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Metabolic Effects of Melatonin on Oxidative Stress and Diabetes Mellitus

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Melatonin, which is synthesized in the pineal gland and other tissues, has a variety of physiological, immunological, and biochemical functions. It is a direct scavenger of free radicals and has indirect antioxidant effects due to its stimulation of the expression and activity of antioxidative enzymes such as glutathione peroxidase, superoxide dismutase and catalase, and NO synthase, in mammalian cells. Melatonin also reduces serum lipid levels in mammalian species, and helps to prevent oxidative stress in diabetic subjects. Long-term melatonin administration to diabetic rats reduced their hyperlipidemia and hyperinsulinemia, and restored their altered ratios of polyunsaturated fatty acid in serum and tissues. It was recently reported that melatonin enhanced insulin-receptor kinase and IRS-1 phosphorylation, suggesting the potential existence of signaling pathway cross-talk between melatonin and insulin. Because TNF-α has been shown to impair insulin action by suppressing insulin receptor–tyrosine kinase activity and its IRS-1 tyrosine phosphorylation in peripheral tissues such as skeletal muscle cells, it was speculated that melatonin might counteract TNF-α–associated insulin resistance in type 2 diabetes. This review will focus on the physiological and metabolic effects of melatonin and highlight its potential use for the treatment of cholesterol/lipid and carbohydrate disorders.

Key Words: Melatonin; oxidative stress; free radicals; diabetes mellitus; insulin; polyunsaturated fatty acids; cholesterol; TNF-α; NOS.

Introduction

Melatonin is synthesized and secreted by the pineal gland, retina, and other vertebrate tissues with a daily rhythmical peak in the dark phase. It has been shown to exhibit a variety of physiological functions including the control of seasonal reproduction, thermoregulation, energy metabolism, circadian rhythm regulation, and sleep control. Melatonin has direct radical scavenging activity and indirect antioxidant effects *(1–4)* as well as antitumor effects *(5,6)*. Melatonin was also shown to reduce serum cholesterol levels in mammalian species *(4,7–9)*, and to prevent oxidative stress in diabetic subjects *(9,10)*. Melatonin reduced the hyperglycemia, hyperinsulinemia, and hyperleptinemia, and restored hepatic Δ-5 desaturase (an insulin-permissive enzyme) activity in type 2 diabetic rats. These animals also exhibited a restoration in their altered polyunsaturated fatty acids (PUFA) levels to near normal values *(10)*. Thus, melatonin may prevent changes in membrane fluidity during lipid peroxidation induced by oxidants *(11)* and diabetes *(10)*. The long-term administration of melatonin to male rats reportedly reduced their visceral fat and plasma leptin and insulin levels to values found in young rats *(12,13)*, suggesting that melatonin might affect insulin sensitivity as well as the development of obesity, although direct evidence for this has not been reported. In mammals, two high-affinity plasma-membrane-bound G protein–coupled receptors (MT1 and MT2) mediate melatonin's effects. MT1 and MT2 receptors are encoded by separate genes and their activation results in the inhibition of adenyl cyclase via a pertussis toxin–sensitive G protein that inhibits adenyl cyclase *(14)*. A recent report suggested the potential existence of signaling pathway cross-talk between melatonin and insulin *(15)*. This review will focus on the physiological and metabolic effects of melatonin and highlight why melatonin has the potential to be an important tool for the treatment of cholesterol and diabetic disorders.

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Melatonin Reduces Hypercholesterolemia

It has been reported that long-term administration of melatonin reduces serum cholesterol levels in mammalian species. This effect was only observed in animal models of hypercholesterolemia such as in diet-induced and genetic hypercholesterolemic rats *(4,7,8,16)*, as well as in middle-aged *(12)* or diabetic animals *(10)*, but not in young and normal rats. When administered for 3 mo to 1-mo-old rats that were fed a normal diet, pharmacological doses of melatonin had no effect on serum total cholesterol levels, although similar doses significantly reduced elevated total cholesterol levels in animals fed a cholesterol-enriched diet. Interestingly, the reduction in HDL cholesterol that was induced by this hypercholesterolemic diet was reversed by pharma-

