Inflammaging, Metabolic Syndrome and Melatonin: A Call for Treatment Studies

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Abstract
The metabolic syndrome (MS) is a collection of risk factors for cardiovascular disease, including obesity, hypertension, hyperinsulinemia, glucose intolerance and dyslipidemia. MS is associated with low-grade inflammation of the white adipose tissue, which can subsequently lead to insulin resistance, impaired glucose tolerance and diabetes. Adipocytes secrete proinflammatory cytokines as well as leptin and trigger a vicious circle which leads to additional weight gain largely as fat. The imbalance between inflammatory and anti-inflammatory signals is crucial to aging. Healthy aging can benefit from melatonin, a compound known to possess direct and indirect antioxidant properties, to have a significant protective effect on mitochondrial function, to enhance circadian rhythm amplitudes, to modulate the immune system and to exhibit neuroprotective actions. Melatonin levels decrease in the course of senescence and are more strongly reduced in diseases related to insulin resistance. This short review article analyzes the multiple protective actions of melatonin that are relevant to the attenuation of inflammatory responses and progression of inflammaging and how melatonin is effective to curtail MS in animal models of hyperadiposity. The clinical data supporting the possible therapeutic use of melatonin in human MS are also reviewed. Since attention has been focused on the development of potent melatonin analogs with prolonged effects (ramelteon, agomelatine, tasimelteon, piromelatine) and in clinical trials these analogs were administered in doses considerably higher than those usually employed for melatonin, clinical trials on melatonin in the range of 50–100 mg/day are needed to further assess its therapeutic value in MS.

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Inflammaging and the Metabolic Syndrome

The metabolic syndrome (MS) is a collection of risk factors for cardiovascular disease, including obesity, hypertension, hyperinsulinemia, glucose intolerance and dyslipidemia. MS is a major clinical challenge with a prevalence of 15–30%, depending on the world region considered [1–3]. MS increases overall cardiovascular mortality by 1.5–2.5 times and, together with neurodegenerative disorders like Alzheimer’s disease, it represents one of the two major public health problems nowadays [4].
There is impressive information indicating that the obesity in MS is associated with low-grade inflammation of the white adipose tissue, which can subsequently lead to insulin resistance, impaired glucose tolerance and diabetes [5, 6]. Adipocytes actively secrete proinflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin (IL)-1β and IL-6 as well as leptin, and trigger a vicious circle which leads to additional weight gain largely as fat. Increased circulating levels of C-reactive protein and other inflammatory biomarkers also support the occurrence of inflammation in obesity [7, 8].

Several studies have shown that altered production of proinflammatory cytokines modulates adipocyte size and number through paracrine mechanisms that exert an important role in the regulation of fat mass [9–11]. The amount of proinflammatory molecules derived from adipose tissue in obese patients diminishes after weight loss [12]. Therefore, the fat cells are both a source as well as a target for TNF-α, IL-1β and IL-6.

The imbalance between inflammatory and anti-inflammatory signals is also a hallmark of aging and contributes to its progression. The term ‘inflammaging’ was introduced to underscore the importance of inflammation in senescence and its role in the development of age-related diseases as MS [13–15]. The levels of inflammatory mediators typically increase with age, even in the absence of acute infection or physiological stress. Such stress leads to inflammatory damage of cellular components, including proteins, lipids and DNA, and contributes to the age-related decline in physiological functions particularly in neural, immune and endocrine cells that regulate homeostasis. Therefore, the functional losses observed during aging include a slowly progressing, persistent type of oxidative stress resulting from the increased production of reactive oxygen species and reactive nitrogen species, which is enhanced by damage to the mitochondria [16, 17].

An age-related proinflammatory tendency is mostly unavoidable because of thymic involution and extended germ exposure, which both lead to the exhaustion of various subforms and developmental stages of leukocytes (details in Hardeland [17]). However, considerable interindividual differences exist in the velocity of these changes and the balance between proinflammatory and anti-inflammatory cytokines [17]. This may in part be due to genetic predispositions [18] as well as to histories of viral load [19], which contribute an immune risk profile (IRP) [20]. In some centenarians, either an ‘inverted IRP’ has been found or a combination of elevated pro- and anti-inflammatory cytokines, two conditions which are believed to represent protective phenotypes [21, 22].

On this background, it has been concluded that an increased tendency to inflammatory responses may place limits on lifespan [22], and that a well-functioning immune system is the strongest predictor of human longevity and healthy aging [23–25]. Therefore, inflammaging is associated with a state of oxidative stress, defined as an excessive production of reactive oxygen species and reactive nitrogen species compared to the level of antioxidants that act in the natural defense systems. Among antioxidants, melatonin has a special place for its antioxidant and anti-inflammatory properties, and partly for its role as a metabolic regulator [26, 27]. As melatonin modulates many processes involved in obesity and related metabolic disorders, it could have a therapeutic benefit in the treatment of obesity.

