SLEEP RESTRICTION FOR ONE WEEK REDUCES INSULIN SENSITIVITY IN HEALTHY MEN

Running title: Insufficient sleep and insulin sensitivity

Orfeu M Buxton¹,², Milena Pavlova²³, Emily W. Reid¹, Wei Wang¹,², Donald C. Simonson¹,², Gail K. Adler¹,²

¹ Department of Medicine, Brigham and Women’s Hospital, Boston, MA
² Harvard Medical School, Boston, MA
³ Department of Neurology, Brigham and Women’s Hospital, Boston, MA

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Corresponding Author:
Orfeu M. Buxton, Ph.D.
Email: Orfeu@HMS.Harvard.edu

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Objective: Short sleep duration is associated with impaired glucose tolerance and an increased risk of diabetes. The effects of sleep restriction on insulin sensitivity have not been established. This study tests the hypothesis that decreasing nighttime sleep duration reduces insulin sensitivity and assesses the effects of a drug, modafinil, that increases alertness during wakefulness.

Research Design and Methods: This twelve-day, inpatient General Clinical Research Center study included twenty healthy men (age 20-35 years, BMI 20-30 kg/m²). Subjects spent 10 hours/night in bed for ≥8 nights including 3 inpatient nights (sleep-replete condition), followed by 5 hours/night of time in bed for 7 nights (sleep-restricted condition). Subjects received modafinil (300 mg/day) or placebo during sleep restriction. Diet and activity were controlled. On the last two days of each condition we assessed glucose metabolism by intravenous glucose tolerance test (IVGTT) and euglycemic hyperinsulinemic clamp. Salivary cortisol, 24-hr urinary catecholamines, and neurobehavioral performance were measured.

Results: IVGTT-derived insulin sensitivity was reduced 20 ± 24% (mean±SD) after sleep restriction (p=0.001), without significant alterations in the insulin secretory response. Similarly, insulin sensitivity assessed by clamp was reduced 11±5.5% (p<0.04) after sleep restriction. Glucose tolerance and the Disposition Index were reduced by sleep restriction. These outcomes were not affected by modafinil treatment. Changes in insulin sensitivity did not correlate with changes in salivary cortisol (increase of 51±8% with sleep restriction, p<0.02), urinary catecholamines or slow wave sleep.

Conclusion: Sleep restriction (5 hrs/night) for one week significantly reduces insulin sensitivity, raising concerns about effects of chronic insufficient sleep on disease processes associated with insulin resistance.

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The average sleep duration in the United States has fallen below 7 hours per night, a drop of about 2 hours per night over the last century and more than 1 hour per night over the last 40 years (1;2). Cross-sectional and longitudinal studies have demonstrated a link between short sleep duration or poor sleep quality and increased risk of obesity (3-7), diabetes (7-11), hypertension (12), cardiovascular disease (13;14), the metabolic syndrome (15), and early mortality (14;16-21). Short-term sleep restriction (4 hours/night for one week in a laboratory setting) impaired glucose tolerance during a frequently sampled intravenous glucose tolerance test (IVGTT) in healthy subjects (22).

In healthy subjects, the mechanisms leading to impaired glucose tolerance with short-term reductions in nightly sleep duration are unclear. Decreases in insulin secretion have been implicated and sleep restriction increases cortisol levels which could influence glucose tolerance (22). Further, insulin resistance has been reported in two very different models of disrupted sleep – sleep apnea (23) and experimental disruption of slow wave sleep (24). In the latter model, the extent of slow wave sleep disruption predicted reductions in insulin sensitivity (24).

Our primary goal was to test the hypothesis that sleep restriction in healthy subjects
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reduces insulin sensitivity as assessed by the hyperinsulinemic euglycemic clamp technique. Insulin secretion was assessed using intravenous glucose tolerance tests. To identify possible mechanisms by which sleep restriction may affect insulin sensitivity, we assessed the relationships between changes in insulin sensitivity and changes in cortisol, catecholamines and slow wave sleep. Further, we tested the ability of modafinil to ameliorate the adverse effects of sleep restriction on insulin sensitivity. Modafinil activates central, wake-promoting dopaminergic and noradrenergic mechanisms (25;26) and ameliorates the adverse effects of sleep deprivation on alertness and performance (27-29), impairments in which have been attributed to reduced brain glucose utilization (30). Thus, we performed hyperinsulinemic euglycemic clamps and intravenous glucose tolerance twice, at baseline in sleep-replete individuals and after 7 nights of sleep restriction (5 hr in bed) in healthy individuals randomized to daily treatment with placebo or modafinil.

