Melatonin supplementation in the management of obesity and obesity-associated disorders: a review of physiological mechanisms and clinical applications

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Abstract

Despite the evolving advances in clinical approaches to obesity and its inherent comorbidities, the therapeutic challenge persists. Among several pharmacological tools already investigated, recent studies suggest that melatonin supplementation could be an efficient therapeutic approach in the context of obesity. In the present review, we have amalgamated the evidence so far available on physiological effects of melatonin supplementation in obesity therapies, addressing its effects upon neuroendocrine systems, cardiometabolic biomarkers and body composition. Most studies herein appraised employed melatonin supplementation at dosages ranging from 1 to 20 mg/day, and most studies followed up participants for periods from 3 weeks to 12 months. Overall, it was observed that melatonin plays an important role in glycaemic homeostasis, in addition to modulation of white adipose tissue activity and lipid metabolism, and mitochondrial activity. Additionally, melatonin increases brown adipose tissue volume and activity, and its antioxidant and anti-inflammatory properties have also been demonstrated. There appears to be a role for melatonin in adiposity reduction; however, several questions remain unanswered, for example melatonin baseline levels in obesity, and whether any seeming hypomelatonaemia or melatonin irresponsiveness could be clarifying factors. Supplementation dosage studies and more thorough clinical trials are needed to ascertain not only the relevance of such findings but also the efficacy of melatonin supplementation.

Keywords: Obesity therapy; weight loss; melatonin; obesity; endocrinology.

1. Introduction

According to results from the National Health and Nutrition Examination Survey 2013–2014 [1], more than 2 in every 3 adults in the United States were overweight or had obesity. In 2017 in England, over 64% of the adult population were overweight or obese [2]. It is estimated that the United Kingdom National Health Service spent £6.1 billion on overweight and obesity-related ill-health in 2014 to 2015 [3]. Most significantly however, numbers are predicted to increase for both countries in the near and far future [4, 5], and the costs associated with obesity are simply unaffordable. It is believed that the exponential increase in obesity in recent times may be explained by likely synergistic interactions amongst increased calorie consumption, low nutritional value of foods, increased sedentariness, reduced time and quality of sleep, and epigenetic susceptibility [6-8].

The relationship between obesity and several other non-communicable diseases is becoming unarguably evident [6, 9]. Even if obese individuals do not have an associated diagnosis of hypertension, type II diabetes mellitus (T2DM) or dyslipidaemia, yet there is an approximate 49% increased risk for coronary heart disease; 7% increased risk for cerebrovascular diseases and 96% increased risk for heart failure [10]. A prospective cohort study demonstrated that every kg of weight gained annually over 10 years was associated with a 49% increased risk of developing T2DM. On the other hand, every kg of weight lost annually over the same period was associated with a 33% lowered risk of developing T2DM [11]. Despite being a well-known disease and the importance of its treatment well elucidated, obesity remains a topic of global interest. The full elucidation of the aetiologies associated with obesity, which include factors as diverse as genetic, epigenetic, environmental [12, 13], psychological and social [14, 15], and the further advancement of effective pharmacological therapies [16] may, in tandem, increase the likelihood of successful management and possibly treatment.

Melatonin, a pineal hormone, is a molecule associated in a way or another with probably all physiological systems, aiming to fulfil its functional integrative roles in central nervous system activity, sleep and wakefulness cycles, energy metabolism and thermoregulation, immune, reproductive, endocrine, cardiovascular, respiratory and excretory systems [17]. This highly pervasive, integrative physiological regulatory role attributed to melatonin adapts the organism to the present environmental challenges, by means of the so-called immediate effects. At the same time, melatonin equips the

organism with the capacity to deal with future predictive events, by means of the so-called prospective effects. Additionally, melatonin synchronizes the organism's physiology and behaviour to the daily (circadian internal zeitgeber) and annual photoperiodical (seasonal synchronizer) cycles.

Whilst the effectiveness of melatonin supplementation on improving sleep quality has been investigated [18-20], its supplementation has also been proposed as beneficial for the treatment of obesity and inherent metabolic dysregulations [21-25]. Such hypothesis is derived from a plethora of experimental studies and from a few clinical studies. Nevertheless, the actual role of melatonin as adjuvant for the treatment of obesity, if any, is far from being fully understood. In the present narrative review, we have identified and further discussed physiological pathways in which melatonin, or the lack of thereof, could be associated with energy metabolism and obesity. We have also attempted to discuss how melatonin supplementation could ameliorate the metabolic disturbances associated with obesity.