Melatonin and Inflammaging

A role of melatonin in attenuating inflammaging and its progression has been especially discussed with regard to options of treatment under conditions of reduced endogenous melatonin levels. Melatonin is one of the hormones known to decline during aging and, even more in a number of age-related diseases, changes that have been particularly documented in humans [26–28]. Interindividual variations observed among elderly persons may be explained, to a certain extent, by differences in the acquisition of melatonin-reducing diseases and disorders. Among these pathological causes of melatonin reduction, neurodegenerative processes have been identified as well as MS-related changes. For instance, decreases in melatonin were observed in coronary heart disease/cardiac syndrome X [28–34] and in diabetes type 2 [35, 36]. In either case, the pathophysiological nexus to inflammation and obesity is well established. Additional evidence from polymorphisms of human melatonin receptor genes indicates that deviations in melatonergic signaling favor the development of prediabetic states, diabetes type 2, elevated cholesterol and coronary heart disease (see [28]). Moreover, insulin resistance was induced in mice by knocking out the melatonin receptor MT1 [37] and also by pinealectomy [38, 39].

Counteractions of inflammaging by melatonin seem to occur at different levels. One of them concerns the correction of metabolic dysregulation (table 1), including the prevention of insulin resistance, an inflammation-promoting change and hallmark of MS [40–43]. Notably, melatonin was effective in suppressing insulin resistance in different models, tissues and methods of induction (table 1).
Although in these studies several regulatory pathways have been found to be modulated by melatonin treatment, the decisive effect at which the relevant routes converge is the reduced serine phosphorylation of IRS-1 (insulin receptor substrate 1), which has sometimes been accompanied by an upregulation of IRS-1 expression. The activated, tyrosine-phosphorylated insulin receptor is known to activate IRS subforms, in particular IRS-1, by tyrosine phosphorylation, a process that is inhibited by serine 307 phosphorylation which causes interruption of insulin signaling [69]. Melatonin and the melatonergic agonist piromelatine have been shown to reverse the blockade of this key step of insulin signal transduction [41, 43, 52]. Persistent insulin sensitivity has in recent

**Table 1. Effects of melatonin in animal models of hyperadiposity**

| Observation                                                                                                                                  | Ref.  |
|----------------------------------------------------------------------------------------------------------------------------------------------|-------|
| In rats fed from weaning with a high-fat diet, melatonin decreased body weight gain, feed efficiency and plasma glucose, leptin and triglyceride levels | 44    |
| In middle-aged rats receiving a high-calorie liquid diet, melatonin reduced weight gain and plasma insulin and leptin levels                  | 45    |
| In high-fat diet-fed mice, melatonin improved insulin sensitivity and glucose tolerance                                                      | 46    |
| In ovariectomized rats, melatonin was effective in reducing obesity                                                                          | 47–49 |
| In olanzapine-treated rats, melatonin was effective in reducing obesity                                                                       | 50    |
| In gold fish, body weight gain and specific growth rate were reduced by melatonin treatment in high-fat-fed rats                               | 51    |
| Melatonin and its analog piromelatonin inhibited weight gain and improved insulin sensitivity in high-fat-fed rats                              | 52    |
| Melatonin reduced body weight gain, visceral adiposity, blood triglyceride and insulin levels, and thioriburatic acid reactive substances under a high-calorie diet in rats | 54    |
| In young male Zucker diabetic fatty rats, melatonin treatment reduced mean weight gain without affecting food intake, decreased in a nonsignificant way blood pressure and improved dyslipidemia | 55    |
| Melatonin improved MS induced by high-fructose intake in rats without affecting food intake                                                   | 56–59 |
| Melatonin and its analog piromelatonin reduced blood pressure in spontaneously hypertensive rats                                            | 60    |
| Melatonin prevented the development of MS in male rats exposed to different light/dark regimens                                                | 61    |
| Melatonin attenuated high-fat diet-induced fatty liver disease in rats                                                                         | 62    |
| Melatonin, given at the time of reperfusion, prevented ventricular arrhythmias in isolated hearts from fructose-fed rats and spontaneously hypertensive rats | 63    |
| Melatonin ameliorated low-grade inflammation and oxidative stress in young Zucker diabetic fatty rats                                           | 64    |
| Protective effects of melatonin against metabolic and reproductive disturbances in polycystic ovary syndrome in rats                            | 65    |
| Melatonin normalized clinical and biochemical parameters of mild inflammation in rats with diet-induced MS syndrome                            | 66    |
| Melatonin counteracted changes in the hypothalamic gene expression of signals regulating feeding behavior in high-fat-fed rats                   | 67    |
| Melatonin reduced obesity and restored adipokine patterns and metabolism in obese (ob/ob) mice                                              | 68    |
years gained a particular relevance to inflammaging of the brain, because insulin resistance was shown to represent an early sign of low-grade neuroinflammation in demen-
tias, such as Alzheimer’s disease, and to aggravate their progression (see [70]).

A further level of action concerns the avoidance of processes that favor or lead to inflammation. This com-
prises calcium overload, excessive nitric oxide release that results in the formation of peroxynitrite, peroxynitrite-
derived free radicals (‘OH, CO₃⁻, NO₂⁻), and, finally, tyrosine nitration as well as mitochondrial dysfunction with its consequence of oxidative stress (summarized in [17, 27]). All these changes are known to initiate low-
grade inflammation in various organs, which is relevant to aging progression and comprises, in the central ner-
vous system, microglia activation and vicious cycles via overexcitation and damage by oxidants that ultimately cause impaired neuronal and astrocytic functions. In vari-
ous animal models, melatonin has been shown to counteract these detrimental processes to a substantial extent, by multiple antiexcitatory actions [17, 71], mitochondrial protection [17, 27, 42, 72–74], reduction of peroxynitrite-
related damage [75] and attenuation of microglia activa-
tion [27, 76, 77]. These effects go far beyond the frequent-
ly discussed direct antioxidant properties of melatonin based on scavenging of free oxygen radicals. In fact, an-
tioxidative protection by melatonin comprises various mechanisms that reduce the formation of free radicals rather than eliminating those already formed, as outlined in the concept of radical avoidance [78].

