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MELATONIN AND THE METABOLIC SYNDROME. PHYSIOPATHOLOGIC AND THERAPEUTICAL IMPLICATIONS.

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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 sequela in animal models of diabetes and obesity. A small number of controlled trials indicate that melatonin is useful to treat the metabolic and cardiovascular comorbilities 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.

Key words: melatonin, metabolic syndrome, circadian rhythms, melatonergic agonists.

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 greater than 102 cm in males and 88 cm in females, triglycerides greater than 150 mg/dL, HDL lower than 40 mg/dL, blood pressure greater than 130 x 85 mmHg and fasting glucose greater than 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 non-stop "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 an average 25% less than 40 years ago. Around 30% of adults report sleeping less than 6 hours per night [2].

Circadian misalignment is associated with increased risk for obesity, diabetes, and cardiovascular disease [3]. Life style 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 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 a high blood pressure and impaired glucose uptake by the muscle on one hand and a high insulin secretion, increase in intra-abdominal and 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 II diabetes. In a large cohort of nurses (Nurse Health Study with more than 70,000 respondents), self-reported short (5 hours or less) and long duration of sleep (9 hours or more) 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 2000 people were followed for over 10 years a short duration of sleep (<5 hours) and difficulty 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 less than 6 hours of 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 non-enzymatic 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 (see for ref. [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 melatonergic 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 feedback to influence the biological clock [13]. An example of such a feedback is given by the experiment depicted in Fig. 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 rats 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 post-translational modifications that give the clock its own natural rhythmicity can be disrupted in obese rats.

(INSERT FIGURE 1 ABOUT HERE)

Results like those depicted in Fig. 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 (see for ref. [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 (for ref. 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 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 an 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 a 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 μg/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, night but not daytime plasma leptin levels, and daytime but not night plasma insulin levels, decreased [32]. In a diet-induced murine model of obesity the effects of a 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 improves insulin sensitivity in high-fat fed rats [39].

We recently examined the effect of melatonin on body weight progression, mean levels and 24-hour pattern of circulating adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in rats fed a high-fat diet [40]. Animals were divided into 3 groups, two fed with a high-fat diet (35% fat) and melatonin (25 μg/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 6 different time intervals throughout a 24 hour 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].

(INSERT FIGURE 2 ABOUT HERE)

Two recent publications support the activity of melatonin in obese rats above described. 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]. Male Wistar rats received for 16 weeks 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 (TBARS). 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

non-significant way blood pressure, and improved significantly 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 non-seasonal 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 (see for ref. [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 up-regulation 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 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 also may 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 TBARS 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).

(INSERT FIGURE 3 ABOUT HERE)

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 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. 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 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 strength circadian rhythmicity in MS (Fig. 4).

(INSERT FIGURE 4 ABOUT HERE)

Melatonin can provide an innovative strategy in MS by combining its effects on circadian rhythmicity with its cytoprotective properties. Melatonin protects against several comorbilities 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 U.S. Food and Drug Administration or the European Medicines Agency (ramelteon, agomelatine) or 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 [70].

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, that 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 ad-on therapy to curtail insulin resistance, dyslipidemia and overweight in obese individuals.

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References

[1] Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486-2497.

[2] National Sleep Foundation: Sleep in America Poll 2003. ,Washington, DC, USA, National Sleep Foundation, 2003.**

[3] Scheer FA, Hilton MF, Mantzoros CS, Shea SA: Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci U S A 2009;106:4453-4458. **

[4] Bixler E: Sleep and society: an epidemiological perspective. Sleep Med 2009;10 Suppl 1:S3-S6.

[5] Buijs RM, Scheer FA, Kreier F, Yi C, Bos N, Goncharuk VD, Kalsbeek A: Chapter 20: Organization of circadian functions: interaction with the body. Prog Brain Res 2006;153:341-360. *** *The link between metabolic syndrome and autonomic circadian disorganization is discussed.*

[6] Ayas NT, White DP, Al Delaimy WK, Manson JE, Stampfer MJ, Speizer FE, Patel S, Hu FB: A prospective study of self-reported sleep duration and incident diabetes in women. Diabetes Care 2003;26:380-384.

