

## SLEEP IN DIABETIC ANIMAL MODELS

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### ABSTRACT

Diabetes, which has been declared a major global health issue, involves the central nervous system and balancing vital functions such as cardiovascular and circadian rhythms. There is increasing evidence that the alarming prevalence of diabetes may be aggravated by endemic voluntary sleep loss. In an attempt to understand the underlying mechanisms involved in the genesis and progression of diabetes, animal models have been developed to mimic the physiological responses involved. These models have greatly assisted research in this field. Major advancements have been made in diabetes research in animal models that have significantly contributed to the understanding of the etiopathology of this disease and its dreaded chronic complications. This review summarizes rodent models used in studying diabetes, focusing on its manifestations in sleep patterns.

**Keywords:** Diabetes; Sleep; Sleep deprivation; Obesity; Animal models; Rats.

### INTRODUCTION

Sleep is a complex behavioral state spanning over one-third of the human life. Although viewed as a passive condition, sleep is a highly active and dynamic process. Until recently, it was believed that sleep was important primarily for restoring brain functions. There is, however, increasing evidence that sleep also modulates metabolic, endocrine and cardiovascular systems (1).

Diabetes is an endocrine disease that was fairly rare at the beginning of the 20<sup>th</sup> century, but it has become a major hurdle for health care worldwide, and it is likely to remain so. This endocrinal disease, characterized by metabolic alterations, usually culminates

in blood hyperosmolarity. Depending on its origin, this pathology may be classified into diabetes insipidus or diabetes mellitus. The pathogenesis of diabetes insipidus is related to the hypothalamic-hypophysis, and it results in insufficient secretion of antidiuretic hormone, leading to polyuria due to electrolytic imbalance, followed by polydipsia. Diabetes mellitus is an endocrine disorder of the carbohydrate metabolism, and it results primarily from inadequate insulin release caused by autoimmune destruction of pancreatic  $\beta$ -cells, characterizing type I diabetes. Type II diabetes is associated with obesity and insulin insensitivity coupled with inadequate compensatory release of insulin.

Intriguingly, a dramatic increase in the incidence of diabetes

seems to develop parallel to self-reported sleep loss, which strongly suggests that a poor sleep pattern could represent a risk factor for diabetes (2,3).

Recent studies show that patients with diabetes experience more sleep problems than non-diabetic subjects (4,5). Indeed, sleep loss may adversely affect glucose tolerance and involve an increased risk of diabetes (2).

Until recently, a reduction in insulin concentrations and the associated increase in circulating glucose concentrations were believed to be the prime peripheral signals linked to diabetes. Excess weight in adults is clearly associated with increased incidence of type II diabetes and impaired glucose tolerance (6).

In patients with obstructive sleep apnea syndrome (OSAS), obesity is very common (7). A clinical study with 494 patients demonstrated that the frequencies of type II diabetes and impaired glucose tolerance in OSAS patients (30 and 20%, respectively) were higher than the prevalence of these clinical disorders in the general population (8).

According to several studies, OSAS is an important cause of excessive sleepiness and shows higher prevalence in adults (9). A random sample of 3201 Swedish men showed that diabetes was associated with frequent complaints about excessive daytime sleepiness (EDS) (12.2%), difficulty in maintaining sleep (21.9%), and insomnia (21.1%) (10). Literature also suggests an association with metabolic syndrome (e.g., obesity, diabetes, insulin resistance) and EDS (9), which is commonly assumed to be the result of disturbed or inadequate sleep.

Sleep disturbances may not be markers of psychosocial stress, but they do represent a primary stressor and could influence HPA axis and sympathetic nervous activation by hypothalamic activation (e.g., through HPA and sympathetic nerve activity, possibly causing insulin resistance and increasing the risk of type II diabetes) (11). Sleep deprivation in rats is associated with increased corticosterone (12) and a stimulatory effect on the hypothalamo-pituitary adrenal (HPA) axis (13).

Since the impact of diabetes on the brain and frequent sleep disturbances have been observed in diabetic patients (1), both clinical and basic research should focus on the mechanisms by which abnormalities in the physiology of the brain in the presence of diabetes occur and on the best ways to prevent chronic brain damage.