METHODS

Study Design. A schematic of this double-blind, placebo-controlled, randomized, clinical study is presented in Figure 1. Procedures were approved by the Human Research Committee of the Brigham and Women’s Hospital and conducted according to the principles expressed in the Declaration of Helsinki. All subjects provided written informed consent.

Subject recruitment and screening. Healthy male subjects were recruited using newspaper ads, flyers, and website postings. Subjects were screened for sleep patterns and medical and psychological history, underwent a physical examination by a licensed physician, and provided blood and urine samples to ensure that hematology and serum chemistry including metabolic and thyroid panels were within normal limits. All subjects passed a urine toxicology screen.

Pre-study conditions. Before the experiment, subjects slept at home for at least 5 days (mean 8.9, range 5-21 days) with 10 hours per night of time in bed (TIB) from 10pm to 8am (±1 hr) in order to enter the experimental portion of the protocol in a nearly ‘sleep-replete’ state, i.e. with similar and presumably minimal amounts of ‘sleep debt’ (31). Subjects called into a time-stamped phone answering system, wore wrist activity monitors (Minimitter, Bend OR), and completed a sleep diary to assure compliance with this schedule.

Inpatient study conditions. Subjects were admitted to the General Clinical Research Center at Brigham and Women’s Hospital for a 12-day inpatient visit. The study began with a 3-day baseline period of 10/hrs night TIB (continued from the pre-test period) and baseline metabolic assessments, after which subjects were scheduled for Sleep Restriction (5 hours/night TIB) for the following 7 nights with the sleep periods centered at the same clock time of 0300. During the periods of wakefulness, subjects were allowed to perform activities such as writing, reading, computer work, board or card games, movie viewing, arts and crafts, listening to or playing music and light stretching. Subjects were observed by research technicians throughout the protocol either in direct interaction or remotely via video. Light levels during sleep periods were essentially complete darkness (<1 lux) and <90 lux during wakefulness, which simulations suggest would lead to a <9 min mean difference of circadian phase between sleep conditions (32). Subjects were randomized to modafinil (100 mg per tablet) or placebo, 2 tablets at 0600, and 1 tablet at 1300 during the 7 days of Sleep Restriction. Post-Sleep Restriction metabolic assessments were performed, and subjects were discharged on day 12 after a recovery night of 10 hours TIB (see Figure 1, Protocol Schema). Metabolic
assessments are described below and consisted of an IVGTT, euglycemic hyperinsulinemic clamp, and collection of saliva and urine for hormone measurements. During each sleep period, scalp surface electrodes (Beckman Instrument Company, Schiller Park, IL) were applied to specific locations on the subject’s face and scalp at least 2 hours prior to the scheduled sleep period for recordings of central (C3 and C4) and occipital (O1 and O2) electroencephalogram (EEG), electrooculogram (EOG: LOC and ROC), electromyogram (EMG), and electrocardiogram (ECG). Data were collected using Vitaport 3 digital sleep recorders (TEMEC) and scored visually in 30-second epochs by registered polysomnographic technologists (33).

**Controlled Diet.** Throughout the inpatient portion of the study, subjects received an isocaloric, controlled-nutrient diet containing 58-60% carbohydrates, 15-17% protein, 25-27% fat (+/- 1%), 800-1000 mg calcium, 100 mEq (+/- 2 mEq) potassium, and 200 mEq (+/- 2 mEq) sodium. Subjects were required to consume all food provided. An identical menu was provided on the day before and the day of each IVGTT and each euglycemic hyperinsulinemic clamp procedure.

**Subjective measures of sleepiness and alertness.** Every three hours during wake periods, subjects completed a short test battery including the Karolinska Sleepiness Scale (KSS) (34) and the Psychomotor Vigilance Task (PVT) (3);(24). The PVT involved a 10-minute visual reaction time (RT) performance test in which the subject was instructed to maintain the fastest possible RT to a simple visual stimulus. Lapses of attention refer to the number of times the subject failed to respond to the signal within 500ms.

**Frequently-sampled Intravenous Glucose Tolerance tests (IVGTT; insulin-modified).** IVGTT tests were performed after an overnight fast on the mornings of days 3 and 10, e.g. following the penultimate night of 10 hours TIB and the penultimate (sixth) night of 5 hours TIB, respectively. Blood samples were drawn via an intravenous catheter every 5 min for 20 minutes starting at T= -20 min. At time 0 (9am), 0.3 g/kg glucose was infused over 1 minute via an intravenous catheter in the non-sampling arm. Blood samples were then taken at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 19, 21, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min. At time 20 min, human insulin (0.02U/kg; Novolin R U-100, Novo Nordisk, Princeton, NJ) was administered intravenously in less than 30 seconds, including saline flush. Minimal Model analyses (Minmod Millennium 2000, R. Bergman) were performed to determine first phase area under the insulin curve from 0 min to 10 min, glucose effectiveness (Sg), and insulin sensitivity (SI). Glucose tolerance (Kg) was calculated as the slope of the natural log of glucose values from minutes 5 through 19.