[7] Mallon L, Broman JE, Hetta J: High incidence of diabetes in men with sleep complaints or short sleep duration: a 12-year follow-up study of a middle-aged population. Diabetes Care 2005;28:2762-2767.

[8] Yaggi HK, Araujo AB, McKinlay JB: Sleep duration as a risk factor for the development of type 2 diabetes. Diabetes Care 2006;29:657-661.

[9] Vincent HK, Innes KE, Vincent KR: Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diabetes Obes Metab 2007;9:813-839.

[10] Sanchez-Barcelo EJ, Mediavilla MD, Tan DX, Reiter RJ: Clinical uses of melatonin: Evaluation of human trials. Curr Med Chem 2010;17:2070-2095. **

[11] Dibner C, Schibler U, Albrecht U: The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol 2010;72:517-549. *** *Circadian clockwork mechanisms are discussed in detail*

[12] Froy O: The relationship between nutrition and circadian rhythms in mammals. Front Neuroendocrinol 2007;28:61-71. *** *How nutrients affect the circadian system is reviewed*

[13] Yanagihara H, Ando H, Hayashi Y, Obi Y, Fujimura A: High-fat feeding exerts minimal effects on rhythmic mRNA expression of clock genes in mouse peripheral tissues. Chronobiol Int 2006;23:905-914.

[14] Cano P, Cardinali DP, Jimenez-Ortega V, Ríos-Lugo MJ, Scacchi PA, Esquifino AI: Effect of a high-fat diet on 24-hour pattern in expression of prolactin and redox pathway enzymes in the rat adenohypophysis. The Open Obesity J 2010;2: 1-9.

[15] Jiménez-Ortega V, Cardinali DP, Fernández-Mateos MP, Rios-Lugo MJ, Scacchi PA, Esquifino AI: Effect of cadmium on 24-hour pattern in expression of redox enzyme and clock genes in medial basal hypothalamus. Biometals 2010;23:327-337.

[16] Cano P, Jiménez-Ortega V, Larrad A, Reyes Toso CF, Cardinali DP, Esquifino AI: Effect of a high-fat diet on 24-hour pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid stimulating hormone and glucose, and pineal melatonin content, in rats. Endocrine 2008;33:118-125. **

[17] Cano P, Cardinali DP, Ríos-Lugo MP, Fernández-Mateos MP, Reyes MP, Esquifino AI: Effect of a high-fat diet on 24-hour pattern of circulating adipocytokines in rats. Obesity 2009;117:1866-1871. **

[18] Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR: Melatonin - a pleiotropic, orchestrating regulator molecule. Progr Neurobiol, in press. *** *Melatonin pleiotropy is analyzed*

[19] Arendt J, Skene DJ: Melatonin as a chronobiotic. Sleep Med Rev 2005;9:25-39. **

[20] Cardinali DP, Furio AM, Reyes MP, Brusco LI: The use of chronobiotics in the resynchronization of the sleep-wake cycle. Cancer Causes Control 2006;17:601-609.

[21] Dawson D, Armstrong SM: Chronobiotics--drugs that shift rhythms. Pharmacol Ther 1996;69:15-36.

[22] Peschke E, Stumpf I, Bazwinsky I, Litvak L, Dralle H, Muhlbauer E: Melatonin and type 2 diabetes - a possible link? J Pineal Res 2007;42:350-358. *** *The link between melatonin and type 2 diabetes is thoroughly examined*

[23] Zaouali MA, Reiter RJ, Padrissa-Altes S, Boncompagni E, Garcia JJ, Ben Abnennebi H, Freitas I, Garcia-Gil FA, Rosello-Catafau J: Melatonin protects steatotic and nonsteatotic liver grafts against cold ischemia and reperfusion injury. J Pineal Res DOI: 10.1111/j.1600-079X.2010.00831.x

[24] Kanter M, Uysal H, Karaca T, Sagmanligil HO: Depression of glucose levels and partial restoration of pancreatic beta-cell damage by melatonin in streptozotocin-induced diabetic rats. Arch Toxicol 2006;80:362-369.