Most of our knowledge concerning the general biochemistry, physiology, endocrinology and pathways involved in genetic disease stems from animal experiments, which ideally should be extrapolated to humans. In most experiments, the animal serves as a substitute for humans and is referred to as an animal model. For instance, major advances in our understanding of *in vivo* mechanisms of insulin resistance at the whole-body and tissue-specific level have been achieved through the use of genetically modified animal models (14). Many animal models have been created to help understand the pathophysiology of diabetes, and several toxins, including streptozotocin, have been used to induce hyperglycaemia in rats and mice by damaging the pancreas. The significance of results from animal experiments depends on suitable animal models that provide data that allow comparisons between relevant biomedical aspects (15).

This metabolic disorder is known to produce alterations in var-

ious organs of the body, including several central nervous system disturbances, such as neurobehavioral and neurotransmitter alterations, autonomic dysfunctions, and adversely affected endocrine functions (1). Until recently, a reduction in insulin concentrations and the associated increase in circulating glucose concentrations were believed to be the prime peripheral signals linked to this disorder. Insulin resistance, as indicated by an impaired biological response to this hormone, has been implicated in the pathogenesis of a metabolic syndrome known as "insulin-resistance syndrome," which is generally accepted to comprise hyperinsulinaemia, glucose intolerance, dyslipidaemia, central obesity, hypertension and sleep disturbances.

## OBESITY

Feeding behavior is dependent upon the integration of metabolic, autonomic, endocrine, and environmental factors coordinated with an appropriate state of cortical arousal (wakefulness). Thus, this behavior is critically dependent on appropriate sleep-wakefulness cycling. Indeed, sleep and metabolism co-morbid disturbances are well-described symptoms of obesity (6).

The International Diabetes Federation considers obesity to be one of the main drivers of the high prevalence of metabolic syndrome, contributing to hyperglycaemia and insulin resistance. This is where a striking association between obesity and Type II diabetes mellitus can be traced (1).

Obesity is the strongest and probably most relevant risk factor for sleep-disordered breathing (especially obstructive sleep apnea syndrome, OSAS) in patients. The mechanisms underlying the effects of obesity on the risk of OSAS may be related to fat deposition in airway anatomy or alterations in upper airway function.

Significant obstructive sleep apnea is present in 40% of obese individuals, and 70% of OSAS patients are obese (7). In addition to increased body weight, fat distribution plays a major role in the development of OSAS. Studies suggest that, among all anthropometric variables, central obesity, rather than more generalized distribution of body fat, is an important risk factor for OSAS in obese subjects (9,6). A significantly greater amount of visceral fat resulting from the action of insulin may be observed in OSAS subjects compared with obese controls (6).

Animal models of diabetes are likely as complex and heterogeneous as human models; insulin resistance predominates in some animals, whereas  $\beta$ -cell failure prevails in others. Models in which glucose intolerance is part of a broader obesity phenotype and models of dyslipidaemia and hypertension may also provide valuable insights into Type II diabetes. However, despite the existence of dozens of different models, sleep patterns have been examined in only a few models. In this review, we depict the animal models that have proven to be of great value in the investigation of diabetes and are currently ubiquitous in the related research.

## SPONTANEOUS ANIMAL MODELS OF DIABETES

### Animal models of type II Diabetes Mellitus

#### *Zucker Fatty Rat*

In the Zucker fatty rat, obesity is genetically transmitted as two mutations of the leptin receptor (16) that culminates in hyperleptinemia, hyperphagia, and hyperinsulinemia (17). In this strain, all homozygous male rats develop diabetes around 10 weeks of age after a prediabetic period during which obesity and insulin resistance are present but blood glucose concentrations are normal (18). This animal model is most often used in the investigation of Type II diabetes due to its similarities with human pathogeny.

This strain also exhibits many of the same respiratory deficits shown by obese humans, including reduced lung volume, reduced chest wall compliance, blunted ventilatory responses to hypercapnia and hypoxia, and narrowed upper airway (19). Obese Zucker rats develop morphologic and mechanical changes in respiratory muscle function that are consistent with chronic overload: the diaphragm becomes weak, and fiber hypertrophy is observed (20).

Obese Zucker rats presented a longer daily period of slow wave sleep (SWS), whereas paradoxical sleep (PS) was shorter in Wistar rats (21). Investigation of sleep patterns in obese Zucker rats compared to lean Zucker rat controls showed that, during the light period, the former presented a SWS period longer than that of the latter (22). Studies focusing on sleep patterns in these rats may well provide evidence on the influence of obesity on sleep.

