

REVIEW ARTICLE

Disrupted chronobiology of sleep and cytoprotection in obesity: possible therapeutic value of melatonin

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Reprinted from *Neuroendocrinol Lett* 2011; **32**(5): 588–606.

Key words: sleep; metabolic syndrome; diabetes; obesity; neuroimmunology; melatonin; cytokines; circadian rhythms; inflammation; free radicals

Act Nerv Super Rediviva 2011; **53**(4): 159–176 ANSR530411R01

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Abstract

From a physiological perspective the sleep-wake cycle can be envisioned as a sequence of three physiological states (wakefulness, non-rapid eye movement, NREM, sleep and REM sleep) which are defined by a particular neuroendocrine-immune profile regulating the metabolic balance, body weight and inflammatory responses. Sleep deprivation and circadian disruption in contemporary “24/7 Society” lead to the predominance of pro-orexic and proinflammatory mechanisms that contribute to a pandemic metabolic syndrome (MS) including obesity, diabetes and atherosclerotic disease. Thus, a successful management of MS may require a drug that besides antagonizing the trigger factors of MS could also correct a disturbed sleep-wake rhythm. This review deals with the analysis of the therapeutic validity of melatonin in MS. Melatonin is an effective chronobiotic agent changing the phase and amplitude of the sleep/wake rhythm and having cytoprotective and immunomodulatory properties useful to prevent a number of MS sequels. Several studies support that melatonin can prevent hyperadiposity in animal models of obesity. Melatonin at a low dose (2–5 mg/day) has been used for improving sleep in patients with insomnia and circadian rhythm sleep disorders. More recently, attention has been focused on the development of potent melatonin analogs with prolonged effects (ramelteon, agomelatine, tasimelteon, TK 301). In clinical trials these analogs were employed in doses considerably higher than those usually employed for melatonin. In view that the relative potencies of the analogs are higher than that of the natural compound, clinical trials employing melatonin doses in the range of 50–100 mg/day are needed to assess its therapeutic value in MS.

INTRODUCTION

The association between sleep and the immune system was first identified in the 1970's, when a sleep-inducing factor was isolated and chemically characterized from human urine as a muramyl peptide derived from bacterial peptoglycan (or “Factor S”) (Krueger *et al* 1984). Subsequently muramyl dipeptide and Factor

S-related peptidoglycans were all shown to induce the release of a key immunoregulatory cytokine, i.e. interleukin (IL)-1, a potent somnogen and central player in the physiological regulation of sleep. IL-1 levels in the brain correlate with sleep proneness (Krueger 2008) (*Figure 1*).

Sleep and the immune system share cytokines as regulatory molecules. These molecules are involved

in both physiological sleep and in the disturbed sleep observed during acute or chronic inflammation. It is feasible that sleep influences the immune system through the action of centrally produced cytokines that are regulated during sleep (see for ref. Pandi-Perumal *et al* 2007).

Obesity is one of the situations associated with a chronic inflammation of the white adipose tissue (Bremer *et al* 2011; Ye & Gimble 2011; Lam *et al* 2011) that shows a high prevalence of sleep disturbances (Bass & Takahashi 2010; Huang *et al* 2011; Hart *et al* 2011; Horne 2011; Huneault *et al* 2011). Inflammation in obesity leads to insulin resistance and impaired glucose tolerance. The adipose tissue is in obesity characterized by an increased production and secretion of inflammatory cytokines like tumor necrosis factor (TNF)- α and IL-6, which have local and systemic effects. Obesity is also a state of chronic oxidative stress, a major mechanism underlying the development of co-morbidities like atherosclerotic disease (Vincent *et al* 2007; Bremer *et al* 2011; Ye & Gimble 2011; Lam *et al* 2011).

Obesity and atherosclerotic disease are components of the metabolic syndrome (MS) that affects 10–25% of the adult population worldwide. There is a general consensus that the cause for such pandemic MS is the surplus of food whereas evolution has rather shaped humans for periods of food scarcity (Stoger 2008; Horne 2011; Huneault *et al* 2011). In addition, a true “environmental mutation” ensued after the availability of artificial illumination and its chronodisruptive properties is affecting homeostasis (Reiter *et al* 2011), including reactive homeostasis (i.e., the mechanisms triggered to maintain a physiological variable within a normal range in face of a perturbation) and predictive homeostasis (the mechanisms that are active in advance to maintain a set point that itself is rhythmic). Predictive homeostasis has evolved as an adaptation to anticipate predictable changes in the environment, such as the light/dark (LD) cycle, food availability, temperature or predator activity, and is the basis of the circadian clock. We have “learned” to adapt to the ever LD cycle such that the body anticipates the coming sleep and activity period. Therefore, life style changes in modern society, such as nocturnality and overly rich diets, have emerged as chronodisruptive events that definitively help to increase the incidence of MS (Reiter *et al* 2011). That we are living in a sleep-deprived society is indicated by the 25% reduction in sleep time that took place during the last 40 years. Presently, around 30% of adults report sleeping less than 6 hours per night (Blanco *et al* 2004; National Center for Health Statistics., 2005).

The objective of this review is to summarize some of the mechanisms implicated in the mutual interaction between sleep and the immune system with a focus in obesity as a state of chronic inflammation. Since there is considerable evidence that circadian misalignment is associated with increased risk for obesity, diabetes and cardiovascular disease (Buijs *et al* 2006; Scheer *et al* 2009; Corbalan-Tutau *et al* 2011), the successful management of MS ideally

requires a drug that besides antagonizing the trigger factors of MS could also correct the disturbed sleep-wake rhythm. Melatonin is an effective chronobiotic able to change the phase and amplitude of the sleep/wake rhythm, and having cytoprotective and immunomodulatory properties useful to prevent a number of MS sequels. Its possible therapeutic value in MS is discussed below.

SLEEP IS A COMPLEX PHENOMENON COMPRISING TWO SUB-STATES

Sleep is an essential process in life. It is a behavioral state defined by: (i) characteristic relaxation of posture; (ii) raised sensory thresholds; (iii) distinctive electroencephalographic (EEG) pattern; and (iv) ready reversibility. One difficulty in understanding sleep is that it is not a unitary state but composed of two sub-states. Based on polysomnographic measures, sleep has been divided into categories of rapid eye movement (REM) sleep and non-REM (NREM) sleep (also called slow wave sleep) (Rechtschaffen & Kales 1968). NREM sleep comprises four stages (stage 1 to stage 4), with stages 3 and 4 being characterized by slow, high amplitude EEG waves in the frequency range below 4-Hz (delta rhythm). Standards for the analysis of sleep were revised in 2007 by the American Academy of Sleep Medicine (Iber *et al* 2007) by renaming NREM as N1, N2 and N3, the latter representing stages 3–4 of former categories.

Sleep alternates between NREM and REM stages approximately every 90–120 min (Beersma 1998) (**Figure 2**). Periods of NREM sleep constitute about 80% of the total sleep time and NREM reaches its greatest depth during the first half of the night. After completion of N3, the next stage does not begin immediately. Instead, the first three stages reverse quickly and are then immediately followed by a period of REM sleep (R, **Figure 2**) (Iber *et al* 2007). REM sleep is defined by a faster EEG activity, rapid horizontal eye movements on electrooculography, vital sign instability and the occurrence of skeletal muscle hypotonia and dysautonomia. The tone in most voluntary muscles is minimal but the diaphragm and the eye muscles are phasically active, giving REM sleep some resemblance to the wake state, for which reason it is sometimes referred to as “paradoxical sleep”.

The length of the NREM and REM stages is not static. The first REM sleep will occur roughly 90 min after falling asleep and will last only 10 min. As night proceeds the length of N3 (stages 3 and 4) of NREM (delta or deep sleep) begins to wane and the length of REM sleep increases up to about 0.5 h in length after a number of cycles (**Figure 2**). After a prolonged period of wake activity (as in humans) the first cycles are characterized by a preponderance of high-voltage, slow wave activity (i.e., the NREM phase is enhanced) while the last cycles show more low-voltage, fast wave activity (i.e., the REM phase is enhanced) (**Figure 2**) (Pace-Schott & Hobson 2002; Parmeggiani & Velluti 2005; Dijk 2009).

