the activity rhythm would result in more regulation of the sleep-wake cycle.

Actigraph studies on T2DM subjects revealed that sleep-wake cycle irregularities are more frequent in these individuals than in the healthy controls. Tsujimura et al. (2009) reported higher scores of night activity in the diabetic group when compared to controls (Tsujimura et al., 2009). Additionally, they reported that higher fasting plasma glucose and HbA1c values were positively correlated with pronounced sleep and circadian rhythm disturbances. Our study also found higher activity level in the diabetic group during night time (measured by L5, related to the least 5 hours of activity, night activity); however, we did not find significant relationships between HbA1c and the circadian variables. Nakanishi-Minami et al. (2012) reported that T2DM subjects tended to go to bed later and wake up later; and had higher daytime sleepiness compared to controls (Nakanishi-Minami et al., 2012). These authors suggested that irregular sleep-wake cycle patters and short and long sleep may enhance dysfunction in the glucose metabolism.

It is well known that meal time exert influence in the circadian regulation (Mendoza, 2007). A variety of behavioral and physiological activities are entrained by food, as it is considered to be a potent synchronizer of peripheral clocks (Hara et al., 2001; Stephan, 2002; Mendoza, 2007; Carneiro et al., 2012). For instance, researchers have demonstrated that the use of glucose only as nourishment is able to synchronize not only the rest-activity rhythm but also the temperature rhythm in rats (Hara et al., 2001; Stephan, 2002; Mendoza, 2007; Carneiro et al., 2012). Wu et al. (2012) in a study with induced T2DM rats, observed that daytime restricted feeding condition shows a more

preeminent effect on the reentrainment of circadian rhythm in the T2DM rats; leading them to suggest that the circadian system in diabetics is unstable and more easily shifted by feeding stimuli (Wu et al., 2012). Studies also have shown that disruptions on circadian rhythms can provoke the development of metabolic disorders, including obesity and T2DM (Turek et al., 2005; Woon et al., 2007). Furthermore, alterations on the rhythm of glucose tolerance have been reported even in first-degree relatives of patients with T2DM (Boden et al., 1999). However, it is not clear whether this alteration exerts influence on the sleep-wake cycle and which mechanisms would be related. Our study has not found significant relationships between HbA1c and the circadian variables using the nonparametric method, perhaps due to the small sample size. More research is required in order to examine associations between glycemic control and the sleep-wake cycle. We believe that these studies should include analysis of the glucose variation throughout the day, as HbA1c reflects the overall glycemic control for the past 2 to 3 months and not the variances occurred during the specific days of analysis. Additionally, studies should record subjects' meal time, as these variables might exert influence mainly on IS and IV, and could further explain the alterations found in this study.

In regards to the light exposition, we have examined the amount of light the subjects were exposed to throughout the week. Studies on the influence of light in circadian rest-activity rhythm regulation have shown that sessions of bright light during the day can increase IS, and decrease IV (Van Someren et al., 1997a). The association between IS and daytime light exposition has also been reported (Van Someren et al., 1996). Although our study did not found differences between groups in light exposition (Lux multiplied by minute) and average of all valid light (Lux); the diabetic group was

exposed to less amount of light, and had lower values for all valid light during the active phase. Moreover, it has been previously reported that ophthalmic diseases, such as cataract, diabetes retinopathy, and glaucoma might affect photic input to the circadian system (Jean-Louis et al., 2008). Therefore, it is possible that the presence of unknown retinopathy or other ophthalmic disease may have influenced the strength of the coupling of the rhythm to this environmental Zeitgeber. When analyzing differences between good and poor glycemic control, it was observed that the poor control was exposed to significant less amount of light. We recommend for futures studies to perform a clinical assessment for ophthalmic disease on T2DM in order to better assess the influence of light on these circadian variables.

Some limitations should be considered in this study. First, this is an observational study and no causality can be assumed; moreover, the lack of differences between good and poor control and associations with HbA1c with the circadian variables may be a function of the limited power of the diabetes sample because of the small sample size.

Conclusions

In summary, we have shown that the sleep-wake cycle is disturbed in T2DM when compared to healthy controls and it is mainly characterized by lesser consistency across days of the daily circadian signal, higher rhythm fragmentation and lower rhythm amplitude. Futures approaches may be developed to better investigate which mechanisms are involved in this sleep-wake cycle deregulation in T2DM, also considering the influence of circadian glucose variations throughout the day and meal times on the coupling of the rest-activity rhythm to zeitgeber and rhythm fragmentation.

Acknowledgments: We acknowledge the financial support for this research from the Clinical Research Molecular Laboratory and the Physical Therapy department, School of Allied Health Professions, Loma Linda University.

References

American Diabetes Association. (2003). Screening for Type 2 Diabetes. *Diabetes Care.* 26: S21-24.

American Diabetes Association (2012). Standards of Medical Care in Diabetes--2012. *Diabetes Care.* 35 (Suppl 1): S11-63.

American Sleep Disorders Association. (1995). Practice parameters for the use of actigraphy in the clinical assessment of sleep disorders. American Sleep Disorders Association. *Sleep.* 18: 285-287.

Bass J, Takahashi J S. (2010). Circadian integration of metabolism and energetics. *Science.* 330: 1349-1354.

Boden G, Chen X, Polansky M. (1999). Disruption of circadian insulin secretion is associated with reduced glucose uptake in first-degree relatives of patients with type 2 diabetes. *Diabetes.* 48: 2182-2188.

Buijs R M, Scheer F A, Kreier F, Yi C, Bos N, Goncharuk V D, Kalsbeek A. (2006). Organization of circadian functions: interaction with the body. *Prog Brain Res.* 153: 341-360.

Calogiuri G, Weydahl A, Carandente F. (2013). Methodological issues for studying the rest-activity cycle and sleep disturbances: a chronobiological approach using actigraphy data. *Biol Res Nurs.* 15: 5-12.

Carneiro B, Fernandes D, Medeiros C, Diniz N, Araujo J. (2012). Daily anticipatory rhythms of behavior and body temperature in response to glucose availability in rats. *Psychology & Neuroscience.* 5.

Carneiro B T, Araujo J F. (2009). The food-entrainable oscillator: a network of interconnected brain structures entrained by humoral signals? *Chronobiol Int.* 26: 1273-1289.

(2012). Food entrainment: major and recent findings. *Front Behav Neurosci.* 6: 83.

Cavalcanti P, Campos T, Araujo J. (2012). Actigraphic analysis of the sleep-wake cycle and physical activity level in patients with stroke: implications for clinical practice. *Chronobiol Int.* 29: 1267-1272.

(2013). Circadian and homeostatic changes of sleep-wake and quality of life in stroke: Implications for neurorehabilitation. *NeuroRehabilitation.* 32: 337-343.

Chiasson J L, Josse R G, Gomis R, Hanefeld M, Karasik A, Laakso M. (2003). Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA.* 290: 486-494.

Contreras S F S, Malcarne V L, Ingram R E and Vaccarino V R. (2004). Reliability and Validity of the Beck Depression and Anxiety Inventories in Caucasian Americans and Latinos. *Hispanic Journal of Behavioral Sciences.* 26: 446-462.

Department of Health and Human Services C f D C a P. (2011). Centers for Disease Control and Prevention. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Atlanta, GA: U.S.

Dijk D J, von Schantz M. (2005). Timing and consolidation of human sleep, wakefulness, and performance by a symphony of oscillators. *J Biol Rhythms.* 20: 279-290.

Gaede P, Vedel P, Larsen N, Jensen G V, Parving H H, Pedersen O. (2003). Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med.* 348: 383-393.

Gangwisch J E. (2009). Epidemiological evidence for the links between sleep, circadian rhythms and metabolism. *Obes Rev.* 10 (Suppl 2): 37-45.

Gottlieb D J, Punjabi N M, Newman A B, Resnick H E, Redline S, Baldwin C M, Nieto F J. (2005). Association of sleep time with diabetes mellitus and impaired glucose tolerance. *Arch Intern Med.* 165: 863-867.

Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M, Shibata S. (2001). Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes Cells.* 6: 269-278.

Hartemann-Heurtier A, Sultan S, Sachon C, Bosquet F, Grimaldi A. (2001). How type 1 diabetic patients with good or poor glycemic control cope with diabetes-related stress. *Diabetes Metab.* 27: 553-559.

Huang Y L, Liu R Y, Wang Q S, Van Someren E J, Xu H, Zhou J N. (2002). Age-associated difference in circadian sleep-wake and rest-activity rhythms. *Physiol Behav.* 76: 597-603.

International Expert Committee. (2009). International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care.* 32: 1327-1334.

Jean-Louis G, Zizi F, Lazzaro D R, Wolintz A H. (2008). Circadian rhythm dysfunction in glaucoma: A hypothesis. *J Circadian Rhythms.* 6: 1.

Juarez D T, Sentell T, Tokumaru S, Goo R, Davis J W, Mau M M. (2012). Factors associated with poor glycemic control or wide glycemic variability among diabetes patients in Hawaii, 2006-2009. *Prev Chronic Dis.* 9: 120065.

Lamond N, Tiggemann M, Dawson D. (2000). Factors predicting sleep disruption in Type II diabetes. *Sleep.* 23: 415-416.

Laposky A D, Bass J, Kohsaka A, Turek F W. (2008). Sleep and circadian rhythms: key components in the regulation of energy metabolism. *FEBS Lett.* 582: 142-151.

Levendowski D. J OR P D, Carper D. L, Berka C, Westbrook P. R. (2007). Assessment of Obstructive Sleep Apnea Risk and Severity in Truck Drivers: Validation of a Screening Questionnaire. *Sleep Diagnosis and Therapy.* 2: 20-26.

Mendoza J. (2007). Circadian clocks: setting time by food. *J Neuroendocrinol.* 19: 127-137.

Nakanishi-Minami T, Kishida K, Funahashi T, Shimomura I. (2012). Sleep-wake cycle irregularities in type 2 diabetics. *Diabetol Metab Syndr.* 4: 18.

Plantinga L, Rao M N, Schillinger D. (2012). Prevalence of self-reported sleep problems among people with diabetes in the United States, 2005-2008. *Prev Chronic Dis.* 9: E76.

Richter P, Werner J, Heerlein A, Kraus A, Sauer H. (1998). On the validity of the Beck Depression Inventory. A review. *Psychopathology.* 31: 160-168.

Scheen A J, Byrne M M, Plat L, Leproult R, Van Cauter E. (1996). Relationships between sleep quality and glucose regulation in normal humans. *Am J Physiol.* 271: E261-270.

Spiegel K, Tasali E, Leproult R, Van Cauter E. (2009). Effects of poor and short sleep on glucose metabolism and obesity risk. *Nat Rev Endocrinol.* 5: 253-261.

Stephan F K. (2002). The "other" circadian system: food as a Zeitgeber. *J Biol Rhythms.* 17: 284-292.

Stokkan K A, Yamazaki S, Tei H, Sakaki Y, Menaker M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science.* 291: 490-493.

Tsujimura T, Matsuo Y, Keyaki T, Sakurada K, Imanishi J. (2009). Correlations of sleep disturbance with the immune system in type 2 diabetes mellitus. *Diabetes Res Clin Pract.* 85: 286-292.

Turek F W, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen D R, Eckel R H, Takahashi J S, Bass J. (2005). Obesity and metabolic syndrome in circadian Clock mutant mice. *Science.* 308: 1043-1045.

UK Prospective Diabetes Study Group. (1998a). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes

(UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 352: 854-865.

(1998b). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 352: 837-853.

Van Cauter E, Blackman J D, Roland D, Spire J P, Refetoff S, Polonsky K S. (1991). Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. *J Clin Invest.* 88: 934-942.

Van Cauter E, Holmback U, Knutson K, Leproult R, Miller A, Nedeltcheva A, Pannain S, Penev P, Tasali E, Spiegel K. (2007). Impact of sleep and sleep loss on neuroendocrine and metabolic function. *Horm Res.* 67 (Suppl 1): 2-9.

van Dam R M. (2003). The epidemiology of lifestyle and risk for type 2 diabetes. *Eur J Epidemiol.* 18: 1115-1125.

Van Someren E J, Hagebeuk E E, Lijzenga C, Scheltens P, de Rooij S E, Jonker C, Pot A M, Mirmiran M, Swaab D F. (1996). Circadian rest-activity rhythm disturbances in Alzheimer's disease. *Biol Psychiatry.* 40: 259-270.

Van Someren E J, Kessler A, Mirmiran M, Swaab D F. (1997a). Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. *Biol Psychiatry.* 41: 955-963.

Van Someren E J, Lijzenga C, Mirmiran M, Swaab D F. (1997b). Long-term fitness training improves the circadian rest-activity rhythm in healthy elderly males. *J Biol Rhythms.* 12: 146-156.

Whitehead D L, Davies A D, Playfer J R, Turnbull C J. (2008). Circadian rest-activity rhythm is altered in Parkinson's disease patients with hallucinations. *Mov Disord.* 23: 1137-1145.

Woon P Y, Kaisaki P J, Braganca J, Bihoreau M T, Levy J C, Farrall M, Gauguier D. (2007). Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. *Proc Natl Acad Sci U S A.* 104: 14412-14417.

Wu T, ZhuGe F, Sun L, Ni Y, Fu O, Gao G, Chen J, Kato H, Fu Z. (2012). Enhanced effect of daytime restricted feeding on the circadian rhythm of streptozotocin-induced type 2 diabetic rats. *Am J Physiol Endocrinol Metab.* 302: E1027-1035.

Young M E, Bray M S. (2007). Potential role for peripheral circadian clock dyssynchrony in the pathogenesis of cardiovascular dysfunction. *Sleep Med.* 8: 656-667.

CHAPTER THREE

CIRCADIAN AND HOMEOSTATIC CONTROL OF THE SLEEP-WAKE CYCLE IN TYPE 2 DIABETES CORRELATED WITH SALIVARY MELATONIN

By [1]Paula Regina Aguiar Cavalcanti, DSc, PT; [1,2]Lee Berk, DrPH, MPH, CLS; [3]Noha Daher, DrPH, MSPH; [4]Tania Campus, Phd, PT; [4]Araujo, John, Phd, MD; [1]Jerrold S. Petrofsky, Phd, J.D.; [1]Everett Lohman, DSc, PT, OCS

[1]Dept. of Physical Therapy, School of Allied Health Professions, Loma Linda University

[2]Dept. Pathology and Humans Anatomy, School of Medicine, Loma Linda University

[3]Dept. of Computing, Research and Statistics, School of Allied Health Professions, Loma Linda University

[4] Neurobiology and Biological Rhythm Laboratory, School of Psychobiology, Federal University of Rio Grande do Norte

Abstract

The purpose of this study was to assess the changes in the circadian and homeostatic control of the sleep-wake cycle in type 2 diabetes mellitus (T2DM) and correlate the findings with salivary melatonin levels. The study cohorts were 21 subjects with a diagnosis of T2DM of more than one year and 21 healthy subjects age and gender matched. Subjects were assessed by the Beck's Depression Inventory (BDI), the Apnea Risk Evaluation System (ARES) questionnaire, hemoglobin A1c (HbA1c), actigraphy and salivary melatonin levels. Melatonin levels at bed time were significantly lower in diabetic subjects than healthy controls (11.7±7.2 pg/mL vs. 24.1±10.8pg/mL; $p<.01$). Actigraphic analysis during the wake phase showed that diabetic subjects had lower levels of activity ($p=.02$). Additionally, there was a significant decrease in sleep duration ($p=.03$), efficiency ($p=.02$); and higher activity counts during the sleep phase ($p=.02$) in the diabetic group when compared to controls. Sleep efficiency was significantly correlated with melatonin collected two hours before bed time ($r=.61$; $p=.047$). Additionally, there were significant inverse relationships between melatonin collected at two hours before bed time and latency ($r=-.87$; $p=.001$), wake after sleep onset ($r=-.69$; $p=.02$) and nocturnal activity ($r=-.67$; $p=.03$). Latency was inversely correlated with melatonin collected at bed time ($r=-.69$; $p=.02$). These composite findings suggest that T2DM presents disturbances in the homeostatic drive to sleep. Moreover, in addition to lower melatonin levels, the decrease in the amplitude of the activity rhythm may also be involved in circadian alterations of the sleep-wake cycle.

