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# Influence of Sleep Duration on Insulin Sensitivity

Brian Perry
*University of Colorado Boulder*

# INFLUENCE OF SLEEP DURATION ON INSULIN SENSITIVITY

BRIAN PERRY

AN HONORS THESIS

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**Committee**

Kenneth P. Wright Jr., Ph.D., Advisor, Department of Integrative Physiology

Leigh Perreault, M.D., UC Denver School of Medicine

David E. Sherwood, Ph.D., Honors Council Member

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**ABTRACT**

**Introduction:**
The metabolic syndrome is reaching epidemic proportions worldwide. Sleep duration has been shown to be important in glucose homeostasis. It has been reported that the specific stages of sleep (most importantly slow wave sleep and rapid eye movement sleep) each play their part in glucose homeostasis. The aim of this thesis was to determine if partial sleep deprivation has an impact on insulin sensitivity. Specifically, we sought to determine if 3 nights of 5h sleep opportunity when compared to 3 nights of 9h sleep opportunity with food being *ad libitum*, would result in decreased insulin sensitivity.

**Methods:**
13 healthy subjects (7 men, 6 women) aged 24.77 ± 4.02 (Mean ± SD), participated in the study. Subjects were admitted to the Clinical Translational Research Center (CTRC) at the University of Colorado Health Sciences Center for the duration of the study. Subjects lived in a hospital room for 14-15 days, depending on which sleep deprivation protocol they were randomized to. Total sleep time (TST) and sleep efficiency (SE) were assessed via actigraphy daily. Plasma glucose and insulin levels were obtained from oral glucose tolerance tests (OGTTs), assessed in the morning following a 9h baseline night, 3 days of 9h sleep per night, and 3 days of 5h sleep per night. Insulin sensitivity was assessed using the Matsuda index and HOMA-IR. Mixed model ANOVA with subject as a random factor and day, time and/or condition as fixed factors was used to analyze data.

**Results:**
Main effects of day ($p<0.000001$) and of subject ($p<0.000001$) were seen for TST. Planned comparisons showed significantly less TST for each day of the 5h sleep opportunity condition, as compared to baseline and each respective 9h day sleep opportunity. Main effects of day ($p<0.001$) and of subject ($p<0.000001$) were also seen for SE. Planned comparisons showed significantly higher SE for each day of the 5h sleep opportunity condition versus the 9h day sleep opportunity. Significant effects were seen for subject ($p<0.00085$), time ($p<0.00$), and significant condition × subject (0.012173) and time × subject ($p<0.00$) interactions for glucose levels. Significant effects were seen for condition ($p<0.015392$), time ($p<0.00$), and significant condition × subject ($p<0.050201$) and time × subject ($p<0.000001$) interactions for insulin levels. Significant main effects were also seen for condition ($p<0.05$) and for subject ($p<0.05$) for the Matsuda index. Planned comparisons revealed a significant reduction in the Matsuda index for the 5h condition versus baseline ($p=0.01$). Planned comparisons showed a significant increase in HOMA-IR for the 5h condition vs. baseline ($p<0.05$).

**Discussion:**
A sleep opportunity of 5h for 3 nights resulted in a reduction in insulin sensitivity when compared to a baseline 9h sleep opportunity. An increase in insulin levels following the sleep restriction condition was associated with normal glucose levels. These results indicate a reduction in insulin sensitivity following sleep restriction of 5h. The study's results on insulin sensitivity are similar to those seen in obese and aging individuals and provide important implications into the effects of sleep restriction on metabolic health.

**Support:**
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**Introduction:**

A voluntary reduction in sleep duration has become a worldwide practice to meet the demands of modern society [13, 21]. Findings from several studies have shown that sleep duration and quality are important in the modulation of glucose metabolism [35, 36, 8, 17, 23, 29]. Additionally, findings from epidemiological studies have indicated that chronic sleep restriction is correlated with an increased risk of type 2 diabetes [26]. Findings from research studies have shown that not only does chronic sleep curtailment have an effect on glucose metabolism, but acute sleep deprivation (i.e. one night) can alter glucose tolerance and insulin sensitivity [37, 8, 30]. In addition to these notable effects, the different stages of sleep, most importantly slow-wave sleep (SWS) [32] and rapid eye movement sleep (REM) [28], are known to play a role in glucose homeostasis during a sleep episode. SWS has been shown to be better preserved than that of REM sleep during a sleep restriction of 4h [30]. Glucose tolerance has been shown to be modulated by sleep, as well as the circadian system, driven by the superchiasmatic nucleus (SCN) (regulator of circadian rhythms [10]) in the hypothalamus [36]. It has been reported that a SCN-lesion disrupts glucose homeostasis and results in the lack of rise in cortisol and glucose levels shortly after awakening (morning arousal) [15]. Due to the circadian influence, several studies have utilized constant glucose infusion, continuous enteral nutrition or an identical meal and interval protocol to determine how the circadian system affects glucose tolerance throughout the day and night, as well as the impact of sleep on glucose tolerance [35, 12, 28].

Insulin sensitivity refers to the ability of insulin to inhibit hepatic glucose production and promote glucose disposal by peripheral tissues [29]. It is commonly

assessed from procedures, such as oral glucose tolerance tests, intravenous glucose tolerance tests, fasting measures, and the hyper-insulinemic-euglycemic clamp (considered the gold standard for the measurement of insulin sensitivity [8]). The hyper-insulinemic-euglycemic clamp involves the infusion of insulin at a set (clamped) level and glucose is infused, as needed to maintain euglycemia (normal glucose levels). A sampling catheter is placed in the contralateral (opposite) arm for blood sampling [8]. If a smaller than normal amount of glucose needs to be infused to maintain euglycemia, then there is an indication of reduced insulin sensitivity. The Matsuda index and homeostatic model of assessment are indices that have been established to determine the insulin sensitivity that is assessed from the OGTT and fasting measures, respectively. Insulin sensitivity has become an important topic in modern day society, due to the increased prevalence of diabetes and sleep restriction. Sleep restriction has been shown to cause a reduction in insulin sensitivity (i.e., more insulin is required to maintain glucose homeostasis) [8, 14, 29, 39]. Acute sleep restriction results in increased glucose and decreased insulin levels, which is indicative of decreased glucose tolerance [29]. Chronic sleep restriction has been shown to be associated with insulin resistance/impaired insulin sensitivity, putting an individual in a pre-diabetic state [29]. This may lead to a permanent state of insulin resistance/impaired insulin sensitivity that is defined as type 2 diabetes. To the best of our knowledge, the current study is the first study to look at insulin sensitivity, with food being *ad libitum*, following a protocol that simulates sleep restriction across a modern work week, in which an individual might encounter in their life.

*Hypothesis:* The present study was designed to determine if 3 nights of 5h sleep opportunity compared to 3 nights of 9h sleep opportunity decreases insulin sensitivity.

**Sleep- Glucose Homeostasis and Metabolism**

*Nocturnal Sleep*

Glucose homeostasis is dependent on both glucose production (from the liver during the post-absorptive state and the gut during the post-prandial state) and utilization [12, 18, 29]. Findings from studies have shown that sleep is important in the modulation and homeostasis of glucose [35, 36, 8, 17, 23, 29]. Specifically, nocturnal sleep provides multiple mechanisms that keep glucose levels relatively stable throughout the sleep episode, even though this period of time is associated with a fasting state [36, 29].