Immunological effects of melatonin represent a third area relevant to inflammaging. In this field, one of the major problems consists of melatonin’s multiple roles as an immune modulatory agent, which comprise both pro-
inflammatory and anti-inflammatory actions, which, consequently, also lead to either a pro-oxidant or anti-
oxidant balance [17, 27, 79]. At first glance, these observa-
tions appear to be contradictory, but they may only re-
fect the conditionality of melatonin’s actions. However, the precise reasons for when melatonin behaves in a pro-
or anti-inflammatory way remain to be identified, al-
though the strength of inflammation and the temporal sequence of initiation and healing processes may play a role. Moreover, changes due to immune remodeling in the course of senescence have to be taken into account. With regard to aging and age-associated diseases, proin-
flammatory/pro-oxidant effects are mainly observed un-
der rheumatic conditions, especially rheumatoid arthritis [80, 81]. However, under other conditions concerning se-
nescence, melatonin’s anti-inflammatory side seems to prevail. In the liver of aged, ovariectomized female rats, melatonin downregulated proinflammatory cytokines, such as TNF-α, IL-1β and IL-6, and upregulated the anti-
inflammatory IL-10 [82]. Corresponding findings were obtained in the dentate gyrus, in conjunction with an up-
regulation of sirtuin 1 [83], which is assumed to also poss-
several anti-inflammatory properties. Reductions of TNF-α and IL-1β and increased levels of IL-10 were also ob-
erved in the liver [84], pancreas [85] and heart [86] of the senescence-accelerated mouse strain SAMP8. Numerous other reports on anti-inflammatory actions of melatonin that were not obtained under conditions of aging, but in brain trauma, ischemia/reperfusion, hemorrhagic shock and various forms of high-grade inflammation including endotoxinemia and sepsis, have been summarized else-
where [17]. The applicability of these results to inflam-
maging and MS remains uncertain, but the data certainly underlie melatonin’s anti-inflammatory potential.

Melatonin-induced changes in gene expression re-
quire detailed analyses beyond the primary signaling pathways transduced by the MT₁ and MT₂ receptors via decreases in cAMP and ERK1/2 activation and modula-
tions by protein-protein interactions [87]. However, from a mechanistic point of view, it is not always possible to discriminate between direct actions and indirect effects via changes in the phase and amplitude of circadian oscil-
lators. While the changes induced in the circadian master clock, the suprachiasmatic nucleus (SCN), are relatively well understood, this is less the case in the numerous pe-
ripheral oscillators, which strongly differ in their depen-
dence on the SCN [88]. Since circadian oscillators are cel-
ular machineries, in which the core oscillators are modu-
lated by accessory oscillator components in an often cell type-specific way [89, 90], tissue-dependent differences can be expected. With regard to the cellular generation of circadian rhythms, oscillators may be assumed to be pres-
ent in any nucleated nonresting cell. Notably, peripheral oscillators exist in cells of particular relevance to MS, such as pancreatic β-cells [91], hepatocytes, adipocytes, car-
diomyocytes [92] and leukocytes [93, 94]. Moreover, the effects of melatonin are known in all these cell types, and the factors involved in metabolic sensing are modulated by this hormone, such as peroxisome proliferator-activ-
ated receptor-γ coactivator-1α (PGC-1α), peroxisome proliferator-activated receptor-γ, phosphoinositide 3-ki-
nase, protein kinase B (Akt), including the accessory osci-
lator components AMP kinase, nicotinamide phos-
phoribosyl transferase and sirtuin 1 [17, 90]. However, the effects of melatonin on all these factors are by far not uniform, but rather often contradictory or, at least, con-

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Therefore, it is of utmost importance to remain in the context of inflammaging and to discriminate between direct effects and indirect actions via circadian central or peripheral oscillators, demands that frequently have not been considered in respective studies. It would also be important to be aware of the fundamental rules of phase dependency of any action on circadian oscillators, which can lead to either up- or downregulations at different circadian times. Cases of direct effects not mediated by oscillators may be present in the induction of antioxidant enzymes in the rat liver and pancreas under inflammatory conditions, where melatonin promotes the expression and nuclear translocation of nuclear factor erythroid 2-related factor 2 that mediates the upregulation of the protective enzymes [95–97]. Correspondingly, melatonin reduced proinflammatory factors such as TNF-α, IL-1β and inducible nitric oxide synthase by suppressing the expression of nuclear factor-κB via recruitment of a histone deacetylase to its promoter [95, 96]. However, it is important to remain aware of the conditionality of melatonin effects on pro- and anti-inflammatory cytokines, which may be either up- or downregulated by this hormone [79]. Importantly, proinflammatory cytokines such as TNF-α, IL-1β and IL-6 have been shown to be reduced in various models of aging, whereas the anti-inflammatory IL-10 was typically stimulated [27]. Whether this would be also the case under conditions of beginning MS at younger age deserves future attention and thorough analysis, especially as the proinflammatory cytokines are usually upregulated by melato-
Melatonin not only affects white adipose tissue, but also increases the recruitment of brown adipocytes and increases their metabolic activity in mammals (see [108]). It has been speculated that the hypertrophic effect and functional activation of brown adipose tissue induced by melatonin can likely be applied 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 animals, in particular insulin resistance, dyslipidemia and obesity.

