Review

MELATONIN AND THE METABOLIC SYNDROME: A TOOL FOR EFFECTIVE THERAPY IN OBESITY-ASSOCIATED ABNORMALITIES?

Frederic Nduhirabandi ¹, Eugene F du Toit ², Amanda Lochner ¹*

¹Division of Medical Physiology, Department of Biomedical Sciences, Faculty of Health Sciences, Stellenbosch University, South Africa; ²School of Medical Science, Griffith University, Australia

* Corresponding address: Prof Amanda Lochner

Department of Biomedical Sciences,
Faculty of Health Sciences,
PO Box 19063
Tygerberg
7505
Republic of South Africa

Tel: 27219389391
Fax: 27219389476
E-mail: alo@sun.ac.za

Key words: Cardiovascular effects, insulin resistance, leptin resistance, melatonin, metabolic syndrome, obesity
Abstract:

The metabolic syndrome is a cluster of metabolic abnormalities associated with increased risk for cardiovascular diseases. Apart from its powerful antioxidant properties, the pineal gland hormone melatonin has recently attracted the interest of various investigators as a multifunctional molecule. Melatonin has been shown to have beneficial effects in cardiovascular disorders including ischaemic heart disease and hypertension. However its role in cardiovascular risk factors including obesity and other related metabolic abnormalities is not yet established, particularly in humans. New emerging data show that melatonin may play an important role in body weight regulation and energy metabolism. This review will address the role of melatonin in the metabolic syndrome focusing on its effects in obesity, insulin resistance and leptin resistance. The overall findings suggest that melatonin should be exploited as a therapeutic tool to prevent or reverse the harmful effects of obesity and its related metabolic disorders.

Introduction

The metabolic syndrome (MetS), also termed syndrome X or insulin resistance syndrome, represents a cluster of metabolic abnormalities including, amongst others, abdominal obesity, insulin resistance, glucose intolerance, atherogenic dyslipidaemia, raised blood pressure and a pro-inflammatory state (Cornier et al. 2008). In addition to an increased risk for cardiovascular diseases and type 2 diabetes, this syndrome is also associated with numerous co-morbidities such as non-alcoholic fatty liver disease, reproductive disorders, obstructive sleep apnoea syndrome, osteoarthritis and some cancers (Pothiwala et al. 2009). MetS patients exhibit also sleep/wake disturbances and other circadian abnormalities (Cardinali et al. 2011, Reiter et al. 2011).
Available evidence indicates that in most developed and developing countries between 20% and 30% of the adult population can be characterized as having MetS (Grundy 2008) with obesity (increased body fat accumulation due to excessive nutrient/energy intake) playing an important role in the development of this condition (Cornier et al. 2008). Previous strategies to combat the prevalence of obesity and the MetS have not yet been successful and it is predicted that up to 58% of the global adult population could be either overweight or obese by 2030 (Kelly et al. 2008). This alarming prevalence and an increased risk for numerous co-morbidities will have serious socio-economic implications for both government and society in the future (Haslam & James 2005).

The pathophysiological mechanisms involved in the development of obesity-induced MetS, from adipose tissue dysregulation to a chronic inflammatory state, are complex and not well understood. In this regard, several mechanisms in connection with increased body fat accumulation and insulin resistance have been proposed including, amongst others, generation of lipid metabolites, inflammation, and cellular stress (oxidative and endoplasmic reticulum stress) (for review see Boden (2011)). Support for a potential role of oxidative stress in the MetS is rapidly increasing (Roberts & Sindhu 2009, Hopps et al. 2010, Otani 2011). A recent USA national survey showed that MetS patients were found to have a low serum antioxidant capacity compared with those without MetS (Beydoun et al. 2011). Therefore, along with other substantial interventions (e.g., sustained lifestyle modification with calorie restriction, increased physical activity and adjuvant drugs), antioxidant supplementation in the management of the MetS or prevention of increased oxidative stress associated with obesity-related alterations are currently receiving much attention.

The pineal gland hormone melatonin has potent antioxidant activities (Korkmaz et al. 2009a). In addition, it has been shown to play a role in metabolic regulation (Korkmaz et al. 2009b, Tan et al. 2011). Convincing evidence exists for the association of circadian system derangement (chronodisruption), sleep deprivation, and melatonin suppression in MetS and obesity (Reiter et
Melatonin: a multifunctional molecule more than an antioxidant

Melatonin or N-acetyl-5-methoxytryptamine is the hormone secreted mainly by the pineal gland which is under the control of the central nervous system via the suprachiasmatic nucleus (SCN) of the hypothalamus. Since the pineal gland is active only in darkness, the levels of melatonin in the pineal gland and in blood are high at night and low during the day (Altun et al. 2002). The biosynthesis and metabolism of melatonin has recently been extensively reviewed (Pandi-Perumal et al. 2006, Hardeland 2008).