**Euglycemic Hyperinsulinemic Clamp Procedure.** Insulin sensitivity was measured using the insulin clamp procedure as previously described (35). Studies were performed on the mornings of days 4 and 11, following the final night of 10 hours TIB and the final (seventh) night of 5 hours TIB, respectively. Following an overnight fast, the subject remained in bed until the completion of the procedure. Intravenous lines were placed in each arm for infusion or blood draw. After a baseline sample was collected, subjects were infused with human insulin (Novolin R) with priming doses of 80 and 60 mU/m²body surface area/minute over the first two 5-min periods, respectively, followed by a constant infusion rate of 40mU/m²minute for 170 minutes. Blood samples were collected every 5 minutes from T=0 to 180 minutes from a catheter placed retrograde in a dorsal vein of the wrist; this hand was placed in a hand warmer thermostatically controlled
at 140 degrees Fahrenheit to arterialize venous blood. Serum glucose levels were determined immediately at the bedside and serum insulin was measured from frozen samples. Dextrose solution (20%) was variably infused to maintain serum glucose levels at 90 mg/dL throughout the clamp procedure. The mean glucose infusion rate over the last 60 min of the clamp (M), after correcting for changes in plasma glucose concentration, and expressed as mg (glucose) \cdot kg^{-1} \cdot minutes^{-1} of infusion) as detailed by Defronzo et al. (36) was determined and used as an indicator of insulin sensitivity.

**Resting Metabolic Rate (RMR).** RMR was estimated from expired gases using a validated and FDA-approved indirect calorimeter (Medgem 100, Healthetech Inc) that estimates RMR in kcal/day (20;37). Assessments were made upon awakening in the sleep replete condition, while subjects were still in bed (i.e., after a 12-hour fast), after a void and at least 10 min of quiet bed rest. The timing was at the same clock time in both conditions, approximately 0820, and the test duration about 12-14 minutes until steady state was attained.

**Saliva and Urine sampling.** Saliva samples for determination of free cortisol levels were collected from 1500-2100 on the last two days of each condition. Twenty-four-hour urine collections were obtained on the last two days of each condition.

**Assays.** Serum glucose during the clamp studies was measured using the Beckman Glucose Analyzer 2 (Beckman Coulter, Chaska, MN) with sensitivity of <10 mg/dL and precision <5%. Serum glucose during the IVGTT was measured using the COBAS Integra 400 (Roche Diagnostics, Indianapolis, IN) with sensitivity of 0.59 mg/dL and precision <4.3% (37). Serum insulin was measured by chemiluminescence immunoassay (Access Immunoassay System, Beckman Coulter, Chaska, MN) with sensitivity 0.03 IU/mL, precision <5.6%. Salivary cortisol was measured using a solid-phase radioimmunoassay (Coat-A-Count, DPC, Los Angeles, CA), with sensitivity <0.02 µg/dL, and precision 4-5%. Urinary norepinephrine and epinephrine was assayed using the LDN CAT RIA kit (Immuno Biological Laboratories, Inc, Minneapolis, MN). The sensitivity of this method is 1.5 ng/mL for norepinephrine and 0.3 ng/mL for epinephrine; the precision is <15% for both assays (38).

**Statistical analyses.** Mixed-effects models were applied to study the effects of the number of nights of sleep restriction and the effects of drug treatment on subjective and objective measures of sleepiness, including self-reported sleepiness and lapses of attention, and on insulin secretion, insulin sensitivity, cortisol levels, urinary norepinephrine and epinephrine, and resting metabolic rate. Treatment and sleep restriction status were considered fixed effects, and random intercepts were added to account for individual variation from the group mean. Data are presented as mean ± SEM unless otherwise indicated.

**RESULTS**

**Subjects.** Twenty healthy men (mean ± SD age: 26.8 ± 5.2 years, BMI: 23.3 ± 3.1 kg/m²) completed the study (11 placebo, 9 active drug). An additional 3 subjects were withdrawn from the study after initiation of drug treatment due to: 1) transient EKG changes in one subject; 2) tachycardia (up to 126 beats per minute) and elevated blood pressure (systolic 145 mm Hg and diastolic 91 mm Hg) in another subject; and 3) tachycardia (up to 127 beats per minute), elevated systolic blood pressure (systolic 147 mm Hg and diastolic 87 mm Hg), and urinary frequency in a third subject. These subjects were otherwise asymptomatic. Unblinding revealed that all three of these subjects had been receiving modafinil. All signs and
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symptoms resolved within a day of stopping the medication.