Evidence for Melatonin’s Therapeutic Value in MS: Clinical Studies

Table 2 summarizes the results of clinical studies on melatonin’s activity relevant to human MS. Medical literature was identified by searching databases (MEDLINE, Embase), including bibliographies from published literature and clinical trial registries/databases. Searches were last updated on March 23, 2016.

Type 2 diabetic patients have low circulating levels of melatonin [36] with a concurrent and expected upregulation of mRNA expression of melatonin membrane receptor [109]. Furthermore, allelic variants for melatonin receptors were associated with the level of fasting blood glucose and/or increased risk of type 2 diabetes [110–112] and with polycystic ovary syndrome [113]. These findings strongly bind melatonin to glucose homeostasis in blood.

Patients with coronary artery disease show decreased melatonin secretion [31–34], and among elderly hypertensive individuals, nocturnal urinary melatonin excretion was inversely associated with the nondipper pattern [114]. In turn, administered melatonin proved capable of reducing nocturnal blood pressure in hypertensives [117–120] and attenuated age-dependent disturbances of cardiovascular rhythms [121]. A meta-analysis of randomized controlled trials suggests that melatonin-controlled release is effective and safe in improving nocturnal hypertension [136]. As a pleiotropic molecule, melatonin may exert its antihypertensive and anti-remodeling effects through its antioxidant and scavenging properties, preserving the availability of nitric oxide and having sympatholytic effects that provide cardiovascular protection in MS.

As well as in animal models, clinical studies have shown that melatonin improves lipid profiles in MS patients. Melatonin treatment (1 mg/kg for 30 days) increased the levels of high-density lipoprotein cholesterol
Table 2. Clinical observations on melatonin relevant to MS