Viewed as nature’s most versatile biological signaling (Pandi-Perumal et al. 2006) and multitasking molecule (Reiter et al. 2010), melatonin is a highly conserved molecule found in almost all groups of organisms (Hardeland & Fuhrberg 1996). Beside its classical role as a chronobiotic factor or endogenous synchronizer participating in regulation of seasonal as well as circadian rhythm (Zawilska et al. 2009), melatonin is also involved in a wide range of physiological functions in humans and animals having anti-excitatory, antioxidant, anti-inflammatory, immunomodulatory and vasomotor effects (for review see Hardeland et al.(2006), Pandi-Perumal et al.(2006)). Many of the biological actions of melatonin are elicited through
activation of membrane (Hardeland et al. 2006, Pandi-Perumal et al. 2008) or nuclear (Wiesenberge et al. 1998, Carlberg 2000) receptors while some of its intracellular actions are receptor-independent (for example, free radical scavenging). Two types of membrane receptors have been identified in mammals: melatonin receptor type 1 (MT1 in rodents or MTNR1A in humans) and type 2 (MT2 in rodents or MTNR1B in humans); they belong to the family of G-protein-coupled receptors (for review see Hardeland et al. 2011) and are increased in type 2 diabetic patients (Peschke et al. 2007).

The antioxidant activity of melatonin is well established (for reviews see Tan et al. 2007, Korkmaz et al. 2009a). Most of studies used melatonin at very high concentrations (1μM to 100mM) compared to its physiological concentrations (10-60 pM and 43-400pM during the day and the night, respectively) (Barrenetxe et al. 2004, Bonnefont-Rousselot & Collin 2010). In both in vitro and in vivo experiments, melatonin administration was able to neutralize a number of toxic reactants including reactive oxygen species (ROS) and free radicals (Reiter et al. 2000, Reiter et al. 2008) and no toxicity was found when melatonin was administered to experimental animals or humans at doses varying between 1mg and 300mg per day (Bonnefont-Rousselot & Collin 2010)). It has also been shown to increase the expression and activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (Vural et al. 2001, Rodriguez et al. 2004) and to increase the efficacy of classic antioxidants such as vitamin E, vitamin C and glutathione (GSH) (Gitto et al. 2001). Furthermore, several melatonin metabolites (e.g., N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK)) which are formed when melatonin neutralizes damaging reactants are themselves free radical scavengers (Tan et al. 2007, Reiter et al. 2008). This would increase the efficacy of melatonin in pathological conditions associated with increased oxidative stress (Korkmaz et al. 2009a, Bonnefont-Rousselot & Collin 2010). Importantly, melatonin treatment protects against mitochondrial dysfunction, the major source of reactive oxygen and nitrogen species (ROS/RNS) (Lopez et al. 2009, Paradies et al. 2010).
Melatonin is a small, highly lipophilic and hydrophilic molecule able to cross all morphological barriers and acts not only in every cell but also within every subcellular compartment (Vural et al. 2001, Rodriguez et al. 2004). These pleiotropic activities have led to the suggestion that it may be used clinically in disease conditions where its circulating levels are reduced such as in cardiovascular diseases (Reiter et al. 2010a) and diabetes (Peschke 2008) as well as in cancer (Srinivasan et al. 2008). The description of the role of melatonin in these conditions is beyond the focus of this review and only the cardiovascular effects of melatonin will be summarized.

**Cardiovascular effects of melatonin**

Endogenous melatonin has been shown to play an important role in the cardiovascular system (CVS) (Dominguez-Rodriguez et al. 2010, Reiter et al. 2010a). As indicated above, melatonin exerts its physiological functions through its chronobiotic, anti-excitatory, antioxidant, anti-inflammatory, immunomodulatory and vasomotor activities (Pandi-Perumal et al. 2006). Indeed, the CVS is connected to the SCN via multisynaptic autonomic neurons, and evidence confirmed that melatonin affects the CVS via these activities (Ruger & Scheer 2009, Dominguez-Rodriguez et al. 2010). Moreover, melatonin receptors (MT1/MT2) have been identified in the heart and arteries (Ekmekcioglu et al. 2003). Melatonin has been reported to influence blood pressure (Kitajima et al. 2001) and heart function (Abete et al. 1997) directly and/or indirectly by affecting cardiovascular risk factors including, amongst others, increased visceral fat accumulation and dyslipidaemia (Agil et al. 2011a, Kozirog et al. 2011, Nduhirabandi et al. 2011). The latter will be discussed in the section on obesity and insulin resistance (see figure 1 and table1). Importantly, subjects with coronary heart disease had impaired nocturnal melatonin secretion (Brugger et al. 1995, Altun et al. 2002) while those with myocardial infarction (Dominguez-Rodriguez et al. 2002) and hypertension (Forman et al. 2010) were found to have low circulating melatonin levels. Interestingly, a recent case-control study done by Samimi-Fard et al. (2011) showed a significant association between single nucleotide
polymorphisms (SNPs) (rs28383653) of melatonin receptor type 1A (MT1A) and coronary artery disease.