According to Van Cauter *et al* [36], during a nocturnal sleep episode of 8h (0:00-0:800), plasma glucose levels remain relatively stable throughout the night. This is very different from the daytime (08:00-16:00) results that show a notable decrease in plasma glucose when subjects were kept awake. Furthermore, the rate of production of glucose and rate of utilization of glucose shows a U-shaped curve throughout the sleep period. These concurrent U-shaped curves show endogenous production of glucose, mainly by the liver, is likely associated with glucose utilization by tissues. Daytime values for rate of production and rate of utilization of glucose are relatively stable and do not show the same nadir as their respective nighttime values. The latter implies that overall glucose homeostasis is modulated by sleep.

*Sleep Stages and Hormones- Roles in Glucose Homeostasis during Sleep*

The adult human sleep cycle is composed of two types of sleep: rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. NREM consists of

stages 1, 2, and the combined stages 3 and 4, known as slow-wave sleep (SWS) [16]. Throughout a normal sleep episode (7-9h), the first part of the night an individual spends relatively more time in SWS. During the later part of the night, more time is spent in REM sleep. Findings from research studies have shown that certain sleep stages, most importantly SWS and REM, impact glucose metabolism during sleep [27, 29, 32, 30, 33].

SWS sleep has been shown to be associated with a decrease in glucose effectiveness (clearance of glucose from the body by non-insulin dependent tissues (i.e., brain) ($S_G$) [21]), an increase in growth hormone release, inhibition of cortisol secretion, decreased sympathetic nervous system activity and increased vagal (parasympathetic) tone. These physiological processes that are impacted by SWS may affect total-body glucose homeostasis [32]. Specifically, growth hormone acts to stimulate gluconeogenesis in the liver and inhibit peripheral tissue uptake of glucose. Therefore, growth hormone produces anti-insulin like effects on total body glucose metabolism.

The decrease in glucose utilization that is observed in the early part of the night is thought to be mainly due to a reduction in cerebral glucose utilization during SWS [29]. As opposed to SWS, REM sleep and wakefulness are associated with an increase in glucose utilization [28]. The increase in glucose utilization towards the end of the sleep episode [36] is consistent with the latter. As opposed to GH, an increase in cortisol secretion occurs during the second half of the sleep episode, a time when REM sleep amounts are high [27]. Previously described, glucose tolerance improves during the second half of a sleep episode, under constant glucose infusion conditions. This improvement in glucose tolerance may be associated with the increased secretion of cortisol, seen in the second half of the sleep episode. According to these findings, the

specific sleep stages, most importantly SWS and REM sleep are thus important in glucose homeostasis during a sleep episode.

*24-hour Glucose and Insulin Profiles*

Glucose tolerance is defined as the ability of insulin to clear (or restore) blood glucose levels to normal after a glucose challenge [12]. It has been shown that glucose tolerance starts to decrease throughout the late afternoon and early evening [35, 36, 12, 14]. Constant glucose infusion protocols (e.g., continuous enteral nutrition protocol over a 24h period or greater, or a 24h study with identical meals at even intervals [35, 12, 28]) have been used to better understand factors that contribute to changes in glucose levels during nocturnal sleep. Constant glucose infusion inhibits endogenous glucose production (EGP); the liver is the main contributor to EGP. Findings from these studies indicate that there is a decrease in glucose utilization by both insulin-dependent (i.e., muscle and adipose tissue) and independent (i.e., brain) tissues during the nocturnal sleep episode. The observed decrease in glucose utilization may be a result of a reduction in insulin sensitivity [35, 12, 28].

Findings from Van Cauter *et al* [37] indicate that there is a reduction in glucose tolerance throughout the day and into the night time, as assessed by blood glucose and the insulin response to 3 identical meals given 6 hours apart from each other (08:00, 14:00, 20:00). Although they found an increase in blood glucose levels in response to each subsequent meal than the previous meal, the insulin secretion rate (ISR) and insulin levels did not compensate in response to the rising glucose levels. The latter may indicate a circadian influence in the set point at which β-cells respond to glucose, with a reduction in the responsiveness in the evening resulting in increased blood glucose concentrations

[37]. Van Cauter *et al* [35] also demonstrated that ISR and glucose levels show a marked increase in response to sleep onset, peak in the middle of a nocturnal sleep episode, and decrease in the second half of the sleep episode during a constant infusion of glucose. These spikes in both the ISR and glucose levels during the sleep episode are juxtaposed to the lower ISR and glucose levels during the daytime, even though the subjects are under a constant infusion protocol. In addition, in response to sleep onset, ISR increased as glucose tolerance decreased during sleep, regardless of when sleep was scheduled. This specific finding indicates that sleep triggers events that result in impaired glucose tolerance. These findings from studies using a constant infusion and identical meal and interval protocol indicate that sleep and circadian rhythms modulate glucose homeostasis.

**Sleep Deprivation- Glucose Homeostasis and Metabolism**

According to the Centers for Disease Control, from 2004-2006, 28.5% of men and women in the U.S. aged 18 or older, reported getting 6 or less hours of sleep per night [25]**.** Additionally, findings from Morselli *et al* [21] show that 44% of US adults obtain less than 7h of sleep per work night. Besides the obvious cognitive impairment, sleep restriction may contribute to the development of obesity and/or insulin resistance [6, 36, 8, 12, 14, 17, 23, 38, 39]. According to Figure 1, acute sleep deprivation (i.e., one night of total sleep deprivation) results in increased levels of glucose and suppressed levels of insulin. Chronic sleep restriction can increase the levels of insulin, even though glucose levels may be normal. These results are indicative of decreased insulin sensitivity, since more insulin is required to clear the same amount of glucose. When these levels are

sustained chronically, it can lead to a pre-diabetic state of impaired glucose tolerance and insulin resistance and eventually lead to an increased risk for developing type-2 diabetes.