2. Melatonin characteristics

2.1. Biosynthesis and Pharmacokinetics of exogenous supplementation

The light/dark environmental information is crucial for the regulation of melatonin endogenous biosynthesis. During the night most melatonin is synthesized, approximately 80%, reaching serum concentrations between 80 and 120 pg/mL [26], whilst during the day its concentration remains lower, around 10 pg/mL or less [27]. Salivary melatonin, a reliable and easily accessible biomarker, shows concentrations at around 4 pg/mL in dim-light melatonin onset (DLMO), which normally occurs between 19:30 and 22:00 in adults and between 19:00 and 21:00 in children (6 to 12 years) [28]. Plasma melatonin concentration during DLMO is about three times the values obtained for salivary melatonin concentration, around ≤10 pg/mL [29].

Melatonin is endogenously synthesised from its precursor tryptophan. Melatonin production in the central nervous system is carried out mainly in pinealocytes, cells of the pineal gland, starting with the hydroxylation of tryptophan to 5-hydroxytryptophan (5-HTP). A subsequent decarboxylation catalysed by the 5-hydroxytryptophan decarboxylase generates serotonin (5-hydroxytryptamine (5-HT)). The arylalkylamine-N-acetyltransferase (AANAT) acetylates serotonin to N-acetylserotonin (NAS), which is methylated by the acetylserotonin-O-methyltransferase (ASMT), producing melatonin.

Exogenous melatonin, on the other hand, is shown to feature rapid absorption and plasma distribution. Its plasma concentration peaks at approximately 50 minutes after oral administration, with half-life of approximately 45 minutes [30]. In healthy volunteers, 10 mg melatonin oral supplementation resulted in mean t1/2 absorption of 6.0 (3.1) min, mean tmax of 40.8 (17.8) min, median (IQR) maximum concentration (Cmax) of 3,550.5 (2,500.5–8,057.5) $pg.mL^{-1}$ and mean t1/2 elimination of 53.7 (7.0) min [31]. The same dosage administered intravenously showed a median Cmax of 389,875.0 (174,775.0–440,362.5) $pg.mL^{-1}$ and mean t1/2 elimination of 39.4 (3.6) min [31]. The rapid increase in plasma concentrations following intravenous as well as oral supplementation has influenced decision-making practice in therapies at secondary and tertiary healthcare levels, given the important antioxidant and hypnotic roles of melatonin, and as an important pharmacological aid in critically ill patients [32, 33].

Lastly, melatonin oral supplementation is metabolised in the liver by the cytochrome P450 CYP1A2 enzyme [34], where a hydroxylation reaction converts melatonin to its main metabolite 6-hydroxymelatonin sulfate, also known as 6-sulfatoxymelatonin [35, 36]. This metabolite, when measured in urine, has been used clinically as a biomarker in the fields of psychiatry [37, 38], gynaecology [22, 39] and sleep medicine [40, 41].

2.2. Mechanism of action and metabolic regulation

Melatonin receptors are G protein-coupled receptors (GPCR), in which two types are found in humans and mammals (MT1 and MT2) and a third form (Mel1C) is found in fish, amphibians and birds [42, 43]. The melatonin MT1 receptor (also called Mel1a, ML1A or MTNR1A) consists of 350 amino acids, whilst the melatonin MT2 receptor (also called Mel1b, ML1B or MTNR1B) consists of 362 amino acids [43]. Currently, melatonergic agonist drugs, such as agomelatine, ramelteon and tasimelteon, have been widely used in psychiatric (e.g. major depression), oncological (e.g. glioblastoma) and sleep diseases (e.g. insomnia) [44-46].

The systematic regulation induced by melatonin, from the binding to its receptors, varies according to each organ [47]. Our research group has recently discussed the effects of melatonin in the ovary and, amongst its various effects, melatonin activation reduces cyclic AMP (cAMP) levels in granulosa cells (GCs), resulting in lowered synthesis of androgenic hormones [22]. Furthermore, in the pancreas, melatonin reduces cAMP as well as cGMP levels, effects that control insulin

and glucose homeostasis [48]. Such regulation, together with the circadian phase control, allows a rhythmic fluctuation in glucose tolerance that is increased in the early hours of the day and reduced at night [49, 50].