cological doses of melatonin. Long-term melatonin administration to diabetic rats significantly reduced their plasma levels of total cholesterol and triglycerides *(10)*. Some plasma LDLs are oxidized in hypercholesterolemic and diabetic animals; oxidized LDLs are not effectively taken up by body cells and thus contribute to the development of hypercholesterolemia. Melatonin treatment was shown to reduce the formation of oxidized LDLs *(3)*. In contrast, acute melatonin administration was shown to have the opposite effect—such treatment increased total cholesterol and triglyceride levels 1 and 4 h after treatment *(17)*. Early studies suggested that melatonin might enhance the catabolism of cholesterol to bile acid *(18)* and/or inhibit cholesterol synthesis and LDL receptor activity *(19)*. Although the mechanism by which melatonin reduces cholesterol levels in hypercholesterolemic animals is still unclear, the above data suggest that it may augment endogenous cholesterol clearance via ligand-dependent transcription factors such as liver X receptors (LXRs).

Melatonin's Antioxidant Effects

The most abundant radicals generated in cells are superoxide anion, hydroxyl radicals, and nitric oxide (NO). High reactivity of these radicals results in the oxidation of normal cellular molecules, resulting in the formation of oxidation products such as lipid peroxides, malonedialdehyde (MDA), and 8-hydroxydeoxyguanosine. Radicals such as these are metabolized into nontoxic compounds by an antioxidative defense system that consists of free-radical scavengers, metal chelators, and radical-metabolizing enzymes. It is widely accepted that melatonin can act as an antioxidant by scavenging free radicals, thereby protecting cells from oxidative damage induced by a variety of free-radical-producing agents and processes. The fact that melatonin has properties that are both hydrophobic and hydrophilic facilitates its diffusion into all cells and subcellular compartments.

Several enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and glutathione reductase (GSH-Red), metabolize radicals or reactive oxygen species into non-radical products in cells *(20, 22)*. At pharmacological levels, melatonin has been shown to enhance the activity of GSH-Px, SOD, and CAT, in animal tissues in which oxidative stress was induced by treatment with streptozotocin (STZ), 7,12-dimethylbenz(*a*)anthracene *(21,23)*, carbon tetrachloride *(21,24)*, or experimental cholestasis *(25)*. Furthermore, chronic melatonin administration (500 μg/kg) increased tissue levels of mRNAs for both mitochondrial Mn-SOD and cytoplasmic Cu/Zn-SOD in Syrian hamsters *(26)*; the protective role of SODs against oxidative damage is well established *(27,28)*.

NO is a free radical that is synthesized by nitric oxide synthase (NOS), and is an important messenger that regulates nervous, immune, and cardiovascular system homeostasis *(29)*. However, elevated NO production has been implicated in the pathogenesis of circular shock and inflammation, as well as neuronal degeneration *(30)*. NO can react with superoxide anion (O_2^-) to produce peroxynitrite ($ONOO^-$), which oxidizes sulfhydryl groups and generates hydroxyl radicals, the latter of which lead to oxidative damage in many tissues including the liver, lung, kidney, and pancreatic β-cells. NOS activity was reported, after peripheral nerve injury, to be induced in neurons that were normally devoid of it, and seemed to act as the killer signal that resulted in the production of neurotoxic levels of NO. Physiological concentrations of melatonin have been shown to inhibit NOS activity in the cerebellum, hypothalamus, gastrointestinal tract *(31)*, and hamster retina *(32)*. Crespo et al. reported that melatonin significantly inhibited lipopolysaccaride (LPS)-induced NO production in the lung and liver in a dose-dependent manner, primarily by inhibiting inducible NO synthase (iNOS) expression *(33)*. Overproduction of NO by LPS treatment was shown to induce the production of iNOS. Activation of iNOS and NO formation may result in IL1β-mediated β-cell impairment. Nonselective inhibition of all NOS isoforms, however, may cause excessive vasoconstriction as a result of the inhibition of endothelial NOS (eNOS), leading to organ ischemia, oxidative stress, and death. Thus, selective inhibitors of the iNOS isoform were shown to prevent NO-mediated oxidative damage. Moreover, the formation of peroxynitrite formation from NO was prevented by melatonin (6 mg/kg ip) but not by constitutive inhibitors of endothelial and neuronal NOSs in STZ-induced diabetic animals *(34)*. These findings suggest that iNOS-derived NO as well as oxygen free radicals play a role in STZ-induced pancreatic β-cell destruction and that inhibitors of peroxynitrite, such as melatonin may offer some protection against such damage *(35)*.