Subjective and objective measures of sleepiness. Sleep restriction increased self-reported sleepiness and objective measures of sleepiness compared with the sleep-replete baseline condition (Table 1, Figure 2); modafinil treatment significantly reduced the deleterious effects of sleep restriction on these measures of sleepiness.

Cortisol and norepinephrine. Salivary cortisol levels (assessed between 1500 and 2100 hrs) were elevated with sleep restriction compared to the baseline sleep-replete condition (Figure 3). The mean increase in cortisol of 0.054 ± 0.01 ng/mL with sleep restriction and placebo was similar to the increase observed with sleep restriction and modafinil treatment (0.066 ± 0.01 ng/mL, p=0.48). Compared to placebo, modafinil treatment significantly increased urinary epinephrine and norepinephrine with decreased sleep duration (Table 1). Changes in urinary catecholamines (sleep restricted minus baseline sleep replete) were 4.98±4.02 µg/day (placebo, t-test, p=0.24) and 18.29±3.31 µg/day (modafinil, t-test, p=0.0006) for norepinephrine and 1.58±1.72 µg/day (placebo, t-test, p=0.39) and 6.52±0.94 µg/day (modafinil, t-test, p=0.0001) for epinephrine.

Energy expenditure. Fasted resting metabolic rate was unchanged from baseline (sleep-replete) to sleep restriction (mean change: 0 ± 44 kcal). There was no effect of drug treatment on the change in RMR (Table 1).

Insulin sensitivity and acute insulin response (IVGTT). Insulin sensitivity assessed by minimal model analysis of IVGTT data was significantly reduced after sleep restriction compared with the sleep-replete baseline condition with no significant effect of modafinil treatment (Table 1, Figure 4E). Fifteen out of twenty subjects had a decrease in insulin sensitivity (SI) with sleep restriction with a mean decrease of (20 ± 24%; F1,18=15.18, p=0.001) (Table 1, Figure 4 E, F). The acute insulin response (AIRg) was not significantly affected by either sleep restriction or drug treatment (Table 1, Figure 4 C). With sleep restriction, the Disposition Index, the product of SI and AIRg, was significantly but slightly reduced (Table 1, Figure 4 D) and glucose tolerance was significantly reduced (change of 0.31 ± 0.13 %•min⁻¹ with modafinil, 0.17± 0.15 %•min⁻¹ with placebo). There were no significant effects of sleep restriction or drug treatment on other minimal model parameters (Table 1).

Insulin sensitivity (euglycemic hyperinsulinemic clamp). Glucose and insulin levels at baseline and during euglycemic hyperinsulinemic clamp protocols were similar between sleep-replete and sleep-restricted conditions and between modafinil and placebo treatments. Fasting insulin levels were 4.5 ± 0.4 µU/mL and increased to 57.8 ± 2.3 µU/mL during the insulin infusions. Serum glucose levels averaged 89.9 ± 0.3 mg/dL during the last 60 minutes of the clamp procedures. The dextrose infusion rate (M) needed to maintain euglycemia during the final hour of the clamp procedure was significantly reduced with sleep restriction compared to the baseline sleep replete condition (Figure 4 G, H); there was no significant effect of drug treatment (Table 1). Ninety per cent of subjects had a decrease in M with sleep restriction. The average decrease for all subjects was 11 ± 5.5% (mean ±SE, F1,18=4.64, p=0.045) relative to the baseline sleep replete condition (Figure 4). Importantly, changes in insulin sensitivity (M) assessed by the clamp procedure correlated with the change in insulin sensitivity (Si) assessed by the IVGTT procedure (r=0.53, p=0.02). Overall, there was a strong correlation of the absolute level of M with Si (r=0.85, p<0.0001).

Changes in slow wave sleep. Sleep restriction resulted in a significant decrease in
total sleep time, but changes in the amount slow wave sleep (NREM stages 3 and 4), previously linked to changes in glucose metabolism (24), were not related to changes in insulin sensitivity (see Table 1).

Predictors of changes in insulin sensitivity. There was no significant linear relationship between BMI and change in SI ($F_{1,17}=1.04$, $p=0.32$) or change in M ($F_{1,17}=1.23$, $p=0.28$), between change in cortisol and change in SI ($F_{1,17}=1.28$, $p=0.27$) or change in M ($F_{1,17}=0$, $p=0.99$), or between change in urinary catecholamine levels and change in either SI ($F_{1,17}=0.04$, $p=0.85$ for norepinephrine, $F_{1,17}=1.79$, $p=0.20$ for epinephrine) or M ($F_{1,17}=0.68$, $p=0.42$ for norepinephrine, $F_{1,17}=0.01$, $p=0.91$ for epinephrine). Similar results were obtained when the analysis was restricted to subjects receiving modafinil (data not shown).