Melatonin has potent cardioprotective properties against ischaemia/reperfusion injury (Tengattini et al. 2008, Dominguez-Rodriguez et al. 2009). The study done by Sahna et al. (2002a) reported a bigger post-ischaemic myocardial infarction in pinealectomized rats compared to non-pinealectomized rats, indicating for the first time the cardioprotective effects of endogenous melatonin. The same study and additional investigations in our laboratory (Lochner et al. 2006, Genade et al. 2008) and elsewhere (Sahna et al. 2002b, Sahna et al. 2005, Petrosillo et al. 2009, Lamont et al. 2011) have confirmed that the short- or long-term administration of melatonin at either physiological or pharmacological doses protected the heart against myocardial ischaemia/reperfusion damage. Furthermore, the beneficial effects of exogenous melatonin on the heart in physiological conditions such as ageing (Petrosillo et al. 2010) as well as in pathophysiological conditions, for example hyperthyroidism (Ghosh et al. 2007), cadmium-induced oxidative damage (Mukherjee et al. 2011) and myocardial hypertrophy (Reiter et al. 2010b) have been demonstrated. However, the mechanism of the actions of melatonin is still complex and not yet fully explored. Besides its direct free radical scavenger and indirect antioxidant activity (Tengattini et al. 2008), the contribution of MT receptors in the cardioprotective properties has also been emphasized (Arvola et al. 2006, Sallinen et al. 2007, Genade et al. 2008, Grossini et al. 2011, Lamont et al. 2011).

Melatonin supplementation has largely been considered as a potential pharmacological agent in non-dipper and individuals with hypertension (Paulis & Simko 2007, Reiter et al. 2009, Grossman et al. 2011, Kozirog et al. 2011). Melatonin was reported to reduce systolic blood pressure along with aortic pulse wave velocity which is regarded as an important indicator of total cardiovascular risk estimation (Yildiz & Akdemir 2009). A recent meta-analysis of randomized controlled trials by Grossman et al. (2011) indicated that melatonin administration (at 2mg: controlled-release preparation) was effective to reduce nocturnal systolic and diastolic
blood pressure in patients with nocturnal hypertension. The regulation and modulation of blood pressure is a complex mechanism with multifactorial aspects (Paulis & Simko 2007). Several reports have indicated a direct effect of melatonin on blood pressure through its anti-adrenergic effects and nitric oxide availability (Kitajima et al. 2001, Paulis & Simko 2007). It appears however, as pointed out by Paulis & Simko (2007), that controversial *in vitro* results complicate the explanation of the overall effect on blood pressure *in vivo* while different vascular responses depending on the type of vascular bed (Cook et al. 2011) have also been reported. For example melatonin administration caused vasoconstriction in isolated coronary arteries (Tunstall et al. 2011) and the renal vascular bed (Cook et al. 2011) as opposed to vasodilatation in the aorta, pulmonary and umbilical vascular bed (Thakor et al. 2010). In contrast to the vasoconstriction observed in porcine isolated coronary arteries (Tunstall et al. 2011), intracoronary infusion of melatonin (70pgmL$^{-1}$ per minute of coronary blood flow) in anaesthetized pigs, was followed by an increased coronary flow and cardiac function through the beta-adrenoreceptors and nitric oxide (Grossini et al. 2011). These inconsistent vasomotor effects could be due to difference in the expression of the type of MT1/MT2 receptor in some vascular regions and involvement of the autonomic nervous system (Grossini et al. 2011).

Although more clinical investigations are required, melatonin appears to be an effective cardioprotectant in non-obese animals in addition to its promising antihypertensive effects. Since obesity is strongly associated with the increased prevalence of cardiovascular disorders (Poirier et al. 2006) which have been linked to derangement of melatonin’s circadian rhythm (Altun et al. 2002, Reiter et al. 2009, Dominguez-Rodriguez & Abreu-Gonzalez 2011), the role of melatonin in obesity/overweight and other related MetS components (insulin resistance, glucose intolerance, atherogenic dyslipidaemia, elevated blood pressure) is the topic of many investigations.
Melatonin and obesity

Melatonin is involved in energy expenditure and body fat mass regulation (Korkmaz et al. 2009b, Tan et al. 2011). Previously reported in seasonal animals (Bartness & Wade 1985), the role of melatonin in body weight regulation was clearly demonstrated by the observation that the reduction in circulating melatonin in pinealectomized rats was followed by an increase in body weight and that intraperitoneal (ip) administration of melatonin (30mgkg⁻¹ per day for 3 weeks) to these pinealectomized animals reversed the body weight gain (Prunet-Marcassus et al. 2003). In non-pathological conditions, melatonin has been shown to directly mediate body weight reduction and loss of adipose tissue: daily oral melatonin supplementation (0.4μgml⁻¹ to 4μgml⁻¹ for 12 weeks) in middle-aged rats increased plasma melatonin and reduced body weight, visceral adiposity, and plasma insulin and leptin levels to youthful levels (Rasmussen et al. 1999, Wolden-Hanson et al. 2000). Similar effects on body weight and visceral fat were observed in young rats (Bojkova et al. 2006, Kassayova et al. 2006). Interestingly, these weight loss effects of melatonin are independent of energy intake (for a review see Tan et al. (2011)).

