

Sleep Disturbances and Glucose Homeostasis

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Abstract

Sleep disturbances, induced by either lifestyle, shift work or sleeping disorders, have become more prevalent in our 24/7 Western society. Sleep disturbances are associated with impaired health including metabolic diseases such as obesity and type 2 diabetes. The question remains whether there is a direct effect of disturbed sleep on glucose homeostasis. Experimental studies under controlled laboratory conditions in both humans and experimental animals revealed that there are differences between the effects of acute or chronic sleep disturbance. Acute sleep restriction clearly leads to glucose intolerance, often combined with insulin resistance. Although glucose intolerance does also occur after chronic sleep disturbance, the changes in insulin can vary, dependent on the body weight changes in the various studies. The underlying mechanism that might cause the changes in glucose homeostasis after sleep disturbance remains unclear, but both the biological clock located in the nucleus suprachiasmaticus as well as orexinergic mechanisms in brain and periphery seem to be involved.

Keywords

Sleep restriction, sleep deprivation, shift work, glucose homeostasis, hyperglycaemia, type 2 diabetes, rats, obstructive sleep apnoea

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Over the past 50 years, average sleep duration in Western societies has decreased by two hours per night.¹ Initially, this decrease in sleep was observed mainly in adulthood, but recent epidemiological studies have shown a similar decrease in children and adolescents.^{2,3} A decrease in sleep includes not only the duration, but also the quality of sleep. Quality of sleep is generally defined in terms of changes in sleep architecture, the content of dreaming and the amount of awakenings.⁴

A decrease in length or quality of sleep has important consequences for an individual's well-being. Sleeping problems are linked to many health and lifestyle problems, such as increased errors, loss of productivity, sleepiness during the day, impaired social activities and an elevated risk of accidents.^{5,6} Disturbed sleep has also been identified as a risk factor for a variety of diseases, including psychiatric disorders such as depression^{7–9} and immune system dysfunctions.^{10–12} These health problems are increasing as sleep loss and decreased sleep quality become more prevalent in our current 24/7 Western society.

Metabolic Consequences of Sleep Disturbances

Sleep disturbances are also linked to metabolic dysfunctioning. Studies in shift workers provided the first (indirect) evidence for a relationship between sleep disturbances and impaired metabolism. Shift work has become more prevalent during the past decades and shows clear negative effects on sleep timing, length and quality.¹³ Shift workers are active during the night and sleep and eat at abnormal hours. They fall asleep in the morning, but are awakened ahead of time owing to their circadian rhythm, causing them to exhibit sleepiness and reduced performance.¹⁴ The shift work-

induced changes, in particular sleep loss and disturbed circadian rhythmicity in eating and energy expenditure are associated with an increased susceptibility to develop obesity, type 2 diabetes and cardiovascular disorders.^{15,16}

There is also a direct correlation between disturbed sleep and obesity, the main risk factor for developing cardiovascular diseases and type 2 diabetes. Evidence for this comes from several epidemiological studies, including those described by Caput and Van Cauter.^{17,18} In addition, the group of Gottlieb and co-workers¹⁹ provided evidence for a direct correlation between disturbed sleep and the increased prevalence of type 2 diabetes, independent of changes in body weight.

Another striking example of the relationship between disturbed sleep and increased risk for metabolic disorders derives from studies of patients with obstructive sleep apnoea (OSA). OSA is characterised by the recurrent collapse of the airway during sleep, which usually leads to arousals to resume breathing. The patients suffer from sleep fragmentation and hypoxemia, causing disturbed sleep architecture and an increased amount of awakenings.^{20,21} OSA primarily changes the quality of sleep, whereas total sleep time is not dramatically altered. Vgontzas and colleagues²² were the first to show that there is a relation between OSA and obesity, type 2 diabetes and cardiovascular disease. In fact, there is a bidirectional relationship: OSA leads to obesity, but obesity also directly affects OSA: weight gain or weight loss leads to a significant worsening or improvement, respectively, of sleep apnoea in adults.^{23,24} OSA is also directly associated with insulin resistance and glucose intolerance, independent of changes in weight.²⁵

Human Studies on Sleep and Glucose Homeostasis

The main point of the above is that disturbed sleep is clearly associated with an increased risk for type 2 diabetes, even when corrected for body mass index (BMI) or fat content of the body.^{19,26} This leads to the question: is there a direct effect of (disturbed) sleep on glucose and insulin regulation? To answer this question, experimental studies under controlled laboratory conditions are required. These studies, both in humans and experimental animals, were recently performed in different labs.

