Review

Ameliorating effects of melatonin on high-fat diet induced non-alcoholic fatty liver diseases and their associated pathologies: A comprehensive review

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is caused by hepatic fat accumulation with a high prevalence globally, especially in Western countries in which individuals have excessive fat consumption. Prolonged intake of high dietary fat causes various diseases due to the imbalance of energy metabolism, which leads to obesity and other pathological conditions. Currently, the exact pathogenesis of NAFLD is still obscure. In this review, the potential etiologies for NAFLD will be discussed, including adipose tissue dysfunction, intrahepatic *de novo* lipogenesis, hepatic fat accumulation, insulin resistance, hepatic inflammation, inflammasome activation, mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum stress. Melatonin is a potent antioxidant and anti-inflammatory molecule. It is also a regulator of lipid and glucose metabolism which is indicated by melatonin's effects on weight loss, reduction of liver weight, blood levels of lipids, glucose and insulin, activities of hepatic enzymes, steatohepatitis, and fibrosis. Melatonin considerably reduces mitochondrial dysfunction and proinflammatory cytokines. Moreover, it downregulates NLRP3 and its associated downstream effectors of caspase-1, IL-1β, and IL-18 proteins. This review will update the molecular mechanisms behind high-fat diet induced hepatic dysfunction and the protective role of melatonin in NAFLD.

Key words: NAFLD, HFD, obesity, oxidative stress, mitochondria, inflammasome, melatonin

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1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a pathological condition caused by the deposition of fat in hepatocytes leading to hepatic steatosis without alcohol consumption (1). The progression of NAFLD is associated with obesity, insulin resistance, type 2 diabetes, hypertension and dyslipidemia, collectively termed as "metabolic syndrome" (2-4). One of the hepatic manifestations of obesity is NAFLD which is primarily caused by high-fat diet (HFD). Most of the NAFLD patients require serious medical attention. Its prevalence in obese patients is around 90% (5) and many of them have metabolic syndrome (6, 7). The lifestyle modification

with diet, exercise and weight loss is the important management for NAFLD patients to improve their liver function (8). Therapeutic targets for NAFLD including improvement of insulin resistance, dyslipidemia, and oxidative stress, may alleviate the pathological progression (5, 8). The natural compounds, such as vitamin E and omega-3 polyunsaturated fatty acids, have showed potential protective effects against NAFLD (5) and with little side effects (9).

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring molecule with multiple functions (10). It is synthesized from various tissues and organs including pineal gland of mammals (11). Various studies have showed that melatonin lowers incidence of obesity, type 2 diabetes, and liver steatosis (12, 13). Its use can influence the proliferation of pancreatic β cells in streptozotocin-induced type 1 diabetic rats with reduced blood glucose levels (14). Pinealectomy results in significant hyperinsulinemia and triglyceride accumulation in the liver owing to reduced melatonin levels (15). Melatonin administration improves lipid metabolism by restoring insulin resistance in type 2 diabetic rats (16, 17). The detailed pathway of NAFLD pathogenesis remains elusive. Studies have shown that hepatic steatosis and NASH (non-alcoholic steatohepatitis) are directly associated with high dietary fat in humans and other animals (18,19). Furthermore, multiple adverse "hits" are associated with dietary fat and fatty liver disease progression and the most accepted “multiple hit” theory elaborates on the pathogenesis of NAFLD (20). In this review, we elaborated on the molecular mechanisms of a high-fat diet (HFD) induced NAFLD and the possible ameliorative effects of melatonin.

2. EPIDEMIOLOGY OF NAFLD

NAFLD is one of the most prevalent diseases worldwide, affecting approximately a quarter of the adult population. The prevalence of NAFLD in Africa is 13%, in the Middle East is 32% and in South America is 30%. The prevalence of NAFLD in the individuals with severe obesity (90%) and/or type 2 diabetes (76%) is higher (2) than lean individuals who only have the prevalence of 16% (4). In Asia, the prevalence of NAFLD vary widely because of the vast geographical area with diverse socioeconomic conditions, diet, and lifestyles which are risk factors of NAFLD (21-24). The incidence of NAFLD in Asian countries ranges from 12.5–38% including 23–26% in China Mainland, 27% in Japan, 12–51% in Korea, 28% in Taiwan, 5–30% in Hong Kong, other regions of South Asia and far East Asia (24). According to a recent epidemiological report, NAFLD affects 9-32% of the general population in India, with a higher prevalence among those who are overweight/obese and those have diabetes or prediabetes (25).

