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# Sleep Duration, Postprandial Metabolic Function, and the Role of Insulin Resistance in Nondiabetic Individuals

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UNIVERSITY OF MIAMI

# SLEEP DURATION, POSTPRANDIAL METABOLIC FUNCTION, AND THE ROLE OF INSULIN RESISTANCE IN NONDIABETIC INDIVIDUALS

By

Karin A. Garcia

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

December 2018

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# SLEEP DURATION, POSTPRANDIAL METABOLIC FUNCTION, AND THE ROLE OF INSULIN RESISTANCE IN NONDIABETIC INDIVIDUALS

Karin A. Garcia

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The influence of sleep duration on metabolic pathogenic pathways associated with Type 2 diabetes mellitus are not well understood but may be operational long before the development of clinical diabetes or even prediabetes is detected. This study was designed to examine in preclinical nondiabetic adults whether the association of insulin sensitivity and postprandial metabolic function is moderated by sleep duration. The sample was comprised of 143 individuals (65% men), aged 18–55 years, who had no diabetes or other diagnosed conditions. Metabolic function outcomes were assessed in response to an OGTT, and two 14-h serial mixed carbohydrate-meal tests administered, over 3 successive in-patient days; the carbohydrate content of the mixed-meals was manipulated to compare a standard-load day with a double-load day (300 vs. 600 kcal/ meal). Sleep duration over 1-week was derived using actigraphy. Quantitative modeling was applied to derive total postprandial insulinemia ($AUC_{INS}$), total postprandial glycemia ($AUC_{GLU}$), β-cell glucose sensitivity (β-GS), early insulin secretion rate sensitivity (ESRS), and potentiation ratio (POT). Study findings indicated that the relationship between insulin sensitivity and postprandial insulin response following a carbohydrate load depended on sleep duration, even after controlling for relevant covariates. Specifically, with more insulin resistance and shorter sleep duration, more elevated postprandial insulin secretion

was observed to the double carbohydrate load condition. These findings reflect a compensatory adaptation of postprandial insulin metabolism in insulin resistance that is heightened with shorter sleep duration. Although there was no moderation of the association of insulin sensitivity with $AUC_{GLU}$ and ESRS by sleep duration, moderation was observed for β-GS and POT. However, the pattern of these relationships suggest that these metabolic parameters do not account for the moderation of the association of insulin sensitivity and postprandial insulinemia by sleep duration. Thus, some mechanism other than that measured in this study is responsible for the differences in insulin metabolic response to high carbohydrate loading in these individuals. Further studies are necessary to delineate whether there are alterations in some aspect of sleep function or architecture beyond sleep duration that may mediate the heightened insulin secretion response to high carbohydrate loading in insulin resistant individuals.

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## TABLE OF CONTENTS


Page

LIST OF TABLES AND FIGURES ..... v

Chapters

1 INTRODUCTION ..... 1
Short Sleep: Prevalence and Contribution to Cardiovascular Risk ..... 1
Sleep Duration and Metabolic Function ..... 3
Measurement of Sleep Duration ..... 6
Measurement of Postprandial Metabolic Function ..... 8
The Present Study ..... 11
Study Aims and Hypothesis ..... 13

2 METHODS ..... 16
Participants ..... 16
Procedures ..... 16
Measures ..... 19

3 DATA ANALYSIS PLAN ..... 24
Preliminary Analyses ..... 24
Primary Analyses ..... 24

4 RESULTS ..... 27
Descriptive Characteristics of the Study Sample ..... 27
Relationship between Insulin Sensitivity and Metabolism (Aim 1) ..... 28
Relationship between Sleep Duration and Metabolism (Aim 2) ..... 29
Association between Insulin Sensitivity and Metabolism Moderated by Sleep Duration (Aim 3 and 4) ..... 30

5 DISCUSSION ..... 35

6 CONCLUSION ..... 48

Bibliography ..... 52
Tables ..... 60
Figures ..... 69

## LIST OF TABLES AND FIGURES

Page

Table 1. Clinical characteristics of the Insulin Sensitive and Insulin Resistant Groups ..... 60

Table 2. Regression Results from Aim 1- Insulin Sensitivity and Carbohydrate Load ..... 62

Table 3. Regression Results from Aim 2- Sleep Duration and Carbohydrate Load.... 63

Table 4. Regression Results from Aim 3- Two-way and Three-way Interactions ..... 64

Table 5. Regression Results from Aim 4- Two-way and Three-way Interactions plus Covariates ..... 66

Figure 1. Interaction between Insulin Sensitivity and Carbohydrate Load for $AUC_{INS}$ by Sleep Group ..... 69

Figure 2. Interaction between Insulin Sensitivity and Carbohydrate Load for β-GS by Sleep Group ..... 70

Figure 3. Interaction between Insulin Sensitivity and Carbohydrate Load for POT by Sleep Group ..... 71

Figure 4. Interaction between Insulin Sensitivity and Sleep Duration for $AUC_{GLU}$ ..... 72

*Chapter 1*

# INTRODUCTION

## Short Sleep: Prevalence and Contribution to CVD Risk

Over the past forty years, there has been an alarming decrease in the number of hours individuals are sleeping per night. Facets of modern day society, including longer work hours and more shift work, have affected both the quality and quantity of sleep. In fact, most adults in recent times are voluntarily restricting the number of hours they sleep from 8.5 hours to less than 7 hours per night (Centers for Disease Control, 2005). According to recent studies in the Center for Disease Control and Prevention's (CDC) Morbidity and Mortality Weekly report, more than one third of American Adults are not getting enough hours of sleep on a regular basis (Centers for Disease Control, 2016). Shortened sleep duration has been linked to several detrimental consequences including excessive daytime sleepiness, fatigue, and overall decline in daytime performance (Kahneman, Krueger, Schkade, Schwarz, & Stone, 2004). Shortened sleep duration has also been linked to disease progression, particularly in the context of cardiometabolic pathophysiology (Van Cauter, Spiegel, Tasali, & Leproult, 2008; Wolk, Gami, Garcia-Touchard, & Somers, 20050.

Short sleep duration and long sleep duration have both been associated with an increased mortality risk (Gallicchio & Kalesan, 2009). It is now well established that chronic sleep deprivation is also associated with an increased risk cardiovascular disease (CVD) mortality, although the mechanisms underlying these associations are not fully understood (Cappuccio, Cooper, D'Ella, Strazzullo, & Miller, 2009; Ferrie et al., 2007;

Meisinger, Heier, Lowel, Schneider, & Doring, 2007). It is thought that the mechanisms relating short sleep duration to adverse health outcomes include reciprocal changes in circulating levels of leptin and ghrelin that in turn would increase appetite, caloric intake, reduce energy expenditure and facilitate the development of obesity and impaired glycemic control with increased cardiovascular risk (Spiegel, Tasali, Penev, & Van Cauter, 2004; Taheri, Lin, Austin, Young, & Mignot, 2004; Cappuccio, Cooper, D'Ella, Strazzullo, & Miller, 2009). Short sleep duration may also affect several factors promoting proinflammatory status, including increased levels of circulating inflammatory cytokines and markers such as C-reactive protein (CRP), thought to be associated with elevated blood pressure and metabolic/endocrine dysfunction (Smagula et al., 2016). Increased cortisol secretion and altered growth hormone metabolism have also been implicated as potential mechanisms in the association between sleep duration and cardiometabolic function (Copinschi, 2005). In addition, low grade inflammation, which is a consequence of short sleep, may have possible implications for CVD and other chronic medical conditions (Miller & Cappuccio, 2007). Many theorize that this association is related to the recent obesity epidemic, as rates of obesity have paralleled in growth with sleep deprivation (Patel & Hu, 2012). Physiological evidence suggests long-term sleep deprivation may influence obesity through effects on sedentary behavior, appetite, and/or thermoregulation (Patel & Hu, 2012; Patel, Malhotra, White, Gottlieb, & Hu, 2006). As the incidence of chronic sleep deprivation worsens, the cardiometabolic disease prevalence will likely be adversely impacted.

**Sleep Duration and Metabolic Function**

Of special interest to both researchers and clinicians are the mechanisms involved that may be mediating a relationship of shortened sleep with cardiometabolic functioning, particularly in preclinical populations before structural and functional alterations occur that would confound investigation. In recent years, substantial evidence has supported the hypothesis that sleep dysfunction is a risk factor for type 2 diabetes mellitus (T2DM), suggesting that alterations in sleep may diminish metabolic function in otherwise healthy individuals (Nock, Larkin, Patel & Redline, 2009). A number of sleep-related factors are linked to poor glycemic control, including sleep duration, sleep quality, insomnia, circadian misalignment, altered sleep architecture, and sleep disordered breathing (SDB) (Grandner, 2014). It has been shown that worsening sleep is associated with a progressive worsening of insulin sensitivity, glucose homeostasis, and other systemic complications, including obesity (Wilcox, 2005). Prior to T2DM diagnosis, a progressive decline in insulin sensitivity (IS) occurs in prediabetes, manifesting itself as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) (Ferrannini, Gastaldelli & Iozzo, 2011). During this phase, there is an upregulation of insulin secretion during fasting and postprandial periods. However, a decline in the ability of the pancreatic β-cell to fully compensate for the insulin resistance results in altered insulin and glucose functioning (Ferrannini, 2010). The influence of sleep dysfunction on these metabolic pathogenic pathways are not well understood, but may be operational long before the development of clinical diabetes or even prediabetes is detected.

Insulin resistance (IR) among non-diabetic subjects has been shown to be associated with subsequent increased risk of T2DM and cardiovascular morbidity, and is

an independent predictor of overall and cardiac mortality (Kent, McNicholas & Ryan, 2015). Several cross-sectional and longitudinal studies suggest that short sleep duration (generally defined as less than 6 hours of sleep) is associated with increased risk of T2DM (Ayas et al., 2003; Chaput et al., 2009; Yaggi, Araujo, and McKinlay, 2006). In fact, experimental studies have shown that restricting sleep duration in healthy young subjects resulted in an acute alteration in glucose metabolism indicated by a decrease in insulin sensitivity, with subsequent reduced glucose tolerance (Buxton et al., 2010; Spiegel, Leproult, and Van Cauter, 1999). Buxton and colleagues (2010) conducted a 12-day inpatient sleep study to test the hypothesis that sleep restriction in healthy subjects reduces insulin sensitivity. Subjects experienced two conditions: the sleep-replete condition, in which participants spent 10 hours per night in bed for approximately 8 nights, followed by the sleep-restricted condition, in which participants were restricted to 5 hours per night in bed for 7 nights (Buxton et al., 2010). Glucose metabolism (measured by intravenous glucose tolerance test (IVGTT) and euglycemic-hyperinsulinemic clamp), salivary cortisol, 24-hour urinary catecholamines, and neurobehavioral performance were measured during the last 2 days of each condition. Results showed that IVGTT-derived insulin sensitivity and clamp-derived insulin sensitivity were both significantly reduced acutely after sleep restriction without significant alterations in the insulin secretory response (Buxton et al., 2010). This finding suggests the possibility that more than one mechanism is contributing to impaired glucose metabolism with sleep restriction. Glucose tolerance was also shown to be acutely reduced by sleep restriction in this study. Changes in insulin sensitivity did not correlate with changes in salivary cortisol, urinary catecholamines, or slow wave sleep, suggesting

that these systems do not mediate the changes in insulin sensitivity induced with moderate sleep restriction. Spiegel, Leproult, & Van Cauter (1999) also examined the effects of sleep deprivation on metabolism in healthy subjects using frequently sampled IVGTT and sleep debt (4 hours per night) and sleep-replete (12 hours night) conditions. Study findings indicated that the sleep debt condition led to impaired glucose metabolism as a result of reductions in glucose tolerance, glucose effectiveness, and acute insulin response to glucose. However, researchers failed to find a significant reduction in insulin sensitivity in this study (Spiegel, Leproult, & Van Cauter, 1990). Several other laboratory studies manipulating sleep duration in healthy adults indicated that a few days of sleep restriction were sufficient to cause a marked reduction of insulin sensitivity, resulting in decreased glucose tolerance (Anothaisintawee, Reutrakul, Van Cauter, & Thakkinstian, 2016; Stamatakis, & Punjabi, 2010).

These findings are consistent with the results of several longitudinal studies that revealed that short sleep (generally less than 6 hours per night) is associated with an increased risk of incident diabetes after adjusting for relevant confounders (Hayashino et al., 2007; Xu et al., 2010). Holliday and colleagues (2013) examined whether short sleep duration predicts future incident CVD or T2DM diagnoses after accounting for baseline health and found that, compared to 7 hours of sleep, less than 6 hours of sleep was associated with incident CVD in individuals with poor health at baseline. Study findings also found the risk of incident T2DM was significantly increased in those with less than 6 hours versus 7 hours sleep, even after excluding those with baseline illness and adjusting for baseline health. These findings suggest a potential mechanistic association between shortened sleep duration and development of T2DM that appears not to be a simple

reflection of pre-existing illness (Holliday et al., 2013). In sum, there is general consensus in the medical community that altered sleep duration and its mental and physiological comorbidities warrant further attention (Van Cauter, Spiegel, Tasali, & Leproult, 2008).