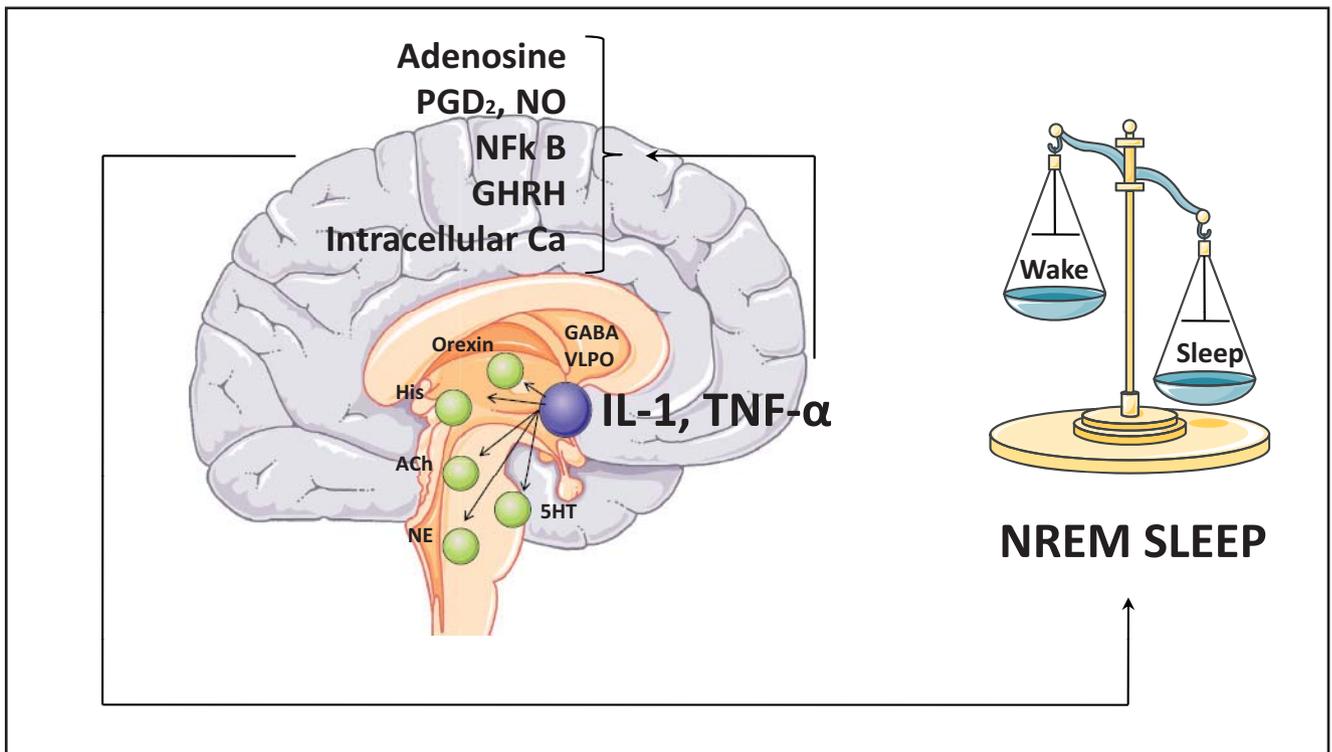


Fig. 1. The increase in IL-1 and TNF- α in the anterior hypothalamus as a result of wakefulness-related neuronal/glial mechanisms activates the activity of the ventrolateral preoptic area (VLPO). The cascade of events triggered include adenosine, growth hormone releasing hormone (GHRH), NOS, PGD₂, Ca²⁺ and other components of the signaling mechanisms leading to activation of NF- κ B. γ -Aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in VLPO. VLPO sends out multiple inhibitory projections that innervate neurons that release wake-promoting neurotransmitters, including orexin neurons in the lateral and posterior hypothalamic areas, NE neurons in the locus coeruleus, histamine neurons in the tuberomammillary nuclei, serotonin (5HT) in raphe nuclei and acetylcholine (ACh) in laterodorsal/pedunculopontine tegmental nuclei. VLPO fires at a rapid rate during sleep with substantial attenuation of firing during wakefulness. Conversely, neurons in wake-promoting centers fire rapidly during wakefulness and are relatively quiescent during sleep, with the exception of the cholinergic neurons, which fire rapidly during REM sleep. It is through this reciprocal interplay of stimulation and inhibition that stable states of sleep and wakefulness are maintained (Saper *et al.* 2005).

The recurrent cycles of NREM and REM sleep are accompanied by major changes in physiology. Indeed, it can be said that we live sequentially in three different physiological states (“or bodies”): that of wakefulness, that of NREM sleep and that of REM sleep. For an average of 8 h of sleep per day, a 76-years-old adult has lived about 50 years in wakefulness, 20 years in NREM sleep and 6 years in REM sleep. Since, as above mentioned, epidemiological data indicate that in our modern society we indulge only about 6 h of sleep per day, for an adult living 76 years, approximately 55 years are lived in wakefulness, 15 years in NREM sleep and 6 years in REM sleep (Pace-Schott & Hobson 2002; Parmeggiani & Velluti 2005). The average 5-year longer wakefulness stage, and the average 5-year shorter NREM stage, have strong negative consequences for health. There is an increasing evidence that obesity, the MS and neurodegenerative diseases can be related to the prevalence of wakefulness in face of NREM sleep loss in contemporary, 24/7 Society (Dijk 2009; Gangwisch 2009; Gimble *et al.* 2009; Reiter *et al.* 2011; Cardinali *et al.* 2011a). To understand this it is necessary to realize that significant physiological differences exist among the three physiological stages above discussed (**Table 1**).

Wakefulness is a catabolic, sympathotonic stage as reflected in every physiological system examined with a predominant activity of the hypothalamic-pituitary axis (HPA) and high cortisol and norepinephrine levels, whereas NREM sleep is an anabolic stage characterized by parasympathetic predominance, with decreases in blood pressure, heart rate and respiratory rate. Occurrence of a pulsatile release of anabolic hormones like growth hormone (GH), insulin and prolactin has been documented in NREM sleep. Indeed, NREM sleep is functionally associated with several cytoprotective processes and, in the brain, several neurotrophic factors are synthesized during this period (Faraguna *et al.* 2008; Gorgulu & Caliyurt 2009; Lee *et al.* 2009). Leptin, a strong satiety factor, is released during NREM sleep whereas ghrelin, a gastrointestinal hormone that induces hunger, is released during wakefulness (Van Cauter *et al.* 2008). The reduced time in NREM sleep together with an increase in wakefulness brought about by the “24/7 Society” have been proposed as probably cause as to why sleep loss lowers the feeling of satiety and promotes additional food intake.

REM sleep is typically an “antihomoeostatic” stage (**Table 1**). The regulatory mechanisms controlling car-

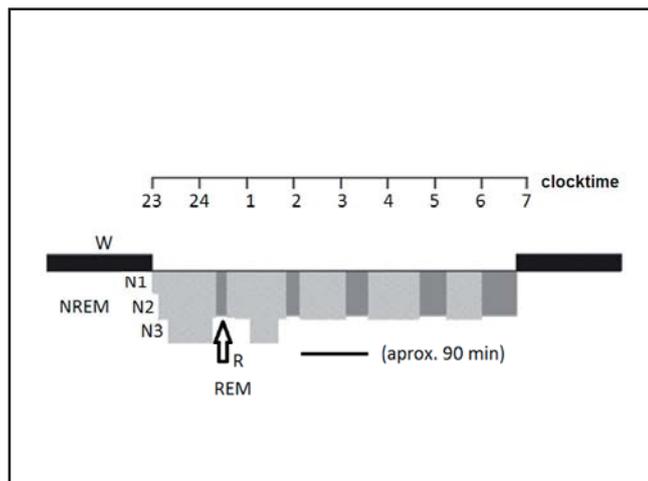


Fig. 2. Based on polysomnographic measures, sleep has been divided into categories of REM and NREM sleep. NREM sleep comprises three stages (stage N1, N2 and N3), with stage N3 being characterized by slow, high amplitude EEG waves (delta rhythm). Sleep alternates between NREM and REM stages approximately every 90 min. W: wakefulness, R: REM sleep.