In experimental studies in humans, most experimenters standardise the experimental protocol by providing standard meals at fixed time points of the day and by requiring the subjects to stay in bed during the sleep restriction hours to minimise activity. Unfortunately, there is still large variation in the experimental set up of the sleep restriction protocols, in particular in the length of the sleep restriction period, which can vary from acute one night of total sleep deprivation up to periods of six nights of partial sleep deprivation. These differences in length of the sleep restriction protocols markedly influenced the outcomes of the different studies.

The first experimental study in humans was published in 1993, when VanHelder and colleagues showed that 60 hours of sleep deprivation led to an increased plasma insulin response without changes in blood glucose during an oral glucose tolerance test (OGTT).²⁷ Similar data after one night of sleep restriction have been found in a recent study by Donga et al.²⁸ in which insulin sensitivity was measured with the gold standard method for measuring insulin sensitivity: a hyperinsulinaemic euglycaemic clamp. In this study, one night of sleep restriction (four hours in bed) was sufficient to develop moderate insulin resistance, reflected by a decreased glucose infusion rate, a reduced glucose disposal rate and increased endogenous glucose production. In this study, the baseline glucose and insulin levels were not different from controls.

In 1999, Spiegel and colleagues showed that six days of partial sleep restriction (subjects were allowed to sleep only four hours per night) led to glucose intolerance reflected by a significant elevation of blood glucose levels during an intravenous glucose tolerance test (IVGTT).²⁹ Surprisingly, there was no reduction in insulin sensitivity. Instead, it was observed that plasma insulin levels were decreased after six days of sleep disturbance, which is in sharp contrast with the data from VanHelder and Donga.^{27,28} However, in a follow-up study from the same lab,³⁰ the authors found that sleep disturbance could indeed lead to reduced insulin sensitivity and glucose intolerance. In this particular study, the subjects were submitted to an IVGTT and sleep disturbance was defined as three nights of experimentally suppressed slow-wave sleep without changes in total sleep time. This also leads to the conclusion that it is not only the quantity, but also the quality of sleep that is important.

Although many of these human data are seemingly in conflict with each other, one can still conclude that sleep disturbance has a marked impact on glucose and insulin homeostasis. When searching for a common denominator, it seems that acute sleep disturbances might lead to insulin resistance and, in some cases, glucose intolerance, whereas longer periods of disturbed sleep have no direct effect on insulin sensitivity, but the glucose intolerance remains. One can speculate that the insulin resistance after acute

total sleep deprivation is secondary to the (stress-induced) elevation of the hypothalamic–pituitary–adrenal (HPA) axis reflected by the elevated cortisol levels in the evening after a night without sleep.³¹ This acute stress response can be less pronounced in the longer sleep restriction studies, where the subjects are still allowed to sleep for a few hours per night. However, Spiegel and colleagues²⁹ still found increased cortisol levels after the six days of sleep restriction, although this increase was less pronounced when compared with the acute response after one night without sleep.

Animal Studies of Sleep and Glucose Homeostasis – Rat Studies

As mentioned above, human studies can vary in length from acute sleep deprivation to a maximum of up to six days of sleep restriction. Animal studies are required for studies of chronic sleep disturbance for investigation of the underlying mechanisms. Most animal studies in (chronic) sleep research are performed in rodents and experimental methods to prevent the animals from sleeping vary from gentle handling to small balance platforms and forced activity. The different methods are discussed below.

The gentle handling method³² is comparable to the sleep restriction protocol in human experiments. The animals, mostly rats, are kept awake by a protocol that includes tapping on the cage, shaking the cage gently or, if required, disturbing the nest. This method is mainly used in acute or short-term sleep deprivation experiments, because the experimenter is required to be present continuously during the experiments. In our laboratory, 12 hours of sleep deprivation by means of gentle handling had no effect on baseline levels of blood glucose, plasma insulin and plasma corticosterone.