DISCUSSION
Sleep restriction to 5 hours/night (TIB) for one week in non-obese, healthy men significantly reduced insulin sensitivity as assessed by two techniques, the euglycemic hyperinsulinemic clamp and the intravenous glucose tolerance test, yet did not affect the acute insulin response to intravenous glucose administration. Sleep restriction led to elevations of afternoon and evening levels of free cortisol, but these increases were not linearly related to changes in insulin sensitivity. The effects of sleep restriction on measures of glucose metabolism and on salivary cortisol were not altered by administration of modafinil, though modafinil did improve subjective and objective measures of sleepiness. These changes in insulin sensitivity support the hypothesis that insufficient sleep duration leads to insulin resistance.

Our finding that sleep restriction leads to a decrease in insulin sensitivity is consistent with earlier studies showing impaired glucose metabolism with altered sleep duration. The earliest direct assessment of the relationship between sleep and glucose metabolism demonstrated that complete sleep deprivation for 3-4 days led to an elevation of glucose levels on an oral glucose tolerance test (39). Spiegel and colleagues from the Van Cauter laboratory (22) performed FSIVGTT in healthy subjects during a sleep-debt condition (4 hrs per night) and the sleep replete condition (12 hrs/night). They found that the sleep-debt condition led to impaired glucose metabolism characterized by 30-40% reductions in glucose tolerance, glucose effectiveness and acute insulin response to glucose, but a non-significant reduction in insulin sensitivity. We also demonstrated impairment in glucose metabolism with sleep restriction (to 5 hrs/night, compared to baseline sleep replete of 10 hrs/night), but the impairment was attributable to a decrease in insulin sensitivity rather than to impairments in insulin secretion or glucose effectiveness. However, we did not observe a compensatory increase in insulin secretion despite the reduction in insulin sensitivity so it is possible that more than one mechanism is contributing to impaired glucose metabolism with sleep restriction in our study. Our results are consistent with recent results in eleven, overweight, middle-aged adults that sleep restriction to 5.5 hours/night with an ad libitum diet reduces insulin sensitivity but does not change insulin secretion on an IVGTT(40). The current study extends from these findings with two techniques for assessing insulin sensitivity, the insulin-modified FSIVGTT and the gold standard euglycemic hyperinsulinemic clamp, with concordant results. In further support of the concept that alterations in sleep may affect insulin sensitivity, Van Cauter and colleagues recently reported that the nearly total suppression of slow wave sleep by acoustic disruption for 3 nights (without changing total sleep duration) reduces insulin sensitivity as well as the acute insulin response (24).
Substantive differences in the current protocol compared to Spiegel and colleagues may account for our different results. While both studies examined the effects of sleep restriction in healthy subjects, the ‘baseline’ sleep replete condition actually came after the sleep debt condition in the Spiegel protocol, so the sleep replete condition may reflect more of a recovery process than the actual baseline for each individual. We believe our ‘sleep replete’ baseline more accurately defines (in experimental and ecological terms) the changes in both sleep and metabolism from sleep replete to sleep restricted conditions. In addition, the current protocol carefully controlled food intake and activity whereas the Spiegel protocol allowed subjects to leave the laboratory each day during sleep-restricted conditions. Also, the ‘dose’ of sleep restriction could influence the results, as Spiegel and colleagues restricted sleep to 4 hours/night whereas we used 5 hours/night (to apply to a greater proportion of the adult population). Finally, the specific procedures to assess glucose metabolism differed between the two studies. The Spiegel study (24) used the tolbutamide-assisted FSIVGTT and this procedure may not have had sufficient sensitivity to detect a significant increase in insulin resistance with sleep restriction.

Previous studies have shown that sleep restriction increases self-reported hunger and appetite for carbohydrate food (41;42). Therefore, to control for potential variations in diet, subjects in the current study consumed a consistent diet throughout the protocol. In addition, subjects maintained a sedentary (but not bed rest) level of activity. Thus, the current study did not allow behavioral changes in diet composition (43), caloric intake, or activity/exercise levels that may have contributed to the association of reduced habitual sleep duration and metabolic dysregulation in previous studies. We observed no change in resting metabolic rate using indirect calorimetry, consistent with a prior report of a nonsignificant change in total energy expenditure with sleep restriction (42).