The effects of melatonin in obesity have been intensively studied in animal models of diet-induced obesity (Nishida et al. 2002, Prunet-Marcassus et al. 2003, Puchalski et al. 2003, Hussein et al. 2007, Sartori et al. 2009, She et al. 2009, Shieh et al. 2009, Rios-Lugo et al. 2010, Agil et al. 2011a, Cardinali et al. 2011, Nduhirabandi et al. 2011). It was shown that the amplitude of the nocturnal pineal (Cano et al. 2008) and serum (Peschke et al. 2006) melatonin peaks decrease significantly in obese animals. Daily melatonin supplementation (4-10mg kg⁻¹ for 8 to 12 weeks) significantly reduced body weight as well as plasma glucose, leptin, triglyceride (TG) and total cholesterol levels of the rat models of high-fat diet-induced obesity (Prunet-Marcassus et al. 2003, She et al. 2009, Rios-Lugo et al. 2010). Using a rat model of high-calorie diet-induced obesity, our laboratory has demonstrated that long-term oral melatonin consumption (4mg kg⁻¹ per day for 16 weeks) starting before the establishment of obesity attenuated weight gain and prevented the development of obesity-induced metabolic
alterations (elevated visceral fat, serum insulin, leptin, TG and reduced high density lipoprotein cholesterol (HDL-C)) (Nduhirabandi et al. 2011). This study also reported for the first time the protective effects of melatonin against the increased susceptibility to myocardial ischaemia and reperfusion damage of the hearts from obese rats. We found that melatonin administration reduced post-ischaemic myocardial infarct size and improved myocardial function recovery via activation of reperfusion injury salvage protein kinases (PKB/Atk and ERK1/2) (Nduhirabandi et al. 2011). However, how melatonin affects the heart in obesity needs further investigation to determine the underlying mechanisms and the role of its direct or indirect actions.

Beside these metabolic effects, the antioxidant activities of melatonin were also demonstrated in animal models of diet-induced obesity: daily melatonin administration to obese rabbits (1mgkg⁻¹ subcutaneously for 4 weeks)(Hussein et al. 2007) or rats (4mgkg⁻¹, ip for 8 weeks) (She et al. 2009) improved the metabolic profile (reduction in body weight gain, blood glucose, triglycerides, cholesterol, low density lipoprotein cholesterol (LDL-C)) and increased the levels of glutathione peroxidase (GSH-Px) and HDL-C. Additionally, melatonin administration reduced oxidative stress as indicated by plasma malondialdehyde (MDA) levels and increased superoxide dismutase (SOD) activity to control levels (She et al. 2009). These changes were also associated with amelioration of blood pressure, heart rate, sympathetic activities and other obesity-related morphological pathologies including disappearance of fatty changes in the liver and kidney as well as atheromatous changes in the blood vessels (Hussein et al. 2007).

Despite the studies on the antioxidant activities of melatonin in experimental obese animals, only few have been done in obese humans. In a recent promising study done by Kozirog et al. (2011), one month of administration of melatonin (5mg per day) to patients with MetS reduced the body mass index (BMI), systolic blood pressure (SBP) and plasma fibrinogen as well as thiobarbituric acid reactive substrates (TBARS) levels. Interestingly, after two months of treatment, there was a further significant improvement of SBP and antioxidative status as indicated by an elevated catalase activity and a decrease in low density lipoprotein cholesterol
(LDL-C) levels (Kozirog et al. 2011). Although no information was given about the levels of circulating melatonin and insulin, this report pointed out, as claimed by other authors, that the levels of melatonin per se are not as important as the melatonin/insulin ratio which correlates negatively with the lipid profile of patients with MetS (Robeva et al. 2008).

The impact of obesity on the overall circulating melatonin levels in humans has been marked by divergences. For example, while the mean nocturnal serum melatonin was reported to be lower in patients with type 2 diabetes (Peschke et al. 2006) and coronary heart disease (see Cardinali et al. (2011)) and higher in severe obesity (Shafii et al. 1997), the study done by Rojdmark et al. (Rojdmark et al. 1991) showed that there were no significant modifications of melatonin secretion and excretion in obesity. Additional studies indicated that circulating melatonin levels in pre-diabetic obese male (Robeva et al. 2006) and male and female obese diabetic (Robeva et al. 2008) patients with MetS did not differ from controls. These discrepancies may be due to various factors including age of patients and severity of obesity (Shafii et al. 1997).

MT receptor-mediated pathways may play an important role in melatonin’s actions in obesity. This was evidenced by studies using the melatonin agonist NEU-P11 (10mg kg⁻¹ per day for 8 weeks, ip) (She et al. 2009) or Ramelteon, a potent selective MT1/MT2 receptor agonist (8 mg kg⁻¹ per day for 8 weeks, oral) (Oxenkrug & Summergrad 2010): it was found that melatonin agonist administration exerts similar regulatory effects as melatonin, namely decreasing body weight and blood pressure. Using MT receptor knock out mice, Muhlbaier et al. (2009) demonstrated an active role for these receptors in the synchronisation of the major organs involved in blood glucose regulation and that they act negatively on insulin secretion. Since MT receptors are also present in adipose tissue (Brydon et al. 2001), it seems that the overall effects of melatonin in obesity are partly mediated through these receptors in addition to activation of the sympathetic nervous system via hypothalamic receptors and subsequent effects on lipolysis and adipose tissue plasticity (Penicaud et al. 2000, Song & Bartness 2001, Bartness et al. 2002).
In vitro melatonin treatment of adipocytes has been shown to possibly inhibit differentiation and limit adipose tissue hypertrophy (Alonso-Vale et al. 2009) by inhibiting fatty acid-induced triglyceride accumulation in cells exposed to physiological levels of oleic acid (Sanchez-Hidalgo et al. 2007). This could explain how in vivo melatonin treatment prevents the increase in circulating triglycerides and eventually body fat accumulation and body weight gain in overweight and obesity (Agil et al. 2011a, Nduhirabandi et al. 2011). The reduction in body weight gain might be due to a significant decrease in fat content as opposed to lean body mass (Wolden-Hanson et al. 2000) and could be related to improvements in the compromised insulin and leptin signaling associated with obesity (Sartori et al. 2009) (see details in the following sections).

