

Melatonin and the Metabolic Syndrome: Physiopathologic and Therapeutical Implications

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Key Words

Melatonin · Metabolic syndrome · Circadian rhythms · Melatonergic agonists

Abstract

Metabolic syndrome (MS) patients exhibit sleep/wake disturbances and other circadian abnormalities, and these may be associated with more rapid weight increase and development of diabetes and atherosclerotic disease. On this basis, the successful management of MS may require an ideal drug that besides antagonizing the trigger factors of MS could also correct the disturbed sleep-wake rhythm. Melatonin is an effective chronobiotic agent able to change the phase and amplitude of circadian rhythms. Melatonin has also significant cytoprotective properties preventing a number of MS sequelae in animal models of diabetes and obesity. A small number of controlled trials indicate that melatonin is useful to treat the metabolic and cardiovascular comorbidities of MS. Whether the recently introduced melatonergic agents (ramelteon, agomelatine, tasimelteon) have the potential for treating sleep disorders in MS patients and, more generally, for arresting the progression of disease, merits further investigation.

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Introduction

Metabolic syndrome (MS) is associated with atherosclerotic disease and affects 10–25% of the adult population worldwide. Clinical definition of MS includes the presence of at least three of the following items: waist circumference >102 cm in males and >88 cm in females, triglycerides >150 mg/dl, HDL <40 mg/dl, blood pressure >130/85 mm Hg and fasting glucose >110 mg/dl [1].

There is a consensus that the cause for MS pandemic prevalence is the surplus of food while evolution has rather shaped humans for periods of food scarcity. In addition, a nonstop '24/7 society' has given rise to a true environmental mutation for which human beings do not have a suitable physiological design to adapt. We are living in a sleep-deprived society with evidence showing that we sleep on average 25% less than 40 years ago. Around 30% of adults report sleeping <6 h per night [2].

Circadian misalignment is associated with increased risk for obesity, diabetes, and cardiovascular disease [3]. Lifestyle changes, such as a tendency to nocturnality (that defines an animal behavior characterized by activity during the night and sleeping during the day) and overly rich diets, are followed by disruption of the sleep/wake cycle

and other circadian rhythms [4]. This changes the balance of the autonomic nervous system function towards a predominance of the sympathetic branch in the thoracic and muscular compartment and a predominance of the parasympathetic branch in the intra-abdominal compartment. The result is high blood pressure and an impaired glucose uptake by the muscle on the one hand, and a high insulin secretion, an increase in intra-abdominal fat and a fatty liver on the other [5].

An increasing number of epidemiological studies have reported an association between short sleep duration and higher risk of developing obesity and type 2 diabetes. In a large cohort of nurses (Nurse Health Study with more than 70,000 respondents), self-reported short (≤ 5 h) and long duration of sleep (≥ 9 h) was associated with symptomatic diabetes with a relative risk of 1.34 for short and 1.35 for long sleepers even after adjusting for confounding factors like diet or physical activity [6]. In a Swedish study in which more than 2,000 people were followed for over 10 years, a short duration of sleep (< 5 h) and difficulty in initiating and maintaining sleep were associated with higher incidence of diabetes in men even after adjusting for confounding factors like age, BMI, snoring, depression and hypertension [7]. In a longitudinal study, a large cohort of men from the Massachusetts Male Aging Study without diabetes at baseline was followed for more than 15 years. Subjects who self-reported < 6 h sleep were twice as likely to develop diabetes. This elevated risk remained after adjusting for factors like age, waist circumference, smoking and education [8].

Obesity is a state of chronic oxidative stress, a major mechanism underlying the development of co-morbidities [9]. Oxidative stress is associated with several indices of adiposity and a low antioxidant defense. Because reactive oxygen species generation is a continuous and physiological phenomenon, cells possess efficient antioxidant systems that protect them from oxidative damage. These defense systems are thought to prevent free radicals from causing irreparable damage by reacting with lipids, proteins and nucleic acids and are controlled in vivo by a wide spectrum of enzymatic and nonenzymatic systems.