Regarding its other physiological effects, the antioxidant capacity of melatonin is mainly associated with increased production of antioxidant enzymes, including superoxide dismutase, catalase, glutathione and others, as well as its antioxidant effect *per se* [51, 52]. The anti-inflammatory role of melatonin is evidenced, amongst several pathways, through its inhibitory effect on NF-kB activity, identified in several diseases with pro-inflammatory aetiology, such as rheumatoid arthritis and osteoarthritis [53]. Melatonin has also been shown to inhibit the NLR Family Pyrin Domain Containing 3 (NLRP3), an important inflammatory mediator known to reduce the inflammatory cascade for the synthesis of pro-inflammatory cytokines [54].

3. Melatonin supplementation and adipose tissue

3.1. Modulation of adipose tissue activity

It has been demonstrated in rats that melatonin appears to induce lipolysis [55, 56], which is further corroborated by findings of reduced lipolysis and increased lipogenesis in pinealectomized rats [57]. Mechanistically, melatonin administration not only reduces total body fat, but more specifically intra-abdominal, retroperitoneal, hepatic and visceral fat in experimental models [58]. The lipolytic mechanisms modulated by melatonin are illustrated in Figure 1.

[Insert figure 1 here]

Several animal studies have provided insights into the roles of melatonin on expanding brown adipose tissue (BAT) volume and metabolic capacity, as confirmed by research employing rats [59, 60], sheep [61], hamsters [62] and rabbits [63]. A recent study employing diet-induced obese rats found that the time of the day in which melatonin was administered had an impact on BAT metabolism, being more effective late in the evening as compared to morning administration [64].

The relationship between melatonin and BAT activity has been investigated in humans [58]. Recently, Halpern *et al* (2019) demonstrated in a small but important proof-of-concept study that the daily administration of 3 mg melatonin for 3 months increased BAT volume in the four patients investigated, who presented melatonin

deficiency due to either radiotherapy or surgical removal of their pineal gland [65]. Putatively, the activity of melatonin upon BAT appears to be mediated by MT2 receptors [66], which were firstly identified in human retina by Dubocovish in 1985 [67] and cloned by Reppert *et al* ten years later [68].

The hypothesis that melatonin supplementation could possibly modulate BAT activity in overweight and obese individuals has been proposed, but to the best of our knowledge no clinical trial has yet been carried out to investigate this specific effect. On those grounds, further research is imperative to elucidate the roles of melatonin upon BAT activity in obesity, since this role has been currently explored as a clinical target in therapies for obesity and other metabolic disorders, including T2DM [69, 70].

3.2. Body composition: weight and fat loss

Melatonin supplementation hindered weight gain in a model of high-fat diet obese rats [71]. In the same study, pinealectomized rats developed not only obesity but also T2DM, with melatonin supplementation partially mitigating the high glucose levels observed. The effects of melatonin supplementation as modulator of body composition have been previously discussed in previous studies [72-74]. In a double-blind randomised clinical trial (RCT), Amstrup *et al* [75] reported that a group of postmenopausal eutrophic women diagnosed with osteopenia and treated with melatonin (1 and 3 mg/day) for 12 months showed improved body composition, measured by dual X-ray absorptiometry, as compared to the women who received placebo [75]. The researchers also found at the end of the 12 months study a 6.9% reduction in fat mass combined with a 5.2% increase in lean mass in the melatonin-receiving group. However, as that study did not report dietary patterns nor levels of physical activity, caution is required before any clinical recommendation can be made.

In an investigation associating melatonin supplementation (10 mg/day 1 h before bedtime) combined with calorie-restricted diet (1,000–1,200 kcal/d for women and 1,400–1,600 kcal/d for men) in adult patients with obesity, the intervention with melatonin was able to enhance weight loss further, from 113.6 to 105.9 kg, as compared to the placebo-receiving group, from 114.4 to 109.8 kg. Additionally, malondialdehyde (MDA) levels, an oxidative stress biomarker, had greater reduction in the melatonin group, from 34.3 to 24.5 nmol/g Hb, when compared to placebo, from 30.1 to 27.4 nmol/g Hb [76]. The apparent reduction of oxidative stress induced by melatonin observed in this study may suggest an important therapeutic approach for

obese patients, since exacerbated oxidative stress induced by obesity is related to a greater risk for insulin resistance and increased accumulation of abdominal fat [77-79].