Key words: type 2 diabetes, sleep-wake cycle, melatonin, HbA1c, actigraphy

Introduction

Among the American diabetic population, sleep complaints are of high incidence and importance (Plantinga et al., 2012). Sleep disturbances are related to an increased risk of developing chronic systemic conditions, such as hypertension (Gangwisch et al., 2006), obesity and cardiovascular disease (Gangwisch et al., 2005; Gangwisch et al., 2006). Furthermore, sleep disorders have been both identified as a risk factor for developing T2DM, as well as associated with worsening the diabetes condition (Knutson et al., 2006; Tasali et al., 2009).

Sleep-wake cycle disturbances in T2DM have been previously reported (Trento et al., 2008; Nakanishi-Minami et al., 2012; Rajendran et al., 2012). Alterations on daytime sleepiness, sleep duration, sleep efficiency, onset latency and decreased quality of sleep have been described (Trento et al., 2008; Nakanishi-Minami et al., 2012; Rajendran et al., 2012). Despite the literature addressing the sleep disturbances, little is known about the involvement of the circadian and homeostatic controls and the extension of synchronizers influence, such as melatonin, on these processes. The organization of the sleep-wake cycle, according to the two-process model proposed by Borbély (Borbely, 1982), is composed of homeostatic and circadian processes. The homeostatic process (process S) is determined by the previous amount of sleep and wakefulness, in which the sleep pressure is greatest at the beginning of sleep and gradually decreases throughout this phase. The circadian process (process C) organizes sleep and wakefulness alternations over the 24 hours of the day. Its control is expressed by the Circadian System (Dijk & von Schantz, 2005). This system aims to temporally organize the physiological processes of the body by specialized neural structures such as the retinohypothalamic track, which is

responsible for transmitting light signals from the retina to the circadian master clock (the suprachiasmatic nucleus -SCN). The SCN generate signals to the pineal gland to regulate melatonin (N-acetyl-5-methoxytryptamine) production (Lewy et al., 1980; Scheer & Czeisler, 2005). In response to light, the excitatory signal from the SCN and consequent suppression of melatonin production are believed to be related to wakefulness promotion during the day. In the dark phase, this inhibition is released and melatonin is produced, leading to sleep promotion (Lewy et al., 1980; Scheer & Czeisler, 2005; Khullar, 2012). In this context, the endogenous circadian melatonin rhythm is of great importance for synchronization of the sleep-wake cycle (Nagtegaal, 2002).

Melatonin concentration in plasma begins to increase approximately 2 h before habitual bedtime, it peaks around the first hours of the morning and after waking, decreases to levels close to those found during the day (Tzischinsky et al., 1993).

Studies have found decreased melatonin levels in T2DM and it has been proposed that melatonin deficiency may be related to increased risk of developing T2DM (Peschke et al., 2006; Mantele et al., 2012; McMullan et al., 2013). Associations between melatonin alterations and insulin, glucose, lipid metabolism and antioxidant capacity have been reported (Nishida, 2005; Robeva et al., 2008) and an experimental analysis has shown a relationship between impaired melatonin levels and disturbances in insulin sensitivity that can be associated with development of T2DM (Peschke et al., 2006). In addition, the use of exogenous melatonin has been suggested as an adjunct to improve glucose control and diabetic complications in T2DM (Garfinkel et al., 2011).

Since there seems to be a complex interaction involving alterations in melatonin production, circadian rhythm disturbances, impaired insulin and glucose metabolism and

T2DM; it is important to further investigate alterations in the sleep-wake cycle and melatonin to identify the steps that can be taken to minimize the consequences of these disturbances, and thus assist in improving the health of this population. Therefore, the aim of this study was to assess the changes in the circadian and homeostatic control of the sleep-wake cycle in T2DM and correlate it with melatonin concentration. To our knowledge, this is the first study to further discuss the sleep-wake cycle in T2DM based on the two-process model to assess the circadian and homeostatic controls; furthermore it is the first study to correlate these variables with melatonin concentration in diabetic subjects.

Materials and Methods

Sample

The sample consisted of 25 subjects with diagnosis of T2DM for more than a year and 21 healthy controls matched for gender and age. The exclusion criteria consisted of: Obstructive sleep apnea (OSA), neurological comorbidities such as Parkinson's and Alzheimer's disease, use of antidepressant or any other drug that may affect alertness or drowsiness (e.g. melatonin, antipsychotics, antidepressants, benzodiazepines, muscle relaxants), depression (assessed by Beck's Depression Inventory as at least having "borderline clinical depression"), incorrect use of the actimeter and report of important routine changes while enrolled in the study. Four diabetics were excluded from the study due to improper use of actimeter, classification of "severe depression" by the Beck's Depression Inventory, and self report of sleep apnea.

Procedures

The Loma Linda University Institutional Review Board reviewed and approved the present study. All participants received proper information on the procedures involved in the study and signed the informed consent. Variables measured included: Gender, age, body mass index (BMI) (calculated based on weight in kilograms divided by height in meters squared) and high blood pressure. In addition, the diabetic group was assessed on duration of the diabetes diagnosis, presence of visual complaint, type of diabetes treatment (oral drug or insulin) and glycemic control through hemoglobin A1c (HbA1c) level.

According to the Center for Disease Control and Prevention, the level of HbA1c < 7.5% is defined as good glycaemic control, a level of HbA1c ≥7.5% to ≤9% is considered fair glycemic control, and poor control is classified when HbA1c> 9% (Hartemann-Heurtier et al., 2001). The blood concentration of hemoglobin A1c (HbA1c) was measured using the DCA Vantage^{TM} Analyzer (Siemens®, Malvern, PA, USA). For analysis purposes, the diabetic group was classified as good (< 7.5%) and fair-poor controls (≥7.5%).

Subjects were screened for risk of OSA through apnea risk evaluation system (ARES) questionnaire. The questionnaire consists of 5 sections including: 1) demographic data, 2) diagnosis of diseases associated with risk for OSA (i.e., high blood pressure, heart disease, diabetes, or stroke), 3) sleepiness, and 4) frequency of snoring or waking up choking, and 5) having been told about stop breathing during sleep. Scores were classified as low, high and very high risk for OSA. Scoring between 4 and 5 is

considered low risk, 6 to 10 high risk and 11 or more, very high risk (Levendowski D. J OR, 2007).

Eleven subjects from the diabetic group and ten subjects from the control group were selected to perform the melatonin analysis. Melatonin was assessed through saliva. The melatonin collection was collected two hours before bed time, immediately before bed time and as wake up in the following morning. In order to analyze melatonin, we used amber-colored vials and the samples were shipped to Salimetrics® (State College, PA, USA) for analysis. A pamphlet with instructions of how to perform the salivary collection was given to subjects including: Collecting saliva in a dim light place, finishing meals or snacks at least 30 min prior to the saliva collection; avoiding eating bananas and chocolate on the collection day; drinking water up to 10 min before each sample collection; no tooth brushing with toothpaste during the sampling period; avoiding drinking coffee, cola and alcohol at least 4 hours prior to the collection; and avoiding taking aspirin or ibutrofen during the collection day (Pandi-Perumal et al., 2007).

Actigraphy was applied to assess the sleep-wake pattern using the Actiwatch 2 (Philips Respironics, Andover, MA, USA). This device was placed on participants' non-dominant wrist. The actimeter was employed over 7 days; and analysis used records from 6 completes days. Activity counts were recorded every minute and actigraphy recordings were accompanied by self-reporting sleep log. The device contained a button to mark events, and participants were instructed to push this button when going to sleep and waking up in the morning. They were also asked not to remove the device during this collection period. Parameters of the sleep-wake cycle were classified in variables of the

wake and sleep phases. Variables of the wake phase were: sum of all valid physical activity counts in average, average activity counts (the average of all valid physical activity counts during wake time), and immobile time. Variables of the sleep phase were the following: sleep duration (amount of time between sleep onset and waking); sleep latency (time elapsed between going to bed and sleep onset), wake after sleep onset-WOSA (amount of time classified as awake during the sleep time) and average activity counts (the average of all valid physical activity counts during sleep time).

Statistical Analysis

Data analysis was performed using SPSS 21.0 software (Armonk, NY, USA). The Kolmogorov-Smirnov test was used to examine the distribution of the continuous variables. The independent *t*-test was used to compare groups in regards to mean age, BMI, ARES score and BDI, as well as, melatonin concentrations, the differences in the wake and sleep variables, mean light exposition per minute and average of all light in Lux. Analysis of covariance was conducted to control for risk of OSA. The Spearman's and Pearson's correlation tests were used to examine the intra-group correlation among wake and sleep variables, melatonin concentration, and HbA1c. The chi-square Fisher's exact test was used to compare proportions of males and females and the presence of hypertension by group. Comparisons between good control and poor control were assessed using Mann-Whitney U test. The level of significance was set at $p<.05$. Actimetry variables were analyzed by Respironics Actiware 5.70.1 software (Philips Respironics, Andover, MA, USA).

Results

There were no significant differences in gender, weight, height and BMI between the diabetic and the control group (Table 1). Also, there was no significant difference in the depression scale measured by BDI (Diabetics 5.7 ± 5.8 vs. Controls 3.5 ± 3.4; p=.13). In regards to the risk for obstructive sleep apnea, the groups were significantly different (p=.009). The diabetic group had higher scores indicating an increased risk for OSA (5.8 ± 2.7 vs. 3.4 ± 2.7; p=.009).

Table 1 - Mean±SD of subjects'characteristics according to group.

Variables	Diabetic Group	Control Group	*p value*
n	21	21	
Gender [a] (%) Male Female	38.1 61.9	42.9 57.1	.50
Age[b] (years)	59.0 ± 8.6	59.5 ± 8.5	.68
Weight[b] (Kg)	77.0 ± 15.0	79.0± 15.3	.33
Height[b] (m)	1.7 ± .1	1.7 ± .1	.91
BMI[b] (kg/m^2)	27.6 ± 7.2	28.8± 4.3	.21
High blood pressure[a] (%)	85.7	14.3	<.01*
Duration of disease (years)	11.7 ± 9.6		
HbA1c[b] (%)	7.7 ± 1.4		
Diabetes treatment (%) Oral drug Insulin	 61.9 38.1		

n, number of subjects per group
[a]Fisher's test comparisons
[b]Independent test comparisons
*significant difference (p<.05)

Salivary melatonin concentrations were measured 2 hours before bed time, at bed time and at wake time in the following morning. There was a significant difference in mean melatonin concentrations at bed time between diabetic subjects and healthy controls (11.7 ± 7.2pg/ml vs. 24.1 ± 10.8pg/ml; p<.01). Additionally, when normalized for baseline and then analyzed for percent change in melatonin level, there was a significant difference between groups between the sample collected two hours before bed time and the sample collected at bed time (31.9 ± 62.7 vs. 174.3 ± 193.3; p=.048) (Fig. 1).

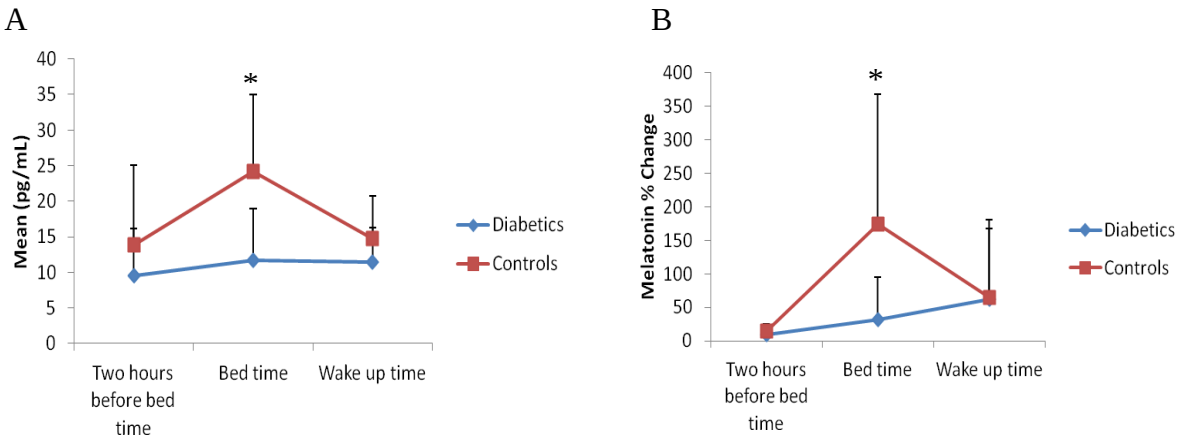


Figure1: A, mean±SD melatonin concentrations in diabetic and control groups; B, mean±SD melatonin percent change in melatonin level in diabetic and control groups. *p<.05

Analysis on actigraphic variables showed that, compared to controls, the diabetic group presented a significant lower level of activity during the wake phase. The sum of all valid physical activity counts (average total activity count) and the average of the activity counts were significantly lower in the diabetic group than in the control group (p=.02 and p=.02 respectively) (Table 2). Moreover, diabetics tended to spend more time

immobile while awake, although there was no significant difference between groups (p=.09) (Table 2).

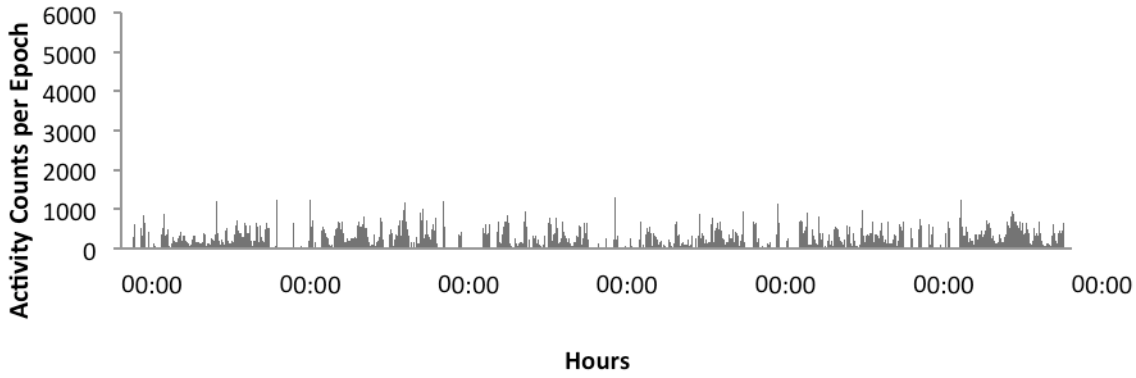
Results on the sleep phase showed that the diabetics slept significantly less hours per night (p=.03), and more inefficiently than the healthy controls (p=.02) (Table 2). On regards to latency, there was no significant difference between groups (p=.60); however, the diabetic group showed higher latency in minutes than controls. There was also a borderline significant difference in wake after sleep onset (WASO) in minutes between groups; the diabetic group spent more time awake after the sleep onset compared to controls (p=.07). In average, the activity counts during sleep was significantly higher in diabetics than in the control group (p=.02). Figure 2 demonstrates the activity pattern during the six days of analysis of a diabetic and a healthy control. It indicates a significant difference between the two groups in relation to total activity (p= .02), with smaller values for diabetics.

Table 2 – Comparison of actigraphic variables in diabetic and control group.