Inasmuch as melatonin has been demonstrated to be an effective sleep regulator by changing the amplitude and timing of the biologic clock (chronobiotic effect) and to have antioxidant properties [e.g. see 10], a consideration of its possible role in the etiology of MS is directly relevant not only for providing new insights into the disease, but also for guiding the therapeutic use of melatonin agonists in the treatment of MS.

Circadian Disorganization after Hyperadiposity

It is known that the mammalian circadian timing system comprises peripheral oscillators located in almost every cell of the body together with a central rhythm generator located in the SCN [11]. The LD cycle, food, ambient temperature and social cues have been identified as synchronizers (or 'Zeitgebers'). An entraining agent can actually reset, or phase shift, the internal clock. Depending on when an organism is exposed to such an entraining agent, circadian rhythms can be advanced, delayed, or not shifted at all. Therefore, involved in adjusting the daily activity pattern to the appropriate time of day is a rhythmic variation in the influence of the Zeitgeber as a resetting factor [11].

At a cellular level, circadian rhythms are driven by the self-regulatory interaction of clock genes and their related proteins. Among these, *Clock*, *Bmal1*, *Per-1-3* and *Cry1-2* play a major role. The heterodimer of the proteins CLOCK:BMAL1 binds E-box elements at the promoter region of *Per1*, *Per2*, *Per3*, *Cry1* and *Cry2*, inducing their transcription. Conversely, PER1-3 and CRY1-2 proteins, by interacting with the CLOCK:BMAL1 heterodimer, operate as negative regulators inhibiting their own transcription. Via clock-controlled genes and their downstream effectors, peripheral circadian clock components directly regulate many aspects of cell physiology, such as membrane trafficking, detoxification, nutrient metabolism, and the cell cycle [11].

There is a large body of evidence that links feeding regimens and food components with the circadian system [12]. 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 [13]. An example of such a feedback is given by the experiment depicted in figure 1. In the anterior pituitary of rats fed a 4% fat diet, the peaks of *Clock* and *Bmal1* expression and those of *Per1* and *Per2* expression were in antiphase, *Per1* and *Per2* peaking at the beginning of the light phase while *Clock* and *Bmal1* peaked during scotophase. Such a reciprocal relation was reported in several peripheral tissues. Maximal expression of *Cry1* and *Cry2* showed a phase delay of about 4 h as compared to *Per1* or *Per2*. Rats fed a 35% fat diet exhibited a disrupted 24 h rhythmicity of *Per1*, *Per2*, *Cry1* and *Cry2* expression without affecting the diurnal rhythmicity of *Clock* or *Bmal1*. In particular, *Per1* and *Per2* rhythmicity was almost inverted by the high-fat diet. The results indicate that the inherent transcription, translation, and posttranslational modifica-