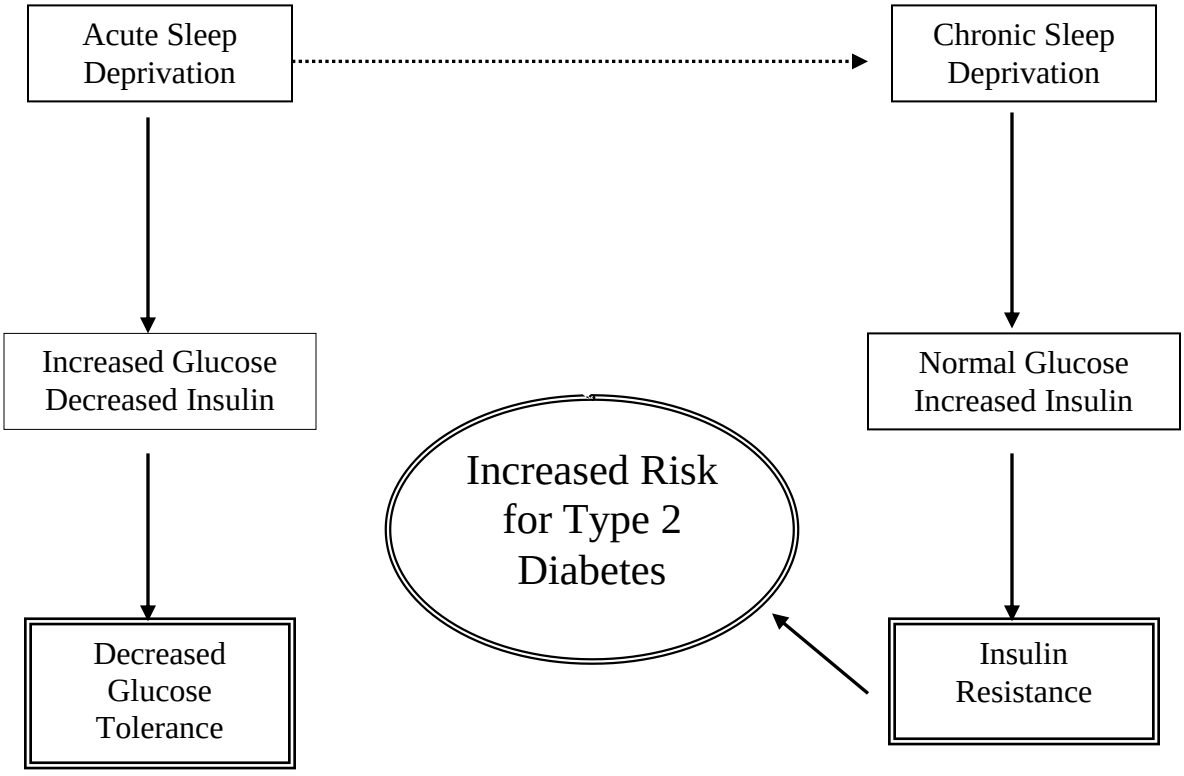


**Figure 1.** A schematic representation of the effects of acute and chronic sleep deprivation. Both pathways subsequently leading to glucose dysregulation. (Adapted from Spiegel et al., 2005 (29))

*Chronic/Total Sleep Deprivation*

Modern society's increasing demands, whether it is work or social, have increased the prevalence of chronic sleep restriction. One example is seen with individuals that undergo shift work (i.e., doctors, nurses, construction workers, etc.) [3]. According to the study by Scheen *et al* [24], disturbances in glucose regulation can result from chronic disturbances in sleep, such as those occurring in shift workers. Experimental sleep

disruption has been shown to disturb glucose regulation. For example, if SWS is experimentally suppressed by nearly 90%, insulin sensitivity is decreased by 25% and reaches a level that is consistent with aging individuals, as well as populations at high risk for diabetes [5].

*Sleep Restriction*

Sleep restriction, as previously noted, has been associated with an increased risk for the development of insulin resistance (characterized by needing higher than normal amounts of insulin to reduce blood glucose levels, following administration of the same amount of exogenous glucose [14]) [8, 14, 39] and impaired glucose tolerance [8, 14, 30, 39], which in turn may result in the development of type 2 diabetes in the future. Specifically, findings from a study by Spiegel *et* al [30] showed that 4h in bed for 6 nights when compared to 12h in bed for 6 nights resulted in negative effects on glucose metabolism and other endocrine functions. Following the 4h sleep duration, the subjects displayed a reduction in glucose tolerance, acute insulin response to glucose (characterized by pancreatic beta-cell responsiveness [21]) and glucose effectiveness.

Furthermore, Donga *et al*. [8] found that with only a single night of sleep restriction with a sleep opportunity of 4h when compared to a control condition of 8.5h of scheduled sleep, subjects displayed a 19-25% reduction in insulin sensitivity in multiple pathways. These pathways consisted of EGP and glucose uptake, as well as peripheral lipolysis, indicated by the increased non-esterified fatty acid (NEFA) levels, due to the reduction of insulin's action on adipose tissue. Sleep restriction results in alterations in the relative proportion of the specific sleep stages. The study by Spiegel *et al* [30] indicated that the proportion of SWS increased when the subjects were only allowed 4h

in bed per night for 6 nights as opposed to 12h in bed per night for 6 nights. This increase in the proportion of SWS may be a compensatory mechanism to maintain stable glucose levels.

**Measurement of Glucose Homeostasis and Insulin Sensitivity**

*Oral Glucose Tolerance Test (OGTT)*

The OGTT is the most commonly used method to evaluate whole body glucose tolerance and provides a clinically relevant, easily translated, metabolic assessment that gives insight into the entire axis under a more physiological setting than does the Intravenous Glucose Tolerance Test (IVGTT). Since, the glucose in an OGTT is given to the subject through ingestion, rather than intravenously, it interacts with the gastrointestinal system and hence, is more translatable to a real life situation. The justification for using an OGTT rather than an IVGTT is that although OGTTs are less precise, IVGTTs are time-consuming and expensive, while OGTTs are much simpler to perform [34]. The OGTT is commonly performed following an over-night fast of 12h [7].

*Homeostatic Model of Assessment of Insulin Resistance (HOMA-IR)*

The homeostatic model of assessment of insulin resistance (HOMA-IR) was used in this thesis to measure whole-body insulin sensitivity. HOMA was first established by Matthews *et al* [19] in 1985. According to the formula, the higher the HOMA, the worse insulin sensitivity the subject displays. Matthews *et al* [19] used HOMA to assess insulin resistance and the deficiency of β-cell function. Spiegel *et al* [31] assessed insulin sensitivity via HOMA, following a sleep restriction of 4h. It was reported that post-

breakfast, HOMA was significantly elevated in the sleep restriction when compared to the fully rested state of 12h.

*The Matsuda Index*

The Matsuda index is used as a measure of whole-body insulin sensitivity. Matsuda & DeFronzo in 1999 [18], concluded that the Matsuda index is a novel estimate of insulin sensitivity that is simple to calculate and provides a reasonable approximation of whole-body insulin sensitivity from an OGTT. Even though HOMA-IR was also used to determine whole-body insulin sensitivity, the equation assumes that the hepatic and peripheral insulin sensitivities are equivalent within an individual [18]. Given this information, the HOMA (primarily liver) and the Matsuda index (liver and muscle) provide different information [18]. Although the Matsuda index serves as a measure of whole-body insulin sensitivity, other indices have been developed to measure specific tissue insulin sensitivity, such as skeletal muscle insulin sensitivity (SMIS) and hepatic (liver) insulin sensitivity (HIS) [1].

The current study was designed to determine if short sleep duration would result in a reduction in insulin sensitivity. Specifically, we hypothesized that 3 nights of 5h sleep opportunity when compared to 3 nights of 9h sleep opportunity, will result in a reduction in insulin sensitivity, as assessed by an oral glucose tolerance test and analyzed via the Matsuda index and HOMA-IR.

## Methods

### *Subjects:*

13 healthy subjects (7 men, 6 women) aged 24.77 ± 4.02 (Mean ± SD), participated in the study. Subjects were recruited via flyers, online advertisements (i.e. Buff Bulletin and Craigslist), and online surveys (i.e. RED Cap). Exclusion criteria consisted of any history of acute/chronic medical or psychiatric disorder, use of prescription medication (other than hormonal contraceptives), BMI outside the range of 18.5 to 24.9, shift work within 6 months of the health screening visit, less than 3 months living at Denver altitude, traveling more than one time zone during the one month prior to the study, and conditions related to sleep disorders, as determined from medical history, sleep history, physical examination, or polysomnogram (first night of study).

Subjects gave written informed consent and the protocol was approved by the Colorado Multiple Institutional Review Board (COMIRB). Subjects were deemed physically healthy after passing a thorough screening visit at the Clinical Translational Research Center on the CU Boulder campus. The screening tests included: medical history, physical examination, blood chemistries, 12-lead EKG, DEXA scan and urine toxicology.

Prior to the start of the study, subjects were asked to keep a consistent self-selected sleep schedule of ~9h for 7 days at home, verified by actigraphy (Actiwatch-L Mini Mitter, Bend, OR), sleep logs and call-ins to a time stamped recorder. Medications, caffeine, alcohol, and nicotine were proscribed 3 days prior to testing. 3 days prior to the start of the study, subjects were asked to consume an outpatient diet provided by the CTRC nutritionists. The energy content of the diet was designed to meet individual daily energy requirements (RMR × an activity factor).