**Measurement of Sleep Duration**

Polysomnography (PSG) is considered to be the gold standard for measuring sleep architecture and other aspects of sleep function (Kushida et al., 2001). Commonly utilized variables obtained from PSG include measures of sleep disordered breathing, oxygenation during sleep, sleep stages, and total sleep time (American Association of Sleep Technologists, 2012). Despite the usefulness of PSG variables, PSG studies are often time and cost intensive to complete as they require individuals to sleep overnight at a sleep center and monitoring, scoring, and interpretation from a team of healthcare providers. As a result, researchers often rely on the use of self-report measures, including questionnaires and sleep logs, to gauge participant sleep quality and quantity (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989; Carney et al., 2012). Measures of subjective sleep can be useful in understanding the effects of sleep on mood and psychological distress (Morin, Gibson, & Wade, 1998; Hall et al., 2000; Glozier et al., 2010). Self-reported subjective sleep measures have also been associated with various health outcomes including obesity, cardiovascular health, and all-cause mortality (Vgontzas & Bixler, 2008; Unruh et al., 2008; Tamakoshi & Ohno, 2004). However, it has been well established in the literature that sleep logs often overestimate sleep latency and underestimate total sleep time (Silva et al., 2007; Lauderdale, Knutson, Yan, Liu, & Rathouz, 2008). Indeed, some patients frequently self-report an inability to sleep at all

over the course of a night even after obtaining a full night's sleep as verified by PSG (Tyron, 2004). Self-report measures of sleep typically do not provide a comprehensive assessment of sleep quality and disturbance and provide little information regarding the biological mechanisms involved during sleep (Carpenter & Andrykowski, 1998).

Measures of sleep duration using daily logs and survey instruments are often used in the literature (Silva et al., 2007; Lauderdale, Knutson, Yan, Liu, & Rathouz, 2008). In the past 20 years, more objective measurement of sleep/wake patterns using actigraphy has been used (Ancoli-Israel et al., 2003). Actigraphs are devices generally placed on the wrist to record movement. Actigraphic-recorded movement is later downloaded and analyzed to measure periods of activity and inactivity that are further analyzed to estimate wake and sleep (Sonia Ancoli-Israel et al., 2003). The advantage of utilizing actigraphy over traditional polysomnography (PSG) is that actigraphy can conveniently record continuously for 24-hours a day for extended periods of time, providing information about habitual sleep patterns (Sonia Ancoli-Israel et al., 2003). Previous studies have typically yielded between 78-95% agreement rates when comparing various aspects of sleep using actigraphy compared with measures using PSG (Kushida et al., 2001). Several review papers have concluded that wrist actigraphy can usefully approximate sleep versus wake during 24 hours and have noted that actigraphy has been used for clinical monitoring of insomnia, circadian sleep/wake disturbances, and periodic limb movement disorder (Broughton, Fleming, & Fleetham, 1996). Therefore, in addition to sleep surveys and logs, PSG assessment provides some unique advantages in characterizing sleep architecture and diagnosing sleep disorders, additional studies have found utility in measuring sleep/wake patterns using actigraphy.

**Measurement of Postprandial Metabolic Function**

In studies of sleep and metabolic function, measures typically have included fasting blood glucose and insulin concentrations and the oral glucose tolerance test (OGTT). These measures have traditionally been used to estimate insulin sensitivity and β-cell insulin secretion using the homeostatic model (HOMA) and gluco-regulatory function (Kent, McNicholas & Ryan, 2015). In contrast to studies of a single oral glucose load test, studies have used mixed-meal assessments to provide more robust information about postprandial metabolism (Mari et al., 2002). It has been suggested that longer-term postprandial assessments that include repeated meal ingestion may better approximate circumstances of daily consumption conditions (Mari et al., 2002; Mari et al., 2005). The use of more long-term assessments using repeated meal ingestion may be thought to approximate free-living conditions (Mari et al., 2005). Although shorter tests may be less costly to implement in clinical investigations, they may fail to reveal aspects of β-cell function that only emerge over extended periods of observation (Mari et al., 2002). Moreover, mixed-meals containing protein and fat calories in addition to carbohydrate calories may differ from meals that contain a glucose load to the exclusion of these macronutrients by stimulating greater insulin secretion, gastric release of incretins, and insulin-mediated glucose uptake, and slowing gastric emptying (Brodovicz et al., 2011)

The relative proportion of macronutrient contents of the meal may also make a difference in metabolic functioning. For example, β-cell strain, dyslipidemia, and CVD have all been linked to diets that are high in carbohydrates (Krauss, Blanche, Rawlings, Fernstrom, & Williams, 2006; Poppitt et al., 2002). Specifically, a high carbohydrate load has been shown to result in increased insulin secretion and fluctuations in glucose.

(Fabbrini et al., 2013; Ferrannini, Natali, Bell, Cavallo-Perin, Lalic, & Mingrone, 1997). In insulin resistant individuals, insulin secretion in response to a meal is typically elevated above normal levels in order to maintain normal glycemic control (Kahn, 2003; Ahre´n & Pacini, 2004). Insulin resistance is a necessary antecedent condition before frank type 2 diabetes is diagnosed and may even precede the development of impaired fasting glucose and glucose intolerance observed in prediabetes (Ferrannini, Gastaldelli, & Iozzo, 2011). Recently, the metabolic responses to a 14-hour serial mixed meal challenge were examined in insulin sensitive (IS) and insulin resistant (IR) nondiabetic individuals without diagnosed cardiovascular disease (Hurwitz et al., 2015). This study characterized the glucose and insulin metabolic regulation in response to two consecutive days wherein mixed meals with a standard carbohydrate load were provided every 3.5 hours on one day and then the same meals were provided on a second day except that the proportion of calories from carbohydrates was doubled. Glycemic regulation was assessed using a deconvolution quantitative modeling approach that describes the dose-response relationship of the insulin secretion rates to the concomitant plasma glucose concentrations (Mari et al., 2002). From this analysis, standard metabolic measures may be derived (e.g., fasting levels, total postprandial insulin secretion, total postprandial glucose), but in addition the methodology permits an estimation of the postprandial functioning of the pancreatic β-cell including β-cell glucose sensitivity variable (β-GS), early insulin secretion rate sensitivity (ESRS), and a second insulin secretion component, known as the potentiation factor (POT). The β-GS quantifies the ability of β-cells to respond to changes in glucose concentration. The ESRS is a measure of postprandial dependence of insulin secretion on rate of initial glucose concentration. Hence, this

measure accounts for the initial fast rise in insulin secretion. The POT reflects the insulin secretion levels that are typically observed following the peak of postprandial glycemia, when glucose is returning to pre-meal levels over 2-4 hours. This measure is dependent on β-cell function and in the presence of nondiabetic insulin resistance would be expected to be more sustained to re-establish basal glycemic level. In sum, these measures describe the postprandial rise and fall of insulin secretion that parallels the rise and fall of glucose concentration.

In a previous study of insulin sensitive and insulin resistant men and women, who were otherwise healthy, we have used mathematical modeling to quantitate postprandial β-cell and insulin secretion function using two 14-hour repeated mixed-meal challenges, over two sequential days (Hurwitz et al., 2015). In these meal challenges, the carbohydrate content of the meals was manipulated so that participants received mixed meals with 300 kcal/meal of carbohydrates on one day and mixed meals with 600 kcal/meal carbohydrates on the other day (Hurwitz et al., 2015). Results showed that β-GS and ESRS were elevated for both the IS and IR groups following the double carbohydrate load versus the standard load. So, doubling the carbohydrate load induced metabolic regulation adjustments in both groups. Notably, the IR group displayed greater β-cell glucose sensitivity than IS group in both standard and double carbohydrate meal challenges. In contrast, the potentiation measure did not differ between groups to the standard carbohydrate loads, and although both groups elevated their potentiation to the double carbohydrate load, the elevation to this meal challenge was not as large as displayed by the IS group. Overall, those with IR showed higher postprandial glycemia when compared with the IS group; however, their glycemic levels were constrained by

insulin hypersecretion. This systemic adaptation appears to be a result of greater β-GS and ESRS.

**The Present Study**

In recent years, the prevalence of chronic sleep duration and its detrimental effects on health and functioning have been elucidated in the literature. Negative effects of sleep deprivation include a wide range of performance tasks, mood, health, and mortality (Bonnet & Arand, 1995; Van Cauter, Spiegel, Tasali, & Leproult, 2008). Shortened sleep duration has also been implicated in the disruption of both acute and long term cardiometabolic functioning (Knutson, 2010).

Previous groups have attempted to experimentally restrict sleep time to examine acute metabolic functioning (Buxton et al., 2010; Spiegel, Leproult, & Van Cauter, 1999). These research groups have demonstrated that sleep loss can increase cardiometabolic risk in the short-term in individuals without clinical diagnoses of cardiometabolic illness (Knutson, 2010). Epidemiologic studies have also been conducted and have provided some insight into the associations between chronic shortened sleep and cardiometabolic risk (Knutson, 2010). However, experimental sleep restriction in the laboratory is solely reflective of acute induced metabolic dysfunction. The conclusion of such findings is that there is a linkage between short sleep duration and metabolic dysfunction. However, in the long-term people may undergo a metabolic adaptation such that repeated shorter sleep duration may not have as dramatic effect on their metabolism. Studies assessing more long-term dynamic biological processes linked with abnormal sleep duration and metabolic dysfunction are required to resolve this issue.

Sleep quantity and quality is typically measured using self-report measures or sleep logs which lack objectivity and include inherent self-report biases. Using objective actigraphic data provides a more convenient and cost-efficient measurement of sleep duration (Ancoli-Israel et al. 2003). The evaluation of metabolic function using repeated meal ingestion and longer assessments of postprandial metabolic effects is also more likely to reveal complex dysfunction, especially in healthy samples (Mari et al., 2005). There is a lack of literature exploring objectively measured sleep duration and metabolic functioning following a dynamic meal challenge in healthy individuals. In addition, there is a lack of understanding of the biological mechanisms involved in the relationship between short sleep duration and metabolic functioning, despite evidence that sleep deprivation and cardiometabolic complications provide ever-increasing burden on societal health systems.

Despite the well-established links between sleep duration with diminished insulin sensitivity and glucose control, the extent to which postprandial metabolic dysregulation is an independent function of sleep duration or insulin resistance is unclear. The present study will examine the sleep duration-metabolic function relationship in nondiabetic men and women, who do not have diagnosed sleep or cardiometabolic conditions and seeks to explore the interrelationship between sleep duration, insulin sensitivity/resistance and postprandial metabolic outcomes. In addition to using the oral glucose tolerance test (OGTT), long-term assessments using repeated meal ingestion over 14 hours will be used to approximate free-living conditions. Such an evaluation could shed light on whether subclinical metabolic alterations linked with sleep duration are operating before cardiometabolic structural and functional changes occur and clinical diagnosis is

rendered. Moreover, if these subclinical pathophysiological interactions are robust, the signs of these relationships should be apparent in men and women who have not been previously diagnosed with a potentially confounding cardiometabolic condition.

**Study Aims and Hypothesis**

Specific Aim 1: To examine the relationship between insulin resistance and indices of postprandial insulin and glucose metabolic regulation in two serial mixed-meal challenge conditions, wherein the meal carbohydrate content was manipulated to compare standard and double carbohydrate load (300 vs. 600 kcal/meal).

*Hypothesis 1:* Greater insulin resistance will be significantly associated with poorer postprandial pancreatic β-cell glucose sensitivity (β-GS), less early secretion rate sensitivity (ESRS) and second phase insulin secretion potentiation (POT), greater total glycemia ($AUC_{GLU}$), and greater total insulinemia ($AUC_{INS}$) in both the standard (300 kcal/meal) and double (600 kcal/meal) carbohydrate load meal challenges.

*Hypothesis 2:* The relationship between insulin resistance and postprandial pancreatic β-cell and metabolic function will depend on the meal carbohydrate challenge, such that the relationship between greater insulin resistance and poorer metabolic function will be stronger following a double (600 kcal/meal) load challenge than a standard (300 kcal/meal) load challenge.

Specific Aim 2: To examine the relationship between sleep duration and indices of postprandial insulin and glucose metabolic regulation in two serial mixed-meal challenge conditions, wherein the meal carbohydrate content was manipulated to compare standard and double carbohydrate load (300 vs. 600 kcal/meal).

*Hypothesis 1:* Shorter sleep will be significantly associated with poorer postprandial pancreatic β-cell glucose sensitivity (β-GS), less early secretion rate sensitivity (ESRS) and second phase insulin secretion potentiation (POT), greater total glycemia ($AUC_{GLU}$), and greater total insulinemia ($AUC_{INS}$) in both the standard (300 kcal/meal) and double (600 kcal/meal) carbohydrate load meal challenges.