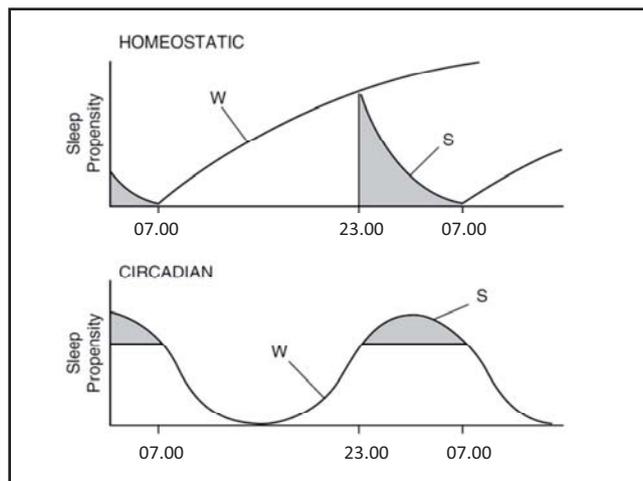


Fig. 3. The two process model of sleep regulation. The S process is the homeostatic pressure for sleep that rises when awake and is only relieved during sleep. C process in this model is a circadian (approximately 24 h) rhythm of sleep propensity that is independent of other factors. W: wakefulness, S: sleep.

diovascular, respiratory and thermoregulatory functions become grossly inefficient, leaving functional the spinal, metameric responses only. Heart rate and blood pressure as well as their variability increase and the respiratory rate becomes irregular (Parmeggiani & Velluti 2005). Awakening from REM sleep yields reports of hallucinoid dreaming, even in subjects who rarely or never recall dreams spontaneously. This indicates that the brain activation of this phase of sleep is sufficiently intense and organized to support complex mental processes and again argues against a rest function for most of the brain in REM sleep. Indeed, several areas of the brain, e.g. the limbic system, are more active in REM sleep than during wakefulness (Hobson 2009).

A significant physiological concomitant of REM sleep is the loss of temperature regulation (Parmeggiani & Velluti 2005). If ambient or core temperature begins to fall during REM sleep, thermoregulatory processes cannot be brought into play and body temperature falls. Thus the notion that we humans are homeothermic animals is not entirely correct. The evolutionary logic of somatic and autonomic disconnection during REM is that if acted, this period of sleep could be damaging for individual's survival.

THE SLEEP/WAKE CYCLE IS THE MOST RELEVANT 24-HOUR CYCLE

In mammals, the circadian system is composed of many individual, tissue-specific cellular clocks (Reddy *et al* 2005; Dibner *et al* 2010). To generate coherent physiological and behavioral responses, the phases of this multitude of cellular clocks are orchestrated by a master circadian pacemaker residing in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Morin & Allen 2006).

The sleep/wake cycle is the most prominent circadian rhythm in humans.

Two interacting processes are interlocked to regulate the timing, duration and depth, or intensity, of the circadian sleep/wake cycle (Borbely 1982; Dijk & Duffy 1999): a homeostatic process (S process, for sleep) that maintains the duration and intensity of sleep within certain boundaries and a circadian component (C process, for circadian) that determines the timing of sleep. In addition an ultradian rhythm of approximately 90 min drives NREM sleep – REM sleep alternancy (**Figures 2 and 3**).

The S process depends on the immediate history: the interval elapsed since the previous sleep episode and the intensity of sleep in that episode. It controls mainly NREM sleep. The drive to enter sleep increases, possibly exponentially, with the time elapsed since the end of the previous sleep episode. The increase in IL-1 and TNF- α in the anterior hypothalamus as a result of wakefulness-related neuronal/glia mechanisms constitutes the biochemical correlate of the "sleep debt" (**Figure 1**). This debt declines exponentially once sleep is initiated. The cyclical nature of sleep and wakefulness equates sleep with other physiological needs such as hunger or thirst (Scheer *et al* 2007; Pandi-Perumal *et al* 2009).

The C process, the circadian component, controls REM sleep via the master oscillator located in the SCN. The circadian system is also known as a wake-promoting system because it determines the timing and strength of wakefulness (Edgar *et al* 1993). The circadian rhythm in the secretion of the pineal hormone melatonin has been shown to be responsible for the sleep rhythm in both normal and blind subjects (i.e., in the absence of the synchronizing effect of light). More specifically, it has been demonstrated that melatonin feedbacks at the SCN to inhibit the circadian signal

Tab. 1. The three physiological states (“bodies”) of our life.

	Vigilia Wakefulness	NREM sleep Sueño lento	Sueño REM REM sleep
	“Active brain in an active body”	“Inactive brain in an active body”	“Hallucinating brain in a paralyzed body”
Neurochemical „microclimate”	Tonic firing of neurons in the locus coeruleus (noradrenergic) and raphe nuclei (serotonergic) driven by orexinergic hypothalamic neurons. Phasic discharge of the pedunculo-pontine nucleus of the pontine tegmentum, PPT (cholinergic)	Inhibition by VLPO area of the arousal systems. Decreased aminergic activity in face of progressive increase of cholinergic activity (tonic firing of PPT neurons). Both responsible for decreased consciousness	Prevalent cholinergic activity (PPT nucleus) concomitant with extreme reduction of aminergic activity. REM sleep and wakefulness are states of cortical activation with different neuromodulation pattern (cholinergic vs. noradrenergic) and different contents of consciousness
Afferent	Thalamocortical circuit in „open gate fashion” so that sensory information can reach the cerebral cortex. Activated dorsolateral prefrontal cortex (working memory)	Thalamo-cortical circuit in “gate closed fashion” (which prevents sensory information to reach the cerebral cortex. 25% decrease in cerebral blood flow and oxygen consumption. Synthesis of neurotrophins	Thalamic activity changes to operation “open gate fashion” as during wakefulness
Efferent	Actively functioning	Episodic muscle activity, hypotonia	Skeletal muscle paralysis as a protective mechanism to prevent the locomotor correlates of a highly activated brain
Content awareness	Attention, logical thinking, memory	Disconnection, episodic memory	Dream activity characterized by vivid hallucinations, illogical thinking and intense emotion
Perception	Externally generated	Absent	Generated internally, preferential activation of the pons and limbic system with deactivation of dorsolateral prefrontal cortex
Physiological pattern in organs and systems	Predominance of sympathetic activity in organs and systems, e.g., Th2 responses. Augmented plasma norepinephrine, cortisol and ghrelin.	Parasympathetic hyperfunction in organs and systems. GH, prolactin, leptin and insulin secretion. Th1 responses	Generalized disconnection of autonomic regulatory system (this prevents expression of dreaming’s emotions). Antihomeostatic physiology

responsible for promoting wakefulness (Sack *et al* 1992; Dijk *et al* 1997; Pandi-Perumal *et al* 2008b).

Studies in humans under constant routine conditions have led to the definition of the so-called “biological night” that corresponds to the period during which melatonin is produced and secreted into the bloodstream. The beginning of the biological night is characterized by onset of the melatonin surge, an accompanying increase in sleep propensity as well as a decrease in core body temperature; the opposite occurs as the biological night and sleep end (Lewy *et al* 2006). Rising nocturnal levels of endogenous melatonin contribute significantly to the nocturnal decline in core body temperature. The inability to fall asleep during the “wake maintenance zone” or “sleep forbidden zone”, occurs just prior to the opening of the “sleep gate” (Lavie 2001). The “sleep gate” is represented by the steep rise in sleepiness that occurs during the late evening and that begins a period characterized by a consistently high degree of sleep propensity. The nocturnal onset of melatonin secretion predictably precedes the opening of the sleep gate by about 2 h and it is believed to initiate a cascade of events culminating 1–2 h later in the “opening of the sleep gate” (Pandi-Perumal *et al* 2008b). Taken together, current findings suggest that the endogenous nocturnal circadian melatonin signal is involved in the

circadian rhythm of sleep propensity by turning off the circadian wakefulness-generating mechanism rather than by actively inducing sleep.

Many studies have described circadian variations in immune parameters such as lymphocyte subpopulations, proliferation, antigen presentation, and cytokine gene expression (Bonnetfont 2010; Bollinger *et al* 2010). The number of lymphocytes and monocytes in the human blood reach maximal values during the night and are lowest after waking. Natural killer (NK) cells, by contrast, reach their highest level in the afternoon, with a normal decrease in number and activity around midnight (Petrovsky & Harrison 1998; Buijs *et al* 2006; Cutolo *et al* 2006). Changes in lymphocyte subset populations can depend on time of day-associated changes in cell proliferation of immunocompetent organs and/or on diurnal modifications in lymphocyte release and traffic among lymphoid organs. A purely neural pathway including as a motor leg the autonomic nervous system innervating the lymph nodes was identified (Cardinali & Esquifino 1998; Logan *et al* 2011). In addition, a hormonal pathway involving the circadian secretion of melatonin also plays a role to induce rhythmicity (Cardinali *et al* 2004).

The 24 h sleep/wake rhythm correlates with specific circadian patterns of circulating cytokines (Table 2) (Dimitrov *et al* 2004; Berger 2008; Lange *et al* 2010; Bollinger

et al 2010). NREM sleep is associated with T helper (Th) 1 responses while during wakefulness Th2 responses are predominant. Th1 cells release mainly interferon (IFN)- γ , aside from other cytokines including IL-2 and TNF- α ; they become activated in response to intracellular viral and bacterial challenges supporting cellular (type 1) responses, like macrophage activation and antigen presentation. Wakefulness is associated with predominance of cytokines characteristic of Th2 immunity e.g. IL-4, IL-5, IL-10 and IL-13, that mediate humoral (type 2) defense via stimulating mast cells, eosinophils and B cells against extracellular pathogens (Berger 2008; Opp 2009; Chrousos 2009; Lange *et al* 2010; Bollinger *et al* 2010). Indeed, type 1–type 2 cytokine balance is crucial for the control of immune function, with type 1 cytokines overall supporting cellular aspects of immune responses and type 2 cytokines moderating the type 1 response (Kidd 2003; Corthay 2009). An excessive production of either cytokine type leads to inflammation and tissue damage on the one hand, and to susceptibility to infection and allergy on the other hand.