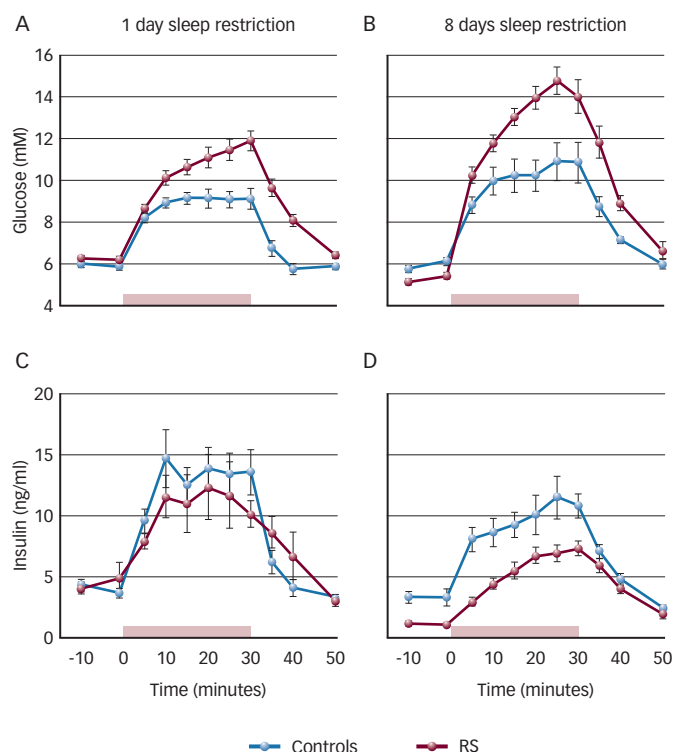
The balance platform method is more commonly used in chronic sleep restriction experiments. Two methods are described in the literature: the flower pot and the single platform method. Both methods are used to deprive rats of rapid eye movement (REM) sleep only and the principle is as follows: to induce REM sleep deprivation, rats are placed inside a water chamber onto a flower pot or a platform of approximately 7.0cm in diameter. The platform is in the water up to 1.0cm of the platform upper surface. The method is based on the loss of muscle tonus that occurs during REM sleep. This means that if the rat enters the REM sleep stage, it loses muscle tonus and will touch the water, after which the rat will wake up.³³ When this method is used to deprive rats of REM sleep for four days, it leads to a decrease in plasma insulin levels, which is secondary to a significant reduction in body weight.³⁴

Another commonly used method in animal sleep restriction research is the disk over water method, extensively described by Rechtschaffen and colleagues.^{35,36} In short, the experimental animal is housed on a horizontal disk above water. The electromyograph (EMG) and the electroencephalograph (EEG) are continuously recorded to detect sleep states. When the experimental rat starts to sleep or enters a certain sleep stage, the disk starts to rotate at a low speed. This causes the rat to wake and forces it to walk to avoid being carried into the water. There is only one study in which this method was used to investigate glucose homeostasis after a longer period of sleep deprivation. In this study by Everson and colleagues, they measured glucose disappearance after a dextrose injection. The data revealed that there was a tendency towards somewhat lowered glucose levels after sleep deprivation.³⁷

Figure 1: Slowly Rotating Drum



Figure 2: Blood Glucose and Plasma Insulin Levels in Response to a 30-minute Intravenous Glucose Infusion After Either One (Graphs A and C) and Eight (Graphs B and D) Days of Restricted Sleep or in Controls



The horizontal pink bars at the bottom of each graph represent the 30 minutes of 15% glucose infusion. Data are average values \pm SEM.³⁹ RS = restricted sleep.