***Protocol:***

Subjects participated in one of two cross over designs (Figures 2 & 3). Subjects experienced both sleep loss and adequate sleep conditions with equal numbers exposed to sleep loss and adequate sleep first. The following protocol outlines assessments relative to the current thesis that is part of a larger study and does not include the full protocol.

Baseline Assessments: Days 1-3 (Figures 2 & 3)

The study began with admission to the CTRC on day 1 (Figures 2 & 3). The three inpatient days of segment A were required to assess baseline measurements for the outcome variables (sleep and insulin sensitivity). Subjects were scheduled to sleep (black bars) and wake (gray bars) at their regularly scheduled times for 3 days. Time of day is plotted as relative time with scheduled wake time arbitrarily assigned a value of 0800h and all other times were referenced to this value. The actual clock hour was determined by the subject's habitual schedule the week prior to entry into the laboratory. Light exposure in the laboratory was a combination of natural sunlight and artificial room lighting during scheduled wakefulness and darkness during scheduled sleep. Food intake for the first three days was the same energy balance diet as the outpatient diet. Wrist actigraphy was continuously measured to assess sleep and wakefulness. On day 2, baseline assessments for fasting glucose levels and oral glucose tolerance testing were performed (indicated by an OGTT in Figures 2 & 3).

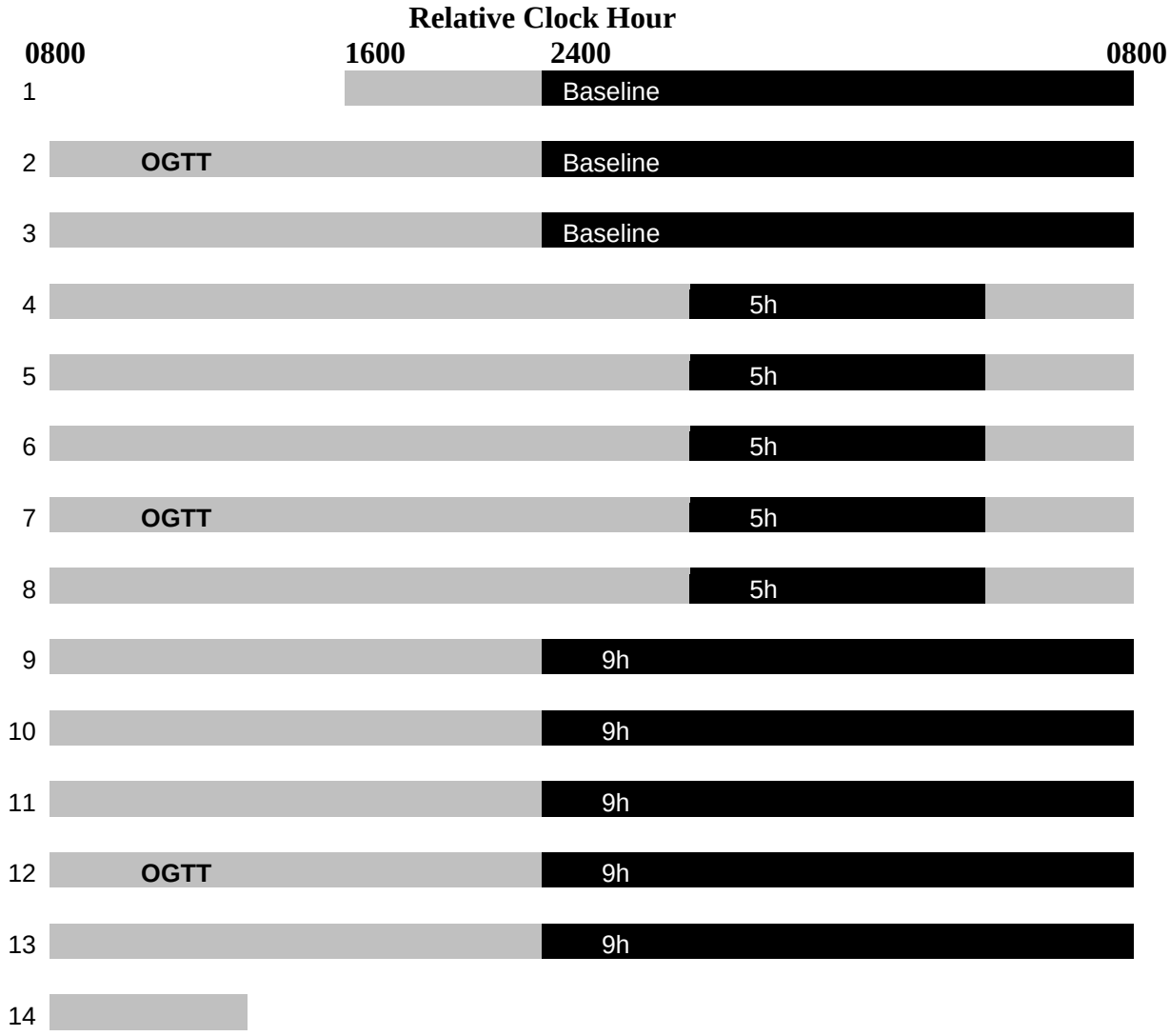


**Figure 2--*14 Day Design***
Sleep Restriction (5h per night) followed by adequate sleep(9h per night)
OGTT = Oral Glucose Tolerance Test

Experimental Manipulation, Chronic Sleep Loss and Adequate Sleep Crossover: Days 4-14/15 (Figures 2 & 3)

Subjects were scheduled to sleep (black bars) for 5h per night for 5 nights (nights 4-8 or nights 9-13, Figures 2 & 3). Meals were provided *ad libitum* except the mornings of the OGTT where fasting was imposed. Overnight fasting glucose levels were assessed

and oral glucose tolerance testing was performed prior to breakfast on days 7 and 12 of the study. A recovery sleep opportunity was provided *ad libitum* prior to discharge for the group that was scheduled to sleep restriction second (Figure 3).

