A Single Night of Partial Sleep Deprivation Induces Insulin Resistance in Multiple Metabolic Pathways in Healthy Subjects

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Background: Subsequent nights with partial sleep restriction result in impaired glucose tolerance, but the effects on insulin sensitivity have not been characterized.

Objective: The aim of this study was to evaluate the effect of a single night of partial sleep restriction on parameters of insulin sensitivity.

Research Design and Methods: Nine healthy subjects (five men, four women) were studied once after a night of normal sleep duration (sleep allowed from 2300 to 0730 h), and once after a night of 4 h of sleep (sleep allowed from 0100 to 0500 h). Sleep characteristics were assessed by polysomnography. Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp studies (from 1130 to 1430 h) with infusion of [6,6-^{2}H$_2$]glucose.

Results: Sleep duration was shorter in the night with sleep restriction than in the unrestricted night (226 ± 11 vs. 454 ± 9 min; $P < 0.0001$). Sleep restriction did not affect basal levels of glucose, nonesterified fatty acids, insulin, or endogenous glucose production. Sleep restriction resulted in increased endogenous glucose production during the hyperinsulinemic clamp study compared to the unrestricted night (4.4 ± 0.3 vs. 3.6 ± 0.2 μmol×kg lean body mass$^{-1} \cdot$ min^{-1}; $P = 0.017$), indicating hepatic insulin resistance. In addition, sleep restriction decreased the glucose disposal rate during the clamp (32.5 ± 3.6 vs. 40.7 ± 5.1 μmol $\cdot$ kg lean body mass$^{-1} \cdot$ min^{-1}; $P = 0009$), reflecting decreased peripheral insulin sensitivity. Accordingly, sleep restriction decreased the rate of glucose infusion by approximately 25% ($P = 0.001$). Sleep restriction increased plasma nonesterified fatty acid levels during the clamp study (68 ± 5 vs. 57 ± 4 μmol/liter; $P = 0.005$).

Conclusions: Partial sleep deprivation during only a single night induces insulin resistance in multiple metabolic pathways in healthy subjects. This physiological observation may be of relevance for variations in glucoregulation in patients with type 1 and type 2 diabetes. (**J Clin Endocrinol Metab** 95: 2963–2968, 2010)

Sleep plays a key role in the homeostasis of normal glucose metabolism (1, 2). In physiological circumstances, glucose metabolism shows a diurnal pattern with intraindividual variations in glucose tolerance: glucose utilization is highest during wake and lowest during non-rapid eye movement (REM) sleep (3).

Reductions in sleep duration result in impaired glucose tolerance. Epidemiological studies documented a strong association between partial sleep restriction and impaired glucose tolerance (4–6). In accordance with these findings, experimental studies showed that sleep restriction to only 4 h of sleep during two or more nights reduced glu-

Abbreviations: CV, Coefficient of variation; NEFA, nonesterified fatty acids; Ra, rate of appearance; Rd, rate of disappearance or disposal; REM, rapid eye movement.

cose tolerance by 40% and reduced the acute insulin response to glucose in healthy subjects by 30% (7, 8). However, the effects of only a single night of partial sleep restriction on insulin sensitivity are unknown. Moreover, previous studies did not include the assessment of insulin sensitivity by the hyperinsulinemic euglycemic clamp method, which is considered to be the gold standard for measurement of insulin sensitivity.

We hypothesized that even a single night of partial sleep deprivation might induce insulin resistance in healthy subjects. Therefore, we compared the effects of a single night of partial sleep restriction and of a night of normal sleep duration on hepatic and peripheral insulin sensitivity in hyperinsulinemic euglycemic clamp conditions combined with tracer dilution of $[6,6-^2H_2]$glucose.

Subjects and Methods

Subjects

Nine healthy lean subjects (five men and four women) were recruited by advertisement. Their weight had been stable for at least 3 months before participation in this study. Subjects were instructed not to alter lifestyle habits during the whole study period. All premenopausal women were studied in the follicular phase of their menstrual cycle.

Exclusion criteria were a body mass index greater than 26 kg/m^2, sleep disorders, habitual sleep duration of less than 6 h or more than 9 h, psychiatric disorders, and use of sleep medication or of medication affecting glucose metabolism.

The study was approved by the medical ethical committee of Leiden University Medical Center, and written informed consent was obtained from all subjects before the study.