The exact mode of action of melatonin in body weight regulation is however complex and not fully elucidated. As previously indicated the divergences in melatonin activities may be explained by differences in experimental protocols. For instance, while body weight reduction following melatonin administration was independent of food intake in middle-aged male rats (Wolden-Hanson et al. 2000) and obese rats (She et al. 2009, Agil et al. 2011a), it was accompanied with a decrease in food consumption in ovariectomized rats (Sanchez-Mateos et al. 2007) and obese rabbits (Hussein et al. 2007). It appears that melatonin’s effects could vary from one study to another depending on the study design: the dose, mode and time of administration as well as the age, sex, species and strain of the animal model. For example, following a prolonged oral melatonin administration to normal rats, serum glucose levels were increased in male and female 48-hours fasted animals (Bojkova et al. 2006), decreased in overnight fasted females (Bojkova et al. 2006) or in both males and females (Kassayova et al. 2006) or unchanged in either sexes of non-fasted animals (Markova et al. 2003). Whether the overall effect of melatonin administration in obesity results from its direct or/and indirect actions requires further research.
Melatonin and insulin resistance

Insulin resistance is characterized by a decreased cellular sensitivity to its effects on glucose uptake, metabolism and storage in peripheral tissues (for more details see Benito (2011), Tesauro & Cardillo (2011)). Although not all overweight/obese persons develop insulin resistance and a normal weight does not equate to insulin sensitivity (McLaughlin et al. 2004), obesity is the most important factor in the etiology of insulin resistance (Cornier et al. 2008). It is generally accepted that hyperinsulinaemia and insulin resistance are the common links between obesity and its vascular complications (for review see Tesauro & Cardillo (2011)).

Melatonin has been implicated in regulation of insulin secretion and glucose/lipid metabolism (Nishida 2005, Peschke 2008, Peschke & Muhlbauer 2010). Studies on pinealectomized animals also gave more insight into the role of melatonin in insulin resistance: in normal rats, pinealectomy induced insulin resistance and glucose intolerance (Lima et al. 1998, Zanquetta et al. 2003). In type 2 diabetic rats, pinealectomy increased plasma insulin significantly (after 21 weeks) and caused accumulation of triglycerides which was followed later (after 35 weeks) by a net decrease in insulin levels (Nishida et al. 2003), reflecting impairment in insulin release from pancreatic β-cells, as seen in patients at an advanced stage of type 2 diabetic mellitus (Kuzuya et al. 2002).

Pineal gland melatonin synthesis is decreased in type 2 diabetic Goto-Kakizaki rats (Frese et al. 2009); long term melatonin consumption (2.5 mgkg⁻¹ per day for 9 weeks) increased plasma melatonin levels with a concomitant reduction in insulin levels in these animals (Peschke et al. 2010). Sartori et al. (2009) found that 8 weeks oral melatonin (100mg kg⁻¹ per day) markedly improved insulin sensitivity and glucose tolerance in mice on a high-fat diet. A similar finding was associated with an increase in hepatic glycogen and improvement in liver steatosis following 2 weeks of melatonin administration (10mgkg⁻¹ per day, ip) in high-fat diet-induced diabetic mice (Shieh et al. 2009). In high fat/high sucrose-fed rats, 8 weeks treatment with melatonin or its agonist NEU-P11 increased insulin sensitivity (She et al. 2009). Besides the
reduction in plasma hyperinsulinaemia, chronic melatonin administration (1.1mgkg\(^{-1}\) per day for 30 weeks, subcutaneously via implanted melatonin-releasing pellets) to rats with type 2 diabetes mellitus reduced the hyperlipidaemia, and hyperleptinaemia (Nishida et al. 2002). This long term melatonin-induced improvement of insulin sensitivity may therefore be linked to a reduced body weight and improved lipid metabolism as it was recently demonstrated in young Zucker diabetic fatty rats (Agil et al. 2011a & b). Although 2 weeks of melatonin administration (1 or 10 mg kg\(^{-1}\) per day, ip starting at 4 weeks) did not affect the body weight of rats fed a high-fructose diet for 6 weeks, it ameliorated insulin resistance (Kitagawa et al. 2011). This was associated with increase in serum adiponectin levels and reduction in leptin levels (Kitagawa et al. 2011). Interestingly, melatonin administration attenuated the levels of circulating free fatty acids (FFA) and serum tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) (Kitagawa et al. 2011), two important factors implicated in the development of insulin resistance and vascular dysfunction in obesity (Benito 2011, Tesauro & Cardillo 2011).