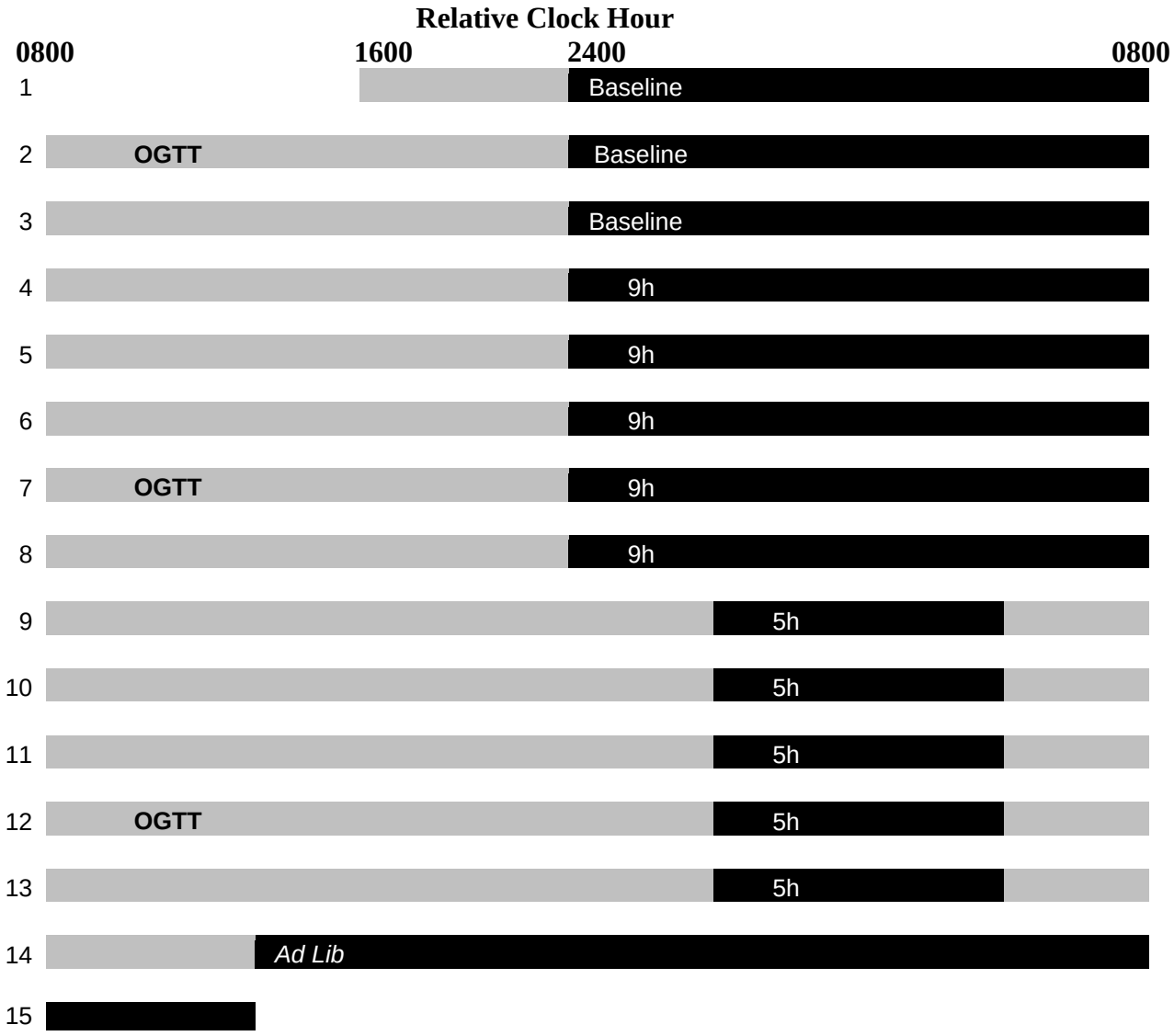


**Figure 3--*15 Day Design***
Adequate Sleep (9h Per Night) followed by sleep restriction (5h per night)
OGTT = Oral Glucose Tolerance Test

***Materials and Measurements*:**

Actigraphy

Subjects were asked to wear an actiwatch (Actiwatch-L Mini Mitter, Bend, OR) for the duration of the study. The actiwatch, which was worn around the wrist of the subject, measured light and activity levels. Actigraphy was used to assess total sleep time (TST) and sleep efficiency (SE) throughout the protocol. Polysomnography (PSG) was performed on select nights during the study, but was not analyzed for this thesis. Actigraphy has been validated as a reasonable estimate of sleep [2, 20].

Oral Glucose Tolerance Test

An OGTT was performed on three study days (days 2, 7, and 12) as previously indicated in the protocol. Glucose and insulin were assessed in blood samples (2 ml) drawn from an indwelling catheter with heplock at t=-30, -15, 0, 30, 60, 90, 120 minutes with 75g glucose ingested at t=0. 2-h post-load glucose levels were assessed to determine normal glucose tolerance (< 140 mg/dl (7.8 mmol/l)) impaired glucose tolerance (>140 mg-199 mg/dl and > 200 mg/dl as diabetes) [4].

***Data Analysis:***

Actigraphy

Actigraphy was analyzed with Actiware 3.3 Mini Mitter software. TST and SE were analyzed and calculated using the medium sensitivity setting. TST was calculated from the bed time to wake time and any sleep bouts that occurred during the night. If for example, the subject had to get up to go to the bathroom (as measured as an increase in activity levels), such awakenings were taken into account. SE was calculated as follows:

$$\mathbf{SE\% = \frac{Total\ Sleep\ Time}{Time\ in\ Bed}}$$

Sleep data were compared using the final baseline day (day 3) as the sleep baseline to account for adaption to sleeping in the laboratory.

The Homeostatic Model of Assessment of Insulin Resistance

According to the formula, the higher the HOMA score, the worse the insulin sensitivity the subject displays. Matthews *et al* [19] used HOMA to investigate the comparison of a subject's fasting glucose and insulin levels with the model to establish the contributions of insulin resistance and the deficiency of β-cell function to a subject's fasting hyperglycemia.

The model was calculated as:

$$\frac{\mathbf{1}}{\mathbf{[22.5/(fasting\ insulin \times (fasting\ glucose/18.01))]}} = \mathbf{HOMA\text{-}IR}$$

The Matsuda Index

According to the formula, the higher the Matsuda index, the better the insulin sensitivity. The index takes the fasting plasma glucose (FPG) (glucose sampled at t=0) and multiplies it by the fasting plasma insulin (FPI) (insulin sampled at t=0). FPG and FPI refer to an individual's glucose and insulin levels during a fasted state (i.e., at least 8h abstaining from eating). The product of FPG and FPI is then multiplied by the product of the mean glucose and insulin concentrations over the course of the OGTT. The square root of the result is then divided into 10,000, which serves as a constant to obtain numbers ranging from 0-12 [18].

The index is calculated as:

$$\frac{\mathbf{10{,}000}}{\sqrt{\mathbf{(FPG \times FPI) \times (Mean\ OGTT\ glucose\ concentration \times Mean\ OGTT\ insulin\ concentration)}}} = \mathbf{Matsuda\ Index}$$

***Statistics:***

Sleep efficiency, total sleep time, Matsuda index, and HOMA-IR were analyzed via the Mixed-Model ANOVA approach. The ANOVA included the following factors: Subject was included as a random factor and order (5h or 9h first) time, and condition day (Baseline, 5h sleep, 9h sleep) were included as fixed factors. Planned comparisons were computed to compare each condition day to the other. Two subjects had missing data for one of their OGTT tests. Analyses were assessed with STATISTICA, version 6.0 (StatSoft Inc).

**Results**

No effects were seen for order of conditions (5h vs. 9h first) for any outcome variable, therefore the factor order was removed from all analyses.

***Actigraphy:***

Mixed-Model analysis revealed significant main effects of condition day ($F=311.30$; $p<0.000001$) and of subject ($F=7.70$; $p<0.000001$) for TST. Individual differences in TST were observed. Planned comparisons showed significantly less TST for each day of the 5h sleep opportunity condition, as compared to baseline and each respective 9h day sleep opportunity (day 1 5h vs. day 1 9h) (Figure 4). No significant differences were found between the 9h condition and baseline. Mixed Model analysis also revealed significant main effects of day ($F=3.08$; $p<0.001$) and of subject ($F=8.85$; $p<0.000001$) for SE. Individual differences in SE were observed. Planned comparisons showed significantly higher SE for each day of the 5h sleep opportunity condition compared to the 9h day sleep opportunity (day 1- 5h versus day 1- 9h) (Figure 5). No

significant SE differences were found between the 5h condition or 9h condition days and baseline day 3.