A further strength of the current study is that all subjects began the study in a similar sleep-replete state prior to the imposition of sleep restriction. Under our experimental conditions there was a deterioration of subjective and objective measures of sleepiness with sleep restriction, consistent with the known effects of sleep restriction. Modafinil administration partially mitigated this effect. However, modafinil treatment had no discernable affect on glucose metabolism. Activation of the hypothalamic-pituitary-adrenal axis and the autonomic nervous system are two key counterregulatory pathways for increasing glucose levels during hypoglycemia. Both these pathways also have been proposed as possible mediators of the impairments in glucose tolerance associated with sleep restriction (22;44-48). In the current study, sleep restriction led to a significant increase in salivary cortisol, and urinary norepinephrine and epinephrine. Catecholamine increases with sleep restriction were amplified by modafinil treatment, consistent with prior reports of increased catecholamine levels with modafinil administration (49). However, we found no association, under our experimental conditions, between changes in insulin sensitivity and changes in hypothalamic-pituitary-adrenal axis function and sympathetic nervous system function, suggesting that these systems do not mediate the changes in insulin sensitivity with moderate sleep restriction.

In the present study, the relatively modest restriction of the sleep period to 5 hrs per night led to a small increase in slow wave sleep (SWS) amount by the third night, reflecting an increase in homeostatic sleep drive. A study that deliberately reduced SWS through acoustic disruption without changing total sleep time led to a reduction in insulin sensitivity (24). In the current study we
observed reductions in insulin sensitivity due to reductions in total sleep time, not to reductions in slow wave sleep. Our experimental design is much more closely related to the type of sleep restriction that occurs in healthy individuals who voluntarily restrict sleep. The limitations of this study include the small sample size that is limited to healthy non-obese men and the lack of a control group that continued the sleep-replete condition of 10 hours/night TIB from baseline through to the end of the study. Future studies are needed to determine the effects of sleep restriction on insulin sensitivity in other populations, including women, obese patients, and individuals with insulin resistance or diabetes. It is unlikely that the protocol alone (in the absence of sleep restriction) would lead to decreased insulin sensitivity because, in other published studies, repeating these intensive glucose metabolism test does not lead to worsening of metabolic function. Other authors, in validating different types of metabolic challenge tests, have demonstrated the reproducibility of the test results especially for insulin sensitivity (50). Furthermore, the careful control of diet and exercise allowed us to focus on effects of sleep restriction. However, sleep restriction increases appetite and increases the desire for high carbohydrate/high fat foods so the control of food intake may have dampened the full effects of sleep restriction on glucose metabolism. In addition, we did not measure circadian phase changes directly. However, using a validated, data-based mathematical model, we estimate a < 9 min variation in circadian phase under the experimental conditions and light levels employed in this study (32). Thus, circadian phase changes are unlikely to be responsible for the differences we found in insulin sensitivity.

Insufficient sleep duration (quantity) has been associated with an increased risk of obesity (3-6;51), type 2 diabetes mellitus (7-11), hypertension (12), cardiovascular disease (13;14), metabolic syndrome (a combination of cardiovascular and metabolic dysfunction) (15), and early mortality (14;16;17;19-21;52). Our finding that reducing sleep increases insulin resistance provides one possible mechanism for these associations. In prospective studies, decreases in the Disposition Index, the product of insulin secretion and insulin sensitivity, are a strong predictor of diabetes onset and worsening of metabolic function pre- and post-diagnosis (53). Our finding that sleep restriction reduces the Disposition Index further supports the hypothesis that sleep restriction contributes to the development of metabolic dysregulation resulting in elevated risk for diabetes. Future studies are needed to determine whether chronic short sleep has detrimental effects on insulin resistance and glucose metabolism and whether short sleep is a risk factor for disease processes associated with insulin resistance.

Author Contributions: OB designed study, collected data, analyzed data, wrote manuscript. MP assisted with study design, collected data, reviewed/edited manuscript; ER collected data, reviewed/edited manuscript; WW analyzed data and performed statistical analyses, reviewed/edited manuscript; DS assisted with study design, assisted with data analysis, reviewed/edited manuscript; GA assisted with study design, collected data, assisted with data analysis, reviewed/edited manuscript