In our laboratory, we use a forced activity paradigm known as the slowly rotating drum to sleep deprive the rats. This method was originally described in 1984 by Borbely and colleagues.³⁸ In our studies, the experimental animals are placed for 20 hours per day in

a rotating drum (see *Figure 1*) that is rotating at a constant speed of 0.4cm/min.³⁹⁻⁴¹ In this way, the animals are allowed to sleep only for (the remaining) four hours per day. We generally include a control group that walks twice the speed (0.8cm/min) but half the time to control for the forced activity in this protocol. Our latest studies focused on the difference between the effects of acute and chronic sleep deprivation on glucose homeostasis and insulin levels.³⁹ To this end, we performed an intravenous glucose tolerance test after one day and eight days of sleep restriction. The data revealed that a reduction in sleep markedly interfered with glucose metabolism.³⁹ Acute sleep restriction was accompanied by elevated blood glucose profiles without any changes in plasma insulin levels during an IVGTT. The effects of chronic sleep reduction are presented in *Figure 2*. The observed glucose intolerance is still present after eight days of sleep restriction, which is similar to the data obtained in most human studies. Baseline insulin levels and glucose-stimulated insulin responses were lower after chronic sleep reduction. This effect on insulin was, similar to the data in humans obtained by Spiegel et al. in 1999,²⁹ secondary to the weight loss after chronic sleep reduction.

Based on the data above, we can conclude that the effects of sleep restriction on glucose homeostasis in experimental animals are remarkably similar in rats and humans. Acute sleep restriction leads to glucose intolerance in combination with unchanged insulin levels (all rat models and some human studies) or insulin resistance (other human studies). The glucose intolerance remains prevalent after chronic sleep restriction, both in humans and rats. Chronic sleep restriction might also lead to a reduction in plasma insulin levels, but this effect seems secondary to a reduction in body weight. The reduction in body weight is typical for experimental studies under controlled conditions and in sharp contrast with epidemiological data that suggest that long-term sleep disturbances are associated with weight gain and the development of obesity and, consequently, of insulin resistance and type 2 diabetes. The insulin resistance that occurs after one night of total sleep deprivation in the (human) experimental studies seems primarily caused by a (stress-induced) activation of the HPA axis, reflected by the elevated cortisol levels under these circumstances.

Possible Mechanisms Underlying Glucose Intolerance

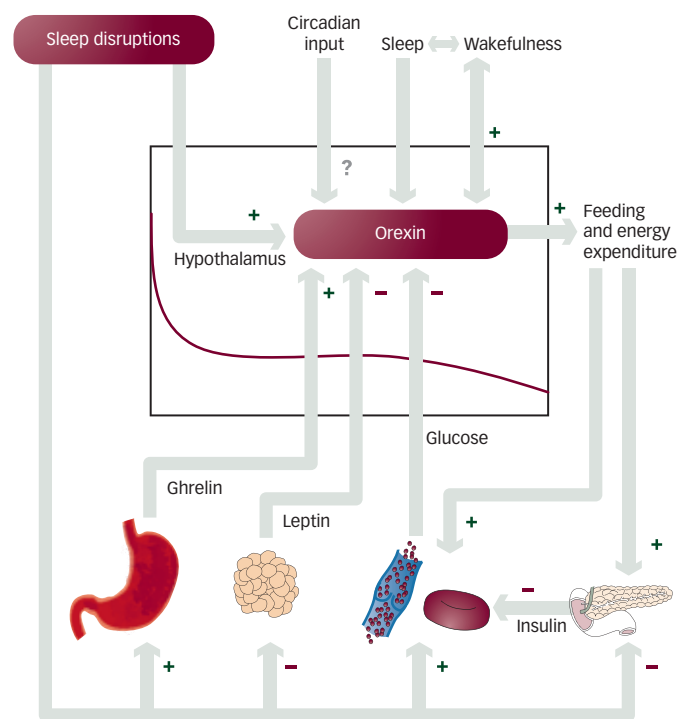
The data above raise the question: what is the cause of the glucose intolerance that occurs after both acute and chronic sleep deprivation? Is it mainly a behavioral effect, related to changes in food intake, physical activity and energy expenditure? Or is it caused by (circadian) disturbances in hormonal outflow and/or the activation of the autonomic nervous system? Or is hyperglycaemia mainly secondary to changes at the level of the central nervous system, in particular at orexinergic neurons in the lateral hypothalamus? Some of these options are discussed below.