*Hypothesis 2:* The relationship between sleep duration and postprandial pancreatic β-cell and metabolic function will depend on the meal carbohydrate challenge, such that the relationship between shorter sleep duration and poorer metabolic function will be stronger following a double (600 kcal/meal) load challenge than a standard (300 kcal/meal) load challenge.

Specific Aim 3: To assess whether the association between insulin resistance and postprandial insulin and glucose metabolic regulation depends on sleep duration in two serial mixed-meal challenge conditions, wherein the meal carbohydrate content was manipulated to compare standard and double carbohydrate load (300 vs. 600 kcal/meal).

*Hypothesis 1:* Associations between insulin sensitivity and postprandial pancreatic β-cell and metabolic function established in previous aim will depend on sleep duration in both the standard (300 kcal/meal) and double (600 kcal/meal) carbohydrate load meal challenges.

*Hypothesis 2:* The relationship between greater insulin resistance and poorer postprandial pancreatic β-cell and metabolic function will be stronger in individuals with shorter sleep duration and effects will be stronger following a double (600 kcal/meal) load challenge than a standard (300 kcal/meal) load challenge.

Specific Aim 4: To assess whether the associations observed in previous aims is independent of key demographic variables (age, gender, education level) and traditional CV risk factors (smoking history, casual systolic blood pressure, HDL, LDL, CRP, total visceral adiposity).

*Hypothesis 1:* All relationships between insulin resistance, sleep duration, and postprandial insulin and glucose metabolic regulation will remain after controlling for key demographic and traditional CV risk factors.

*Chapter 2*

# METHODS

## Participants

The data were collected in the Carbohydrate Loading, Insulin Resistance and Cardiometabolic Risk study, which has examined cardiometabolic function in response to meals with low and high carbohydrate content in 143 insulin sensitive and insulin resistant healthy, nondiabetic adult participants (Hurwitz et al., 2015). Participants were recruited via flyer advertisement and chain-referral from Miami-Dade, Broward, and other South Florida counties.

Inclusion/exclusion criteria. Study eligibility included participants who: 1) were aged 18-55 years; 2) had no nicotine use in the past year, no history of substance or alcohol dependency in the ten years pre-study entry, and negative urine toxicology screen; 3) were taking no prescribed cardiovascular, carbohydrate, endocrine, or psychiatric medication; 4) had no history of diagnosed cardiovascular, metabolic, or endocrine disorder; and 5) for women, were not pregnant, with regular menstrual cycling (26-35 days) for the 3 months before study entry. The protocol was approved by the Institutional Review Board of the University of Miami and informed consent was obtained prior to inclusion.

### Procedures

All examination and interviewer-administered questionnaires were conducted by trained and certified study personal following a standardized protocol. The protocol consisted of three separate assessment sessions, which included: 1) screening session to

confirm study eligibility; 2) euglycemic hyperinsulinemic clamp to measure insulin sensitivity; and 3) 3-day/night in-patient laboratory stay to evaluate postprandial metabolic function over two meal challenge days (standard vs. double carbohydrate load). The procedures of the initial three sessions will be briefly summarized below.

Following telephone screening of participants, in the first assessment session, collection of standard demographic, anthropometric and personal medical information, and casual blood pressure, urine toxicology, and fasting comprehensive and CBC chemistry panels, and glycosylated hemoglobin (HbA1c) were obtained. Persons who met the study eligibility criteria were invited to participate in the next two sessions. In the second assessment session, fasting blood sampling for lipid profile, insulin, glucose and C-reactive protein, was followed by a 150-min euglycemic hyperinsulinemic procedure (insulin infusion rate 40 mU.min.m2) to clamp glucose at within 5% of fasting levels (Hurwitz et al., 2015; Goldstein et al., 2001). The euglycemic hyperinsulinemic method provides a measure of insulin-mediated glucose uptake as an index of insulin sensitivity.

The third assessment session was a 3-day, overnight in-patient laboratory stay wherein four meals per day were provided. The first day of the overnight stay included administration of the standard 75-g, 3-hour OGTT (National Diabetes Data Group, 1979). Subsequently, visceral (VAT) and subcutaneous (SAT) abdominal fat was measured using multi-slice computed tomography performed by Siemens Somaton-Sensation-16 scanner (Siemens, Malvern, PA) (Mari et al., 2005). On days 2 and 3, subjects were given a meal every 3.5 hours over 14-hour periods. Meals were given at 8:00 am, 11:30 am, 3:00 pm and 6:30 pm, a snack was provided at 9:00 pm, and an overnight fast ensued. The meal carbohydrate content was manipulated so that on one day subjects received a

'standard' level of carbohydrate intake per meal (as administered in the OGTT e.g., 300 kcal) and on the other day received a 'double' carbohydrate content per meal (e.g., 600 kcal). The sequence (SEQ) of standard and double carbohydrate days was randomized. The calories from fats and proteins per meal were kept identical throughout the study. The macronutrient content per meal was adjusted on the basis of the subjects' body surface area and sex, approximating the U.S. national criteria prescribed for normal daily consumption (i.e., 50% carbohydrate, 35% fat and 15% protein) (USDA Economic Research Service, 2002). Macronutrient content was also tailored to a sedentary to light daily energy utilization as participants were not very active during their lab stay. The average daily caloric intake for the cohort met the current average U.S. dietary intake of about 3000 kcal/day for men and 2300 kcal/day for women (Wright & Wang, 2010). Carbohydrate calories were provided from OGTT drink and white rice (75% drink/25% rice ratio), whereas fat and protein calories were provided by hamburger and sausage. Total daily sodium intake was 1/3 of the recommended daily allowance (Centers for Disease Control, 2009). Blood samples were timed and taken before and after meal consumption (-15 and 0 minutes and 0, 15, 30, 60, 90, 120 150 and 180 minutes respectively). These samples were assayed to derive measures of fasting/pre-meal and postprandial levels of glucose, insulin, C-peptide, and other constituents, using methods described elsewhere (Hurwitz et al., 2015). These measures were used to derive indices of β-cell function and overall glucose and insulin metabolism from the OGTT and the multiple meal test days using mathematical modeling as detailed previously (Mari et al., 2002).

**Measures**

Fasting and Postprandial Metabolic Function Tests. Fasting plasma glucose, insulin, FFA, and triglyceride levels were obtained for each of the visit 3 days. Estimates of postprandial glucose and insulin metabolic function were determined using the Oral Glucose Tolerance Test (OGTT) standard procedures as per the National Diabetes Data Group. Oral glucose (75 gm i.e., equivalent to 300 calories) was administered as a solution in a concentration of 25.3 gm/dL of flavored water, consumed within 5 min. For the OGTT and the meals, baseline blood samples are obtained (at -15 and 0 minutes) before glucose/food was consumed. To measure glucose, insulin, FFA and triglyceride levels, blood samples were obtained at 15, 30, 60, 90, 120, 150, and 180 minutes after consumption. On visit#3 days 2 & 3 the OGTT volume administered was adjusted on the basis the participant's sex, body weight and estimated daily energy utilization.

β-Cell Function. β-cell function was assessed using a model describing the relationship between insulin secretion and glucose concentration obtained from both the OGTT and the repeated meal tests. In this model, insulin secretion is represented as the sum of two components. The first component is a dose-response function of the relationship of insulin secretion and absolute glucose concentration. β-cell glucose sensitivity (β-GS) is a parameter of the dose response and is the mean slope over the observed glucose range. Potentiation factor (POT), which represents differences in glucose-induced insulin secretion between early and late phases of the multiple meal tests, modulates the dose-response and encompasses prolonged hyperglycemia, non-glucose substrates, gastrointestinal hormones, and neural modulation along with other potentiating mechanisms. POT is quantified as a ratio wherein the denominator is the

mean of the POT values across the initial 20 minute post-session onset. The numerator of this ratio is the mean of the POT values across 1 to 14 hours post-session onset. The second insulin secretion parameter is Early Rate Sensitivity (ESRS). This measure is an index of the initial post-prandial insulin secretion that is a function of the rate of the immediately preceding glycemic change. Using the values of glucose and C-peptide derived from the blood samples, a deconvolution analysis was undertaken. This modeling analysis yielded values every 5 minutes, from which the insulin secretion rate per value was computed.

Sleep Duration. Subjects were instructed to wear the Mini-Mitter Actiwatch wrist-watch style actigraph (Mini-Mitter, Sunriver, OR) following Visit 2 for one week. Participants were instructed to wear the actigraph on the non-dominant wrist and the actigraph was used to derive 24-hour objective estimates of physical activity and sleep parameters. The Actiwatch contains a calibrated accelerometer and 64K memory storage apparatus, housed in a casing that, in size and shape, resembles a wrist watch. The specially designed accelerometer samples movement/activity at a rate of 32 times/sec. The wireless wrist-watch style of the actigraph was chosen as it provided a relatively inexpensive, unobtrusive objective activity and sleep monitoring method that was well tolerated by subjects over multiple recording nights. Although the device was placed on the wrist and hence was more sensitive to arm movement, the measures have been validated previously and provide adequate assessment of whole body movements. The Actiwatch was designed to interface with a PC via a specially designed Reader/Interface unit. Windows-style software that accompanies the Actiwatch was used to program the recording unit, download data into storage, and engage a scoring algorithm to provide

measures of various sleep and activity parameters. Actigraphy counts from the one week of use prior to visit 3 used measures of non-movement scored as sleep used to derive measures of sleep duration using the scoring procedures available using the Mini-Mitter software.

Lipid Profile, Triglycerides, Free Fatty Acids and CRP. Free fatty acids, triglycerides and cholesterols (serum total cholesterol, triglycerides, LDL cholesterol, VLDL-cholesterol, HDL-cholesterol) were derived from a fasting blood sample. Plasma free fatty acids (FFA) were measured by an enzymatic colorimetric method (WAKO Diagnostics, Richmond, VA), which relies upon the acylation of coenzyme A (CoA) by the fatty acids. The peroxidation of hydrogen peroxide that is generated forms a condensate that was measured spectrophotometrically at 550 nm. The linear range of the assay is 0-2.0 mmol/L, and the normal range in a previous healthy cohort was 0.50 ± 0.24 mmol/L. Intra-assay CV was < 2.7% and inter-assay CV was < 5.6%. Cholesterol was measured in plasma after release from its esters by an ester hydrolase; the free cholesterol was then oxidized by cholesterol oxidase, producing hydrogen peroxide that, when combined with 4 aminoantipyrine and phenol, forms a chromophore in an amount that was directly proportional to the cholesterol concentration and was quantitated photometrically at 540 nm. The triglyceride level was measured in plasma after hydrolysis by lipoprotein lipase to glycerol and fatty acids. Glycerol is enzymatically phosphorylated and then oxidized to release hydrogen peroxide, which is peroxidized to form a quinoneimine chromophore that can be read photometrically at 490 nm. After centrifugation, the HDL-cholesterol remains in the supernatant and was measured. Dextran sulfate (50,000 MW) and magnesium precipitate the low density lipoproteins

(LDL) and very low density lipoproteins (VLDL). The LDL-cholesterol was calculated according to the Friedewald method. This method correlates well with ultra-centrifugally derived LDL-cholesterol values up to triglyceride values of 400 mg/dl. Respectively, intra- and inter-assay CVs for cholesterol were < 2.5% and < 3.5%, and for triglycerides were < 3.9% and < 1.1%. C-reactive protein (CRP), an index of systemic inflammation, was measured using a high-sensitivity assay. Diluted serum was incubated with polystyrene particles coated with monoclonal antibodies to CRP to produce agglutination and light scattering in proportion to the concentration of antigen. The serum standard was diluted to produce a standard curve from 0.08 to 6 mg/L. The intra- and inter-assay CVs for CRP were < 4.4% and < 5.7%.

Abdominal Fat. Abdominal fat was measured by multislice CT using a Siemens Somaton-Sensation-16 scanner (Siemens, Malvern, PA) using a seeding program with a Siemens WorkStream Wizard workstation. Subcutaneous adipose tissue fat and visceral adipose tissue fat were derived in kilograms using a triangulation formula multiplied by 0.9391 mg/mL.

Covariates. Standard questionnaires and interviews were used to collect information on age, gender, smoking history, and education level. Ethnicity was not included as a covariate as the majority of the group was of Hispanic/Latino origin (77%). Education will be treated as a continuous variable. Basic anthropometric information was also collected to obtain measures of blood pressure, cholesterol, and inflammation. Blood pressure will be examined using casual systolic blood pressure. Cholesterol will be examined by including both the ratio of total cholesterol (TC) with HDL and triglyceride

levels. Inflammatory status will be examined using CRP. Central fat deposition will be indexed by total visceral adiposity volume.