Study design

Actigraphy (Actiwatch AW7; Cambridge Neurotechnology, Cambridge, UK) was performed to objectively measure habitual sleep duration during 7 d before the actual study, including one weekend. In addition, self-reported sleep duration and sleep quality were assessed using validated questionnaires (Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, and Berlin Questionnaire) (9–11).

The subjects were studied on 3 d, separated by intervals of at least 3 wk. Subjects kept a detailed diary of their diet and physical activity for 3 d before each study day and were asked to maintain a standardized schedule of bedtimes and mealtimes in accordance with their usual habits. They were admitted to our clinical research center the night before each study day, and spent 8.5 h in bed from 2300 to 0730 h on all three occasions. Subjects fasted throughout these nights from 2200 h. The first study day was included to let the subjects become accustomed to sleeping in our clinical research center. Subjects were randomly assigned to sleep deprivation on either the second (n = 4) or third (n = 5) occasion.

During the night of sleep restriction, subjects spent 8.5 h in bed but were only allowed to sleep from 0100 to 0500 h. They were allowed to read or watch movies in an upward position during the awake hours, and their wakefulness was monitored and assured if necessary.

The rationale for essentially broken sleep deprivation from 2300 to 0100 h and from 0500 to 0730 h, as opposed to sleep deprivation from 2300 to 0300 h or from 0300 to 0730 h, was that in both conditions, the time in bed was centered at the same time, *i.e.* approximately 0300 h. Slow-wave sleep (*i.e.* stage III of non-REM sleep) is thought to play the most important role in metabolic, hormonal, and neurophysiological changes during sleep. Slow-wave sleep mainly occurs during the first part of the night, whereas REM sleep predominantly occurs during the latter part of the night (12). We used broken sleep deprivation to achieve a more equal compression of both non-REM and REM sleep stages. Moreover, we used the same experimental conditions for partial sleep deprivation as previously used in other studies (7, 13) to enable comparison of the results.

Polysomnography

Sleep recordings were performed using a portable polysomnography recorder (Titanium; Embla Systems, Inc., Broomfield, CO). Sleep was visually scored for each of the three nights according to the guidelines of the American Association of Sleep Medicine (14). For this study, the duration of wake, stage I, II, III, and REM sleep was noted. Total sleep duration was the sum of sleep stages I, II, III, and REM.

Hyperinsulinemic euglycemic clamp studies

Hyperinsulinemic euglycemic clamp studies were performed the day after the second and third nights. After an overnight fast, a catheter was inserted into an antecubital vein for infusion of isotopes, glucose, and insulin, and a sampling catheter was inserted into a dorsal hand vein of the contralateral arm. For all blood samples, the heated hand technique was used to obtain arterialized blood (15). A primed (17.6 μmol · kg^{-1}), continuous (0.22 μmol · kg^{-1} · min^{-1}) infusion of $[6,6-^2H_2]$glucose (Cambridge Isotope Laboratory, Andover, MA) was started at 0830 h, both after the night of normal sleep duration and after the night of partial sleep deprivation, after basal blood samples had been taken for determination of background glucose enrichment. Labeled glucose was infused by a Pilot C syringe pump (Fresenius Vial; Fresenius Kabi, Brezins, France). Blood samples were obtained after 160, 170, and 180 min of $[6,6-^2H_2]$glucose infusion for assessment of glucose kinetics in the basal state and concentrations of glucose, insulin, and plasma nonesterified fatty acids (NEFA).

Subsequently, infusion of insulin was started, using the method of DeFronzo *et al.* (16) while the infusion of $[6,6-^2H_2]$glucose continued. Briefly, the infusion of insulin consisted of a primed (80 mU · m^{-2} · min^{-1} for 5 min and subsequently 40 mU · m^{-2} · min^{-1} for 5 min), followed by continuous (20 mU · m^{-2} · min^{-1}) infusion of insulin (Actrapid; Novo Nordisk, Alphen a/d Rijn, The Netherlands), dissolved in sterile NaCl 0.9%, using a Pilot C syringe pump. A variable infusion of glucose 20% enriched with 3% $[6,6-^2H_2]$glucose was started 4 min after the start of insulin infusion. Plasma glucose concentrations were measured at intervals of 5 min with a bedside calibrated glucose analyzer (Accu-Chek; Roche, Mannheim, Germany) and the infusion rate of glucose 20% was adjusted to keep the plasma glucose levels constant at 5.0 mmol/liter during the clamp study. Blood samples were obtained after 160, 170, and 180 min of combined insulin and $[6,6-^2H_2]$glucose infusion for assessment of glucose kinetics and of concentrations of glucose, insulin, and plasma NEFA.