The biological clock, located in the suprachiasmatic nucleus (SCN), has a marked effect on glucose homeostasis. There are clear differences in the glucose and insulin responses to an IVGTT in both rats and humans at different times of the circadian clock.⁴² This circadian rhythmicity in glucose regulation disappears when the SCN is lesioned.⁴³ One can state that the biological clock not only influences the sleep-wake cycle, but also prepares the glucose regulatory mechanisms for the changes in energy uptake and

expenditure at different time points of the day/night cycle. These circadian systems are tightly integrated and problems can occur when our daily activities are not in synchrony with our sleep-wake cycle, for example, during shift work and periods of disturbed sleep.¹⁶ For example, when rats are forced to be active in their non-active phase, they eat at the wrong time of the day and consequently increase in body weight compared with controls.⁴⁴ This weight gain can be prevented when the animals are only allowed to eat in the (normal) active phase and not in the shift work period.⁴⁵

Hormonal influences also seem to be involved in the effects of disturbed and restricted sleep in glucose metabolism. In humans, sleep deprivation has been shown to decrease plasma leptin and to increase plasma ghrelin levels, leading to increased hunger and appetite.⁴⁶ At a central level, the orexinergic system in the brain also seems to have a role. Orexin is involved in food intake, energy expenditure and the sleep-wake cycle, and could therefore be an interesting link between sleep disturbances and the increased prevalence of obesity and type 2 diabetes.^{47,48} For example, narcolepsia is a disease that is characterised by reduced orexin levels in the central nervous system causing less consolidated wake periods, leading to a sudden appearance of sleep periods. Narcolepsia is associated with an increased frequency of type 2 diabetes.⁴⁹ Peripheral orexin also seems to be involved as orexin receptors have been found on the pancreas and other peripheral organs.⁵⁰ It is also known that orexin has a direct role in the regulation of glucose homeostasis. Tsuneki and colleagues showed that orexin neurons directly respond to the nutritional status of an individual and are modulated by metabolic signals, such as glucose, leptin and ghrelin.^{51,52} Studies in orexin knockout mice point to a contribution of orexin in the age-related development of impaired glucose tolerance, independent of obesity.⁵³ Finally, it is known that orexin is directly involved in the regulation of energy expenditure. Increased orexin levels correspond with increased physical activity and increased non-exercise thermogenesis (NEAT) and, consequently, glucose utilisation. Taken together, these data indicate that orexin might serve as a crucial factor in the relationship between sleep loss, circadian rhythms, physical activity and the effects on glucose metabolism (see *Figure 3*).

Figure 3: Overview of the Connections Between Sleep, Sleep Loss, Orexin and Metabolic Hormones



In summary, sleep disturbances are directly associated with changes in glucose homeostasis. Experimental studies under controlled laboratory conditions in both humans and experimental animals revealed that there are differences between the effects of acute or chronic sleep disturbance. Acute sleep restriction clearly leads to glucose intolerance often combined with insulin resistance. Glucose intolerance also occurs after chronic sleep disturbance but the changes in insulin can vary, depending on the body weight changes in the various studies. The underlying mechanism that might cause the changes in glucose homeostasis after sleep disturbance remain unclear, but both the biological clock located in the nucleus suprachiasmaticus as well as orexinergic mechanisms in the brain and periphery seem to be involved. ■