*Chapter 3*

# DATA ANALYSIS PLAN

## Preliminary Analysis

Preliminary statistical analyses will include descriptive statistics, assessment of distributions, outlier detection, box plots, and tests of normality. SPSS version 22.0 will be used for data preparation and descriptive analysis.

Descriptive statistics will be calculated (e.g. mean and standard deviation) for all demographic and biological variables included in the analyses. The t-student test will be used to examine significant differences by insulin resistant status in demographic and biological continuous variables. The chi-square test of independence will be used to test differences among categorical variables.

## Primary Analysis

Statistical analyses are outlined in correspondence to each specific aim.

Analysis of aim 1: The association between insulin sensitivity and metabolic parameters, including pancreatic β-cell (β-GS) and metabolic function (ESRS, POT, AUCGLU, and AUCINS), in two serial mixed-meal challenge conditions will be examined using a series of moderation analyses. Insulin sensitivity will be entered into each model as a continuous independent variable. The meal challenge condition will be entered as a dichotomous variable (1 for 300 kcal/meal condition and 0 for 600 kcal/meal condition). Moderation analyses will include the aforementioned main effects as well as the interaction term (insulin sensitivity*meal challenge condition) of the two main effects.

The interaction will be probed for significance. Unstandardized regression coefficients (b) and p-values for each model will be presented in Table 2. Statistical tests will be two-sided at a significance level of 0.05.

Analysis of aim 2: The association between sleep duration and metabolic parameters, including pancreatic β-cell (β-GS) and metabolic function (ESRS, POT, AUCGLU, and AUCINS), in two serial mixed-meal challenge conditions will be examined using a series of moderation analyses. Sleep duration will be entered into each model as a continuous independent variable. The meal challenge condition will be entered as a dichotomous variable (1 for 300 kcal/meal condition and 0 for 600 kcal/meal condition). Moderation analyses will include the aforementioned main effects as well as the interaction term (sleep duration*meal challenge condition) of the two main effects. The interaction will be probed for significance. Unstandardized regression coefficients (b) and p-values for each model will be presented in Table 3. Statistical tests will be two-sided at a significance level of 0.05.

Analysis of aim 3: A series of moderation analyses will be constructed to identify the influence of sleep duration on the relationship between insulin sensitivity and postprandial metabolic function. Metabolic parameters that were established as significant during previous analyses mentioned above will be included in these analyses. Three-way moderation analyses will include the main effects from previous analyses, the interaction term for sleep duration and insulin sensitivity and the three-way interaction term for sleep duration, insulin sensitivity, and meal challenge condition. Unstandardized regression coefficients (b) and p-values for each model will be presented in Table 4.

Analysis of aim 4: Aim 4 will use the models created to examine aim 3 to test whether the effects remain after covariates are entered into the model. Covariates that will be included in the analysis of aim 4 will include the demographic variables (age, gender, and education level), as well as traditional CV risk factors (smoking history, casual systolic blood pressure, TC/HDL, triglycerides, CRP, and total visceral adiposity).

All significant three-way interactions will be probed via posthoc analyses that decompose significant two-way interaction between insulin sensitivity and carbohydrate load for different levels of sleep duration. Sleep duration will be split into three groups (short, average, and long sleepers) by using the mean, one standard deviation above and one standard deviation below the mean as cutoffs.

*Chapter 4*

# RESULTS

## Descriptive Characteristics of the Study Sample

The sample consisted of 143 persons, aged 18 to 55 years, with a mean age of 39 years. The overall cohort included about 65% men, were comprised mostly of minority race/ethnicity (~90%) and, on average, had low total family income but had accrued some college education. As per enrollment criteria, none of the participants were current smokers, although the sample included about 14% who were former smokers. Blood pressure measures indicated that all participants were normotensive. Based on body mass index, 39% of the sample were overweight and 41% were obese. Average sleep duration was 422.4 minutes or approximately 7 hours with an average sleep onset latency of 13 minutes, and average WASO of 69 minutes, and an average sleep fragmentation index of 34.5 %. Of the total sample, 79 persons (55%) were classified as insulin sensitive (IS) and 64 as insulin resistant (IR) by the euglycemic hyperinsulinemia clamp. Participant characteristics contrasting persons classified with IR ($M \leq 4.5$ mg·min$^{-1}$·kg$^{-1}$) with persons with IS in the normative range ($M > 4.5$ mg·min$^{-1}$·kg$^{-1}$) can be found in Table 1. Both IS and IR subjects exhibited comparable sex, ethnicity/race and former smoking habits composition, with no significant differences on age and socioeconomic status. IR subject had markedly greater total adiposity than IS subjects on average. Similarly, IR subjects compared with IS subjects displayed greater blood pressure, triglycerides, LDL-c and TC/HDL-c ratio, and lower HDL-c. Those with IR displayed, on average, about 2.9-fold more diminished insulin sensitivity than those classified IS. As can be seen in this table, although fasting glucose was significantly greater in the IR than IS subjects by

about 4%, fasting insulin was about 63% greater in the IR subjects than the IS subjects. In response to the standard OGTT, $AUC_{GLU}$ was comparable between IS and IR subjects, although IR subjects evidenced larger postprandial glycemia (~23%). In contrast, persons with IR displayed, on average, about 111% greater postprandial insulin secretion, indexed by $AUC_{INS}$. No significant difference between IS and IR groups was observed in β-GS or ESRS, although the groups differed in POT ratio; POT was more diminished in the IR subjects. In sum, subjects with IR appeared to display subclinical cardiometabolic abnormalities that suggests elevated CVD and T2DM risk.

Both IS and IR subjects exhibited comparable sleep duration and sleep onset latency. The IR group spent more time snoozing after waking, although those classified as IS had a significantly higher WASO. These findings suggest that the IR individuals spent more time in bed after waking in the morning and the IS individuals spent more time awake after the initial onset of sleep. The IS individuals also had a slightly higher fragmentation index than the IR group, a finding that trended toward significance.

**Relationship between insulin sensitivity and metabolism (Aim 1)**

For Aim 1, mixed linear modeling analyses, as described above, were used to examine the relationships between insulin sensitivity and postprandial insulin and glucose metabolic regulation by carbohydrate load. Results from Aim 1 are summarized in Table 2. As can be seen in this table, insulin sensitivity was significantly inversely related to both $AUC_{INS}$ and $AUC_{GLU}$. Insulin sensitivity was also significantly positively associated with POT, and inversely associated with ESRS, but not with significantly associated with β-GS. In addition, there was a significant main effect for carbohydrate load for $AUC_{INS}$, $AUC_{GLU}$ as well as for POT and β-GS, but not for ESRS. Significant interactions between

insulin sensitivity and carbohydrate load were observed for $AUC_{INS}$, $AUC_{GLU}$ and POT, but not for β-GS or ESRS.

Therefore, analyses suggest that in response to a double carbohydrate load, persons with more diminished insulin sensitivity displayed more elevated $AUC_{INS}$, which was not sufficient to bring postprandial glycemia, indexed by $AUC_{GLU}$, to the levels displayed by persons with higher insulin sensitivity. However, the difference in $AUC_{GLU}$ between persons with higher and lower insulin sensitivity to the double carbohydrate load was markedly less than the difference in postprandial insulinemia between persons with higher and lower insulin sensitivity. This finding illustrates the adaptive compensation of insulin secretion evidenced by the more insulin resistant subjects. The findings also showed that more insulin resistant persons did not elevate POT as much as more insulin sensitive persons in response to the double carbohydrate load. In sum, these findings confirm the carbohydrate load manipulation effect distinguishing more insulin sensitive from more insulin resistant persons in terms of postprandial $AUC_{INS}$, $AUC_{GLU}$, and POT regulation.

**Relationship between sleep duration and metabolism (Aim 2)**

Results from Aim 2 are summarized in Table 3. As can be seen in this table, there were no significant main effects for sleep duration for any metabolic outcomes. There were significant main effects for carbohydrate load for $AUC_{INS,}$ $AUC_{GLU}$, POT, and β-GS, but not for ESRS, as was reported in Aim 1. There were no significant interactions between sleep duration and carbohydrate load for any of the outcome variables. In sum, when sleep duration was assessed without consideration of insulin sensitivity, sleep

duration was not significantly related to postprandial metabolic function to the carbohydrate load manipulation.

**Association between insulin sensitivity and metabolism moderated by sleep duration (Aim 3 and 4)**

The results for Aim 3 are displayed in Table 4. For this aim, the significant effects observed in these models are reported below and then any significant interaction will be described using post hoc analyses. The model for Aim 3 contained all previous relationships examined in Aim 1 and Aim 2, with the addition of one two 2-way interaction (insulin sensitivity*sleep duration) and one 3-way interaction (insulin sensitivity*sleep duration*carbohydrate load). In this larger model, significant 2-way interactions between insulin sensitivity and carbohydrate load, and between insulin sensitivity and sleep duration for $AUC_{GLU}$ were observed. The insulin sensitivity interaction with carbohydrate load, as in Aim 1, reflects the inverse relationship between insulin sensitivity and postprandial glycemia. The insulin sensitivity interaction with sleep duration reflects a significant, positive relationship between insulin sensitivity and $AUC_{GLU}$ that depends on sleep duration. The 3-way interaction was not significant for $AUC_{GLU}$; therefore, the significant two-way interaction between sleep duration and insulin sensitivity was interpreted as the highest order interaction. As seen in Figure 4, there is a different pattern of postprandial glycemia for insulin sensitive versus insulin resistant individuals as a function of sleep duration for insulin sensitive versus insulin sensitive individuals. For those with more insulin sensitivity, glycemia is lower when sleep duration is shorter and becomes higher when sleep duration is longer. In contrast,

for those with more insulin resistance, glycemia is higher when sleep duration is shorter but glycemia is lower when sleep duration is longer.

The 3-way interaction was significant for $AUC_{INS}$ and β-GS. For ESRS, the only significant finding was the main effect, indicating the inverse association previously observed in Aim 1 between insulin sensitivity and ESRS. In contrast, for POT, a significant 2-way interaction was found between insulin sensitivity and carbohydrate load, reflecting the deficit in POT in persons with more insulin resistance. Moreover, a trend toward significance for POT ($P$=.054) was found for the 3-way interaction among insulin sensitivity, sleep duration and carbohydrate load. Table 5 displays the same analytic models as displayed in Table 4, but all analyses included covariates. As can be seen in Table 5, the findings were unchanged when controlling for these parameters.

Post hoc analyses were conducted for metabolic outcome variables that demonstrated significant 3-way interactions between insulin sensitivity, sleep duration, and carbohydrate load (i.e., $AUC_{INS}$, β-GS, and POT) to better understand these interrelationships. These analyses decomposed significant 2-way interactions between insulin sensitivity and carbohydrate load for $AUC_{INS}$, β-GS and POT, by testing the simple effect of different levels of sleep (i.e., short sleepers, average sleepers, and long sleepers). Sleep duration was mean centered and values of one standard deviation above were used to derive the long sleep group, the average was used for the average sleep group, and values of one standard deviation below were used to create the short sleep group for these analyses. The three different sleep time points are as follows: 6 hours (short sleep), 7 hours (average sleep), and 8 hours (long sleep). Results from post hoc analyses for each outcome variable are described below and plotted in Figures 1-3.

Simple slope analyses for $AUC_{INS}$ were conducted examining the 2-way interaction between insulin sensitivity and carbohydrate load for the short, average, and long sleepers. For short sleepers and average sleepers, there was a significant interaction, indicating that the strength of the relationship between insulin sensitivity and $AUC_{INS}$ was significantly different depending on carbohydrate load in these two groups (t=-4.52, $P<.001$; t=-5.12, $P<.001$). As can be seen in Figure 1, with shorter sleep duration, the amount of insulin secreted increases following a double carbohydrate load but not following a standard carbohydrate load. However, there was no significant interaction between insulin sensitivity and carbohydrate load for long sleepers, indicating that the strength of the relationship between insulin sensitivity and $AUC_{INS}$ does not depend on carbohydrate load for long sleepers.

Post hoc analyses for β-GS were conducted examining the 2-way interaction between insulin sensitivity and carbohydrate load for short, average, and long sleepers. In contrast to the 3-way interaction findings for $AUC_{INS}$ described above, β-GS demonstrated no significant interaction between insulin sensitivity and carbohydrate load for short sleepers or average sleepers, indicating that the strength of the relationship between insulin sensitivity and β-GS was not significantly different depending on carbohydrate load in these 2 groups. Thus, for short and average sleepers greater insulin resistance was associated with greater β-GS. However, as can be seen in Figure 2, there was a significant interaction between insulin sensitivity and carbohydrate load for long sleepers (t=2.12, $P=.04$), indicating that the strength of the relationship between insulin sensitivity and β-GS depends on carbohydrate load for long sleepers. Specifically for

long sleepers, the relationship between insulin sensitivity and β-GS becomes weaker following a double carbohydrate load but not for a standard carbohydrate load.