On a cellular level, insulin resistance is associated with abnormal or compromised intracellular insulin signaling cascade. This cascade principally includes binding of insulin to insulin receptor (IR), tyrosine phosphorylation of insulin receptor substrate (IRS) proteins and activation of phosphotidylinositol-3-kinase (PI-3K), protein kinase B (PKB/Akt) and protein kinase C (PKC) isoforms (for details see Benito (2011)). Melatonin (1nM) treatment has been shown to stimulate glucose transport in skeletal muscle via the phosphorylation and activation of IRS-I and PI-3K respectively (Ha et al. 2006). It was further demonstrated that melatonin improves glucose homeostasis by restoring the vascular actions of insulin which were characterized by increased phosphorylation of Akt and endothelial nitric oxide synthase (eNOS) in aortic tissue (Sartori et al. 2009). In addition to the phosphorylation of Akt and PKC-\(\zeta\), Shieh et al. (2009) indicated that melatonin (1nM) stimulated glycogen synthesis and increased the phosphorylation of glycogen synthase kinase 3 \(\beta\) (GSK3-\(\beta\)) in hepatic cells. More interestingly, the effects of melatonin could be blocked by using the non-selective MT1/MT2 antagonist, luzindole, or the MT2 selective antagonist, 4-phenyl-2-propionamido tetrалine (4P-PDOT) (Ha et
al. 2006, Shieh et al. 2009), suggesting MT receptor involvement. It is not clear however how activation of the high affinity MT receptors which are G-protein linked leads to stimulation of the IRS-1/PI-3K pathway and the role of PKC-ζ in this regard (see figure 2). In addition, the role of PKB/Akt is not clear in view of the different results that have been reported showing its activation in skeletal muscle cells (Ha et al. 2006) as opposed to its inactivation in hepatic cells (Shieh et al. 2009).

Using adipose tissue of the female fruit bat, *Cynopterus sphinx* (seasonal animal), Banerjee et al. (2011) have shown that melatonin treatment (100ngml⁻¹ and 500pgml⁻¹) together with insulin increased the glucose uptake compared to control cells. There was however no correlation between glucose uptake and the protein expression of glucose transporter 4 (GLUT-4) in these cells (Banerjee et al. 2011). In this regard, additional exploration of GLUT4-translocation could give more insight in the results obtained. Although a decrease in GLUT-4 gene expression was reported following melatonin treatment (1μM for 14 days) in human brown adipocyte cells lines (PAZ6) (Brydon et al. 2001), Zanquetta et al. (2003) found that 30 days of calorie restriction or melatonin replacement (50μg100g⁻¹ per day i.p.) to pinealectomised rats was accompanied by increased plasma membrane GLUT-4 protein content in white adipose tissue to values similar to those of control rats. Importantly, Gosh and coworkers (2007) found that melatonin protected against oxidative damage and restored expression of GLUT-4 gene in the hyperthyroid rat heart, establishing the ability of antioxidants to reverse oxidative stress-mediated metabolic derangements. We have recently observed that acute melatonin (100nM) treatment enhances the insulin action on glucose uptake of normal isolated adult cardiomyocytes (Nduhirabandi et al. unpublished data). However, further experiments are needed to fully explore the role of melatonin in glucose homeostasis.

Further support for a role of melatonin in the regulation of energy metabolism came from the recent finding that removal of the melatonin receptor type I (MT1) significantly impairs the ability of mice to metabolize glucose and probably induces insulin resistance in these animals.
Contreras-Alcantara et al. 2010). Epidemiological studies have also revealed that variants near/in the melatonin receptor type 1B (MTNR1B) are associated with elevated plasma fasting glucose levels (Kan et al. 2010) and impaired insulin secretion (Tam et al. 2010).

In addition to the above, melatonin receptors MT1 and MT2 are also expressed in pancreatic islets (Peschke et al. 2000) and as insulin levels exhibit a nocturnal drop, its production has been suggested to be controlled, at least in part, by melatonin (Mulder et al. 2009). Indeed, melatonin administration inhibits insulin secretion in rat pancreatic islets (Picinato et al. 2002, Peschke 2008) and could explain why melatonin reduced the fasting insulin levels (Puchalski et al. 2003, She et al. 2009). Importantly, a recent case study done by (Nieuwenhuis et al. 2009) demonstrated a potentially important role of melatonin in diabetes by successfully treating a 40-year-old woman with insulin-dependent diabetes mellitus (IDDM) with phototherapy. Indeed, the exposure to the light during phototherapy could suppress melatonin production as expected and this could lead to an increase in insulin secretion which is low in IDDM and causing a subsequent improvement in glucose metabolism.