Variables	Diabetic Group		Control Group		
	Mean	SD	Mean	SD	*p*
Wake phase					
Average total activity[a]	219927.3	56702.7	275444.0	91789.6	.02*
Average activity counts[a]	261.8	62.0	325.6	97.4	.02*
Immobile time[a] (min)	132.9	81.7	99.4	34.1	.09
Total Exposure[a] (Lux by minute)	424654.1	263551.6	478923.9	414253.4	.61
Average light[a] (Lux)	477.5	297.2	531.7	431.1	.64
Sleep phase					
Sleep duration[a] (min)	320.9	66.0	363.7	56.2	.03*
Efficiency[a] (%)	68.7	12.4	77.1	9.3	.02*
Latency[a] (min)	16.9	16.2	14.6	11.7	.60
WASO[a,b] (min)	122.9	60.5	89.0	55.9	.07
Average activity counts[a]	19.8	13.8	9.6	5.3	.02*

[a]Independent t test comparisons
[b]WASO = wake after sleep onset
*significant difference ($p<.05$)

A



B

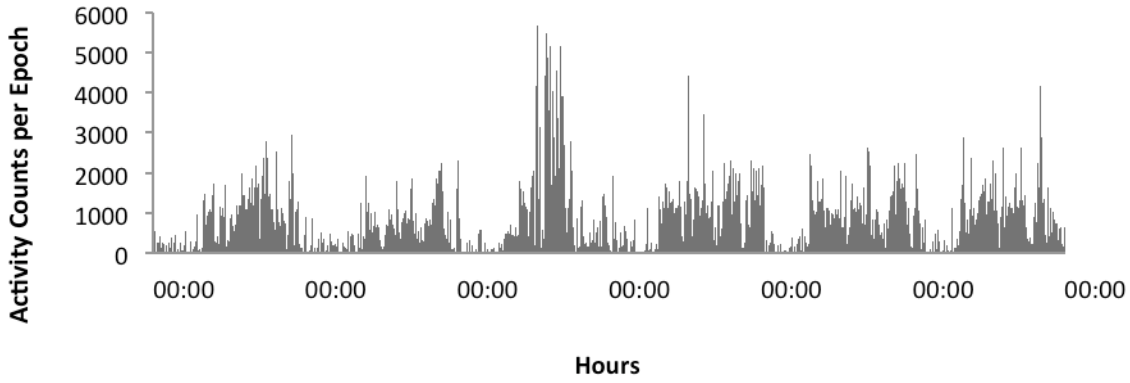


Figure 2 – Activity count per epoch in the six days of analysis. A, activity level of a diabetic subject; and B, activity level of a healthy control.

There were no significant differences between groups in the sum of all light exposition (Lux multiplied by minute) (424654.1 ± 263551.6 vs. 478923.9 ± 414253.4; p=.61) and average of all valid light (Lux) (477.5 ± 297.2 vs. 531.7 ± 431.1; p=.64) during the wake interval (Table 2). Based on the diabetic subjects classified as good (< 7.5%) and fair-poor controls (≥7.5%), the poor controls were significantly less exposed to light per minute than good controls (548322.3 ± 257660.0 vs. 288619.2 ± 203310.3; p=.02); and there was a significantly less amount of all valid light in Lux for the poor controls, when compared to good controls (617.8 ± 298.0 vs. 323.2 ± 217.2; p=.02).

Actigraphic variables comparisons between good and fair-poor controls revealed

that there were no significant differences in these variables (p>.05; data not shown), with the exception to latency, in which the poor controls demonstrated longer latency time in minutes compared to good controls (Min:Max; Median) (0.6: 24.0; 4.6 vs. 3:5, 7.0; 28.4; p=.005).

The corresponding parameters of the sleep-wake cycle of the day of melatonin collection was individually analyzed and correlated with melatonin concentrations. In the diabetic group, there were no significant correlations between melatonin concentrations and variables of the wake phase (p>.05; data not shown). When analyzing correlations with variables of the sleep phase, sleep efficiency was significantly correlated with melatonin collected two hours before bed time (ρ=.61; p=.047). Additionally, there were significant inverse associations between melatonin collected at two hours before bed time and latency (ρ=-.87; p=.001), WASO (ρ=-.69; p=.02) and nocturnal activity (ρ =-.67; p=.03). Also, there was a significant inverse relationship between latency and melatonin collected at bed time (ρ =-.69; p=.02) (Table 3).

Table 3: Correlation coefficients and p values recorded when analyzing the correlation between melatonin concentrations and variables of the sleep phase.

Sleep phase variables	M1		M2		M3	
	ρ	*p*	ρ	*p*	ρ	*p*
Efficiency (%)[a]	.61	.047*	.35	.30	.57	.06
Latency (min)[b]	-.87	.001*	-.69	.02*	-.54	.09
WASO[a]	-.69	.02*	-.52	.10	-.60	.05
Average activity counts[a]	-.67	.03*	-.56	.07	-.55	.08

M1, melatonin collected two hours before bed time
M2, melatonin collected at bed time
M3, melatonin collected at wake up in the morning
[a]Pearson's Correlation
[b]Spearman's Correlation
*significant difference (p<.05)

Discussion

This study found alterations in the homeostatic and circadian controls of the sleep-wake cycle on T2DM that were significantly related to melatonin concentrations. Actigraphic analysis during the wake phase demonstrated that diabetic subjects showed lower daily activity. Additionally, there was a significant decrease in sleep duration and efficiency; and higher activity counts during the sleep phase in the diabetic group when compared to controls. These findings suggest that T2DM presents disturbances in the homeostatic drive to sleep; moreover, in addition to the lower melatonin amplitude observed in these subjects, the decrease in the amplitude of the activity rhythm may also be involved in circadian alterations of the sleep-wake cycle.

Melatonin concentrations were significantly lower at bed time in the diabetic group when compared to controls. Although we did not detect significant differences in melatonin levels between groups when analyzing concentrations at 2 hours before bed time and at wake up in the morning, the diabetic subjects had lower melatonin levels in all three measurements. These findings reveal a possible alteration in the amplitude of the melatonin rhythm; which was previously reported by other studies (Arendt et al., 1982; Tutuncu et al., 2005; Mantele et al., 2012). Tutuncu et al., 2005, observed significant lower values of melatonin and disturbances in melatonin rhythm in T2DM (Tutuncu et al., 2005). These authors suggested that autonomic neuropathy found in diabetic patients is the most important complication with concerning the decreased in melatonin secretion (Tutuncu et al., 2005). A recent study by McMullan et al., 2013, reported that the decrease in melatonin secretion is associated with an increased chance in developing T2DM (McMullan et al., 2013). Some studies have suggested the relationship between

impaired melatonin levels and signaling being involved in the disturbance of insulin sensitivity, and as a consequence, leading to T2DM (Peschke et al., 2006; Mantele et al., 2012).

Melatonin appears to influence the sleep-wake cycle by interaction with the circadian and homeostatic processes. Exogenous melatonin is able to shift the circadian rhythm to earlier or later based on the time of the day that it is provided (process C) (Lewy et al., 1998). On the other hand, melatonin may also influence the homeostatic drive to sleep due to its "hypnotic" property (process S) (Mendelson, 1997). Furthermore, the rapid increase in melatonin in blood is responsible for the abrupt increase in sleep propensity and inhibition of neural activities in the SCN, which suppresses the circadian drive (Lavie, 1997). Similarly to T2DM, studies on Alzheimer disease also observed decrease in melatonin amplitude and irregularities on melatonin patterns (Cardinali et al., 2002). It has been implied that the signal strength to clock resetting by the melatonin rhythm has been disrupted, as well as the internal synchronizing time cue (Cardinali, 2010). Changes in melatonin secretion observed in Alzheimer patients might be responsible for sleep disruption, nightly restlessness and sundowing (Cardinali et al., 2002). In our study, it was not possible to assess the entire melatonin rhythm to better analyze endogenous circadian signaling; however, the decrease in melatonin amplitude may be affecting the circadian and homeostatic processes, influencing the suppression of the circadian drive, and at the same time decreasing the homeostatic drive to sleep. Moreover, these dysfunctions may be related to the sleep disturbances found in our study.

Analysis during the wake phase demonstrated that the level of physical activity in T2DM was significantly lower when compared to the control group. These results

suggest a decrease in the amplitude of the activity rhythm in the diabetic group, most likely due to the increased sedentary behavior. It has been previously reported that lower amplitude might decrease the stimuli sent to structures that regulate the circadian rhythm (Winget et al., 1972), and possibly affecting the circadian drive. Immobilized healthy subjects that were induced to forced hypokinesis by resting in bed resulted in disrupted sleep and reduced amplitude of the circadian body temperature rhythm; and as a consequence, it leaded to dessynchonization with the environment (Winget et al., 1972). The decrease in the amplitude of the rest-activity rhythm was previously identified in Parkinson's disease (Whitehead et al., 2008), Alzheimer (Van Someren et al., 1996) and stroke (Cavalcanti, 2012; Cavalcanti, 2013), but to our knowledge, it has not been reported in T2DM. Physical activity has a dual function that should be further considered for diabetes. It is a synchronizer for the sleep-wake cycle; and it is also important for prevention and treatment of diabetes, mainly due to its potential to reduce insulin resistance (Gill & Malkova, 2006; Ross & Despres, 2009; Zuo et al., 2012). Perhaps the decrease in the activity rhythm observed in T2DM may be one of the factors influencing the disturbances in the circadian function, and in addition, together with the decrease in melatonin concentrations, may further disrupt the glucose and insulin metabolism. In line with this explanation, studies have shown a relationship between the combination of low physical activity and insufficient amount of sleep and insulin resistance and reduced glucose tolerance (Nedeltcheva et al., 2009a; Zuo et al., 2012). Zuo et al., 2012 observed that adequate sleep duration together with different types of physical activity may assist the decrease of insulin resistance (Zuo et al., 2012). Moreover, Bromley et al., 2012 suggested that one of the factors mediating chronic inadequate sleep and metabolic

morbidity is related to physical activity decrease (Bromley et al., 2012). Furthermore, studies have shown associations between exercise and quality of sleep (Paparrigopoulos et al., 2010), sleep restriction and decreased physical activity (Nedeltcheva et al., 2009a; Bromley et al., 2012), insomnia, and decreased physical activity in prediabetic subjects (Chasens & Yang, 2012). Finally, sleep disturbances are considered risk factor for developing T2DM (McMullan et al., 2013). Further analysis should be considered to further assess the influence of physical activity on sleep-wake cycle synchronization as well as on melatonin amplitude, and glucose and insulin metabolism in T2DM.

In regards to the relationship between physical activity and melatonin concentrations, we did not find a significant association. Few studies have assessed the role of physical activity on melatonin levels, but we found no studies analyzing this relationship in T2DM. A study on shift worker nurses reported similar results (Grundy et al., 2009); however, another study found an inverse association between sedentary behavior and physical activity during the biological relevant timeframe (3PM to 7AM) and melatonin levels on urinary samples (McPherson et al., 2011). Although, results on the relationship between melatonin concentrations and physical exercise have been conflicting, growing sources of evidences have demonstrated direct and indirect benefits from melatonin on physical activity and on diabetes. Previous studies indicated that melatonin influences physical performance (Escames et al., 2012), preserves glycogen stores (Kaya et al., 2006), reduces oxidative stress and inflammation in cardiac and skeletal muscle (Escames et al., 2006; Mukherjee et al., 2010), protects against heart damage by acute exercise (Veneroso et al., 2009), has protective effects toward obesity-related metabolic complications (Cardinali et al., 2011), improves glycemic control (Agil

et al., 2012), protects pancreatic b-cells against functional overcharge and, as a consequence, against the development of T2DM (Peschke et al., 2012). Furthermore, a study on T2DM suggested that alteration on melatonin rhythm amplitude may be functionally related to changes in insulin secretion (Mantele et al., 2012). Associations between physical activity and melatonin concentrations on T2DM, as well as the influence of these variables on the circadian and homeostatic drive should be further explored. Futures studies should examine the responses of different levels of physical activity at different times of the day on the circadian rhythm synchronization, while analyzing changes in melatonin concentrations on T2DM.

The sleep phase assessment of diabetic subjects showed that they slept significantly less, with less efficiency and had higher scores of activity counts than healthy controls. These findings suggest they may have a disturbance in the homeostatic drive to sleep. Similar results on these variables were previously reported by Trento et al., 2008 who reported lower sleep maintenance, lower efficiency, and more movement while in bed in the T2DM group (Trento et al., 2008). The authors argued that sleep disturbances found in this population could be related to impaired glucose tolerance and consequences of the disease itself (Trento et al., 2008). Tsujimura et al., 2009 also reported increased activity when subjects were in bed and long wake episodes during sleep time. They found an inverse relationship between activity counts while in bed and wake episodes with HbA1c level (Tsujimura et al., 2009). Our study did not find a significant difference between diabetic and controls regarding WOSA. In regards to the relationship between HbA1c and actigraphs parameters, we only found a positive correlation between HbA1c and onset latency. These findings are similar to those

reported by Rajendran et al. who did not find relationships between HbA1c and sleep dysfunction (Rajendran et al., 2012). More studies need to be conducted to assess the correlations between glucose tolerance and sleep-wake cycle in order to better direct therapeutic procedures that might help to achieve a better glycemic control.

The analysis of correlation between melatonin concentration and sleep parameters demonstrated that melatonin collected two hours before bed time was positively correlated with sleep efficiency. Additionally, inverse associations were found between melatonin collected two hours before bed time and WASO and nocturnal activity. Onset latency was found to be inversely correlated with melatonin collected two hours before bed time and at bed time. Similar results on the analysis of correlation between melatonin and sleep parameters have been previously reported. Increased melatonin concentration correlated with decrease onset latency, increase sleep efficiency and decrease nocturnal activity during sleep (Waldhauser et al., 1990; Nunes et al., 2008). A study by Waldhauser et al., 1990 indicated that the administration of exogenous melatonin in young healthy volunteers improves sleep latency, number of awakenings, and sleep efficiency (Waldhauser et al., 1990). Similarly to our findings, this study found no association between melatonin and with total sleep time. Garfinkel et al., 2011 assessing the effect of exogenous administration of melatonin in T2DM with insomnia found improvement on sleep maintenance and glycemic control (Garfinkel et al., 2011). They found improvements in sleep efficiency, WASO, and number of awakenings with prolonged-release melatonin when compared to placebo (Garfinkel et al., 2011). This study supports the use of melatonin to improve sleep maintenance in T2DM with insomnia, also suggesting that exogenous melatonin may also be beneficial to increase

glycemic control (Garfinkel et al., 2011). Based on these findings and on the hypothesis that endogenous melatonin have a direct effect on sleep regulation (Tzischinsky et al., 1993), the decrease in melatonin amplitude observed in T2DM, might be one of the factors influencing the homeostatic control that lead to impairment on sleep maintenance.

Light is the most important synchronizer for the circadian system and directly influences melatonin amplitude (Lewy et al., 1980; Nagtegaal, 2002). High Light intensities (2500 Lux) produce suppression of melatonin in short period of time (Lewy et al., 1980; Pandi-Perumal et al., 2007); bright light exposition during the day may increase melatonin amplitude at night; while light exposure during night time decrease endogenous melatonin and may contribute to the incidence of diabetes (Hikichi et al., 2011). Results of our study indicated no significant differences between groups in mean light exposure and average of total amount of light in Lux recorded during the wake phase. Also, there were no significant correlations between these variables and melatonin concentration in the diabetic group. One of the possible reasons for the lack of correlations might be related to disturbances in the strength of the light signal to reset the clock. Ophthalmic disturbances can impair the synchronization by light and have been previously reported in diabetic subjects as possibly disturbing photic input to the circadian system (Brainard et al., 2001). Hikichi et al., 2011 reported that diabetic patients with retinopathy presented more apparent alteration of melatonin when compared to diabetic subjects without retinopathy (Hikichi et al., 2011). These authors suggested that dysfunctional retinal light perception may be associated with the decrease melatonin levels found in diabetic subjects (Hikichi et al., 2011). Although we have not clinically

assessed presence of retinopathy in our sample, around 76% of our subjects reported having some source of ophthalmic complaint. Possibly, the visual disturbances reported in our sample may also be related to the alteration in melatonin amplitude. In aging process, the decrease in the photic stimuli is related to aging changes in retinal function that can lead to less short-wavelength light stimuli, what is indicated to be imperative for circadian light input (Herljevic et al., 2005). This reduction in the circadian response to light may result in a weaker circadian drive (Cajochen et al., 2006). Perhaps, diabetic subjects with increased photic resistance can benefit from stimuli from other zeitgebers, such as meal time and physical activity to assist the entrainment to changes in the light-dark cycle and compensate the possible decrease in the circadian drive due to ophthalmic dysfunction.