Assays

Serum concentrations of glucose were measured using a fully automated Modular P 800 analyzer (Roche/Hitachi, Mannheim, Germany) with intraassay variations of 1%. Serum insulin concentrations were measured by enzyme-labeled chemiluminescent immunometric assay (Immulite 2500; Siemens, Munich, Germany) with an intraassay coefficient of variation (CV) of 4%. Cortisol was analyzed on a Modular E-170 immunoanalyzer of Roche Diagnostics (Mannheim, Germany). Intra- and interassay CV were less than 5% at all measured levels. Glucagon was analyzed using an immunoradiometric assay of Millipore (Billerica, MA; formerly known as Linco) with a functional sensitivity of 20 pg/ml. Intraassay CV was less than 12%, and interassay CV was less than 15%. NEFA were determined spectrophotometrically by enzymatic colorimetric acyl-coenzyme A synthase/acyl-coenzyme A oxidase assay (WAKO Chemicals, Neuss, Germany) with intraassay CV of 2.7%. Enrichment of plasma $[6,6-^2H_2]$glucose was determined in a single analytical run using gas chromatography coupled to mass spectrometry, as described previously (17). All isotope enrichments were measured on a gas chromatograph mass spectrometer (model 6890/5973; Hewlett-Packard, Palo Alto, CA).

Calculations

An isotopic steady state was achieved during the final 30 min of the basal period and the final 30 min of the hyperinsulinemic clamp study. Therefore, the rates of appearance (Ra) and disappearance (Rd) of glucose were calculated as the tracer infusion rates divided by the tracer-to-tracee ratios (18). Endogenous glucose production during the basal steady state is equal to Ra of glucose, whereas endogenous glucose production during the hyperinsulinemic clamp study was calculated as the difference between Ra and the glucose infusion rates.

Statistical analysis

Data are presented as mean ± SEM. Differences between the effects of the night of normal sleep duration and sleep restriction were analyzed by Student's t test for paired samples. All analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL). Significance was accepted at $P < 0.05$.

Results

Clinical characteristics

Mean age of the subjects (five men, four women) was 44.6 ± 4.9 yr; mean weight was 72.4 ± 2.6 kg; mean height was 174.6 ± 1.4 cm; and mean body mass index was 23.8 ± 0.8 kg/m². All subjects had normal results on validated questionnaires on sleep characteristics (9–11). Self-reported sleep duration and recorded habitual sleep duration by actigraphy were not different (450 ± 20 vs. 476 ± 11 min; $P = 0.19$).

The effects of partial sleep restriction on polysomnographic parameters (Table 1)

Measured sleep duration was considerably shorter in the night with partial sleep restriction, compared with the night with normal sleep duration ($P < 0.001$). The proportion of stage III sleep was higher in the sleep-deprived night ($P = 0.007$), whereas the subjects had relatively less stage II sleep ($P = 0.006$). The percentages of REM sleep and wake time during sleep did not differ significantly between nights.

TABLE 1. The effects of a night of normal sleep duration and a night with sleep restricted to 4 h on sleep parameters, basal and insulin-stimulated glucose, and fatty acid metabolism measured the following morning in nine healthy subjects