| Finding                                      | Subjects                        | Design            | Study duration | Treatment | Measured                                      | Results                                                                 | Ref. |
|----------------------------------------------|---------------------------------|-------------------|----------------|-----------|-----------------------------------------------|-------------------------------------------------------------------------|------|
| Low plasma melatonin levels in type 2 diabetic patients | 36 type 2 diabetic patients and 13 age-matched healthy subjects | Observational study | Unquoted       | None      | Serum melatonin levels measured by RIA between 02.00–04.00 and 16.00–18.00 h. Cardiovascular reflex tests, HRV, and 24-hour BP monitoring | Nocturnal melatonin levels and the nocturnal melatonin surge were significantly lower in the diabetic group. A negative correlation occurred between nocturnal melatonin levels and the degree of systolic BP decrease at night Patients with autonomic neuropathy showed decreased melatonin levels both at night and during day when compared to healthy controls. In patients with autonomic neuropathy, nocturnal melatonin levels were positively correlated with nocturnal high- and low-frequency components of HRV and systolic and diastolic BP at night | 36   |
| Increased number of melatonin receptors in type 2 diabetic patients | Surgical specimens of pancreas obtained from 25 patients who underwent partial or total pancreatectomy because of cancer or severe chronic pancreatitis | Observational study | Unquoted       | None      | Real-time PCR and immunocytochemistry of MT₁ and MT₂ receptors | The existence of the melatonin MT₁ and MT₂ receptors in human pancreatic tissue and in islets of Langerhans was assessed. mRNA transcript levels of melatonin receptors and their immunocytochemical expression were significantly higher in type 2 diabetic patients. Transcripts of the nuclear orphan receptors were also higher in human pancreatic tissue and islets of type 2 diabetic patients | 109  |
| Melatonin receptor gene polymorphism associated with high risk of type 2 diabetes | 36,610 individuals of European descent | Observational study | Unquoted       | None      | Leading association signals in ten genome-wide association scans | The strongest signal was observed at rs10830963, where each G allele (frequency 0.30 in HapMap CEU) was associated with an increase of 0.07 mmol/l in fasting glucose levels and reduced β-cell function as measured by homeostasis model assessment. The same allele was associated with an increased risk of type 2 diabetes | 110  |
| Genetics of MT₂ receptor associated with left ventricular function in hypertensive patients | 605 patients with arterial hypertension and cardiac ejection fraction ≤40% | Observational study | Unquoted       | None      | SNPs of MT₂ Cardiac parameters assessed by echocardiography | Analysis of SNPs rs10830962, rs4753426, rs12804291, rs10830963 and rs3781638 revealed two haplotypes, 1 and 2, with frequencies of 0.402 and 0.277, respectively. Carriers with haplotype 1 showed a higher mean 24-hour systolic BP. Haplotype 2 was significantly related to cardiac ejection fraction with an absolute increase of 1.8% in carriers vs. noncarriers | 111  |
| Genetics of MT₂ receptor associated with increased risk of impaired fasting glucose in youth with obesity | 346 Caucasians, 218 African-Americans, and 217 Hispanics, obese children and adolescents | Observational study | Variable       | None      | Oral glucose tolerance test. Evaluation of insulin secretion by the oral minimal model | The MTNR1B rs10830963 variant was associated with higher fasting glucose levels and lower dynamic β-cell response in Caucasians and Hispanics and conferred an increased risk of showing impaired fasting glucose on Caucasians, African-Americans and Hispanics | 112  |
| Melatonin receptor gene polymorphisms in polycystic ovary syndrome | 789 participants (Han Chinese) | Observational study | Unquoted       | Unquoted  | Genotypes were obtained by sequencing | An association was detected between MTNR1B rs2119882 and polycystic ovary syndrome, suggesting that the MTNR gene may indicate increased susceptibility to polycystic ovary syndrome in Chinese. No significant association was found for rs10830963. CC genotype carriers had higher levels of clinical and metabolic features of polycystic ovary syndrome than the TC and TT genotypes. A significant difference in transmission of allele C of rs2119882 was found between obese and nonobese women with polycystic ovary syndrome | 113  |
| Low melatonin production in coronary disease independent of β-adrenoceptor blockade | 48 male patients with angiographically documented severe coronary artery disease. 18 age-matched men, with no evidence of coronary sclerosis, served as controls | Observational cross-sectional study | Unquoted       | 24 patients took β-blockers daily in therapeutic dosages | aMT6s was measured by RIA from overnight urine | Urinary aMT6s concentration was significantly decreased in patients, and β-blocker treatment did not suppress melatonin production further | 31   |
| Finding | Subjects | Design | Study duration | Treatment | Measured | Results | Ref. |
|---------|----------|--------|----------------|-----------|----------|---------|-----|
| Low melatonin production in coronary disease independent of β-adrenoceptor blockade | Three groups of individuals were studied: (a) 24 healthy subjects; (b) 32 patients with chronic, stable, coronary disease; (c) 27 patients with unstable angina | Observational cross-sectional study | Unquoted | 24 patients with chronic coronary disease and 14 patients with unstable angina received β-blockers daily in therapeutic dosages | aMT6s was measured by RIA from overnight urine | Urinary aMT6s excretion was significantly lower in unstable angina patients than in healthy subjects or in patients with stable angina. aMT6s correlated negatively with age in healthy subjects, but not in coronary patients. aMT6s excretion in patients treated with β-adrenoceptor blockers did not differ significantly from coronary patients not receiving β-blockers | 32 |
| Low melatonin production in myocardial infarction | 25 patients diagnosed with acute myocardial infarction, and 25 patients with no evidence of coronary artery disease were studied | Observational cross-sectional study | Unquoted | Unquoted | Levels of melatonin, glutathione peroxidase and lipid peroxidation in serum samples collected at 10.00 h (light period) and 03.00 h (dark period) | A reduced nocturnal elevation of melatonin was found in the acute myocardial infarction group. Glutathione peroxidase levels were lower after acute myocardial infarction and did not show diurnal variations. In the control group, lipid peroxidation levels presented a light/dark pattern, but in the acute myocardial infarction group diurnal variations of this parameter were lost | 33 |
| Low melatonin production in coronary disease | 16 patients with angiographically documented coronary disease and 9 healthy controls | Observational cross-sectional study | Unquoted | Unquoted | Melatonin levels measured by RIA in serum samples collected every 2 h between 22.00 and 08.00 h | A large interindividual variation in the pattern of melatonin secretion was seen in both groups. Patients with coronary disease secreted less nocturnal melatonin at 02.00, 04.00 and 08.00 h than control subjects. Peak time of melatonin secretion was observed earlier in patients with coronary disease (02.00 vs. 03.45 h) | 34 |