To prevent overactivity, the type 1–type 2 cytokine balance is tightly regulated by mutual inhibition and via a complex neuroendocrine control. Indeed, several reports indicate that the shift towards Th1 mediated immune defense occurring in NREM sleep is driven by the neuroendocrine environment (see for ref. Krueger 2008; Opp 2009; Rector *et al* 2009). The circadian peak of the ratio of IFN- γ /IL-10 in whole blood samples during nocturnal sleep is abolished by administering glucocorticoids the preceding evening. Thus, the suppression of endogenous cortisol release and the increase in melatonin secretion, both driven by the SCN during early sleep,

seem to play a promoting role for Th1 shift. In addition, NREM sleep that is dominant during the early part of nocturnal sleep promotes the release of GH and prolactin which supports Th1 cell-mediated immunity. In summary, GH, prolactin and melatonin are known to shift the type 1–type 2 balance toward type 1, whereas cortisol and norepinephrine can shift it toward type 2 responses (Opp 2009; Chrousos 2009).

Several studies have investigated the changes in cytokine levels that occur during the 24 h sleep–wake cycle in humans (**Table 2**) (see for ref. Pandi–Perumal *et al* 2007; Opp 2009). Plasma TNF- α levels peak during the dark phase of cycle, and this circadian rhythm of TNF- α release is disrupted by sleep pathology, e.g. obstructive sleep apnea. Plasma IL-1 β levels also have a diurnal variation, being highest at the onset of NREM sleep. Both intrahypothalamic IL-1 and TNF- α regulate sleep (**Figure 1**). The levels of other cytokines (including IL-2, IL-6, IL-10 and IL-12) and the proliferation of T cells in response to mitogens also change during the 24-h cycle. The production of macrophage-related cytokines (such as TNF- α) increases during sleep (in response to *in vitro* stimulation) in parallel with the rise in monocyte numbers in the blood. The production of T-cell-related cytokines (such as IL-2) increases during sleep, independent of migratory changes in T-cell distribution (Pandi-Perumal *et al* 2007; Opp 2009). All these diurnal changes could be specific to the effects of sleep or associated with the circadian oscillator. To dissociate both effects, experimental procedures such as forced desynchrony are needed.

SLEEP DEPRIVATION STUDIES PROVIDE CLUES ON THE IMMUNOREGULATORY EFFECT OF SLEEP

Sleep deprivation is associated with a shift of the type 1–type 2 cytokine balance toward type 2 activity, as found in healthy subjects acutely deprived of sleep and in chronic sleep deficits occurring in obesity, insomnia, diabetes, asthma, alcoholism, stress and during the course of aging (Opp 2009; Wang 2009; Tsujimura *et al* 2009; Yehuda *et al* 2009; van Mark *et al* 2010; Orzel-Gryglewska 2010; Irwin *et al* 2010; Motivala 2011; Zielinski & Krueger 2011; Lange & Born 2011). A consistent finding is that pro-inflammatory cytokines are elevated in all these groups (Irwin *et al* 2003; Okun *et al* 2004; Vgontzas *et al* 2004). Sleep apneics and narcoleptics have higher TNF- α levels compared to controls while abstinent alcoholics and people partially deprived of sleep show elevations of both TNF- α and IL-6. Together with increased levels of inflammatory cytokines, insomniacs show decreased T-helper (CD3⁺, CD4⁺), T-cytotoxic (CD8⁺) cell numbers and decreased NK cell activity (Irwin *et al* 2003; Savard *et al* 2003). Experimentally-induced sleep deprivation has been found to alter the diurnal pattern of cellular and humoral immune functions (Dinges *et al* 1995; Heiser *et al* 2000) and to decrease overall immune function (Redwine *et al* 2000) in normal adults.

IL-6 is one of the cytokines whose levels fluctuate in response to partial sleep deprivation. IL-6 is produced

Tab. 2. Maxima in 24-h rhythm of some circulating cytokines and antibodies in healthy volunteers (see for ref. Pandi-Perumal *et al* 2007; Opp 2009).

Variable	Maximum at:
IL-1	1300 & 0030 h
IL-10	1830 h
IL-2	0100 h
IL-6	2200 h
IL-10	0730 & 1930 h
IL-12	1800 h
IFN γ	0230 h
TNF- α	0300 h
IgA	1200-1500 h
IgG	1400-1600 h
IgM	1500-1800 h
IgE in asthma	0730-1600 h
C3 Complement	1200-1930 h
C-reactive protein in rheumatoid arthritis	2330 h

by multiple sources including monocytes, fibroblasts, endothelial cells, smooth muscle cells, and the adipose tissue (Vgontzas *et al* 2005). IL-6 regulates systemic inflammation by stimulating production of acute phase reactants by the liver. It also stimulates both B-cell maturation into plasma cells and T-cell differentiation to cytotoxic T cells. IL-6 has a circadian rhythm, peaking at night, with lower levels during the day. In healthy men, the levels of IL-6 increased with peak values occurring 2.5 h after sleep onset (Redwine *et al* 2000). During partial sleep deprivation, the nocturnal increase of IL-6 was delayed and did not occur until sleep was allowed. Hence, like GH secretion, sleep, rather than a circadian pacemaker, influences nocturnal IL-6 secretion (Redwine *et al* 2000).

To study the role of nocturnal sleep on normal immune regulation in a design to assess acute sleep loss rather than excessive sleep loss, normal volunteers slept two consecutive regular sleep-wake cycles or remained awake for 24 hours followed by recovery sleep (Born *et al* 1997; Marshall & Born 2002). No alteration in the absolute production of IL-1 β and TNF- α between the two experimental conditions was found; however, the expected decrease of IL-1 β and TNF- α during sleep was blocked when subjects were kept awake. Hence, there was an increase in the nocturnal production of both cytokines during the sleep deprivation period. Other studies evaluating sleep restriction, found a delayed nocturnal release of sleep-associated cytokines, IL-1, IL-6 and TNF- α , with subsequent recuperation of normal levels on recovery nights (Moldofsky & Dickstein 1999; Redwine *et al* 2000; Vgontzas *et al* 2005). This suggests that cytokines depend more on the activity of the sleep homeostat than on the circadian oscillator.

Deep sleep has an inhibitory influence on the hypothalamic-pituitary-adrenal (HPA) axis, in contrast to activation of the HPA axis or administration of glucocorticoids, which has been reported to lead to arousal and sleeplessness (Chrousos 2009). Not surprisingly then, insomnia, the most common sleep disorder, is associated with a 24-hour increase of corticotropin and cortisol secretion, consistent with a disorder of CNS hyperarousal. Clearly sleep deprivation, by removing the prevalent parasympathetic component of NREM sleep (**Table 1**), co-exists with the activation of the HPA with elevated glucocorticoid and catecholamine plasma levels (McEwen 2006; Chrousos 2009).

CYTOKINES HAVE AN ACTIVE ROLE IN SLEEP REGULATION

The criteria to be fulfilled for a presumed sleep regulatory molecule were outlined by Krueger and co-workers as follows: "(1) the molecule should induce physiological sleep; (2) the substance and its receptors should be present in the organism; (3) the concentration or turnover of the substance or its receptor should vary with the circadian rhythm; (4) induction of the substance

should induce sleep; (5) inactivation of the substance or its receptor should reduce spontaneous sleep; (6) inactivation of the substance should reduce sleep induced by somnogenic stimuli; and (7) other biological actions of the substance should be separable, in part, from its sleep-promoting actions" (Krueger 2008).

Cytokines as immune-mediators match those criteria (**Table 3**). They are multifunctional pleiotropic proteins that are involved not only in the immune response but also in a variety of physiological and pathological processes in the CNS (Wu *et al* 2009; Ziebell & Morganti-Kossmann 2010). Cytokines exert their effect on the CNS both directly and indirectly. Direct action means that cytokines themselves are present in the brain, in and/or around the various neuronal and glial cells, while secondary effects that are the result of cytokine action on other targets represent the indirect pathways. The various cytokines directly affecting the CNS have two possible origins, namely the periphery and the brain.

