# Sleep science

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Abstracts

3. Moreno CRC, Carvalho FA, Matuzaki LA, Louzada FM. Effects of irregular working hours on sleep and alertness in Brazilian truck drivers [abstract]. Sleep. 2002; 25:399.

Chapter in a book

4. Andersen, ML; Bittencourt, LR. Fisiologia do sono. In: Tufik S, editor. Medicina e Biologia do Sono. 1a ed. São Paulo: Manole; 2007. p. 48-58.

Official publications

5. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. 2nd ed. Geneva: WHO; 2003. p. 1-24.

Thesis

6. Bittencourt L. Avaliação da Variabilidade do Índice de apnéia e hipopnéia em pacientes portadores da Síndrome da Apnéia e Hipopnéia do Sono Obstrutiva [tese]. São Paulo: Universidade Federal de São Paulo;1999.

Electronic publications

7. Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm.

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8. Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc., c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/

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# SLEEP-RELATED MOVEMENT DISORDERS

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## ABSTRACT

Movement disorders or disturbances (MD) comprise an important subset of sleep medicine. Among the known types of MDs, two are considered to be of great importance: disorders related to periodic limb movements in sleep (PLMS) and restless leg syndrome (RLS), which usually occur during sleep or at the transition between waking and sleep. These two problems tend to be associated and are frequently referred to by the common acronym RLS/PLMS. In addition to these, sleep related cramps, sleep bruxism and rhythmic movements make up the range of sleep-related movement disorders listed in the International Classification of Sleep Disorders. The present paper reviews the epidemiological, etiological, diagnostic and therapeutic aspects of these diseases.

**Keywords:** sleep; sleep disorders; movement disorders; restless legs syndrome; periodic limb movements.

## INTRODUCTION

Sleep disorders are common but often neglected causes of human suffering. Due to the structuring of the sleep medicine and sleep societies spread throughout the world, there have been international efforts to analyze the mounting literature on the subject. The second edition of the International Classification of Sleep Disorders has recently been completed and published (ICSD-2). Periodic Limb Movement Disorder (PLMD) and Restless Legs Syndrome (RLS) are the primary themes of the chapter dealing with sleep-related movement disorders (SRMD).

Today, there are 72,027 articles containing the word "sleep" indexed in the ISI Web of Science. Among the 50 most frequently cited articles is one that for the first time defined the criteria for diagnosing RLS in operational terms, with almost 500 citations (1). The present paper offers a review of the diagnostic and therapeutic aspects of SRMD.

## PERIODIC LIMB MOVEMENT DISORDER

Originally termed nocturnal myoclonus, this disorder was described by Symonds (2) in 1953 as a manifestation of epilepsy. In 1972, Lugaresi and collaborators (3) recorded the first map of periodic limb movements (PLM) using polysomnography (PSG).

These events are characterized by periodic, stereotyped movements, similar to the Babinski reflex. They involve stretching of the great toe, generally associated with a partial flexion of the foot

and leg, occasionally reaching the thigh and hip. Such movements primarily involve the legs, although less frequently they may also affect the arms and trunk. The movements may vary from night to night, simultaneously involving both legs, but may be predominant in one limb or the other.

The periodicity of the movements is quite marked, suggesting the existence of some underlying mechanism, such as a pacemaker. Four burst in a sequence is necessary to characterized PLM. The interval between the movements varies between 5 and 90 seconds, but 70% occur at intervals of 30 seconds or less (Figure 1). Each contraction lasts from 0.5 to 5 seconds and induces a pronounced increase in the electromyographic register at least five times above the baseline.

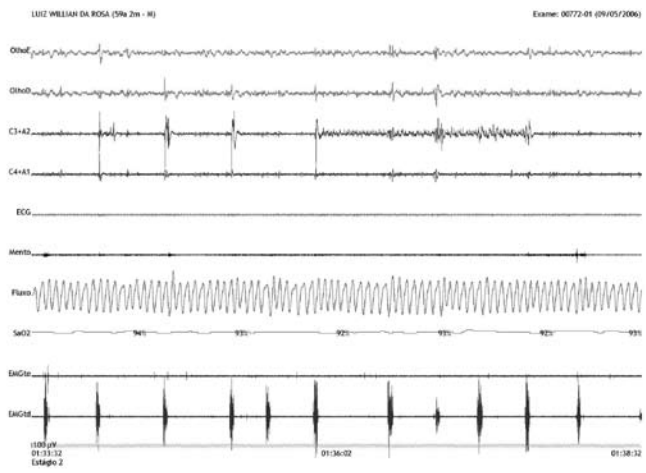

**Figure 1. PLMS polysomnographic register during 5 minutes with predominance of the right anterior tibial muscle in the electromiography (EMGtd).**

## MECHANISMS

Frequently, each PLM is followed by a cortical excitation visible in the sleep electroencephalogram (EEG) or by a short autonomic activation. This excitation in the EEG can be expressed as short cortical awakenings, with the emergence of alpha waves for 3 to 15 seconds, micro-awakenings, K-complexes, K-alpha complexes (4) and delta wave surges. The autonomic activation is expressed as an increase in cardiac and respiratory frequency. In sporadic cases, PLMS is associated with light or fragmented sleep (5), as well as complaints of insomnia. Intense movements can promote full arousal, with restoration of consciousness and occasionally difficulty in returning to sleep, thus leading to insomnia.

Changes in the sleep EEG can match the movement, follow it or precede it. This variety of temporal associations suggests the existence of a central process that generates the cortical excitation and the movement, e.g., a pacemaker. PLM, therefore, instead of being the reason for sleep instability, may just be an epiphenomenon of the normal periodic excitation process which is observed in the sleep EEG, termed cyclic alternating pattern (CAP) (6). CAP is characterized by a recurrent sequence of excitatory activity (phase A) and inhibitory activity (phase B) in the EEG in cycles of 20 to 40 seconds.

Terzano and Parrino state that CAP is the expression of a basic excitation modulator. Such a modulator emerges in normal non-REM (NREM) sleep, causing the emergence of K-complexes and other excitatory phasic events in the EEG, coupled with autonomic excitation represented by fluctuations in blood pressure and cardiorespiratory frequency (7). In normal individuals, up to 50% of NREM sleep can show CAP. Increases in the percentage of NREM sleep with CAP have been linked to sleep instability (8,9). Phase A of CAP would represent "permission" for the occurrence of PLM. It was observed that in twelve cases of PLMS followed by complaints of insomnia, 94% of the contractions occurred during the excitation cycles in the EEG in phase A, most at an interval of one second from the onset of the excitation period. Nevertheless, PLM episodes were not always concomitant with phase A. Thus, it can be concluded that phase A allows the occurrence of a PLM episode but does not cause it. The effect of sleep upon the PLM generator seems to be inhibitory. Spinal cord dissection, either partial or full, prevents central inhibition from reaching the legs and increases the probability of a PLM episode, both in paraplegic (10) individuals and in models of spinal cord injury in rats (11). In cases of PLMS, subjects show a reduction in the sleep period and greater sleep fragmentation. In addition, NREM sleep duration in the presence of CAP is 15% longer than in controls. The fact that CAP is responsible for PLM periodicity but does not induce contractions leads to the conclusion that the causes of the contractions are the same as those underlying RLS, including changes in iron metabolism (12).

## EPIDEMIOLOGY

In adults, the prevalence of PLMS varies from 5% to 11%; in elderly people, it may reach as high as 30% (13). In children, the prevalence is around 6%, but this increases in children with associated diseases. PLMS can occur as an isolated condition or associated with other sleep disorders. RLS is followed by PLMS in 80-90% of the cases, and such a high degree of co-occurrence has led some authors to raise the hypothesis that RLS/PLM is a single disorder, where PLMs represent the painless form fruste – or precursor – of RLS (14).

The prevalence of PLMS increases with age, regardless of its association with other sleep disorders. When other sleep disorders are present, the prevalence may reach 80%, as happens among RLS sufferers. Greater prevalence is also observed in patients with narcolepsy, REM behavioral disorders (70%) (15) and sleep apneas. PLMs may occur either associated with apneas or as independent episodes. In patients suffering from severe apnea associated with a significant number of PLMs, apnea should be treated before evaluating the clinical relevance of the PLMD (16).

## DIAGNOSIS

Since PLM episodes occur during sleep, they usually are not noticed by the patient. The search for a diagnosis occurs mainly as a function of the close association with RLS. PLMS diagnosis

is conducted primarily in the PSG, but screening for cases can be performed using actigraphy (17). The severity of the disorder is given by the PLM index (PLMI; Table 1), which is obtained by dividing the number of PLMs by the number of sleeping hours observed in the PSG. Non-periodic movements, PLMs inducing to awakening and PLMs during wakefulness, which may indicate the presence of RLS, are also reported in the PSG.

Table 1. PLMS severity indices

| DISORDER DEGREE OF SEVERITY | NUMBER OF PLMS PER HOUR |
|---|---|
| Normal | up to 5/hour |
| Mild | from 5 to 24/hour |
| Moderate | from 25 to 50/hour |
| Severe | More than 50/hour |

## TREATMENT

Since many patients with PLMS are asymptomatic, the need for treatment is controversial. PLMS can be an incidental finding in the PSG, and treatment will not always resolve the symptoms that motivated the investigation, typically insomnia or excessive diurnal sleepiness. One of the reasons for the persistence of symptoms despite treatment might be the fact that PLMS occurs as an epiphenomenon of excessive central excitability, with awakenings and EEG activation in phase A of CAP (18). Thus, eliminating PLMs would not eliminate either awakenings or the sleep instability shown by the increased CAP; thus, it would not result in any symptomatic improvement. Since no criteria have been established in clinical studies to determine which cases should be treated, the decision depends on clinical judgement. When PLMS is associated with sleep obstructive apnea-hypoapnea syndrome, treating apneas with CPAP masks reduces PLMI (19). Treatment for PLMS is the same as for RLS and effectively reduces PLMI. Upon performing a therapeutic test, evaluation of the symptomatic benefits obtained with a reduction in PMLI should guide the decision about proceeding with treatment for PLMS.

## RESTLESS LEG SYNDROME

Restless leg syndrome (RLS) is a common neurological condition that involves motor and sensory symptoms that follow a circadian pattern. It is characterized by an almost irresistible urge to move the legs and is associated with countless unpleasant symptoms that are engendered or exacerbated by rest and relieved by walking or moving the legs. In 1672, RLS was described by Thomas Willis (20), but only in 1945 was the condition understood as it is today. Karl Ekbom (21) observed that 5% of patients showed restless leg symptoms and described the characteristics of the syndrome. Even so, until the 1990's it was considered an uncommon disorder.

## EPIDEMIOLOGY

In 1994, Lavigne and Montplaisir (22) published data on 2,019 Canadians showing a prevalence of 12% and confirming that RLS was indeed a common disorder, though not frequently diagnosed. The percentage of RLS was substantially larger among French Canadians compared to British Canadians, suggesting a genetic component. Most studies on prevalence have been conducted only in recent years, and it has been found that prevalence varies widely as a function of population characteristics and the methods applied. Studies conducted in other countries show that prevalence is estimated between 7% and 11%.

Most surveys show that the prevalence of RLS increases with age. Nevertheless, a bimodal distribution has been observed: the onset of RLS symptoms usually occurs before the patient is 30 years old when a genetic component is present, but later in the absence of such a component (23). In addition, the prevalence is higher among females (24-26). Studies have also demonstrated that during pregnancy, the prevalence of RLS ranges from 19% (27,28) to 26% (29). The prevalence of RLS in children has not been well demonstrated. It has been observed that RLS is prevalent in adults who experienced childhood pains; in addition, the parents of children who have experienced childhood pains show a higher prevalence of RLS than control parents (30).

## ETIOLOGY

Despite progress in studies related to RLS and advanced methods that can be used to study the disorder, the cause for the disorder still remains unexplained in most cases. In view of this, the syndrome has been classified as follows:

### 1. Idiopathic

It is estimated that 60-80% of the cases are classified as idiopathic or cryptogenic and that half of them are hereditary in nature. In a study conducted with twelve pairs of monozygotic twins, it was seen that the RLS symptoms were concurrent in 10 pairs (31). Despite the high rate of concurrence, the age of onset of symptoms and the severity of such symptoms varied among the pairs. In all likelihood, the syndrome is not caused by a single genetic fault, but by a hereditary complex, as seen in other disorders like Alzheimer's disease.

### 2. Secondary

Association with several health conditions (e.g., pregnancy, uremia, iron deficiency or polyneuropathies) leads to classification of the disease as secondary RLS. There are at least two proven causal mechanisms (Chart 1):

#### *2.1. Alterations in iron metabolism in the CNS*

Iron metabolism can play an important role in RLS secondary to pregnancy, anemia, gastric surgery and renal disease, and under these conditions the symptoms usually recede with iron supplementation (32). A clear relationship has been observed between low concentrations of ferritin and symptoms of RLS, particularly when ferritin levels are measured in the cerebrospinal fluid. Anal-

ysis of the substantia nigra in cases of RLS submitted to autopsy compared to that of controls without RLS shows a complex pattern of abnormalities: iron, H-ferritin and two primary iron carriers are reduced, while ferritin levels are increased, as expected in cases of iron deficiency. Nevertheless, the number of transferrin receptors is reduced, contrary to the expected response to iron deficiency (33). This suggests that in the presence of RLS, iron deficiency in the substantia nigra is likely to be associated with abnormalities in regulation of the transferrin receptor.

**Chart 1. Causes of Secondary RLS**

- Uremia (34)
- Diabetes
- Anemias
- Sleep disorders
  - Periodic limbs movements
  - Narcolepsy (35)
  - REM sleep behavior disorders
  - Sleep apneas (36)
  - Insomnia
  - Hypersomnia
- Reumathoid arthritis (37)
- Iron deficiency (38,39)
- Ferritin deficit (40)
- Folate deficit
- Myelopathies
- Polyneuropathies (41)
- Radiculopathies
- Multiple sclerosis
- Fibromialgies (42)
- Parkinson's Disease
- Post-polio Syndrome
- Neoplasies
- Attention-deficit hyperactivity disorder (43-46)
- Peripheral vascular insufficiency
- Peripheral venous insufficiency
- Drugs
  - Caffeine
  - Alcohol (47)
  - Antidepressants
  - Dopamine inhibitors (48)
  - Lithium carbonate
  - Neuroleptic agents

### *2.2. Dopaminergic neurotransmission*

The optimal response to treatment of RLS with dopaminergic medication indicates the involvement of both the dopamine neurotransmitter and dopaminergic receptors in RLS pathophysiology. Iron is an important cofactor for tyrosine hydroxylase, an enzyme that inhibits dopamine synthesis and plays an important role in the functioning of $D_2$ receptors.

Worsening of RLS symptoms in the evening seems to be modulated by circadian factors. At night, a reduction in dopamine levels can be observed (49). Nevertheless, the only circadian marker significantly correlated with RLS so far is melatonin. Apparently, melatonin exacerbates symptoms in the evening and at night due to its inhibitory effect on central dopamine secretion (50).

## CLINICAL PICTURE

Patients experience a unilateral or bilateral unpleasant sensation, which is many times impossible to describe, but is sometimes described as twinges, shocks, electrical current, burning, parenthesies or pain. At the onset of the episode, discomfort is felt mainly deep inside the calf. As the disorder progresses, the feet, ankles and knees are also frequently affected. Other parts of the body (51), such as the arms (52), trunk and face, may also be impaired (53). Another characteristic of RLS is that rest, even in wakefulness, evokes motor and sensory symptoms. The concept of rest includes both physical immobility and reduction of alertness levels. Over 80% of patients have difficulty in falling and/or remaining asleep.

Circadian factors and immobility are RLS facilitators. Thus, going to bed in an attempt to fall asleep triggers discomfort. Moving the legs relieves the symptoms, if not completely, then at least partially or temporarily. Patients with RLS usually sleep better at daybreak. A large number of patients with RLS complain about fatigue and sleepiness and report that they do not have refreshing sleep. On the other hand, other do not have any complaints about fatigue or sleepiness. Results of studies conducted by Allen and collaborators (54) suggest that high levels of hypocretin in the CNS of patients with this disorder maintain the alert state, but their findings have not been confirmed in subsequent studies (55). Depression and anxiety symptoms, difficulty sleeping with a partner sharing the same bed and deterioration of quality of life are frequently reported by these patients (56).

## DIAGNOSIS

### 1. Medical history

Diagnosis demands a description of the clinical history of sensory symptoms experienced by the patient. In 1995, the essential criteria for diagnosis were standardized by the international group of studies on RLS (IRLSSG). In 2003 (57), the IRLSSG reviewed and updated these criteria. The essential criteria should be utilized to confirm diagnosis, but ancillary criteria also provide useful clues (Chart 2). Diagnosis in children has its own peculiarities in view of the difficulty that children usually have in describing the sensations experienced in their legs (Chart 3).

**Chart 2. Criteria for diagnosing RLS in adults (58)**

ESSENTIAL CRITERIA

1. Urge to move the legs (with or without an unpleasant sensation; other parts of the body may also be involved, such as the arms).
2. Rest triggers or worsens symptoms.
3. Movement makes the unpleasant sensation vanish.
4. Symptoms get worse in the evening or at night.

ANCILLARY CRITERIA

1. Family history (50% of the idyopathic cases have a positive family history).
2. Response – at least at the beginning of treatment – to levodopa or dopamine receptor agonists at doses significantly lower than those used to treat Parkinson's disease.
3. Periodic limb movements during wakefulness or during sleep occur in at least 85% of patients with RLS.

ASSOCIATED CRITERIA

1. Variable clinical course, but typically chronic and progressive.
2. Normal physical tests in idiopathic/familial forms.
3. Sleep disorder is a common complaint in most patients affected.