[25] Nishida S, Segawa T, Murai I, Nakagawa S: Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Delta-5 desaturase activity. J Pineal Res 2002;32:26-33.

[26] Paskaloglu K, Sener G, Ayangolu-Dulger G: Melatonin treatment protects against diabetes-induced functional and biochemical changes in rat aorta and corpus cavernosum. Eur J Pharmacol 2004;499:345-354.

[27] Cam M, Yavuz O, Guven A, Ercan F, Bukan N, Ustundag N: Protective effects of chronic melatonin treatment against renal injury in streptozotocin-induced diabetic rats. J Pineal Res 2003;35:212-220.

[28] Reyes Toso C, Linares LM, Ricci C, Obaya-Naredo D, Pinto JM, Rodríguez R, Cardinali DP: Melatonin restores endothelium-dependent relaxation in aortic rings of pancreatectomized rats. J Pineal Res 2005;39:386-391.

[29] Tan DX, Manchester LC, Reiter RJ, Qi W, Kim SJ, El Sokkary GH: Ischemia/reperfusion-induced arrhythmias in the isolated rat heart: prevention by melatonin. J Pineal Res 1998;25:184-191.

[30] Castagnino HE, Lago N, Centrella JM, Calligaris SD, Farina S, Sarchi MI, Cardinali DP: Cytoprotection by melatonin and growth hormone in early rat myocardial infarction as revealed by Feulgen DNA staining. Neuro Endocrinol Lett 2002;23:391-395.

[31] Prunet-Marcassus B, Desbazeille M, Bros A, Louche K, Delagrange P, Renard P, Casteilla L, Penicaud L: Melatonin reduces body weight gain in Sprague Dawley rats with diet-induced obesity. Endocrinology 2003;144:5347-5352. **

[32] Puchalski SS, Green JN, Rasmussen DD: Melatonin effect on rat body weight regulation in response to high-fat diet at middle age. Endocrine 2003;21:163-167. **

[33] Sartori C, Dessen P, Mathieu C, Monney A, Bloch J, Nicod P, Scherrer U, Duplain H: Melatonin improves glucose homeostasis and endothelial vascular function in high-fat diet-fed insulin-resistant mice. Endocrinology 2009;150:5311-5317. **

[34] Ladizesky MG, Boggio V, Albornoz LE, Castrillón P, Mautalen CA, Cardinali DP: Melatonin increases oestradiol-induced bone formation in ovariectomized rats. J Pineal Res 2003;34:143-151.

[35] Sanchez-Mateos S, Alonso-Gonzalez C, Gonzalez A, Martinez-Campa CM, Mediavilla MD, Cos S, Sanchez-Barcelo EJ: Melatonin and estradiol effects on food intake, body weight, and leptin in ovariectomized rats. Maturitas 2007;58:91-101.

[36] Hussein MR, Ahmed OG, Hassan AF, Ahmed MA: Intake of melatonin is associated with amelioration of physiological changes, both metabolic and morphological pathologies associated with obesity: an animal model. Int J Exp Pathol 2007;88:19-29.

[37] Raskind MA, Burke BL, Crites NJ, Tapp AM, Rasmussen DD: Olanzapine-induced weight gain and increased visceral adiposity is blocked by melatonin replacement therapy in rats. Neuropsychopharmacology 2007;32:284-288.

[38] She M, Deng X, Guo Z, Laudon M, Hu Z, Liao D, Hu X, Luo Y, Shen Q, Su Z, Yin W: NEU-P11, a novel melatonin agonist, inhibits weight gain and improves insulin sensitivity in high-fat/high-sucrose-fed rats. Pharmacol Res 2009;59:248-253.