This study has several limitations. First, it is an observational study and we can not draw conclusions on causality. Second, only approximately half of our sample had the melatonin concentrations analyzed, and the collection was done for only one night. Third, it is possible that our subjects did not completely follow our instructions on melatonin collections at home, and it might as well have influenced the results observed. Fourth, because melatonin concentrations were measured only three times, it was not possible to show the complete melatonin rhythm over 24 hours period.

Conclusions

In summary, in this study we found disturbances in the circadian and homeostatic control of the sleep-wake cycle in T2DM. Additionally, we identified associations between salivary melatonin concentration and sleep efficiency, onset latency, WASO and

night activity during sleep phase. Further studies should address whether stimulating other zeitgebers, such as physical activity and meal time, as well as interventions using sleep hygiene, in conjunction with or instead of exogenous melatonin, would alter any of these sleep-wake parameters and therefore compensate the impaired sleep-wake cycle due to altered circadian and homeostatic drives.

Acknowledgments: We acknowledge the financial support for this research from the Clinical Research Molecular Laboratory and the Physical Therapy department, School of Allied Health Professions, Loma Linda University.

References

Agil A, Rosado I, Ruiz R, Figueroa A, Zen N, Fernandez-Vazquez G. (2012). Melatonin improves glucose homeostasis in young Zucker diabetic fatty rats. *J Pineal Res.* 52: 203-210.

Arendt J, Hampton S, English J, Kwasowski P, Marks V. (1982). 24-hour profiles of melatonin, cortisol, insulin, C-peptide and GIP following a meal and subsequent fasting. *Clin Endocrinol (Oxf).* 16: 89-95.

Borbely A A. (1982). A two process model of sleep regulation. *Hum Neurobiol.* 1: 195-204.

Brainard G C, Hanifin J P, Greeson J M, Byrne B, Glickman G, Gerner E, Rollag M D. (2001). Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J Neurosci.* 21: 6405-6412.

Bromley L E, Booth J N, 3rd, Kilkus J M, Imperial J G, Penev P D. (2012). Sleep restriction decreases the physical activity of adults at risk for type 2 diabetes. *Sleep.* 35: 977-984.

Cajochen C, Munch M, Knoblauch V, Blatter K, Wirz-Justice A. (2006). Age-related changes in the circadian and homeostatic regulation of human sleep. *Chronobiol Int.* 23: 461-474.

Cardinali D P, Brusco L I, Liberczuk C, Furio A M. (2002). The use of melatonin in Alzheimer's disease. *Neuro Endocrinol Lett.* 23 Suppl 1: 20-23.

Cardinali D P, Cano P, Jimenez-Ortega V, Esquifino A I. (2011). Melatonin and the metabolic syndrome: physiopathologic and therapeutical implications. *Neuroendocrinology.* 93: 133-142.

Cardinali D P S, P. A. . (2010). Chronophysiology of Melatonin: Therapeutical Implications. *The Open Neuroendocrinology Journal.* 3: 72-84.

Cavalcanti P, Campos, T, Araujo, J. (2012). Actigraphic analysis of the sleep-wake cycle and physical activity level in patients with stroke: implications for clinical practice. *Chronobiol Int.* 29: 1267-1272.

Cavalcanti P, Campos, T, Araujo, J. (2013). Circadian and homeostatic changes of sleep-wake and quality of life in stroke: Implications for neurorehabilitation. *NeuroRehabilitation.* 32: 337-343.

Chasens E R, Yang K. (2012). Insomnia and physical activity in adults with prediabetes. *Clin Nurs Res.* 21: 294-308.

Dijk D J, von Schantz M. (2005). Timing and consolidation of human sleep, wakefulness, and performance by a symphony of oscillators. *J Biol Rhythms.* 20: 279-290.

Escames G, Lopez L C, Tapias V, Utrilla P, Reiter R J, Hitos A B, Leon J, Rodriguez M I, Acuna-Castroviejo D. (2006). Melatonin counteracts inducible mitochondrial nitric oxide synthase-dependent mitochondrial dysfunction in skeletal muscle of septic mice. *J Pineal Res.* 40: 71-78.

Escames G, Ozturk G, Bano-Otalora B, Pozo M J, Madrid J A, Reiter R J, Serrano E, Concepcion M, Acuna-Castroviejo D. (2012). Exercise and melatonin in humans: reciprocal benefits. *J Pineal Res.* 52: 1-11.

Gangwisch J E, Heymsfield S B, Boden-Albala B, Buijs R M, Kreier F, Pickering T G, Rundle A G, Zammit G K, Malaspina D. (2006). Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. *Hypertension.* 47: 833-839.

Gangwisch J E, Malaspina D, Boden-Albala B, Heymsfield S B. (2005). Inadequate sleep as a risk factor for obesity: analyses of the NHANES I. *Sleep.* 28: 1289-1296.

Garfinkel D, Zorin M, Wainstein J, Matas Z, Laudon M, Zisapel N. (2011). Efficacy and safety of prolonged-release melatonin in insomnia patients with diabetes: a randomized, double-blind, crossover study. *Diabetes Metab Syndr Obes.* 4: 307-313.

Gill J M, Malkova D. (2006). Physical activity, fitness and cardiovascular disease risk in adults: interactions with insulin resistance and obesity. *Clin Sci (Lond).* 110: 409-425.

Grundy A, Sanchez M, Richardson H, Tranmer J, Borugian M, Graham C H, Aronson K J. (2009). Light intensity exposure, sleep duration, physical activity, and biomarkers of melatonin among rotating shift nurses. *Chronobiol Int.* 26: 1443-1461.

Hartemann-Heurtier A, Sultan S, Sachon C, Bosquet F, Grimaldi A. (2001). How type 1 diabetic patients with good or poor glycemic control cope with diabetes-related stress. *Diabetes Metab.* 27: 553-559.

Herljevic M, Middleton B, Thapan K, Skene D J. (2005). Light-induced melatonin suppression: age-related reduction in response to short wavelength light. *Exp Gerontol.* 40: 237-242.

Hikichi T, Tateda N, Miura T. (2011). Alteration of melatonin secretion in patients with type 2 diabetes and proliferative diabetic retinopathy. *Clin Ophthalmol.* 5: 655-660.

Kaya O, Gokdemir K, Kilic M, Baltaci A K. (2006). Melatonin supplementation to rats subjected to acute swimming exercise: Its effect on plasma lactate levels and relation with zinc. *Neuro Endocrinol Lett.* 27: 263-266.

Khullar A. (2012). The Role of Melatonin in the Circadian Rhythm Sleep-Wake Cycle. *Psychiatric Times.* 29.

Knutson K L, Ryden A M, Mander B A, Van Cauter E. (2006). Role of sleep duration and quality in the risk and severity of type 2 diabetes mellitus. *Arch Intern Med.* 166: 1768-1774.

Lavie P. (1997). Melatonin: role in gating nocturnal rise in sleep propensity. *J Biol Rhythms.* 12: 657-665.

Levendowski D. J OR P D, Carper D. L, Berka C, Westbrook P. R. (2007). Assessment of Obstructive Sleep Apnea Risk and Severity in Truck Drivers: Validation of a Screening Questionnaire. *Sleep Diagnosis and Therapy.* 2: 20-26.

Lewy A J, Bauer V K, Ahmed S, Thomas K H, Cutler N L, Singer C M, Moffit M T, Sack R L. (1998). The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. *Chronobiol Int.* 15: 71-83.

Lewy A J, Wehr T A, Goodwin F K, Newsome D A, Markey S P. (1980). Light suppresses melatonin secretion in humans. *Science.* 210: 1267-1269.

Mantele S, Otway D T, Middleton B, Bretschneider S, Wright J, Robertson M D, Skene D J, Johnston J D. (2012). Daily rhythms of plasma melatonin, but not plasma leptin or leptin mRNA, vary between lean, obese and type 2 diabetic men. *PLoS One.* 7: e37123.

McMullan C J, Schernhammer E S, Rimm E B, Hu F B, Forman J P. (2013). Melatonin secretion and the incidence of type 2 diabetes. *JAMA.* 309: 1388-1396.

McPherson M, Janssen I, Grundy A, Tranmer J, Richardson H, Aronson K J. (2011). Physical activity, sedentary behavior, and melatonin among rotating shift nurses. *J Occup Environ Med.* 53: 716-721.

Mendelson W B. (1997). Efficacy of melatonin as a hypnotic agent. *J Biol Rhythms.* 12: 651-656.

Mukherjee D, Roy S G, Bandyopadhyay A, Chattopadhyay A, Basu A, Mitra E, Ghosh A K, Reiter R J, Bandyopadhyay D. (2010). Melatonin protects against isoproterenol-induced myocardial injury in the rat: antioxidative mechanisms. *J Pineal Res.* 48: 251-262.

Nagtegaal J E (2002). Chronobiological, clinical and pharmacological aspects of melatonin in human circadian rhythm dysfunction. *Treatise on pineal gland and*

melatonin. C Haldar, M Singaravel and S K Maitra. Enfield, NH, Science Publishers, Inc.**:** 461–489.

Nakanishi-Minami T, Kishida K, Funahashi T, Shimomura I. (2012). Sleep-wake cycle irregularities in type 2 diabetics. *Diabetol Metab Syndr.* 4: 18.

Nedeltcheva A V, Kessler L, Imperial J, Penev P D. (2009). Exposure to recurrent sleep restriction in the setting of high caloric intake and physical inactivity results in increased insulin resistance and reduced glucose tolerance. *J Clin Endocrinol Metab.* 94: 3242-3250.

Nishida S. (2005). Metabolic effects of melatonin on oxidative stress and diabetes mellitus. *Endocrine.* 27: 131-136.

Nunes D M, Mota R M, Machado M O, Pereira E D, Bruin V M, Bruin P F. (2008). Effect of melatonin administration on subjective sleep quality in chronic obstructive pulmonary disease. *Braz J Med Biol Res.* 41: 926-931.

Pandi-Perumal S R, Smits M, Spence W, Srinivasan V, Cardinali D P, Lowe A D, Kayumov L. (2007). Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* 31: 1-11.

Paparrigopoulos T, Tzavara C, Theleritis C, Soldatos C, Tountas Y. (2010). Physical activity may promote sleep in cardiac patients suffering from insomnia. *Int J Cardiol.* 143: 209-211.

Peschke E, Frese T, Chankiewitz E, Peschke D, Preiss U, Schneyer U, Spessert R, Muhlbauer E. (2006). Diabetic Goto Kakizaki rats as well as type 2 diabetic patients show a decreased diurnal serum melatonin level and an increased pancreatic melatonin-receptor status. *J Pineal Res.* 40: 135-143.

Peschke E, Hofmann K, Ponicke K, Wedekind D, Muhlbauer E. (2012). Catecholamines are the key for explaining the biological relevance of insulin-melatonin antagonisms in type 1 and type 2 diabetes. *J Pineal Res.* 52: 389-396.

Plantinga L, Rao M N, Schillinger D. (2012). Prevalence of self-reported sleep problems among people with diabetes in the United States, 2005-2008. *Prev Chronic Dis.* 9: E76.

Rajendran A, Parthsarathy S, Tamilselvan B, Seshadri K G, Shuaib M. (2012). Prevalence and correlates of disordered sleep in southeast asian indians with type 2 diabetes. *Diabetes Metab J.* 36: 70-76.

Robeva R, Kirilov G, Tomova A, Kumanov P. (2008). Melatonin-insulin interactions in patients with metabolic syndrome. *J Pineal Res.* 44: 52-56.

Ross R, Despres J P. (2009). Abdominal obesity, insulin resistance, and the metabolic syndrome: contribution of physical activity/exercise. *Obesity (Silver Spring).* 17 Suppl 3: S1-2.

Scheer F A, Czeisler C A. (2005). Melatonin, sleep, and circadian rhythms. *Sleep Med Rev.* 9: 5-9.

Tasali E, Leproult R, Spiegel K. (2009). Reduced sleep duration or quality: relationships with insulin resistance and type 2 diabetes. *Prog Cardiovasc Dis.* 51: 381-391.

Trento M, Broglio F, Riganti F, Basile M, Borgo E, Kucich C, Passera P, Tibaldi P, Tomelini M, Cavallo F, Ghigo E, Porta M. (2008). Sleep abnormalities in type 2 diabetes may be associated with glycemic control. *Acta Diabetol.* 45: 225-229.

Tsujimura T, Matsuo Y, Keyaki T, Sakurada K, Imanishi J. (2009). Correlations of sleep disturbance with the immune system in type 2 diabetes mellitus. *Diabetes Res Clin Pract.* 85: 286-292.

Tutuncu N B, Batur M K, Yildirir A, Tutuncu T, Deger A, Koray Z, Erbas B, Kabakci G, Aksoyek S, Erbas T. (2005). Melatonin levels decrease in type 2 diabetic patients with cardiac autonomic neuropathy. *J Pineal Res.* 39: 43-49.

Tzischinsky O, Shlitner A, Lavie P. (1993). The association between the nocturnal sleep gate and nocturnal onset of urinary 6-sulfatoxymelatonin. *J Biol Rhythms.* 8: 199-209.

van Someren E J, Hagebeuk E E, Lijzenga C, Scheltens P, de Rooij S E, Jonker C, Pot A M, Mirmiran M, Swaab D F. (1996). Circadian rest-activity rhythm disturbances in Alzheimer's disease. *Biol Psychiatry.* 40: 259-270.

Veneroso C, Tunon M J, Gonzalez-Gallego J, Collado P S. (2009). Melatonin reduces cardiac inflammatory injury induced by acute exercise. *J Pineal Res.* 47: 184-191.

Waldhauser F, Saletu B, Trinchard-Lugan I. (1990). Sleep laboratory investigations on hypnotic properties of melatonin. *Psychopharmacology (Berl).* 100: 222-226.

Whitehead D L, Davies A D, Playfer J R, Turnbull C J. (2008). Circadian rest-activity rhythm is altered in Parkinson's disease patients with hallucinations. *Mov Disord.* 23: 1137-1145.

Winget C M, Vernikos-Danellis J, Cronin S E, Leach C S, Rambaut P C, Mack P B. (1972). Circadian rhythm asynchrony in man during hypokinesis. *J Appl Physiol.* 33: 640-643.

Zuo H, Shi Z, Yuan B, Dai Y, Hu G, Wu G, Hussain A. (2012). Interaction between physical activity and sleep duration in relation to insulin resistance among non-diabetic Chinese adults. *BMC Public Health.* 12: 247.

CHAPTER FOUR

DISCUSSION

According to the nonparametric methodology approach, it was found that diabetics presented lower IS (p=.03), higher IV (p=.046) and lower rhythm amplitude (p=.02) when compared to healthy controls. Similar results were observed by Van Someren et al. (1996) in Alzheimer patients. Based in this study, lower values of IS represents less consistency across days of the daily circadian signal; also, revealing a potential disturbance in the strength of its coupling to stable zeitgebers. In regards to IV, the diabetic group showed significantly higher values when compared to controls and higher values of IV are indicative of more fragmented rhythm. Similar results were observed in Parkinson's disease by Whitehead et al. (2008). This study reported that on the Parkinson's group, it was found a more fragmented rest-activity rhythm with constants transitions from high to low periods of activity. In T2DM, studies have shown that nocturia is a significant variable that is positively correlated with difficulty to maintain sleep (Lamond et al., 2000) and it might as well contribute to increase IV in the diabetic population. Lower peak of physical activity and lower rhythm amplitude were also observed in Parkinson's disease and stroke. Motor alterations on stroke patients could be influencing the expression of this variable (Cavalcanti, 2012). Similarly, we observed lower levels of physical activity and low rhythm amplitude in the diabetic group. This finding could be related to the increased sedentary activity commonly found in these individuals.