**Chart 3. Criteria for diagnosing RLS in individuals under 12 years of age**

1. Criteria are the same as those for adults, as long as the child is able to clearly explain the uncomfortable symptoms felt in the legs.
2. If the child shows the essential criteria as those for adults but has difficulty in reporting the sensation of discomfort in the legs, then at least two of the following findings must be confirmed:
   a. Disturbed sleep for his/her age
   b. Parents or siblings with well defined RLS
   c. Periodic limb movements registered in the PSG at a rate of 5 or more PLMs per hour.

### 2. Lab tests

The most useful lab tests are those involving iron metabolism. Serum ferritin is the primary indication that low iron reserves participate in RLS genesis. Another important aspect is the evaluation of renal function for detection of renal insufficiency and glycemia, in order to confirm the presence of diabetes mellitus.

### 3. Immobilization test

This test was developed for the purpose of inducing and exacerbating RLS motor and sensory manifestations in wakefulness, thus allowing their quantification. The test is run for one hour, before the patient's main sleep period, usually before the PSG. Motor manifestations are obtained through a register of the electromyography run on the right and left anterior tibial muscles. A high rate of periodic limb movements corroborates RLS diagnosis.

### 4. Polysomnography

RLS diagnosis is based primarily on the patient's clinical history. PSG, however, may contribute to detect the presence of PLMs, which occur in 80-90% of cases. The movements are measured through electromyography of the anterior tibial muscles. For the activity to be considered periodic, it should occur in sequences of at least four contractions lasting from 0.5 to 5 seconds, with a minimum interval of 5 seconds and a maximum interval of 90 seconds. Some publications, however, accept intervals between 4 and 120 seconds.

### 5. Actigraphy

The actigraph is worn on the wrist, like a watch, and registers the presence or absence of movements every minute for periods that may extend for weeks. Periods of immobility are correlated with sleep. The validity of the method for PLMS diagnosis, however, has not been well established.

## DIFFERENTIAL DIAGNOSIS

Among the several manifestations that can be confounded with RLS are positional discomfort, cramps, "painful legs and moving toes syndrome," pains in the legs, acathisia induced by neuroleptic agents, intermittent vascular claudication, peripheral neuropathy and myelopathy.

Positional discomfort may occur when a patient is lying in bed, but may be relieved by changing position, without the need for making repetitive movements or wandering around. Cramps may be exacerbated at night and relieved with movement, as happens in RLS, but since they are localized and painful, they are usually easy to differentiate. In the "painful legs and moving toes syndrome," described in 1971 (59), the pain is typically intense (similar to that experienced in causalgia), affecting one or both feet and causing a burning sensation and frequent movement of the toes. Such symptoms may occur any time of the day and are not relieved by walking around. Several pains in the legs must be distinguished from RLS, such as neuropathic, vascular and traumatic pains. RLS can be triggered by dopaminergic antagonists, as well as by neuroleptic-induced acathisia. In acathisia, however, the difference lies in the absence of a circadian pattern and in relief with levodopa.

## TREATMENT

Treatment of RLS can be pharmacological or not, and should be individualized according to the situation when the syndrome occurs also in subjects with no comorbidity. When the patient has an ongoing condition such as pregnancy, breast-feeding or any other condition associated with end-stage renal disease, treatment of RLS demands specific approaches.

### 1. Non-Pharmacological

Before anything else, it is essential to investigate and exclude manageable medical conditions that induce RLS and also ensure that the patient is not using antidopaminergic or antidepressant agents.

Adequate measures concerning sleep hygiene should be imple-

mented before starting the pharmacological treatment. Patients should avoid consuming caffeine, alcohol, nicotine and substantial meals right before bedtime; they should also maintain a regular bedtime schedule and gradually reduce night activities (60).

If the serum ferritin concentration is below 45–50 mcg/L, iron levels should be restored. In these cases, a combination of ferrous sulfate and vitamin C is indicated. Iron levels should be monitored through the control of ferritin levels every 3 or 4 months.

**2. Pharmacological**

Four categories of drugs are normally prescribed to treat RLS: dopaminergic agents, anticonvulsive agents, opioid substances and benzodiazepines.

*2.1. Dopaminergic drugs*

*2.1.a) Dopaminergic precursors*

Levodopa or lovodopa-benserazide. Levodopa was the first dopaminergic agent used to treat RLS and is still the initial choice. Nevertheless, its side effects with prolonged use are such that they make the use of levodopa as a standard drug impracticable.

*2.1.b) Dopaminergic agonists*

Dopaminergic agonists may be ergotaminic or non-ergotaminic. Ergotaminic agonists (e.g., bromocriptine, pergolide and cabergoline) are associated with frequent side effects. Non-ergotaminic dopaminergic agonists (e.g., such as ropinirole and pramipexol) are currently considered first line drugs for treating RLS, as they are more effective and do not cause as many side effects as dopamine precursors.

Pramipexol has high affinity for the D3 dopamine receptor and shows sustained efficacy in over 90% of patients with RLS (61). Nausea and orthostatic hypotension on the first day of administration are common side effects. Ropinirole, a dopaminergic agonist similar to pramipexol, is well tolerated by patients and has a 24-hour half-life. It is metabolized in the liver, and is therefore indicated in cases of RLS caused by renal insufficiency.

*2.1.c) Side effects of dopaminergic drugs*

Dopaminergic precursors are effective, although many times they can trigger side effects that make their use impracticable. Among these effects are nausea, vomiting, taquicardy, postural hypotension, rebound and symptom exacerbation.

Morning rebound is characterized by recurrence of RLS symptoms in the morning period as a result of the drug having been taken the previous night at bedtime. It occurs while the circulating drug is eliminated, which happens within a time period comparable with the drug half-life.

Augmentation (exacerbation) (62) is an increase in symptoms, an intriguing phenomenon that is peculiar to RLS. This term is used to describe the occurrence of exacerbated symptoms over a larger period, that is, two or three hours prior to the time when the symptoms used to emerge prior to taking the drug started, in at least five out of seven days. Augmentation is less prevalent with the use of dopaminergic agonists than with levodopa; even so, the phenomenon can be observed in more than 32% of patients undergoing long term treatment with the drug (63).

*2.2. Anticonvulsants:*

Within this category, the preferred drug is gabapentin, in view of its greater effectiveness and fewer side effects, including light sleepiness that can be desirable in cases of insomnia. It is most useful in managing the augmentation phenomenon. It is also the first choice in cases of RLS associated with painful neuropathy, as well as in patients that describe pain as an RLS sensorial component.

*2.3. Benzodiazepines:*

The effect of such drugs upon RLS symptoms is not significant, and they are typically used as a coadjuvant therapy to help sleep maintenance in patients with RLS. They are used when dopaminergic agents show stimulating effects that exacerbate insomnia. Clonazepam is preferred in view of its extended half-life.

*2.4. Opioids:*

Opioids are classified into two groups according to their potency: low potency opioids (e.g., codein and tramadol) and high potency opioids (e.g., morphine, methadone and oxycodone). Opioids are well tolerated, and their efficacy lasts for extended periods. Addiction and abuse are quite rare, even in the case of methadone, which is used in cases of RLS associated with painful diabetic neuropathy (64).

## THERAPEUTIC SCHEMES

Symptom regularity and severity may vary depending on the patient, indicating the need for individualized control (65). Therapeutic approaches can be devised for three levels of RLS (Chart 4):

- *Intermmitent*: when symptoms cause a degree of discomfort sufficient to require treatment, but their occurrence is not frequent enough to justify daily use of medication.
- *Daily*: when symptoms cause a degree of discomfort sufficient to require treatment and are so frequent that they require daily use of medication.
- *Refractory*: when the patient under treatment with a dopaminergic agonist shows one or more of the following reactions:

a) inadequate initial response despite dose adequacy;

b) inadequate response over the course of time despite an increase in dosage;

c) intolerable side effects;

d) side effects, like augmentation, which are not controlled with preventive additional doses.

Chart 4. Approach of RLS.

| | Non-Pharmacological | Pharmacological |
|---|---|---|
| **Intermittent** | • Sleep hygiene<br>• Assessment and treatment of primary disease<br>• Withdraw of alcohol, caffeine, nicotine.<br>• Withdraw of drugs witch cause RLS.<br>• Iron replacement when necessary | • Dopaminergic agonists<br>• Dopaminergic precursors<br>• Benzodiazepines<br>• Low potency opioids |
| **Daily** | • Sleep hygiene | • Dopaminergic agonists<br>• Gabapentin<br>• Low potency opioids |
| **Refractory** | • Sleep hygiene | • Change between dopaminergic agonists<br>• Change dopaminergic agonists by gabapentin<br>• Keep dopaminergic agonists and add gabapentin<br>• Add low potency opioids<br>• Change to high potency opioids |

## CONCLUSION

Not much attention has been given to RLS, but it is a disease that may impair quality of life and must, therefore, be treated. The use of dopaminergic agonists has simplified control of this disease.

### Sleep-Related Cramps

Sleep-related cramps are characterized by painful sensations caused by sudden and intense involuntary contractions of muscles, usually in the calf, occurring during sleep. The cramp can be relieved by strongly stretching the affected muscle and sometimes by local massage, application of heat or movement of the affected limb. Nearly every adult over the age of 50 has experienced cramps at least once in their lifetime. Both the prevalence and frequency of the number of nights in which such episodes occur usually increase with age. Among children and adolescents, about 7% have been reported to have sleep-related leg cramps, but children under 8 almost never experience this kind of discomfort. Among older people, 33% above the age of 60 and 50% above the age of 80 report experiencing cramps at least once every two months, and 6% of adults above the age of 60 experience cramps every night (66). In studies conducted in the city of São Paulo in 1987 and 1995, it was found that the prevalence of nocturnal cramps ranged from 2.6% to 5.8% in samples comprising 1,000 adults, but frequency was not studied (67). Predisposing factors are: diabetes mellitus, peripheral vascular disease and metabolic diseases. The disorder is associated with prior vigorous exercise, electrolytic disorders, endocrine diseases, neuromuscular diseases and medications such as oral contraceptives. Sleep-related cramps occur in about 40% of pregnant women and generally resolve after delivery. No familiar pattern is known. Complications include muscular pain, insomnia and occasional diurnal fatigue. A large majority of cases of nocturnal cramps seems to be idiopathic, with no relation to other diseases (68). RLS registers intense non-periodic electromyographic activity of the gastrocnemius muscle. The episodes occur during sleep, and no specific physiologic changes are observed.

### Sleep-Related Bruxism

Bruxism or nocturnal tooth grinding is characterized by grinding or clenching of the teeth during sleep, usually associated with sleep arousals. Contraction of masticatory muscles can be isolated and sustained, termed tonic contraction, or can occur through a series of phasic muscle contractions, termed rhythmic. Friction may lead to abnormal wear of the teeth, tooth pain, jaw muscle pain or temporal headache. Sleep-related bruxism may also result in sleep disruption. Severe cases usually lead to limitation of jaw movements. Bruxism may also occur during the waking period, but a correlation with sleep bruxism is not known. Bruxism is termed primary when there is not a clear triggering cause, and secondary bruxism may be associated with the use of psychoactive medications, recreational drugs or other diseases (e.g., infantile cerebral paralysis and mental retardation). There is a correlation between bruxism episodes and stressful situations or anxiety related to current life events. It seems to occur most frequently among highly motivated or vigilant individuals. The use of cigarettes and caffeine in hours before sleep contributes to increased tooth grinding.

Sleep-related bruxism is frequently reported in childhood but decreases with age; nevertheless, in some subjects tooth grinding may be observed every night across their life span. The prevalence in children ranges from 14% to 17%; in adolescents and young adults, it is around 12%; in adults, it is around 8%; and in older people, it is around 3%. Between 20% and 50% of subjects have at least one member in the family showing a history of bruxism.

Bruxism episodes are preceded by autonomic cardiac activation 4-8 minutes before the masticatory activity starts. Four seconds before that, an increase in cerebral activity is registered in the PSG and, in 90% of the cases, alpha waves with microawkenings are registered in the EEG. One second before the onset of a bruxism episode, an increase in the cardiac frequency and in the suprahyoid muscular tonus occurs, followed by a contraction of the masseter muscles and tooth grinding (69). Electromyography of the masseter muscles shows either a phasic activity pattern with a frequency varying from 0.25 to 2 seconds, a sustained tonic activity lasting for more than two seconds or otherwise a mixed patttern. It could be said that a new episode of bruxism is distinguished when at least three seconds have elapsed without the occurrence of any muscular activity. Movements caused by tooth grinding can be video-monitored. Bruxism may occur during any sleep stage, but over 80% of typical episodes occur in stages 1 and 2 of NREM sleep, while less than 10% occur during REM sleep. Rarely is bruxism predominant in REM sleep. The disorder is diagnosed as bruxism when at least four episodes per hour of sleep or 25 individual surges of muscular activity per hour of sleep occur, with at least two audible tooth grinding episodes and no

abnormal activity being registered in the EEG.

Treatment remains controversial. The literature is concentrated mainly on the use of bite guards to avoid tooth wear, but evidence cited in an updated study performed by Cochrane in October, 2007, was considered insufficient to ensure that dental devices were effective for treating bruxism (70). Other therapeutic attempts remain inconclusive. Despite the success of dopaminergic agents for treating MDs, only one study so far has investigated their effect on bruxism, demonstrating that L-Dopa reduces the number and the intensity of masticatory episodes (71). In view of this lack of evidence, but based on experience with hundreds of cases, the authors think that bruxism can be considered to be another DM that adequately responds to dopaminergic medications, mainly pramipexol.

## CONCLUSION

Knowledge about the mechanisms of the disorders reviewed here enables the prevention of undesirable effects caused by erroneous treatments (e.g., the use of hypnotic drugs to treat RLS), which may perpetuate and aggravate the suffering of individuals with misdiagnosed conditions.

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Sleep science

# 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$^{th}$ 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 ß-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 various 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 ß-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 ß-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$^{ob}$ (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$^{ob}$ 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 ß-cell mass (19).

Leptin has been shown to inhibit choline acetyltransferase (ChAT), the sympathetic enzyme that produces acethylcholine, 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 ß 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 ß 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 ß-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 autossomic 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 ß-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 ß 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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Sleep science

# PROFESSIONAL DRIVERS AND WORKING TIME: JOURNEY SPAN, REST, AND ACCIDENTS

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***Running Title:*** *Professional Drivers and Working Time*

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## ABSTRACT

Shift work is a reality in many sectors of industrial societies. Unfortunately, it is associated with several problems. Within this context, we highlight fatigue caused by extended working hours and the resulting increase in the risk of accidents. This is also a reality on Brazilian roads, with increasingly alarming rates of accidents involving intercity drivers. A significant number of such accidents is related to sleepiness caused by disruptions in the circadian rhythm. The necessity to drive for many consecutive hours without a pause, extended working hours, and mainly driving in the early hours of the morning may affect the driver's wakefulness state and performance. This may be due to a lack of synchrony with the temperature curve, as well as melatonin levels. To minimize the risk of accidents, work schedules should include regular pauses during the work journey. Moreover, such schedules should prevent professional drivers from working for more than 10 consecutive hours, as the risk of accidents increases significantly after the 8th hour at the wheel.

**Keywords:** Shift work; Accidents; Intercity drivers; Circadian rhythm; Fatigue.

## INTRODUCTION

Society-induced pressure for productivity and performance has caused a continuous work schedule, the so-called "24-hour society" (1). This implies a series of adaptations regarding the physical and psychological structures of humans, which may not always reach satisfactory levels.

In a 24-hour industrial society, the effects of work activities on sleep and general health have been the object of extensive investigation in the last decades (2). Night workers, for example, have less refreshing diurnal sleep, show lower alertness and performance levels, and also exhibit higher rates of accidents compared to daily workers (3,4).

Particularly for individuals whose profession involves driving, this is a problem that deserves special attention due to the alarming rates of accidents and their respective costs to society (5,6). According to a study conducted by Instituto de Pesquisas Econômicas Aplicadas (7) (Institute of Applied Economic Research), the

average cost of an accident involving injured people amounts to R$90.000,00, and one involving fatal casualties amounts to R$421.000,00. This study was based on data collected between 2004 and 2005, and found that the total cost of traffic accidents reaches R$24.6 billion – R$8.1 billion for accidents occurring on Brazilian federal roads and R$16.5 billion for those occurring on Brazilian estate roads. The government agency (DENATRAN) responsible for traffic administration nationwide estimates that 34,000 people die each year as a consequence of road accidents. Similarly, data from the Ministry of Health (SVS/MS) indicates that in Brazil 35,674 people die each year due to traffic accidents; such deaths represent 28% of total deaths in Brazil and 81% of these deaths are males.

The highest risk of accidents does not always occur during the peak traffic hours, but rather tends to occur when people experience a decline in the body/core temperature curve, which usually occurs between 12:30-02:00 pm and 10:00 pm-06:00 am. The period between 03:30 am-05:30 am represents the critical hours most associated with sleepiness. Traffic accidents are related to fatigue and sleepiness, and are a consequence of the increasing pressure for productivity and excessively long work journeys (8). The latter, coupled with inadequate shift organization, can contribute to an increased potential for road accidents (9).

Shift work usually triggers sleep deficit, which is called acute or chronic sleep deprivation. The conditions involved may increase fatigue and the risk of making mistakes. Nevertheless, financial needs frequently cause drivers to perform under suboptimal conditions (10). Unfortunately, extended work journeys without pauses for rest or consecutive extended work journeys and consuming chemical substances or other drugs to help maintain alertness are becoming quite common.

In an attempt to minimize the negative effects of such conditions, some strategies, such as exposure to light and consumption of products containing caffeine and other substances, such as melatonin, as suggested by Goh et al., have been used to promote adaptation to work (11).

Other less complicated strategies, such as taking naps, have also been suggested. In this respect, pauses during the work journey have shown to be quite effective for improving performance and reducing risk rates, thus contributing to increased safety (12).

Nevertheless, it is important to point out that great efforts have been made to develop models which would help estimate the benefits of work schedules that substantially contribute to reducing the incidence of road accidents and, at the same time, improve the quality of life for night and shift workers (3,4).