[39] Rios-Lugo MJ, Cano P, Jimenez-Ortega V, Fernandez-Mateos MP, Scacchi PA, Cardinali DP, Esquifino AI: Melatonin effect on plasma adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in normal and high fat-fed rats. J Pineal Res 2010,49:342–348. **

[40] Nduhirabandi F, Du Toit EF, Blackhurst D, Marais D, Lochner A: Chronic melatonin consumption prevents obesity-related metabolic abnormalities and protects the heart against myocardial ischemia and reperfusion injury in a prediabetic model of diet-induced obesity. J Pineal Res DOI:10.1111/j.1600-079X.2010.00826.x

[41] Agil A, Navarro-Alarcon M, Ruiz R, Abuhamadah S, El Mir MY, Vazquez GF: Beneficial effects of melatonin on obesity and lipid profile in young Zucker diabetic fatty rats. J Pineal Res DOI:10.1111/j.1600-079X.2010.00830.x

[42] Bartness TJ, Demas GE, Song CK: Seasonal changes in adiposity: the roles of the photoperiod, melatonin and other hormones, and sympathetic nervous system. Exp Biol Med (Maywood) 2002;227:363-376.

[43] Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ: Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. Obes Rev DOI: 10.1111/j.1467-789X.2010.00756.x

[44] Tutuncu NB, Batur MK, Yildirir A, Tutuncu T, Deger A, Koray Z, Erbas B, Kabakci G, Aksoyek S, Erbas T: Melatonin levels decrease in type 2 diabetic patients with cardiac autonomic neuropathy. J Pineal Res 2005;39:43-49.

[45] Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Erdos MR, Sanna S, Hottenga JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bonnycastle LL, Buchanan TA, Cao A, Cervino A, Coin L, Collins FS, Crisponi L, de Geus EJ, Dehghan A, Deloukas P, Doney AS, Elliott P, Freimer N, Gateva V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naitza S, Orru M, Palmer CN, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Sijbrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuomi T, Tuomilehto J, Uitterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemsen G, Witteman JC, Yuan X, Zhao JH, Zeggini E, Schlessinger D, Sandhu M, Boomsma DI, Uda M, Spector TD, Penninx BW, Altshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Waeber G, Fox CS, Peltonen L, Groop LC, Mooser V, Cupples LA, Thorsteinsdottir U, Boehnke M, Barroso I, Van Duijn C, Dupuis J, Watanabe RM, Stefansson K, McCarthy MI, Wareham NJ, Meigs JB, Abecasis GR: Variants in MTNR1B influence fasting glucose levels. Nat Genet 2009;41:77-81. *** *The novel finding that variants in the gene encoding melatonin receptor are associated with increased risk of type 2 diabetes is made*

[46] Sakotnik A, Liebmann PM, Stoschitzky K, Lercher P, Schauenstein K, Klein W, Eber B: Decreased melatonin synthesis in patients with coronary artery disease. Eur Heart J 1999;20:1314-1317.

[47] Yaprak M, Altun A, Vardar A, Aktoz M, Ciftci S, Ozbay G: Decreased nocturnal synthesis of melatonin in patients with coronary artery disease. Int J Cardiol 2003;89:103-107.

[48] Girotti L, Lago M, Ianovsky O, Carbajales J, Elizari MV, Brusco LI, Cardinali DP: Low urinary 6-sulphatoxymelatonin levels in patients with coronary artery disease. J Pineal Res 2000;29:138-142.

[49] Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia MJ, Sanchez J, Marrero F, Armas-Trujillo D: Decreased nocturnal melatonin levels during acute myocardial infarction. J Pineal Res 2002;33:248-252.