- Van Cauter E, Knutson K, Leproult R, Spiegel K, The impact of sleep deprivation on hormones and metabolism, *Medscape Neurol Neurosurg*, 2005;7(1).
- Horne J, Short sleep is a questionable risk factor for obesity and related disorders: statistical versus clinical significance, *Biol Psychol*, 2008;77(3):266–76.
- Van Cauter E, Knutson KL, Sleep and the epidemic of obesity in children and adults, *Eur J Endocrinol*, 2008;159(Suppl. 1):S59–66.
- Vandekerckhove M, Cluydts R, The emotional brain and sleep: an intimate relationship, *Sleep Med Rev*, 2010;14(4):219–26.
- Banks S, Dinges DF, Behavioral and physiological consequences of sleep restriction, *J Clin Sleep Med*, 2007;3(5):519–28.
- Horne J, Reyner L, Vehicle accidents related to sleep: a review, *Occup Environ Med*, 1999;56(5):289–94.
- Breslau N, Roth T, Rosenthal L, Andreski P, Sleep disturbance and psychiatric disorders: a longitudinal epidemiological study of young adults, *Biol Psychiatry*, 1996;39(6):411–8.
- Ford DE, Kamerow DB, Epidemiologic study of sleep disturbances and psychiatric disorders. An opportunity for prevention?, *JAMA*, 1989;262(11):1479–84.
- Neckelmann D, Mykletun A, Dahl AA, Chronic insomnia as a risk factor for developing anxiety and depression, *Sleep*, 2007;30(7):873–80.
- Imeri L, Opp MR, How (and why) the immune system makes us sleep, *Nat Rev Neurosci*, 2009;10(3):199–210.
- Irwin M, Effects of sleep and sleep loss on immunity and cytokines, *Brain Behav Immun*, 2002;16(5):503–12.
- Opp MR, Toth LA, Neural-immune interactions in the regulation of sleep, *Front Biosci*, 2003;8:d768–79.
- Dumont M, Montplaisir J, Infante-Rivard C, Sleep quality of former night-shift workers, *Int J Occup Environ Health*, 1997;3(Suppl. 2):S10–S4.
- Akerstedt T, Psychological and psychophysiological effects of shift work, *Scand J Work Environ Health*, 1990;16(Suppl. 1):67–73.
- Akerstedt T, Kecklund G, Johansson SE, Shift work and mortality, *Chronobiol Int*, 2004;21(6):1055–61.
- Knutsson A, Health disorders of shift workers, *Occup Med*, 2003;53(2):103–8.
- Chaput JP, Despres JP, Bouchard C, Tremblay A, Association of sleep duration with type 2 diabetes and impaired glucose tolerance, *Diabetologia*, 2007;50(11):2298–304.
- Van Cauter E, Holmback U, Knutson K, et al., Impact of sleep and sleep loss on neuroendocrine and metabolic function, *Horm Res*, 2007;67(Suppl. 1):2–9.
- Gottlieb DJ, Punjabi NM, Newman AB, et al., Association of sleep time with diabetes mellitus and impaired glucose tolerance, *Arch Intern Med*, 2005;165(8):863–7.
- Bandla HP, Gozal D, Dynamic changes in EEG spectra during obstructive apnea in children, *Pediatr Pulmonol*, 2000;29(5):359–65.
- Svanborg E, Guilleminault C, EEG frequency changes during sleep apneas, *Sleep*, 1996;19(3):248–54.
- Vgontzas AN, Bixler EO, Chrousos GP, Metabolic disturbances in obesity versus sleep apnoea: the importance of visceral obesity and insulin resistance, *J Intern Med*, 2003;254(1):32–44.
- Peppard PE, Young T, Palta M, et al., Longitudinal study of moderate weight change and sleep-disordered breathing, *JAMA*, 2000;284(23):3015–21.
- Pillar G, Shehadeh N, Abdominal fat and sleep apnea: the chicken or the egg? *Diabetes Care*, 2008;31(Suppl. 2):S303–9.
- Punjabi NM, Sorokin JD, Katzel LI, et al., Sleep-disordered breathing and insulin resistance in middle-aged and overweight men, *Am J Respir Crit Care Med*, 2002;165(5):677–82.
- Chaput JP, Despres JP, Bouchard C, et al., Sleep duration as a risk factor for the development of type 2 diabetes or impaired glucose tolerance: analyses of the Quebec Family Study, *Sleep Med*, 2009;10(8):919–24.
- VanHelder T, Symons JD, Radomski MW, Effects of sleep deprivation and exercise on glucose tolerance, *Aviat Space Environ Med*, 1993;64(6):487–92.
- Donga E, van Dijk M, van Dijk JG, et al., A single night of partial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects, *J Clin Endocrinol Metab*, 2010;95(6):2963–8.
- Spiegel K, Leproult R, Van Cauter E, Impact of sleep debt on metabolic and endocrine function, *Lancet*, 1999;354(9188):1435–9.
- Tasali E, Leproult R, Ehrmann DA, Van Cauter E, Slow-wave sleep and the risk of type 2 diabetes in humans, *Proc Natl Acad Sci U S A*, 2008;105(3):1044–9.
- Leproult R, Copinschi G, Buxton O, Van Cauter E, Sleep loss results in an elevation of cortisol levels the next evening, *Sleep*, 1997;20(10):865–70.
- Van der Borgh K, Ferrari F, Klauke K, et al., Hippocampal cell proliferation across the day: increase by running wheel activity, but no effect of sleep and wakefulness, *Behav Brain*