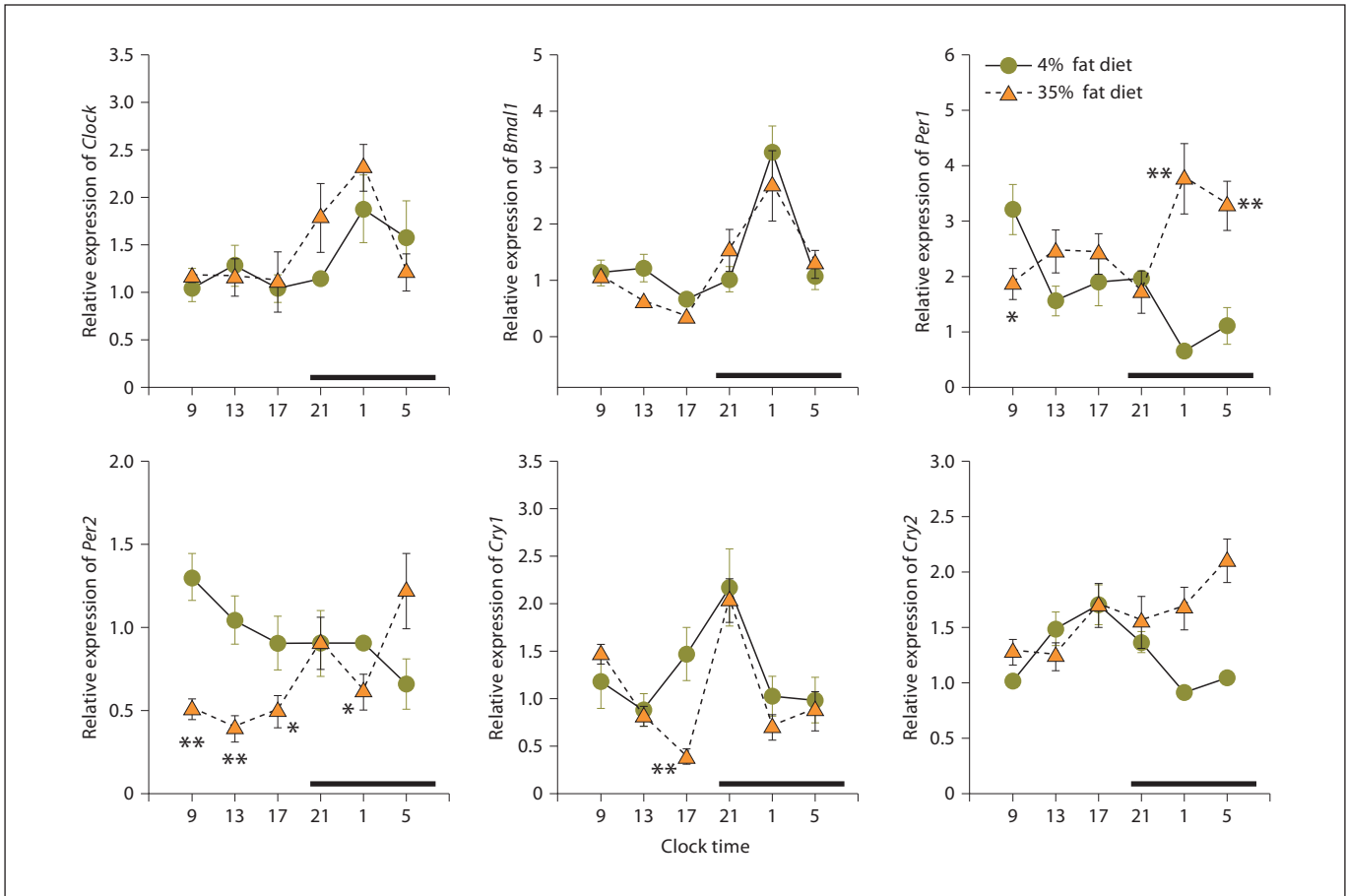


Fig. 1. Effect of a high-fat diet on 24 h changes in expression of clock genes in the anterior pituitary of rats. Rats were fed a 4% fat diet or a 35% fat diet for 11 weeks. Groups of 6–8 rats were killed by decapitation at six different time intervals throughout a 24 h cycle. Shown are the means \pm SEM of mRNA determination as measured by triplicate real-time PCR analyses of RNA samples.

** $p < 0.01$, * $p < 0.05$ as compared to rats fed a 4% fat diet (Student's t test). The data are unpublished results from an experiment reported earlier [14, see this reference for experimental details]. The sequence of primers used for PCR analysis is described elsewhere [15].

tions that give the clock its own natural rhythmicity can be disrupted in obese rats.

Results like those depicted in figure 1 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 [12]. Our own data in obese rats fed a 35% fat diet indicate a significant disruption of 24 h changes in circulating prolactin, LH, TSH, testosterone, corticosterone, insulin, leptin, ghrelin, adiponectin, tumor necrosis factor- α , interleukin-1 and -6, and monocyte chemoattractant protein-1 [16, 17]. A decrease in amplitude of the 24 h rhythm in pineal melatonin content was also observed [16], underlining the significant effects that obesity has on circadian organization [12].