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**FIGURE LEGENDS**

**Figure 1. Protocol Schema.** (See Methods for detailed description.)

**Figure 2. Subjective sleepiness and lapses of attention under sleep restriction conditions.** Effects of sleep restriction on subjective sleepiness (top panel) and lapses of attention (bottom panel). Subjective sleepiness and objective lapses of attention in sleep-replete subjects were assessed after 10 hours of Time in Bed per night and after restricting sleep to 5 hours/night over one week (Days 1-6 averages) in subjects randomized to placebo (red circles) or modafinil administration (green triangles). Subjective sleepiness is defined as mean deviation from baseline Karolinska Sleepiness Scale (KSS). Lapses of attention are defined as reaction times >500 ms and are quantified as the absolute deviation from baseline (lapses/test, per day). For self-reported sleepiness, we noted significant main effects of sleep restriction (p<0.0001), treatment (p<0.0001), and their interaction p<0.0001). With modafinil administration, self-reported sleepiness was significantly reduced compared with placebo after the first and second nights of sleep restriction only (p=0.0276). For lapses of attention, there was a significant main effect of number of nights of sleep restriction (p=0.0012), a borderline effect for treatment (p=0.0647), and their interaction was non-significant (p=0.1488). With modafinil administration, lapses of attention were significantly reduced compared with placebo during the daytime testing after the first and second nights of sleep restriction only (p=0.0141); after the second and third nights, the borderline p-values were 0.0779 and 0.0770.

**Figure 3. Salivary (free) cortisol levels with sleep restriction.** Salivary cortisol levels were assessed hourly from 1500 to 2100 under sleep-replete conditions (10 hrs/night Time In Bed, filled circles), and under sleep-restricted conditions (5 hrs/night Time In Bed for 1 week) in subjects receiving placebo (red circles) or modafinil (green triangles). Salivary cortisol levels (mean ± SD) from 1500-2100 were significantly affected by sleep duration only: 0.13 ± 0.03 ng/ml for the sleep-replete condition and 0.17 ± 0.04 ng/ml for the sleep restricted condition (p<0.0001; Table 1). Identical (mixed composition) dinners (D) were served just after the 1800 saliva sample and finished before 1840.
Figure 4. Effects of sleep restriction on glucose metabolism

Panels A-B. Mean glucose levels (±SE) from IVGTT during the baseline sleep-replete condition (10 hours/night of Time In Bed; black line) and following sleep restriction for 1 week (5 hours/night of Time In Bed) in subjects receiving A, placebo, red line or B, modafinil, green line. Left arrow: glucose infusion at time = 0 min; right arrow: insulin infusion at time = 20 min. 