## BIOLOGICAL AND CIRCADIAN RHYTHMS

According to Menna-Barreto (13), environmental cycles, such as day and night and the seasons of the year, are examples of factors capable of establishing synchrony between the body's functions and the external environment. The bodily functions that are repeated with a specific periodicity are called biological rhythms. These cycles can be circadian (period of approximately 24 hours), ultradian (more frequent than one cycle in 20 hours), and infradian (less frequent than one cycle in 28 hours).

According to Menna-Barreto (13), our body establishes a series of temporal relations with environmental processes and maintains a series of independent processes, as observed in the production of certain hormones. Regular work schedules may act as an environmental synchronizer. The morning shift (6 am to 2 pm) leads workers to wake up earlier than office employees (who work from 9 am to 6 pm). Day/night and noise/stillness cycles are examples of synchronizers that affect human being internal temporal organization.

An abrupt change in the scheduled work period, as occurs when there is a change in shifts, brings about a change in some biological rhythms, such as the sleep-wake cycle and body/core temperature, triggering an "internal disruption" (13). When such disruption occurs, it is usually due to changes in work shifts or intercontinental trips. Thus, 'jet lag' is a result of an abrupt change in time zone, and may cause discomfort, fatigue, and difficulty in falling asleep. To accelerate synchronization and minimize the impact of jet lag, melatonin and light pulses have been utilized (14). Shift workers who perform on a rotating system display symptoms similar to those generated by jet lag, and are called "shift lag."

Another aspect that changes or has a direct influence on adaptation to the work journey is the chronotype. Horne and Ostberg (15) determined that the number of morning people is greater than that of evening people, who have a higher level of tolerance to shift work. Nevertheless, experience with shift work and the development of studies and clinical research in the field of sleep have demonstrated that people above 55-60 years of age show a reduction in their total sleep time (TST). Thus, a prevalence of morning people can be observed among older individuals. As such, investigation into chronotype may not reflect a person's actual characteristics (biotype), as these data may change or be masked as an individual ages. Thus, an elderly individual may be considered a morning person as a result of a reduction in his/her TST, which did not occur in earlier years.

Several authors have investigated the impact of shift work on the circadian rhythm. Dahlgren et al. (16) found that there is a significant reduction in cortisol secretion in worn-out individuals. Danel and Touitou (17) demonstrated the extremely negative impact of alcohol on biological rhythms. Pasqua and Moreno (18) observed that the seasons of the year exert a negative influence on the nutritional habits of workers. Finally, lab and field studies conducted by James et al. (14) demonstrated that light and dark promote circadian adaptations, and that such factors should be considered in the observation of any other phenomenon or variable, as they are powerful agents, capable of altering biological rhythms.

According to Arendt (19), the circadian pattern of melatonin production usually begins late at night, simultaneous with the onset of sleepiness and a reduction in core temperature. The peak melatonin production occurs between 2:00 am and 4:00 am. As per Cagnacci et al. (20), the light-dark cycle is the major synchronizer of our biological clock, exerting a direct influence upon melatonin production, which is physiologically involved in the regulation of our core temperature. Menna-Barreto (21) observed that the core temperature shows a reduction during sleep, dropping to its minimum value around 04:00 am and reaching its maximum around 06:00 pm (Figure 1).

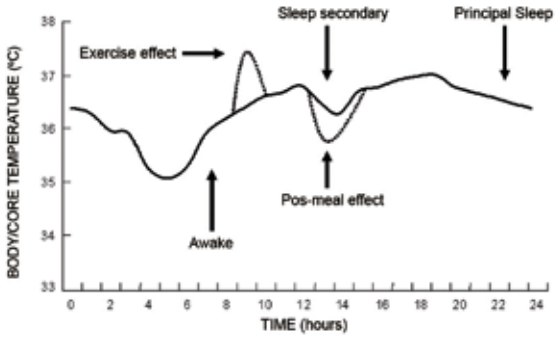


**Figure 1: Body/Core Temperature Hypothetical Curve (21)**
**Source: University of São Paulo. Institute of Biomedical Sciences. GMDRB –Multidisciplinary Development Group and Biological Rhythms. Body/Core temperature hypothetical curve. São Paulo: USP. [quoted on June 12, 2006]. Available on: http://www.crono.icb.usp.br.**

In the context of this article, it is interesting to note that according to Winget et al. (22), there are several performance components that are influenced by the circadian rhythm, such as reaction time, psychomotor coordination, and cognitive processing, all of which are critical for driving. The same authors demonstrated that there are other factors that may influence the variation in circadian performance and which may, consequently, affect drivers. Among these factors are work load, psychological stress, motivation, and the chronotype in itself. In general, all these factors are influenced by the light-dark cycle, as well as by cycles of melatonin release and body/core temperature.

## WORK SCHEDULES

Work schedules are schemes that allow maximal productivity. A poorly organized work schedule may cause acute or chronic sleep deprivation, as well as a series of other disturbances that may affect the worker's performance (1,5), particularly if that worker is a professional driver.

Professional drivers face serious problems concerning work schedules and working conditions.

According to several studies (1,5,23), it is recommended that a work schedule:

- Allows the worker to have a pause for rest every two or three hours during the working time;
- Provides conditions for the worker to have light stimulus so sleepiness is minimized;
- Includes a rotation between shifts occurring clockwise;
- Organizes work such that night shifts are less frequent than day shifts, as the former induces greater fatigue and sleepiness; and
- Minimizes extended work journeys, as after being awake for more than 19 hours, an individual feels the same sensation as felt in the initial stage of drunkenness. Data show that after the 9th hour of work the risk of accidents increases significantly.

Well planned work schedules may offer a great contribution to the driver's psychophysical balance, minimizing the effects caused by fatigue and, consequently, reducing the risk of accidents. Thus, the work schedule should be carefully planned, taking into consideration principles and theories involving shift work.

## WORK JOURNEY TOTAL TIME

Despite the planning of work schedules, there can be a necessity for long working hours in order to accomplish certain tasks, and thus, an increase in the relative risk of accidents and other similar events can be observed in several areas, including roads.

It is important to point out that few studies have investigated the risk of accidents as a function of the number of consecutive hours the individual is at the wheel, as most accident records do not contain such information. In this regard, two studies were distinct. Pokorny et al. (24) investigated the risk of accidents involving bus drivers, and observed that the peak risk occurs at the beginning of their shift, around the 3rd and 4th hour, followed by a period of relatively low risk until the 8th hour, when risk increases again.

In the study conducted by Hamelin with truck drivers, it was observed that the risk of accidents as a function of the number of consecutive hours driving was higher in the first 4 hours (25). Additionally, for individuals driving for more than 12 consecutive hours, there is an exponential increase in the risk of accidents. Thus, a potential strategy would be to include pauses between the risk periods, that is, for a driver who is supposed to work for 9 hours, pauses for rest should take place after 2 and 4 hours of driving, with a final pause between the 6th and 7th hours, close to the end of the working day.

Compiling the results of several studies, Folkard (9) found a substantial reduction (approximately 30%) in the relative risk during the second half of an eight-hour shift. Extending the work journey for more than 8 hours resulted in an exponential increase in the relative risk of accidents, which may double when comparing the 12th and 8th hours (Figure 2).

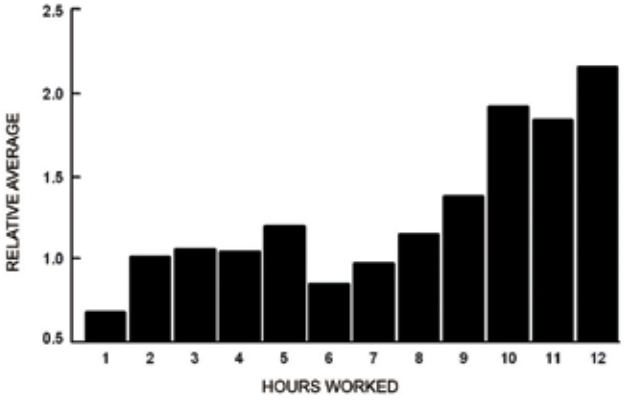


**Figure 2: Relative average risk over the number of hours worked (26).**

A study by Rajaratnam and Arendt noted that a reduction in motor and cognitive capacities are among the consequences of sleep deprivation (2). Williamson and Feyer observed that staying awake for more than 17-19 hours impairs the driver's performance in the same way as if the driver's blood alcohol concentration was around 5% (26). For some tasks, including driving, remaining

awake for 20-25 hours results in a 10% decrease in performance (Figure 3).

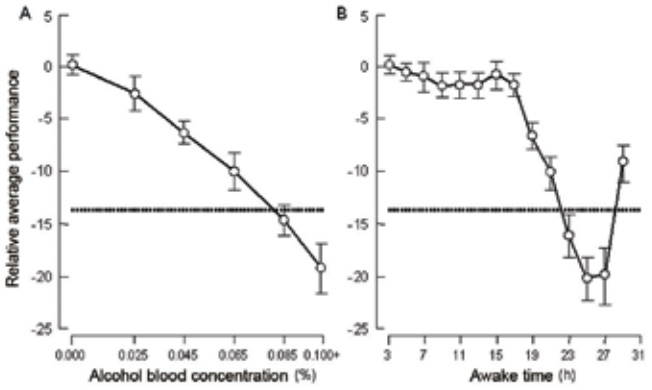


**Figure 3: Comparison between the effects of alcohol concentration in the blood and wakefulness during performance (2).**

It was also observed that the time of day and number of hours an individual remains awake both influence the alertness state (4). Figure 4 shows that the alertness state reaches its lowest level between 3:30 am and 6:00 am. It also shows an almost linear decrease with relation to the time the individual remains awake; such time is coincidental with the reduction in the body/core temperature curve, which is also associated with peaks of melatonin, a hormone that induces sleep, release. Thus, extended work journeys framed into specific schedules may substantially increase the risk of accidents.

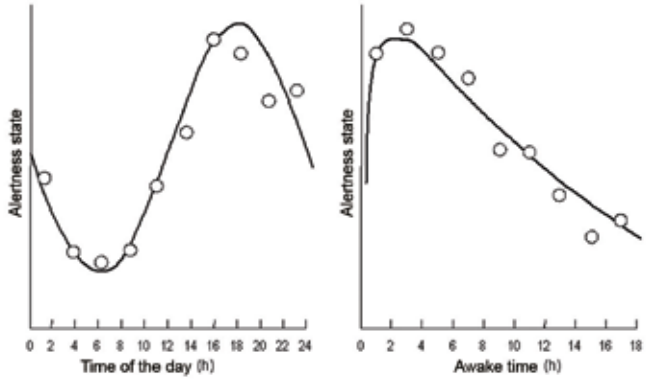


**Figure 4: Influence of the time of the day and the number of hours the individual remains awake upon his/her alertness state (4).**

## SLEEPINESS, NUMBER OF HOURS WORKED, AND ACCIDENTS INVOLVING TRUCK DRIVERS AND BUS DRIVERS

According to Folkard (9), changes in environmental conditions, particularly light levels and temperature, exert a clear influence on our "biological clock". They are partially responsible for the circadian rhythms that dictate biochemical and physiological processes, specifically the transition between sleep and wakefulness.

Folkard observed that the risk of accidents increases significantly at dawn or in the early morning (02:00–04:00) (9). He also observed a secondary peak in the early afternoon, which is attributed to a reduction in body/core temperature; such a reduction may be brought about by the performance of a monotonous, unending task (Figure 5). The study conducted by Lavie indicates that sleepiness was higher at dawn or in the early morning (between 02:00 and 05:00) (27). Therefore, an association between road accidents and sleepiness has been observed by several authors (28).

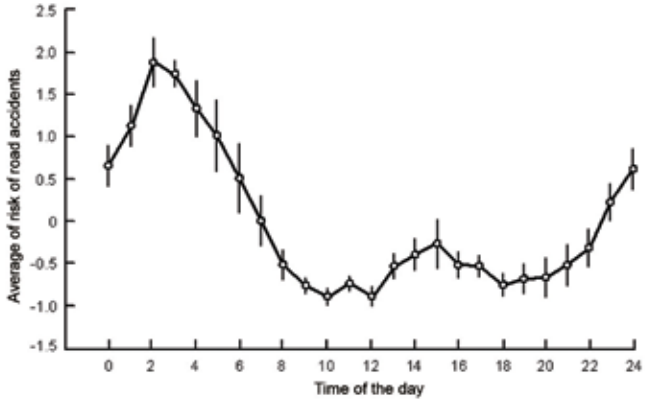


**Figure 5: Average estimates on risk of road accidents along the 24 hours of the day (9).**

As per Philip et al., fatigue is a complex state characterized by a deficit in the alertness level and a reduction in physical and mental performance, frequently accompanied by sleepiness (8). The main factors that generate fatigue are the time of the day (between 00:00 and 06:00 am), number of hours the individual has remained awake (more than 17h from the main sleep period), and number of hours performing a task without any break.

In a study conducted by Souza et al. with 260 truck drivers, it was found that in Brazil 85% of the drivers work for more than 11 hours straight, and 43,2% drive for more than 16 consecutive hours (29). Even more troublesome, 23,8% of drivers sleep less than 5 hours on working days, and 13,1% have been involved in accidents in the last five years. These are alarming figures, as they clearly demonstrate how the need to stop for rest is neglected and how heavy the professional driver work load is.

An extended work journey may significantly increase fatigue levels. In a study conducted by Phillip et al., young drivers (18-24 years old) were submitted to sleep deprivation and simple reaction tests (30). An increase in the reaction time equivalent to 650 ms was observed when compared to control conditions (without sleep deprivation), which represents an increase of 23 meters in the distance for braking at a speed of 75 miles (120 km) per hour.

According to Akerstedt, professional drivers (bus and truck drivers) are typically affected by sleep disorders, and 20-30% of road accidents involving this population are caused by sleepiness while driving, as a consequence of this type of disorder (31). In a study conducted with 3,268 professional drivers, Howard et al. (32) found a high prevalence of excessive sleepiness (24%) and sleep disorders. According to Garbarino et al., between 19 and 21% of road accidents were due to sleepiness while driving (33).

Estimates on traffic accidents related to sleepiness vary widely. In a study conducted by McCartt et al., the conclusion was that 55% of the drivers felt sleepy during the working time, and 3% fell asleep while driving (34). According to Hakkanen and Sum-

mala, 40% of wagon truck drivers reported problems in maintaining alertness state after driving for periods of 5 consecutive hours, and approximately 20% confessed that they took more than one involuntary nap at the wheel (35). Interviews with long-haul wagon truck drivers showed that 47% fell asleep while driving (36). Two additional Brazilian studies show important and alarming data. Mello et al. (37) demonstrated that 16% of interstate bus drivers confessed that they sometimes fall asleep while driving (an average of 8 naps per journey), and Santos et al. (5) demonstrated that 48% of interstate bus drivers from a single company of mass transport felt fatigue and tiredness during their working time. In Van Den Berg and Landstrom's opinion, the difference between the drivers' testimonies on sleepiness while driving and the statistics on accidents related to sleep should be carefully analyzed, since sleepiness is broadly identified as the main cause of accidents in transport operations (31). Sleepiness symptoms (sight problems, yawning, difficulties in maintaining the alertness state, and concentration on the task) are well known and have been frequently investigated; thus, continuing to drive after manifestation of such symptoms is not justified (38). The symptoms emerging from a state of chronic fatigue in truck drivers were investigated by Milosevic, who observed that the most common were pains in the legs and back, sleepiness, irritation, moroseness in performing activities, pains throughout the body, and problems related to eyesight (38).

In order to prevent drivers from falling asleep during the work journey, many strategies, such as exposure to light (39), consumption of caffeine (40), balancing the work and rest periods (10), scheduled naps (41), and consumption of energetic beverages (42) have been studied. Nevertheless, the safest preventive measure is to stop driving (12), as the drivers should be aware of the risk involved in continuing to drive when they feel sleepy.

## PAUSES AND STRATEGIES FOR RECOVERING WORK CAPACITY

Regular pauses are recommended to prevent accidents during performance of extended or continuous activities (43). Several studies have investigated the effect of pauses on the potential risk in function of the task time duration.

In a study conducted by Tucker et al. (12), an eight-hour work journey was analyzed, and a 15-minute pause was introduced after each continuous 2 hour working period. Every 2 worked hours the number of errors committed at every 30 minutes was calculated. It was observed that the risk increased substantially in a regular pattern, and that it doubled in the last 30-minute period preceding the pause. It was not observed that this trend is different for daily and night shifts, nor for the three consecutive 2-hour periods within an 8-hour work shift.

Some studies suggest that short and frequent pauses (10 min/hour) can improve work performance. In the study conducted by Phillip et al., where a pause for rest was introduced every hour and a half (90 minutes), no significant differences were found in the reaction time when comparing control conditions (without sleep deprivation) to the results after a 9-hour journey subsequent to a full night's sleep (20). The result was attributed to the model that included pauses, which can minimize the effects of fatigue.

European traffic regulations recommend a driving period shorter than 10 hours within a 24 hour, and a driving period shorter than 4.5 consecutive hours. Such recommendations, compared to the study conducted by Souza et al., where 85% of the truck drivers worked more than 11 hours per day, call for deep reflection and consistent actions (29). In Brazil, the Law Project n° 2.660/1996, whose purpose is to regulate the limit of consecutive hours truck and bus drivers should work, has followed the procedures of the National Congress for more than 10 years. Nevertheless, the driving time and work suggested in the above law project or in other work schemes do not match what is recommended by researchers and scientists from several countries.

Thus, strategies that may help reduce the negative impacts caused by sleep deprivation have been proposed by several scholars. Purnell et al. observed that for a surveillance task, a 20-minute nap taken on the first working night significantly improved the response time at the end of the work shift (44). Matsumoto and Harada also demonstrated that short naps were effective at lessening the effects of sleepiness, consequently improving performance (41). Naps can, thus, be beneficial for counteracting sleepiness at the wheel (45). They may have a short duration (10 minutes), which significantly improves the alertness state in the short term, or a long duration (20-30 minutes), which is quite effective for improving general performance and reducing fatigue (6).