Actigraph studies on T2DM subjects revealed that sleep-wake cycle irregularities are more frequent in diabetic individuals than in the healthy controls. Tsujimura et al. (2009) reported higher scores of night activity in the diabetic group when compared to controls (Tsujimura et al., 2009). Additionally, they reported that higher fasting plasma glucose and HbA1c values were positively correlated with pronounced sleep and circadian rhythm disturbances. Our study also found higher activity level in the diabetic group during night time (measured by L5, related to the least 5 hours of activity, night activity); however, we did not find significant relationships between HbA1c and the circadian variables. Nakanishi-Minami et al., 2012 reported that T2DM subjects tended to go to bed later and wake up later; and had higher daytime sleepiness compared to controls (Nakanishi-Minami et al., 2012). These authors suggested that irregular sleep-wake cycle patters and short and long sleep may enhance dysfunction in the glucose metabolism.

The sleep phase assessment of diabetic subjects showed that they slept significantly less, with less efficiency and had higher scores of activity counts than healthy controls. These findings suggest they may have a disturbance in the homeostatic drive to sleep. Similar results on these variables were previously reported by Trento et al. (Trento et al., 2008). They reported lower sleep maintenance, lower efficiency, and more movement while in bed in the T2DM group (Trento et al., 2008). The authors argued that sleep disturbances found in this population could be related to impaired glucose tolerance and consequences of the disease itself (Trento et al., 2008). Tsujimura et al. (Tsujimura et al., 2009) also reported increased activity when subjects were in bed and long wake episodes during sleep time. They found an inverse relationship between activity counts

while in bed and wake episodes with HbA1c level (Tsujimura et al., 2009). Our study did not find a significant difference between diabetic and controls regarding WOSA. In regards to the relationship between HbA1c and actigraphs parameters, we only found a positive correlation between HbA1c and onset latency. These findings are similar to those reported by Rajendran et al. who did not find relationships between HbA1c and sleep dysfunction (Rajendran et al., 2012). More studies need to be conducted to assess the correlations between glucose tolerance and sleep-wake cycle in order to better direct therapeutic procedures that might help to achieve a better glycemic control.

Sleep duration has been the focus of many studies on sleep disturbances in T2DM. Moreover, it has been suggested that patients with T2DM sleep less than the general population (Buxton et al., 2010). Sleep duration higher than 8 hours or less than 7 hours per night, have a moderate increased risk of all-cause mortality, as well as cardiovascular disease, and developing symptomatic diabetes (Phillips et al., 2006). Results from the Sleep Heart Health Study showed that sleeping 6 hours or less and sleeping 9 hours or more per night have a greater prevalence of T2DM and impaired glucose tolerance when compared to individuals sleeping 7–8 hours per night (Gottlieb et al., 2005). A study by Chasens et al., 2009, reported that the mean sleep duration of the diabetes group was 5.5 hours per night (Chasens et al., 2009). These authors described this group as experiencing chronic sleep deprivation (Chasens et al., 2009). Our study also observed similar results. It was found that the diabetic group slept on average 5.3 hours per night during the seven days of assessment; perhaps also experiencing chronic sleep deprivation. Sleep deprivation has been described as linked with high glucose levels due to reduced glucose metabolism, increased insulin resistance and high cortisol

concentrations (Vgontzas et al., 1999; Gottlieb et al., 2005; Nedeltcheva et al., 2009a; Spiegel et al., 2009; Buxton et al., 2010). Additionally, Sleep disruption may also be associated with diabetes. A study reported that males who slept less than 5 hours per night were twice as likely to develop diabetes than those who slept 7 hours per night (Ayas et al., 2003; Yaggi et al., 2006). In a case-control study of T2DM, short sleep duration and snoring frequency were associated with increased incident of T2DM (McMullan et al., 2013). In our study, we have not found an association between sleep duration and HbA1c; however, experimental analysis on healthy subjects found that restricting sleep to 4h for a period of two or more nights was able to reduce glucose tolerance by 40%, while also reducing acute insulin response to glucose by 30% (Spiegel et al., 1999; Spiegel et al., 2005). Another study showed that even one night of sleep restriction has negative effects on glucose metabolism in healthy individuals (Donga et al., 2010).

In regards to sleep quality, Rajendranl et al., 2012 reported a high percentage of T2DM with poor quality of sleep (PSQI score ≥5) (Rajendran et al., 2012). This finding is in line with our results in which the diabetic group showed significantly poorer quality of sleep compared to controls. Additionally, we found significant correlation between glycemic control and quality of sleep. In a study conducted by Knutson et al., 2006, involving 161 African Americans with T2DM, an association was observed between lower sleep quality and poorer glucose control even after controlling for age, sex, BMI, insulin use, and the presence of major complications of diabetes (Knutson et al., 2006). Tsai et al., 2011, also demonstrated that poor sleep quality is significantly correlated with worse glycemic control in patients with T2DM (Tsai et al., 2012). Similarly, Knutson et

al., 2006 reported that sleep quality and duration were significant predictors of HbA1c. These authors suggested the development of interventions that focus on optimizing sleep duration and quality in order to improve glucose control in patients with T2DM (Knutson et al., 2006).

Melatonin concentrations were significantly lower at bed time in the diabetic group when compared to controls. Although we did not detect significant differences in melatonin levels between groups when analyzing concentrations at 2 hours before bed time and at wake up in the morning, the diabetic subjects had lower melatonin levels in all three measurements. These findings reveal a possible alteration in the amplitude of the melatonin rhythm; which was previously reported by other studies (Arendt et al., 1982; Tutuncu et al., 2005; Mantele et al., 2012). Tutuncu et al. (Tutuncu et al., 2005) observed significant lower values of melatonin and disturbances in melatonin rhythm in T2DM. These authors suggested that autonomic neuropathy found in diabetic patients is the most important complication with concerning the decreased in melatonin secretion (Tutuncu et al., 2005). A recent study by McMullan et al. (McMullan et al., 2013) reported that the decrease in melatonin secretion is associated with an increased chance in developing T2DM. Some studies have suggested the relationship between impaired melatonin levels and signaling being involved in the disturbance of insulin sensitivity, and as a consequence, leading to T2DM (Peschke et al., 2006; Mantele et al., 2012).

Our study observed a strong significant inverse association between quality of sleep and percent change between melatonin collected at bed time and at wake up in the following morning; meaning that as lower the difference between the melatonin before bed time and at awakening, the poorer the quality of sleep. Studies have previously

reported administration of exogenous melatonin and improvement in quality of sleep (Nunes et al., 2008; Luthringer et al., 2009). Garfinkel et al., 2011 using exogenous melatonin found improvements in sleep maintenance assessed through actimetry in diabetic subjects (Garfinkel et al., 2011).

In summary, we have shown that the sleep-wake cycle is disturbed in T2DM when compared to healthy controls and it is mainly characterized by less consistency across days of the daily circadian signal, higher rhythm fragmentation and lower rhythm amplitude. Additionally, we found disturbances in the circadian and homeostatic control of the sleep-wake cycle in T2DM and we identified associations between salivary melatonin concentration and sleep efficiency, onset latency, WASO and night activity during sleep phase.

Limitations and Suggestions for Futures Studies

Some limitations should be considered in this study. First, this is an observational study and no causality can be assumed. Second, the lack of differences between good and poor control and associations with HbA1c with the circadian variables may be a function of the limited power of the diabetes sample because of the small sample size. Third, only approximately half of our sample had the melatonin concentrations analyzed, and the collection was done for only one night. Fourth, it is possible that our subjects did not completely follow our instructions on melatonin collections at home, and it might as well have influenced the results observed. Finally, because melatonin concentrations were measured only three times, it was not possible to show the complete melatonin rhythm over 24 hours period.

Further studies should address whether stimulating other zeitgebers, such as physical activity and meal time, as well as interventions using sleep hygiene, in conjunction with or instead of exogenous melatonin, would alter any of the sleep-wake parameters and therefore compensate the impaired sleep-wake cycle due to altered circadian and homeostatic drives. Moreover, more studies are needed to test if increasing the amplitude of the activity rhythm would result in more regulation of the sleep-wake cycle, while analyzing melatonin amplitude, glucose and insulin metabolism in T2DM.

Futures approaches may also further examine associations between glycemic control and the sleep-wake cycle with approaches that consider the influence of circadian glucose variations throughout the day and meal times on the coupling of the rest-activity rhythm to zeitgeber and rhythm fragmentation.

Perhaps, diabetic subjects with increased photic resistance can benefit from stimuli from other zeitgebers, such as meal time and physical activity to assist the entrainment to changes in the light-dark cycle and compensate the possible decrease in the circadian drive due to ophthalmic dysfunction. Therefore, it is also recommend for futures studies to perform a clinical assessment for ophthalmic disease on T2DM in order to better assess the influence of light on these circadian variables.

CHAPTER FIVE

CONCLUSIONS

The present study examined the sleep-wake cycle variations in T2DM using the nonparametric methodological approach and the Two-process Model Theory to analyze the circadian and homeostatic drives. This study found alterations in the homeostatic and circadian controls of the sleep-wake cycle in T2DM that were significantly related to melatonin concentrations. Actigraphic analysis during the wake phase demonstrated that diabetic subjects showed lower daily activity. The findings revealed that T2DM individuals demonstrate lower IS, indicating a lesser consistency across days of the daily circadian signal; higher IV, showing greater variability within each 24-hour period; and lower peak activity levels, indicated by lower activity rhythm amplitude compared to healthy, age matched subjects. Additionally, there was a significant decrease in sleep duration and efficiency; and higher activity counts during the sleep phase in the diabetic group when compared to controls. These data together suggest that T2DM exhibit a dysfunction in the sleep-wake cycle due to alterations in the circadian function as well as in the homeostatic capacity to maintain sleep. Moreover, in addition to the lower melatonin amplitude observed in these subjects, the decrease in the amplitude of the activity rhythm may also be involved in the circadian alterations of the sleep-wake cycle.

REFERENCES

Agil A, Rosado I, Ruiz R, Figueroa A, Zen N, Fernandez-Vazquez G. (2012). Melatonin improves glucose homeostasis in young Zucker diabetic fatty rats. *J Pineal Res.* 52: 203-210.

American Diabetes Association. (2003). Screening for Type 2 Diabetes. *Diabetes Care.* 26: S21-24.

American Diabetes Association (2010). Summary of revisions for the 2010 Clinical Practice Recommendations. *Diabetes Care.* 33 Suppl 1: S3.

American Diabetes Association (2012). Standards of Medical Care in Diabetes--2012. *Diabetes Care.* 35 (Suppl 1): S11-63.

American Sleep Disorders Association. (1995). Practice parameters for the use of actigraphy in the clinical assessment of sleep disorders. American Sleep Disorders Association. *Sleep.* 18: 285-287.

Arendt J, Hampton S, English J, Kwasowski P, Marks V. (1982). 24-hour profiles of melatonin, cortisol, insulin, C-peptide and GIP following a meal and subsequent fasting. *Clin Endocrinol (Oxf).* 16: 89-95.

Ayas N T, White D P, Al-Delaimy W K, Manson J E, Stampfer M J, Speizer F E, Patel S, Hu F B. (2003). A prospective study of self-reported sleep duration and incident diabetes in women. *Diabetes Care.* 26: 380-384.

Bass J, Takahashi J S. (2010). Circadian integration of metabolism and energetics. *Science.* 330: 1349-1354.

Beersma D G, Gordijn M C. (2007). Circadian control of the sleep-wake cycle. *Physiol Behav.* 90: 190-195.

Boden G, Chen X, Polansky M. (1999). Disruption of circadian insulin secretion is associated with reduced glucose uptake in first-degree relatives of patients with type 2 diabetes. *Diabetes.* 48: 2182-2188.

Booth J N, Bromley L E, Darukhanavala A P, Whitmore H R, Imperial J G, Penev P D. (2012). Reduced physical activity in adults at risk for type 2 diabetes who curtail their sleep. *Obesity (Silver Spring).* 20: 278-284.

Borbely A A. (1982). A two process model of sleep regulation. *Hum Neurobiol.* 1: 195-204.

Borbely A A, Achermann P. (1999). Sleep homeostasis and models of sleep regulation. *J Biol Rhythms.* 14: 557-568.

Brainard G C, Hanifin J P, Greeson J M, Byrne B, Glickman G, Gerner E, Rollag M D. (2001). Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J Neurosci.* 21: 6405-6412.

Brandstaetter R. (2004). Circadian lessons from peripheral clocks: is the time of the mammalian pacemaker up? *Proc Natl Acad Sci U S A.* 101: 5699-5700.

Bromley L E, Booth J N, 3rd, Kilkus J M, Imperial J G, Penev P D. (2012). Sleep restriction decreases the physical activity of adults at risk for type 2 diabetes. *Sleep.* 35: 977-984.

Buijs R M, Scheer F A, Kreier F, Yi C, Bos N, Goncharuk V D, Kalsbeek A. (2006). Organization of circadian functions: interaction with the body. *Prog Brain Res.* 153: 341-360.

Buxton O M, Lee C W, L'Hermite-Baleriaux M, Turek F W, Van Cauter E. (2003). Exercise elicits phase shifts and acute alterations of melatonin that vary with circadian phase. *Am J Physiol Regul Integr Comp Physiol.* 284: R714-724.

Buxton O M, Pavlova M, Reid E W, Wang W, Simonson D C, Adler G K. (2010). Sleep restriction for 1 week reduces insulin sensitivity in healthy men. *Diabetes.* 59: 2126-2133.

Cajochen C, Munch M, Knoblauch V, Blatter K, Wirz-Justice A. (2006). Age-related changes in the circadian and homeostatic regulation of human sleep. *Chronobiol Int.* 23: 461-474.

Calogiuri G, Weydahl A, Carandente F. (2013). Methodological issues for studying the rest-activity cycle and sleep disturbances: a chronobiological approach using actigraphy data. *Biol Res Nurs.* 15: 5-12.

Cardinali D P, Brusco L I, Liberczuk C, Furio A M. (2002). The use of melatonin in Alzheimer's disease. *Neuro Endocrinol Lett.* 23 Suppl 1: 20-23.

Cardinali D P, Cano P, Jimenez-Ortega V, Esquifino A I. (2011). Melatonin and the metabolic syndrome: physiopathologic and therapeutical implications. *Neuroendocrinology.* 93: 133-142.

Cardinali D P S, P. A. . (2010). Chronophysiology of Melatonin: Therapeutical Implications. *The Open Neuroendocrinology Journal.* 3: 72-84.

Carneiro B, Fernandes D, Medeiros C, Diniz N, Araujo J. (2012). Daily anticipatory rhythms of behavior and body temperature in response to glucose availability in rats. *Psychology & Neuroscience.* 5.

Carneiro B T, Araujo J F. (2009). The food-entrainable oscillator: a network of interconnected brain structures entrained by humoral signals? *Chronobiol Int.* 26: 1273-1289.

Carneiro B T, Araujo, J F. (2012). Food entrainment: major and recent findings. *Front Behav Neurosci.* 6: 83.

Cavalcanti P, Campos, T, Araujo, J. (2012). Actigraphic analysis of the sleep-wake cycle and physical activity level in patients with stroke: implications for clinical practice. *Chronobiol Int.* 29: 1267-1272.

Cavalcanti P, Campos, T, Araujo, J. (2013). Circadian and homeostatic changes of sleep-wake and quality of life in stroke: Implications for neurorehabilitation. *NeuroRehabilitation.* 32: 337-343.