Walecka-Kapica *et al* [80] found a significant reduction in body mass index (BMI) in postmenopausal women (mean age 56.9 ± 5.3) with high (from 29.62 to 27.88 kg/m²; $p<0.001$) and normal (from 22.07 to 21.86 kg/m²; $p<0.05$) body weight under combined therapy of melatonin supplementation (5 mg/day for 24 weeks) and a balanced diet of 1,500 kcal/d. In addition to this effect on body composition, participants who received melatonin supplementation showed improved sleep quality, measured by the insomnia severity index (ISI). In their investigation [80] and in another one published by the same research group [81], a significant relationship was reported between decreased levels of 6-sulfatoxymelatonin and higher BMI in postmenopausal overweight women. We recently emphasized the importance of 6-sulfatoxymelatonin as a biomarker in the field of gynaecology [22] and, in conjunction with the findings of Walecka-Kapica *et al*, we reemphasize its usefulness as a measurable tool in clinical practice, as is in the case of women suffering with hormone dysregulation. In another study involving postmenopausal overweight women, melatonin supplementation at a dosage of 5 mg/day associated with fluoxetine at 20 mg/day for 24 weeks resulted in improved sleep quality, in addition to reduced appetite and BMI, from 30.9 to 26.3 kg/m² [82].

In 2014, Romo-Nava *et al* investigated in a RCT the effects of melatonin supplementation in patients suffering with bipolar disorder or schizophrenia and treated with second-generation antipsychotics [83], which are known by weight gain as one of their textbook side effects [84]. After eight weeks of placebo or melatonin supplementation at 5 mg/day, the researchers found that the placebo-receiving group gained significantly more weight (2.2 kg) than the melatonin-receiving group (1.5 kg). Olanzapine is one of the various second-generation antipsychotic drugs currently available [84], and most interestingly, Modabbernia *et al* [85] found in a RCT a protective effect of melatonin supplementation against weight gain induced by olanzapine treatment. Schizophrenia patients treated with olanzapine and supplemented with melatonin at the dosage of 3 mg/day for 8 weeks gained 2.2 kg body weight, as compared to the 5.4 kg body weight gain observed in the matched placebo-supplemented group [85]. The studies of Amstrup [75], Romo-Nava [83] and Modabbernia [85] did not report data on dietary intake or levels of physical activity.

The question whether melatonin has a direct effect upon fat levels, or whether the changes in body weight observed were the result of an indirect pathway associated with decreased appetite or increased metabolic rate, remain unanswered. Further studies are needed to fully elucidate the role of melatonin in protecting against weight gain induced by antipsychotic pharmacotherapy.

In the opposite direction to what has so far been discussed in this section, other studies have shown that melatonin supplementation have not ameliorated body composition in obese women [86], neither in diabetic [87] nor in metabolic syndrome [88] patients. The clinical trials that have investigated the effects of melatonin supplementation on body composition appraised in the present study are summarized in Table 1. Several studies have not reported data on dietary intake nor levels of physical activity and are noticeably different in their methodologies. For those reasons, additional comprehensive clinical trials are required to further elucidate melatonin actions in energy metabolism.

[Insert table 1 here]

Two recent meta-analyses have appraised clinical trials focusing on body composition and melatonin supplementation. In the meta-analysis of Mostafavi and colleagues published in 2017 [89], which covered seven clinical trials and a total of 244 participants based on Mostafavi's inclusion criteria, melatonin supplementation alone was ineffective in inducing weight loss, but showed a positive effect when combined with other strategies. A more recent meta-analysis, published in 2019, showed that melatonin supplementation alone improved selected lipid biomarkers but was ineffective in inducing weight loss [90]. Based on their inclusion criteria, that meta-analysis selected 6 clinical trials and a sample of 338 individuals for effects on weight loss. The authors themselves recognised a few limitations in their selection of clinical trials, such as low sample size, lack of clarity in the blinding process of the studies appraised and selection of participants, as well as heterogeneity attributed to the differences in characteristics, including range of diseases, amongst the selected studies.