Lenné et al. (46) quoted several studies that recommend the adoption of short naps as a way to reduce sleepiness and, consequently, the rate of accidents. Two important factors should be considered:

- The first is sleep inertia (reduction in performance and / or in alertness state immediately after arousal, characterized by hypoalertness and sleepiness transient state), which may substantially reduce the benefit of naps;
- The second is the possibility that the nap takes place in a noisy environment, which may not bring the same benefits as in a quiet environment.

Thus, pauses during the work journey are extremely important, and recommended. It should be considered, though, that only well planned pauses, not just a random nap, can help improve the driver's performance along the work journey, contributing to the recovery of alertness and performance levels.

## CONCLUSION

Educational and informational programs have been proposed to increase awareness about the risks of driving while tired. Nevertheless, such programs haven't been enough, and a lot more work should be done.

Meanwhile, the driver must acknowledge the sleepiness symptoms (eyesight problems. yawning, difficulty to remain mentally alert and concentrated on the job, pains in the legs and back, irritability, and slow performance), in order to avoid taking risks and performing the job under unsafe conditions. As for the employers, it is extremely important that they orient their employees (drivers) so that they avoid driving when sleepy and /or weary, develop sensible work schedules, and check to ensure drivers do not have

any sleep disorders that may impair sleep efficiency, which is critical for the process of physical and cognitive recovery.

According to the National Sleep Foundation (NSF), an optimal alertness level, which encourages good performance throughout the journey, depends on the fulfillment of two conditions:

a) satisfaction of the biological need to sleep, which implies sleeping the necessary number of hours free from disturbances and sleep fragmentation;

b) synchronizing the waking period with the circadian biological clock, thus avoiding waking up too early.

The NSF believes that several problems are responsible for the fact that drivers rarely sleep an average of 8 hours between their work shifts. These problems are related to other activities involved, such as eating, vehicle replenishing, etc, and so it recommends:

- A minimum of a 10-hour rest period within a 24-hour period;
- Reducing the work journey in the early hours, when the risk of accidents is maximized;
- Including an educational program on how to get refreshing sleep and how to recognize signs of a reduction in alertness levels;
- Encouraging drivers to have an effective medical examination to check for sleep disturbances and excessive sleepiness, and also recommend them to avoid consuming drugs that change sleep efficiency patterns.

Extended work journeys without adequate recovery routines, many times imposed by the need to achieve the targets intended, significantly increase the risk of accidents on Brazilian roads. Coupled with these factors are several other chronic external problems that affect the professional driver's performance, among them poor maintenance of our roads, problems related to the signaling system and layout of the roads, and intense traffic due to lack of alternatives for cargo flow.

In summary, the establishment of well-balanced work journeys that would ensure the driver's physical and psychological recovery, including pauses long enough to restore alertness and performance levels, critical for the task performed, would minimize the risk of accidents and improve the quality of life of professional drivers. In addition, it would help reduce the high costs of accidents currently incurred and the loss of Brazilian lives.

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Sleep science

# SLEEP DISTURBANCES AND GENDER DIFFERENCES IN SCHIZOPHRENIA

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***Running Title:*** *Sleep in schizophrenia*

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## ABSTRACT

Objective: The objective of this study was to evaluate sleep disorders and their relationship to clinical variables in schizophrenia. Subjects and Methods: In this cross-sectional study with ambulatory patients suffering from schizophrenia, sleep quality was evaluated with the Pittsburgh Sleep Quality Index (PSQI), and excessive daytime sleepiness by the Epworth Sleepiness Scale (ESS). Results: Eighty-two patients (42 male and 40 female) aged 17 to 59 years (mean age 32.2±9.8) were studied. Poor sleep quality (PSQI>6), exhibited by 41 patients (51.3%), was independently associated with the female gender (OR=2.98; CI=1.13-7.83). Excessive daytime sleepiness (ESS>10) was found in 20 patients (24.7%), and ESS scores tended to correlate with treatment duration (P=0.07). Conclusion: In schizophrenia, poor sleep quality, present in 50% of the patients studied, is associated with the female gender. We suggest that physical and cognitive-behavior therapy to improve sleep quality should be initiated intensively in women with schizophrenia.

**Keywords:** Sleep; Schizophrenia; Epworth Sleepiness Scale.

## INTRODUCTION

Sleep is an active state vital for physical, mental, and emotional well-being, and is important for optimal cognitive functioning (1). Sleep problems are described as difficulty in falling or staying asleep, in staying awake, or in adhering to a consistent sleep-wake schedule. Poor sleep quality and excessive daytime sleepiness are known to occur in both schizophrenia (2) and Parkinson's disease (3), suggesting that dopamine may play a role in regulating the sleep-wake cycle (4). Administration of antipsychotic drugs that have effects on neurotransmitter systems, including histamine, acetylcholine, serotonin, norepinephrine and dopamine, might play a role in the sleep-wake cycle. Antipsychotic therapy in schizophrenia can minimize sleep problems. Conversely, sedative effects associated with these medications have the ability to disrupt sleep and wake patterns. Randomized controlled trials comparing the effects of these medications upon sleep have rarely been conducted (5).

It has already been shown that sleep quality is related to quality of life (6), and altered sleep has been associated with a failure to consolidate learning (7). Although the characteristics of sleep in patients with schizophrenia have been described (8), subjective sleepiness (previously associated with deficits in pre-frontal activation (9) can potentially affect cognition and has rarely been investigated. Other relevant aspects of the relationship between sleep disorders and clinical variables, particularly gender differ-

ences, have not been studied.

Although schizophrenia affects both genders with the same frequency, women tend to have a better clinical pre-morbid state, develop the disease at a later age, and show a different symptom profile, with less florid psychotic symptoms (10,11). These findings have been attributed to different structural brain abnormalities. A better response to typical antipsychotic drugs in patients undergoing the premenopausal phase has been associated with the presence of estrogen (12). Understanding gender differences in schizophrenia is critical to guide therapeutic decisions.

The purpose of this study was to evaluate sleep quality, excessive daytime sleepiness and its relationship to the severity of comorbidity among patients with schizophrenia.

## SUBJECTS AND METHODS

### Study design

This was a cross-sectional study involving a sample of 82 ambulatory schizophrenia patients consecutively recruited from an outpatient hospital-based clinic. Assessment included the Structured Clinical Interview from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). All subjects met the DSM-IV criteria for schizophrenia (13), and had not undergone recent hospitalizations or changes in medication in the preceding three months. Mini-Mental State (MMSE) (14) above 18 was required to join the study. None of the subjects were using sleep-promoting drugs, such as benzodiazepines or antidepressants. The antipsychotic treatment as well had not undergone any changes during the preceding three months. All data were collected simultaneously during a four-month period. The pertinent protocol was approved by the local Research Ethics Committee, and written informed consent was obtained from all patients involved.

### Assessment procedures

A socio-demographic and clinical questionnaire was applied by a medical staff comprising three professionals. Clinical data were further confirmed by a chart review. Subjective sleep quality was evaluated by the Pittsburgh Sleep Quality Index (PSQI) (15). The PSQI has seven components, each one dealing with a major aspect of sleep: 1) subjective sleep quality; 2) sleep onset latency; 3) sleep duration; 4) sleep efficiency; 5) presence of sleep disturbances; 6) use of hypnotic-sedative medication; and 7) presence of daytime disturbances, as an indication of daytime alertness. Component six always scored zero because the patients who used hypnotic-sedative medication were not included in the study. Individuals showing total PSQI scoring six or above were considered poor sleepers.

Excessive daytime sleepiness (EDS) was assessed by the Epworth Sleepiness Scale (ESS), a validated questionnaire containing eight items that query about dozing expectation in eight hypothetical situations. Dozing probability ratings range from zero (no probability) to three (high probability) (16). An ESS scoring 10 or above indicates EDS.

### Statistical analysis

Data were examined for normality using the Kolmogorov-Smirnov test. ANOVA and Mann-Whitney test were used to assess gender differences. Pearson's correlation test was used to compare scores of behavior and clinical variables. A logistic regression analysis was performed to estimate the effect of clinical variables in the presence of poor sleep quality (PSQI>6) and excessive daytime sleepiness (ESS>10). Further adjustments were made using variables derived from the bivariate analysis (P<0.1). The statistical analysis was done with the Statistic Package for Social Sciences (SPSS- Norusis, 1993) software for Windows. The level of significance was set at p<0.05.

## RESULTS

Eighty-two subjects of both genders (42 male) aged 17 to 59 years (mean age 32.2±9.8 years) with schizophrenia were evaluated. Patients were either in use of traditional antipsychotic medication (haloperidol or fluphenazine) or atypical antipsychotic medication (risperidone or olanzapine). Therapy duration ranged from 3 to 72 months (mean duration 4.96±4.99). Clinical and demographic data are depicted in Table 1. Women were of older age, had undergone treatment for longer periods, and had lower levels of education (Table 1). Poor sleep quality (PSQI>6) could be observed in 41 (51.3%) of the cases, and prevailed among female patients (mean 7.08±2.86); mean among male patients was 5.15±2.67 (Mann-Whitney, P= 0.002).

**Table 1: Demographic data of 82 patients with the diagnosis of schizophrenia according to gender**

| | | Male N=42 | Female N=40 | Test, p value |
|---|---|---|---|---|
| Age (y) | Range<br>Mean±SD | 18-59<br>31.81±9.90 | 17-56<br>38.95±9.86 | ANOVA, 0.002** |
| Treatment duration (m) | Range<br>Mean±SD | 3-20<br>5.57 ±5.42 | 4-72<br>10.14 ±13.27 | ANOVA, 0.008** |
| Years of education (y) | Range<br>Mean±SD | 1-5<br>3.56 ±1.42 | 1-5<br>2.56 ±1.68 | ANOVA, 0.04* |
| PSQI | Range<br>Mean±SD | 1-12<br>5.15 ±2.67 | 2-12<br>7.08± 2.86 | Mann-Whitney, 0.002** |
| ESS | Range<br>Mean±SD | 0-18<br>6.83± 5.06 | 0-15<br>5.83 ±4.18 | Mann-Whitney, 0.48 |

Abbreviations: y=years; m=months; PSQI=Pittsburgh Sleep Quality Index; ESS=Epworth Sleepiness Scale.

The patient's age was directly correlated with treatment duration and inversely to years of education (Table 2). PSQI scores were inversely correlated with level of education, indicating that patients with a higher level of education had better sleep quality. ESS scores showed a trend of correlation with treatment duration (p=0.07).

**Table 2: Pearson correlation test between clinical variables and scores of behavioral scales in 82 patients with schizophrenia**

| | Age | Treatment duration | Years of education | PSQI |
|---|---|---|---|---|
| **Treatment duration** | r=0.359** p= 0.001 | | | |
| **Years of education** | r=-0.250* p=0.03 | r=-0.150 p= 0.20 | | |
| **PSQI scores** | r=0.113 p=0.31 | r=0.088 p=0.44 | r=-0.356** p=0.002 | |
| **ESS scores** | r=-0.081 p=0.47 | r=-0.204 p=0.07 | r=0.058 p=0.62 | r=0.010 p=0.92 |

**Abbreviations: PSQI= Pittsburgh Sleep Quality Index; ESS= Epworth Sleepiness Scale. *=p<0.05; **=p<0.01**

Poor sleep quality was associated with the female gender (OR= 2.79; CI= 1.12-6.90) and remained so after controlling for years of education (OR= 2.98; CI= 1.13-7.83) (Table 2). Excessive daytime sleepiness (ESS>10) was found in 20 patients (24.7%), and was not associated with any of the measures studied (Table 3).

**Table 3: Logistic regression analysis between clinical variables, poor sleep quality (PSQI>6) and excessive daytime sleepiness (ESS>10)**

| | PSQI>6 N=41 (51.3%) | ESS>10 N=20 (24.7%) |
|---|---|---|
| **Gender** | 2.79 (1.12-6.90)* | 0.60 (0.21-1.68) |
| **Age** | 1.00 (0.96-1.05) | 0.99 (0.95-1.04) |
| **Treatment duration** | 1.02 (0.97-1.07) | 1.00 (0.96-1.05) |
| **Years of education** | 0.66 (0.49-0.90) | 1.09 (0.79-1.52) |

**Abbreviations: PSQI= Pittsburgh sleep quality index; ESS= Epworth sleepiness scale.**

## DISCUSSION

The results of our study show that poor sleep quality is frequent and independently associated with the female gender in patients with schizophrenia. According to our data, a more vigorous therapy, particularly the use of physical therapy or cognitive behavior therapy for sleep disorders, could be used in female patients. Gender differences in schizophrenia have been extensively described. It has been observed that male cases tend to develop earlier, and severe forms of the disease cause greater deterioration (17). In contrast, schizophrenia onset in the female sex occurs later and presents more severe positive and affective symptoms (11). Anxiety-depressive symptoms and low self-perception have also been described in association with the female gender in schizophrenia (18). Our findings related to poor sleep quality in women may be explained by the presence of increased mood behavior disorders in such patients. Interestingly, sleep quality was not correlated with age and treatment duration, but was correlated with the level of education. In this study, women were older and had a lower level of education. Lower school performance levels among women can be explained by lower pressure for educational achievement, particularly due to different cultural pressures exerted upon men and women. It has been previously demonstrated that lower school performance levels predict poor outcomes (19). As sleep quality was not related to age or to comorbidity severity, two factors frequently associated with sleep disorders, we postulate that sleep disorders commonly found in schizophrenia are directly connected to mental illness.

We found that nearly a quarter of patients presented excessive daytime sleepiness. In our study, a trend of association between therapy duration and excessive daytime sleepiness was found, so it is possible that chronic antipsychotic therapy contributes to sleepiness. Personality disorders have already been identified as an important clinical factor that determines excessive daytime sleepiness (20). Establishing causes for daytime sleepiness may be a difficult task, considering that in other medical situations it has been demonstrated that several clinical conditions, such as diabetes (21), sleep apnea syndrome and daily social activities (22), can interfere with the sleep-wake cycle. To our knowledge, studies on the prevalence of sleepiness and its association with clinical factors or therapeutic measures in schizophrenia are still missing. This is an important subject, and modafinil, a drug that reduces sleepiness, has been associated with general schizophrenia improvement (23).

In short, poor sleep quality, shown by half of patients studied, prevails among female patients. We suggest that physical and cognitive-behavior therapy with the purpose of improving sleep quality should be initiated intensively in women with schizophrenia.

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Sleep science

# EVALUATION OF TIMING AND RESPONSES TO PHYSIOTHERAPEUTIC TREATMENT

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***Running title:*** *Chronotype and Physiotherapeutic treatment*

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## ABSTRACT

In several treatment regimens, the recognition of chronobiology contributes to the therapeutic process through the effective use of temporization protocols. The purpose of the present study was to evaluate the relationship between the response to physiotherapeutic treatment and the time of day when such treatment was performed, as well as the chronotype of orthopedic and rheumatologic patients in a clinical physiotherapy school. The population studied was treated in the morning and evening periods. The patients were divided into three groups of pathologies with similar treatments, which were as follows: syndrome of shoulder impact (n=33), knee artrosis (n=17), and lombalgia (n=23). At the end of ten treatment sessions, data concerning pain, percentage of subjective improvement, chronotype, and age were compared. At the end of the study, it was observed that the time of day when treatment was performed influenced the results of individuals treated in the evenings but had no influence on the individuals treated in the morning. In addition, the evening schedule was the most well suited for intermediate individuals.

**Key words:** Physiotherapy, Chronobiology, Chronotype, Chronotherapy.

## INTRODUCTION

The functions of the human body are rhythmic and typically follow a 24 hour cycle (1). These cycles, called circadian rhythms ('approximately one day'), represent a critical adaptation of the human body to environmental stimuli (2).

People prefer different schedules to perform their activities. Some feel good waking up very early; others feel better waking up later. Some prefer practicing physical activities in the evening, whereas others do not feel good practicing physical exercises at such late times. The biological concept determining these extreme individual differences in temporal preference is called chronotype (3).

It is known that, in several treatment regimens, chronobiology can contribute to the course of therapeutics through effective temporization protocols (4). In the case of physiotherapy, the determination of an optimal schedule for physiotherapeutic treatment could lead to the patient's early release and even to a better outcome.

Some studies have reported that synchronization with the environment and the maintenance of an internal temporal order is necessary for human physiological fitness and normal behavior (5). Therefore, disturbances in both internal and external temporal states may lead to health problems.

The purpose of the present study was to evaluate the relationship between the response to physiotherapeutic treatment and

the time of day when such treatment was performed. In addition, the chronotype of orthopedic and rheumatologic patients under treatment was compared for their responses to treatment performed in the morning period (during the day) versus that performed in the evening.

## SUBJECTS AND METHODS

The population studied comprised all the orthopedic/rheumatologic patients treated both in the morning and evening periods in a clinical physiotherapy school located in Santa Catarina, Brazil, for a period of one year. Patients whose clinical diagnoses included syndromes of shoulder impact, knee artrosis, and lombalgia were included in the sample.

Criteria for excluding patients from the study were as follows: 1) patients who had not attended two or more consecutive treatment sessions; 2) a serious evaluation/revaluation error in the patient's records had been committed, thus preventing a comparison between the initial and final data; 3) the patient was under 18 years old.

The evaluations were based on the patient's records and the Horne and Ostberg's questionnaire on morningness-eveningness (HO) (6). The records included data on evaluation, treatment, and revaluation of patients in relation to the following variables: subjective degree of pain intensity and patient's subjective improvement.

A 10 cm visual analog scale (EAV) was utilized for measuring subjective pain. In brief, the patient was instructed to draw a vertical line at the point corresponding to the pain that he/she felt. For performing the comparison calculation, the value (in centimeters) for the revaluation was deducted from the value found in the initial evaluation.