	Normal sleep duration	Partial sleep deprivation	*P*-value
Sleep parameters			
Total sleep time (min)	454 ± 9	226 ± 11	**0.000**
Stage I (%)	9.6 ± 1.1	10.7 ± 2.3	0.490
Stage II (%)	41.3 ± 2.5	34.5 ± 3.4	**0.006**
Stage III (slow-wave sleep) (%)	25.4 ± 1.8	33.5 ± 3.1	**0.007**
REM sleep (%)	23.7 ± 1.7	21.3 ± 3.0	0.364
Wake time during sleep (%)	6.8 ± 1.5	6.8 ± 2.2	1.0
Basal values			
Glucose (mmol/liter)	4.8 ± 0.18	4.8 ± 0.18	0.32
Insulin (mU/liter)	3.1 ± 0.5	3.6 ± 0.6	0.18
NEFA (μmol/liter)	630 ± 50	620 ± 50	0.76
Endogenous glucose production (μmol · kg LBM^{-1} · min^{-1})	16.7 ± 0.4	16.7 ± 0.5	0.87
Glucagon (pg/ml)	36 ± 4	35 ± 4	0.888
Cortisol (nmol/liter)	349 ± 59	395 ± 59	0.308
Hyperinsulinemic euglycemic clamp study			
Glucose (mmol/liter)	5.0 ± 0.11	4.9 ± 0.9	0.10
Insulin (mU/liter)	22.0 ± 1.5	21.4 ± 1.8	0.30
NEFA (μmol/liter)	57 ± 4	68 ± 5	**0.005**
Endogenous glucose production (μmol · kg LBM^{-1} · min^{-1})	3.6 ± 0.2	4.4 ± 0.3	**0.017**
Glucose Rd (μmol · kg LBM^{-1} · min^{-1})	40.7 ± 5.1	32.5 ± 3.6	**0.009**
Glucose infusion rate (μmol · kg LBM^{-1} · min^{-1})	36.9 ± 5.1	27.8 ± 3.7	**0.001**
Glucagon (pg/ml)	24 ± 3	25 ± 3	0.590
Cortisol (nmol/liter)	353 ± 47	410 ± 48	0.321

Data are presented as mean ± sem. Significant differences are shown in *bold*. LBM, Lean body mass.
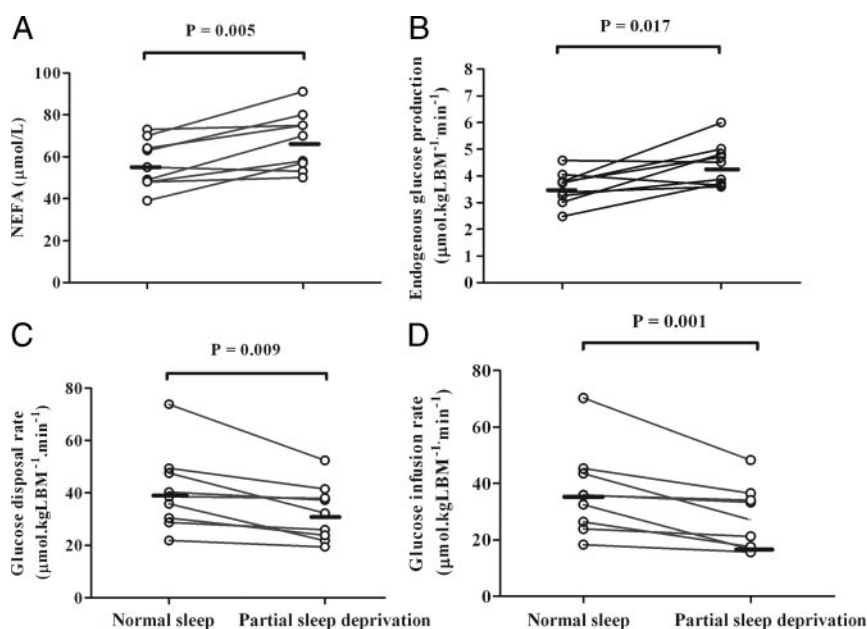
FIG. 1. A, Individual values obtained during steady state of the hyperinsulinemic euglycemic clamp studies of NEFA; B, endogenous glucose production; C, glucose disposal rate; D, the glucose infusion rate after a night of normal sleep duration versus after a night of partial sleep deprivation in healthy subjects (n = 9). *Black horizontal lines* represent the mean of the values of 9 subjects. There were significant differences ($P < 0.05$) for all these parameters after partial sleep deprivation.

The effects of partial sleep restriction on basal metabolic parameters (Table 1)

Compared with normal sleep, partial sleep deprivation did not alter basal levels of glucose, NEFA, insulin, glucagon, or cortisol measured the following morning. In addition, sleep restriction did not affect basal endogenous glucose production assessed by primed, continuous infusion of $[6,6-^2H_2]$glucose.