| Low melatonin production in elderly hypertensives | 141 elderly hypertensives | Observational cross-sectional study | Unquoted | Unquoted | Overnight urinary melatonin excretion, ambulatory blood pressure and actigraphic physical activity | When participants were divided into two groups (high- and low-melatonin groups) by the cutoff value for identifying the top tertile, the characteristics, except for age, did not significantly differ between the two groups. In a multivariate analysis after adjustment for age, diabetes and daytime activity, the odds ratio for the nondipper pattern in the high-melatonin group was significantly lower than that in the low-melatonin group. The mean percentage systolic blood pressure nocturnal fall, adjusted for the former covariates, was significantly higher in the high-melatonin group than the low-melatonin group. Among elderly hypertensive individuals, nocturnal urinary melatonin excretion was significantly and inversely associated with the nondipper pattern | 114 |
| Melatonin treatment decreases nocturnal BP in type 1 adolescent diabetics | 11 normotensive adolescents with type 1 diabetes and 10 healthy controls | Randomized placebo-controlled double-blind crossover study | 2 weeks | 5 mg melatonin or placebo taken at bedtime p.o. | BP every 20 min for 24 h by an ambulatory device on the day before and on the last day of each treatment. Sleep measures were recorded by a diary and a wrist activity meter | Exogenous melatonin given to healthy normotensive adults reduces BP in the patients with type 1 diabetes, the decline in diastolic BP during sleep was significantly greater on melatonin than on placebo. No significant drug effect was present in the controls. No significant side effects were noted | 115 |
| Melatonin treatment decreases nocturnal BP in type 1 adolescent diabetics | 9 normotensive adolescents with type 1 diabetes and 8 healthy controls | Open-label trial | 1 week | 10 mg melatonin taken at bedtime p.o. | BP was measured every 20 min by ambulatory monitoring device for 24 h before treatment onset and on the last treatment day; sleep was monitored by diary and wrist actigraphy | In patients with diabetes, the mean BP during sleep was lower on melatonin than before treatment. In controls, there was no significant effect of melatonin on BP. There was no significant effect of sleep duration or number of awakenings on the BP responses | 116 |
| Melatonin treatment decreased high nocturnal BP in hypertensives | 16 mm with untreated essential hypertension | Randomized placebo-controlled double-blind crossover study | 3 weeks | Acute (single) and repeated (daily for 3 weeks) oral melatonin (2.5 mg) intake 1 h before sleep | BP was measured every 20 min by ambulatory monitoring device for 24 h before treatment onset and on the last treatment day | Repeated melatonin intake reduced systolic and diastolic BP during sleep by 6 and 4 mm Hg, respectively. The treatment did not affect the heart rate. The day-night amplitudes of the rhythms in systolic and diastolic BP were increased by 15 and 25%, respectively. A single dose of melatonin had no effect on BP. Repeated (but not acute) melatonin also improved sleep | 117 |
| Finding | Subjects | Design | Study duration | Treatment | Measured | Results | Ref. |
|---------|----------|--------|----------------|-----------|----------|---------|------|
| Melatonin treatment decreased high nocturnal BP in hypertensives | 18 women, 47–63 years of age with normal BP \( (n = 9) \) or treated essential hypertension \( (n = 9) \) | Randomized double-blind study | 6 weeks | Slow-release melatonin pill (3 mg) or placebo 1 h before going to bed | Ambulatory BP was recorded for 41 h at baseline at the end of each treatment period | Melatonin treatment did not influence diurnal BP but did significantly decrease nocturnal systolic, diastolic and mean BP without modifying the heart rate. The effect was inversely related to the day-night difference in BP | 118 |
| Melatonin treatment decreased high nocturnal BP in hypertensives | 38 treated hypertensive patients with confirmed nocturnal hypertension according to repeated 24-hour ambulatory BP monitoring | Randomized double-blind study | 4 weeks | controlled release melatonin 2 mg or placebo 2 h before bedtime | Ambulatory BP was recorded for 24 h | Melatonin treatment reduced nocturnal systolic BP significantly from 136 to 130 mm Hg, and diastolic BP from 72 to 69 mm Hg, whereas placebo had no effect on nocturnal BP. The reduction in nocturnal systolic BP was significantly greater with melatonin than with placebo and was most prominent between 02.00 and 05.00 h. Nocturnal BP control in treated patients with nocturnal hypertension | 119 |
| Melatonin treatment decreased high nocturnal BP in type 2 diabetic hypertensives | 60 dipper and 64 nondipper patients | Open-label trial | 8 weeks | 3 mg melatonin p.o. at bedtime was administered to 32 dipper and 34 nondipper patients for 4 weeks. Then the same patients received 5 mg of melatonin for the next 4 weeks. 28 dippers and 30 nondippers did not receive melatonin | Ambulatory BP was recorded for 24 h | Melatonin treatment reduced systolic BP significantly from 136 to 130 mm Hg, and diastolic BP from 72 to 69 mm Hg, whereas placebo had no effect on nocturnal BP. The reduction in nocturnal systolic BP was significantly greater with melatonin than with placebo and was most prominent between 02.00 and 05.00 h. Nocturnal BP control in treated patients with nocturnal hypertension | 120 |
| Melatonin treatment attenuated age-dependent disturbances of cardiovascular rhythms | 97 normotensive and hypertensive volunteers (63–91 years old) | Placebo-controlled trial | 3 weeks | 1.5 mg melatonin or placebo p.o. each day at 22.30 h for 2 weeks | Systolic and diastolic BP and HR were measured using semiautomated devices at 03.00, 08.00, 11.00, 14.00, 17.00, 23.00 h each day of the 1st and the 3rd week | The 24-hour HR rhythm was monophasic with a steeper increase in the morning. The daily systolic and diastolic BP rhythms were bimodal. In reference to previously reported data of younger subjects, mean BP was elevated, particularly the nocturnal fall was less pronounced. Also, the overall systolic BP variability was higher as was the percentage of the 12-hour component. Both values and also the systolic and diastolic BP were reduced during melatonin treatment. The hypertensive effect of melatonin was most pronounced between 03.00 and 08.00 h. Melatonin not only has a direct hypotensive effect, it also stabilizes the internal temporal order enhancing the circadian component and the synchronization between rhythms of different physiological functions | 121 |
| Melatonin treatment prevented catecholamine-induced hypercoagulability | 45 healthy young men | Placebo-controlled trial | 1 h | A single oral dose of either 3 mg melatonin \( (n = 24) \) or placebo \( (n = 21) \). One hour thereafter, they underwent a standardized short-term psychosocial stressor | Plasma levels of clotting factor VII activity (FVII:C), FVIII:C, fibrinogen, D-dimer, and catecholamines were measured at rest, immediately after stress, and 20 and 60 min after stress | Compared with the melatonin group, the placebo group showed a greater increase in absolute D-dimer levels from rest to immediately after stress and a significant recovery of D-dimer levels from immediately after stress to 60 min thereafter. Stress-induced changes in FVII:C, FVIII:C, fibrinogen, and catecholamines did not significantly differ between groups. Oral melatonin attenuated the stress-induced elevation in the sensitive coagulation activation marker D-dimer without affecting catecholamine activity | 122 |
| Melatonin inhibited human platelet aggregation | 22 healthy young men | In vitro study | Unquoted | \( 10^{-9} \) to \( 10^{-5} \) M melatonin | Platelet aggregation and TxB2 production in PRP | Melatonin inhibited in a dose-dependent way ADP-induced platelet aggregation with individual inhibitions 40% or more at \( 10^{-6} \) to \( 10^{-5} \) M concentrations and a higher global inhibition at 18.00 h. TxB2 production elicited by AA in the evening was inhibited significantly in a concentration-related manner by \( 10^{-9} \) to \( 10^{-5} \) M melatonin, while during the morning hours the inhibition was significant only at \( 10^{-6} \) M or higher melatonin concentrations. Melatonin depression of TxB2 generation was about 2-fold greater at 18.00 h than at 08.30 h. | 123 |
Aspartate aminotransferase and γ-glutamyl transpeptidase (GGT)