#### *OLETF Rat*

The Otsuka-Long-Evans-Tokushima Fatty (OLETF) rat is a spontaneously diabetic rat with polyuria, polydipsia and mild obesity, and it was discovered in 1984 in an outbred colony of Long-Evans rats. This strain develops an increase in body weight following weaning accompanied by high plasma insulin and hyperplasia of  $\beta$ -cells in the pancreatic islets. There are two essential mutations related to the induction of diabetic phenotype in OLETF rats (23). These diabetogenic genes have been assigned to chromosome X (Odb1) and 14 (Odb2) (23).

Studies have suggested that cholecystokinin (CCK)-A receptor gene expression in these rats is absent (24). This hormone is a gastrointestinal and brain octapeptide that modulates a variety of behavioral responses. Furthermore, CCK has been reported to modulate the circadian rhythm of vasopressin and oxytocin release (25). Therefore, the circadian rhythm of the activity is probably affected by a dysfunction in the CCK-A receptor in OLETF rats (26). A study conducted with rats showed that intraperitoneal injection of CCK promotes PS and increases slow-wave activity with an electroencephalogram (27), which is generally thought to be an indicator of sleep intensity. In addition, OLETF rats showed a decrease in large movement during the dark period and in the circadian rhythm compared to LETO (non-diabetic strain, Long-Evans-Tokushima-Otsuka) rats (26).

#### *C57BL/6J Mouse*

The C57BL/6J (B6) is a normal mouse strain susceptible to diabetes/obesity when maintained on a high-fat diet. Thus, the B6 mouse provides a glimpse of obesity at its onset as a result of the

interaction between the nutritional content of the diet and genetic variables. The development of diabetes and obesity in this strain closely parallels the progression of common forms of the human disease. As in humans, diet-induced diabetes and obesity in the B6 mouse are characterized by selective deposition of fat in the abdomen (28). In addition, studies using this strain have shown several abnormalities in autonomic nervous system, beta cell, and adipocyte function.

Although very few studies have examined sleep patterns in B6 mice after induction of diabetes, this strain is often used in animal experimentation. A recent study using B6 mice reported that hypoxic exposure caused overall sleep loss (29), thus suggesting that intermittent hypoxia may lead to more significant disruptions of sleep patterns in patients with OSAS than in patients with recurrent non-hypoxic arousals.

#### *C57BL/6J-Lep<sup>ob</sup> (ob/ob) Mouse*

The ob/ob mouse originated from a spontaneous mutation of the leptin gene in the C57BL/6J (B6) strain (30), which led to a complete impairment of leptin signaling (31). Lep<sup>ob</sup> mutation in the B6 strain by standard background produces juvenile onset obesity as well as hyperinsulinemia with increasing insulin resistance. However, hyperglycemia is relatively mild and transient. This remission from chronic hyperglycemia is correlated to a sustained hypertrophy of pancreatic islets primarily caused by hyperplasia of the  $\beta$ -cell mass (19).

Leptin has been shown to inhibit choline acetyltransferase (ChAT), the sympathetic enzyme that produces acetylcholine, suggesting that cholinergic modulation of sleep and breathing may be altered in leptin-deficient ob/ob mice (32). In addition, recent studies have shown that sleep disordered breathing may induce and further propagate the insulin resistance state in the presence of obesity (33). For example, ob/ob mice exposed to continuous hypoxia also showed decreased glucose and insulin levels (34). Indeed, intermittent hypoxemia in obese mice induces a progressive state of insulin resistance, with insulin levels 5 to 7 times higher than those in control mice (34).

### Animal models of type I diabetes

Type I diabetes mellitus in humans is characterized by specific destruction of pancreatic  $\beta$  cells, which is commonly associated with immune-mediated damage. Although the damage may occur unnoticed over the years, at clinical presentation, there is little surviving  $\beta$  cell mass, and the disorder progresses to absolute insulinopaenia. Because the diseased pancreas in humans is inaccessible, it is worth noting that all the above data were obtained from autopsies, either at the onset of diabetes or at advanced stages of the condition. Consequently, they do not provide any idea of the sequence of events involving the different cell types, thus justifying the need for animal models.

The most commonly used animal that spontaneously develops diseases bearing similarities with human type I diabetes is the non-obese diabetic (NOD) mouse.