For measuring the percentage of patient's subjective improvement, the individual was asked the following question: "What is, in your opinion, the percentage of your improvement (from zero to 100%) since you have started the physiotherapeutic treatment?" This value (according to the patient's own opinion) was taken as the patient's subjective improvement score.

The daily period for treating the morning group ranged from 8:00 to 11:00 h and from 18:30 to 21:30 h for the evening group. The HO was delivered to patients on their first visit, and they were briefed on every item in the questionnaire and of its respective contribution to the research. The participants answered the questionnaire at home and returned it upon the following visit. For the purposes of analysis, at the end of ten visits, the patient's revaluation was performed based on the same parameters used for the initial evaluation. The physiotherapeutic procedures utilized for treatment of each pathology group were the following:

- Group A (syndrome of shoulder impact): continuous ultrasound therapy with intensity ranging from 0.5 to 0.7 W/cm$^2$, strengthening of the rotator cuff with the use of thera-band®, and upper limbs proprioception.

- Group B (knee artrosis): application of shortwave therapy, exercises for strengthening lower limbs with leg warmers, and exercises for stretching lower limbs.

- Group C (Lombalgia): application of shortwave deep heat on the lombar spine, exercises for stretching the trunk back muscles, exercises for stretching the lower limbs, lombar and cervical spine pompages, and final relaxation.

Data were analyzed in a descriptive way so as to allow a comparison. In addition, a cluster analysis was conducted in order to group similar individuals under measured variables, such as chronotype, pain before and after treatment, absolute pain, self-evaluation, age, and HO scores. PAST ® software was utilized for these analyses.

In the revaluation, Spearman's linear correlation index was utilized to correlate the values with respect to pain, and the percentage of subjective improvement was calculated with the use of the software mentioned above. This test was performed with a 5% significance level and a 95% confidence level. A Wilcoxon test for dependent samples was utilized to check the existence of two similar populations and performed with a 5% significance level and 95% confidence level, according to Triola (7). Statdisk® software was utilized in this analysis.

The individuals who agreed to participate in the study signed a Free and Informed Consent Form. The present study was approved by the UNISUL Ethics Committee under register number 05.182.4.08.III.

## RESULTS

A total of 211 patients participated in the present study, including 140 females (66.35%) and 71 males (33.65%). The average age was 43.41 years (± 14.33 years); the minimum age was 18.50 years; and the maximum age was 77.83 years. Out of this population, there were 88 intermediate individuals (I), 83 moderate morning-oriented individuals (MM), 21 moderate evening-oriented individuals (ME), 16 extreme morning-oriented individuals (EM), and 3 extreme evening-oriented individuals (EE) according to Horne and Ostberg's classification.

Considering these inclusion and exclusion criteria, the 74 samples were composed of 33 individuals that were classified under Group A, 18 under Group B, and 23 under Group C (Table 1).

**Table 1 - Distribution of patients according to pathologies with similar treatments and chronotypes.**

| Chronotype | Group A | Group B | Group C |
|---|---|---|---|
| MM | 20 | 7 | 11 |
| EM | 3 | 2 | 0 |
| I | 9 | 6 | 11 |
| ME | 1 | 3 | 1 |

**MM = moderate morning-oriented; EM = extreme morning-oriented; I = intermediate; ME = moderate evening-oriented**

In regard to chronotype, 50% of the male patients and 62% of the female patients were morning-oriented (MM and EM). A larger number of evening-oriented subjects were found among the male population in the present study (Table 2). The male group showed an average HO score of 56.8 (±11.15), and the female group showed and average score of 58.42 (±8.8). The Wilcoxon test showed a significant difference between these two groups ($p < 0.001$).

Table 2 - Distribution of patients according to chronotype and sex.

| Chronotype | Male | | Female | |
|---|---|---|---|---|
| | n | % | n | % |
| MM | 9 | 37,5 | 29 | 58 |
| EM | 3 | 12,5 | 2 | 4 |
| I | 9 | 37,5 | 17 | 34 |
| ME | 3 | 12,5 | 2 | 4 |

MM = moderate morning-oriented; EM = extreme morning-oriented; I = intermediate; ME = moderate evening-oriented

The HO average score for individuals aged below the median (median = 45.88 years; n = 37) was 54.46 (±10.06), and the average score for those aged above the median was 61.32 (±7.82). The Wilcoxon test confirmed the existence of a difference between these two groups ($p < 0.001$). The first group mentioned included 15 morning-oriented individuals (EM and MM) and 5 evening-oriented individuals (ME). The second group included 28 morning-oriented individuals (EM and MM), and no evening-oriented individuals. The morning-oriented individuals corresponded to 40.54% of the first group and to 75.67% of the second group (EM and MM).

In addition, in the sample studied (n = 74), the older participants were predominantly morning-oriented individuals. Hence, when considering the median as the dividing factor, the younger group included around 40% of morning-oriented individuals, while the other group included 75% of morning-oriented individuals and no evening-oriented individual.

Male and female individuals reported similar complaints related to pain at the beginning of treatment. The percentage of male individuals who reported feeling 'almost no pain' was higher than the percentage of female individuals who reported the same (8.3% in the male group and 2% in the female group). Among the patients who reported 'severe pain', the percentage of male individuals was higher than the percentage of female individuals (50% in the male group and 44% in the female group), according to the qualitative pain classification mentioned before.

Despite the apparent similarity existing between the values found in the male and female groups, the Wilcoxon test showed a significant difference between the two groups ($p < 0.001$).

At the end of treatment, both groups showed similar variations. The male group maintained a higher percentage of individuals who reported feeling 'almost no pain' compared to the female group (41% in the male group and 36% in the female group). However, there was an inversion in the percentage of male and female individuals in the group that reported 'severe pain'. In this group, the rates of male individuals were lower than those of female individuals (4% in the male group and 10% in the female).

According to the EAV, the average pain among male individuals was 5.7 (±2.8), and the average pain among female individuals was 6.1 (±2.6). The minimum and maximum values were 0.0 and 10 for both sexes, respectively; the mode for the male sex was equal to 7, and that for the female sex was 5.5. In the revaluation, the average pain for the male sex was 2.2 (±2.3), and that for the female sex was 2.8 (±2.6). For the male sex, the minimum and maximum values were 0.0 and 9.5, respectively, and the respective values for the females were 0.0 and 10. The mode was equal to 0.0 for both sexes.

The percentage scores for subjective improvement showed a significant difference for the group of female patients who were treated in the morning compared to the female group who was treated in the evening ($p<0.001$). Nevertheless, 27 female patients included in the group treated in the evening (65.85%) showed a subjective improvement of 70% or above. On the other hand, in the groups treated in the morning (7MM, 1 EM, and 1I), only 3 individuals (33.33%) showed such a high percentage of subjective improvement (2MM).

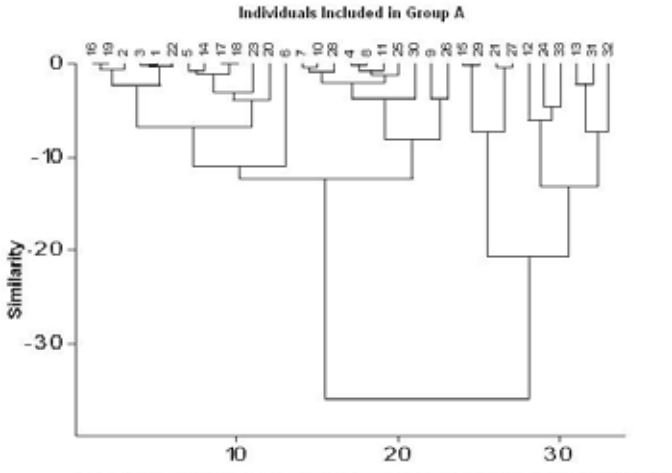
In the male group, the percentages of subjective improvement were also significantly different among those treated in the morning compared to those treated in the evening ($p<0.001$). Nevertheless, similar to the results of the female group, the majority of male patients treated in the evening (72.22%) showed a subjective improvement of 70% or above. However, in the morning groups (2 MM, 2 EM and 2 I), only 4 patients (66.67%) showed such a high percentage of subjective improvement.

By applying the Spearman's linear correlation test with a 95% confidence level ($\alpha=0.05$) and by comparing the value of the pain upon revaluation and the patient's subjective improvement, a negative correlation from moderate to severe could be observed among the female patients ($r_s = -0.57$; $p < 0.000$), and the same was observed in relation to male patients ($r_s = -0.56$; $p < 0.01$). Such analysis indicates the existence of a strong correlation between pain at the end of treatment and the value for the patient's subjective improvement. When comparing the group of patients who received treatment in the evening, the same negative correlation could be observed ($r_s = -0.57$; $p < 0.000$). A strong negative correlation could also be observed in the group of individuals treated during the daily period ($r_s = -0.69$; $p=0.004$).

It was also observed among the male individuals that there was a larger percentage of subjects who reported feeling 'severe pain' compared to female individuals (50% and 44%, respectively). However, during the revaluation, the number of male individuals who reported feeling 'almost no pain' was larger than the number of female patients (41.7% and 36%, respectively). Since the statistical test showed a significant difference, it appears that the female individuals in the present study were more prone to pain than the male individuals.

In the dendrogram analysis, the individuals included in groups A, B, and C were grouped according to the degree of pain present at the time of revaluation and the percentage of subjective improvement. In Group A, the treatment time did not seem to exert any influence upon moderate morning-oriented individuals. The best time for treating ME individuals was the daily schedule. For I individuals, the best time was the evening schedule. In the case of the single ME individual in this group, no influence concerning the time of the day when treatment was performed could be observed. In Group B, the schedule did not seem to have any influence upon morning-oriented individuals. In regard to I individuals, the best time was the evening schedule, and the same was observed concerning ME individuals. In Group C, the schedule seemed to have almost no influence on MM individuals. For the I and ME individuals included in this group, the best time was the evening schedule (Figures 1, 2, and 3).

Figure 1.

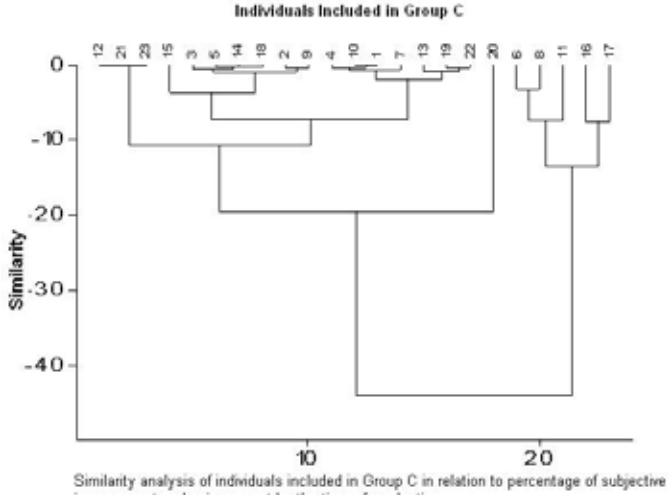



Similarity analysis of individuals included in Group A in relation to pain present by the time of revaluation and percentage of subjective improvement

Figure 2.


Similarity analysis of individuals included in Group B in relation to percentage of subjective improvement and pain present by the time of revaluation

Figure 3.




Similarity analysis of individuals included in Group C in relation to percentage of subjective improvement and pain present by the time of revaluation

## DISCUSSION

The results of the present study corroborate other findings related to the Brazilian population selected. Such results confirm that morning preference prevails among the female sex when compared to the male sex. Therefore, the HO values for the female sex were higher than those for the male sex (8). As observed in the literature, the results of the present study showed that, as a person gets older, the tendency for morning preference increases significantly (9).

Some studies show consistent differences in the perception of pain according to sex (10). However, some authors point out that this evidence may be related to the fact that the pain stimulus was induced experimentally (11,12).

Thus, the sample used in the present study corroborates several other studies, which show that the degree of pain felt by the female sex is higher than that felt by the male sex. As a significant difference was observed in the correlation between pain at the end of the treatment and the percentage of subjective improvement assessed by patients, one could consider that this result is a good indicator of the response to treatment.

By summarizing the findings relative to the three groups studied, it can be observed that, on the whole, the time of the day when treatment was performed did not have any influence upon morning and evening individuals. Nevertheless, intermediate individuals showed a better response to evening treatment than to daily treatment.

The hypothesis that morning-oriented patients treated during the day might obtain a better response to treatment than those treated in the evening has not been confirmed. In general, the hypothesis that morning-oriented patients treated in the evening would obtain a better response to treatment than those treated during the day was confirmed. In addition, the hypothesis that intermediate patients would obtain the same response to treatment if they were treated either during the day or in the evening has not been confirmed, as such individuals showed a better response to treatment performed in the evening.

In general, when comparing the response to treatment time, it was observed that the evening schedule provided better results. We believe that, with strict control over some variables involved (treatment, social and labor influences, etc.) it will be possible to reaffirm the findings of the present study. No doubt, the difficulty of evaluating patients showing identical pathologies represented one of the limitations to the present study, as it does not allow an evaluation of the sample as a whole. Thus, the degree of variation in the responses to treatment among patients treated in the evening or morning cannot be assessed.

It is quite clear from the literature that the signs and symptoms of certain diseases may undergo daily, monthly, and/or yearly changes. These changes may be utilized to identify the causes for some disease states and their corresponding treatments (13,14). In view of this relationship, there is no doubt that chronobiology contributes to therapeutic treatments through effective temporally optimized protocols (8). In order to allow the development of such treatment regiments, further studies addressing the influence of circadian rhythms should thus be conducted.

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Sleep science

# SLEEP-WAKE CYCLE PATTERN, SLEEP QUALITY AND COMPLAINTS ABOUT SLEEP DISTURBANCES MADE BY INPATIENTS

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***Running title:*** *Sleep in hospitalized patients*

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## ABSTRACT

**Objectives:** Comparing the sleep-wake cycle, sleep quality, and sleep-related complaints of patients in a private general hospital to those patients in a public general hospital

**Methods:** Transversal study conducted with a sample comprising 50 patients in a public hospital and 42 patients in a private hospital. Protocols: Pittsburgh Sleep Quality Index, Questionnaire on Sleep Habits and medical records. The Student's t-Test was utilized for independent samples and for Person's correlation.

**Results**: The sleep quality averages for patients in the private hospital and in the public hospital were 5.3 ± 2.9 and 7.04 ± 4.2, respectively, with a significant difference between them ($t = 2.2$; $p < 0.05$). Overall, 74% of patients in the public hospital and 69% of patients in the private hospital showed excessive daily sleepiness. Disturbed sleep during the night for medication was the most frequent complaint in relation to the hospital environment. Only a few complaints were made by patients, and the ones that were reported were seldom acted on by the health care professionals.

**Conclusions**: Environmental and individual factors should be considered in the etiology, predisposition and maintenance of sleep disturbances in patients treated in general hospitals.

**Key words:** Inpatients, Sleep, Sleep disorders, Hospitals, Behavioral Medicine, Health.

## INTRODUCTION

The prevalence of sleep disorders has shown a marked increase lately, affecting between 30 and 50% of the general population (1). These disorders are the result of health and/or behavioral problems, such as harmful sleep habits (2,3). However, those who suffer from sleep disorders do not give too much attention to the problem, and few patients seek professional help or mention the problem during a clinical examination (4,5).

In general hospital inpatients, the frequency of sleep disorders

is up to twice as high as that of the population at large (6,7), and there is a tendency to ignore sleep disturbances or complaints of sleep disturbances (8). Some of the most frequent disturbances are sleep fragmentation and reduced night sleep during the hospitalization period. The amount of the total sleep period can range from 1 to 15 hours (4), resulting in a variation in the distribution of non-REM (Rapid eye movements) and REM sleep stages (9,10).

The high frequency of sleep disorders in hospitalized patients and their indifference to this affliction can be attributed to two factors:

*1) individual:* presence of clinical pathologies, seriousness of the disease, pain, use of painkillers, sedation and duration of the hospital stay (11); emotional alterations or changes, among them anxiety, depression and stress (12,13).

*2) environmental:* hospital's physical structure or hospital environment (noise, unsuitable environmental temperature, excessive light) (14); hospital work routine (interruptions for medication during the night); lack of an adequate hospital structure to maintain the sleep-wake cycle pattern and provide good sleep quality to patients (14).

Most published studies focus mainly on environmental factors (noise and work routine) involved in predisposing, triggering and maintaining sleep disorders and disturbances in ICU patients. The influence of these factors, however, remains controversial (14).

The purpose of this study was to compare the sleep-wake cycle patterns and the sleep quality of patients in a public general hospital to those in a private general hospital; additionally, the sleep complaints at each facility were evaluated.

## METHODS

### Study and sample type

A transversal study was conducted in both a private and public hospital in the city of Natal, Brazil with a convenience sample (nonrandomized allocation). All patients agreed to participate in the study, and 100% completed it. A total of 42 patients (21 female and 21 male), mean age 53.05 ± 20.2 years, were interviewed at the private hospital, and 50 patients (28 male and 22 female), mean age 47.3 ± 16.9 years, were interviewed at the public institution.

### Instruments

The instruments used were demographic-social characteristics (with the purpose of recording age, education level, and marital status), the Pittsburgh Sleep Quality Index, the Sleep Habits Questionnaire and medical records for accessing complaints on sleep disturbances.

Sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI) (15). This questionnaire comprises seven items whose summed scores result in the sleep quality index. The maximum value is 20; values above 5 characterize poor sleep quality; values above 10 point to a clinical diagnosis of sleep disturbances or disorders. This instrument allows a retrospective assessment of sleep quality, as the items are related to the patient's sleep habits one month after hospital admission. The Portuguese validated version was used to avoid possible measuring biases (16).