3. NAFLD ASSOCIATED PATHOLOGIES

NAFLD is associated to many liver diseases, including simple hepatic steatosis to non-alcoholic steatohepatitis (NASH). The pathogenesis of NASH is multiples and is characterized by the irreversible development of fibrosis and cirrhosis of the liver. The deposition of fat in hepatocytes, particularly triglycerides (TG) and fatty acids contributes to NAFLD as the hepatic manifestation of metabolic syndrome or insulin resistance syndrome (26, 27). While the processes underlying NAFLD pathogenesis remain unknown, “double-hit” hypothesis is usually used to explain the comprehensive paradigm for NAFLD progression. The major cause of steatosis is insulin resistance (IR), which causes hepatic *de novo* lipogenesis (DNL) and fatty acid (FA) transport impairment. Endoplasmic reticulum stress, autophagy disruption,

mitochondrial malfunction, hepatocyte apoptosis, and an elevation in inflammatory responses are among the events or multiple strikes in the second hit (20). Overall, evidence suggests that NAFLD development requires numerous interconnected mechanisms. Fat deposition in the liver (first hit) increases susceptibility to risk factors (second hit), leading to the progression of NAFLD to more severe NASH, cirrhosis, and hepatocyte cancer. The "multiple hit" hypothesis is also accepted since a complex interplay between many events in correlation with genetic predisposition, provides a realistic explanation of the mechanisms underlying NAFLD (28).

3.1. Effect of high-fat diet on the pathogenesis of NAFLD.

High dietary saturated fats and carbohydrates promote NAFLD. A high-fat diet (HFD) causes NAFLD in various animal models (29, 30). In body, the lipids are converted to triglycerides in the intestine and form chylomicrons for delivery to the tissues including muscle and adipose tissue. Chylomicrons are broken down into fatty acids by the action lipoprotein lipases (LPLs) in target tissues. Adipose tissue absorbs and stores some of these free fatty acids (FFA) and around 33 –36% of total FFAs are delivered to liver for further metabolism (31). Hepatic steatosis can occur after few days of HFD consumption in animals and humans (32). The primary source of hepatic lipid accumulation in NAFLD is circulating FFAs, primarily derived from adipocyte lipolysis and dietary fat (33). Pancreatic lipase hydrolyzes dietary triglyceride, emulsified in the intestinal lumen by bile acid, to create sn2-monoacylglycerol and FFA products (34). Enterocytes re-synthesize and assemble the lipid to triglyceride after emulsification. Triglyceride is packaged into chylomicrons and secreted into the lymphatic system before reaching the plasma. A substantial amount of chylomicron with triglycerides are extracted by muscle and adipose tissue. In the case of HFD, insulin resistance induces higher lipolysis ratio in adipose tissue, leading to a rise in blood FFA, which is absorbed by the liver and converted to triglyceride stored in lipid droplets. Fat accumulation causes liver damage (20). High FFAs, particularly saturated fatty acids (SFAs), induce lipotoxic process in animal or in NAFLD patients. SFAs are the most harmful dietary lipids to cause organ damage. SFAs in hepatocytes induce death receptor signalling and ER stress leading to intrinsic mitochondrial apoptosis, activation of toll-like receptors, inflammasome formation, and autophagy inhibition (35, 36). Individuals with NASH have high absorption of SFAs and cholesterol and low absorption of dietary polyunsaturated fatty acids (PUFA) (37). Moreover, Toshimitsu *et al*. showed the lower level of PUFAs and, higher level of SFAs in individuals with fatty liver and NASH compared to healthy individuals (38). In addition, individuals subjected to an SFA diet exhibited the increased insulin resistance, hepatic steatosis, and inflammation compared to PUFA-fed individuals (39).

3.2. Adipose tissue dysfunction after a period of HFD consumption in NAFLD.

Under normal condition, adipose tissue is sensitive to insulin for lipid metabolism. High levels of FFA causes lipid accumulation, insulin resistance and dysregulation of lipolysis (40). Adipocyte dysfunction is related to the severity of liver injury and cardiac disease in NASH (41-43). NASH causes the hepatic and peripheral insulin resistance to promote hepatic FFA flux, steatosis, and inflammation (40).