4. Musculoskeletal metabolism and lean body mass

The adipose tissue and skeletal muscle are the two largest insulin-responsive tissues in the human body, and responsible for the secretion of adipokines and myokines, respectively [91]. Both families of bioactive molecules act in synergy to regulate a vast range of biochemical pathways involved in energy metabolism and lipid storage, and it is widely accepted that disturbances in the lean/fat mass ratio lead to several metabolic diseases. Muscle mass is protective against not only obesity but also other metabolic disturbances, including sarcopenic obesity [92], which is characterized by impaired function and strength of skeletal muscle [93]. The main aggravating factors in sarcopenic obesity include exacerbated oxidative stress and skeletal muscle inflammation [94]. On those grounds, an adequate programme of physical activity associated with specific nutritional interventions that focus on muscle protection is a determining strategy in sarcopenic obesity [92, 95]. The previously described antioxidant properties of melatonin could positively influence myocyte metabolism, in which redox signalling molecules modulate several physiological processes [96]. The study of Amstrup *et al* [75], referred earlier, found a small but important increase of 2.6% in lean body mass, adjusted for BMI, in the melatonin group as compared to placebo. A shortfall, however, is that Amstrup did not report physical activity patterns or dietary habits.

More recently, Rondanelli *et al* investigated in a RCT the effects of melatonin supplementation at the dosage of 1 mg/day, associated or not with essential amino acid supplementation at the dosage of 4 g/day for 4 weeks, in a total of 159 elderly hospitalized sarcopenic patients, segregated in that study into 4 groups [97]. The researchers found that melatonin-only supplementation worsened protein metabolism by decreasing albumin levels, but also that melatonin when combined with essential amino acid supplementation improved fat-free mass [97]. A few limitations in the design of Rondanelli's study may be identified. Firstly, plasma albumin *per se* is not the best biomarker for muscle status [98], a fact that the authors themselves appreciate in their study. Secondly but most importantly, despite the randomisation been made by software, it is an unfortunate fact that gender distribution was not homogeneous across the four groups: the ratio women/men was 2.4 in the placebo group, 6.0 in the melatonin-only group, 3.4 in the essential amino acid-only group, and 1.4 in the essential amino acid plus melatonin group. It is possible to speculate that the differences in gender distribution across the four groups may have influenced the results on body composition and lean body mass, and on those grounds the observed

effects of melatonin supplementation may not be a true representation of its actual metabolic potential for that population.

Other studies have investigated the roles of melatonin on muscle homeostasis by focusing on its antioxidant and anti-inflammatory actions, as well as its indirect effects by means of ameliorating chronoregulation [99, 100]. However, further clinical trials are needed to thoroughly investigate the effects of melatonin supplementation not only on musculoskeletal metabolism, but also in other systems with low fat content, including skin, blood cells, bones, and internal organs in general.

5. Cardiovascular and lipid profile outcomes

A few review papers have reappraised the effects of melatonin supplementation in the field of cardiology [101-103]. As an adjuvant antihypertensive agent, melatonin has been proposed as a modulator of oxidative stress at vascular endothelium level [104]. In a small population of healthy volunteers, the acute effects of a single 3 mg dose of melatonin were tested. As a result, forearm blood flow and vascular conductance were increased 45 minutes after its ingestion, as compared to the same individuals taking placebo a few days later [105]. Such effects may be explained by activation of MT2 receptors and subsequent vasodilation of the capillary bed [47, 106].

Scheer *et al* [107] found that melatonin supplementation at the dosage of 2.5 mg/day 1 hour before bedtime for three weeks reduced systolic blood pressure by 6 mmHg and diastolic blood pressure by 4 mmHg in a group of 16 men suffering with untreated essential hypertension. Along similar lines, Koziróg *et al* [88] observed that melatonin supplementation at the dosage of 5 mg/day 2 hours before bedtime for two months reduced systolic blood pressure by nearly 13 mmHg and diastolic blood pressure by 6.5 mmHg in a group of metabolic syndrome patients unresponsive to positive lifestyle interventions.

Corroborating the evidence referred above, a recently published systematic review and meta-analysis of RCTs showed a small but statistically significant effect of melatonin supplementation on blood pressure in patients with metabolic disorders [108]. The authors found that overall 2 to 10 mg/day of melatonin supplementation during intervention periods ranging from 4 to 12 weeks were effective in reducing systolic blood pressure by 0.87 mmHg, and diastolic blood pressure by 0.85 mmHg, in patients with metabolic disorders [108].