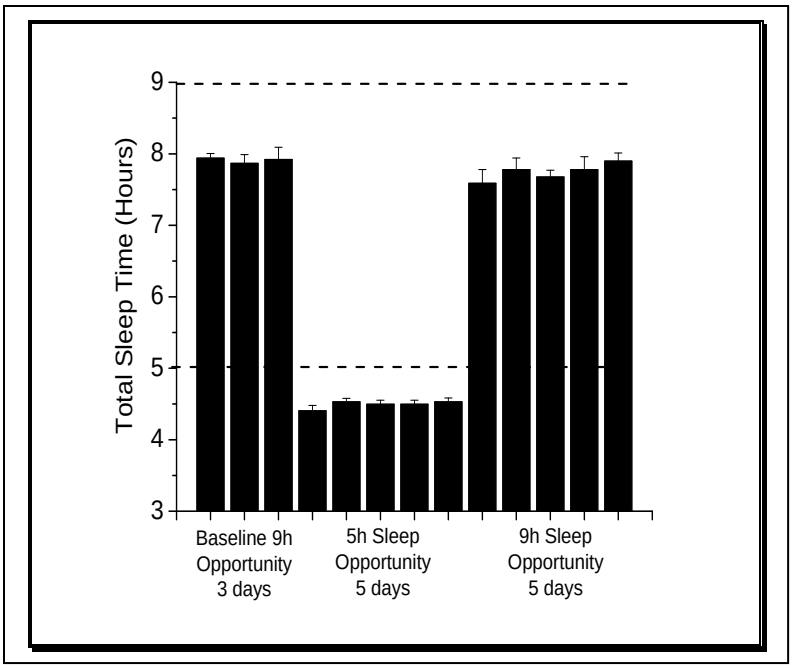


**Figure 4.** Total Sleep Time (TST) profile over baseline, sleep restriction (5h sleep opportunity) and 9h sleep opportunity.

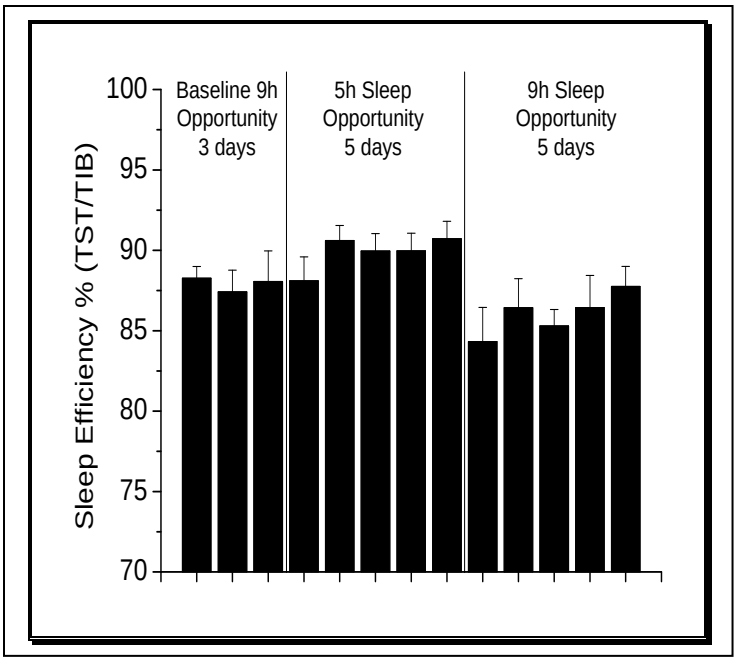


**Figure 5.** Sleep efficiency (SE) profile over baseline, sleep restriction (5h sleep opportunity) and 9h sleep opportunity.

***Glucose and Insulin Levels during the OGTT:***

Significant effects of subject ($F=3.33601$; $p<0.00085$), time ($F=34.93969$; $p<0.00$), and significant condition × subject ($F=1.90798$; 0.012173) and time × subject ($F=4.22417$; $p<0.00$) interactions for glucose levels were observed. A significant effect of condition ($F=4.9554$; $p<0.015392$), time ($F=40.78414$; $p<0.00$), and significant condition × subject ($F=1.6226$; $p<0.050201$) and time × subject ($F=2.58181$; $p<0.000001$) interactions for insulin levels were also found. As seen in Figure 6, glucose

levels are similar among the three conditions, yet to maintain "normal" glucose levels during the 5h sleep restriction OGTT, insulin levels were higher.

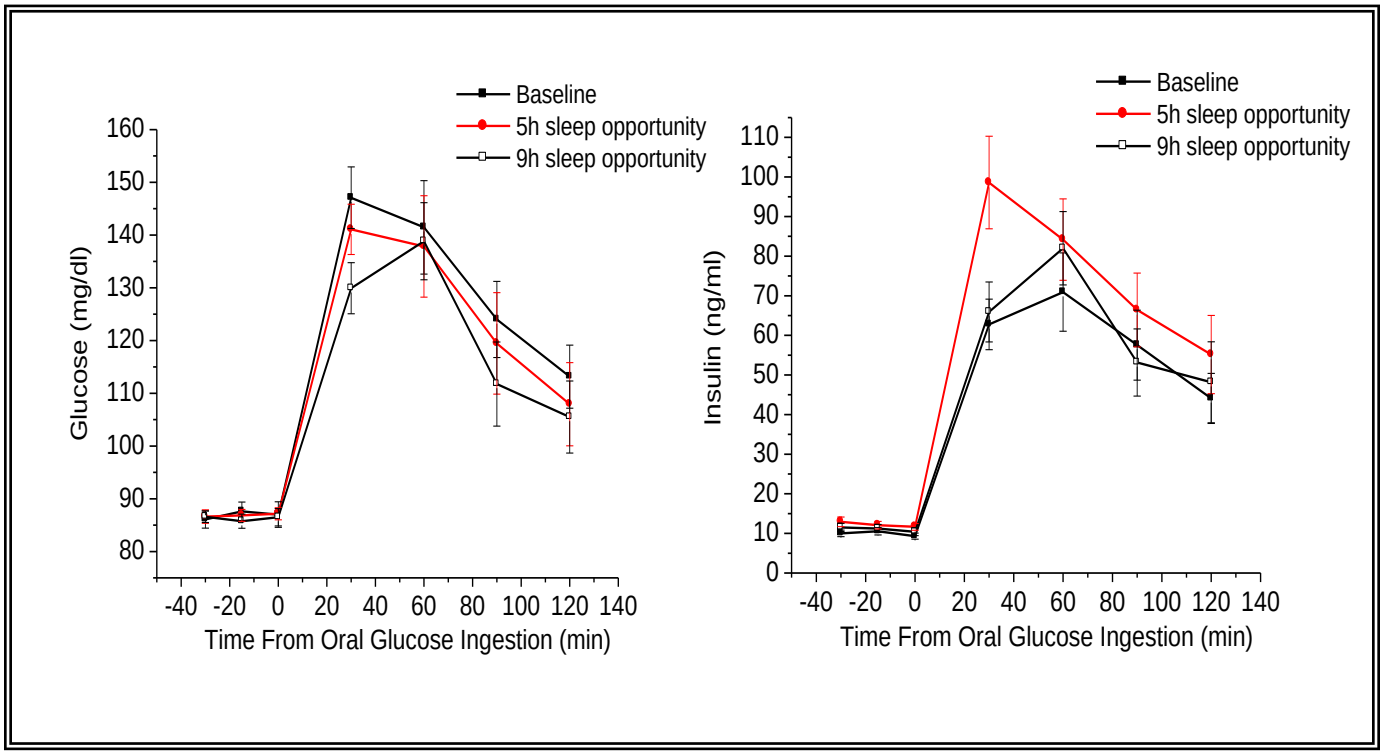


**Figure 6**. Plasma glucose (left panel) and insulin (right panel) levels during the OGTT.

***The Matsuda Index:***

Significant main effects were seen for condition ($F=4.20$; $p<0.05$) and for subject ($F=5.66$; $p<0.05$) for the Matsuda index. The significant subject effect indicates inter-individual variability in Matsuda index scores during the OGTT (Appendix B). Planned comparisons revealed a significant reduction in the Matsuda index for the 5h condition versus baseline (Figure 7). The comparison between the 5h and 9h was not significant

(p=0.12). As seen in (Appendix B), the 5h condition showed worse insulin sensitivity compared to baseline and the 9h condition.