Obesity is an important cause of the increased global incidence of NAFLD, the exact link between obesity and NAFLD is unknown. Expansion of the peripheral adipose depot may act as

a buffer, shielding the liver from excessive FFA flux. Lipodystrophy is an example of a collection of diseases characterized by partial or complete loss of adipose tissue but severe insulin resistance, ectopic fat deposition, NAFL, and NASH (41). Even metabolically healthy obese individuals have a significantly higher risk of NAFLD (42), implying that obesity is a potential risk factor for NAFLD independent of insulin sensitivity. Adiponectin secretion is also reduced in dysfunctional adipose tissue, this insulin-sensitizing adipokine is paradoxically reduced in obesity. This adipokine increased FFA oxidation and decreased FFA inflow, gluconeogenesis, and DNL (43). It also exhibits anti-inflammatory and antifibrotic characteristics in the liver, reducing the upregulation of hepatic stellate cells (HSC) (44-46) and suppressing pro-inflammatory cytokines [e.g., tumor necrosis factor-α and interleukin (IL)-6] (47, 48). The frequency of hepatic steatosis, necroinflammation, and fibrosis correlates with the circulating adiponectin level in patients with NAFLD and NASH (49). Pioglitazone is an insulin sensitizing drug, significantly improves NASH histology (50). Adiponectin therapy also improves NASH in rodent models (51); however, human studies are required to confirm the results from animal studies (52).

3.3. Intrahepatic *de novo* lipogenesis and hepatic fat accumulation in NAFLD.

DNL is the second most important intrahepatic FFA source, derived from nonlipid precursors such as glucose and fructose. The elevated DNL is another characteristic of NAFLD. The DNL in NAFLD patients has 3.5-fold rise compared to healthy controls (53, 54). Interestingly, although adipose-derived FFA contributes to the predominance of liver triglyceride, but it has a less elevated level than that of DNL in NAFLD (53). Insulin promotes lipid synthesis by transcription and stimulation of sterol regulatory element-binding protein-1c (SERBP-1c), which is a crucial regulator of lipogenesis (55), and insulin also stimulates DNL while at the same time does not reduce hepatic gluconeogenesis even in insulin-resistant conditions such as T2DM, obesity, and NAFLD (56, 57). A variety of insulin-dependent (58-60) and independent mechanisms regulate hepatic lipid and glucose metabolism while the significant roles of these differentiated regulations on hepatic lipid and glucose metabolism are complicated (61). The primary source for DNL is dietary glucose (57, 62). The increased level of carbohydrates increases liver lipid content by DNL (62). The dietary carbohydrates directly enter hepatocytes via portal circulation, whereas dietary lipids indirectly via lymphatic and systemic circulation. Therefore, dietary lipid has a limited contribution to intrahepatic triglyceride of NAFLD patients (63). Insulin resistance in skeletal muscle may also influence steatosis by using postprandial glucose for hepatic DNL but not store it as the peripheral glycogen (64, 65). A major population survey showed that skeletal muscle mass was inversely related to NAFLD and directly connected to the resolution of baseline NAFLD (66). Generally, lipid accumulation occurs in the liver of NAFLD patients as esterified FFA or triglycerides. Alternatively, FFA can enter β-oxidation, or triglycerides can be transported from the liver in the form of very low-density lipoprotein (VLDL). In NAFLD, steatosis is caused by an imbalance in the lipid input and synthesis vs disposal mechanisms. There are three major sources of FFA in liver including 59% from circulating FFA; 26% from DNL via nonlipid precursors (such as glucose and fructose), and 14% comes from the diet (63).