[50] Scheer FA, Van Montfrans GA, Van Someren EJ, Mairuhu G, Buijs RM: Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. Hypertension 2004;43:192-197. **

[51] Cagnacci A, Cannoletta M, Renzi A, Baldassari F, Arangino S, Volpe A: Prolonged melatonin administration decreases nocturnal blood pressure in women. Am J Hypertens 2005;18:1614-1618. **

[52] Grossman E, Laudon M, Yalcin R, Zengil H, Peleg E, Sharabi Y, Kamari Y, Shen-Orr Z, Zisapel N: Melatonin reduces night blood pressure in patients with nocturnal hypertension. Am J Med 2006;119:898-902. **

[53] Cavallo A, Daniels SR, Dolan LM, Bean JA, Khoury JC: Blood pressure-lowering effect of melatonin in type 1 diabetes. J Pineal Res 2004;36:262-266. **

[54] Tamura H, Nakamura Y, Narimatsu A, Yamagata Y, Takasaki A, Reiter RJ, Sugino N: Melatonin treatment in peri- and postmenopausal women elevates serum high-density lipoprotein cholesterol levels without influencing total cholesterol levels. J Pineal Res 2008;45:101-105

[55] Hussain SA: Effect of melatonin on cholesterol absorption in rats. J Pineal Res 2007;42:267-271.

[56] Muller-Wieland D, Behnke B, Koopmann K, Krone W: Melatonin inhibits LDL receptor activity and cholesterol synthesis in freshly isolated human mononuclear leukocytes. Biochem Biophys Res Commun 1994;203:416-421.

[57] Esquifino A, Agrasal C, Velazquez E, Villanua MA, Cardinali DP: Effect of melatonin on serum cholesterol and phospholipid levels, and on prolactin, thyroid-stimulating hormone and thyroid hormone levels, in hyperprolactinemic rats. Life Sci 1997;61:1051-1058.

[58] Pan M, Song YL, Xu JM, Gan HZ: Melatonin ameliorates nonalcoholic fatty liver induced by high-fat diet in rats. J Pineal Res 2006;41:79-84.

[59] Wirtz PH, Bartschi C, Spillmann M, Ehlert U, von Kanel R: Effect of oral melatonin on the procoagulant response to acute psychosocial stress in healthy men: a randomized placebo-controlled study. J Pineal Res 2008;44:358-365. **

[60] Wirtz PH, Spillmann M, Bartschi C, Ehlert U, von Kanel R: Oral melatonin reduces blood coagulation activity: a placebo-controlled study in healthy young men. J Pineal Res 2008;44:127-133. **

[61] Del Zar MM, Martinuzzo M, Cardinali DP, Carreras LO, Vacas MI: Diurnal variation in melatonin effect on adenosine triphosphate and serotonin release by human platelets. Acta Endocrinol (Copenh) 1990;123:453-458.

[62] Vacas MI, Del Zar MM, Martinuzzo M, Falcon C, Carreras LO, Cardinali DP: Inhibition of human platelet aggregation and thromboxane B_2 production by melatonin. Correlation with plasma melatonin levels. J Pineal Res 1991;11:135-139.

[63] Kozirog M, Poliwczak AR, Duchnowicz P, Koter-Michalak M, Sikora J, Broncel M: Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. J Pineal Res DOI:10.1111/j.1600-079X.2010.00835.x

[64] Young ME: The circadian clock within the heart: potential influence on myocardial gene expression, metabolism, and function. Am J Physiol Heart Circ Physiol 2006;290:H1-16.

[65] Lewy AJ, Emens J, Jackman A, Yuhas K: Circadian uses of melatonin in humans. Chronobiol Int 2006;23:403-412. **

[66] Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burillo S: Melatonin: reducing the toxicity and increasing the efficacy of drugs. J Pharm Pharmacol 2002;54:1299-1321. **

[67] Weishaupt JH, Bartels C, Polking E, Dietrich J, Rohde G, Poeggeler B, Mertens N, Sperling S, Bohn M, Huther G, Schneider A, Bach A, Siren AL, Hardeland R, Bahr M, Nave KA, Ehrenreich H: Reduced oxidative damage in ALS by high-dose enteral melatonin treatment. J Pineal Res 2006;41:313-323.

[68] Ferguson SA, Rajaratnam SM, Dawson D: Melatonin agonists and insomnia. Expert Rev Neurother 2010;10:305-318. ¨¨

[69] Oxenkrug GF, Summergrad P: Ramelteon attenuates age-associated hypertension and weight gain in spontaneously hypertensive rats. Ann N Y Acad Sci 2010;1199:114-120.