The effects of partial sleep restriction on metabolic parameters during hyperinsulinemic euglycemic clamp conditions (Table 1 and Figure 1)

Steady-state glucose and insulin levels did not differ between the two clamp studies. Sleep restriction resulted in an increase of endogenous glucose production of approximately 22% ($P = 0.017$) during the clamp conditions, indicating hepatic insulin resistance. In addition, sleep restriction decreased the rate of glucose disposal (Rd) during the clamp by approximately 20% ($P = 0.009$), reflecting decreased peripheral insulin sensitivity. Accordingly, the rate of infusion of glucose, necessary to maintain plasma glucose levels during the hyperinsulinemic clamp study, was approximately 25% lower after the night of reduced sleep duration than after the night of normal sleep duration ($P = 0.001$). Finally, the night of partial sleep restriction induced an increase of approximately 19% in plasma NEFA levels ($P = 0.005$) during the clamp study, indicating decreased insulin sensitivity of lipolysis. There

were no differences in glucagon or cortisol levels between the steady-state conditions of both clamp studies.

Discussion

The aim of the present study was to assess the effects of partial sleep deprivation during a single night on insulin sensitivity in healthy subjects. The results indicate that partial sleep restriction during only a single night reduces insulin sensitivity by 19–25% of hepatic and peripheral glucose metabolism, as well as of peripheral lipolysis, reflected by NEFA levels. Therefore, a single night of sleep restriction to 4 h induces insulin resistance of multiple metabolic pathways in healthy subjects.

The results of the present study stress the importance of sleep duration as a physiological determinant for insulin sensitivity. The study is unique in several ways. First, the finding that a single night of shortened sleep is sufficient to reduce insulin sensitivity in healthy men and women is novel. Second, this is the first study detailing the effects of sleep restriction on parameters of insulin sensitivity documented during hyperinsulinemic euglycemic clamp conditions with isotope dilution of a glucose tracer. Third, we document that insulin sensitivity is reduced in different metabolic pathways, *i.e.* endogenous glucose production, glucose uptake, and lipolysis.

The current study extends the observations of previous experimental studies on the effects of sleep deprivation on glucose metabolism, summarized in Table 2. These studies covered a range of sleep deprivation between 1 h of restriction of sleep during multiple subsequent nights, restriction to 4 h of sleep during subsequent nights, and finally complete deprivation of sleep. There was no significant effect on glucose tolerance if sleep was reduced by only 1 h (19). More severe restriction of sleep duration to 4 h per night during multiple subsequent nights resulted in decreased glucose tolerance (7, 20). Total sleep deprivation also decreased glucose tolerance (21, 22). Apparently, there is a threshold of a minimum duration of sleep, which is required for maintenance of normal glucose tolerance. Moreover, based on our findings after only a single night of partial sleep deprivation, it is tempting to speculate that the negative effects of multiple nights of partial sleep restriction on glucose tolerance can be reproduced, at least in part, by only a single night of sleep deprivation.

TABLE 2. Summary of experimental studies on the effects of sleep deprivation on glucose metabolism

First author, year (Ref.)	Sample size	Sleep deprivation	Insulin sensitivity	Results
VanHelder, 1993 (22)	10 healthy males	Total sleep deprivation for 60 h	OGTT	No differences in plasma glucose response to OGTT 20% increase in resting plasma insulin levels after sleep deprivation
Spiegel, 1999 (7)	11 healthy males aged 18–27 yr	Partial sleep deprivation for 6 nights, bedtime 4 h/night	IVGTT 24-h profile glucose and hormone concentrations	40% decrease in glucose tolerance after sleep deprivation
González-Ortiz, 2000 (21)	7 healthy males, 7 healthy females	Total sleep deprivation for 24 h	Insulin suppression test with octreotide	18% increase in steady state glucose concentration after sleep deprivation
Zielinski, 2008 (19)	40 healthy adults aged 50–70 yr	Time in bed restriction for 8 wk, total sleep time 1 h less per night	OGTT	No significant effect on glucose tolerance
Nedeltcheva, 2009 (20)	6 healthy males, 5 healthy females, mean age 39 yr	Partial sleep deprivation 14 nights, bedtime 5.5 h/night	OGTT, IVGTT	10% increase in 2-h glucose levels and 17.5% decrease in insulin sensitivity
Present study	5 healthy men, 4 healthy women, aged 23–62 yr	Partial sleep deprivation 1 night, bedtime 4 h/night	Hyperinsulinemic euglycemic clamp [6,6-^{2}H$_2$] glucose infusion	19–25% decrease in insulin sensitivity in multiple metabolic pathways

OGTT, Oral glucose tolerance test; IVGTT, iv glucose tolerance test.