BMI, plasma alanine aminotransferase, aspartate aminotransferase, γ-glutamyl-transpeptidase, alkaline phosphatase, cholesterol, triglycerides, glucose and melatonin

Randomized controlled study

42 patients with histological evidence of nonalcoholic steatohepatitis

Randomized placebo-controlled study

30 patients were allocated to 2 × 5 mg/day melatonin p.o., and 12 patients were allocated to placebo

BMI, plasma alanine aminotransferase, aspartate aminotransferase, γ-glutamyl-transpeptidase, alkaline phosphatase, cholesterol, triglycerides, glucose and melatonin

Aspartate aminotransferase and γ-glutamyl transpeptidase decreased significantly in melanin-treated patients only. Plasma levels of triglycerides and glucose as well as BMI in controls and melanin-treated patients were not significantly different from baseline.

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### Table 2 (continued)

| Finding | Subjects | Design | Study duration | Treatment | Measured | Results | Ref. |
|---------|----------|--------|----------------|-----------|----------|---------|------|
| Melatonin treatment improved enzymatic profile in patients with nonalcoholic liver steatosis | 42 patients with histological evidence (liver biopsy) of nonalcoholic steatohepatitis (follow-up of the previous study) | Randomized placebo-controlled study | 24 weeks | 30 patients were allocated to 2 × 5 mg/day melatonin p.o., and 12 patients were allocated to placebo | BMI, plasma alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, cholesterol, triglycerides, glucose and melatonin | Aspartate aminotransferase and γ-glutamyl transpeptidase decreased significantly in melatonin-treated patients only. Plasma levels of triglycerides and glucose as well as BMI in controls and melatonin-treated patients were not significantly different from baseline | 132 |
| Melatonin alone or in combination with metformin improved glycemic control in type 2 diabetic patients | 46 type 2 diabetic patients | Placebo-controlled, double-blind trial | 90 days | Patients were allocated to 3 groups: (a) single daily oral doses of both 10 mg melatonin and 50 mg zinc acetate alone; (b) 10 mg melatonin and 50 mg zinc acetate in addition to the regularly used metformin; (c) placebo, all given at bedtime | Fasting plasma glucose, glycated hemoglobin (HbA1C) and serum C-peptide before starting the treatment and after 30 and 90 days of treatment | Daily administration of melatonin and zinc improved the impaired fasting and postprandial glycemic control and decreased the level of glycated hemoglobin. The addition of this treatment regimen in combination with metformin improved the tissue responses to this oral hypoglycemic agent | 133 |
| Melatonin treatment ameliorated oxidative stress and inflammatory parameters of obese women | 44 obese women | Randomized double-blind, placebo-controlled trial | 40 days | 6 mg melatonin p.o. at bedtime | Serum TNF-α, IL-6, hsCRP, TAC, and MDA levels | In the melatonin group, mean serum TNF-α, IL-6, hsCRP and MDA levels decreased significantly, whilst in the placebo group the decrease in these values was not statistically significant. The mean TAC level increased in the melatonin group, whereas it decreased slightly in the placebo group. Melatonin decreased significantly TNF-α and IL-6 levels | 134 |
| Acute melatonin administration in healthy women impaired glucose tolerance | 21 healthy young women | Randomized double-blind, placebo-controlled trial | 4 nonconsecutive days | 5 mg melatonin p.o. | Glucose tolerance was assessed by oral glucose tolerance tests 15 min after melatonin or placebo administration on 4 occasions: in the morning (09.00 h) and evening (21.00 h) | Melatonin administration significantly impaired glucose tolerance. The effect of melatonin on insulin response depended on the time of day. In the morning, melatonin decreased glucose tolerance primarily by decreasing insulin release, while in the evening, by decreasing insulin sensitivity | 135 |

AA = Arachidonic acid; aMT6s = 6-sulfatoxymelatonin; BP = blood pressure; hsCRP = human serum C-reactive protein; HR = heart rate; HRV = heart rate variability; MDA = malondialdehyde; PRP = platelet-rich plasma; RIA = radioimmunoassay; SNP = single nucleotide polymorphism; TAC = total antioxidant capacity; TBARS = thiobarbituric acid reactive substances; TxB2 = thromboxane B2.
in peri- and postmenopausal women [137]. Several mechanisms may explain the hypolipidemic effects of melatonin, such as reduced intestinal absorption of cholesterol [138] or inhibiting cholesterol biosynthesis [139].