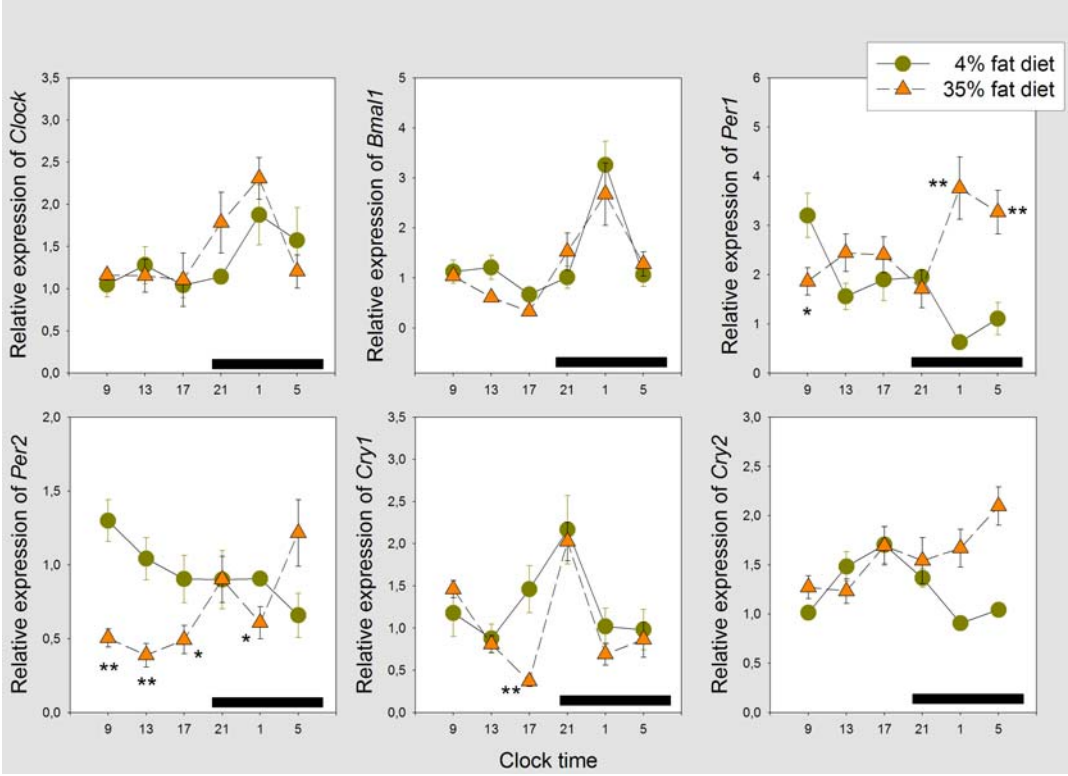

Figure 1.
Effect of a high-fat diet on 24-h changes in expression of clock genes in the anterior pituitary of rats. Rats were fed a 4% fat diet or a 35% fat diet for 11 weeks. Groups of 6-8 rats were killed by decapitation at 6 different time intervals throughout a 24 h cycle. Shown are the means ± S.E.M. 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 ref. for experimental details). The sequence of primers used for PCR analysis is described elsewhere [15].