However, in the case of type 2 diabetes, as pointed out by Peschke & Muhlbauer (2010), the exploitation of melatonin’s inhibitory effect on insulin secretion as a potential therapy to reduce hyperinsulinaemia requires prudence and more large clinical studies. The implication of catecholamines as causal factor controlling the biological relevance of melatonin-insulin interaction has recently been demonstrated in type 1 and 2 diabetes (Peschke et al. 2011). It was observed that catecholamines (noradrenaline and adrenaline) and melatonin levels were reduced in type 2 diabetic GK rats (characterized by high insulin levels) and elevated in type 1 diabetic rats (associated with reduced insulin levels) (Peschke et al. 2011). The role of catecholamines in these pathological conditions could be explained by their observed (high or low) levels taking into consideration the fact that catecholamines decrease insulin secretion and stimulate melatonin synthesis (Peschke et al. 2011).
Melatonin and leptin resistance

Leptin is an adipocyte hormone that has a central role in regulating food intake, body weight and energy expenditure and leptin resistance refers to the inability of elevated circulating leptin levels to reduce common obesity. As a predisposing factor for diet-induced obesity (Scarpace & Zhang 2009), leptin resistance is associated with insulin resistance and an increased pro-inflammatory state (Lago et al. 2008). Pinealectomy increases circulating leptin (Baydas et al. 2001) while exogenous melatonin decreases serum leptin levels in both pinealectomized (Canpolat et al. 2001) and intact rat models of diet-induced obesity (Wolden-Hanson et al. 2000) before decreasing plasma insulin levels (Puchalski et al. 2003). These observations suggest a secondary modulatory effect of leptin on insulin in body weight reduction (Morrison et al. 2009). On the other hand, opposite results (increased leptin levels) have also been reported following melatonin administration to normal and pinealectomized rats (3 mg kg⁻¹ per day, ip, for 6 months) (Baltaci & Mogulkoc 2007) and to male C57BL/6 adult mice (10 µg ml⁻¹ in drinking water, for 1 month) (Song & Chen 2009). In this regard, surprisingly, the study done by Baltaci and Mogulkoc (2007) showed that pinealectomy decreased body weight gain and leptin levels. Furthermore, other studies have shown that melatonin had no effect on leptin levels in menopausal women (Cagnacci et al. 2002), ovariectomized rats (Sanchez-Mateos et al. 2007) and obese horse (Buff et al. 2005). However, as expected, in a rat model of high-fructose diet induced MetS (Kitagawa et al. 2011) and in young Zucker diabetic fatty rats (Agil et al. 2011b) melatonin administration reduced serum leptin levels. Apart from differences in experimental designs and animal models, the causes of these controversial results remain to be clarified.

At a molecular level, the mechanisms of leptin resistance and impaired leptin signaling includes amongst other things increased activity of suppressor of cytokine signaling 3 (SOCS3) (Bjorbaek et al. 1999, Emilsson et al. 1999) which is a member of a family of proteins which inhibits the JAK-STAT signaling cascade (Myers et al. 2008). Melatonin may act initially on hypothalamic leptin and insulin receptor sensitivity (as these hormones do under normal
conditions) and may consequently relay information about peripheral fat stores to central effectors in the hypothalamus to modify food intake and energy expenditure (Song & Bartness 2001). In this regard, melatonin, leptin and insulin have been found to activate the same intracellular signaling pathways namely PI3K and STAT-3 (Carvalheira et al. 2001, Anhe et al. 2004, Picinato et al. 2008). As a consequence, melatonin may attenuate or reverse the insulin resistance in obesity by mimicking the actions of insulin and leptin signaling via cross-talk between these pathways. Concerning the effects of leptin administration on melatonin, it was shown that leptin administration (50ngml⁻¹) suppressed melatonin secretion (by pineal explants) during long days and stimulated its secretion during short days in seasonal breeding animals (Zieba et al. 2011). While further evidence is required in diet-induced obesity, it appears that an intricate relationship exists between leptin, melatonin and insulin, synchronized in circadian fashion and having profound effects on metabolism.

**Conclusion**

Although its experimental relevance is still to be validated in the clinical setting, several studies have demonstrated the potential beneficial actions of melatonin in the metabolic syndrome and obesity (see table 1 and figure 1). Apart from its antioxidant actions, the overall metabolic actions of melatonin are the result of its pleiotropic activities associated with multiple signaling in areas of the central nervous system and in peripheral organs (Hardeland et al. 2011). In view of the beneficial actions of melatonin on obesity-related metabolic disorders (increased body fat accumulation, glucose intolerance, atherogenic dyslipidaemia, and raised blood pressure), it would seem that melatonin treatment should indeed be exploited in the future as a tool for effective management of obesity-induced complications, including cardiovascular disease. Melatonin is an affordable, cheap and nontoxic molecule with exceptional potential to have a profound effect on public health (Reiter & Korkmaz 2008, Sanchez-Barcelo et al. 2010).
Future perspectives

The research on the role of melatonin and its effects on the pathophysiological mechanisms of obesity and metabolic syndrome is a recent, large and growing field. From a circadian system derangement to metabolic disorders, it involves various aspects including epidemiological, physiological and genetic factors. It is clear however, that extensive clinical trials are required to establish the clinical potential of melatonin treatment in a number of pathologies. It appears that the big challenge is the enormous variation of dosage of melatonin administered in experimental animals where low and high doses both seem to be effective. In this regard, the extrapolation of experimental findings to humans will be one of the first obstacles, but this is facilitated by the no-toxicity of melatonin even at high dosage.

Acknowledgements:

We would like to thank Prof Barbara Huisamen for her valuable comments on the manuscript.