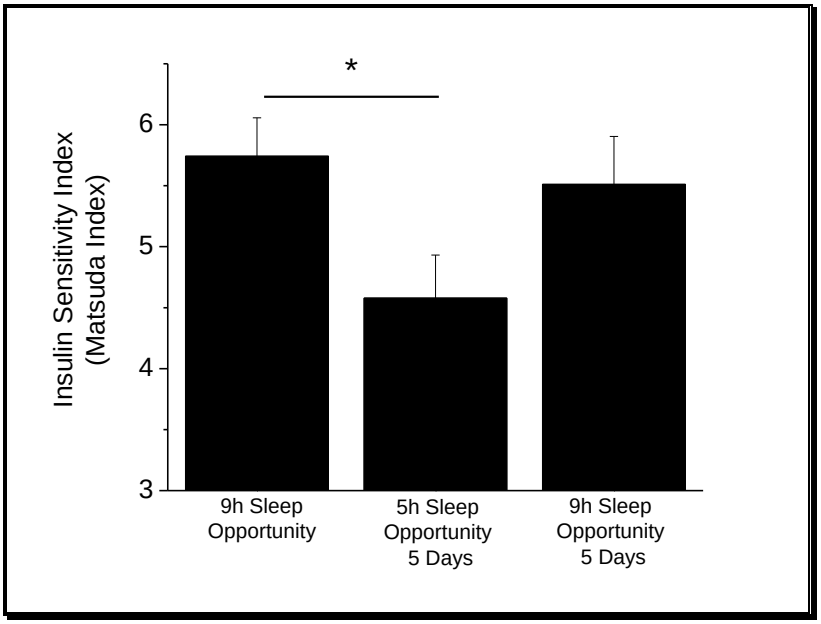


**Figure 7.** Insulin sensitivity was found to be significantly worse following the 5h sleep restriction compared to the baseline sleep opportunity of 9h (p=0.01).

***HOMA-IR:***

No significant effects of condition or subject were observed for HOMA-IR, however planned comparisons showed a significant increase in HOMA-IR for the 5h sleep condition vs. baseline ($p<0.05$) (Figure 8).

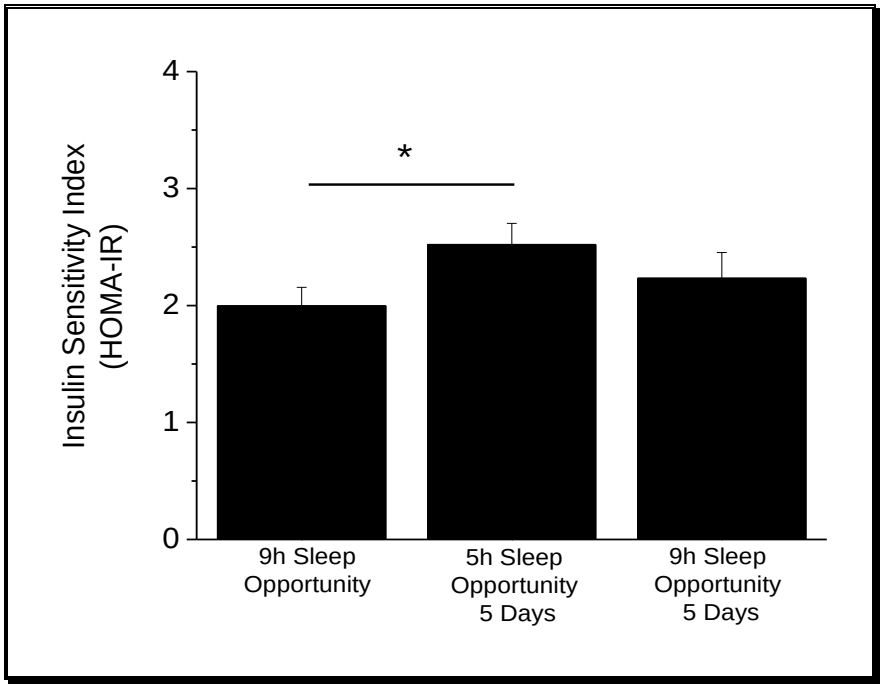


**Figure 8.** Insulin sensitivity was found to be significantly worse following the 5h sleep restriction compared to the baseline sleep opportunity of 9h ($p<0.05$).

**Discussion**

This study is the first of its kind to provide food *ad libitum* while looking at insulin sensitivity during days of sleep restriction versus days of a 9h adequate sleep opportunity. The study was designed to mimic a traditional work week of inadequate sleep. The comparison between the 5h and 9h sleep conditions revealed that the sleep restriction condition resulted in a reduction in insulin sensitivity. This finding is consistent with previous findings of impaired insulin sensitivity following sleep restriction of 4h [8, 17]. Donga *et al* [8] found that in healthy lean adults, following sleep restriction of 4h for 1 night, insulin sensitivity (assessed by the hyper-insulinemic-euglycemic clamp) was decreased from a baseline night of 8.5h. Following the sleep restriction night, glucose infusion rate was significantly decreased ($p<0.001$) from baseline. Additionally, Spiegel *et al* [31] found that in healthy adults, sleep restriction of 4h for 6 nights decreased insulin sensitivity. Specifically, HOMA was significantly higher in the sleep restricted group when compared to the 12h sleep opportunity group ($1399 \pm 153$ vs. $1954 \pm 188$) for the area under the curve ($p=0.026$), following breakfast at 09:00.

In the current study, glucose levels during the OGTT were similar between sleep conditions. Although glucose levels were similar, higher amounts of insulin were needed in the sleep restriction condition to maintain these similar glucose levels. This finding and the results from the Matsuda index and HOMA-IR are indicative of decreased insulin sensitivity. The physiological meaningfulness of the change in insulin sensitivity observed in the current study can be considered by comparing the observed changes to the effects of aging and obesity on insulin sensitivity. We found sleep restriction to lower

the Matsuda index in healthy young adults from an average of 5.74 to 4.58. The 4.58 Matsuda index is similar to that reported average Matsuda index of 5.06 (Men- 4.43; Women- 5.32) for subjects as 50-95 years old [22] and that of 4.98 for older (73.74 ± 11.23) lean individuals [11]. In addition to aging, a study by Gniuli *et al* [9] found that obese individuals display worse insulin sensitivity, assessed by HOMA-IR. The current study's results for insulin sensitivity show that the 2.52 HOMA-IR for the 5h sleep condition fell between two quartiles of overweight subjects (second quartile: BMI 26.71; HOMA-IR=1.57, third quartile: BMI 37.94; HOMA-IR=3.82) in the study by Gniuli *et al* [9].

***Strengths:***

The present study was designed to be well controlled with subjects being in a hospital setting. The study was designed as a counter-balanced, crossover, within-subjects design, so each subject served as their own control. Since, subjects were provided food *ad libitum* and the sleep restriction protocol was equated to a modern work week, the findings have important implications for the general working population.

***Limitations and Future Research:***

One limitation of the study is that the controlled laboratory conditions do not mimic daily life. Since sleep restriction produces performance impairments similar to alcohol intoxication, it is problematic to assess effects of experimentally induced sleep restriction under real world conditions to obtain more translatable findings. Future studies could look at this variable of insulin sensitivity under different protocols, such as different degrees and durations of sleep restriction. One could also assess the effects of sleep restriction on insulin sensitivity of aging and overweight adults to better understand the importance of sleep for overall metabolic health.