3.4. HFD induced insulin resistance on NAFLD.

Insulin usually lowers glucose level by inhibiting gluconeogenesis and glycogenolysis as well as stimulating glycogen synthesis and lipogenesis (67). It also promotes lipolysis, fatty acid esterification and lipid storage. As a result, hepatic insulin signaling is critical for maintaining energy balance through regulation of glucose and lipid metabolism. It is still obscure whether insulin resistance is the effect of hepatic fat accumulation or it is the causative factor of it. Adipose tissue dysfunction, DNL, and fatty acid metabolic disorder are caused by insulin resistance with the liver fat accumulation (65). Furthermore, insulin inhibits lipase, a rate-limiting enzyme for triglycerides lipolysis in adipocytes, and insulin-induced lipolysis is suppressed by insulin resistance in the adipose tissue. The substantial levels of FFA are transported from adipose tissue to the liver under insulin resistance, resulting in ectopic intracellular FFA accumulation. HFD causes various insulin resistance syndromes including metabolic disorders, abdominal obesity, hypertension, and cardiac disease. Epidemiological and experimental data have shown that insulin resistance and its complications are higher in subjects with long-term of HFD than those with normal diet. Moreover, adipose tissue is the storage site for lipids which are from excessive energy intake. After excessive lipid accumulation, adipose tissue secretes many inflammatory cytokines such as TNF-α, IL-6, and MCP-1. These pro-inflammatory cytokines regulate adipocyte responsiveness to insulin (68). The ability of HFD to induce insulin resistance and hepatic steatosis has been well studied in animals. HFD induced hepatic insulin resistance in the adult male Wistar rat has been reported by Kraegen *et al.* (69). They measured insulin levels after isocaloric HFD or high-starch feeding (59% and 10% cal) for 3 weeks. HFD-fed rats showed more glucose intolerance than starch-fed control rats (69). Furthermore, HFD feeding in C57BL/6J mice also caused obesity, insulin resistance, NASH, and liver cancer (70) and these observations have been confirmed by Nakamura and Terauchi (71). HFD-fed mice had higher fasting insulin and leptin levels, whereas plasma adiponectin level was significantly lower than in controls. A recent study found that the c-Jun N-terminal kinase (JNK) pathway influences insulin signaling and lipid-mediated metabolic stress (72). Hepatic p-JNK protein level rises sharply following HFD feeding (73), which promotes hepatic lipid accumulation and liver damage (74). Furthermore, the activation of genes encoding the lipogenic transcription factor SREBP-1c down-regulates genes related to glucose metabolism in animal models of insulin resistance (75). Lipogenic pathway was activated during insulin resistance through insulin receptor activation (76, 77). This implies that multiple other downstream intracellular signaling pathways are involved. One of these downstream pathways is the mammalian target of rapamycin complex 1 (mTORC1). When mTORC1 is inhibited, lipogenesis is reduced, while lipogenesis is stimulated under insulin resistance (78). All the evidence shows that insulin resistance is the primary regulator of HFD-induced NAFLD pathways.

3.5. NAFLD induced hepatic inflammation, immune cells, and inflammasome activation.

High levels of FFAs, insulin resistance, gut-generated endotoxins, and adipose tissue dysfunction in NAFLD induce a severe pro-inflammatory state in the liver, which promotes NASH and fibrosis. Patients with NASH have higher tumor necrosis factor-alpha (TNF-α) level in their hepatic and adipose tissues than obese controls, which corresponds with fibrosis complexity (79). NASH patients showed persistent activation or overexpression of the

transcription factor nuclear factor kappa light chain enhancer of activated B cells (NF-κB), an essential regulator of the acute inflammatory response, has been discovered (80). Hepatic inflammation is triggered and amplified by various immunological responses (81). Hepatocyte injury induces the secretion of host biomolecules known as damage-associated molecular patterns (DAMPs). These molecules can stimulate inflammation by inducing local macrophages, Kupffer cells (KC), and pattern recognition receptors like the toll-like receptor (TLR) family. TNF-α, IL-1β, IL-6, and C–C motif ligand 2 and 5 are among the pro-inflammatory cytokines produced by activated KC, which aggravate hepatocyte damage and cell death, primarily by apoptosis (80). Transforming growth factor β (TGF β) and platelet-derived growth factors are also secreted by KC, providing further stimulation to HSC, which induces fibrosis by upregulating smooth muscle actin, desmin, and type I collagen. Gut-derived bacterial compounds, such as lipopolysaccharide (LPS), can also activate KC and HSC, through pathogen-associated molecular patterns (PAMPs). In experimental mouse models of NASH, suppressing TLR-4, the LPS receptor, and TLR-9, which binds bacterial DNA, has been shown to diminish liver inflammation (82, 83). Inflammasomes are multiprotein intracellular complexes in response to cell injury or infections to recruit IL-1β and IL-18, which are also activated by DAMPs, PAMPs, and KC. Inflammasomes directly relate to several acute and chronic liver illnesses (84). In mouse models of NASH, FFAs activate inflammasome and sensitize hepatocytes to LPS (85). In contrast, inflammasome-deficient animals are protected from diet-induced NASH and fibrosis (86). Neutrophil infiltration is found in the hepatocyte of NASH patients, which promotes macrophage aggregation and injury of the cell by elevated reactive oxygen species (ROS) and inflammatory substances like myeloperoxidase and elastase (87).