Simple slope analyses for POT were conducted examining the two-way interaction between insulin sensitivity and carbohydrate load for short, average, and long sleepers. For short sleepers and average sleepers, there was a significant interaction, indicating that the strength of the relationship between insulin sensitivity and POT was significantly different depending on carbohydrate load in these 2 groups (t=3.12, *P*=.002; t=2.74, *P*=.007). As can be seen in Figure 3, with shorter sleep duration, the relationship between insulin sensitivity and POT was weaker following a standard carbohydrate load but not following a double carbohydrate load. However, there was no significant interaction between insulin sensitivity and carbohydrate load for long sleepers, indicating that the strength of the relationship between insulin resistance and POT does not depend on carbohydrate load.

In sum, we found that decreased insulin sensitivity results in increased $AUC_{INS}$, a result that is exacerbated following a double carbohydrate load when compared to a standard carbohydrate load. As seen in Figure 1, for both short and average sleepers, greater insulin resistance is related to greater insulin response, a response that was stronger following a double versus a standard carbohydrate load. Of note, the overall postprandial insulinemia for short and average sleepers with more diminished insulin sensitivity following a double carbohydrate load was greater than the overall response for average and long sleepers with more diminished insulin sensitivity. Post hoc findings for β-GS, as seen in Figure 2, indicate that for insulin resistant long sleepers following a double carbohydrate load, β-GS was lower than for average and short sleepers who have

more diminished insulin sensitivity. Figure 3 describes the findings for POT, which showed that a double carbohydrate load resulted in a significant elevation of POT in all individuals. Notably, following a standard carbohydrate load, insulin sensitive shorter sleepers did not elevate POT as much as did the longer sleepers, whereas insulin resistant shorter sleepers appeared to increase POT more than the longer sleepers.

## *Chapter 5*

## DISCUSSION

Shortened sleep duration in otherwise healthy individuals has been linked to obesity and metabolic pathophysiology, and ultimately frank disease (Buxton et al., 2010; Copinschi, 2004; Knutson, 2010; Cappuccio et al., 2011; Grandner et al., 2010). The influence of sleep duration on these metabolic pathogenic pathways are not well understood, but may be operational long before the development of clinical diabetes or even prediabetes is detected (Hurwitz et al., 2015; Knutson, Spiegel, Penev, & Van Cauter, 2007; Knutson & Van Cauter, 2008; Mesarwi, Polak, Jun & Polotsky, 2013). With progression toward prediabetes and T2DM diagnosis, there is a gradual worsening of insulin mediated glucose uptake indexed by measures of insulin sensitivity (Brodovicz et al., 2011; Fonseca, 2009; Tabák, Herder, Rathmann, Brunner & Kivimäk, 2012; Guillausseau et al., 2008). While insulin sensitivity declines in persons at diabetes risk, there are adaptive alterations in metabolic function that occur to compensate, resulting in glycemic regulation within a normative range (Fu, Gilbert & Liu, 2013; Van Leeuwen et al., 2010; Cerf, 2013; Knutson et al., 2010; Grandner et al., 2010). We hypothesized that if the relationship between sleep duration and early subclinical metabolic pathophysiology were robust, then we should observe that shorter habitual sleep duration would be associated with metabolic adaptations postprandial response that serve to maintain glycemic regulation within relative normative ranges. Hence, this study evaluated in the context of the range of insulin sensitivity, sleep duration and its inter-relationship with postprandial insulin and glucose AUC, β-GS, ESRS and POT in response to standard and double carbohydrate load to 4 meal challenges carried out over a 14-hour period across two days of in-patient stay. We hypothesized that these

relationships would be stronger in response to double carbohydrate load meals, wherein the metabolic demand was substantially elevated. The extent to which alterations in sleep duration in prediabetic individuals account for metabolic dysfunction is unclear, perhaps obscured by mechanisms of metabolic adaptation. The main finding of the present study showed that the relationship between insulin sensitivity and postprandial insulin response following a carbohydrate load depended on sleep duration, such that with more elevated carbohydrate load and greater insulin resistance, shorter sleep duration was associated with more elevated postprandial insulin secretion. This finding was not observed when the relationship of sleep duration with study outcomes was examined, but only revealed when analyses considered both sleep duration and insulin sensitivity.

**Insulin Sensitivity and Metabolic Alterations**

Results from Aim 1 demonstrated the expected relationship between insulin sensitivity and altered metabolic functioning for $AUC_{INS}$, $AUC_{GLU}$, and POT. While ESRS was greater with more insulin resistance, all other metabolic outcomes were found to be significantly affected by the double carbohydrate load compared to the standard carbohydrate load as reported previously (Hurwitz et al., 2015). In sum, the double carbohydrate load induced an elevation in insulin secretion that was more than two-fold the insulin secretory response observed in the standard carbohydrate load. The consequence was that postprandial glycemia for the double carbohydrate load was within normative ranges and comparable for insulin sensitive and insulin resistant individuals, although glycemia was still somewhat elevated in persons with more insulin resistance. Thus, with greater insulin resistance, postprandial glycemia was greater with high carbohydrate loading. Our previous paper discusses these findings in more depth and

reviews the possible metabolic basis for the observed differences in insulin resistant compared with sensitive persons (Hurwitz et al., 2015). Although the present study is novel in the manner in which predictors and outcomes were measured and examined, the previous literature, in large part, found similar results. We found that individuals who are more insulin resistant compensate by secreting more postprandial insulin; this finding is not only well established in the literature but is suggestive of a relationship between insulin resistance and relative intolerance to carbohydrate excess, even in the presence of normal glucose tolerance as measured by conventional diagnostic criteria (Van Leeuwen et al., 2010; Knutson et al., 2010; Grandner et al., 2010).

**Sleep Duration and Metabolic Alteration**

On average, the cohort sleep duration was within the normal range when compared to other actigraphy studies assessing healthy groups (Grandner, Patel, Gehrman, Perlis, & Pack, 2010; Cespedes et al., 2016; Lauderdale et al., 2008; Girschik et al., 2012). In contrast to the literature linking sleep duration and metabolic functioning, results from Aim 2 did not demonstrate any significant relationships between sleep duration and postprandial metabolic response, even following a high carbohydrate load. Previous evidence suggesting that shortened sleep duration was associated with obesity and T2DM risk came mainly from two sources: 1) experimental studies of acute sleep restriction in laboratory settings; and 2) larger retrospective and prospective epidemiological studies examining the association of self-reported sleep duration (Grandner et al., 2010; Copinschi, Leproult, & Spiegel, 2014; Najafian, Mohamadifard, Sadri, & Rahmati, 2013; Cappuccio et al., 2011; Lee, Ng, & Chin, 2017). The landmark experimental study by Spiegel and colleagues (1999) examined young healthy normal-

weighted men who were successively subjected to three baseline nights with 8 hours in bed, six nights of sleep curtailment with 4 hours in bed, and six nights of sleep extension with 12 hours in bed, under strictly controlled conditions of physical activity and caloric intake. Results showed that sleep curtailment was associated with marked alterations of glucose metabolism. Specifically, acute insulin response, insulin sensitivity, glucose tolerance and glucose effectiveness were all markedly reduced in response to an intravenous glucose infusion in the restricted sleep condition when compared with the fully rested condition (Grandner et al., 2010). Several other experimental studies demonstrated deleterious effects of sleep restriction on glucose metabolism in laboratory-based investigations performed in healthy young subjects using either intravenous glucose tolerance test or euglycemic-hyperinsulinemic clamp to measure fasting insulin, glucose, or insulin sensitivity as outcomes (Copinschi, 2005; Reutrakul & Van Cauter, 2014).

In addition to experimental studies, several large cross-sectional and prospective population studies have demonstrated increased risk of developing T2DM for self-reported short sleepers (Reutrakul & Van Cauter, 2014; Knutson, 2010). These large observational studies have reported cross-sectional associations between short sleep duration and increased prevalence of diabetes or impaired glucose tolerance (Knutson, 2010; Knutson and Van Cauter, 2008). These studies found that shorter sleep durations (≤5h or ≤6h per night) increased the odds of diabetes; most studies relied on self-reported sleep duration. Another cross-sectional study (Chaput, Després, Bouchard & Tremblay, 2007) found an increased risk of prevalent diabetes for those self-reporting sleep duration less than 7 hours (OR 1.58, 95% CI 1.13, 2.31). In this study, the short sleepers also

demonstrated higher fasting plasma glucose, fasting plasma insulin, and HOMA insulin resistance index (Chaput et al., 2007). One study that did use wrist actigraphy to assess sleep function found greater sleep fragmentation in those with T2DM compared to healthy controls; however, there were no differences related to sleep duration (Knutson 2010; Trento et al., 2008).

A prospective meta-analysis of 107,756 participants concluded that the relative risk of the development of T2DM was 1.28 for self-reported short sleepers (≤5–6 h per night) after adjusting for possible confounders (Copinschi, 2004). The Nurses Health Study found an increased risk of incident diabetes in individuals reporting sleep durations of 5 hours or less over 10 years, even after controlling for relevant covariates such as BMI, shiftwork, hypertension, exercise and depression (Ayas et al., 2003). A prospective study conducted in Sweden followed 1,187 nondiabetic men and women free 12-years, and found that men who reported sleep duration of 5 hours or less had a significantly greater risk of developing T2DM (Mallon, Broman, & Hetta, 2005). Another prospective study from Sweden that followed over 600 women for 32 years did not find a relationship between self-reported sleep duration and incidence of diabetes over a 32-year period was not associated with the self-reported sleep duration at baseline (Björkelund et al., 2005). The Massachusetts Male Aging Study reported that a sleep duration of 6 hours or less per night was associated with twice the risk of developing diabetes in men after adjustment for relevant covariates (Yaggi, Araujo, & McKinlay, 2006). Finally, an examination of the First National Health and Nutrition Examination Survey found that those who self-reported sleep duration of five hours or less were at an increased risk of developing T2DM (Gangwisch et al., 2007). In sum, it appears that the majority of these studies,

which notably included a variety of different patient populations, consistently reported that short sleep may increase the risk of developing T2DM; however, nearly all of these epidemiologic studies relied on self-reported measures of sleep duration (Knutson and Van Cauter, 2008).

Several observational studies have also examined the association between self-reported sleep and obesity (Knutson, 2010). Numerous cross-sectional analyses have demonstrated significant associations between short sleep duration and increased prevalence of obesity or higher BMI (Patel & Hu, 2008; Knutson & Van Cauter, 2008; Marshall, Glozier, & Grunstein, 2008). Cappuccio and colleauges (2008) found that short sleep duration significantly predicted obesity in adults (pooled odds ratio [OR] was 1.55, 95% CI: 1.43–1.68); specifically, results showed BMI was a 0.35 $kg/m^2$ lower for every additional hour of sleep on average. Several international prospective studies also found relationships between self-reported shorter sleep duration and eventual weight gain in both men and women (Chaput, Després, Bouchard, & Tremblay, 2008; López-García et al., 2008; Watanabe, Kikuchi, Tanaka, & Takahashi, 2010). Thus, this literature suggests that sleep duration is associated with weight gain and higher BMI, which is related to the development of obesity and associated cardiometabolic disruption (Knutson et al., 2010).

In light of the findings, which appear to demonstrate a sleep duration-metabolic dysfunction relationship in persons with obesity and T2DM, one would expect that this same relationship would be found in preclinical samples. However, our results show no direct relationships between sleep duration and metabolic regulation. Based on the previous literature, it may be concluded that previously found relationships between sleep duration and metabolism are due to secondary complications of advanced disease

progression i.e., that this relationship strengthens with disease progression. Alternatively, it is possible that the relationship between sleep duration and metabolic function either is not present in healthy, nondiabetics or that there is possibly one or more factors suppressing the observation of this relationship in our study.

The present study differs from previous literature in that we did not experimentally manipulate sleep, or correlate sleep function prospectively with the obesity, T2DM or with future metabolic function. Our study measured sleep duration objectively using at-home actigraphy for approximately 1 week to estimate typical sleep patterns, rather than relying on self-reported measures of sleep. Previous studies that objectively measured sleep typically have used polysomnography, which is costly and often results in studies with small cohorts. Only a few studies have measured sleep duration using actigraphy in healthy, preclinical populations to investigate inter-relationships among insulin resistance and indices of metabolic function (Knutson et al., 2010; Lauderdale et al., 2009; Trent et al, 2008). A subset from the Coronary Artery Risk Development in Young Adults (CARDIA) cohort used actigraphy to measure sleep and found that those higher BMI was related to shorter average sleep durations when compared to longer sleep durations (Lauderdale et al., 2009). Another study found that nurses who slept 6 hours or less per night gained more weight over 16 years than those sleeping 7 hours after adjusting for age and baseline BMI (Patel, Malhotra, White, Gottlieb & Hu, 2006). However, as mentioned above, Trent and colleagues (2008) used actigraphy but did not find any significant relationship between sleep duration and metabolic outcomes. It is possible that other studies that reported weak or no sleep duration-metabolic function associations may not have suffered from some confounding

or suppression due to unaccounted variables or to insufficient metabolic challenge. These previous studies did not examine carbohydrate load meal challenges; the use of 14-hour meal challenge testing with manipulation of carbohydrate load may have permitted relationships to be observed in the present study that were not readily apparent under standard carb load conditions, when metabolic adaptations have greater capacity to compensate for diminished preclinical metabolic function. In addition, previous studies also often evaluated estimates of insulin sensitivity as a metabolic outcome rather than as a moderating variable and as such were not able to report the interactive influences of insulin sensitivity on metabolic dysfunction (Nedeltcheva, Kessler, Imperial & Penev, 2009; Van Cauter et al., 2008; Spiegel et al., 1999); Spiegel et al., 2004; Grandner et al., 2010; Copinschi et al., 2014).