Melatonin Use in Experimental Hyperadiposity

Melatonin, which occurs ubiquitously in nature, is a remarkable molecule with diverse physiological functions [e.g. see 18]. Melatonin is involved in the seasonal control of reproduction, sleep regulation, immune mechanisms and regulation of circadian rhythmicity. In addition, at pharmacological doses, presumably via antioxidant and anti-inflammatory effects, melatonin inhibits tumor growth. As the prototype of the chronobiotic class of drugs [19–21], melatonin restores the phase and amplitude of circadian rhythmicity by interaction with MT_1 and MT_2 melatonin receptors expressed in the hypothalamic SCN and other brain regions.

A number of studies indicate that melatonin has the ability to reduce obesity, type 2 diabetes and liver steatosis [e.g. see 22]. In a recent study, the *in vitro* addition of melatonin improved nonsteatotic and steatotic liver graft preservation, limiting their risk against cold ischemia-reperfusion injury [23]. Since chronic organ-donor shortage has required the acceptance of steatotic livers for transplantation purposes despite the higher risk of graft dysfunction, the potential application of this observation is obvious.

Melatonin treatment induces regeneration/proliferation of β cells in the pancreas which leads to a decrement in blood glucose in streptozotocin-induced type 1 diabetic rats [24]. Loss of circulating melatonin via pinealectomy results in marked hyperinsulinemia and accumulation of triglycerides in the liver. Long-term administration of melatonin improves lipid metabolism in type 2 diabetic rats through restored insulin resistance [25].

Melatonin treatment not only exerted a hypoglycemic effect in diabetic rats [24], but also improved a number of diabetes complications like the cardiovascular ones [26–28]. Melatonin infusion reduced arrhythmias induced by experimental ischemia of the isolated rat heart [29] and has a cytoprotective effect at the early phase of a myocardial infarction, at a time oxidative damage was minimal [30].

A number of studies were addressed to assess whether melatonin could effectively reduce adiposity in obese rats. In one of them [31], rats fed from weaning with a high-fat diet until they were overweight were then treated for 3 weeks with melatonin (30 mg/kg) 1 h before lights out. The treatment decreased body weight gain and feed efficiency by about half. Melatonin decreased plasma glucose, leptin and triglyceride levels [31].

In an experiment designed to examine whether melatonin altered consumption of a liquid diet with high-fat content in middle-aged rats, 10-month-old rats received this high caloric liquid diet containing either melatonin (0.2 μ g/ml) or vehicle [32]. The animals receiving melatonin gained 4% body weight during the first 2 weeks and then stabilized, whereas rats receiving vehicle continued to gain for an additional week. In melatonin-treated rats, nighttime but not daytime plasma leptin levels, and daytime but not nighttime plasma insulin levels decreased [32]. In a diet-induced murine model of obesity, the effects of an 8-week-long oral treatment with melatonin on insulin and glucose tolerance were assessed [33]. In high-fat diet-fed mice, but not in normal chow-fed control mice, melatonin significantly improved insulin sensitivity and glucose tolerance, as evidenced by a higher rate of

glucose infusion to maintain euglycemia during hyperinsulinemic clamp studies and an attenuated hyperglycemic response to a glucose challenge [33].

Other animal models in which melatonin was shown to be effective to reduce obesity include the ovariectomized rat [34, 35], the type 2 diabetic (OLETF) rat [36], high-fat fed rabbits [37] and olanzapine-treated rats [38]. Not only melatonin but also its analog NEU-P11 inhibited weight gain and improved insulin sensitivity in high-fat fed rats [39].

We recently examined the effect of melatonin on body weight progression, mean levels and 24 h pattern of circulating adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in rats fed a high-fat diet [40]. Animals were divided into three groups, two fed with a high-fat diet (35% fat) and melatonin (25 μ g/ml) or vehicle in drinking water for 11 weeks, while a third group was given a normal diet (4% fat). At the end of experiments, groups of rats were killed at six different time intervals throughout a 24 h period. In high-fat fed rats, melatonin attenuated body weight increase, hyperglycemia and hyperinsulinemia, as well as the increase in mean plasma adiponectin, leptin, triglycerides and cholesterol levels (fig. 2). The high-fat diet disrupted normal 24 h patterns of circulating adiponectin, insulin and cholesterol, the effects on insulin and cholesterol being counteracted by melatonin [40].