It has been demonstrated that not only blood pressure can be influenced by melatonin supplementation, but so can lipid profile and antioxidant status. Koziróg *et al* [88], apart from their findings on blood pressure described earlier, also found significantly lowered low-density lipoprotein cholesterol (LDL-c) concentration, from 149.7 mg/dL to 139.9 mg/dL. In the same study, the authors found reduced levels of total cholesterol, from 233.4 mg/dL to 220.7 mg/dL, and triglycerides, from 201.9 mg/dL to 166.6 mg/dL. Such findings however, although clinically relevant, did not reach statistical significance. Antioxidant capacity was also found to be improved in that study, evidenced by significantly increased levels of catalase and decreased levels of thiobarbituric acid reactive substrates (TBARS) [88].

A recently published systematic review and meta-analysis of RCTs investigating the effects of melatonin supplementation on lipid profile showed that supplementation at dosages higher than 8 mg/day for periods longer than 8 weeks significantly improved lipid profile, including reduced triglyceride levels by 31.5 mg/dL and reduced total cholesterol levels by 18.5 mg/dL [109]. In the same study, small changes were observed in LDL-c and high-density lipoprotein cholesterol (HDL-c) levels, but which did not reach statistical significance. The population investigated in that systematic review was varied, including not only healthy volunteers but also patients suffering with non-alcoholic steatohepatitis, metabolic syndrome, hypercholesterolemia, schizophrenia and bipolar disorder [109].

6. Biochemical modulation of hepatic parameters

Obesity is directly related to higher prevalence of non-alcoholic fatty liver disease (NAFLD). Whilst the prevalence of NAFLD in the general population is 15 to 30%, its prevalence in obese individuals ranges from 50 to 90%, in which the higher the BMI the higher the prevalence [110]. Several experimental [111-113] and review studies [114-117] have reported favourable effects of melatonin administration against liver diseases, attributed mainly to the well-described antioxidant, anti-inflammatory and DNA-protective roles of melatonin.

Correspondingly, a recent meta-analysis of RCTs reported appealing results of melatonin supplementation on the traditional clinical panel used in the management of liver diseases, such as a reduction in gamma-glutamyl transferase (GGT) (-33 IU/L) and alkaline phosphatase (ALP) (-8 IU/L) levels [118]. On the other hand, no significant difference was detected in the levels of alanine aminotransferase (ALT), whilst there

was an increase in aspartate aminotransferase (AST) (+2 IU/L) levels but with an absence of clinical significance. The patients included in this meta-analysis had a diagnosis of NAFLD or non-alcoholic steatohepatitis.

In a RCT [119] investigating patients with NAFLD (n = 24) supplemented with 6 mg/day melatonin for 12 weeks significantly reduced levels of ALT (60 to 41 IU/L) and AST (35 to 30 IU/L), in comparison with the placebo group (n = 21). Whilst GGT levels did not change significantly, the authors have considered a tendency for reduction (35 to 31 IU/L, p = 0.07). Interestingly, when comparing baseline *vs*. end of intervention, the melatonin-receiving group showed in average decreased body weight by 1.3 kg (85.5 to 84.2 kg) and abdominal circumference by 2 cm (107 to 105 cm), without changes in calorie intake. Notwithstanding the favourable results herein described, no evidence yet available corroborates melatonin supplementation for the management of liver cirrhosis, an advanced stage of NAFLD.

7. Glucose homeostasis and insulin sensitivity

Melatonin is known to participate in the intracellular processes that regulate insulin secretion and blood glucose homeostasis by its binding to MT1 and MT2 receptors in the pancreatic beta cell [120, 121]. It is also known that genetic variants of the MTNR1B gene, which encodes the MT2 melatonin receptor, have been associated with the development of insulin resistance and T2DM [122, 123]. Experimental studies further corroborate the properties of melatonin supplementation in improving insulin sensitivity and consequently glycaemic control [124-126]. Similarly, in humans the effects are also promising. A recent RCT [127] investigating melatonin therapy for 12 weeks in T2DM patients with coronary heart disease found that melatonin significantly decreased fasting plasma glucose (−29 *vs*.−5.6 mg/dL), serum insulin (−2.2 *vs*. +0.7 μIU/mL), and improved the homeostasis model of assessment-estimated insulin resistance (HOMA-IR) (−1.0 *vs*. +0.01), as compared to the placebo group.