**Insulin Resistance, Sleep Duration, and Metabolic Alteration**

Recall, in Aim 2, we found no significant association of sleep duration with postprandial metabolic outcomes, or moderation by carbohydrate load. In contrast, in Aim 3, we discovered relationships between postprandial measures of $AUC_{INS}$, ß-GS, and POT with insulin sensitivity that were moderated by sleep duration. Moreover, as seen in Aim 4, these results remained significant after controlling for relevant covariates including age, sex, education, blood pressure, triglycerides, cholesterol, and total visceral adiposity. Post hoc analyses were used to further understand the inter-relationships for these significant 3-way interactions among insulin sensitivity, sleep duration, and carbohydrate load. As illustrated in Figure 1, for $AUC_{INS}$, the relationship between total insulinemia and insulin resistance was different depending on carbohydrate load. With more diminished insulin sensitivity, higher postprandial insulinemia was observed across

sleep duration, and for both standard and double carbohydrate load conditions. Notably, however, with shorter sleep, there was greater difference in $AUC_{INS}$ response to the double than standard carbohydrate load for the insulin resistant than insulin sensitive group. Hence, short sleeping insulin resistant persons displayed greater postprandial insulinemia, particularly when challenged with the double carbohydrate load; whereas, short sleeping insulin sensitive persons displayed little or no difference in postprandial insulinemia between the standard and double carbohydrate load meals. These results appear to contradict the landmark study by Speigel et al. (1999), who examined postprandial metabolic response following experimental sleep restriction in healthy adults. The study found that, following sleep restriction, glycemic regulation was substantially worsened to a standardized breakfast despite similar elevation in insulin secretory response (Speigel et al., 1999). This apparent decline in insulin mediated glucose uptake to sleep curtailment was suggested as possible evidence that shortened sleep duration may be an etiological mechanism of the development of insulin resistance. It should be noted, Speigel and colleagues were studying acute not chronic alteration in sleep duration; it is unknown whether there is an adaptation in metabolic regulation in persons whose sleep is chronically shortened. Sleep deprivation studies have been criticized for lacking ecological validity because of differences in function that may exist between sleep in the laboratory and sleep in one's home. Our study clearly differs from the Speigel study in that our measures of sleep function were observed for about one week in study participants so called "natural environment". Although still cross-sectional, such a measure would reflect more habitual sleep function. Thus, our measures of shorter and longer sleep duration have greater likelihood of reflecting more chronic sleep

duration tendencies. We reasoned that it is also more likely that if a metabolic adaptation to chronically shortened sleep does occur in persons with altered sleep duration, its influence on metabolic function would be operational and our results would reflect this effect. Therefore, it is possible that the differences between the Speigel study and our study may simply reflect differences in the metabolic function of persons not adapted to shortened sleep compared with the metabolic function of persons adapted to shortened sleep.

As illustrated in Figure 2 for ß-GS, there appears a significant 3-way relationship among insulin sensitivity, sleep duration and carbohydrate load. For both short and average sleepers, when faced with the double carbohydrate load condition, there appears to be a consistent increase in ß-GS across insulin sensitivity level; as shown in Figure 2, ß-GS increases more for insulin resistant than insulin sensitive persons (Hurwitz et al., 2015). However, this pattern differs for long sleepers. Specifically, for the insulin resistant longer sleepers, there is little or no difference in ß-GS for standard and double carbohydrate load conditions. In contrast, the increased response to double carbohydrate loading in ß-GS observed in the insulin sensitive short and average sleepers was significant. Therefore, the insulin resistant long sleepers do not demonstrate the elevation in ß-GS to the double carbohydrate load seen in the insulin sensitive persons with shorter sleep duration.

As illustrated in Figure 3, the elevation in POT to the double carbohydrate load meals was greater in the insulin sensitive than insulin resistant persons across sleep duration. Notably, the significant interaction between insulin sensitivity and POT by carbohydrate load occurred in short and average sleepers. Specifically, with shorter sleep

duration the relationship between insulin sensitivity and POT was dampened in the standard carbohydrate load condition, but strengthened in the double carbohydrate load condition. Thus, to the standard carbohydrate load, shorter sleeping persons, who are more insulin sensitive, displayed slightly less POT; in contrast, shorter sleeping persons, who are more insulin resistant, displayed slightly less POT to the double carbohydrate load condition.

Although we found differences on β-GS functioning only in long sleepers, recent literature alludes to a possible U-shaped relationship between sleep duration and metabolism in patients with frank T2DM. A large cross-sectional study of 4,870 Japanese participants revealed a U-shaped relationship between sleep duration and glycemic control, compared to those with 6.5-7.4 h/night of self-reported sleep, higher HbA1c levels (i.e., poorer glycemic control) was observed in patients with sleep duration less than 5.5 h/night and with greater than or equal to 8.5 h/night (Ohkuma et al., 2012). More recent epidemiological studies have also suggested that short and long sleep durations were associated with an increased HbA1c compared to normal sleep (Lee et al., 2017). Taking these studies into account, the present findings linking greater insulin resistance to reduced β-GS following a double carbohydrate load in longer sleepers but not in average or shorter sleepers may suggest some alteration in pancreatic glucose-sensing mechanisms in these persons. It is not clear why this may be happening in long sleepers and not in shorter sleepers. Further studies are necessary to evaluate the impact of prolonged sleeping to determine the underlying basis for this apparent metabolic alteration.

Previously, we found that the augmented insulin secretory response to a double carbohydrate load was partially mediated by increased β-GS and POT (Hurwitz et al., 2015). Both glucose-induced (i.e., persistence of raised glycemia) and non–glucose-induced (i.e., incretin effects) mechanisms were posited to be responsible for the elevation in POT (Holst, Vilsbøll, & Deacon, 2009). Others have shown that glucose-induced potentiation is enhanced and incretin-induced potentiation is depressed in hyperglycemic patients (Tura, Muscelli, Gastaldelli, Ferrannini, & Mari, 2014). It is not clear whether the deficit in POT elevation to high carbohydrate loading observed with greater insulin resistance was a consequence of one or both of these mechanisms, or due to some deficit in CNS-pancreatic communication or of the pancreas insulin synthesis or secretory mechanisms. Our analyses of POT functioning indicated a sluggish response for more insulin sensitive shorter sleepers after a standard carbohydrate load; although these individuals have the capacity to elevate their POT, as evidenced by the double carbohydrate load responses across all sleep groups, this elevation in POT in shorter sleepers appears not to be as vigorous when carbohydrate loading is within standard ranges. One possible mechanism may be that mechanisms triggering an up-regulation of pancreatic insulin secretion response upon the evaluation of the glycemic concentration following postprandial absorption of a meal are down-regulated when prevailing glycemia is within a normative range but become fully engaged when there is a more persistent elevation in glycemia beyond this range. Notably, the significant two-way interaction between insulin sensitivity and sleep duration for $AUC_{GLU}$ may provide some support for this interpretation. The finding indicated that sleep duration moderates the relationship between insulin sensitivity and glycemia, such that insulin sensitive

individuals displayed greater postprandial glycemia if they were longer duration sleepers. This finding supports the notion that when persons are insulin sensitive but sleep duration is more prolonged the glycemic regulation is not as good as when sleep is shorter. Thus, the finding that POT is greater in these insulin sensitive longer sleeping individuals suggests that this postprandial response is triggered by these elevated glycemic levels. In contrast, the insulin resistant individuals displayed the opposite pattern of glycemic regulation. Specifically, these persons showed greater postprandial glycemia if their sleep duration was shorter compared with more prolonged sleeping counterparts. These findings correspond with the observed postprandial insulinemia, which was greater in the insulin resistant shorter sleepers than longer sleepers to the double carbohydrate loading condition. Moreover, these insulin resistant shorter sleepers display an elevation in β-GS during these meals that possibly reflects an attempt by these individuals to compensate for their more elevated glycemia. Notably, the insulin resistant short sleepers are not able to elevate their POT in response to the double carbohydrate loading, which supports the hypothesis that this adaptational mechanism may be deficient in these individuals. Further studies are necessary to replicate these findings and elucidate possible mechanisms underlying the dynamic relationships among sleep duration, insulin sensitivity, and postprandial glycemia in mediating the adaptation of insulin secretion in response to elevations in carbohydrate load.

## *Chapter 6*

# CONCLUSION

The present findings replicate previous relationships in the literature that link shortened sleep and insulin resistance with metabolic alterations, but add novel findings due to the use of a more objectively measured sleep methodology, use of the gold-standard measure of insulin sensitivity, use of carbohydrate load manipulation to assess dynamic long-term metabolic function, and use of quantitative modeling of metabolic outcomes to derive indices of pancreatic function. Our results showed that significant changes in insulinemia due to meal provocation resulted in glycemia regulated to within normative ranges, regardless of prevailing insulin sensitivity. Of note, those with greater insulin resistance demonstrated more elevated postprandial insulinemia as a metabolic adaptation to this condition, and as such, their postprandial glycemia was not significantly moderated by sleep duration. However, the major finding of this study, which has not been reported previously, is that with shorter sleep duration, postprandial pancreatic mechanisms mediating insulin secretion are substantially elevated in more insulin resistant persons; thus, in these persons, sufficient insulin is secreted to manage a high carbohydrate load. Moreover, our findings suggest that there are more nuanced alterations in pancreatic ß-GS and POT that are apparent in shorter and longer sleepers. However, further investigation is required to determine whether there is any metabolic impact or clinical relevance of these alterations. It is of some importance, however, that the observed differences in sleep duration moderation of β-GS and POT appear in a different pattern than the observed sleep duration moderation of $AUC_{INS}$. Moreover, there was no moderation of ESRS by sleep duration. Therefore, because the patterns of

relationships among sleep duration and β-GS, ESRS and POT are dissimilar from the patterns observed between sleep duration and $AUC_{INS}$, it may be concluded that altered β-GS, ESRS and POT function does not likely account for the observed heightened insulin response in insulin resistant short sleepers. Thus, some mechanism other than that measured in this study is responsible for the differences in insulin metabolic response to high carbohydrate loading in these individuals. Further studies are necessary to delineate whether there are alterations in some aspect of sleep function or architecture beyond sleep duration that may mediate the heightened insulin secretion response to high carbohydrate loading in insulin resistant individuals.

**Strengths and Limitations**

Strengths of our study include our rigorous methodology for the objective measurement of sleep and metabolic functioning, and manipulation of meal composition, permitting assessment of long-term metabolic function more reflective of daily life. Our study was conducted over a 3-day/night inpatient laboratory stay, during which participants were closely monitored and data were meticulously collected. Our study utilized the gold-standard for measuring insulin sensitivity, the euglycemic clamp; habitual sleep was more objectively measured using actigraphy, and the manipulation of carbohydrate loads using repeated meal challenges permitted the derivation of measures of pancreatic postprandial functioning. Moreover, analyses comprehensively controlled for covariates, indicating significant study findings were independent of sex, age, education, smoking status, and subclinical measures of CVD risk including blood pressure, triglycerides, cholesterol profile, and CRP.

Our study was not without limitations. The study cohort represented primarily Hispanic/Latino adults of various subgroups living in the US. Thus, the study did not include enough individuals of other ethnic groups, such as African-Americans or Caucasians, to allow for comparisons across ethnicities. Our study was also limited by its cross-sectional design, making it impossible to infer temporal precedence or infer that altered sleep duration caused the observed differences in metabolic function.

Nevertheless, our study provides significant insight on possible preclinical markers of metabolic disease development in otherwise healthy normal men and women, and sheds light on the possible moderating role of sleep duration and insulin sensitivity in the progression of metabolic dysregulation in preclinical diabetic pathophysiology. In addition, the present findings demonstrate the importance of examining postprandial alterations rather than fasting insulin and glucose function to better understand possible dysfunction in preclinical samples. Previous studies have illustrated relationships between sleep duration and metabolic function but with small to moderate effect sizes (Copinschi, 2004; Knutson, 2010; Cappuccio et al., 2011; Grandner et al., 2010). Many of these studies include diabetic or other clinical populations in the sample, which may have afforded them better opportunity to observe the predictive association of sleep duration with altered metabolic function. Our findings suggest that previous small to moderate effect sizes may be a consequence of the failure to account for insulin sensitivity, which plays a moderating role in the sleep duration-metabolic function relationship. The present findings add to the literature by showing that the relationship between shortened sleep duration and early alterations in postprandial metabolic function is present in preclinical individuals without clinically diagnosed CVD or metabolic

disease. An additional study strength pertained to the use of the long-term double carbohydrate load challenges because most of our study differences in sleep moderation effects were not observed when standard meal compositions were employed.