Melatonin's Effects on Diabetes/Insulin Action

As noted above, there is a link between diabetes and oxidative stress. Hyperglycemia (and oxidized lipids) leads to the enhanced production of free radicals as well as to alterations in endogenous antioxidants, resulting in tissue and cellular dysfunction. An increasing number of studies report that melatonin ameliorated diabetes- and chemically induced oxidative stress. The early studies of Montilla et al. showed that melatonin reduced hyperglycemia and hyperlipidemia in STZ-induced diabetic rats *(9)*. When melatonin was injected 3 d before diabetes induction and daily thereafter for 8 wk, it significantly increased oxidative parameters in the plasma and erythrocytes such as MDA, reduced glutathione, glycemia, lipids, HbA1c, and plasma fructosamine. With regard to the reductions in plasma HDL-c and GSH content of erythrocytes seen in these animals, melatonin returned their levels to normal. These results suggested that melatonin protected against oxidative stress and reduced the severity of STZ-induced diabetes. However, when administered after the induction of diabetes, melatonin had no effect on

diabetic blood glucose levels, even though it reduced glucose levels when administered a few days before STZ injection. Melatonin was also shown to protect rat β cells from the damaging effects of STZ. Melatonin treatment (200 μg/kg/d, ip) for 3 d prior to the induction of diabetes and daily thereafter for 4 wk restored islet morphology and β-cell insulin levels, and elevated the significantly reduced glutathione peroxidase activity in pancreatic tissue *(36)*. These data suggest that melatonin treatment may be potentially therapeutic in human diabetics.

In contrast to type 1, type 2 diabetes usually develops as a result of a combination of peripheral insulin resistance and impaired insulin secretion. Although genetic and environmental factors are probably involved, insulin resistance most often develops in response to the ingestion of a high-fat diet with a particular fatty-acid composition *(37–39)*, as well as hypertriglyceridemia. In light of similarities between insulin resistance and other inflammatory states, insulin resistance is increasingly being classified as a chronic, low-level inflammatory state that is mediated by such pro-inflammatory cytokines such as TNF-α. Indeed, TNF-α has been shown to impair insulin action by suppressing insulin receptor–tyrosine kinase activity and IRS-1 tyrosine phosphorylation in peripheral tissues such as skeletal muscle cells *(40–42)*. TNF-α also suppresses the expression of genes that encode proteins that affect the diabetic state such as adiponectin, long-chain fatty acyl-CoA synthetase, peroxisome proliferator-activated receptor-γ (PPARγ), and GLUT4 *(42)*. It is not surprising, therefore, that elevated plasma TNF-α levels are highly associated with insulin resistance in humans and animals *(43,44)*.

Melatonin has also been shown to have immunomodulatory effects, and its administration to humans and animals was shown to lower circulating TNF-α and IL-1 levels and suppress inflammation (*45, 46)*. A recent study showed that melatonin induced rapid tyrosine phosphorylation and activation of the insulin receptor β-subunit tyrosine kinase in the rat hypothalamic suprachiasmatic region *(15)*. In humans and rats, melatonin receptors are primarily located in the suprachiasmatic nucleus (SCN) and pars tuberalis. The SCN directly controls the circadian rhythm that controls plasma glucose concentration. The infusion of melatonin into the lateral ventricle results in the phosphorylation of insulin receptors in a dose-dependent manner (10 pg–1 ng), which is followed by the transient tyrosine phosphorylation of IRS-1 and its association with PI3 kinase and SH2-containing phosphotyrosin phosphatase. Melatonin also induces downstream AKT serine phosphorylation and p42MAPK (mitogen-activated protein kinase) phosphorylation. These data suggest that melatonin may be able to influence the insulin-signaling pathway and thereby help to control basal glucose levels. Recently, we and others reported that long-term melatonin treatment lowered plasma levels of triglycerides, total cholesterol, insulin, and leptin in type 2 diabetic rats. Such treatment also resulted in an approx 50% reduction in plasma TNF-α levels. Thus, melatonin appears to act in a manner similar to thiazolidinediones, which are insulin-sensitizing agents that are specific PPAR-γ agonists.