An adapted Sleep Habits Questionnaire was used to assess the patient's sleep-wake cycle pattern based on the number of sleep and waking hours recorded during the hospital stay. This questionnaire consists of five questions about the hospital sleep environment and nine questions about the patient's sleep during hospitalization (naps, sleepiness, insomnia and parasomnia). The original version of the questionnaire was developed with the purpose of evaluating sleep habits in school sites; it contained 32 questions about home conditions, health, sleep (sleep-wake pattern, occurrence of arousals during the night, naps and sleep disturbances and disorders shown by the patients and by family members), consumption of psychostimulant substances and other activities developed when they were at home (17).

### Procedures

The instruments used were applied after the examiner read and explained each question and the proper answering procedure. If a question was not understood, the interviewer repeated it. If it was not understood, it was repeated once again. If no answer was forthcoming, the examiner proceeded to the next question.

The study was approved by Rio Grande do Norte Federal University Ethics Committee. After being informed on the purpose of the research, the patients were invited to participate in the study. Written, informed consent was obtained from all participants, whose privacy was respected and maintained throughout the experiment.

### Statistical analysis

In order to characterize the sample, a data descriptive analysis (means, standard deviations and absolute and relative frequencies) was performed. The Student's t-test for independent samples was used to compare data on patients from the public and the private hospital, and Pearson's correlation was applied to assess the correlations between sleep variables and individual characteristics at a significance level of 5%. SPSS software was used in all tests.

## RESULTS

In both hospitals, the patients who participated in the present study went to bed early and slept an average of 7 hours (Table 1). The private hospital inpatients slept longer and fell asleep earlier than those in the public hospital ($t = 8.42$, $p < 0.001$; $t = 9.48$, $p < 0.0001$, respectively). Although the patients' sleep quality was poor in both hospitals, the private hospital patients had a better sleep quality compared to that of public hospital patients ($t=2.2$; $p<0.05$) (Table 1).

**Table 1: Parameters of sleep of inpatients at a public and a private hospital based on the Sleep Habits Questionnaire.**

| Sleep Characteristics | Public Hospital | Private Hospital |
|---|---|---|
| Sleep Onset | 22:13 ± 106 min | 22:06 ± 90 min* |
| Sleep duration | 7h ± 115 min | 7h43min ± 89min* |
| PSQI | 7.04 ± 4.2 | 5.3 ± 2.9* |

*** Statistically significant differences between the hospitals** *(t-test*=2.2; p<0.005*)*

In both hospitals, no correlation was found between patients' ages and PSQI scores ($r = -0.121$; $p > 0.05$).

We found that male patients at the private hospital went to sleep earlier than their male counterparts at the public hospital, while female patients at the public hospital went to sleep earlier compared to the females at the private institution. Both male and female patients at the public hospital slept fewer hours compared to their private hospital counterparts. All of these results were not statistically significant. (Table 2).

**Table 2: Parameters of sleep of male and female inpatients at a public and at a private hospital based on the Sleep Habits Questionnaire.**

| Sleep Characteristics by sex | Public Hospital | Private Hospital |
|---|---|---|
| Sleep Onset/Men | 22:15 ± 1:20 | 22:01±01:50 |
| Sleep Onset/Women | 21:49 ± 1:48 | 22:03±01:10 |
| Sleep Duration / Men | 06h56min± 115min | 07h37min± 138min |
| Sleep Durtion / Women | 07h32min±117min | 7h47min±78min |
| PSQI / Men | 7.75±4.2 | 5.1±2.8* |
| PSQI / Women | 6.14±4.2 | 5.5±3.0 |

*** Statistically significant differences between the hospitals** *(t-test* **=2.4; p<0.05)**

Regarding variables related to sleep-wake cycle patterns, no differences in sex distribution were observed in patients at either hospital.

Analysis of male and female patients' sleep quality at both hospitals showed that all patients had poor sleep quality (Table 2). Significant differences in sleep quality were found only between male patients at both the private and the public hospital ($t = 2.4$; $p < 0.05$), with private hospital patients enjoying a better sleep quality.

The mean hospital stays of patients at the private and public hospitals were 7 and 10 days, respectively. An indirect and significant correlation was observed between hospital stay and PSQI scores of public hospital patients, with worse scores recorded during the first days of hospitalization ($r=-0.325$; $p<0.05$).

When data were separated by sex for the two hospitals, the male and female patients at the public hospital were hospitalized for 9.3 and 10.7 days, respectively, while the hospital stay of their male and female counterparts at the private hospital was 6.9 and 3.5 days. No statistically significant correlation was found between hospital stay duration and PSQI scores (private hospital: male $r=-.354$, $p>0.05$; female $r=-.107$, $p>0.05$).

With respect to hospital environment, there were numerous complaints concerning excessive noise. At the public hospital, 16% of the patients complained about excessive noise, while 28% reported little noise. At the private hospital, 14% complained about excessive noise in their room, while only 4% reported little noise. In the private hospital, an average of two patients occupied each room, while the public hospital average was six patients.

Around 48% of the public hospital patients and 64.3% of the private hospital patients had their night sleep interrupted for medication. Moreover, these patients displayed excessive daytime sleepiness (74% at the public hospital and 69% at the private hospital) with frequent daytime dozing (79.2 and 74% in the private and the public hospital, respectively).

Interrupted sleep was more frequent among women at the public hospital (women=59% and men=42.9%) and more frequent among men at the private hospital (men=76.2% and women=52.3%). Concurrently, these patients displayed excessive daytime sleepiness (men=75% and women=72.8% at the public hospital; men=81% and women=57% at the private institution), with daytime dozing (men=79% and women=63.3% at the public hospital and 71.4% of the private institution).

The results indicated that the patients who dozed frequently had lower PSQI scores ($r=-0.330$; $p<0.05$). The frequency of sleep complaints from public and private hospital inpatients and assessment of these complaints in regards to sex distribution are presented in Tables 3 and 4, respectively.

**Table 3: Frequency of sleep complaints of inpatients at a public and at a private hospital.**

| Hospital | Complaint | Register of the complaint | Professional intervention | Medical records |
|---|---|---|---|---|
| Public Hospital | 40% | 20% | 10% | 16% |
| Private Hospital | 45% | 26.2% | 19% | 7.3% |

**Table 4: Frequency of sleep complaints of male and female inpatients at a public and at a private hospital.**

| Hospital | Sex | Complaint | Register of the complaint | Professional intervention | Medical records |
|---|---|---|---|---|---|
| Public Hospital | Men | 42.9% | 25% | 17.9% | 17.9% |
| | Women | 36.4% | 13.6% | did not occur | 13% |
| Private Hospital | Men | 47.6% | 33.3% | 19.5% | 9.5% |
| | Women | 28.6% | 19.5% | 19.5% | 4.8% |

## DISCUSSION

According to the present study, the inpatients at both hospitals had irregular sleep-wake patterns. This finding corroborates a previous study in which hospitalized patients displayed irregular sleep and wake cycle patterns and longer daytime sleep (18).

The present study showed that 48% of those patients hospitalized in the public hospital and 64.3% of in the private institution had their sleep interrupted during the night for medication. At the same time, patients at both hospitals experienced excessive daytime sleepiness, longer daytime sleep and night time sleep fragmentation due to the fact that patients were awakened during the night to take their medication. Excessive daytime sleepiness is directly related to the amount of night time sleep; that is, when there is total or partial sleep deprivation during the night time sleep period, there is a corresponding increase in daytime sleep (19).

The patients' sleep quality at both hospitals was poor (scores above 5). Hublin et al. (18) suggest that inpatients have poor sleep quality as a result of hospitalization. However, the private hospital patients had comparatively better sleep quality. This may be due to the following factors: 1. hospital stay duration – the public hospital patients were hospitalized for a longer period of time (10 days) than those at the private hospital (7 days); 2. hospital environment – in the public hospital, there was a larger number of patients in the same room (6 patients) than in the private hospital (2 patients);

3. interruption of night time sleep for medication. It should be pointed out that a longer hospital stay coupled with an unsuitable hospital environment tends to worsen sleep quality (11).

Concomitantly, we found that private hospital patients experienced more frequent daytime dozing (79.2%) compared to those at the public hospital (74%). We observed an inverse correlation between dozing frequency and PSQI scores: patients with more frequent daytime dozing had lower PSQI scores. Given that dozing is a way to replace fragmented sleep, it can result in a better sleep quality.

When we assessed the patient's sleep pattern characteristics by sex, no statistically significant differences for sleep onset or duration were found. A statistically significant difference was found only between male patients from the private and the public hospital with regard to the sleep quality variable, showing that the former had better sleep quality. This finding also confirms the previous suggestion that sleep quality might have been better because of hospital stay duration (the men at the private hospital were hospitalized for a shorter period of time than those at the public institution). Private hospital patients also dozed more frequently (81% versus 79% for public hospital patients). Although the female patients at the private institution were hospitalized for fewer days (3.5) and dozed more frequently when compared to their female counterparts at the public facility, no statistically significant differences were found concerning sleep quality.

More than two-thirds of the patients hospitalized at both hospitals reported that they did not seek medical help. This indicates an indifference to their sleep disorders. At the same time, medical intervention in the case of sleep complaints was performed in only 20% of the cases. Shocat et al. (8) indicated that recognition of sleep complaints by health professionals is low. According to their study, only one-third of patients with insomnia report their sleep disorders, and only 5% of doctors assess their patients' sleep patterns. These results corroborate the data mentioned in the present study, in which only 10 and 19% of professionals at the public and the private hospital, respectively, assess sleep disorder complaints.

As it was observed, the hospital context caused sleep pattern changes through environmental (number of patients per room and excessive noise, among others) and individual alterations (almost no attempt to register the complaint of disturbed or altered sleep). In order to overcome this, a Sleep Hygiene Program has been suggested for hospitals, along with a rigorous assessment of sleep complaints by the medical team. These measures would promote shorter hospital stays, a reduction in the indiscriminate use of hypnotic drugs and a consequent reduction in treatment costs.

A limitation to the present study was the general or habitual nature of the data, which were obtained from subjective protocols where patients may have over- or underestimated the assessment of their sleep pattern during their hospital stay. Sleep data were not evaluated based on objective records, such as those obtained by polysomnography. Furthermore, no continual daily assessment was carried out.

In conclusion, both individual and environmental factors must be considered in the etiology, predisposition and maintenance of sleep disturbances in general hospital inpatients.

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Sleep science

# SLEEP DEPRIVATION REDUCES RAT HYPERHOMOCYSTEINEMIA INDUCED BY A HYPERLIPIDIC DIET

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## ABSTRACT

**Objective:** Several clinical and experimental studies have shown an association between hyperhomocysteinemia and cardiovascular diseases (CVD). Long-term exposure to a hyperlipidic diet induces tissue fatty acid accumulation and increases circulating lipid concentrations, which raises the risk for CVD. Sleep debt is also considered to be an important factor enhancing cardiovascular risk. Since no information concerning homocysteine (Hcy) levels resulting from a hyperlipidic diet is currently available, our objective was to investigate changes in Hcy concentrations in sleep deprived rats fed a hyperlipidic diet. **Subjects and Methods:** Rats were maintained for 65 days on a high-fat diet, whereas control animals received regular food ad libitum. After this period, a sub-group of these animals was submitted to sleep deprivation (SD). Homocysteine, thiobarbituric acid reactive substances (TBARS), vitamin B6, folate, and the lipid profile were measured. **Results:** Hcy concentrations were significantly higher in hyperlipidic fed rats. Sleep deprivation reduces high levels of this amino acid as well as triacylglicerols and TBARS levels. **Conclusions:** The present study shows for the first time that plasma Hcy concentrations in rats increase as a result of a hyperlipidic diet consumption. Metabolic changes induced by SD interfered in CVD-related factors even after the use of a hyperlipidic diet.

**Keywords:** hyperhomocysteinemia; hyperlipidic diet; rats; sleep deprivation.

## INTRODUCTION

Homocysteine (Hcy) is a sulfur-containing amino acid derived from methionine, and there are several possible metabolic pathways for its clearance from the system: remethylation to form methionine via either cobalamin-dependent methionine synthase (using N5-methyltetrahydrofolate as a methyl donor) or betaine-homocysteine methyltransferase (using betaine as a methyl donor); catabolism through the transsulfuration pathway, ultimately forming cysteine; or export to the extracellular space (1,2). Hcy

metabolism and plasma concentration are regulated by vitamin B6, vitamin B12, and folic acid levels (2). An increasing number of current clinical and experimental studies have shown an association between deficiencies in dietary sources of vitamins (mainly folate and vitamin B12) and hyperhomocysteinemia (3).

Hyperhomocysteinemia is associated with cardiovascular, renal, and neurodegenerative diseases (2). Total plasma Hcy values of approximately 10 µmol/L for men and 8 µmol/L for women constitute the normal range (4). However, even a small increase in total plasma Hcy is associated with an increased risk of coronary artery disease both for men and women (5,6). Moreover, a number of studies have demonstrated that smoking, excessive alcohol consumption/alcohol abuse, obesity, type II diabetes, and an unhealthy diet contribute to mild hyperhomocysteinemia (2,4).

Long-term exposure to a hyperlipidic high fat diet induced marked tissue fatty acid accumulation and increased circulating lipid concentrations, which may influence or negatively impact cell function and contribute to the risk of cardiovascular disease (7). Moreover, a hyperlipidic diet may result in an increased heart rate and is generally believed to favor obesity and hypertension (8). Despite the great body of evidence relating Hcy to cardiovascular disease (and particularly associating Hcy with obesity and hypertension), no information is available concerning the causal relationship between a hyperlipidic diet and high Hcy levels. This is of particular interest since the significant change in current nutritional habits has been accompanied by an increased incidence of obesity and cardiovascular disease.

Furthermore, previous studies conducted by our group showed that sleep deprivation (SD) reduces Hcy levels in both young and aged rats (9,10). This unexpected effect was observed in regularly fed rats with Hcy levels within the normal range. We wondered if SD under hyperhomocysteinemic conditions would have the same effect. Therefore, our objective was to verify both the changes in total plasma Hcy concentration associated with a palatable hyperlipidic diet (HD) and the response to SD in rats.

## METHODS AND MATERIALS

### Animals

Male Wistar rats aged 21 days at the beginning of the experiment, which was conducted at the Department of Psychobiology from the Universidade Federal de São Paulo, were housed in standard polypropylene cages in a temperature-controlled (23 ± 1ºC) room with a 12:12 h light-dark cycle (lights on at 07.00 am). All procedures adopted in the present study comply with the Guide for Care and Use of Laboratory Animals.

Each animal was weighed weekly during the experiment between 0800 and 0900 h. The animals were also weighed after the end of the SD procedure.

### Hyperlipidic Diet

The experimental animals were maintained on a palatable, high-fat diet for 65 days, whereas control animals had access to regular food ad libitum. The hyperlipidic diet consisted of commercial rat chow plus peanuts, milk chocolate, and sweet biscuit in a proportion of 3:2:2:1 (11). All components were powdered and mixed, and pellets were produced. The caloric density of the diet was determined with an adiabatic calorimeter (IKA-C400). The composition of both diets is shown in Table 1.

**Table 1: Diet composition**

| | Hyperlipidic Diet (g/Kg) | Normal diet (g/Kg) |
|---|---|---|
| **Proteins** | 190 | 190 |
| **Carbohydrates** | 470 | 560 |
| **Lipids** | 160 | 35 |
| **Cellulose** | 30 | 45 |
| **Minerals and vitamins** | 50 | 50 |
| **Total:** | 21.40 kJ/g | 17.03 kJ/g |

After 65 days on their respective diets, the animals were sacrificed by decapitation. Blood was collected in pre-cooled tubes containing ethylene diamine tetra acetic acid (EDTA) or heparin as an anticoagulant, and it was centrifuged at 900 g for 6 minutes at 4ºC. Another tube free of anticoagulant was used to obtain samples for cholesterol and triacylglycerol analysis. Plasma and serum were extracted, transferred to microtubes, and stored at -80°C until biochemical assays were performed.

Another group of male Wistar rats was maintained on a high-fat diet for 65 days, whereas control animals were kept on regular feed ad libitum. After this period, a subgroup from each diet group was submitted to SD for 96 h using the platform technique. Control counterparts were allowed to sleep. During the SD procedure, all animals received a normal diet, because the feeder on the SD apparatus only permits this kind of food.

The final groups in the experiment were: normal diet (N = 8), hyperlipid diet (N = 8), sleep-deprived and normal diet (N = 8), and sleep-deprived and hyperlipidic diet (N = 8).

### Plasma Hcy

Total plasma Hcy values were determined by high-performance liquid chromatography (HPLC) with fluorimetric detection and isocratic elution (12). This methodology involves three steps: the reduction of thiol groups using tris (carboxyethyl) phosphine, protein precipitation, and derivatization with 7-fluorobenzene-2-oxy-1,3-diazolic-4-ammonium sulfate (SBD-F). The HPLC system used was a Shimadzu apparatus with an SIL-10Dvp automatic sample injector and an RF-10AXL fluorescence detector. Chromatographic separation was performed using a Prodigy Phenomenex ODS2 column (3.2 mm x 150 mm, with 5µm microparticles). The fluorescence of the separated compounds was detected with a detector adjusted for excitation at 385 nm and emission at 515 nm. Total Hcy content was calculated with a calibration curve using known Hcy concentrations and cystamine as the internal standard. The intra-assay coefficients of variation (CV) for Hcy ranged from 1.1 to 1.8%, and the inter-assay CV was 5.6% (12).

### Serum Lipids

Total cholesterol, HDL, LDL, VLDL, and triacylglycerol concentrations were assessed using colorimetric automatic procedures routinely performed in our clinical laboratory (Advia 16/50, Bayer

Diagnostics Corporation) using commercial kits (Dialab®).