Panels C-D. Mean insulin levels (±SE) from IVGTT. Panels E-H. IVGTT parameters were calculated using Minmod Millennium software. Glucose and insulin data from insulin-modified IVGTT procedures under sleep-replete (filled symbols) and sleep-restricted conditions (open symbols) are shown. E. Acute Insulin Response (first phase; AIRg); F. Disposition Index. G. Insulin sensitivity (SI) from IVGTT. H. Relative changes in insulin sensitivity (SI) from IVGTT expressed as percent change from baseline sleep replete condition in subjects randomized to placebo (red circles) or modafinil administration (green triangles). Panels I-J: I. Insulin sensitivity (M) from euglycemic hyperinsulinemic clamp procedure, and J. Relative changes in insulin sensitivity (M) depicted as in Panel F. There was no significant effect of drug administration on any metabolic parameters (see Table 1).
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<td>2.48 (0.37)</td>
<td>5.10 (0.73)</td>
<td>0.92 (0.18)</td>
</tr>
<tr>
<td>5 hours vs. sleep restriction condition</td>
<td>mean (SE)</td>
<td>268.2 (3.5)</td>
<td>502.2 (8.9)</td>
<td>274.0 (1.4)</td>
<td>467.4 (10.8)</td>
</tr>
<tr>
<td>10 hours</td>
<td>mean (SE)</td>
<td>78.6 (3.4)</td>
<td>75.2 (5.9)</td>
<td>75.4 (3.2)</td>
<td>65.2 (7.4)</td>
</tr>
<tr>
<td>sleepiness KSS (1-9 scale)</td>
<td>mean (SE)</td>
<td>4.61 (0.55)</td>
<td>5.70 (0.88)</td>
<td>4.81 (0.78)</td>
<td>6.74 (0.94)</td>
</tr>
<tr>
<td>performance PVT lapses (lapses/test/day)</td>
<td>mean (SE)</td>
<td>4.09 (0.45)</td>
<td>2.48 (0.37)</td>
<td>5.10 (0.73)</td>
<td>0.92 (0.18)</td>
</tr>
<tr>
<td>sleep duration Total Sleep Time (minutes)</td>
<td>mean (SE)</td>
<td>4.09 (0.45)</td>
<td>2.48 (0.37)</td>
<td>5.10 (0.73)</td>
<td>0.92 (0.18)</td>
</tr>
<tr>
<td>slow wave sleep Mean (minutes)</td>
<td>mean (SE)</td>
<td>4.09 (0.45)</td>
<td>2.48 (0.37)</td>
<td>5.10 (0.73)</td>
<td>0.92 (0.18)</td>
</tr>
<tr>
<td>FSIVGTT insulin sensitivity, SI ([(mU/l)^-1 • min^-1])</td>
<td>mean (SE)</td>
<td>4.61 (0.55)</td>
<td>5.70 (0.88)</td>
<td>4.81 (0.78)</td>
<td>6.74 (0.94)</td>
</tr>
<tr>
<td>insulin response, AIRG (mU•l^-1•min^-1)</td>
<td>mean (SE)</td>
<td>549.2 (127.2)</td>
<td>514.0 (106.3)</td>
<td>532.3 (94.1)</td>
<td>566.7 (112.8)</td>
</tr>
<tr>
<td>disposition index, DI (unitless)</td>
<td>mean (SE)</td>
<td>2034.2 (231.6)</td>
<td>2500.6 (391.9)</td>
<td>2079.5 (254.1)</td>
<td>2993.7 (431.6)</td>
</tr>
<tr>
<td>glucose tolerance (K_G; %/min)</td>
<td>mean (SE)</td>
<td>-1.984 (0.137)</td>
<td>-2.295 (0.160)</td>
<td>-2.120 (0.162)</td>
<td>-2.465 (0.261)</td>
</tr>
<tr>
<td>glucose effectiveness, SG (min^-1)</td>
<td>mean (SE)</td>
<td>0.017 (0.002)</td>
<td>0.020 (0.002)</td>
<td>0.021 (0.002)</td>
<td>0.023 (0.003)</td>
</tr>
<tr>
<td>clamp insulin sensitivity (M, mg/kg/min)</td>
<td>mean (SE)</td>
<td>6.15 (0.66)</td>
<td>7.11 (0.86)</td>
<td>6.95 (0.81)</td>
<td>7.39 (0.79)</td>
</tr>
<tr>
<td>cortisol salivary cortisol (ng/ml)</td>
<td>mean (SE)</td>
<td>0.179 (0.017)</td>
<td>0.112 (0.013)</td>
<td>0.180 (0.015)</td>
<td>0.125 (0.010)</td>
</tr>
<tr>
<td>catecholamines norepinephrine (µg/ml)</td>
<td>mean (SE)</td>
<td>48.0 (4.5)</td>
<td>29.7 (4.3)</td>
<td>32.5 (4.4)</td>
<td>27.5 (4.7)</td>
</tr>
<tr>
<td>epinephrine (µg/day)</td>
<td>mean (SE)</td>
<td>13.5 (1.2)</td>
<td>7.0 (0.8)</td>
<td>9.0 (2.1)</td>
<td>7.4 (1.1)</td>
</tr>
<tr>
<td>RMR resting metabolic rate (kcal)</td>
<td>mean (SE)</td>
<td>1931.4 (77.4)</td>
<td>1928.6 (79.1)</td>
<td>1851.0 (86.4)</td>
<td>1853.0 (116.6)</td>
</tr>
</tbody>
</table>
Insufficient sleep and insulin sensitivity

Figure 1

Study Procedures

Wrist actigraphy monitoring and sleep diaries

urine, saliva sampling

Neurobehavioral testing during wake

Resting Metabolic Rate, waketime

Day of Sleep Restriction

Sleep Condition (Time In Bed)

Sleep Replete
(10 hrs/night)
for 1 week at home

Sleep Replete
(10 hrs/night)
3 nights

Sleep Restriction
(5 hrs/night)
for 7 nights

Recovery
(10 hrs)
for 1 night

(randomization)
Placebo or modafinil (300 mg/day)
Figure 2

**Lapses of Attention**
Deviation from Baseline
(Mean Lapses/Test)

**Subjective Sleepiness**
Mean Deviation from Baseline KSS Rating

<table>
<thead>
<tr>
<th>Day of Sleep Restriction</th>
<th>Day of Sleep Restriction</th>
</tr>
</thead>
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<tr>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
Figure 3

![Graph showing cortisol levels over time]

**Salivary cortisol mean ± SE (ng/ml)**

**Clock time (hour)**

- 15:00
- 17:00
- 19:00
- 21:00

Insufficient sleep and insulin sensitivity
Figure 4

**Insufficient sleep and insulin sensitivity**

- **Glucose Response (IVGTT)**
- **Insulin Response, First Phase (IVGTT)**
- **Insulin Response, Disposition Index (IVGTT)**
- **Insulin Sensitivity (IVGTT)**
- **Insulin Sensitivity (Euglycemic Hyperinsulinemic Clamp)**