- Res, 2006;167(1):36–41.
33. Cohen HB, Dement WC, Sleep: changes in threshold to electroconvulsive shock in rats after deprivation of "paradoxical" phase, *Science*, 1965;150(701):1318–9.
 34. Hipolide DC, Suchecki D, Pimentel de Carvalho PA, et al., Paradoxical sleep deprivation and sleep recovery: effects on the hypothalamic-pituitary-adrenal axis activity, energy balance and body composition of rats, *J Neuroendocrinol*, 2006;18(4):231–8.
 35. Rechtschaffen A, Bergmann BM, Sleep deprivation in the rat by the disk-over-water method, *Behav Brain Res*, 1995;69(1–2):55–63.
 36. Rechtschaffen A, Bergmann BM, Sleep deprivation in the rat: an update of the 1989 paper, *Sleep*, 2002;25(1):18–24.
 37. Everson CA, Bergmann BM, Rechtschaffen A, Sleep deprivation in the rat: III. Total sleep deprivation, *Sleep*, 1989;12(1):13–21.
 38. Borbely AA, Tobler I, Hanagasioglu M, Effect of sleep deprivation on sleep and EEG power spectra in the rat, *Behav Brain Res*, 1984;14(3):171–82.
 39. Barf RP, Meerlo P, Scheurink AJ, Chronic sleep disturbance impairs glucose homeostasis in rats, *Int J Endocrinol*, 2010;2010:819414.
 40. Novati A, Roman V, Cetin T, et al., Chronically restricted sleep leads to depression-like changes in neurotransmitter receptor sensitivity and neuroendocrine stress reactivity in rats, *Sleep*, 2008;31(11):1579–85.
 41. Roman V, Walstra I, Luiten PG, Meerlo P, Too little sleep gradually desensitizes the serotonin 1A receptor system, *Sleep*, 2005;28(12):1505–10.
 42. Kalsbeek A, Yi CX, La Fleur SE, Fliers E, The hypothalamic clock and its control of glucose homeostasis, *Trends Endocrinol Metab*, 2010;21(7):402–10.
 43. La Fleur SE, Kalsbeek A, Wortel J, et al., A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus, *Diabetes*, 2001;50(6):1237–43.
 44. Salgado-Delgado R, Angeles-Castellanos M, Buijs MR, Escobar C, Internal desynchronization in a model of night-work by forced activity in rats, *Neuroscience*, 2008;154(3):922–31.
 45. Salgado-Delgado R, Angeles-Castellanos M, Saderi N, et al., Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work, *Endocrinology*, 2010;151(3):1019–29.
 46. Spiegel K, Tasali E, Penev P, et al., Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite, *Ann Intern Med*, 2004;141(11):846–50.
 47. Sakurai T, Roles of orexins in regulation of feeding and wakefulness, *Neuroreport*, 2002;13(8):987–95.
 48. Sakurai T, The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness, *Nat Rev Neurosci*, 2007;8(3):171–81.
 49. Honda Y, Doi Y, Ninomiya R, Ninomiya C, Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients, *Sleep*, 1986;9(1):254–9.
 50. Adegate E, Fernandez-Cabezudo M, Hameed R, et al., Orexin-1 receptor co-localizes with pancreatic hormones in islet cells and modulates the outcome of streptozotocin-induced diabetes mellitus, *PLoS One*, 2010;5(1):e8587.
 51. Tsuneki H, Wada T, Sasaoka T, Role of orexin in the regulation of glucose homeostasis, *Acta Physiol*, 2010;198(3):335–48.
 52. Yi CX, Serlie MJ, Ackermans MT, et al., A major role for perifornical orexin neurons in the control of glucose metabolism in rats, *Diabetes*, 2009;58(9):1998–2005.
 53. Tsuneki H, Murata S, Anzawa Y, et al., Age-related insulin resistance in hypothalamus and peripheral tissues of orexin knockout mice, *Diabetologia*, 2008;51(4):657–67.