A recent systematic review and meta-analysis of RCTs investigating the effects of melatonin supplementation at dosages ranging from 3 to 10 mg/day during periods ranging from 4 to 24 weeks upon glycaemic control showed significant reduction in fasting glucose levels and significant elevation of the quantitative insulin sensitivity check index (QUICKI) [128]. The same study however did not find significant effects on insulin levels, HOMA-IR, nor haemoglobin A1c levels. The population investigated

in that systematic review included patients suffering with endocrine disorders including T2DM, metabolic syndrome, non-alcoholic steatohepatitis, as well as neuropsychiatric disorders [128].

Whilst previous reviews discussed promising results of melatonin administration on metabolic parameters [21, 103], other studies have observed impairments in glucose levels. Working on an acute intravenous glucose tolerance test, Cagnacci *et al* [129] noted that 1 mg melatonin reduced insulin-dependent, but not insulin-independent, glucose utilisation in postmenopausal women. In an oral glucose tolerance test study, a single 5 mg dose of melatonin reduced glucose tolerance when administered in the morning, as well as in the evening, in healthy women (n = 21) [130]. Furthermore, morning melatonin supplementation increased the plasma glucose incremental area under the curve (AUC) by 186% and Cmax by 21%. When melatonin was administered in the evening, glucose AUC and Cmax were raised by 54% and 27%, respectively.

However, in order to properly understand these apparent contradictory results it is necessary to understand melatonin modes of action and its effects [17, 23]. In diurnal active species, humans for example, night is the rest/fasting daily phase and the expected physiological immediate effects of melatonin include induction of sleep, inhibition of appetite and metabolic adaptation to the fasting state, which includes hepatic insulin resistance, inhibition of insulin release, gluconeogenesis, amongst others. In the studies cited above [129, 130], following melatonin administration, its immediate effects were measured either in the morning or in the evening and, as expected, the immediate effects of melatonin are inhibition of insulin release and insulin resistance. However, it should be considered that in health physiological conditions melatonin is at its lowest levels during the day. This indicates that, when considering long-term melatonin therapy, its administration should preferentially be nocturnal, since to date, no chronic study has yet observed such impairments in glucose and insulin homeostasis attributed to melatonin supplementation.

Impaired melatonin function has been described in rare diseases characterized by severe insulin resistance, for example Rabson–Mendenhall syndrome [131], which suggests a protective role for melatonin over pancreatic activity. The aetiology of Rabson–Mendenhall syndrome is associated with extreme hyperinsulinemia induced by mutations in the insulin receptor gene, and the main pathophysiological manifestations include abnormal glucose homeostasis, pineal hyperplasia, elevated

levels of urinary melatonin metabolite [132, 133], dysmorphism of the head, face and nails, alongside the development of acanthosis nigricans [133, 134]. Regarding the latter, it was recently described that melatonin supplementation at the dosage of 3 mg/day for 12 weeks for obese patients improved insulin sensitivity and inflammatory biomarkers, and reduced the intensity of acanthosis nigricans of the neck and axilla [135], a manifestation strongly associated with metabolic syndrome in adults [136] and children [137].

8. Mitochondrial modulation: oxidative stress and antioxidant potential

One of the remarkable physiological roles of melatonin is its capacity to mitigate oxidative stress and to balance antioxidant and oxidant molecules around the mitochondrion, a cellular source of several antioxidant enzymes and reactive oxygen species [138, 139]. Several experimental studies have already sought to understand the antioxidant potential of melatonin in various diseases, including Alzheimer's disease [140], cardiovascular disease [141], obesity and T2DM [142]. In the clinical field, supplementation with 5 mg of melatonin for 30 days in elderly T2DM patients improved the oxidative response by increasing the erythrocytic activity of superoxide dismutase-1 and decreasing oxidative biomarkers, such as the erythrocytic concentration of MDA [87].

The protective effects of melatonin on mitochondrial oxidation-reduction modulation appear to be promising in individuals affected by cardiometabolic disorders. Raygan *et al* [127], working with patients with coronary disease and receiving melatonin at a dosage of 10 mg/day for 12 weeks, found increased levels of glutathione and concomitantly decreased levels of oxidative biomarkers, such as MDA and protein carbonyl concentrations, compared to the placebo group. Furthermore, the melatonin-receiving group showed increased nitric oxide levels, whilst this parameter was reduced in the placebo group, which can be seen as a positive effect of melatonin upon vascular tonus. Given that increased oxidative stress is a major underlying factor in the pathogenesis of obesity and obesity-related diseases, and that melatonin supplementation may at least partially mitigate such metabolic dysregulations, further clinical attention should be considered regarding the latter so as to contribute to the treatment of those conditions.