### Plasma folate and vitamin B6

Folate and vitamin B6 plasma concentrations were determined by HPLC analysis according to the method described by Sharma & Dakshinamurti (13). Samples were extracted using metaphosphoric acid (10%), and folate and vitamin B6 solutions were used as standards. The chromatographic procedure was performed using the same apparatus used for Hcy, but a SPD-10VP UV-VIS detector and a Phenomenex Bondclone C18 (10 µm, 300 x 3.9 mm) were used as well.

### TBARS determination

Plasma lipid peroxidation was determined by the detection of thiobarbituric acid reactive substances (TBARS) using the methodology described by Ohkawa et al. (14). This methodology is based on the formation of a chromophoric compound after the reaction of malondyaldehyde with thiobarbituric acid spectrophotometrically measured at 535 nm.

### Sleep Deprivation

The experimental group was submitted to SD using the modified multiple platform method. This involved placing the rats inside a tiled water tank (123 x 44 x 44 cm) containing 14 circular platforms, 6.5 cm in diameter, with the water level being 1 cm below their upper surface (15). The rats could thus move around inside the tank by jumping from one platform to another. When they reached the sleep paradoxical phase, muscle atonia commenced and they fell into the water, which woke them. Throughout the study, the experimental room was maintained under a controlled temperature (23 ± 1ºC) with a 12:12 h light-dark cycle (lights on at 0700 am). Food and water were provided ad libitum; chow pellets and water bottles were placed on a grid located on the top of the tank. The water in the tank was changed daily throughout the SD period.

After the SD procedure, the animals were sacrificed by decapitation. Blood was collected for biochemical determinations as previously described.

### Statistical analysis

The results for each variable were compared using a two-way ANOVA followed by the Tukey Test for pairwise comparisons (Statistica for Windows 1997, StatSoft, Inc., Tulsa, OK, USA). The level of significance was set at $p \leq 0.05$.

## RESULTS

We found that rats maintained on a 65-day hyperlipidic diet showed higher volumes of fat deposits inside the abdominal cavity than controls regardless of SD. No significant increases in body weight were observed before the beginning of the SD period (416.8±37.8 g vs. 413.3±52.4 g for hyperlipidic diet vs. normal food, respectively). Rats from the sleep-deprived group exhibited lower body weight at the end of the SD period regardless of diet (normal food group: 414.4±29.2 g vs. 379.4±33.6 g and hyperlipidic diet group: 415.0±30.7 g vs. 378.9±27.1 g).

The analysis of total cholesterol levels revealed two main effects of group ($F(1,36)=18.26$; $p<0.0001$) and diet ($F(1,36)=26.82$; $p<0.0001$). Rats fed the hyperlipidic diet exhibited higher total cholesterol levels than those fed normal food (Table 2). Furthermore, sleep-deprived rats had higher cholesterol levels than controls. The same effects were present for LDL levels (group: $F(1,36)=46.85$; $p<0.0001$ and diet: $F(1,36)=15.46$; $p<0.001$) (Table 2).

**Table 2: Biochemical parameters.**

| | ND Control | HD Control | ND Sleep deprived | HD Sleep deprived |
|---|---|---|---|---|
| Total Cholesterol (mg/dL) | 57.9±7.4 | 67.7±7.9* | 65.0±10.2# | 86.1±11.6*# |
| LDL (mg/dL) | 15.2±6.9 | 22.2±6.3* | 36.7±6.4# | 28.0±5.5*# |
| HDL (mg/dL) | 25.3±2.7 | 27.3±4 | 27.8±5.2 | 37.7±6.5§ |
| VLDL (mg/dL) | 17.4±4.5 | 18.2±4.1 | 9.2±1.1# | 11.7±1.7# |
| Triacylglycerol (mg/dL) | 87.0±22.4 | 91,1±20.5 | 46.1±5.2# | 58.7±8.4# |
| Homocysteine (µmol/L) | 6.59±1.24 | 13.76±3.89* | 5.59±0.88 | 7.22±1.72 |
| Folate (µmol/L) | 0.0198±0.009 | 0.0180±0.01 | 0.0216±0.009 | 0.0203±0.009 |
| Vitamin B6 (mmol/L) | 2.88±1.49 | 2.87±1.56 | 3.38±2.50 | 3.81±2.07 |
| Lipid peroxidation (µmol TBARS) | 2.48±0.79 | 1.78±1.17 | 0.38±0.51# | 1.01±0.97# |

Results are shown as mean ± standard deviation. ND – normal diet; HD – hyperlipidic diet. *Different from respective normal diet group. #Different from respective sleep deprived group. §Different from all other groups.

HDL cholesterol levels in sleep-deprived rats on a hyperlipidic diet were higher than those in all other groups studied (group vs. diet interaction $F(1,36)=6.75$; $p<0.01$; Table 2). Although the hyperlipidic diet did not induce hypertriacylglicerolemia, SD reduced both triacylglycerol and VLDL levels in both diet groups ($F(1,36)=52.73$; $p<0.0001$) (Table 2).

Sleep deprivation reduces TBARS levels independent of diet (group vs. diet interaction: $F(1,30)=4.51$; $p<0.05$; Table 2). Homocysteine concentrations were significantly higher in rats on hyperlipidic than normal diets (Mean 13.75 µmol/L vs. 6.59 µmol/L; group vs. diet interaction: $F(1,36)=15.03$; $p<0.001$;

Table 2). Curiously, 96 hours of SD significantly reduced Hcy levels and brought those of hyperhomocysteinemic rats to normal concentrations (13.75 µmol/L to 7.21 µmol/L).

No differences in folate or vitamin B6 were observed between groups ($p = 0.86$ and $p = 0.24$, respectively).

## DISCUSSION

Consumption of a palatable hyperlipidic diet by rats for 65 days produced hyperhomocysteinemia. This finding is of great interest since Western people are consuming increasing amounts of lipids and carbohydrates as part of their regular diet and the incidence of cardiovascular problems is rising every year. Higher Hcy levels induced by hyperlipidic diets have been previously described in humans who showed a positive correlation between Hcy and the consumption of fat and calories (16). This is, however, the first description of hyperhomocysteinemia in rats as a consequence of this kind of diet.

Mild hyperhomocysteinemia in rats usually results from methionine-rich or folate-deficient diets (17) that affect Hcy metabolism by up-regulating the conversion of Hcy back to methionine or cysteine. It is difficult to explain how a hyperlipidic diet leads to higher Hcy concentrations, since neither folate levels (the peanuts added to the diet provided an extra 145 µg of folic acid per 100 g of peanuts) nor methionine content changed with this kind of diet. In fact, folate concentrations in hyperlipidic-diet rats were very similar to those of normally fed animals; the same was true of vitamin B6 levels (Table 2). Taken together, these results suggest that hyperhomocysteinemia was not the result of mechanisms related to the impairment of remethylation or transulfuration pathways due to changes in the availability of vitamins.

High fat diets impair glucose metabolism, stimulate abnormal glucose production, and lead to hyperinsulinemia and insulin resistance (18). Cholesterol levels are also affected by high fat diets (11,19). Hyperhomocysteinemia has been associated with abnormal glucose and cholesterol metabolism in both humans (20-22) and animals (23,24). Although there are no apparent causal relationships between these findings, the hyperlipidic diet rats in our study that presented hyperhomocysteinemia also showed high levels of total cholesterol and LDL.

It is interesting that data from the Hordaland Homocysteine Study suggest that high saturated fat intake in humans is associated with higher total Hcy concentrations (25). In that cross-sectional, population-based study of 5917 subjects in two age groups (47-19 and 71-74 years old), an approximately 15 gram increase in saturated fat intake was associated with a 6% increase in Hcy concentrations after adjustment for age, sex, energy intake, and other factors. In that study, this association is of the same magnitude as that predicted between saturated fat intake and LDL cholesterol (26).

Homocysteine concentrations in sleep-deprived rats maintained on normal food were lower but not statistically significantly different from those of control rats. However, previous work conducted by our group showed lower concentrations of Hcy levels after SD (10,27). In fact, the higher values presented by hyperlipidic-diet rats interfered with the distribution of the values obtained for the other groups and thereby precluded statistical significance. In light of this, no statistically significant difference between hyperlipidic- and control-diet rats could be observed. Interestingly, however, SD reduced Hcy levels to normal values in rats with high Hcy concentrations.

Notably, some parameters related to lipid metabolism either did not change after SD or changed in the opposite direction. For example, SD produced an increase in both total and LDL cholesterol. HDL cholesterol levels did not change after SD in animals that received the normal diet, but they were higher in HD sleep-deprived animals.

Since SD produces important metabolic alterations related to increased risk for cardiovascular disease (28-31), it would be interesting to establish the mechanisms that decrease Hcy concentrations in rats. It is important to emphasize that this decrease brought Hcy concentrations to normal values despite the fact that animals were maintained on a hyperlipidic diet. Reduction in Hcy concentrations is considered a valuable tool for decreasing cardiovascular risk in humans (32-35). Because of this, the decrease in Hcy concentrations associated with SD in rats warrants further investigation.

SD in humans has been associated with altered Hcy concentrations (36,37). In these studies, however, changes occurred in opposite directions. Sleep-deprived shift workers had higher Hcy concentrations than regular-schedule controls (18 vs 8 mol/L). Patients suffering from sleep apnea also presented high Hcy concentrations (37-41). These patients were submitted to several episodes of hypoxia/re-oxygenation, which may explain their Hcy augmentation (37). For the shift workers, changes in feeding patterns may be related to both hyperhomocysteinemia and changes in other cardiovascular risk factors. All parameters analyzed showed variations associated with increased cardiovascular risk (36).

Sleep deprivation reduced the levels of TBARS in both groups. This finding was already observed by our group (27), and it is probably related to difficulty in reaching the necessary amount of food during the SD period (42). Some authors have shown that caloric restriction reduces oxidative stress; in this sense, the hyperlipidic diet did not prevent SD from lowering levels of TBARS, besides being less effective.

The present study shows that some CV risk factors in rats were reduced after SD even when rats were fed a hyperlipidic diet. The reason for this reduction remains to be elucidated. We believe that the higher energy intake induced by SD may produce a metabolic status characterized by hypermethylation, which in turn, may decrease Hcy availability.

The regulation of the genetic expression is tightly controlled and well balanced in the body by different epigenetic mechanisms, such as DNA methylation and histone modification. DNA methylation occurring after embryogenesis is seen primarily as an irreversible event, and even small changes in genomic DNA methylation may have biological relevance. Several factors influencing DNA methylation, including Hcy, have already been identified.

Sleep deprivation is considered to produce a hypermetabolic status. Cirelli and co-workers analyzed the expression of more than 26,000 transcripts in the cerebral cortex after SD in rats (43) and found an increase in the expression of 75 genes. Among these were genes related to metabolism, the immune response, and stress. Since

Hcy is a product of the methylation pathway, changes in its levels may be considered a biological marker of gene expression.

This may, in turn, suggest molecular changes induced by SD.

In conclusion, rats fed a hyperlipidic diet showed increased plasma Hcy concentrations that were decreased after sleep deprivation. Continued research on the role of methylation reactions during SD will lead to greater understanding of the possible mechanisms responsible for these findings.

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Sleep science

# TYPE OF DENTAL OCCLUSION IN CHILDREN AND ADOLESCENTS PRESENTING SLEEP DISORDERS

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## ABSTRACT

**Objective:** To carry out a retrospective evaluation on the characteristics of dental occlusion in children and adolescents who were referred for polysomnography due to complaints about snoring and sleep disorders.

**Subjects and Methods:** In the present descriptive study, out of the 101 patients who were referred for polysomnographic evaluation (PSG), 60 patients (45 male and 15 female) were consecutively evaluated. The instruments used in the evaluation were clinical data, medical records, and PSG.

**Results:** The sample average age was 9.2±4.7 years, and the BMI was 21.0 ± 7.7 kg/m2. According to the PSG, the population evaluated showed the following sleep abnormalities: OSAS was detected in 65% (n=39) of the subjects, snoring in 18.33% (n=11), and contractions of the mentonian region muscles in 26.66% (n=16). According to the clinical evaluation, 49 of the patients showed oral breathing; Class I occurred in 21.66% (n=13) of the patients, Class II in 68.33% (n=41), and Class III in 10% (n=6). A significant correlation was observed between OSAS, and Class II (p=0.05) and between OSAS and Class III (p=0.009).

**Conclusion:** According to the methodology applied, it was found that, in children and adolescents evaluated in the present study, a significant correlation between OSAS and dental occlusion type Class II could be observed. Considering the limited number of subjects included in the sample, prospective studies with larger samples should be conducted.

**Keywords:** Oral breathing; Malocclusion; Sleep disorders; OSAS; Disordered breath during sleep.

## INTRODUCTION

Among the sleep disorders, the obstructive sleep apnea syndrome (OSAS) should be highlightened. This is a complex multifactorial condition generated by a combination of anatomical and physiological factors and also considered a risk factor for cardiovascular diseases (1,2). Among such factors, those related to the face skeletal morphology should be pointed out (1). This is proven by the good performance of orthodontic appliances used by children showing sleep disorders (3,4) particularly nonobese children (5). Such appliances also normalize cardiovascular alterations related to the neurovegetative system (6) except in the case of sleep bruxism, whose etiology is different and not yet well known (7).

OSAS is considered a public health problem, and it is currently underestimated, particularly in children having orthodontic problems or craniofacial abnormalities (8).

Children with nasopharyngeal obstruction usually develop a certain level of alteration in the facial morphology and in the mandibular growth, depending on the severity of the problem and the time of the disease onset (9,10). Oral respirators tend to narrow the pharynx due to a poor craniofacial development and may cause OSAS (11,12). Snoring is another abnormality frequently shown by these patients (10,13).

Cephalometry performed in children carrying OSAS shows different craniofacial characteristics, such as maxilomandibular micrognatia and/or retrognatia, commitment of facial vertical development, and reduction in the posterior airway space (14). Studies conducted with patients in orthodontic clinics show that OSAS diagnosis seems to be related to cross bite, anterior open bite, and absence of labial sealing, although no predictive pattern exists (15,16). On the other hand, the sheer evaluation of the facial profile cannot always predict respiratory abnormalities related to sleep (17).

Enlargement of the palatine and pharyngeal tonsils is also a problem frequently found in othorhinolaryngology clinics and is considered the main cause of OSAS in children (18). Incidence of structural alterations in the nasal cavity, such as nasal septal deviation and hypertrophy of the inferior nasal cornets, is quite common among such patients and should be approached concurrently.

Despite the relevance of the problem, so far only a few studies on the orthodontic characteristics of patients who complain about sleep disorders have been conducted in our community. Thus, the present retrospective and descriptive study aimed at evaluating dental occlusion in children and adolescents who were submitted to polysomnography (PSG) due to their complaints of sleep disorders.

## CASUISTICS AND METHOD

A retrospective analysis of the registers concerning children and adolescents who were submitted to PSG was conducted at Sleep Institute linked to the Universidade Federal de São Paulo in order to assess respiratory disorders (in view of complaints about snoring and sleep disorders) in the period from January to December 2003.

Exclusion criteria: children and adolescents carrying craniofacial malformations or neurological disorders or those who were regularly submitted to PSG for treatment control.

The assessment included the following:

a) Analysis of medical records on clinic of origin and prior clinical diagnoses;

b) General data: age, sex, weight, height, and body mass index (BMI);

c) Type of dental occlusion, which was based on molar and canine relationships (Angle's classification), and

d) PSG, which was performed with the use of a device adequate for collecting sleep variables; such variables were then recorded through a computerized sleep amplifier and preamplifier systems (Sonolab Meditron, São Paulo-BR) with a sampling frequency of 256 Hz/s per channel.

### Data analysis

Data are shown as average and standard deviation. Clinic parameters (BMI, Angle's classification, and oral breathing) were correlated with data obtained from the PSG (apnea, snoring, and mentonian region muscles) by applying Chi-square tests (Fisher's exact test for short frequencies). OSAS and snoring were considered separately and evaluated based on the criteria previously described (AASM, 2005). Electromyography of the mentonian region (EMG) was utilized to evaluate the contractions of orofacial muscles.

## RESULTS

Out of the 101 children and adolescents who were submitted to PSG in the period of study, 60 (45 male and 15 female) complied with the inclusion criteria. The average age was 9.2±4.7 years, and the average BMI was 21.0±7.7 kg/m2.

Based on PSG results, the patients showed the following sleep abnormalities: OSAS in 65% (n=39), snoring in 18.33% (n=11), and increase of EMG of the mentonian muscles in 26.66% (n=16).

The dental evaluation showed that 49 patients showed oral breathing; within that type, Class I occurred in 21.66% of the patients (n=13), Class II in 68.33% (n=41), and Class III in 10% (n=6).

Despite the small number of patients evaluated, statistically significant correlations could be found between OSAS and Class II and between OSAS and Class III. Data related to frequency and correlations are shown in Table 1.

**Table 1 – Table of frequencies and correlations of clinical and polysomnographic data concerning children and adolescents who complain about sleep disorders.**

| | OSAS n = 39 | Snoring n = 11 | Increase of Mentonian EMG n = 16 | Oral breathing n = 49 |
|---|---|---|---|---|
| Class I n = 13 | (n=08) p= 0.77(X2) | (n=02) p=0.55 | (n=03) p=0.52 | (n=10) p=0.62 |
| Class II n = 41 | (n=30) p=0.05 | (n=07) p=0.71 | (n=11) p=0.97 | (n=33) p=0.73 |
| Class III n = 06 | (n=01) p=0.02 | (n=02) p=0.30 | (n=02) p=0.51 | (n=06) p=0.22 |
| Oral breathing n = 49 | (n=30) p=0.20 | (n=11) p=0.08 | (n=12) p=0.89 | ——— |
| Increased of Mentonian EMG | (n=10) p=0.80 | (n=01) p=0.14 | ——— | ——— |

**Chi-Square Test (Fisher's Exact Test when necessary).**

## DISCUSSION

The significant correlation existing between complaints about obstructive sleep apnea syndrome and dental occlusion Class II (p=0.05) corroborates the importance the scientific literature gives to orthodontic problems. The dental arch morphologic characteristics are closely related to clinic disorders (e.g., oral breathing coupled with sleep-related breathing disorders (8). The careful evaluation of dental occlusion or craniofacial problems helps OSAS prognosis (5) in addition to correcting or preventing other dental problems. Today, the effectiveness of oral appliances to control OSAS cannot be denied (4), particularly when the severity of the problem and the presence of associated morbidities, such as obesity, are taken into consideration (14). In addition, there is the fact that patients having oral breathing concomitantly show an anterior open bite and/or mandibular retrognathism and are thus more susceptible to OSAS (13).