Catecholamine-induced hypercoagulability in acute stress that contributes to the growth of thrombus after rupture of coronary plaque was prevented by the administration of melatonin [122]. This was probably mediated by the reported inhibitory effects of melatonin on platelet aggregation [123–125]. In light of these results, melatonin may have a protective effect in reducing atherothrombotic risk in MS.

Several studies support the beneficial role of melatonin in patients with MS. Melatonin treatment ameliorated MS in obese patients [126, 127] as well as in bipolar and schizophrenic patients after treatment with second-generation antipsychotics [128–130]. Melatonin administration normalized MS in elderly hypertensive patients [140] and improved the enzymatic profile in patients with alcoholic liver steatosis [131, 132]. The combination of melatonin and zinc acetate, when used alone or in combination with metformin improved glycemic control in type 2 diabetic patients [133], and an inverse relationship between urinary 6-sulfatoxy melatonin excretion and insulin levels and insulin resistance was reported in healthy women in the Nurses’ Health Study cohort [141]. However, a recent placebo-controlled single-blind study including 21 healthy women reported that melatonin (5 mg) decreased glucose tolerance [135]. Further studies are needed to clarify this controversy.

Overall, the results discussed above suggest that melatonin therapy may be beneficial for patients with MS. Undoubtedly, more studies are needed to evaluate an appropriate time/duration of treatment/dose relationship in the administration of melatonin in patients with MS.

As with many diseases, particularly those related to MS, hypertension, cardiovascular disease, obesity, diabetes, etc., the evidence supports the hypothesis that metabolic rhythm attenuation and/or disruption contribute to the etiology of the disease. Diabetes mellitus, a significant risk factor for developing heart disease and/or MS in humans, is associated with a phase change in the cardiac circadian clock [142]. Actually, metformin, a diabetes medication commonly used under Clinical Commissioning Group guidelines, increases the circadian amplitude of the metabolic sensor AMP kinase and modulates liver casein kinase 1α (CK1α) and muscle CK1ε, two regulators of the respective circadian core oscillators, effects that influence the expression and temporal patterns of several clock components and key genes of energy metabolism [143].

Conclusions

Melatonin can provide an innovative strategy in MS by combining its effects on the circadian rhythm with its cytoprotective properties. Melatonin protects against several MS comorbidities, such as diabetes and concomitant oxypektatic-mediated damage, inflammation, microvascular disease and atherothrombotic risk. At an early stage of the treatment of MS, a nondrug approach such as changing the lifestyle, low-fat diet and exercise is commonly recommended. Patients who are refractory to these changes are treated with antihypertensive drugs (antidiabetic, lipid-lowering drugs) that can have significant side effects.

Melatonin may thus be beneficial from the initial phases of MS treatment. It has a high safety profile and shows a reduced toxicity, thus differing from many pharmaceutical agents used in MS patients. Moreover, melatonin is usually remarkably well tolerated at very high doses [144]. As melatonin is a short-lived molecule that has a limited duration of action (half-life from 0.54 to 0.67 h, analogs with a high affinity for melatonin receptors and a longer duration of action have been synthesized to treat circadian disorders [145]. To what extent the new melatonergic agents approved by the US Food and Drug Administration or the European Medicines Agency (ramelteon, agomelatine, tasimelteon) share the protective activity of melatonin in MS remains to be defined. There is evidence that ramelteon given daily in drinking water (8 mg/kg) for 8 weeks to spontaneously hypertensive male Wistar-Kyoto rats significantly attenuated systolic blood pressure and body weight gain associated with age [146]. In addition, an investigational melatonergic agonist, piromelatine (NEU-P11), has been reported to be similarly effective or even superior to melatonin in improving some MS-associated parameters [52, 60, 147].

Given higher binding affinities, longer half-life and high relative potencies of the various melatonin agonists, studies using 2 or 3 mg/day of melatonin are probably inadequate to provide an adequate comparison with the effects of the natural compound. Doses that considerably exceed those usually applied have been found to be safe, e.g. in the treatment of ALS patients who received either 60 mg/day orally for up to 13 months [148] or 300 mg/day enterally for up to 2 years [144]. In a phase I dose escalation study in healthy volunteers to assess the tolerability and pharmacokinetics of 20-, 30-, 50-, and 100-mg oral doses of melatonin, no adverse effects after oral melatonin other than mild transient drowsiness, with no effects on sleeping patterns, were seen [149]. Therefore,
Further clinical trials using dosages of melatonin in the range of 50–100 mg/day appear to be reasonable and are warranted. The priorities for populations, outcomes, and durations of these studies must be defined.

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