Chaput J P, Despres J P, Bouchard C, Tremblay A. (2008). The association between sleep duration and weight gain in adults: a 6-year prospective study from the Quebec Family Study. *Sleep.* 31: 517-523.

Chasens E R, Sereika S M, Weaver T E, Umlauf M G. (2007). Daytime sleepiness, exercise, and physical function in older adults. *J Sleep Res.* 16: 60-65.

Chasens E R, Umlauf M G, Weaver T E. (2009). Sleepiness, physical activity, and functional outcomes in veterans with type 2 diabetes. *Appl Nurs Res.* 22: 176-182.

Chasens E R, Yang K. (2012). Insomnia and physical activity in adults with prediabetes. *Clin Nurs Res.* 21: 294-308.

Chiasson J L, Josse R G, Gomis R, Hanefeld M, Karasik A, Laakso M. (2003). Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA.* 290: 486-494.

Contreras S F S, Malcarne V L, Ingram R E and Vaccarino V R. (2004). Reliability and Validity of the Beck Depression and Anxiety Inventories in Caucasian Americans and Latinos. *Hispanic Journal of Behavioral Sciences.* 26: 446-462.

Department of Health and Human Services C f D C a P. (2011). Centers for Disease Control and Prevention. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Atlanta, GA: U.S.

Dijk D J, von Schantz M. (2005). Timing and consolidation of human sleep, wakefulness, and performance by a symphony of oscillators. *J Biol Rhythms.* 20: 279-290.

Donga E, van Dijk M, van Dijk J G, Biermasz N R, Lammers G J, van Kralingen K W, Corssmit E P, Romijn J A. (2010). A single night of partial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects. *J Clin Endocrinol Metab.* 95: 2963-2968.

Escames G, Lopez L C, Tapias V, Utrilla P, Reiter R J, Hitos A B, Leon J, Rodriguez M I, Acuna-Castroviejo D. (2006). Melatonin counteracts inducible mitochondrial nitric oxide synthase-dependent mitochondrial dysfunction in skeletal muscle of septic mice. *J Pineal Res.* 40: 71-78.

Escames G, Ozturk G, Bano-Otalora B, Pozo M J, Madrid J A, Reiter R J, Serrano E, Concepcion M, Acuna-Castroviejo D. (2012). Exercise and melatonin in humans: reciprocal benefits. *J Pineal Res.* 52: 1-11.

Frye M A, Grunze H, Suppes T, McElroy S L, Keck P E, Jr., Walden J, Leverich G S, Altshuler L L, Nakelsky S, Hwang S, Mintz J, Post R M. (2007). A placebo-controlled evaluation of adjunctive modafinil in the treatment of bipolar depression. *Am J Psychiatry.* 164: 1242-1249.

Gaede P, Vedel P, Larsen N, Jensen G V, Parving H H, Pedersen O. (2003). Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med.* 348: 383-393.

Gangwisch J E. (2009). Epidemiological evidence for the links between sleep, circadian rhythms and metabolism. *Obes Rev.* 10 (Suppl 2): 37-45.

Gangwisch J E, Heymsfield S B, Boden-Albala B, Buijs R M, Kreier F, Pickering T G, Rundle A G, Zammit G K, Malaspina D. (2006). Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. *Hypertension.* 47: 833-839.

Gangwisch J E, Malaspina D, Boden-Albala B, Heymsfield S B. (2005). Inadequate sleep as a risk factor for obesity: analyses of the NHANES I. *Sleep.* 28: 1289-1296.

Garfinkel D, Zorin M, Wainstein J, Matas Z, Laudon M, Zisapel N. (2011). Efficacy and safety of prolonged-release melatonin in insomnia patients with diabetes: a randomized, double-blind, crossover study. *Diabetes Metab Syndr Obes.* 4: 307-313.

Gatfield D, Le Martelot G, Vejnar C E, Gerlach D, Schaad O, Fleury-Olela F, Ruskeepaa A L, Oresic M, Esau C C, Zdobnov E M, Schibler U. (2009). Integration of microRNA miR-122 in hepatic circadian gene expression. *Genes Dev.* 23: 1313-1326.

Gill J M, Malkova D. (2006). Physical activity, fitness and cardiovascular disease risk in adults: interactions with insulin resistance and obesity. *Clin Sci (Lond).* 110: 409-425.

Gottlieb D J, Punjabi N M, Newman A B, Resnick H E, Redline S, Baldwin C M, Nieto F J. (2005). Association of sleep time with diabetes mellitus and impaired glucose tolerance. *Arch Intern Med.* 165: 863-867.

Grundy A, Sanchez M, Richardson H, Tranmer J, Borugian M, Graham C H, Aronson K J. (2009). Light intensity exposure, sleep duration, physical activity, and biomarkers of melatonin among rotating shift nurses. *Chronobiol Int.* 26: 1443-1461.

Hall M H, Muldoon M F, Jennings J R, Buysse D J, Flory J D, Manuck S B. (2008). Self-reported sleep duration is associated with the metabolic syndrome in midlife adults. *Sleep.* 31: 635-643.

Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M, Shibata S. (2001). Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes Cells.* 6: 269-278.

Hartemann-Heurtier A, Sultan S, Sachon C, Bosquet F, Grimaldi A. (2001). How type 1 diabetic patients with good or poor glycemic control cope with diabetes-related stress. *Diabetes Metab.* 27: 553-559.

Hasler G, Buysse D J, Klaghofer R, Gamma A, Ajdacic V, Eich D, Rossler W, Angst J. (2004). The association between short sleep duration and obesity in young adults: a 13-year prospective study. *Sleep.* 27: 661-666.

Hayashino Y, Fukuhara S, Suzukamo Y, Okamura T, Tanaka T, Ueshima H. (2007). Relation between sleep quality and quantity, quality of life, and risk of developing diabetes in healthy workers in Japan: the High-risk and Population Strategy for Occupational Health Promotion (HIPOP-OHP) Study. *BMC Public Health.* 7: 129.

Herljevic M, Middleton B, Thapan K, Skene D J. (2005). Light-induced melatonin suppression: age-related reduction in response to short wavelength light. *Exp Gerontol.* 40: 237-242.

Hikichi T, Tateda N, Miura T. (2011). Alteration of melatonin secretion in patients with type 2 diabetes and proliferative diabetic retinopathy. *Clin Ophthalmol.* 5: 655-660.

Huang Y L, Liu R Y, Wang Q S, Van Someren E J, Xu H, Zhou J N. (2002). Age-associated difference in circadian sleep-wake and rest-activity rhythms. *Physiol Behav.* 76: 597-603.

Imaki M, Hatanaka Y, Ogawa Y, Yoshida Y, Tanada S. (2002). An epidemiological study on relationship between the hours of sleep and life style factors in Japanese factory workers. *J Physiol Anthropol Appl Human Sci.* 21: 115-120.

International Expert Committee. (2009). International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care.* 32: 1327-1334.

Ip M S, Lam B, Ng M M, Lam W K, Tsang K W, Lam K S. (2002). Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med.* 165: 670-676.

Jean-Louis G, Zizi F, Lazzaro D R, Wolintz A H. (2008). Circadian rhythm dysfunction in glaucoma: A hypothesis. *J Circadian Rhythms.* 6: 1.

Jennings J R, Muldoon M F, Hall M, Buysse D J, Manuck S B. (2007). Self-reported sleep quality is associated with the metabolic syndrome. *Sleep.* 30: 219-223.

Juarez D T, Sentell T, Tokumaru S, Goo R, Davis J W, Mau M M. (2012). Factors associated with poor glycemic control or wide glycemic variability among diabetes patients in Hawaii, 2006-2009. *Prev Chronic Dis.* 9: 120065.

Kahn S E. (2003). The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia.* 46: 3-19.

Kashyap S R, Defronzo R A. (2007). The insulin resistance syndrome: physiological considerations. *Diab Vasc Dis Res.* 4: 13-19.

Kaya O, Gokdemir K, Kilic M, Baltaci A K. (2006). Melatonin supplementation to rats subjected to acute swimming exercise: Its effect on plasma lactate levels and relation with zinc. *Neuro Endocrinol Lett.* 27: 263-266.

Khullar A. (2012). The Role of Melatonin in the Circadian Rhythm Sleep-Wake Cycle. *Psychiatric Times.* 29.

Knutson K L, Ryden A M, Mander B A, Van Cauter E. (2006). Role of sleep duration and quality in the risk and severity of type 2 diabetes mellitus. *Arch Intern Med.* 166: 1768-1774.

Kohsaka A, Bass J. (2007). A sense of time: how molecular clocks organize metabolism. *Trends Endocrinol Metab.* 18: 4-11.

Lamond N, Tiggemann M, Dawson D. (2000). Factors predicting sleep disruption in Type II diabetes. *Sleep.* 23: 415-416.

Laposky A D, Bass J, Kohsaka A, Turek F W. (2008). Sleep and circadian rhythms: key components in the regulation of energy metabolism. *FEBS Lett.* 582: 142-151.

Lauderdale D S, Knutson K L, Yan L L, Liu K, Rathouz P J. (2008). Self-reported and measured sleep duration: how similar are they? *Epidemiology.* 19: 838-845.

Lavie P. (1997). Melatonin: role in gating nocturnal rise in sleep propensity. *J Biol Rhythms.* 12: 657-665.

Levendowski D. J OR P D, Carper D. L, Berka C, Westbrook P. R. (2007). Assessment of Obstructive Sleep Apnea Risk and Severity in Truck Drivers: Validation of a Screening Questionnaire. *Sleep Diagnosis and Therapy.* 2: 20-26.

Lewy A J, Bauer V K, Ahmed S, Thomas K H, Cutler N L, Singer C M, Moffit M T, Sack R L. (1998). The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. *Chronobiol Int.* 15: 71-83.

Lewy A J, Wehr T A, Goodwin F K, Newsome D A, Markey S P. (1980). Light suppresses melatonin secretion in humans. *Science.* 210: 1267-1269.

Liu X, Uchiyama M, Kim K, Okawa M, Shibui K, Kudo Y, Doi Y, Minowa M, Ogihara R. (2000). Sleep loss and daytime sleepiness in the general adult population of Japan. *Psychiatry Res.* 93: 1-11.

Luthringer R, Muzet M, Zisapel N, Staner L. (2009). The effect of prolonged-release melatonin on sleep measures and psychomotor performance in elderly patients with insomnia. *Int Clin Psychopharmacol.* 24: 239-249.

Mantele S, Otway D T, Middleton B, Bretschneider S, Wright J, Robertson M D, Skene D J, Johnston J D. (2012). Daily rhythms of plasma melatonin, but not plasma leptin or leptin mRNA, vary between lean, obese and type 2 diabetic men. *PLoS One.* 7: e37123.

McMullan C J, Schernhammer E S, Rimm E B, Hu F B, Forman J P. (2013). Melatonin secretion and the incidence of type 2 diabetes. *JAMA.* 309: 1388-1396.

McPherson M, Janssen I, Grundy A, Tranmer J, Richardson H, Aronson K J. (2011). Physical activity, sedentary behavior, and melatonin among rotating shift nurses. *J Occup Environ Med.* 53: 716-721.

Mendelson W B. (1997). Efficacy of melatonin as a hypnotic agent. *J Biol Rhythms.* 12: 651-656.

Mendoza J. (2007). Circadian clocks: setting time by food. *J Neuroendocrinol.* 19: 127-137.

Miyazaki T, Hashimoto S, Masubuchi S, Honma S, Honma K I. (2001). Phase-advance shifts of human circadian pacemaker are accelerated by daytime physical exercise. *Am J Physiol Regul Integr Comp Physiol.* 281: R197-205.

Mukherjee D, Roy S G, Bandyopadhyay A, Chattopadhyay A, Basu A, Mitra E, Ghosh A K, Reiter R J, Bandyopadhyay D. (2010). Melatonin protects against

isoproterenol-induced myocardial injury in the rat: antioxidative mechanisms. *J Pineal Res.* 48: 251-262.

Nagtegaal J E (2002). Chronobiological, clinical and pharmacological aspects of melatonin in human circadian rhythm dysfunction. *Treatise on pineal gland and melatonin*. C Haldar, M Singaravel and S K Maitra. Enfield, NH, Science Publishers, Inc.**:** 461–489.

Nakajima H, Kaneita Y, Yokoyama E, Harano S, Tamaki T, Ibuka E, Kaneko A, Takahashi I, Umeda T, Nakaji S, Ohida T. (2008). Association between sleep duration and hemoglobin A1c level. *Sleep Med.* 9: 745-752.

Nakanishi-Minami T, Kishida K, Funahashi T, Shimomura I. (2012). Sleep-wake cycle irregularities in type 2 diabetics. *Diabetol Metab Syndr.* 4: 18.

Nedeltcheva A V, Kessler L, Imperial J, Penev P D. (2009a). Exposure to recurrent sleep restriction in the setting of high caloric intake and physical inactivity results in increased insulin resistance and reduced glucose tolerance. *J Clin Endocrinol Metab.* 94: 3242-3250.

Nedeltcheva A V, Kilkus J M, Imperial J, Kasza K, Schoeller D A, Penev P D. (2009b). Sleep curtailment is accompanied by increased intake of calories from snacks. *Am J Clin Nutr.* 89: 126-133.

Nilsson P M, Nilsson J A, Hedblad B, Berglund G. (2001). Sleep disturbance in association with elevated pulse rate for prediction of mortality--consequences of mental strain? *J Intern Med.* 250: 521-529.

Nilsson P M, Roost M, Engstrom G, Hedblad B, Berglund G. (2004). Incidence of diabetes in middle-aged men is related to sleep disturbances. *Diabetes Care.* 27: 2464-2469.

Nishida S. (2005). Metabolic effects of melatonin on oxidative stress and diabetes mellitus. *Endocrine.* 27: 131-136.

Nunes D M, Mota R M, Machado M O, Pereira E D, Bruin V M, Bruin P F. (2008). Effect of melatonin administration on subjective sleep quality in chronic obstructive pulmonary disease. *Braz J Med Biol Res.* 41: 926-931.

O'Connor P J, Youngstedt S D. (1995). Influence of exercise on human sleep. *Exerc Sport Sci Rev.* 23: 105-134.

Ohida T, Kamal A M, Uchiyama M, Kim K, Takemura S, Sone T, Ishii T. (2001). The influence of lifestyle and health status factors on sleep loss among the Japanese general population. *Sleep.* 24: 333-338.

Pandi-Perumal S R, Smits M, Spence W, Srinivasan V, Cardinali D P, Lowe A D, Kayumov L. (2007). Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* 31: 1-11.

Paparrigopoulos T, Tzavara C, Theleritis C, Soldatos C, Tountas Y. (2010). Physical activity may promote sleep in cardiac patients suffering from insomnia. *Int J Cardiol.* 143: 209-211.

Patel S R, Blackwell T, Redline S, Ancoli-Israel S, Cauley J A, Hillier T A, Lewis C E, Orwoll E S, Stefanick M L, Taylor B C, Yaffe K, Stone K L, Osteoporotic Fractures in Men Research G, Study of Osteoporotic Fractures Research G. (2008). The association between sleep duration and obesity in older adults. *Int J Obes (Lond).* 32: 1825-1834.

Patel S R, Malhotra A, White D P, Gottlieb D J, Hu F B. (2006). Association between reduced sleep and weight gain in women. *Am J Epidemiol.* 164: 947-954.

Patel S R, Palmer L J, Larkin E K, Jenny N S, White D P, Redline S. (2004). Relationship between obstructive sleep apnea and diurnal leptin rhythms. *Sleep.* 27: 235-239.

Peschke E, Frese T, Chankiewitz E, Peschke D, Preiss U, Schneyer U, Spessert R, Muhlbauer E. (2006). Diabetic Goto Kakizaki rats as well as type 2 diabetic patients show a decreased diurnal serum melatonin level and an increased pancreatic melatonin-receptor status. *J Pineal Res.* 40: 135-143.