Our data also indicate that insulin sensitivity is not fixed in healthy subjects, but, rather, depends on the duration of sleep in the preceding night. These observations extend previous studies that documented consistent effects of short-term variations in dietary macronutrient composition, dietary caloric content, and short-term fasting on insulin sensitivity in healthy subjects (17, 23, 24). Other studies have indicated effects of exercise in preceding days on insulin sensitivity (25). Therefore, environmental factors including previous sleep duration, exercise, dietary macronutrient composition, and content modulate insulin sensitivity in healthy subjects.

The current study was aimed at assessing the effects of a single night of sleep deprivation on parameters of insulin sensitivity. Unfortunately, the study was not designed to elucidate the underlying mechanisms of this effect of partial sleep deprivation. We observed no effects of partial sleep deprivation on cortisol and glucagon levels. Schmid *et al.* (26) documented that a single night of sleep restriction to 4.5 h reduced basal plasma glucagon and cortisol levels the following morning but did not affect basal glucose, insulin, C-peptide epinephrine, norepinephrine, or GH levels. Apparently, endocrine changes after a single night of partial sleep deprivation do not provide a simple explanation for the induction of insulin resistance. Spiegel *et al.* (7) documented that subsequent nights of partial sleep deprivation increased

cortisol levels during 24 h, whereas Nedeltcheva *et al.* (20) did not find a major effect on cortisol levels. Nedeltcheva *et al.* documented that prolonged partial sleep deprivation induced a modest increase in 24-h epinephrine and nighttime norepinephrine levels. In accordance, Irwin *et al.* (27) documented that plasma levels of epinephrine increased in association with nocturnal awakening. Therefore, sequential nights of partial sleep deprivation induce modest changes in endocrine homeostasis and in glucose tolerance, but the relation between these phenomena is uncertain. Alternatively, it is possible that partial sleep deprivation decreases insulin sensitivity by altering the activity of the autonomous nervous system. However, this is a difficult area of investigation. Previous studies have documented an increased sympathetic tone derived from recordings of heart rate variability after sleep deprivation for a single night or consecutive nights (13, 28). However, it is not known to what extent partial sleep loss induces comparable increases in sympathetic activity at all peripheral sites. Consequently, the relationship between elevated sympathovagal balance at the level of the heart and the sympathetic outflow to liver, muscles, and adipose tissue is uncertain (13).

What are the implications of the present observations? Sleep duration has shortened considerably in Western societies in the past decades (5, 29). Simultaneously, there has been an increase in the prevalence of insulin resistance

and type 2 diabetes. The current study and others (Table 2) indicate that shortened sleep duration is a factor that contributes to glucose intolerance and, even after a single night, to insulin resistance.

It is also tempting to speculate to what extent variations in sleep duration may be involved in the intraindividual variations in glucose levels that are present especially in patients with type 1 diabetes. At present, it is unclear whether interventions aimed at optimization of sleep duration may be beneficial in stabilizing glucose levels in patients with diabetes.

In conclusion, the present study indicates that partial sleep restriction, even during a single night, decreases insulin sensitivity of multiple metabolic pathways in healthy subjects.