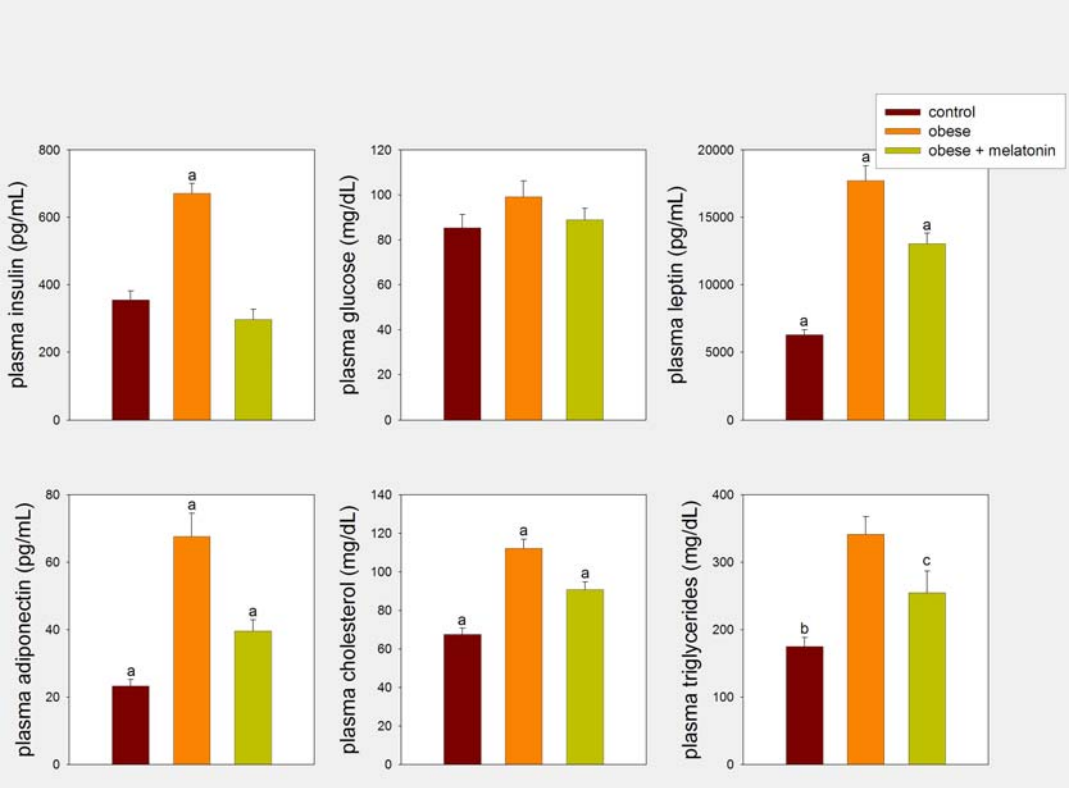

Figure 2. Mean 24 h levels of circulating insulin, glucose, leptin, adiponectin, cholesterol and triglycerides of rats fed a 4% fat diet or a 35% fat diet and melatonin (25 μg/mL) or vehicle in drinking water for 11 weeks. Shown are the means ± S.E.M. Letters indicate the existence of significant differences after a one-way ANOVA followed by a Student-Newman-Keuls´ multiple comparisons test. [a] P< 0.01 vs. the 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].