Peschke E, Hofmann K, Ponicke K, Wedekind D, Muhlbauer E. (2012). Catecholamines are the key for explaining the biological relevance of insulin-melatonin antagonisms in type 1 and type 2 diabetes. *J Pineal Res.* 52: 389-396.

Phillips B, Hening W, Britz P, Mannino D. (2006). Prevalence and correlates of restless legs syndrome: results from the 2005 National Sleep Foundation Poll. *Chest.* 129: 76-80.

Plantinga L, Rao M N, Schillinger D. (2012). Prevalence of self-reported sleep problems among people with diabetes in the United States, 2005-2008. *Prev Chronic Dis.* 9: E76.

Rajendran A, Parthsarathy S, Tamilselvan B, Seshadri K G, Shuaib M. (2012). Prevalence and correlates of disordered sleep in southeast asian indians with type 2 diabetes. *Diabetes Metab J.* 36: 70-76.

Richter P, Werner J, Heerlein A, Kraus A, Sauer H. (1998). On the validity of the Beck Depression Inventory. A review. *Psychopathology.* 31: 160-168.

Robeva R, Kirilov G, Tomova A, Kumanov P. (2008). Melatonin-insulin interactions in patients with metabolic syndrome. *J Pineal Res.* 44: 52-56.

Ross R, Despres J P. (2009). Abdominal obesity, insulin resistance, and the metabolic syndrome: contribution of physical activity/exercise. *Obesity (Silver Spring).* 17 Suppl 3: S1-2.

Ruger M, Scheer F A. (2009). Effects of circadian disruption on the cardiometabolic system. *Rev Endocr Metab Disord.* 10: 245-260.

Scheen A J, Byrne M M, Plat L, Leproult R, Van Cauter E. (1996). Relationships between sleep quality and glucose regulation in normal humans. *Am J Physiol.* 271: E261-270.

Scheer F A, Czeisler C A. (2005). Melatonin, sleep, and circadian rhythms. *Sleep Med Rev.* 9: 5-9.

Scheer F A, Hilton M F, Mantzoros C S, Shea S A. (2009). Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci U S A.* 106: 4453-4458.

Shapiro C M, Flanigan M J. (1993). ABC of sleep disorders. Function of sleep. *BMJ.* 306: 383-385.

Shephard R J. (2003). Limits to the measurement of habitual physical activity by questionnaires. *Br J Sports Med.* 37: 197-206; discussion 206.

Simon C, Brandenberger G, Saini J, Ehrhart J, Follenius M. (1994). Slow oscillations of plasma glucose and insulin secretion rate are amplified during sleep in humans under continuous enteral nutrition. *Sleep.* 17: 333-338.

Skomro R P, Ludwig S, Salamon E, Kryger M H. (2001). Sleep complaints and restless legs syndrome in adult type 2 diabetics. *Sleep Med.* 2: 417-422.

Spiegel K, Knutson K, Leproult R, Tasali E, Van Cauter E. (2005). Sleep loss: a novel risk factor for insulin resistance and Type 2 diabetes. *J Appl Physiol.* 99: 2008-2019.

Spiegel K, Leproult R, Van Cauter E. (1999). Impact of sleep debt on metabolic and endocrine function. *Lancet.* 354: 1435-1439.

Spiegel K, Tasali E, Leproult R, Van Cauter E. (2009). Effects of poor and short sleep on glucose metabolism and obesity risk. *Nat Rev Endocrinol.* 5: 253-261.

Stephan F K. (2002). The "other" circadian system: food as a Zeitgeber. *J Biol Rhythms.* 17: 284-292.

Stokkan K A, Yamazaki S, Tei H, Sakaki Y, Menaker M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science.* 291: 490-493.

Tasali E, Leproult R, Spiegel K. (2009). Reduced sleep duration or quality: relationships with insulin resistance and type 2 diabetes. *Prog Cardiovasc Dis.* 51: 381-391.

Trento M, Broglio F, Riganti F, Basile M, Borgo E, Kucich C, Passera P, Tibaldi P, Tomelini M, Cavallo F, Ghigo E, Porta M. (2008). Sleep abnormalities in type 2 diabetes may be associated with glycemic control. *Acta Diabetol.* 45: 225-229.

Tsai Y W, Kann N H, Tung T H, Chao Y J, Lin C J, Chang K C, Chang S S, Chen J Y. (2012). Impact of subjective sleep quality on glycemic control in type 2 diabetes mellitus. *Fam Pract.* 29: 30-35.

Tsujimura T, Matsuo Y, Keyaki T, Sakurada K, Imanishi J. (2009). Correlations of sleep disturbance with the immune system in type 2 diabetes mellitus. *Diabetes Res Clin Pract.* 85: 286-292.

Turek F W, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen D R, Eckel R H, Takahashi J S, Bass J. (2005). Obesity and metabolic syndrome in circadian Clock mutant mice. *Science.* 308: 1043-1045.

Tutuncu N B, Batur M K, Yildirir A, Tutuncu T, Deger A, Koray Z, Erbas B, Kabakci G, Aksoyek S, Erbas T. (2005). Melatonin levels decrease in type 2 diabetic patients with cardiac autonomic neuropathy. *J Pineal Res.* 39: 43-49.

Tzischinsky O, Shlitner A, Lavie P. (1993). The association between the nocturnal sleep gate and nocturnal onset of urinary 6-sulfatoxymelatonin. *J Biol Rhythms.* 8: 199-209.

UK Prospective Diabetes Study Group. (1998a). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 352: 854-865.

(1998b). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 352: 837-853.

Van Cauter E, Blackman J D, Roland D, Spire J P, Refetoff S, Polonsky K S. (1991). Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. *J Clin Invest.* 88: 934-942.

Van Cauter E, Holmback U, Knutson K, Leproult R, Miller A, Nedeltcheva A, Pannain S, Penev P, Tasali E, Spiegel K. (2007a). Impact of sleep and sleep loss on neuroendocrine and metabolic function. *Horm Res.* 67 (Suppl 1): 2-9.

(2007b). Impact of sleep and sleep loss on neuroendocrine and metabolic function. *Horm Res.* 67 Suppl 1: 2-9.

van Dam R M. (2003). The epidemiology of lifestyle and risk for type 2 diabetes. *Eur J Epidemiol.* 18: 1115-1125.

Van Reeth O, Sturis J, Byrne M M, Blackman J D, L'Hermite-Baleriaux M, Leproult R, Oliner C, Refetoff S, Turek F W, Van Cauter E. (1994). Nocturnal exercise phase delays circadian rhythms of melatonin and thyrotropin secretion in normal men. *Am J Physiol.* 266: E964-974.

Van Someren E J, Hagebeuk E E, Lijzenga C, Scheltens P, de Rooij S E, Jonker C, Pot A M, Mirmiran M, Swaab D F. (1996). Circadian rest-activity rhythm disturbances in Alzheimer's disease. *Biol Psychiatry.* 40: 259-270.

Van Someren E J, Kessler A, Mirmiran M, Swaab D F. (1997a). Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. *Biol Psychiatry.* 41: 955-963.

Van Someren E J, Lijzenga C, Mirmiran M, Swaab D F. (1997b). Long-term fitness training improves the circadian rest-activity rhythm in healthy elderly males. *J Biol Rhythms.* 12: 146-156.

Veneroso C, Tunon M J, Gonzalez-Gallego J, Collado P S. (2009). Melatonin reduces cardiac inflammatory injury induced by acute exercise. *J Pineal Res.* 47: 184-191.

Vgontzas A N, Mastorakos G, Bixler E O, Kales A, Gold P W, Chrousos G P. (1999). Sleep deprivation effects on the activity of the hypothalamic-pituitary-adrenal and growth axes: potential clinical implications. *Clin Endocrinol (Oxf).* 51: 205-215.

Vuori I, Urponen H, Hasan J, Partinen M. (1988). Epidemiology of exercise effects on sleep. *Acta Physiol Scand Suppl.* 574: 3-7.

Waldhauser F, Saletu B, Trinchard-Lugan I. (1990). Sleep laboratory investigations on hypnotic properties of melatonin. *Psychopharmacology (Berl).* 100: 222-226.

Waxman A. (2004). WHO global strategy on diet, physical activity and health. *Food Nutr Bull.* 25: 292-302.

Weaver T E, Laizner A M, Evans L K, Maislin G, Chugh D K, Lyon K, Smith P L, Schwartz A R, Redline S, Pack A I, Dinges D F. (1997). An instrument to measure functional status outcomes for disorders of excessive sleepiness. *Sleep.* 20: 835-843.

Whitehead D L, Davies A D, Playfer J R, Turnbull C J. (2008). Circadian rest-activity rhythm is altered in Parkinson's disease patients with hallucinations. *Mov Disord.* 23: 1137-1145.

Wild S, Roglic G, Green A, Sicree R, King H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care.* 27: 1047-1053.

Winget C M, Vernikos-Danellis J, Cronin S E, Leach C S, Rambaut P C, Mack P B. (1972). Circadian rhythm asynchrony in man during hypokinesis. *J Appl Physiol.* 33: 640-643.

Woon P Y, Kaisaki P J, Braganca J, Bihoreau M T, Levy J C, Farrall M, Gauguier D. (2007). Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. *Proc Natl Acad Sci U S A.* 104: 14412-14417.

Wu T, ZhuGe F, Sun L, Ni Y, Fu O, Gao G, Chen J, Kato H, Fu Z. (2012). Enhanced effect of daytime restricted feeding on the circadian rhythm of streptozotocin-induced type 2 diabetic rats. *Am J Physiol Endocrinol Metab.* 302: E1027-1035.

Yach D, Stuckler D, Brownell K D. (2006). Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat Med.* 12: 62-66.

Yaggi H K, Araujo A B, McKinlay J B. (2006). Sleep duration as a risk factor for the development of type 2 diabetes. *Diabetes Care.* 29: 657-661.

Young M E, Bray M S. (2007). Potential role for peripheral circadian clock dyssynchrony in the pathogenesis of cardiovascular dysfunction. *Sleep Med.* 8: 656-667.

Youngstedt S D, O'Connor P J, Dishman R K. (1997). The effects of acute exercise on sleep: a quantitative synthesis. *Sleep.* 20: 203-214.

Youngstedt S D, Perlis M L, O'Brien P M, Palmer C R, Smith M T, Orff H J, Kripke D F. (2003). No association of sleep with total daily physical activity in normal sleepers. *Physiol Behav.* 78: 395-401.

Zuo H, Shi Z, Yuan B, Dai Y, Hu G, Wu G, Hussain A. (2012). Interaction between physical activity and sleep duration in relation to insulin resistance among non-diabetic Chinese adults. *BMC Public Health.* 12: 247.

APPENDIX A

INFORMED CONSENT FORM

LOMA LINDA UNIVERSITY
School of Allied Health Professions

INFORMED CONSENT

TITLE: Does Physical Therapy Rehabilitation Modulate The Sleep-Wake Pattern in Stroke Patients?

PRINCIPAL INVESTIGATOR: Lee Berk, Dr PH, MPH, CLS

1. WHY IS THIS STUDY BEING DONE?

The purpose of the study is to evaluate the influence of physical therapy rehabilitation on sleep on stroke patients. Also, to analyze the sleep in diabetes individuals.

The rationale for this study is that physical activity level may be involved in the sleep quality in stroke patients, also physically active people report better quality of sleep, but no study has observed the effect of physical activity in the expression of sleep cycle in stroke individuals. Also, little is known about the implications of sleep disturbances in diabetes individuals.

You are invited to participate in this research study because you are a stroke patient admitted at a Rehabilitation Center, a diabetes individual or a healthy person between 30 to 85 years old.

Approximately 50 subjects will participate in this study at about 3 study centers throughout the United States/world, with around 20 subjects at LLU. Participation in this study may last up to 6 weeks.

2. HOW WILL I BE INVOLVED?

Participation in this study involves the following:

- Evaluation of your level of motor impairment and spasticity if you are a stroke subject.
- Evaluation of your Hemoglobin A1c (a measure of the average glucose concentration for the last 6 weeks) through fingerstick of 2 drops of whole blood; sensitivity to touch using a soft nylon fiber and a glucose log throughout the day while participating in the study if you are a diabetic subject.

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 10/16/12 Void after 8/6/2013
#5120185 Chair R L Rigsby MD

A Seventh-day Adventist Institution
DEPARTMENT OF PHYSICAL THERAPY | Nichol Hall, Loma Linda, California 92350
(909) 558-4632 · (800) 422-4558 · *fax* (909) 558-0459 · www.llu.edu/llu/sahp/pt

- Analysis of your mental cognition.
- Evaluation of how you function in your daily activities.
- Evaluation of your level of attention and alertness through a device similar to a cell phone in which you will press a button every time you see a dot on the screen for about 10 minutes.
- Evaluation of your rest-activity cycle through a watch like device called Actiwatch 2®. You will use this instrument in your non affected wrist continuously for a period of one week. In the lateral side of this device, there is a button and you will press this button every time you go to bed at night and as soon as you wake up in the morning. We will ask you not to remove the device any time during the week. A Sleep Log will also be given to you. On this log you will write your bedtime and wake up time in the morning at the same time that you push the button on the actiwatch.
- Assessment of how you sleep using an instrument called Sleep Profiler. It is an instrument that will be placed on your forehead at the time you go to bed to sleep. You will sleep with this device and you take it off as soon as you wake up in the morning. You will use this equipment for two consecutive nights.
- We will also analyze a hormone called melatonin, and for that we will collect samples of your saliva (one table spoon) at three time periods: two hours before you go to bed, at the time you go to bed, and as soon as you wake up in the morning.
- There will be also a packet containing questionnaires that you may answer at home and bring back upon your next scheduled visit to the rehab center. Among these questionnaires are included: assessment of your quality of sleep; if you have any problem breathing during your sleep; your daytime sleepiness; your tendency towards being rather a morning person (lark) or an evening person (owl); your current level of physical activity; evaluation of depression; quality of life; religio-spiritual experience; and your perceived stress level.
- All of the tests and questionnaires listed above will be performed before you start the physical therapy program at Rehabilitation Center and 4 weeks later if you are the stroke subject.

You will be allowing Lee Berk, Dr PH, MPH, CLS and designees to collect demographic data from your medical record, including results of your functional evaluation performed by your physician and/or physical therapist.

If you agree to participate, you will be responsible for bring the Actiwatch 2®, the Sleep Profiler and the packet containing all the answered questionnaires back to the Rehabilitation Center, by the time of your next schedule visit.

3. WHAT ARE THE REASONABLY FORESEEABLE RISKS OR DISCOMFORTS I MIGHT HAVE?

Participating in this study exposes you to minimal physical risk. You may find it uncomfortable to wear the actiwatch device for a week, to use the Sleep Profiler while you sleep and to collect the saliva samples.

4. WILL THERE BE ANY BENEFIT TO ME OR OTHERS?

Although you are not likely to benefit directly from this study, you may find useful to have a sleep assessment. Also, scientific information we learn from the study may benefit the understanding of how we can increase the quality of sleep in stroke patients and diabetes individuals.

5. WHAT ARE MY RIGHTS AS A SUBJECT?

Participation in this study is voluntary. Your decision whether or not to participate or withdraw at any time from the study will not affect your ongoing medical care/relationship to your doctors and will not involve any penalty or loss of benefits to which you are otherwise entitled.

Likewise, the investigator may withdraw you from the study for any reason without your agreement or may stop the study entirely.

6. HOW WILL INFORMATION ABOUT ME BE KEPT CONFIDENTIAL?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will not be identified by name in any publications describing the results of this study. All data collected will be kept in a secured password computer in room A117 at Nichol Hall, Loma Linda University.

Your rights regarding permission to use your health information are described on the attached "Authorization for Use of Protected Health Information" form.