As large, hydrophilic proteins, cytokines can only cross the blood-brain barrier at leaky points (the circumventricular organs) or via specific active transport mechanisms. Cytokines act at the level of the organum vasculosum laminae terminalis, a circumventricular organ located at the anterior wall of the third ventricle. IL-1 binds to cells located on the vascular side of this circumventricular structure, thereby inducing synthesis and release of second messenger systems, such as nitric oxide (NO) synthase (NOS)/NO and the cyclooxygenase (COX)/prostaglandin (PG) systems. It must be noted that a central compartment for cytokines exists and that there are data indicating that an increase in peripheral cytokines evokes a mirror increase in brain levels of cytokines (Krueger 2008). Cytokines that originate in peripheral immune organs can also affect sensory peripheral autonomic terminals, even in healthy, basal conditions.

Imbalances in the effects of somnogenic and anti-somnogenic cytokines can be involved in sleep disturbances. From the cytokines in the sleep producing group, the most extensively studied and best established are IL-1 β and TNF- α , proinflammatory cytokines that produce fever in addition to sleep. The febrile effects of these cytokines can be blocked without altering their sporific actions.

Besides having circadian rhythms with similar nocturnal peaks, TNF- α and IL-1 β act cooperatively in animals to prolong NREM sleep (**Figure 1**). Although the exact mechanisms of the somnogenic or anti-somnogenic effects of cytokines have not yet been fully elucidated, the cascade has also been shown to involve other factors, such as adenosine, growth hormone releasing hormone (GHRH), NOS, PG like PGD₂, Ca²⁺ and other components of the signaling mechanisms leading to activation of the transcription nuclear factor (NF) κ B (Mills & Dimsdale 2004; Krueger 2008) (**Figure 1**).

Administration of TNF- α or IL-1 β increases the amplitude of slow wave EEG, while that of IL-10 and

IL-4, inhibits NREM. Interestingly, these cytokines influence each other's effects on sleep. Pre-treatment with a fragment of the receptor for IL-1 β (type 1 IL-1r, IL-1r1) attenuates TNF-induced NREM enhancement, and TNF antagonists inhibit IL-1 β -induced increases in NREM duration (Krueger 2008).

TNF- α and IL-1 β both stimulate the transcriptional activity of NF- κ B and enhance sleep. Factors that inhibit NF- κ B activation, such as IL-4, IL-10 and inhibitor of NF- κ B inhibit sleep. NF- κ B itself promotes the production on TNF- α and IL-1 β , forming a positive feedback loop, possibly to promote the homeostatic drive for sleep. This is supported by the finding that sleep deprivation causes increased levels of NF- κ B in the CNS (Bryant *et al* 2004) (**Figure 1**).

As already mentioned, IL-1 levels in the brain correlated with sleep propensity, being highest at sleep onset, and together with other proinflammatory cytokines were able to regulate physiologic body temperature and appetite (Szelenyi 2001). Other cytokines that are reported to increase NREM sleep include IL-2, IL6, IL-8, IL-15 and IL-18 (**Table 3**).

Cytokines that disrupt NREM sleep (stages 3 and 4) have been less examined, but the best established are IL-4 and IL-10. These cytokines function by inhibiting the production of IL-1 and TNF- α , probably via inhibition of NF κ B activation (Krueger 2008). Intracerebral injections in rabbits of a cell permeable inhibitor peptide of NF κ B inhibit both spontaneous and IL-1 β induced sleep (Kubota *et al* 2000). Also intracerebral

injections of TNF- α and IL-1 inhibitors in rabbits significantly reduced spontaneous NREM sleep, whereas pretreatment with inhibitors of these cytokines significantly attenuated sleep rebound after sleep deprivation. IL-4 has been shown in animals to reduce slow wave sleep (Krueger 2008). IL-10, produced by lymphocytes and monocytes, inhibits the production of TNF- α , and inhibits slow wave sleep in rabbits. In contrast, IL-10 knockout mice have increased slow wave sleep (Toth & Opp 2001). Other cytokines reported to have sleep inhibiting effects are IL-13 and transforming growth factor (TGF)- β (**Table 3**).

In the last years a number of studies have started to unravel the basis for the circadian modulation by immune factors on the circadian system itself (for ref. see Coogan & Wyse 2008). Several reports indicate a possible immune feedback regulation of the circadian clock. For example, immunosuppressant drugs such as cyclosporine affect the phase of locomotor activity (Marpegan *et al* 2004) and of hormone secretion (Selgas *et al* 1998; Esquifino *et al* 1999). Moreover, immune-related transcription factors are present and active in the SCN and its activity is partially necessary for light-induced phase shifts (Marpegan *et al* 2004). In a recent study on the endogenous expression of the proinflammatory cytokine IL-1 β and its signaling receptor IL-1R1 in the murine SCN only IL-1R1 displayed temporal regulation (Beynon & Coogan 2010).

CIRCADIAN DISORGANIZATION AND INFLAMMATION IN HIGH FAT-FED RATS

There is a large body of evidence that links feeding regimens and food components with the circadian system (Froy 2007). A high-fat diet, that contributes to insulin resistance, impaired glucose metabolism, type 2 diabetes mellitus, stroke, and coronary artery disease can feed back to influence the biological clock (Yanagihara *et al* 2006). This could explain why the circadian oscillation of many hormones involved in metabolism, such as corticosterone, insulin, glucagon, adiponectin, leptin, and ghrelin, becomes disrupted in the development of MS and obesity (Froy 2007).

Disorders of circadian rhythms have been reported to be correlated with the development of metabolic diseases (Green *et al* 2008). Disturbances in circadian rhythms promote glucose intolerance in humans (Scheer *et al* 2009) and obesity and type 2 diabetes are more prevalent in shift workers with circadian rhythm disturbances and sleep-deprivation (Knutson *et al* 2006; Sharma & Kavuru 2010). Reduction of smoking, regular exercise practices, healthy diet, weight reduction, diabetes control and changes in lifestyle are associated with lower risk of MS (King *et al* 1998).

That the high-fat diet did interfere with the circadian signaling regulating gene expression was indicated by the results of a recent study using rat anterior pituitary (Cardinali *et al* 2011a). The normal antiphase expression of the circadian clock genes *Bmal1* and *Per1* and *Per2* became disrupted in obese animals. In particular, *Per1*,

Tab. 3. Cytokines active on sleep (see for ref. Pandi-Perumal *et al.* 2007; Opp 2009).

Prosomnogenic Cytokines	Antisomnogenic Cytokines
IL-1	IL-4 IL-4
TNF- α	IL-10 IL-10
IL-2	IL-13 IL-13
IL-6	Receptores solubles TNF TNF soluble receptors
IL-8	Receptores solubles IL-1 IL-1 soluble receptors
IL-15	IGF-1 IGF-1
IL-18	Transforming growth factor β
Epidermal growth factor (EGF)	
Fibroblast growth factor	
Nerve growth factor (NGF)	
Brain-derived neurotrophic factor (BDNF)	
Neutrophins 3 and 4	
Glial derived neurotrophic factor	
IFN (alpha and beta)	
Granulocyte-macrophage colony stimulating factor GM-CSF)	

Per2, *Cry1* and *Cry2* rhythmicity was almost inverted by the high-fat diet, indicating that the inherent transcription, translation, and post-translational modifications that give the clock its own natural rhythmicity can be severely disrupted in obese rats (Cardinali *et al* 2011a). In a similar group of animals a significant disruption of the 24-h pattern of plasma TSH, LH, testosterone and prolactin, and a decreased amplitude of pineal melatonin rhythm were found (Cano *et al* 2008). Plasma corticosterone levels increased significantly in high-fat fed rats and their 24 h variation became blunted. In obese animals, a significant hyperglycemia developed, individual plasma glucose values correlating with circulating corticosterone in high-fat fed rats only. Altogether these results underlie the significant effects that obesity has on circadian organization of hormone secretion (Cano *et al* 2008).

A high-fat diet contributes to insulin resistance, impaired glucose metabolism, type 2 diabetes mellitus, stroke, and coronary artery disease. As far as glucose metabolism is concerned, dietary fat not only lowers glucose uptake but also stimulates inappropriate glucose production, resulting in elevations in both circulating insulin and glucose. High-fat diets decrease the number of insulin receptors in liver, skeletal muscle, and adipose tissue, decrease glucose uptake into skeletal muscle and adipose tissue, and decrease hepatic glycolysis and glycogen synthesis. One of the factors accounting for insulin resistance in high-fat fed animals is the elevation of glucocorticoid production that antagonizes most of insulin's actions. Indeed the effects of increased glucocorticoid levels mimic those of a high-fat diet, as suggested by the significant correlation between circulating corticosterone and glucose levels found in high-fat fed rats only (Cano *et al* 2008).