Two recent publications support the activity of melatonin in the obese rats described above. In one of them, the effect of chronic melatonin administration on the development of MS syndrome as well as ischemia-reperfusion injury was examined in a rat model of diet-induced obesity [41]. For 16 weeks, male Wistar rats received a control diet, a control diet with melatonin (4 mg/kg/day administered in the drinking water), a high-calorie diet or a high-calorie diet with melatonin. Melatonin treatment reduced the body weight gain, visceral adiposity, blood triglyceride and insulin levels and thiobarbituric acid-reactive substrate. It also reduced the size of heart infarcts and increased percentage recovery of heart functional performance with activation of the reperfusion injury salvage kinases pathway [41].

In another study, the effects of melatonin on obesity and obesity-associated systolic hypertension and dyslipidemia were examined in young male Zucker diabetic fatty rats, an experimental model of the MS [42]. Animals received melatonin (10 mg/kg/day in drinking water) or vehicle for 6 weeks. Melatonin treatment reduced mean weight gain without affecting food intake, decreased in a nonsignificant way blood pressure and improved signifi-

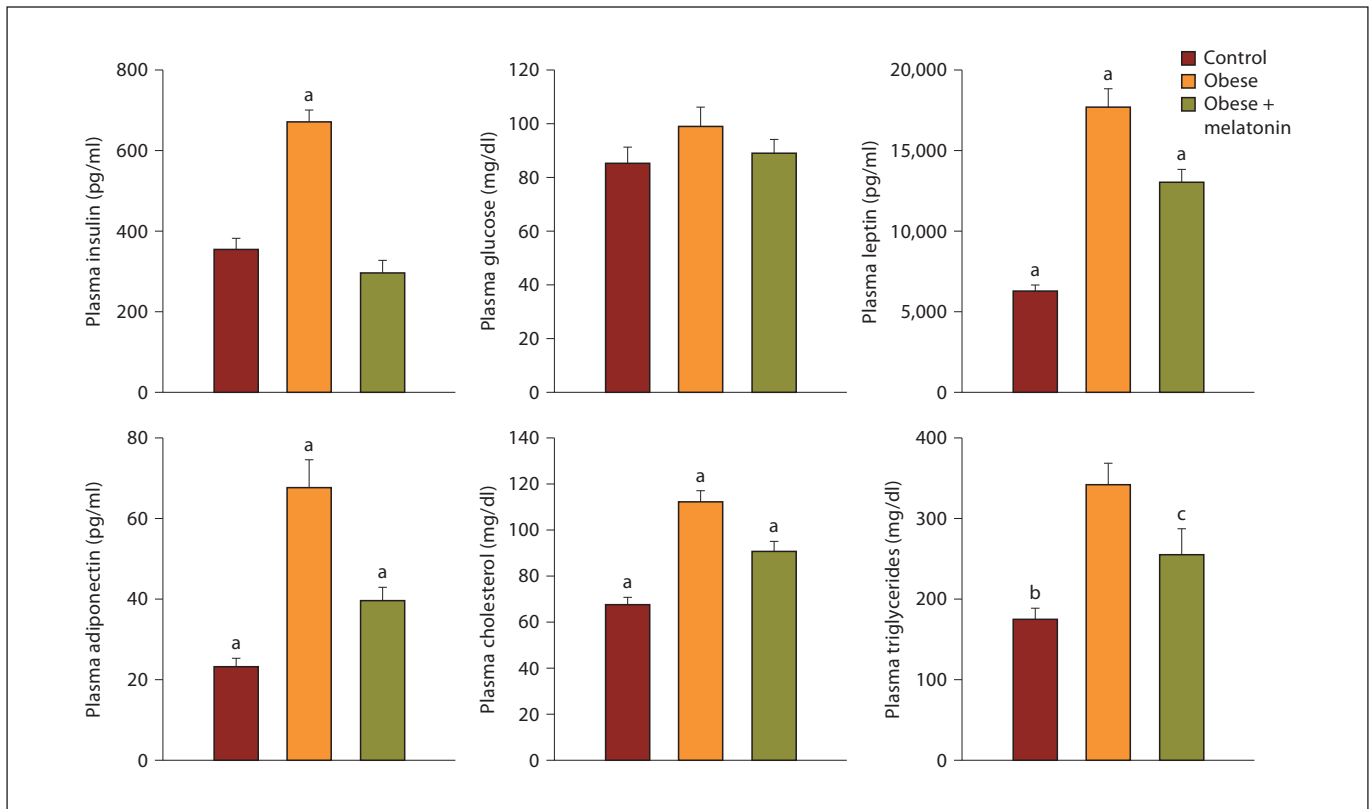


Fig. 2. Mean 24 h levels of circulating insulin, glucose, leptin, adiponectin, cholesterol and triglycerides of rats fed a 4% fat diet or a 35% fat diet and melatonin (25 μ g/ml) or vehicle in drinking water for 11 weeks. Shown are the means \pm SEM. Letters indicate the existence of significant differences after a one-way ANOVA