7. WHAT COSTS ARE INVOLVED?

There is no cost to you for participating in this study.

8. WILL I BE PAID TO PARTICIPATE IN THIS STUDY?

- You will not be paid for study participation; however, you will receive a $25.00 upon completing this study as expression of our appreciation for helping with this study.
- In order to receive such payments, you may be asked to provide your home address and/or your Social Security number.

9. WHO DO I CALL IF I HAVE QUESTIONS?

If you wish to contact an impartial third party not associated with this study regarding any questions about your rights or to report a complaint you may have about the study, you may contact the Office of Patient Relations, Loma Linda University Medical Center, Loma Linda, CA 92354, phone (909) 558-4647, e-mail patientrelations@llu.edu for information and assistance.

10. SUBJECT'S STATEMENT OF CONSENT

- I have read the contents of the consent form and have listened to the verbal explanation given by the investigator.
- My questions concerning this study have been answered to my satisfaction.
- Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors from their responsibilities.
- I may call Lee Berk or his designees during routine office hours at (909) 558-5828 or during non-office hours at (909) 558-5828 and leave a message.
- I hereby give voluntary consent to participate in this study.

I understand I will be given a copy of this consent form after signing it or after verbally consenting it (due to be physically unable to sign the consent) with a witness signature.

_____ Signature of Subject	_____ Printed Name of Subject
_____ Date	

Subject is unable to sign because _____.

_____
Printed name of Subject

I attest that the above named subject has indicated their consent to participate in this study.

_____ _____
Signature of Witness Printed Name of Witness

_____
Date

11. INVESTIGATOR'S STATEMENT

I attest that the requirements for informed consent for the medical research project described in this form have been satisfied, that I have discussed the research project with the subject and explained to him or her in non-technical terms all of the information contained in this informed consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the subject to ask questions and that all questions asked were answered. I will provide the subject with a signed and dated copy of this consent form.

_____ _____
Signature of Investigator Printed Name of Investigator

_____
Date

APPENDIX B

BECK'S DEPRESSION INVENTORY

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
- 0 I do not feel sad.
- 1 I feel sad
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad and unhappy that I can't stand it.

2.
- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel the future is hopeless and that things cannot improve.

3.
- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

4.
- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

5.
- 0 I don't feel particularly guilty
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6.
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7.
- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

8.
- 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

9.
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10.
- 0 I don't cry any more than usual.
- 1 I cry more now than I used to.
- 2 I cry all the time now.
- 3 I used to be able to cry, but now I can't cry even though I want to.

11.

0 I am no more irritated by things than I ever was.
1 I am slightly more irritated now than usual.
2 I am quite annoyed or irritated a good deal of the time.
3 I feel irritated all the time.

12.

0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all of my interest in other people.

13.

0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty in making decisions more than I used to.
3 I can't make decisions at all anymore.

14.

0 I don't feel that I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel there are permanent changes in my appearance that make me look unattractive
3 I believe that I look ugly.

15.

0 I can work about as well as before.
1 It takes an extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

16.

0 I can sleep as well as usual.
1 I don't sleep as well as I used to.
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

17.

0 I don't get more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

18.

0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all anymore.

19.

0 I haven't lost much weight, if any, lately.
1 I have lost more than five pounds.
2 I have lost more than ten pounds.
3 I have lost more than fifteen pounds.

20.

0	I am no more worried about my health than usual.
1	I am worried about physical problems like aches, pains, upset stomach, or constipation.
2	I am very worried about physical problems and it's hard to think of much else.
3	I am so worried about my physical problems that I cannot think of anything else.

21.

0	I have not noticed any recent change in my interest in sex.
1	I am less interested in sex than I used to be.
2	I have almost no interest in sex.
3	I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

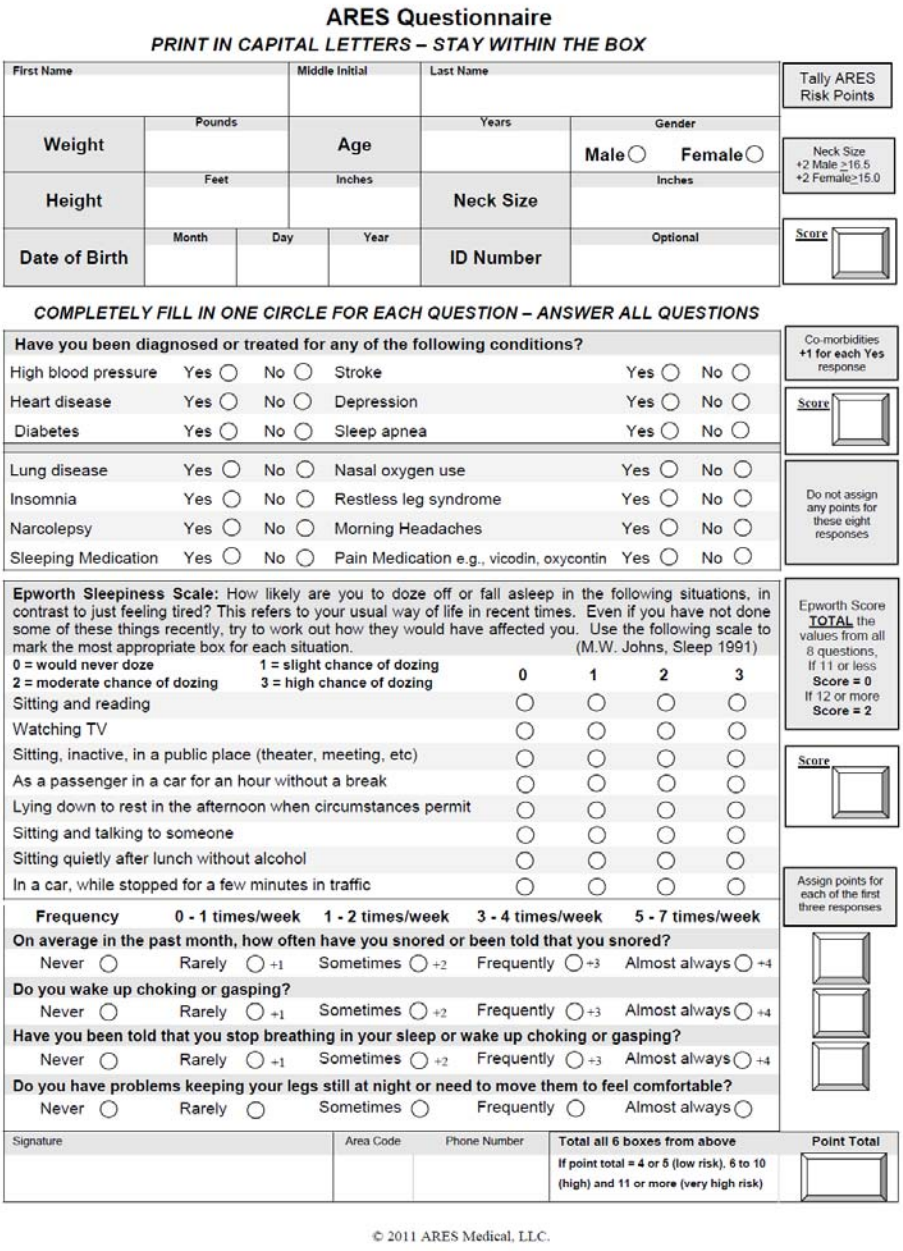
Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

Total Score	Levels of Depression
1-10	These ups and downs are considered normal
11-16	Mild mood disturbance
17-20	Borderline clinical depression
21-30	Moderate depression
31-40	Severe depression
over 40	Extreme depression

A PERSISTENT SCORE OF 17 OR ABOVE INDICATES THAT YOU MAY NEED MEDICAL TREATMENT. IF YOU HAVE ANY CARDIAC CONCERNS, PLEASE CONTACT CARDIOVASCULAR INTERVENTIONS, P.A. at 407-894-4880

APPENDIX C

ARES QUESTIONNAIRE



ARES Questionnaire

PRINT IN CAPITAL LETTERS – STAY WITHIN THE BOX

First Name	Middle Initial	Last Name	Tally ARES Risk Points

Weight	Pounds	**Age**	Years	Gender	Neck Size +2 Male ≥16.5 +2 Female≥15.0
				Male ◯ Female ◯	
Height	Feet / Inches		**Neck Size**	Inches	
Date of Birth	Month / Day / Year		**ID Number**	Optional	Score

COMPLETELY FILL IN ONE CIRCLE FOR EACH QUESTION – ANSWER ALL QUESTIONS

Have you been diagnosed or treated for any of the following conditions?

Co-morbidities **+1 for each Yes** response

Score

High blood pressure	Yes ◯	No ◯	Stroke	Yes ◯	No ◯
Heart disease	Yes ◯	No ◯	Depression	Yes ◯	No ◯
Diabetes	Yes ◯	No ◯	Sleep apnea	Yes ◯	No ◯
Lung disease	Yes ◯	No ◯	Nasal oxygen use	Yes ◯	No ◯
Insomnia	Yes ◯	No ◯	Restless leg syndrome	Yes ◯	No ◯
Narcolepsy	Yes ◯	No ◯	Morning Headaches	Yes ◯	No ◯
Sleeping Medication	Yes ◯	No ◯	Pain Medication e.g., vicodin, oxycontin	Yes ◯	No ◯

Do not assign any points for these eight responses

Epworth Sleepiness Scale: How likely are you to doze off or fall asleep in the following situations, in contrast to just feeling tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently, try to work out how they would have affected you. Use the following scale to mark the most appropriate box for each situation. (M.W. Johns, Sleep 1991)

0 = would never doze **1 = slight chance of dozing**
2 = moderate chance of dozing **3 = high chance of dozing**

	0	1	2	3
Sitting and reading	◯	◯	◯	◯
Watching TV	◯	◯	◯	◯
Sitting, inactive, in a public place (theater, meeting, etc)	◯	◯	◯	◯
As a passenger in a car for an hour without a break	◯	◯	◯	◯
Lying down to rest in the afternoon when circumstances permit	◯	◯	◯	◯
Sitting and talking to someone	◯	◯	◯	◯
Sitting quietly after lunch without alcohol	◯	◯	◯	◯
In a car, while stopped for a few minutes in traffic	◯	◯	◯	◯

Epworth Score **TOTAL** the values from all 8 questions, If 11 or less **Score = 0** If 12 or more **Score = 2**

Score

Assign points for each of the first three responses

Frequency	**0 - 1 times/week**	**1 - 2 times/week**	**3 - 4 times/week**	**5 - 7 times/week**

On average in the past month, how often have you snored or been told that you snored?
Never ◯ Rarely ◯ +1 Sometimes ◯ +2 Frequently ◯ +3 Almost always ◯ +4

Do you wake up choking or gasping?
Never ◯ Rarely ◯ +1 Sometimes ◯ +2 Frequently ◯ +3 Almost always ◯ +4

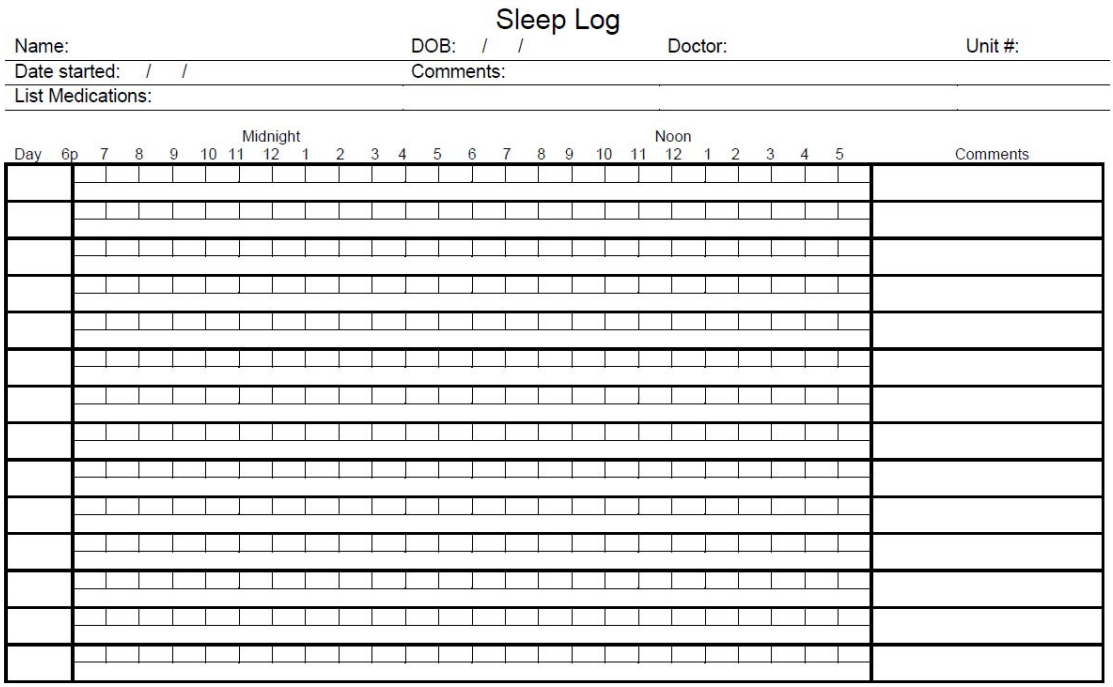
Have you been told that you stop breathing in your sleep or wake up choking or gasping?
Never ◯ Rarely ◯ +1 Sometimes ◯ +2 Frequently ◯ +3 Almost always ◯ +4

Do you have problems keeping your legs still at night or need to move them to feel comfortable?
Never ◯ Rarely ◯ Sometimes ◯ Frequently ◯ Almost always ◯

Signature	Area Code	Phone Number	**Total all 6 boxes from above**	**Point Total**
			If point total = 4 or 5 (low risk), 6 to 10 (high) and 11 or more (very high risk)	

APPENDIX D

SLEEP LOG



APPENDIX E

INSTRUCTIONS FOR THE ACTIWATCH DEVICE

Instructions for the Actiwatch Device

• The Actiwatch should be used as a regular watch and should be placed on your non dominant wrist.

•Please keep the Actiwatch on your wrist during the period requested by the researcher.

• Don't remove the Actiwatch from your wrist, unless it is really necessary.

• Be careful not to immerse the Actiwatch in water below the depth of 39 inches for a period longer than 30 minutes.

• The Actiwatch is a very sensitive device, please do not to open or hit.

• Please press the button placed on the left side on each day, when you go to bed and as soon as you wake up in the morning.

• Please, don't forget to bring the Actiwatch with you on your next visit.

Thank you!

APPENDIX F

INSTRUCTIONS FOR SALIVA MELATONIN COLLECTION

Instructions for Saliva Melatonin Collection

Recommendations Prior to Collection:

- Finish your meal or snack 30 min prior the saliva collection.
- Avoid eating bananas and chocolate on the collection day.
- Drinking water is allowed until 10 min before each sample collection.
- Avoid drinking coffee, cola and alcohol at least 4 hours prior to the collection.
- If possible, avoid aspirin or ibutrofen during the collection day.

Steps:

1) 2 hours before going to sleep, prepare the saliva kit to collect your first saliva sample.

2) Wash your hands and dry using a clean towel.

3) Rinse your mouth with water **10 minutes** prior to collection. **Except** samples taken in the morning upon awakening.

No tooth brushing with toothpaste is allowed during the sampling period.

4) Prepare your room to be in a dim light. The salivary collection needs to be done in a place with low light.

5) When you are ready to start the collection, place the straw in your mouth as shown in the picture. Allow saliva to pool in the mouth.

6) With head tilted forward, drool down into the straw and continue to collect the saliva in the vial until it is half filled.

7) When you are finished, place the vial in the freezer.

Please repeat each step, at all 3 times of collection:

1°) **2 hours before going to bed.**

2°) **Right before going to bed.**

3°) **As soon as you wake up in the morning.**

Don't forget to bring all three vials on your next visit!
Thank you!!