In conclusion, results from the present study provide formative support for the hypothesis that shortened sleep may be etiologically associated with the development of insulin resistance and metabolic pathophysiology resulting in T2DM. Future directions should examine the extent of metabolic dysfunction in individuals with chronic sleep deprivation related to habitual carbohydrate consumption to further ascertain the underlying mechanisms that may link sleep function and subclinical cardiometabolic pathophysiology.

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**Table 1. Clinical Characteristics of the Insulin Sensitive and Insulin Resistant Groups (n = 143).[abc]**

| | IS (n=64) | IR (n=79) | p-value |
|---|---|---|---|
| Demographics | | | |
| Age (years) | 37.2 ± 1 | 39.6 ± 1 | ns |
| Sex (% men) | 64 | 66 | ns |
| Ethnicity/Race (%) | | | |
| Black | 14.4 | 17.7 | ns |
| Hispanic white | 70.3 | 77.2 | |
| Non-Hispanic White | 10.9 | 2.5 | |
| Family annual income ($k) | 13.5 ± 1.8 | 14.6 ± 2.1 | ns |
| Education (years) | 13.1 ± 0.3 | 13.4 ± 0.3 | ns |
| Former Smoker (%) | 10.9 | 19.0 | ns |
| BMI ($kg/m^2$) | 26.3 ± 0.4 | 31.7 ± 0.5 | **** |
| TAT (L) | 9.0 ± 0.5 | 14.9 ± 0.5 | ns |
| VAT (L) | 2.8 ± 0.2 | 4.8 ± 0.2 | ns |
| SAT (L) | 6.1 ± 0.3 | 10.1 ± 0.4 | * |
| Cardiometabolic Function | | | |
| SBP (mmHg) | 114.2 ± 1.5 | 119.7 ± 1.5 | ns |
| DBP (mmHg) | 78.4 ± 1.5 | 84.2 ± 1.2 | ns |
| LDL-c (mg/dl) | 114.4 ± 3.8 | 122.4 ± 3.3 | ns |
| HDL-c (mg/dl) | 49.6 ± 1.6 | 40.4 ± 1.2 | *** |
| Total cholesterol/HDL ratio | 3.9 ± 0.2 | 5.0 ± 0.2 | ns |
| Triglycerides (mg/dl) | 98.9 ± 5.9 | 166.2 ± 2.5 | *** |
| Metabolic Outcomes | | | |
| $HbA_{1c}$ (%) | 5.3 ± 0.04 | 5.5 ± 0.05 | * |
| Insulin sensitivity ($mg \cdot min^{-1} \cdot kg^{-1}$) | 7.8 ± 0.3 | 2.7 ± 0.1 | *** |
| FPG (mmol/L) | 5.0 ± 0.1 | 5.2 ± 0.1 | ** |
| FPI (pmol/L) | 67.9 ± 4.5 | 110.4 ± 6.1 | *** |
| $AUC_{INS}$ ($pmol \cdot L^{-1} \cdot h^{-1}$) | 15343.9 ± 960.2 | 32397.0 ± 2246.3 | *** |
| $AUC_{GLU}$ ($mol \cdot L^{-1} \cdot . h^{-1}$) | 331.3 ± 6.5 | 409.0 ± 8.9 | *** |
| β-GS ($pmol \cdot min^{-1} \cdot m^{-2} \cdot [mmol/L]^{-1}$) | 115.7 ± 73.2 | 113.8 ± 57.0 | ns |
| ESRS ($nmol \cdot m^{-2} \cdot [mmol/L]^{-1}$) | 1316.8 ± 88.5 | 1320.7 ± 99.6 | ns |
| POT (ratio) | 1.6 ± 0.07 | 1.4 ± 0.05 | * |
| Sleep Variables | | | |
| Sleep Duration (min) | 432.2 ± 10.2 | 412.3 ± 10.3 | ns |
| Sleep Onset Latency (min) | 12.1 ± 2.1 | 14.0 ± 2.5 | ns |
| WASO (min) | 73.4 ± 4.7 | 64.9 ± 3.8 | *** |
| Fragmentation Index (%) | 35.3 ± 2.0 | 33.4 ± 1.5 | * |

[a] Data are mean ± SE unless otherwise indicated.

[b] Abbreviations are: IS, insulin sensitive; IR, insulin resistant; BMI, body mass index; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; $HbA_{1c}$, glycosylated hemoglobin; FPI, fasting plasma insulin; FPG, fasting plasma glucose; $AUC_{INS}$, total insulinemia; $AUC_{GLU}$, total glycemia; β-GS, beta cell glucose sensitivity; ESRS, early

secretion rate sensitivity, POT, potentiation; WASO, wake after sleep onset.

[c] *$P$<0.05; **$P$< 0.01; ***$P$< 0.001; ns, not significant

**Table 2. Regression Results from Aim 1- Insulin Sensitivity and Carbohydrate Load**

| | | B | t-value | p-value |
|---|---|---|---|---|
| **$AUC_{INS}$** | | | | |
| | Intercept | 89056.20 | 20.20 | .000** |
| | Carbohydrate Load | -33187.77 | -9.26 | .000** |
| | Insulin Sensitivity | -12184.98 | -8.77 | .000** |
| | CL x IS | 5483.09 | 4.86 | .000** |
| **$AUC_{GLU}$** | | | | |
| | Intercept | 91575.51 | 70.04 | .000** |
| | Carbohydrate Load | -6442.32 | -5.19 | .000** |
| | Insulin Sensitivity | -3446.17 | -8.37 | .000** |
| | CL x IS | 1354.12 | 3.47 | .001** |
| **B-GS** | | | | |
| | Intercept | 122.37 | 35.36 | .000** |
| | Carbohydrate Load | -19.75 | -6.49 | .000** |
| | Insulin Sensitivity | -1.61 | -1.48 | .141 |
| | CL x IS | -0.99 | -1.04 | .302 |
| **ESRS** | | | | |
| | Intercept | 1366.96 | 22.83 | .000** |
| | Carbohydrate Load | -83.63 | -1.34 | .182 |
| | Insulin Sensitivity | -67.73 | -3.59 | .000** |
| | CL x IS | 6.22 | 0.32 | .752 |
| **POT** | | | | |
| | Intercept | 2.70 | 35.82 | .000** |
| | Carbohydrate Load | -0.81 | -8.75 | .000** |
| | Insulin Sensitivity | 0.12 | 5.02 | .000** |
| | CL x IS | -0.07 | -2.49 | .014** |

**Table 3. Regression Results from Aim 2- Sleep Duration and Carbohydrate Load**

| | | B | t-value | p-value |
|---|---|---|---|---|
| **$AUC_{INS}$** | | | | |
| | Intercept | 90260.19 | 16.84 | .000** |
| | Carbohydrate Load | -33305.13 | -8.17 | .000** |
| | Sleep Duration | -130.54 | -1.68 | .096 |
| | CL x SD | 41.72 | 0.70 | .483 |
| **$AUC_{GLU}$** | | | | |
| | Intercept | 91829.82 | 58.68 | .000** |
| | Carbohydrate Load | -6549.51 | -4.83 | .000** |
| | Sleep Duration | -28.75 | -1.26 | .208 |
| | CL x SD | 4.24 | 0.22 | .830 |
| **B-GS** | | | | |
| | Intercept | 121.72 | 33.70 | .000** |
| | Carbohydrate Load | -18.68 | -5.99 | .000** |
| | Sleep Duration | -0.05 | -1.01 | .315 |
| | CL x SD | 0.03 | 0.70 | .484 |
| **ESRS** | | | | |
| | Intercept | 1378.54 | 21.29 | .000** |
| | Carbohydrate Load | -91.16 | -1.41 | .160 |
| | Sleep Duration | -1.17 | -1.25 | .214 |
| | CL x SD | 0.80 | 0.85 | .397 |
| **POT** | | | | |
| | Intercept | 2.68 | 32.69 | .000** |
| | Carbohydrate Load | -0.78 | -8.07 | .000** |
| | Sleep Duration | -0.0002 | -0.15 | .885 |
| | CL x SD | 0.0006 | 0.43 | .671 |

**Table 4. Regression Results from Aim 3- Two-way and Three-way Interactions**

| | | B | t-value | p-value |
|---|---|---|---|---|
| **$AUC_{INS}$** | | | | |
| | **Intercept** | 87190.27 | 18.80 | .000** |
| | **Carbohydrate Load** | -31538.85 | -8.31 | .000** |
| | **Insulin Sensitivity** | -13431.75 | -8.97 | .000** |
| | **Sleep Duration** | -14.64 | -0.22 | .830 |
| | **CL x IS** | 6259.45 | 5.11 | .000** |
| | **SD x IS** | 68.26 | 2.72 | .007** |
| | **CL x SD** | -16.47 | -0.30 | .763 |
| | **CL x SD x IS** | -42.49 | -2.07 | .041** |
| **$AUC_{GLU}$** | | | | |
| | Intercept | 91092.17 | 66.08 | .000** |
| | **Carbohydrate Load** | -6260.29 | -4.70 | .000** |
| | **Insulin Sensitivity** | -3644.23 | -8.18 | .000** |
| | **Sleep Duration** | 1.51 | 0.08 | .941 |
| | **CL x IS** | 1450.74 | 3.37 | .001** |
| | **SD x IS** | 15.49 | 2.07 | .039** |
| | **CL x SD** | -7.75 | -0.40 | .693 |
| | **CL x SD x IS** | -6.02 | -0.84 | .835 |
| **B-GS** | | | | |
| | Intercept | 120.85 | 33.27 | .000** |
| | **Carbohydrate Load** | -17.58 | -5.62 | .000** |
| | **Insulin Sensitivity** | -2.01 | -1.71 | .088 |
| | **Sleep Duration** | -0.03 | -0.57 | .569 |
| | **CL x IS** | -0.50 | -0.49 | .623 |
| | **SD X IS** | 0.02 | 1.18 | .238 |
| | **CL x SD** | 0.02 | 0.46 | .064 |
| | **CL x SD x IS** | -0.04 | -2.12 | .036** |
| **ESRS** | | | | |
| | Intercept | 1372.77 | 21.58 | .000** |
| | **Carbohydrate Load** | -79.34 | -1.20 | .237 |
| | **Insulin Sensitivity** | -65.91 | -3.21 | .002** |
| | **Sleep Duration** | -0.72 | -0.77 | .44` |
| | **CL x IS** | 2.78 | 0.13 | .896 |
| | **SD x IS** | 0.04 | 0.11 | .909 |
| | **CL x SD** | 0.63 | 0.66 | .513 |
| | **CL x SD x IS** | -0.37 | -1.04 | .301 |
| **POT** | | | | |
| | **Intercept** | 2.77 | 29.81 | .000** |
| | **Carbohydrate Load** | -0.90 | -7.31 | .000** |
| | **Insulin Sensitivity** | 0.14 | 4.60 | .000** |
| | **Sleep Duration** | -0.001 | -0.91 | .362 |
| | **CL x IS** | -0.10 | -2.41 | .017** |

| | | | |
|---|---|---|---|
| **SD x IS** | -0.003 | -0.53 | .596 |
| **CL x SD** | 0.002 | 0.98 | .331 |
| **CL x SD x IS** | 0.001 | 1.80 | .059** |