Type 2 diabetic rats show the characteristic alterations in their proportion of fatty acids. Thus, they exhibit significant increases in their proportion of 20:3*n*-6 fatty acids and reductions in their proportion of 20:4*n*-6 fatty acids *(10, 47)*; these changes are primarily due to a reduction in Δ-5 desaturase activity in their liver microsomes. Melatonin restored Δ-5 desaturase activity in the liver of these animals and thereby reduced their elevated 20:3(*n*-6)/20:4(*n*-6) fatty acid ratio and restored their proportions of lipid fraction MUFAs and PUFAs *(10)*. The physicochemical properties of cell membranes are largely determined by the nature of the fatty acids within their phospholipid bilayer, which, in turn, influences a variety of cellular functions including hormonal responsiveness. Indeed, a close relationship was reported between the fatty-acid composition of skeletal-muscle phospholipids and insulin sensitivity in diabetic subjects *(48)*. Insulin sensitivity was shown to be reduced by low levels of PUFAs that had 20–22 carbon chains (arachidonic acid in particular), and high levels of MUFAs. Furthermore, changing the fatty-acid composition of phospholipids in cultured cells altered their insulin-binding characteristics *(48)*. Increasing the levels of PUFAs within the cell membrane increases membrane fluidity as well as the number of insulin receptors *(49,50)*; the opposite effects are seen when the concentration of saturated fatty acids in the membrane increases. Therefore, the restoration of PUFA levels by long-term melatonin administration might gradually result in an improvement in insulin sensitivity, which might in turn lead to a recovery of suppressed hepatic Δ-5 desaturase activity, a reduction in the 20:3*n*-6/20:4*n*-6 fatty-acid ratio, and an increase in membrane long-chain PUFAs *(51)*. Long-term melatonin treatment increased plasma PUFA levels in hypercholesterolemic rats. Melatonin also prevented changes in microsomal membrane fluidity during lipid peroxidation that were induced by the addition of $FeCl_3$, ADP, and NADPH *(11)*.

Pinealectomy, resulting in the loss of the nocturnal melatonin surge, was shown to affect insulin and glucose metabolism. Rodriguez et al. reported that pinealectomy increased plasma glucose and glucagon levels and reduced circulating insulin levels in normal rats; melatonin administration restored all of these changes to their normal values *(52)*. They also found that pinealectomy lowered insulin binding and increased glucagon binding to liver membranes by affecting receptor numbers rather than binding affinity; as above, melatonin treatment restored these binding parameters to normal. The degradation kinetics for insulin and glucagon were not influenced by pinealectomy or pinealectomy followed by melatonin treatment. These findings suggest that the pineal gland modulates circulating insulin and glucagon levels as well liver insulin and glucagon re-

ceptor concentrations. We reported that pinealectomized, type 2 diabetic rats displayed severe hyperinsulinemia, worsened insulin sensitivity, and an accumulation of liver triglycerides *(53)*. Furthermore, insulin secretory capacity, as determined by a glucose tolerance test, was impaired in these animals, as was the ability of their adipocytes to take up glucose, the latter of which may have been due reduced levels of GLUT-4 *(54)*. It is noteworthy that a deficiency in melatonin was reported to result in a reduction and increase in plasma insulin levels in normal and diabetic pinealectomized rats, respectively; the underlying mechanism of these effects has yet to be elucidated.

Recently, Derlacz et al. reported that melatonin enhanced glucose and lactate formation in primary cultures of rabbit kidney-cortex tubules. These changes were accompanied by an enhancement in alanine and glycerol consumption, suggesting that these cells were utilizing alanine as a substrate for glucose production *(55)*. Since luzindole, a melatonin antagonist, attenuated this effect, it is likely that melatonin exerted its effects by first binding to membrane-bound melatonin receptors (subtype ML1).

Cagnacci et al. *(23)* reported that endogenous melatonin seemed to reduce basal blood glucose levels as well as experimentally induced hyperglycemia in nocturnal animals *(9,56)*. In contrast, nighttime melatonin was reported to be associated with elevations in plasma glucose, insulin, and triacylglycerol in diurnal animals *(57)*. Furthermore, the exogenous administration of melatonin to these animals reduced their glucose tolerance and insulin sensitivity *(23)*.

Concluding Remarks

Melatonin plays an important role in a variety of physiological, immunological, and biochemical functions in animals. It was also shown to have direct and indirect antioxidant effects. Chronic melatonin administration in obese and diabetic rats reduced their hypertriglyceridemia, hyperinsulinemia, and plasma TNF-α levels, and restored their PUFA levels to normal. Thus, melatonin may yet prove to be an efficacious compound for the treatment of these conditions in humans.

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