#### *The NOD Mouse*

The NOD mouse was developed by selectively breeding offspring from a laboratory strain. Insulin is present when the mice

are 4-5 weeks old, followed by subclinical  $\beta$ -cell destruction and decreased circulating insulin concentration followed by the onset of type I diabetes at 12 and 30 weeks of age (33). Nevertheless, unlike human diabetes, ketoacidosis is relatively mild, and affected animals can survive for weeks without the administration of insulin.

The origins of autoimmunity remain unknown; it is known, however, that various autoimmune diseases are characterized by a reduction in the number of lymphocytes. Since lymphopenia may facilitate the destructive process that characterizes autoimmunity and the NOD strain proved to be more susceptible to the effects of sleep loss than the Swiss strain, a recent study conducted in our lab suggests that sleep deprivation should be considered a risk factor for the onset of autoimmune disorders (35).

### Animal models of diabetes insipidus

Animal models of genetic hormone deficiency are useful as models for physiological studies of hormone deficiency and hormone action. The human model is appropriately limited by constraints of human studies; thus, engineered animal models of specific diseases, such as familial neurohypophysial diabetes insipidus, are required.

#### *The Brattleboro rat*

Brattleboro rats are mutants of the Long-Evans strain and present deletion of a pair of the gene basis that codifies vasopressin, a hormone secreted by hypothalamic cells and stored in the posterior hypophysis. This recessive autosomic heritage results in direct alterations in the central nervous system, and it is responsible for the development of central diabetes insipidus in the adult homozygote. Such animals present a severe polydipsia and polyuria syndrome that, in homozygous Brattleboro rats, manifests at the beginning of the weaning period, when such animals display preference to water over milk (36).

A significant 38% reduction in the duration of PS was observed in the homozygote of the Brattleboro rat when compared to the Long-Evans strain (37).

The sleep loss in diabetic rats could also be attributable to the hereditary effects of the absence of vasopressin in animals bearing diabetes insipidus, such as the Brattleboro rat, since increases close to 65% in the duration of PS were observed in these animals after infusion of enough water to keep them hydrated (37). This result corroborates the hypothesis that the Brattleboro rat's sleep was interrupted several times in order for the rat to drink water (37).

### CHEMICALLY INDUCED DIABETES IN ANIMAL MODELS

Drugs that induce diabetes in animal models by exerting a direct toxic effect on the pancreas are very practical and simple to use in medical research. The most common substance used in diabetes induction in animals is streptozotocin, which induces insulin deficiency and selective pancreatic  $\beta$ -cell toxicity (38).

#### Streptozotocin

Streptozotocin (STR) is a metabolite of the soil organism *Streptomyces achromogenes*, which is used to induce both insulin

dependent (type I diabetes) and non-insulin dependent diabetes mellitus (type II diabetes). STR may be administered in multiple low doses, and this treatment is predominantly mediated by the activation of immune mechanisms (38).

Sleep disorders are often associated with metabolic dysfunction such as diabetes. As in diabetic patients, sleep loss was observed in rats after injection of STR (39). This data suggest that, following pharmacological destruction of pancreatic  $\beta$  insulin-producing cells, the daily duration of SWS is significantly reduced by 34% and PS, by 43% (39).

The sleep loss observed in these diabetic animals could be a consequence of distinct factors. Previous findings show that chronic intracerebroventricular infusion of insulin in normal rats induces an increase in the daily duration of SWS (40) and that chronic intravenous infusion of this hormone in rats made diabetic by STR resulted in a restoration of SWS, whereas PS remained unaltered (39,40). These studies support the hypothesis that insulin has properties in the induction of sleep and that there is a possible direct action of insulin on brain cells.

### CONCLUSIONS

In short, impairments in glucose metabolism have been a pandemic disease which, once allowed to evolve into chronic complications, may result in morbidity and mortality. Sleep disturbances are among the most prevalent impairments and may also have severe long-term effects upon health, including an increased risk of diabetes. Chronic sleep loss is a consequence of voluntary bedtime restriction and an endemic condition that affects millions. The reciprocal relationship intensifies this metabolic disorder and aggravates sleep disturbances observed in diabetic patients. Consistent with the systemic impacts of diabetes and sleep deprivation, general physiological imbalance has been reported in diabetic patients, with the immune system affected and hormonal patterns altered.

In order to elucidate alternatives for the eventual cure of human diabetes, mouse and rat models have been the primary tools for investigating the human physiology, biochemistry and pathology of diabetes.

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