Via a number of secreted proteins called adipocytokines including hormones, cytokines, growth factors, complement factors and matrix proteins, the adipose tissue participates in the regulation of body weight homeostasis, glucose and lipid metabolism, immunity and inflammation (Padilha *et al* 2011; Pala *et al* 2011). This prompted studies to examine whether the significant disruption of 24-h hormonal pattern seen in high-fat fed rats co-exists with changes in the daily pattern of circulating adipocytokines (Cano *et al* 2009). In high-fat fed rats increased circulating levels of leptin and decreased plasma ghrelin, together with signs of insulin resistance (i.e. hyperglycemia and increased insulin levels) occurred. Concomitantly, the increased mean levels of plasma IL-1, IL-6, TNF- α and monocyte chemoattractant protein (MCP)-1, supported the occurrence of inflammation in high-fat fed rats. The normal daily pattern of plasma insulin, leptin, ghrelin, adiponectin, TNF- α , MCP-1, IL-1 and IL-6 became disrupted in experimentally obese rats (Cano *et al* 2009).

The adipose tissue in obesity characterized by an increased production and secretion of inflammatory molecules like TNF- α and IL-6, which may have local and systemic effects. The amounts of TNF- α and IL-6

are positively correlated with body fat and decrease in obese patients after weight loss (Moschen *et al* 2010; Tilg & Kaser 2011; Moschen *et al* 2011).

The decrease in amplitude of the nocturnal melatonin peak in high-fat fed rats is a strong indication of the disruptive effect of a high-fat diet on the SCN circadian oscillator. Reduction in amplitude of circadian rhythms like that reported for melatonin has been attributed to fatness. For example, in rats susceptible to obesity (Osborne-Mendel rats) the amplitude of rhythm of leptin and insulin was about 50 % that in rats relatively resistant to obesity (Ishihara *et al* 2004). Diurnal rhythms of blood pressure and heart rate were abolished as early as day 1 of ad libitum high-fat feeding, before significant changes in body weight were evident (Carroll *et al* 2005). Thus, food intake by itself appears to be a significant influence on the circadian apparatus (Cardinali *et al* 2011a).

MELATONIN ROLE IN OBESITY

Basic physiology of melatonin

Melatonin is the major secretory product of the pineal gland released every day at night. In all mammals, circulating melatonin is synthesized primarily in the pineal gland (Claustrat *et al* 2005). In addition, melatonin is also locally found in various cells, tissues and organs including lymphocytes (Carrillo-Vico *et al* 2004), human and murine bone marrow (Tan *et al* 1999; Conti *et al* 2000), the thymus (Naranjo *et al* 2007), the gastrointestinal tract (Raikhlin & Kvetnoy 1976), skin (Sloiminski *et al* 2005) and the eyes (Lundmark *et al* 2006), where it plays either an autocrine or paracrine role (Tan *et al* 2003).

Both in animals and in human beings, melatonin participates in diverse physiological functions signaling not only the length of the night (and thus the time of the day or the season of the year) but also enhancing free radical scavenging, the immune response and cytoprotective processes (Hardeland *et al* 2011). Melatonin is a powerful antioxidant that scavenges superoxide radicals as well as other ROS and radical nitrogen species (RNS) and that gives rise to a cascade of metabolites that share its antioxidant properties (Reiter *et al* 2009). Melatonin also acts indirectly to promote gene expression of antioxidant enzymes and to inhibit gene expression of prooxidant enzymes (Jimenez-Ortega *et al* 2009; Hardeland *et al* 2011). Melatonin has significant anti-inflammatory properties presumably by decreasing the synthesis of proinflammatory cytokines like TNF- α and by suppressing inducible NOS (iNOS) gene expression (Cuzzocrea *et al* 1998). Melatonin also exerts a strong antiapoptotic effect (Sainz *et al* 2003).

Circulating melatonin binds to albumin (Cardinali *et al* 1972; Partridge & Mietus 1980) and is metabolized mainly in the liver where it is hydroxylated in the C6 position by cytochrome P₄₅₀ monooxygenases (CYP2A and CYP1A) (Hartter *et al* 2001; Facciola *et al* 2001). Melatonin is then conjugated with sulphate to form 6-sulfatoxymelatonin, the main melatonin metabolite found in urine.

Melatonin is also metabolized in tissues by oxidative pyrrole-ring cleavage into kynuramine derivatives. The primary cleavage product is *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK), which is deformed, either by arylamine formamidase or hemoperoxidase to *N*¹-acetyl-5-methoxykynuramine (AMK) (Hardeland *et al* 2009). It has been proposed that AFMK is the primitive and primary active metabolite of melatonin (Tan *et al* 2007). Melatonin is also converted into cyclic 3-hydroxymelatonin in a process that directly scavenges two hydroxyl radicals (Tan *et al* 2007).

In humans, orally administered melatonin exhibits extensive first-pass metabolism and its absolute bioavailability is reported to be around 15% with a wide range of variability (Di *et al* 1997; DeMuro *et al* 2000). The wide variations in melatonin's bioavailability is attributed to inter-individual variations in the expression of and activity of CYP2A and CYP1A (Hartter *et al* 2001).

Melatonin exerts many physiological actions by acting on membrane and nuclear receptors although other actions are receptor-independent (e.g., scavenging of free radicals or interaction with cytoplasmic proteins) (Pandi-Perumal *et al* 2008b; Reiter *et al* 2009). The two melatonin receptors cloned so far (MT₁ and MT₂) are membrane receptors that have seven membrane domains and belong to the superfamily of G-protein coupled receptors (Dubocovich *et al* 2010). Melatonin receptor activation induces a variety of responses that are mediated both by pertussis-sensitive and insensitive G_i proteins. In the cytoplasm melatonin interacts with proteins like calmodulin and tubulin (Benitez-King 2006). Nuclear receptors of retinoic acid receptor superfamily (RZR/ROR α) have been identified in several cells, among them in human lymphocytes and monocytes (Carrillo-Vico *et al* 2006).

Melatonin and ROS generation in acute inflammation

Because ROS generation is a continuous and physiological phenomenon, cells possess efficient antioxidant systems that protect them from oxidative damage. Data accumulated in the last decade strongly indicate that melatonin plays an important role in this defense (Reiter *et al* 2009; Hardeland *et al* 2011). The regulation of enzymes involved in the redox pathway is one of the ways by which melatonin exerts its antioxidant effects. This action is complementary to the non-enzymatic, radical scavenger effect that melatonin and some of its metabolites (notably AFMK and AMK) have to scavenge ROS, RNS and organic radicals (Reiter *et al* 2009; Hardeland *et al* 2011).

More recently, mitochondrial effects of melatonin have come into focus, including safeguarding of respiratory electron flux, reduction of oxidant formation (by lowering electron leakage) and inhibition of opening of the mitochondrial permeability transition pore (Acuña Castroviejo *et al* 2011; Srinivasan *et al* 2011c). These effects of melatonin and its metabolites are rather unique. For example, the MT₁ / MT₂ melatonergic agonist ramelteon displays no relevant antioxidant activity (Mathes *et al* 2008).

Inflammation is a complex phenomenon that involves numerous mediators triggering physiological defense (Lumeng & Saltiel 2011). Most of the proinflammatory stimuli in inflamed tissues and migratory cells activate both COX-2 and iNOS enzymes (Vane 1998). This overproduction of PGs and NO plays important roles in acute inflammation, being responsible for blood vessel dilatation as well as for local and systemic symptoms of fever, pain and edema (Kiefer *et al* 2002). Therefore, since both COX-2 and iNOS are inducible forms up-regulated in response to inflammatory responses, they have been the focus of interest for understanding melatonin's role in pathophysiology of inflammation (Srinivasan *et al* 2010).

One of the earliest studies on melatonin role in inflammation was that of Costantino *et al* 1998) who used zymosan-activated plasma to induce paw inflammation. Zymosan activated plasma or complement trigger the production of ROS and RNS (Cuzzocrea *et al* 1997). Injection of zymosan-activated plasma in rat's paws readily evokes an inflammatory reaction as evidenced by the presence of edema within 30 min that reached a maximum at 3 h (Costantino *et al* 1998). Nitrite/nitrate levels were found to be elevated at 3 h after injection. Local melatonin administration (62.5 or 125 μ g/paw) significantly reduced zymosan-activated plasma-induced edema in a dose dependent manner. Melatonin treatment also reduced nitrite/nitrate ratio, myeloperoxidase (MPO) activity and malonaldehyde levels (Costantino *et al* 1998).