9. Therapy of obesity: open questions about melatonin supplementation as a possible tool

Historically and still currently, several pharmacological agents have been employed for the management and or treatment of obesity. For example, Franz *et al* [143] in their meta-analysis found an average of 5% to 9% weight loss during the first 6 months of interventions based on low calorie diets, combined or not with orlistat or sibutramine, but the weight losses observed tended to plateau after 6 months, and in several instances were not maintained. Additionally, although paramount for improvement in body composition and overall health, various research groups have independently found very similar results: physical activity only has very little effect on BMI [144, 145]. The overall consensus amongst practitioners is that the triad physical activity + dietary improvement + weight loss medication is much more effective than any of these strategies alone for long term, maintained weight loss.

It may well be possible that melatonin supplementation is an effective adjuvant in therapies for obesity where the affected individuals present lower levels of melatonin to begin with, or are somehow irresponsive to their endogenous melatonin. Further long-term trials recruiting obese subjects investigating the variables nutrition, exercise, melatonin supplementation and its biomarkers would be extremely helpful to expand our knowledge of melatonin as potential adjuvant in the treatment of obesity. Finally, some open questions on the therapeutic applicability of melatonin supplementation in the management of obesity and its inherent comorbidities are presented (Table 2).

[Insert table 2 here]

10. Safety of supplementation

Firstly, upon analyses of experimental models, hyper doses of melatonin showed no signs of toxicity and lethality [146, 147]. Such safety has also been demonstrated in pregnant rats, demonstrating an absence of toxicity for both the mother and the foetus in doses up to 200 mg/kg/day [148].

In humans, several studies have deemed melatonin supplementation to be safe, and no dramatic side effects or toxicological issues have been reported within the dosages investigated [149-151]. Melatonin supplementation at a dosage of 50 mg/day—a dose considered high—for 2 weeks to elderly patients suffering with Parkinson's disease was deemed to be safe, with 2 out of 40 subjects reporting

daytime tiredness as a side effect [20]. A meta-analysis of 8 RCT supplementing 20 mg/day of melatonin to cancer patients showed no significant side effects attributed to melatonin [152]. Another recent meta-analysis, involving children and adolescents affected by sleep onset insomnia, showed that melatonin supplementation was a well-tolerated agent at doses of 1 to 6 mg/day [153]. The most common adverse effects seen are dizziness, headache, nausea, daytime sleepiness, transient episodes of numbness and stomach pain.

Alarmingly however, even though as not the therapeutic target of our hypothesis, three recent reports of infant death have been associated with melatonin [154, 155]. It is worth mentioning that other factors might have, in a way or another, contributed to the deaths, and also that the melatonin concentrations detected in the peripheral blood of the victims were incredibly high, 13 ng/mL and 210 ng/mL in the report of Labay *et al* [154] and 1,400 ng/mL in the report of Shimomura *et al* [155]. What appears to be cases of melatonin overdose justify the development of more detailed overdosage studies in animal models.

11. Conclusion

Melatonin is a potential, multifaceted candidate for the management of obesity due to its biological effects on insulin and adipose tissue metabolism, lipolysis and mitochondrial capacity, added to its antioxidant and anti-inflammatory properties. Melatonin supplementation has been proposed as an adjuvant in the treatment of obesity and associated metabolic diseases including T2DM, NAFLD, hypertension and dyslipidaemia. RCTs appraised in the present review employed in average 1 to 20 mg/day of melatonin supplementation for periods ranging from 3 weeks to 12 months; however, not all trials found evidence of weight loss.

It remains to be elucidated whether melatonin supplementation for the treatment of obesity is effective only for individuals who show hypomelatonaemia or melatonin irresponsiveness at baseline. If so, one begs the question as to why melatonin levels are low or inefficient to begin with. Viewed collectively, the proposed role of melatonin as a weight loss aid is still on debate, and so is its safety. Further well-designed RCTs will test the reliability of the evidence so far available.

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