It is known that orofacial abnormalities lead to pharyngeal obstruction and may be detected by cephalometry (15). It is also known that differences between cephalometric data referring to patients with OSAS and the healthy population have been detected (16). The cephalometric analysis is considered an important method for evaluating craniofacial characteristics, and it also provides data on posterior airway space, tongue length, and position of the hyoid bone, whose condition may contribute to OSAS (15). Nevertheless, these examinations were not the objective of the present study; its main purpose was to conduct a clinical evaluation of the dental arches, which is a relatively simple procedure for an initial assessment. Studies of this nature are absolutely necessary to alert practitioners, physicians and dentists, as well as public health authorities, on the relevance of the problem.

Since this is a retrospective study and no independent electrodes were used to evaluate the masseter muscles, sleep bruxism rates have not been evaluated (BS) (19). Data on the mentonian region are not adequate to evaluate BS. However, it is interesting to observe that BS is another movement abnormality also common in childhood (20) and may be related to OSAS (21). Occlusion factors have been the object of many studies on bruxism etiology, but today it is known that the origin of sleep bruxism is predominantly central (22). In the present study, we found that some family members had complaints about such a disorder, but data with respect to this issue have not been evaluated. The mentonian region EMG is usually utilized to evaluate muscular contractions and is not adequate to evaluate BS. However, in the present study, it did not show any statistical association with the type of dental occlusion ($p=0.52$, 0.97, and 0.51 for Classes I, II and III, respectively).

The present study, though having limitations due to the fact that it is a descriptive study and was conducted with a reduced number of subjects, is quite relevant because it is one of the first to be developed in our community that tries to quantify the clinical relationship that exists between dental occlusion and sleep disorders in children and adolescents. Prospective studies should be developed with a larger number of individuals in order to define the correlations that exist among different types of sleep disorders, oral breathing and type of dental occlusion.

## CONCLUSION

As per the methodology applied in the present study, it was observed that non-treated children and adolescents framed into Class II profile show greater predisposition to OSAS when compared to those framed into Classes I and III profiles. However, it is important to take into consideration the reduced number of individuals who participated in this sample, and studies with larger samples should be conducted for confirmation of the results obtained in the present study.

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Sleep science

# THE ADOLESCENCE SLEEP PHASE DELAY: CAUSES, CONSEQUENCES AND POSSIBLE INTERVENTIONS

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## ABSTRACT

Adolescence is marked by, among other things, an increase in daily sleepiness as a consequence of insufficient sleep, more intense during school days. Partial sleep deprivation results from the tendency of adolescents to delay their sleeping and waking times. This behavior is known as the phase delay, and is in conflict with the students' morning school schedules. Excessive sleepiness during the day impairs their concentration and their capacity to learn. The purpose of this article was to discuss the role of sleep in consolidating learning. In addition, it also shows that the phase delay, in contrast to what was believed some decades ago, is caused not just by changes in the adolescent's habits but also by physiological changes that occur during puberal development. Lastly, this article presents strategies for intervention that aim to reduce excessive daily sleepiness. Such interventions involve increasing awareness in the school community of the importance of sleep habits, introducing educational programs on sleep in elementary school, and discussing the adequacy of school schedules.

**Keywords:** Adolescents; Phase delay; Excessive daily sleepiness; School performance.

## EXCESSIVE DAILY SLEEPINESS: THE TIP OF THE "ICEBERG"

A typical adolescent school life involves struggling to wake up early, suffering a reduced concentration level due to excessive sleepiness, and napping during classes, particularly in the morning period. In the last two decades, groups of researchers in several countries, including Brazil (1-3), have dedicated their attention to this problem, and the results of their research have provided a foundation for understanding this phenomenon, its consequences, and the possible interventions that could minimize its undesirable effects.

Usually, adolescents experience excessive daily sleepiness because they get an insufficient amount of sleep. An adolescent requires an average amount of daily sleep in the range of 8.5 to 9.25 hours. Studies of adolescents in several countries have shown that the student's average sleep period is reduced during school days to around 7 hours (4) due to a tendency of students to delay

bedtime while maintaining the same waking time to adhere to school schedules. A partial sleep deprivation that averages 1-2 hours per day during school days is enough to trigger symptoms of sleepiness, leading to a reduced school performance (5,6). On weekends, the sleep duration period is extended as a result of the so-called rebound effect from the sleep deprivation they are subjected to during the week.

It can be thus observed that the adolescent's sleep-wake cycle shows an irregular pattern that is characterized by a reduction in the sleep period during school days and an extension in the sleep period during weekends (7). The irregularity that results from partial sleep deprivation is associated with a reduction in concentration levels and alterations in mood (8).

The importance of cultivating healthy sleep habits in early childhood has long been acknowledged. In his book on thoughts about education, John Locke (9) argues that nothing contributes more to the child's development and health than sleep. The author emphasizes the importance of establishing a routine from an early age so that the child cultivates good sleep habits. Nevertheless, a careful analysis of results of studies conducted in the last 20 years shows that, in contrast to what has been thought, the problem does not derive exclusively from the lack of limits and discipline that should be imposed on adolescents. The difficulty in promoting earlier sleeping and waking times results from changes that take place in the adolescent's body during puberty. Through mechanisms not yet well understood, these changes in the adolescent physiology trigger the so-called phase delay: the tendency of the body to delay its biological rhythms, including sleeping and waking times.

It is already known that excessive sleepiness impairs concentration and learning capacity. It has been shown recently that there is a relationship between sleep and memory (10,11). Several sleep phases participate in the learning consolidation process and not just the paradoxical sleep as was previously believed (12).

Sleep deprivation alters the functioning of several brain regions, including the prefrontal cortex. This brain region is closely associated with the working memory and with other more complex cognitive functions, such as judgement and the decision making process (13).

Thus, changes in the sleep-wake cycle jeopardize learning in two ways: they reduce concentration and the capacity for quick learning, and they impair consolidation of what has been learned.

Sleep deprivation can also promote alterations in mood, exacerbate symptoms of psychiatric disorders such as depression (14), and cause metabolic changes, which increase appetite and, consequently, body mass (15). Irregular sleep patterns and/or insufficient sleep are associated with an increase in the number of medical appointments among children and adolescents (16).

Despite the need to overcome the biological/social dichotomy in studies that analyze behavior, this dichotomy is frequently present in works that seek an understanding of the origins of the phase delay.

For many years, the phase delay was attributed exclusively to social factors (16): greater independence to organize their own schedules, access to technology (TV, video games, computers, and the Internet), and a greater demand for social outings and events.

In the early 1990s, it was demonstrated that pubertal stages of development were associated with a delay in the expression of biological rhythms. According to the classification proposed by Tanner (17), more mature adolescents have more delayed sleeping times than less mature adolescents, regardless of the age factor (1,18).

The identification of a biological factor for determining the phase delay demanded a reevaluation of the issue. An article has been recently published that developed the idea that this tendency to delay sleep time is part of the human species maturational calendar. The magnitude of the delay increases as puberty progresses until a reversion around the twenties, regardless of the changes in habits of young adults that may appear upon entering university or the job market. The authors of this article suggest that this reversion marks the end of adolescence (19).

In order to identify the possible social factors involved in the expression of the biological rhythmicity during adolescence, studies with rural populations, some of which did not have electric power at home, have been conducted (20,21). One of these studies compared the sleep patterns of adolescents living in the same community and attending the same school. Those who had electric power at home went to bed later than those who did not have electric power at home. These results complement recent studies conducted in native communities (22) that reinforce the major influence exerted by social factors upon the phase delay. The magnitude of this delay in urban populations, particularly those that have access to technological resources, is greater and, consequently, sleep deprivation during school days is also greater.

We can thus conclude that changes associated with pubertal maturation render the individual more susceptible to delays in sleep time. It is as if the same stimuli have a different effect depending on the function of the puberal stage. In more advanced puberal stages, the adolescent's body is more sensitive to stimuli capable of promoting delays in the biological rhythms.

## PHASE DELAY PHYSIOLOGICAL BASES

"Do teenagers sleep late because they go to the disco or do they go to the disco because they cannot sleep until late?" (19).

Subjacent to the question asked by Roenneberg and collaborators lays a discussion on the factors involved in the adolescence phase delay. In attempt to answer this question, it is necessary to discuss the mechanisms behind the control of circadian rhythmicity in humans.

Like with other organic behaviors and functions, sleeping and waking times are controlled by the Circadian Temporization System (CTS). This system consists of a set of structures comprising the suprachiasmatic nuclei, which are small neuron clusters localized in the hypothalamus. Today, these nuclei are recognized as elements that make up the mammalian CTS. This temporization system, commonly known as the "biological clock", generates a regular circadian rhythm even when the body is isolated from environmental cycles and under normal conditions accomplishes the synchronization between endogenous rhythms and environmental cues (23). When adjusting to the day/night cycle, the result of such synchronization is the presence of behavioral and endocrine rhythms and also of multiple variables that have a

24-hour duration. These circadian rhythms allow us to adjust our behaviors to social schedules.

A clear circadian rhythm is the secretion of the melatonin hormone by the pineal gland. This gland, which was seen by Descartes as the part of the human body associated with the soul (24), is an important time signal for our body. By varying melatonin levels, low during the day and high at night, the pineal gland communicates to the body the time of day. Such signaling creates in our body the so-called biological night that is characterized by high melatonin levels, a drop in body temperature and, in humans, the occurrence of a sleep episode. Light stimuli, even of low intensity (25), can drastically reduce melatonin secretion, changing the biological night.

In the last two decades, several studies have shown the importance of the day/night cycle for the adjustment of our biological rhythms (26). It was once believed that the social schedules of humans would seriously affect this adjustment. Today, however, it is known that a large part of the effects that social schedules have is exerted through changes in light intensity. In other words, when we set the clock to ring one hour earlier, we are advancing the light stimuli sent to the CTS by one hour. In the same way, when we decide to participate in a night event, we are increasing the light stimuli during a period when normally our eyes would be closed. By using electric power to produce artificial light, contemporary society has shortened the environmental night. As a result, the biological night and the night sleep have been shortened.

Individual differences in sleep-wake cycles can be noticed even in the early months of life. These differences are reflected in their preferred sleeping and waking up times. People who prefer waking up early and going to sleep early are called "morning-types." Those who prefer going to sleep late and waking up late are called "evening-types." This observation indicates that people establish different temporal relations between their own rhythms and the environmental cycles (27). This characteristic constitutes what is called a chronotype. For example, some people wake up one hour after daybreak. Others, if possible, prefer waking up four or five hours after daybreak. Without intending to resume the nature/culture dichotomy, it is necessary to point out that there are studies that show that there is an influence of some pairs of genes on the determination of chronotype: we are born with a trend to be morning people or evening people. This result does not minimize the critical influence of social interactions; on the contrary, it helps us understand them. The adolescence phase delay is associated with this trend: morning children become less emphatic morning adolescents; evening children become still more emphatic evening adolescents and thus face greater difficulties in adapting to school schedules.

With all the knowledge that is currently available, it is a likely possibility that that the phase delay originates from changes in the temporization system that are triggered by hormone modifications associated with puberty. The endogenously generated circadian rhythms do not have a precise 24-hour period; in humans, they usually extend for more than 24 hours (28). The expression of a circadian rhythm that is adjusted to the 24-hour environmental cycles (day/night, social interaction) depends on the body's interaction with these cycles. Recent studies have shown that evening-type individuals have a longer endogenous period compared to morning-type individuals (28). We could say that individuals with an endogenous period shorter than 24 hours tend to be morning people, whereas individuals with an endogenous period longer than 24 hours tend to be evening people. One hypothesis that might explain the origin of the phase delay is that a change in the endogenous period occurs during puberty. Hormonal changes would modify the CTS functioning speed, which would suffer a reduction, thus resulting in a longer endogenous period.

Another approach to investigating changes in the phase delay is based on a widely accepted model that suggests that the tendency to feel sleepy results from the interaction between the homeostatic (S) and circadian (C) processes (29). The homeostatic process (S) is associated with the number of wakefulness hours: the longer we remain awake, the greater the tendency to feel sleepy. Accordingly, the sleep trend starts low at the beginning of the day and increases as the day progresses, reaching a maximum after 14 or 16 waking hours, after which we usually fall asleep. The homeostatic process does not on its own explain the complexity of our sleep habits. For example, often we are more attentive at the end of the afternoon than soon after waking up; this fact contradicts the tendency to accumulate sleepiness throughout the day and points to the existence of another mechanism that acts in concert, called the circadian process (C). The circadian process dictates that the tendency to sleep increases throughout the night, regardless of the number of waking hours. The relationship between the S and C processes makes us feel a strong tendency to sleep after sunset, as a result from the action of both processes; the outcome is an extended sleep episode during the night. After a few hours of sleep, when the tendency to sleep generated by process S is reduced, we remain asleep due to the action of process C, which is in full operation at daybreak.

The results of studies conducted in recent years suggest that the puberal maturing modifies process S (30). During this phase of life, a larger number of waking hours are necessary to trigger the feeling of sleepiness, explaining the fact that adolescents have an increased ability to remain awake for extended periods of time.

The two processes mentioned above are subject to continuous modulation performed by several environmental stimuli. A boring, non-stimulating class can increase sleepiness levels, whereas a highly motivating activity can mask high sleepiness levels. As a result, many educators interpret signs of sleepiness as a reflex of the activities developed at school and disregard the physiological mechanisms underlying attention control.

A third way of explaining the physiological mechanisms behind phase delay is a change in the CTS sensitivity to light (31). The adjustment between endogenous rhythms and environmental cycles results from advances and delays in the circadian rhythms. Light stimuli between sunset and the end of the night delay circadian rhythms, whereas light stimuli in the early morning hours advance these rhythms. One possibility is that the adolescent's body is susceptible to delays due to an increased sensitivity of the temporization system to light at the beginning of the night or a reduced sensitivity in the early morning. Since the physiological mechanisms behind the delaying and advancing processes are the same, the possibility that the change occurs in only one of the components should be acknowledged.

Nightly social activities favor exposure to light at moments

when the STC responds by promoting a delay in the biological rhythms and, consequently, delaying waking time. This delay reduces the exposure to light in the early morning, which contributes to the advance of biological rhythms. As a result, the time of exposure to light becomes unbalanced at moments when the STC is delayed or advanced.

During vacation, when there is no obligation to comply with school schedules, the adolescent's eyes remain closed during most of the time when the CTS is susceptible to advances. Waking up after midday and, in some situations, at sunset, the adolescent remains awake and exposed to artificial light only at moments when the CTS is delayed. Such a behavior that is adopted for several weeks causes a significant delay. Often, after classes resume, a 12-hour inversion in sleeping and waking times is necessary: instead of waking up at 06:00pm, the adolescent will wake up at 06:00am. Adaptation does not take place immediately, and brings about the consequences already described.

## THE SLEEP PHASE DELAY AT SCHOOL: POSSIBLE INTERVENTIONS

Based on the information provided above, schools need to incorporate into their pedagogical proposal measures that would reduce the impact of phase delay on the student's performance.

The first step would be to reconsider the school's temporal organization, in particular its class schedules, and systematize the results of possible interventions that aim to reduce the student's daily sleepiness and thereby improve performance. This systematization would include, in addition to an educational evaluation, a tracking of the sleep-wake cycle patterns and of some correlated sleep behaviors before and after the implementation of the change. Today, we have available questionnaires and tests to evaluate these interventions in a simple and inexpensive way.

The changes we propose are apparently simple modifications, such as delaying the beginning of morning classes. Nevertheless, changing class schedules involves the participation of the whole community – parents, teachers, and transport service providers – and should be discussed and planned before implementation. This change has been implemented in some North-American schools (32) and also in some Israeli institutions.

Another intervention relates to the teaching of sciences. It is a consensus among educators that access to information is crucial for the students to develop healthier habits, in particular in scientific areas, such as those related to sex, psychotropic drugs, and nutrition. The same access should be delivered for sleep habits. By knowing the aspects of the sleep-wake cycle physiology and the temporization system, the students will be able to develop healthier sleep habits. In some countries, this concern has started to yield results in elementary and high school institutions. In the US, in early 2004, the National Center on Sleep Disorders Research (NCSDR) and the NIH Scientific Education Sector launched a program of activities on Sleep and Biological Rhythms for high school students. This program complies with the country's educational guidelines and includes printed material for teachers, interactive activities and computer simulations. In Brazil, the texts of the Parâmetros Curriculares Nacionais (PCNs) (National Curricular Parameters) for elementary school include proposals on the theme in the module Human Body and Health. Science educational books have gradually incorporated this subject into their units.

A third intervention is related to the characteristics of the temporization system. Light stimuli after sunset contribute to delaying the circadian rhythms. Light stimuli after daybreak – even one or two hours before daybreak – advance circadian rhythms. Increasing the exposure to light in the early morning hours may help students to forward their rhythms. When the students resume classes after vacation, the first morning classes should be attended in open spaces or in well-lighted areas to accelerate the adjustment process and thus promote a higher alertness level and a lower sleepiness level during the day. Only a few studies have evaluated the effect of such an intervention, however (33).

We understand that the discussion about school schedules should, at a given moment, go through the considerations approached in the present article, whether under a more academic viewpoint, that is, incorporating contemporary knowledge, or under a practical viewpoint, that is, improving the conditions of the learning process in the school environment.

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