Acknowledgments

References

1. **Scheen AJ, Van Cauter E** 1998 The roles of time of day and sleep quality in modulating glucose regulation: clinical implications. Horm Res 49:191–201
2. **Knutson KL, Spiegel K, Penev P, Van Cauter E** 2007 The metabolic consequences of sleep deprivation. Sleep Med Rev 11:163–178
3. **Van Cauter E, Blackman JD, Roland D, Spire JP, Refetoff S, Polonsky KS** 1991 Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. J Clin Invest 88:934–942
4. **Gottlieb DJ, Punjabi NM, Newman AB, Resnick HE, Redline S, Baldwin CM, Nieto FJ** 2005 Association of sleep time with diabetes mellitus and impaired glucose tolerance. Arch Intern Med 165:863–867
5. **Ayas NT, White DP, Al-Delaimy WK, Manson JE, Stampfer MJ, Speizer FE, Patel S, Hu FB** 2003 A prospective study of self-reported sleep duration and incident diabetes in women. Diabetes Care 26:380–384
6. **Yaggi HK, Araujo AB, McKinlay JB** 2006 Sleep duration as a risk factor for the development of type 2 diabetes. Diabetes Care 29:657–661
7. **Spiegel K, Leproult R, Van Cauter E** 1999 Impact of sleep debt on metabolic and endocrine function. Lancet 354:1435–1439
8. **Spiegel K, Knutson K, Leproult R, Tasali E, Van Cauter E** 2005 Sleep loss: a novel risk factor for insulin resistance and type 2 diabetes. J Appl Physiol 99:2008–2019
9. **Buysse DJ, Reynolds 3rd CF, Monk TH, Berman SR, Kupfer DJ** 1989 The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 28:193–213
10. **Johns MW** 1991 A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14:540–545
11. **Netzer NC, Stoohs RA, Netzer CM, Clark K, Strohl KP** 1999 Using the Berlin Questionnaire to identify patients at risk for the sleep apnea syndrome. Ann Intern Med 131:485–491
12. **Tasali E, Leproult R, Ehrmann DA, Van Cauter E** 2008 Slow-wave sleep and the risk of type 2 diabetes in humans. Proc Natl Acad Sci USA 105:1044–1049
13. **Spiegel K, Leproult R, L'hermite-Balériaux M, Copinschi G, Penev PD, Van Cauter E** 2004 Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. J Clin Endocrinol Metab 89:5762–5771
14. **Iber C** 2007 The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. In: Ancoli-Israel S, Chesson AL, Quan SF, eds. Westchester, IL: American Academy of Sleep Medicine
15. **Copeland KC, Kenney FA, Nair KS** 1992 Heated dorsal hand vein sampling for metabolic studies: a reappraisal. Am J Physiol 263:E1010–E1014
16. **DeFronzo RA, Tobin JD, Andres R** 1979 Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 237:E214–E223
17. **Bisschop PH, de Metz J, Ackermans MT, Endert E, Pijl H, Kuipers F, Meijer AJ, Sauerwein HP, Romijn JA** 2001 Dietary fat content alters insulin-mediated glucose metabolism in healthy men. Am J Clin Nutr 73:554–559
18. **Rosenblatt J, Wolfe RR** 1988 Calculation of substrate flux using stable isotopes. Am J Physiol 254:E526–E531
19. **Zielinski MR, Kline CE, Kripke DF, Bogan RK, Youngstedt SD** 2008 No effect of 8-week time in bed restriction on glucose tolerance in older long sleepers. J Sleep Res 17:412–419
20. **Nedeltcheva AV, Kessler L, Imperial J, Penev PD** 2009 Exposure to recurrent sleep restriction in the setting of high caloric intake and physical inactivity results in increased insulin resistance and reduced glucose tolerance. J Clin Endocrinol Metab 94:3242–3250
21. **González-Ortiz M, Martínez-Abundis E, Balcázar-Muñoz BR, Pascoe-González S** 2000 Effect of sleep deprivation on insulin sensitivity and cortisol concentration in healthy subjects. Diabetes Nutr Metab 13:80–83
22. **VanHelder T, Symons JD, Radomski MW** 1993 Effects of sleep deprivation and exercise on glucose tolerance. Aviat Space Environ Med 64:487–492
23. **Klein S, Sakurai Y, Romijn JA, Carroll RM** 1993 Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. Am J Physiol 265:E801–E806
24. **van der Crabben SN, Allick G, Ackermans MT, Endert E, Romijn JA, Sauerwein HP** 2008 Prolonged fasting induces peripheral insulin resistance, which is not ameliorated by high-dose salicylate. J Clin Endocrinol Metab 93:638–641
25. **Dela F, Mikines KJ, von Linstow M, Secher NH, Galbo H** 1992 Effect of training on insulin-mediated glucose uptake in human muscle. Am J Physiol 263:E1134–E1143
26. **Schmid SM, Jauch-Chara K, Hallschmid M, Schultes B** 2009 Mild sleep restriction acutely reduces plasma glucagon levels in healthy men. J Clin Endocrinol Metab 94:5169–5173
27. **Irwin M, Thompson J, Miller C, Gillin JC, Ziegler M** 1999 Effects of sleep and sleep deprivation on catecholamine and interleukin-2 levels in humans: clinical implications. J Clin Endocrinol Metab 84:1979–1985
28. **Tochikubo O, Ikeda A, Miyajima E, Ishii M** 1996 Effects of insufficient sleep on blood pressure monitored by a new multibiomedical recorder. Hypertension 27:1318–1324
29. **Basner M, Fomberstein KM, Razavi FM, Banks S, William JH, Rosa RR, Dinges DF** 2007 American time use survey: sleep time and its relationship to waking activities. Sleep 30:1085–1095