Early data pointed out to an inhibitory effect of melatonin on COX (Cardinali *et al* 1980). Indeed melatonin readily inhibits COX-2 (Cuzzocrea *et al* 1999) and iNOS (Pozo *et al* 1994; Cuzzocrea *et al* 1997). Other possible mechanisms for melatonin's anti-inflammatory effects included activation of NF- κ B (Mohan *et al* 1995) and inhibition of neutrophil infiltration (Cuzzocrea *et al* 1997).

The anti-inflammatory actions of melatonin and its metabolites AFMK and AMK were evaluated in lipopolysaccharide (LPS) activated RAW 264.7 macrophages (Mayo *et al* 2005). Melatonin addition at millimolar concentrations significantly inhibited COX-2 and iNOS activity and thus the increase of PGE₂. Melatonin also inhibited LPS-induced increase of COX-2 protein expression. Both AFMK and AMK shared the effect of melatonin to prevent the increase in COX-2 induced by LPS in macrophages. Thus melatonin and its metabolites AFMK or AMK may function as modulatory agents during the inflammatory process and have the potential to be a new class of anti-inflammatory agents (Mayo *et al* 2005). Melatonin suppresses the expression of the proinflammatory genes COX-2 and iNOS by a common transcriptional mechanism involving inhibition of p300 histone acetyltransferase activity, thereby suppressing p52 acetylation, binding and transactivation (Deng *et al* 2006).

In another study aiming to define the effect of melatonin on inflammation-related gene expression in LPS-stimulated human peripheral blood mononuclear cells melatonin pretreatment of LPS-stimulated cells sup-

pressed the expression of 23 genes more than twofold. Interestingly, melatonin showed a suppressive effect on the expression of CC chemokine subfamily genes; in particular the expression and secretion of CCL2 and CCL5 (Park *et al* 2007).

The formation of potent oxidants catalyzed by MPO has been implicated in the pathogenesis of various diseases including obesity, atherosclerosis, asthma, arthritis and cancer (Pattison & Davies 2006) and so far no effective inhibitors has been identified for MPO. (Galijasevic *et al* 2008) demonstrated that melatonin serves as a potent inhibitor of MPO under physiological-like conditions. In the presence of chloride, melatonin inactivated MPO at two points in the peroxidase cycle through binding to MPO to form an inactive complex, melatonin-MPO-Cl, and accelerating MPO compound II formation, an inactive form of MPO (Galijasevic *et al* 2008). This dual regulation by melatonin is unique and may represent a new means through which melatonin can control MPO and its downstream inflammatory pathways.

Melatonin and chronic inflammation in obesity

Melatonin treatment in rats has the ability to reduce obesity, type 2 diabetes and liver steatosis (Pan *et al* 2006; Stumpf *et al* 2009). Moreover, melatonin treatment induces regeneration/proliferation of β -cells in pancreas which leads to a decrement in blood glucose in streptozotocin-induced type 1 diabetic rats (Kanter *et al* 2006). Loss of circulating melatonin via pinealectomy results in marked hyperinsulinemia and accumulation of triglycerides in the liver (Nishida *et al* 2003). Long-term administration of melatonin improves lipid metabolism in type 2 diabetic rats through restored insulin resistance (Nishida *et al* 2002). Melatonin increases liver glycogen content in rats (Mazepa *et al* 2000) and in high fat diet-induced diabetic mice the intraperitoneal injection of 10 mg/kg melatonin ameliorated glucose utilization and insulin sensitivity with an increase in hepatic glycogen and improvement in liver steatosis via a PKC ζ -Akt-glycogen synthase kinase 3 β pathway (Shieh *et al* 2009).

Table 4 summarizes the results of recent studies in models of obesity in rats. Generally melatonin was very effective to reverse hyperadiposity in animals. The reasons for the decrease in body weight by melatonin in the absence of significant differences in food intake deserve to be explored. A key piece of evidence in this respect is the observation that melatonin plays a fundamental role in the seasonal changes of adiposity of Siberian hamsters by increasing the activity of the sympathetic nervous system innervating white fat, thereby increasing lipolysis (Bartness *et al* 2002). Whether or not a similar mechanism is also operative in a non-seasonal species like the laboratory rat remains to be defined.

Melatonin not only affects white adipose tissue but also increases recruitment of brown adipocytes and augments their metabolic activity in mammals (see for ref. Tan *et al* 2011). It was speculated that the hypertrophic effect and functional activation of brown adipose tissue

induced by melatonin may likely apply to treatment of human obesity. Collectively, the results indicate that the administration of melatonin effectively counteracts some of the disrupting effects seen in diet-induced obesity in rats, in particular, insulin resistance, dyslipidemia and overweight (**Table 4**).

CLINICAL STUDIES USING MELATONIN IN MS

Table 5 summarizes available evidence on melatonin involvement in MS. Low levels of circulating melatonin occur in type 2 diabetic patients (Tutuncu *et al* 2005), concomitantly with up-regulation of melatonin membrane receptor mRNA expression (Peschke *et al* 2007). In addition, variants in the gene encoding melatonin receptor were associated with fasting blood glucose level and/or the increased risk of type 2 diabetes (Prokopenko *et al* 2009; Dietrich *et al* 2011; Kim *et al* 2011). These clinical results indicate that melatonin may participate in blood glucose homeostasis and the low levels of melatonin might be related to the development of type 2 diabetes.

Nocturnal secretion of melatonin was lower in patients with coronary artery disease (Sakotnik *et al* 1999; Girotti *et al* 2000; Dominguez-Rodriguez *et al* 2002; Yaprak *et al* 2003). Nighttime melatonin supplementation reduced nocturnal blood pressure in otherwise untreated hypertensive men (Scheer *et al* 2004), nondipping women (Cagnacci *et al* 2005), patients with nocturnal hypertension (Grossman *et al* 2006) and in adolescents with type 1 diabetes mellitus (Cavallo *et al* 2004).

In humans catecholamine-induced hypercoagulability with acute stress contributing to thrombus growth after coronary plaque rupture was prevented by the administration of melatonin (Wirtz *et al* 2008a,b). Platelet aggregation *in vitro* was inhibited by melatonin via a time-dependent, dose-response effect (Del Zar *et al* 1990; Vacas *et al* 1991). The discussed findings provide support for a protective effect of melatonin in reducing the atherothrombotic risk in MS.

MELATONIN VS. MELATONIN ANALOGS

As melatonin exhibits both hypnotic and chronobiotic properties, it has been used for treatment of age-related insomnia as well as of other primary and secondary insomnia (Zhdanova *et al* 2001; Leger *et al* 2004). A recent consensus of the British Association for Psychopharmacology on evidence-based treatment of insomnia, parasomnia and circadian rhythm sleep disorders concluded that melatonin is the first choice treatment when a hypnotic is indicated in patients over 55 yr (Wilson *et al* 2010). Melatonin has also been successfully used for treatment of sleep problems related to perturbations of the circadian time keeping system like those caused by jet-lag, shift-work disorder or delayed sleep phase syndrome (Arendt *et al* 1997; Zhdanova *et al* 2001; Pandi-Perumal *et al* 2008a; Srinivasan *et al* 2010).

Since melatonin has a short half life (less than 30 min) its efficacy in promoting and maintaining sleep has not been uniform in the studies undertaken so far.

Thus the need for the development of prolonged release preparations of melatonin or of melatonin agonists with a longer duration of action on sleep regulatory structures in the brain arose (Turek & Gillette 2004). Slow release forms of melatonin (e.g., Circadin[®], a 2 mg-preparation developed by Neurim, Tel Aviv, Israel, and approved by the European Medicines Agency in 2007) and the melatonin analogs ramelteon, agomelatine, tasimelteon and TK-301 are examples of this strategy.

Ramelteon (Rozerem[®], Takeda Pharmaceuticals, Japan) is a melatonergic hypnotic analog approved by the FDA for treatment of insomnia in 2005. It is a selective agonist for MT₁/MT₂ receptors without significant affinity for other receptor sites (Kato *et al* 2005; Miyamoto 2009). *In vitro* binding studies have shown that ramelteon affinity for MT₁ and MT₂ receptors is 3–16 times higher than that of melatonin.

Agomelatine (Valdoxan[®], Servier, France) is a recently introduced melatonergic antidepressant, acts on both MT₁ and MT₂ melatonergic receptors with a similar affinity to that of melatonin (IC₅₀ 1.3×10⁻¹⁰ and 4.7×10⁻¹⁰ M, respectively) and also acts as an antagonist to 5-HT_{2C} receptors at a 3 orders of magnitude greater concentration (IC₅₀ 2.7×10⁻⁷ M) (Millan *et al* 2003). Agomelatine has been licensed by EMEA for treatment of major depressive disorder at doses of 25–50 mg/day.