followed by a Student-Newman-Keuls' multiple comparisons test. ^a $p < 0.01$ vs. remaining groups. ^b $p < 0.01$ vs. obese and $p < 0.05$ vs. obese + melatonin. ^c $p < 0.01$ vs. obese and $p < 0.05$ vs. control [data redrawn from 40].

cantly dyslipidemia, as shown by reduced triglyceride levels, elevated high-density-lipoprotein (HDL) cholesterol and reduced low-density-lipoprotein (LDL) cholesterol levels [42].

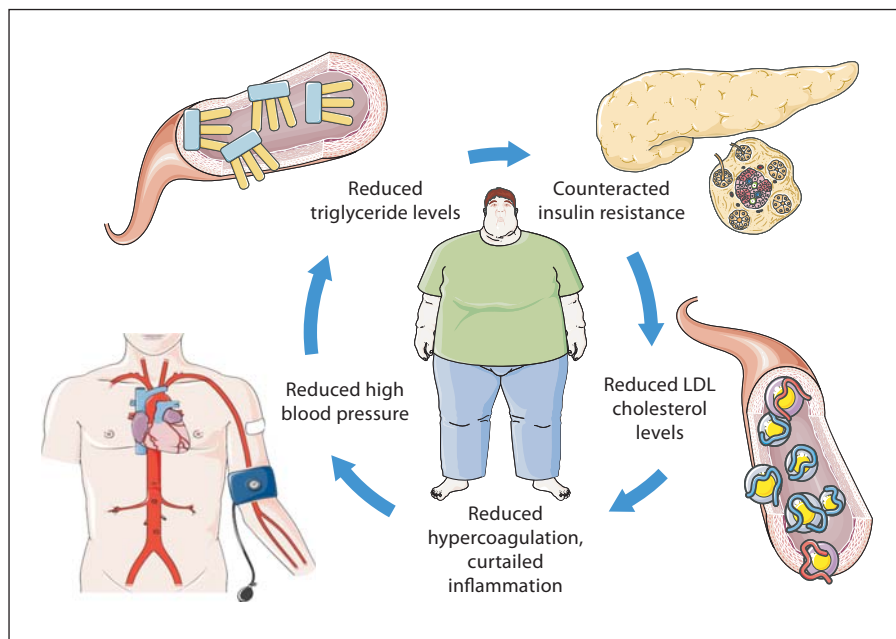
The reasons for the decrease in body weight by melatonin in the absence of significant differences in food intake deserve to be further explored. A key piece of evidence in this respect is the observation that melatonin plays a fundamental role in the seasonal changes of adiposity of Siberian hamsters by increasing the activity of the sympathetic nervous system innervating white fat, thereby increasing lipolysis [43]. Whether or not a similar mechanism is also operative in a nonseasonal species like the laboratory rat remains to be defined. Alternately, the weight-loss-promoting effect of melatonin may be attributable to an increase in energy expenditure by brown adipose tissue [e.g. see 44]. Collectively, these 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. It should be noted that there is a critical need for studies on melatonin effects on MS phenotype in primates, since all animal studies demonstrating effects of melatonin on metabolism have been conducted in nocturnal species. Established MS models like the rhesus monkey and marmoset would be very helpful in this respect.