In conclusion, we have shown that a sleep restriction period of 5h for 5 days results in a reduction in insulin sensitivity from baseline. The current study indicates that sleep restriction in healthy young adults impairs insulin sensitivity to a level comparable to that found in aging and in obese individuals, indicating that sleep restriction induced physiologically and clinically meaningful changes in metabolism.

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## Appendix A- Actigraphy

| 1 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 8:26 | 8:16 | 8:31 | 7:22 | 7:34 | 7:48 | 8:07 | 8:17 | 4:33 | 4:14 | 4:36 | 4:29 | 4:34 |
| | SE% | 93.9 | 91.9 | 94.6 | 81.9 | 84.1 | 86.7 | 90.2 | 92 | 91 | 84.7 | 92 | 89.7 | 91.3 |

| 2 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 7:37 | 7:21 | 7:53 | 5:59 | 6:53 | 7:26 | 6:01 | 7:19 | 3:53 | 4:14 | 4:03 | 4:30 | 4:13 |
| | SE% | 84.6 | 81.7 | 87.6 | 66.5 | 76.5 | 82.6 | 66.9 | 81.3 | 77.7 | 84.7 | 81 | 90 | 85.2 |

| 3 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 8:06 | 8:02 | 8:07 | 7:17 | 7:29 | 7:47 | 7:42 | 7:25 | 4:23 | 4:31 | 4:08 | 4:15 | 4:27 |
| | SE% | 90 | 89.3 | 90.2 | 80.9 | 83.1 | 86.5 | 85.6 | 82.4 | 87.7 | 90.3 | 82.7 | 85 | 89 |

| 4 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 7:52 | 7:07 | 8:10 | 4:21 | 4:32 | 4:35 | 4:24 | 4:36 | 7:46 | 8:46 | 7:25 | 7:54 | 8:31 |
| | SE% | 87.4 | 79.1 | 91.2 | 87 | 90.7 | 91.7 | 88 | 92.9 | 86.3 | 97.4 | 82.4 | 87.8 | 94.6 |

| 5 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 7:41 | 8:11 | 6:48 | 4:10 | 4:37 | 4:33 | 4:13 | 4:35 | 8:03 | 7:28 | 7:21 | 8:17 | 7:44 |
| | SE% | 86 | 90.9 | 75.6 | 83.3 | 92.3 | 91 | 84.3 | 91.7 | 89.4 | 83 | 81.7 | 92 | 85.9 |

| 6 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 7:44 | 7:42 | 8:11 | 7:24 | 7:50 | 7:36 | 7:43 | 7:33 | 4:19 | 4:34 | 4:38 | 4:42 | 4:08 |
| | SE% | 85.9 | 85.6 | 90.9 | 82.2 | 87 | 84.4 | 85.7 | 83.9 | 86.3 | 91.3 | 92.7 | 94 | 82.7 |

| 7 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 8:10 | 7:54 | 6:40 | 4:22 | 4:39 | 4:35 | 4:36 | 4:41 | MD | 7:46 | 7:43 | 7:55 | 7:31 |
| | SE% | 90.7 | 87.8 | 74.1 | 87.3 | 93 | 91.7 | 92 | 93.7 | MD | 86.3 | 85.7 | 88 | 83.5 |

| 8 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 7:43 | 7:45 | 8:01 | 4:26 | 4:28 | 4:24 | 4:09 | 4:22 | 7:25 | 7:11 | 7:14 | 7:10 | 7:56 |
| | SE% | 85.7 | 86.1 | 89.1 | 88.7 | 89.3 | 88 | 83.6 | 87.3 | 82.4 | 79.8 | 80.4 | 79.6 | 88.1 |

| 9 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 8:04 | 8:30 | 8:39 | 7:58 | 8:25 | 8:24 | 8:23 | 8:27 | 4:46 | 4:50 | 4:43 | 4:43 | 4:30 |
| | SE% | 89.6 | 94.4 | 96.3 | 88.5 | 93.5 | 93.3 | 93.1 | 93.9 | 95.3 | 96.7 | 94.3 | 94.3 | 90 |

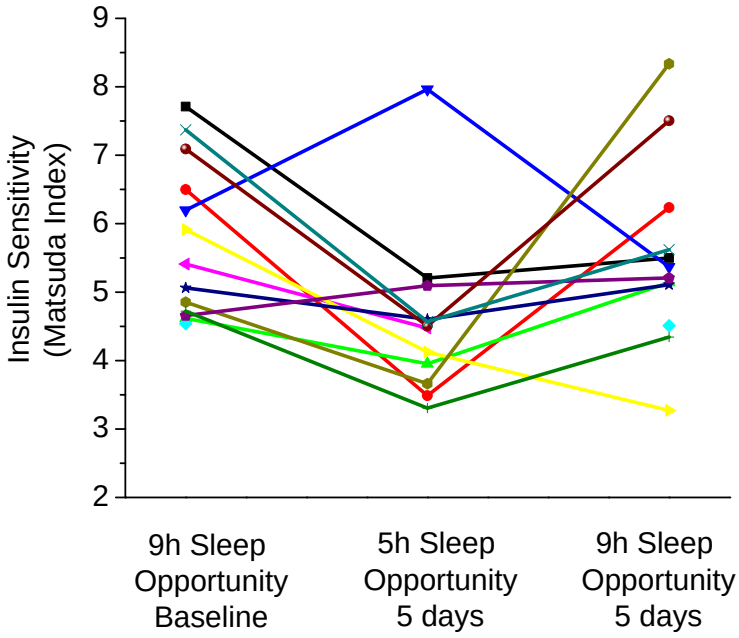
| 10 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 7:52 | 7:27 | 8:00 | 4:27 | 4:33 | 4:31 | 4:32 | 4:51 | 7:37 | 7:26 | 7:49 | 7:24 | 7:40 |
| | SE% | 87.4 | 82.8 | 88.9 | 89 | 91 | 90.3 | 90.7 | 97 | 84.6 | 82.6 | 86.9 | 82.2 | 85.2 |

| 11 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 8:08 | 8:31 | 8:06 | 4:52 | 4:34 | 4:31 | 4:48 | 4:43 | 8:49 | 8:53 | 8:12 | 8:30 | 7:59 |
| | SE% | 90.4 | 94.6 | 90 | 97.3 | 91.3 | 90.3 | 96 | 94.3 | 98 | 98.7 | 91.1 | 94.4 | 88.7 |

| 12 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 7:50 | 7:29 | 7:22 | 7:25 | 7:32 | 7:27 | 8:09 | 7:58 | 4:39 | 4:25 | 4:33 | 4:27 | 4:37 |
| | SE% | 87 | 83.1 | 81.9 | 82.4 | 83.7 | 82.8 | 90.6 | 88.5 | 93 | 88.3 | 91 | 89 | 92.3 |

| 13 | | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 | DAY 12 | DAY 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TST | 8:00 | 8:01 | 8:30 | 7:59 | 7:54 | 7:36 | 7:53 | 8:21 | 4:05 | 4:43 | 4:38 | 4:39 | 4:36 |
| | SE% | 88.9 | 89.1 | 94.4 | 88.7 | 87.8 | 84.4 | 87.6 | 92.8 | 81.7 | 94.3 | 92.7 | 93 | 92 |

## Appendix B- Matsuda Index: Differences Between Conditions




Differences in insulin sensitivity for individual subjects at baseline and following sleep restriction (5h) and adequate sleep (9h) opportunities. Note large individual differences at baseline and following 9h sleep. The variance in insulin sensitivity in the 5h sleep condition appears to be reduced when excluding the one subject who is an outlier.