Tasimelteon, [VES-162] is a MT₁/MT₂ agonist developed by Vanda Pharmaceuticals that completed phase III trial in 2010. In animal studies, tasimelteon exhibited the circadian phase shifting properties of melatonin (Vachharajani *et al* 2003). In clinical studies involving healthy human subjects, tasimelteon was administered at doses of 10 to 100 mg/day (Rajaratnam *et al* 2009). The FDA granted tasimelteon orphan drug designation status for blind individuals without light perception with non-24-hour sleep-wake disorder in 2010.

TIK-301 (formerly LY-156,735) has been in a phase II clinical trial in the USA since 2002. Originally it was developed by Eli Lilly and Company and called LY-156,735. In 2007 Tikvah Pharmaceuticals took over the development and named it TIK-301. It is a chlorinated derivative of melatonin with MT₁/MT₂ agonist activity and 5HT_{2C} antagonist activity. TIK-301 pharmacokinetics, pharmacodynamics and safety have been examined in a placebo controlled study using 20 to 100 mg/day doses in healthy volunteers (Mulchahey *et al* 2004). The FDA granted TIK-301 orphan drug designation in 2004, to use as a treatment for circadian rhythm sleep disorder in blind individuals without light perception and individuals with tardive dyskinesia.

As shown by the binding affinities, half-life and relative potencies of the different melatonin agonists it is clear that studies using 2–5 mg melatonin/day are probably unsuitable to give appropriate comparison with the effect of ramelteon, agomelatine, tasimelteon or TIK-301, which in addition to being generally more potent than the native molecule are employed in considerably higher amounts (Cardinali *et al* 2011b).

Melatonin has a high safety profile and it is usually remarkably well tolerated. In some studies melatonin has been administered to patients at large doses. Melatonin (10 mg/day) decreased IL-6 levels in patients with cancer (Neri *et al* 1998) and 300 mg/day decreased oxidative stress in patients with amyotrophic lateral sclerosis (Weishaupt *et al* 2006). In children with muscular dystrophy, 70 mg/day of melatonin reduced cytokines and lipid peroxidation (Chahbouni *et al* 2010). Doses of 80 mg melatonin hourly for 4 h were given to healthy men with no undesirable effects other than drowsiness (Waldhauser *et al* 1984). In healthy women given 300 mg melatonin/day for 4 months there were no side effects (Voordouw *et al* 1992). Therefore, further studies employing melatonin doses in the 100 mg/day are needed to clarify the potential implication of the native melatonin compound in humans. From animal studies it is clear that a number of potentially useful effects of melatonin, like those in neurodegenerative disorders or in the metabolic syndrome, need high doses of melatonin to become apparent (Cardinali *et al* 2010; Srinivasan *et al* 2011a,b). If one expects melatonin to be effective in improving health, especially in aged people, it is likely that the low doses of melatonin employed so far will not be very beneficial.

CONCLUSIONS

The existence of a daily rhythm affecting heart rate, blood pressure, platelet and endothelial function, among other components of the cardiovascular system, has been known for decades (Takeda & Maemura 2010). Epidemiological studies reported a morning peak regarding the incidents of cardiovascular events, such as ischemic strokes, myocardial infarction, sudden cardiac death and ventricular arrhythmias. Circadian clocks exist in cardiomyocytes, vascular smooth muscle cells and endothelial cells (Takeda & Maemura 2010). Circadian clocks within individual cells of the cardiovascular system have the potential to influence cardiovascular function by allowing anticipation of the onset of neurohumoral stimuli (e.g. increased sympathetic nervous stimulation before awakening), thereby ensuring an appropriately rapid response (Young 2006). Diabetes mellitus, a major risk factor for the development of heart disease in humans, is associated with a phase shift in the cardiac circadian clock (Oishi *et al* 2004; Young 2006). Therefore, a chronobiologic-cytoprotective therapy can be useful in patients showing the MS phenotype.

Melatonin can be the basis for such strategy in MS because it combines effects on circadian rhythmicity with strong cytoprotective properties. As discussed above the doses of melatonin employed should be higher than those usually used for treating sleep disorders. At high doses melatonin may protect against several comorbidities of MS, including diabetes and concomitant oxyradical-mediated damage, inflammation, microvascular disease and atherothrombotic risk. Although understanding of the melatonin 's action in

Tab. 4. Effects of melatonin in animals models of hyperadiposity.

Observation	Reference (s)
In rats fed from weaning with a high-fat diet melatonin decreased body weight gain, feed efficiency and plasma glucose, leptin and triglyceride levels	(Prunet-Marcassus <i>et al.</i> 2003)
In middle-aged rats receiving a high caloric liquid diet, melatonin reduced weight gain and plasma insulin and leptin levels	(Puchalski <i>et al.</i> 2003)
In high-fat diet-fed mice, melatonin improved insulin sensitivity and glucose tolerance	(Sartori <i>et al.</i> 2009)
In ovariectomized rats, melatonin was effective to reduce obesity	(Ladizesky <i>et al.</i> 2003; Sanchez-Mateos <i>et al.</i> 2007; Ciortea <i>et al.</i> 2011)
In type 2 diabetic (OLETF) rats, melatonin was effective to reduce obesity	(Hussein <i>et al.</i> 2007)
In olanzapine-treated rats, melatonin was effective to reduce obesity	(Raskind <i>et al.</i> 2007)
Melatonin and its analog NEU-P11 inhibited weight gain and improves insulin sensitivity in high-fat fed rats	(She <i>et al.</i> 2009)
In high-fat fed rats, melatonin attenuated body weight increase, the increase in plasma glucose, insulin, adiponectin, leptin, triglycerides and cholesterol levels, and counteracted disrupted 24 h patterns	(Rios-Lugo <i>et al.</i> 2010)
Melatonin reduced body weight gain, visceral adiposity, blood triglyceride and insulin levels and TBARS under a high calorie diet in rats. It also reduced the size of heart infarcts and increased percentage recovery of heart function	(Nduhirabandi <i>et al.</i> 2011)
In young male Zucker diabetic fatty rats melatonin treatment reduced mean weight gain without affecting food intake, decreased in a non-significant way blood pressure, and improved dyslipidemia.	(Agil <i>et al.</i> 2011)

Tab. 5. Effects of melatonin in human MS.

Observation	Reference (s)
Low levels of plasma melatonin in type 2 diabetic patients	(Tutuncu <i>et al.</i> 2005)
Up-regulation of melatonin membrane receptor mRNA expression in type 2 diabetic patients	(Peschke <i>et al.</i> 2007)
Polymorphisms in gene encoding melatonin receptor are associated with increased risk of type 2 diabetes	(Prokopenko <i>et al.</i> 2009; Dietrich <i>et al.</i> 2011; Kim <i>et al.</i> 2011)
Nocturnal secretion of melatonin is lower in patients with coronary artery disease	(Sakotnik <i>et al.</i> 1999; Girotti <i>et al.</i> 2000; Dominguez-Rodriguez <i>et al.</i> 2002; Yaprak <i>et al.</i> 2003)
Bedtime melatonin reduced nocturnal blood pressure in hypertensive men	(Scheer <i>et al.</i> 2004; Grossman <i>et al.</i> 2006)
Bedtime melatonin reduced nocturnal blood pressure in nondipping hypertensive women	(Cagnacci <i>et al.</i> 2005)
Bedtime melatonin decreased blood pressure and improved serum lipid profile and antioxidative status in MS patients	(Kozirog <i>et al.</i> 2011)
Bedtime melatonin reduced nocturnal blood pressure in adolescents with type 1 diabetes mellitus	(Cavallo <i>et al.</i> 2004)
Bedtime melatonin prevented catecholamine-induced hypercoagulability after stress in humans	(Wirtz <i>et al.</i> 2008a,b)
Human platelet aggregation <i>in vitro</i> inhibited by melatonin via a time-dependent effect	(Del Zar <i>et al.</i> 1990; Vacas <i>et al.</i> 1991)

the pathogenesis of MS is yet inconclusive, studies so far points out that melatonin through its immunomodulatory, antioxidant and antiapoptotic actions may exert beneficial effects on the MS phenotype. Melatonin also acts specifically at the mitochondrial level protecting the electron leakage and respiratory chain failure thereby augmenting the respiratory efficiency. The results support the concept that melatonin can be a useful add-on therapy to curtail insulin resistance, dyslipidemia and overweight in obese individuals.

ACKNOWLEDGEMENTS

Studies in authors' laboratories were supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT 2007-01045) and the University of Buenos Aires (M006). DPC is a Research Career Awardee from the Argentine Research Council (CONICET) and Professor Emeritus, University of Buenos Aires. ESP and PS are Research Career Awardees from CONICET.

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