**Table 5. Regression Results from Aim 4- Two-way and Three-way Interactions plus Covariates**

| | | B | t-value | p-value |
|---|---|---|---|---|
| **$AUC_{INS}$** | | | | |
| | **Intercept** | 128527.69 | 2.31 | .022** |
| | **Carbohydrate Load** | -31692.60 | -8.29 | .000** |
| | **Insulin Sensitivity** | -10621.05 | -6.14 | .000** |
| | **Sleep Duration** | -3.63 | -0.05 | .958 |
| | **CL x IS** | 6286.72 | 5.11 | .000** |
| | **SD x IS** | 67.73 | 2.78 | .006** |
| | **CL x SD** | -18.28 | -0.33 | .745 |
| | **CL x SD x IS** | -42.10 | -2.04 | .043** |
| | **Sex** | -15888.70 | -1.57 | .120 |
| | **Age** | -1127.38 | -2.09 | .039** |
| | **Education** | 922.44 | 0.62 | .539 |
| | **SBP** | -49.74 | -0.16 | .871 |
| | **Triglycerides** | -27817.60 | -1.27 | .207 |
| | **Cholesterol/HDL ratio** | 5106.37 | 1.34 | .184 |
| | **CRP** | 3688.47 | 2.51 | .014** |
| | **Smoker Status** | 12675.47 | 0.53 | .600 |
| | **VATVol** | 6378.40 | 2.04 | .044** |
| **$AUC_{GLU}$** | | | | |
| | Intercept | 92801.62 | 5.68 | .000** |
| | **Carbohydrate Load** | -6342.55 | -4.73 | .000** |
| | **Insulin Sensitivity** | -2835.34 | -5.43 | .000** |
| | **Sleep Duration** | -1.23 | -0.06 | .953 |
| | **CL x IS** | 1465.32 | 3.39 | .001** |
| | **SD x IS** | 14.64 | 1.97 | .050** |
| | **CL x SD** | -8.71 | -0.44 | .658 |
| | **CL x SD x IS** | -5.81 | -0.80 | .423 |
| | **Sex** | 1282.78 | 0.43 | .668 |
| | **Age** | -116.04 | -0.73 | .467 |
| | **Education** | -99.59 | -0.23 | .821 |
| | **SBP** | -20.70 | -0.23 | .818 |
| | **Triglycerides** | -4049.58 | -0.63 | .531 |
| | **Cholesterol/HDL ratio** | 1147.80 | 1.02 | .309 |
| | **CRP** | 736.27 | 1.70 | .092 |
| | **Smoker Status** | 18124.39 | 2.56 | .012** |
| | **VATVol** | 1735.67 | 1.87 | .062 |

| | | | | |
|---|---|---|---|---|
| **B-GS** | | | | |
| | **Intercept** | 123.25 | 2.69 | .008** |
| | **Carbohydrate Load** | -17.60 | -5.58 | .000** |
| | **Insulin Sensitivity** | -0.48 | -0.34 | .738 |
| | **Sleep Duration** | 0.001 | 0.02 | .984 |
| | **CL x IS** | -0.50 | -0.49 | .627 |
| | **SD x IS** | 0.02 | 1.10 | .274 |
| | **CL x SD** | 0.02 | 0.45 | .652 |
| | **CL x SD x IS** | -0.04 | -2.10 | .037** |
| | **Sex** | -2.85 | -0.34 | .734 |
| | **Age** | -0.8 | -1.80 | .075 |
| | **Education** | 0.17 | 0.14 | .892 |
| | **SBP** | 0.26 | 1.04 | .303 |
| | **Triglycerides** | -13.62 | -0.75 | .452 |
| | **Cholesterol/HDL ratio** | -0.26 | -0.08 | .934 |
| | **CRP** | 1.20 | 0.99 | .322 |
| | **Smoker Status** | -7.69 | -0.39 | .699 |
| | **VATVol** | 5.81 | 2.25 | .026** |
| **ESRS** | | | | |
| | Intercept | 2181.31 | 2.85 | .005** |
| | **Carbohydrate Load** | -75.39 | -1.14 | .257 |
| | **Insulin Sensitivity** | -53.14 | -2.15 | .033** |
| | **Sleep Duration** | -0.42 | -0.42 | .673 |
| | **CL x IS** | 2.08 | 0.10 | .923 |
| | **CL x SD** | 0.01 | 0.02 | .984 |
| | **CL x SD x IS** | 0.68 | 0.70 | .485 |
| | **Sex** | -0.38 | -1.06 | .290 |
| | **Age** | -5.59 | -0.04 | .968 |
| | **Education** | -7.57 | -1.02 | .312 |
| | **SBP** | -14.33 | -0.69 | .489 |
| | **Triglycerides** | -2.81 | -0.67 | .506 |
| | **Cholesterol/HDL ratio** | -102.45 | -0.34 | .736 |
| | **CRP** | -13.98 | -0.27 | .791 |
| | **Smoker Status** | 40.37 | 1.99 | .049** |
| | **VATVol** | 67.84 | 0.20 | .839 |

| | | | | |
|---|---|---|---|---|
| **POT** | | | | |
| | **Intercept** | 1.11 | 1.22 | .224 |
| | **Carbohydrate Load** | -0.88 | -7.18 | .000** |
| | **Insulin Sensitivity** | 0.14 | 4.35 | .000** |
| | **Sleep Duration** | -0.001 | -0.56 | .576 |
| | **CL x IS** | -0.10 | -2.54 | .012** |
| | **SD x IS** | -0.0004 | -0.94 | .347 |
| | **CL x SD** | 0.002 | 1.13 | .262 |
| | **CL x SD x IS** | 0.001 | 1.74 | .059# |
| | **Sex** | -0.62 | -3.74 | .000** |
| | **Age** | 0.005 | 0.62 | .534 |
| | **Education** | 0.01 | 0.49 | .622 |
| | **SBP** | 0.01 | 2.49 | .014** |
| | **Triglycerides** | 0.12 | 0.35 | .727 |
| | **Cholesterol/HDL ratio** | 0.02 | 0.25 | .802 |
| | **CRP** | -0.002 | -0.08 | .940 |
| | **Smoker Status** | -0.57 | -1.45 | .150 |
| | **VATVol** | -0.07 | -1.39 | .166 |

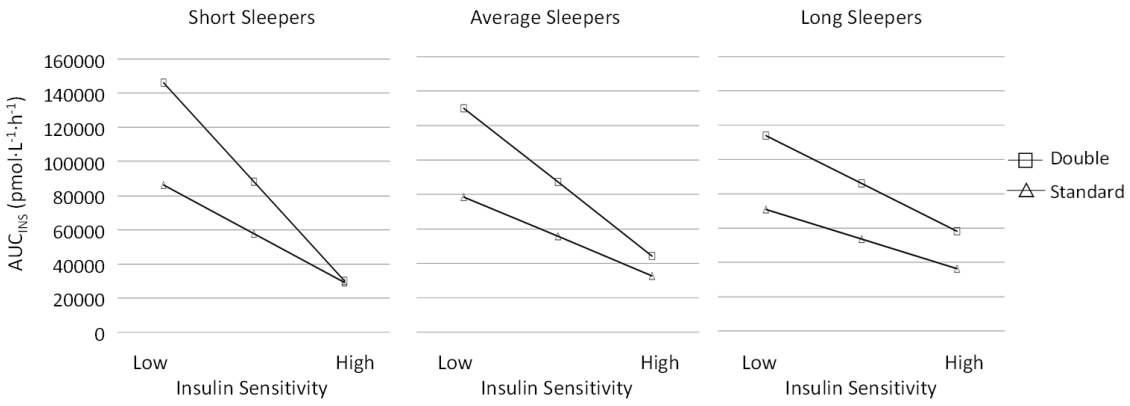


**Figure 1** —Interaction between Insulin Sensitivity and Carbohydrate Load for $AUC_{INS}$ by Sleep Group. Double and Standard Refer to Carbohydrate Load.

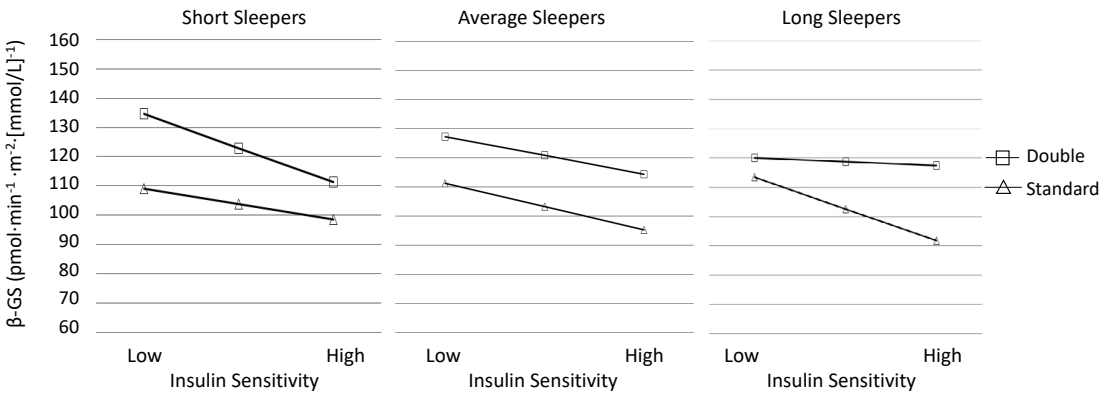


**Figure 2** —Interaction between Insulin Sensitivity and Carbohydrate Load for β-GS by Sleep Group. Double and Standard Refer to Carbohydrate Load.

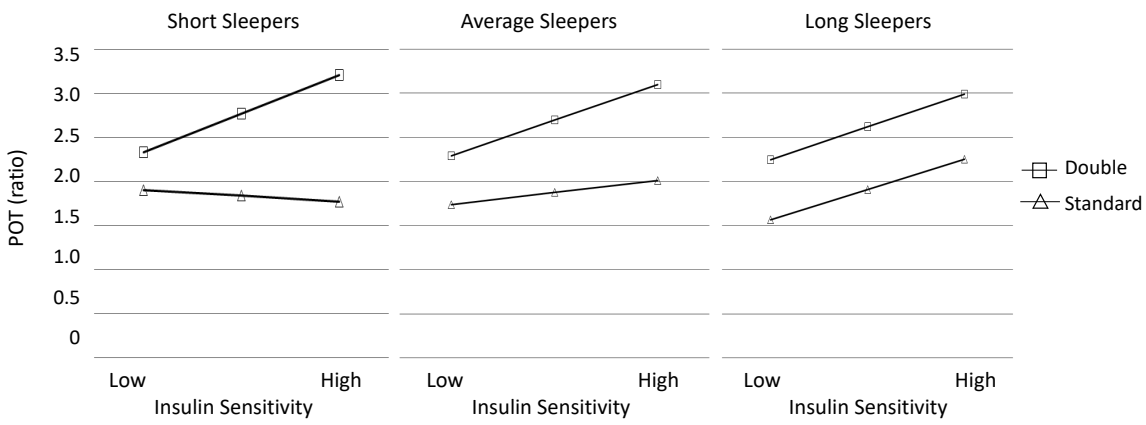


**Figure 3** —Interaction between Insulin Sensitivity and Carbohydrate Load for POT by Sleep Group. Double and Standard Refer to Carbohydrate Load.

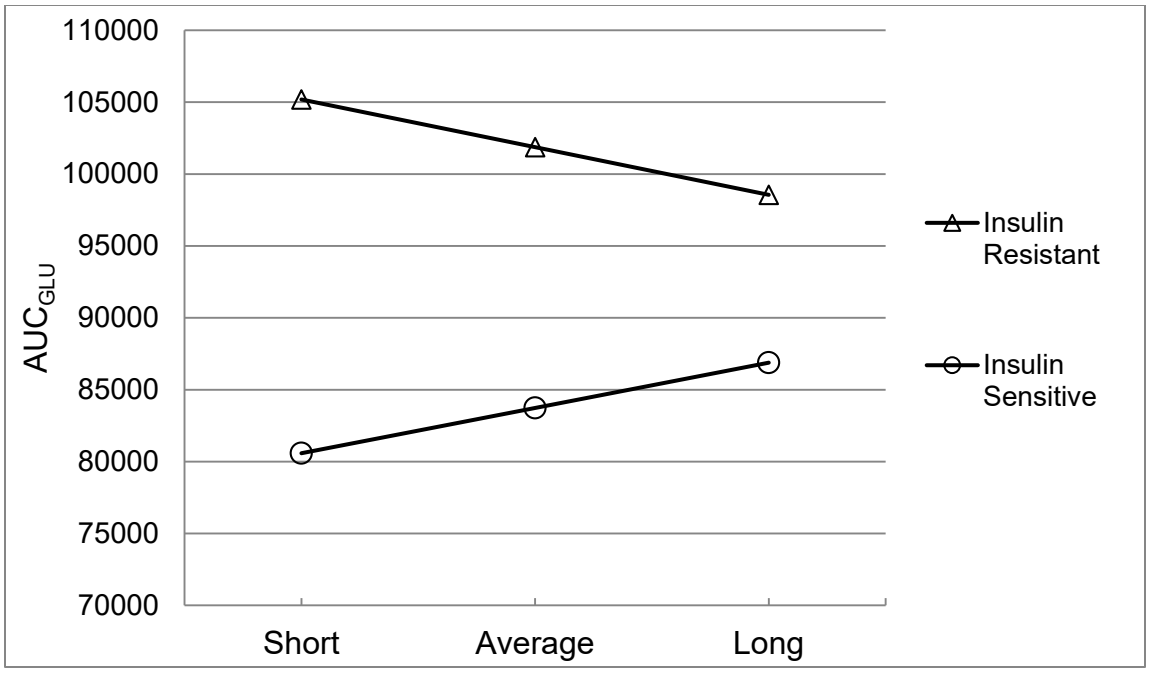


**Figure 4**- Interaction between Insulin Sensitivity and Sleep Duration for $AUC_{GLU}$.