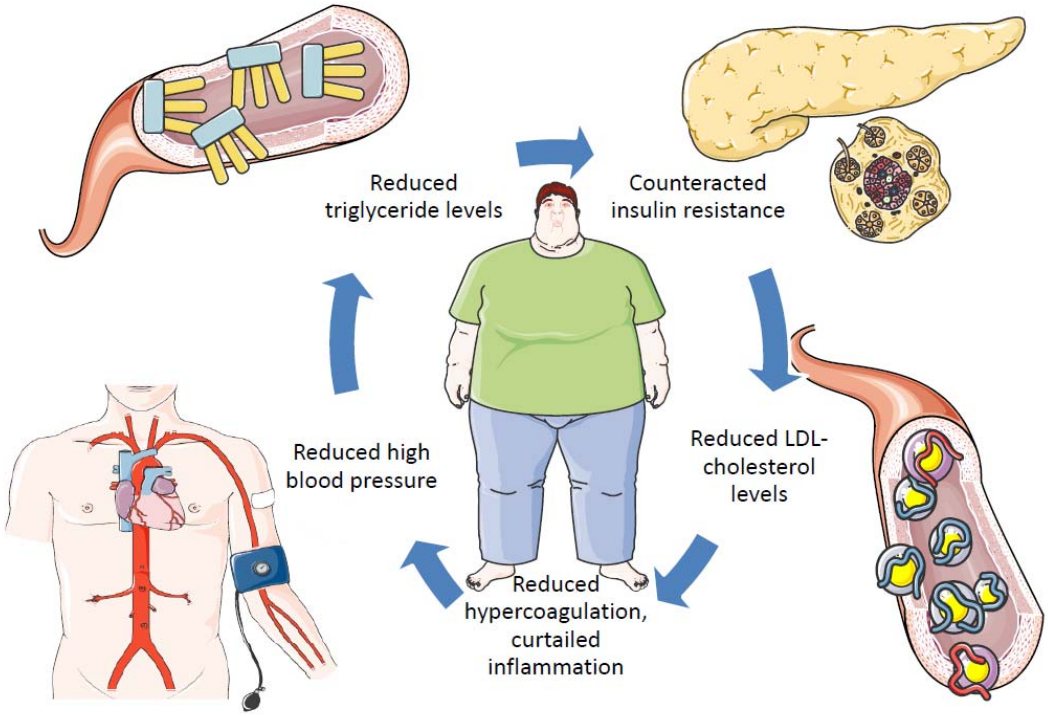

Figure 3. Effects of melatonin in MS. Melatonin treatment counteracts the three main conditions associated with MS, i.e.,central obesity, insulin resistance (type II diabetes) and high blood pressure. Melatonin also reversed the higher than normal triglyceride and LDL-cholesterol levels, hypercoagulation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

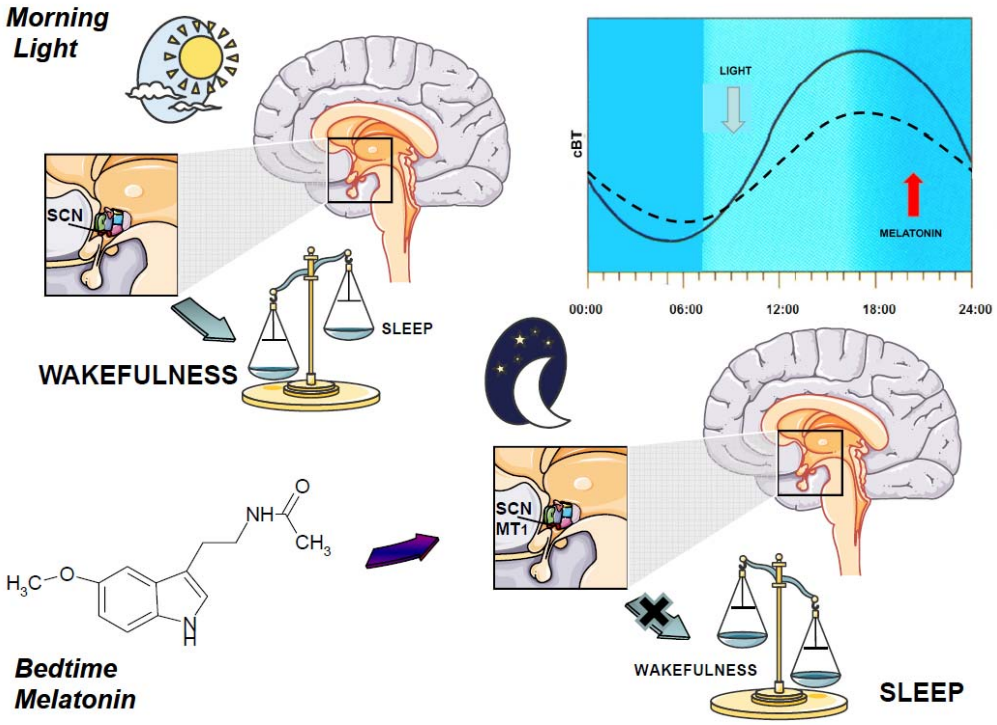

Figure 4. The combined use of melatonin and bright light to augment the amplitude and synchronize endogenous circadian rhythmicity in MS. Melatonin administration at bedtime together with bright light in the morning potentiate to augment the amplitude of sleep/wake cycle and other circadian rhythms,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