Conflict of interest: There is no conflict of interest.
REFERENCES


**TABLE 1 POTENTIAL MELATONIN’S EFFECTS IN OBESITY-RELATED METABOLIC ALTERATIONS**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment</th>
<th>Treatment Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>↑</td>
<td>↓</td>
<td>(Hussein et al. 2007, Agil et al. 2011a, Kozirog et al. 2011)</td>
</tr>
<tr>
<td>VLDL</td>
<td>↑</td>
<td>↓</td>
<td>(Hoyos et al. 2000)</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>↓ or ↑ (?)</td>
<td>↑ or ↓ (?)</td>
<td>↑ (Agil et al. 2011b, Kitagawa et al. 2011) or ↓ (Rios-Lugo et al. 2010) (?)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>↑</td>
<td>↓</td>
<td>(Nishida 2005, Oztekin et al. 2006, Kitagawa et al. 2011)</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen secretion</td>
<td>↑</td>
<td>↓</td>
<td>(Bekyarova et al. 2010)</td>
</tr>
<tr>
<td>TG &amp; VLDL release</td>
<td>↑</td>
<td>↓</td>
<td>(Hussain 2007)</td>
</tr>
<tr>
<td>Glucose release</td>
<td>↑</td>
<td>↓</td>
<td>(Kaya et al. 2010)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>↑</td>
<td>↓</td>
<td>(Bekyarova et al. 2010)</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>↓</td>
<td>↑</td>
<td>(Shieh et al. 2009)</td>
</tr>
<tr>
<td>Liver weight</td>
<td>↑</td>
<td>↓</td>
<td>(Pan et al. 2006)</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity (glucose uptake)</td>
<td>↓</td>
<td>↑</td>
<td>(Sartori et al. 2009, Srivastava &amp; Krishna 2010)</td>
</tr>
<tr>
<td>FFA /TG uptake</td>
<td>↑</td>
<td>↓</td>
<td>(Dauchy et al. 2003)</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>↓</td>
<td>↑</td>
<td>(Mazepa et al. 2000)</td>
</tr>
<tr>
<td>Heart &amp; Vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>↑</td>
<td>↓</td>
<td>(Paulis &amp; Simko 2007, Kozirog et al. 2011)</td>
</tr>
<tr>
<td>Heart rate</td>
<td>↑</td>
<td>↓</td>
<td>(Hussein et al. 2007)</td>
</tr>
<tr>
<td>Endothelial dysfunction</td>
<td>↑</td>
<td>↓</td>
<td>(Sartori et al. 2009)</td>
</tr>
<tr>
<td>Cardiomyopathy /Hypertrophy</td>
<td>↑</td>
<td>↓</td>
<td>(Bertuglia &amp; Reiter 2007, Ghosh et al. 2007)</td>
</tr>
</tbody>
</table>
Table 1 Only some references have been indicated in this table as evidence of the potential effects of melatonin in metabolic syndrome. ↓: decreases or attenuates; ↑: increases or elevates; (?) needs more studies/unusual model of obesity; FFA: free fatty acids; TNF-α: tumor necrosis factor-alpha; VLDL: very low density lipoprotein; HDL-C: high density lipoprotein-cholesterol; LDL-C: Low density lipoprotein-cholesterol; TG: triglyceride.
LEGEND FOR FIGURES

Figure 1 Melatonin improves obesity-induced metabolic alterations by reducing circulating free fatty acids (FFA) and hyperglycaemia and dyslipidaemia as well as hyperinsulinaemia with a concomitant increased in high density lipoprotein-cholesterol (HDL-C) and adiponectin. In addition, melatonin improves leptin and insulin resistance and hypertension. These effects are still complex and interconnected and may lead to improvement of coronary heart disease (CHD), heart failure and arrhythmias. As evidence, we showed that melatonin administration reduced myocardial ischaemia reperfusion (I/R) damage by reducing infarct size, improving post-ischaemic myocardial function recovery via activation of reperfusion injury salvage protein kinases (PKB/Atk and ERK1/2). ↓: reduction, ↑: increase, ?: no information available, FFA: free fatty acids; LDL-C: Low density lipoprotein-cholesterol; TG: triglyceride, GLUT: glucose transport, SOD: superoxide dismutase, BW: body weight, Visc fat: visceral fat, HW: heart weight, TBARS: thiobarbituric acid reactive substrates, GSH-Px: glutathione peroxidase (References see in the text).

Figure 2 Melatonin administration was associated with a significant increase in glycogen synthesis in hepatic cells and improved glucose homeostasis in skeletal muscle by activation of eNOs (endothelial nitric oxide synthase). How melatonin stimulated phosphorylation of IRS remains unknown. The effect of melatonin in glucose uptake remains unclear. The mitogen-activated protein kinase (MAPK) pathway was not included in the figure. ?: no available data, IR: insulin receptor, IRS-1: insulin receptor substrate 1, PI-3K: phosphatidylinositol-3-kinase, PKC-ζ: Protein kinase C -zeta, GSK-3β: Glycogen synthase kinase -3 beta, GLUT-4: glucose transporter -4, Mel: melatonin, MT1/2: melatonin receptor 1, 2, Ins: Insulin. (References see in the text).
Figure 1 Hypothetical representation of the beneficial actions of melatonin in obesity and its related cardiovascular complications.
Figure 2 Simplified representation of the effect of melatonin on insulin signaling pathway in peripheral tissues