Clinical Studies Using Melatonin in MS

Low levels of circulating melatonin occur in type 2 diabetic patients [45], concomitantly with upregulation of melatonin membrane receptor mRNA expression [22]. 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 [46]. These clinical results indicate that melatonin may participate in

Fig. 3. Effects of melatonin in MS. Melatonin treatment counteracts the three main conditions associated with MS, i.e. central obesity, insulin resistance (type 2 diabetes) and high blood pressure. Melatonin also reversed the higher than normal triglyceride and LDL cholesterol levels, hypercoagulation and inflammation that are also associated with MS.



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 [47–50]. Nighttime melatonin supplementation reduced nocturnal blood pressure in otherwise untreated hypertensive men [51], nondipping women [52], patients with nocturnal hypertension [53] and in adolescents with type 1 diabetes mellitus [54].

Improvement in lipid profile after melatonin treatment has been observed in human studies. Melatonin treatment (1 mg/kg, 30 days) elevated HDL cholesterol levels in peri- and postmenopausal women [55]. Indeed, as shown in animal studies, several mechanisms can be responsible for the hypolipidemic effects of melatonin, e.g. decrease in intestinal cholesterol absorption [56], inhibition of cholesterol biosynthesis and interaction with LDL cholesterol receptors [57] or augmentation of lecithin-cholesterol acyltransferase-mediated cholesterol esterification [58]. Melatonin ameliorates nonalcoholic fatty liver induced by a high-fat diet in rats that may also affect serum lipids [59].

In humans, catecholamine-induced hypercoagulability with acute stress contributing to thrombus growth after coronary plaque rupture was prevented by the administration of melatonin [60, 61]. Platelet aggregation *in vitro* was inhibited by melatonin via a time-dependent,

dose-response effect [62, 63]. The discussed findings provide support for a protective effect of melatonin in reducing the atherothrombotic risk in MS.

A recent open-label study on the effect of melatonin on MS included 33 healthy volunteers and 30 patients with MS [64]. As compared to controls, patients with MS had significantly higher body mass index values and total cholesterol, LDL cholesterol, triglycerides, systolic and diastolic blood pressure, glycemia, fibrinogen, and erythrocyte thiobarbituric acid-reactive substrate levels. They also had lower levels of HDL cholesterol levels and of erythrocyte activities of catalase, glutathione peroxidase and superoxide dismutase. Melatonin given daily at a 5-mg dose for 2 months decreased significantly high blood pressure and improved the serum lipid profile and the antioxidative status.

Collectively, the above discussed results suggest that melatonin therapy may be of benefit for patients with MS, particularly with arterial hypertension (fig. 3).

Chronobiologic Therapy in MS

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. Epidemiological studies reported a morning peak regarding the incidents of cardiovascular

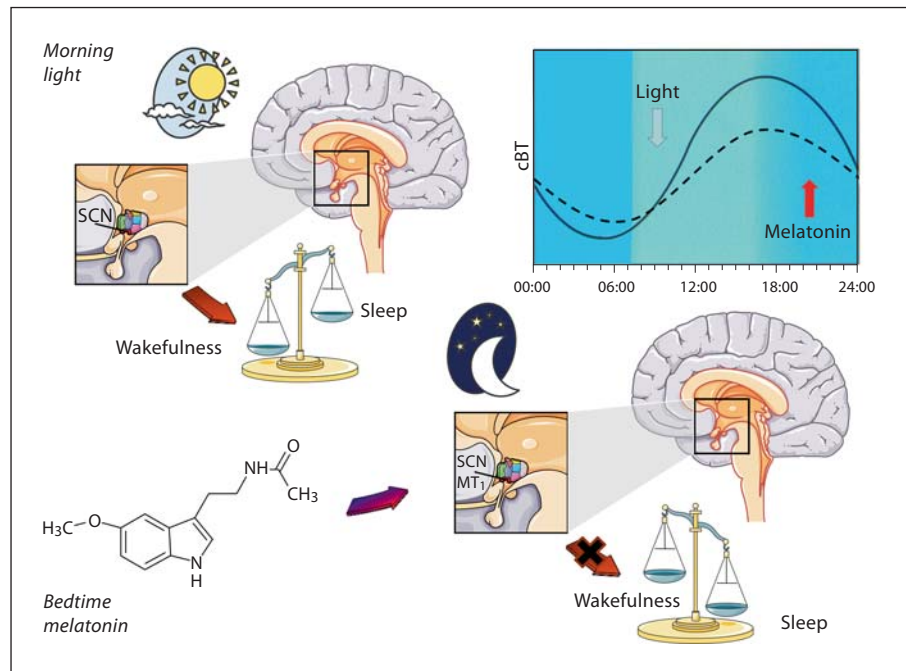


Fig. 4. Combined use of melatonin and bright light to augment the amplitude and synchronize endogenous circadian rhythmicity in MS. Melatonin administration at bedtime together with bright light in the morning potentiate to augment the amplitude of sleep/wake cycle and other circadian rhythms, e.g. core body temperature (cBT). Melatonin activity is achieved via MT_1 receptors located in SCN.

events, such as ischemic stroke, myocardial infarction, sudden cardiac death and ventricular arrhythmias. Circadian clocks exist in cardiomyocytes, vascular smooth muscle cells and endothelial cells. 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 [65]. 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 [65].

Therefore, the combined use of melatonin and bright light to augment the amplitude and synchronize endogenous circadian rhythmicity seems to be warranted (fig. 4). Following their respective phase-response curves [66], the timing of melatonin and light therapy is critical. The combined administration of bright light in the morning and melatonin at bedtime is an ideal adjuvant treatment to restore and strengthen circadian rhythmicity in MS (fig. 4).

Melatonin can provide an innovative strategy in MS by combining its effects on circadian rhythmicity with its cytoprotective properties. Melatonin protects against several comorbidities of MS, including diabetes and concomitant oxyradical-mediated damage, inflammation, microvascular disease and atherothrombotic risk.

At an early stage of MS treatment, a nonpharmacological approach as lifestyle modification, a low-fat diet, and physical exercise are commonly recommended. Patients who are refractory to these changes are treated with drugs (hypotensive, lipid-lowering, antidiabetic drugs) that may have significant side effects. Melatonin has a high safety profile and it reduces the toxicity of many pharmaceutical agents [67]. In addition, it is usually remarkably well tolerated [10], e.g. very high doses (300 mg melatonin/day) were given orally for up to 2 years to amyotrophic lateral sclerosis patients and found to be safe [68].

As melatonin is a short-lived molecule having a limited duration of action (half-life 0.54–0.67 h), analogs with a high affinity for melatonin receptors and a longer duration of action have been synthesized with a potential therapeutic efficacy to treat circadian disorders [69]. To what extent the new melatonergic agents approved by the US Food and Drug Administration or the European Medicines Agency (ramelteon, agomelatine) or those that are in the process of being approved (tasimelteon) share the protective activity of melatonin in MS remains to be defined. A recent study indicates that ramelteon given in drinking water (8 mg/kg/day) for 8 weeks to spontaneously hypertensive Wistar-Kyoto male rats significantly attenuated age-associated increase of systolic blood pressure and age-associated body weight gain [69].

Conclusion

Obesity and insulin resistance represent a problem of utmost clinical significance worldwide. Insulin-resistant states are characterized by the inability of insulin to induce proper signal transduction leading to defective glucose uptake in skeletal muscle tissue and impaired insulin-mediated effects. A high-fat diet, which contributes to insulin resistance, aggravates type 2 diabetes mellitus, stroke, and coronary artery disease and can feed back to influence the biological clock. The results support the concept that melatonin can be a useful add-on therapy to curtail insulin resistance, dyslipidemia and overweight in obese individuals.

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