3.6. Mitochondrial dysfunction on NAFLD.

In addition to regulating the tricarboxylic acid cycle, ATP synthesis by oxidative phosphorylation, mitochondria are the major sites of FFA oxidation in hepatocytes (88). Mice fed with HFD containing lard displayed the altered cardiolipin acyl chain composition in hepatocytes, and this event is followed by declined 3.5-fold respiratory enzyme complex I and III activates (89). Excessive palmitate intake has also affected cardiolipin production, which is associated with cytochrome c release from the mitochondria (90, 91). Compared with healthy controls, patients with NAFLD have elevated mitochondrial biogenesis, mitochondrial mass, and maximal respiration rate for regulating high lipid deposits (92). This adaptation of mitochondria eventually will cause uncoupling, increased ROS production, and oxidative stress, resulting in NASH and severe insulin resistance in hepatocytes (93). Increased lipid peroxidation (94) and TNF-α secretion lead to hepatic damage (92). The progression of NASH may instigate the hepatic sensitivity to TNF-α caused by mitochondrial cholesterol storage and glutathione depletion (93). The NASH may also relate to the impaired mitophagy, which is the selective autophagic clearance of damaged mitochondria. In healthy individuals, mitophagy helps prevent cell death by reducing oxidative stress and maintaining mitochondrial bioenergetics. However, most of the symptoms of metabolic syndrome, such as obesity, insulin resistance, and dyslipidemia, compromise this function (95, 96). The severity of liver disease and oxidative stress correlate with mitophagy, which has been significantly hindered in patients with NASH compared to patients with steatosis (97, 52).

3.7. Oxidative stress and endoplasmic reticulum stress in NAFLD.

The antioxidant defence system is mainly activated by oxidative stress. These antioxidant pathways are altered in NAFLD. The NAFLD patients have higher activities of the antioxidant enzymes SOD and GPX than the healthy controls (98). Profibrotic and pro-inflammatory genes are often overexpressed in hepatic stellate cells that lack the glutathione peroxidase 7 (GPX7) isoform in response to FFA exposure. Overexpression of GPX7 in these cells reduces ROS production and the expression of profibrotic and pro-inflammatory genes. GPX7 deficiency promotes choline-deficient, L-amino-defined, high-fat diet-induced NASH fibrosis (99). Data from patient liver biopsies and mice model of NASH show an elevated glutaminase 1 (GLS1) expression. GLS1 inhibition reduces hepatic triglyceride accumulation in mice fed with a methionine choline-deficient (MCD) diet, restores the exportation of VLDL triglyceride and lowers ROS generation. GLS1 inhibition is also linked to a reduction in lipid peroxidation (100). Paraoxonase-1 is an antioxidant enzyme in the liver that hydrolyses peroxides and lactones. In a cohort of 81 NAFLD patients, low levels of serum paraoxonase-1 were observed, which reflects higher oxidative stress (101). The peroxisomal antioxidant enzyme catalase is crucial for protecting cells from oxidative damage by reducing H_2O_2 concentration. In HFD-fed with catalase deficient mice, lipid accumulation and oxidative stress are exacerbated (102).

The endoplasmic reticulum (ER) regulates calcium homeostasis, lipid production, secretion, and the folding process of transmembrane proteins. Higher FFAs induce ER stress by influencing lipid production of the organelle membranes that stimulate many transcription factors and kinases (103). Numerous pathological conditions, such as inflammation, photodamage, cardiovascular disease, cancer, and metabolic disease, have been linked to ER stress (104-106). NAFLD has been linked to ER dysfunction (107). Additionally, the ER is the primary site for lipid synthesis in hepatocytes. Sterol regulatory element binding proteins and transcription factors in the ER membrane are associated with the DNL process. The liver stores triglycerides by acyltransferase enzymes present in ER (108). VLDL is secreted by hepatocytes and it is assembled in the ER before being transported to the Golgi apparatus (109). Therefore, ER is where most lipid production occurs, and ER stress response has become an essential factor in the development of NAFLD. Saturated fatty acid accumulation alters ER homeostasis, which causes ER stress with progression of NAFLD. ER stress are present in the steatotic livers of mice with HFD (110). Additionally, aberrant lipid changes in hepatocytes may directly impact calcium signaling, which can impair protein translation and cause cell death (111).

4. THERAPEUTIC ROLE OF MELATONIN ON NAFLD

Recently, melatonin has been shown to improve metabolic disorders in preclinical studies. Melatonin is a free radical scavenger and a regulator of insulin sensitivity, lipid level, glucose metabolism, and the pathological change in NAFLD (112, 113). Currently, there is no specific treatment on NAFLD. Exercise and weight loss are the alternative ways to improve insulin sensitivity and reduce obesity. Different antioxidants, hepatoprotective medicines, hypolipidemic drugs, angiotensin receptor blockers, and insulin sensitizers have also been used to reduce the symptoms of NAFLD (114). Melatonin may be a suitable therapeutic molecule in the HFD-induced NAFLD since other antioxidants including vitamin E, vitamin C, and betaine (which generates glutathione as a major hepatic antioxidant), showed some successful in treatment of

NAFLD/NASH (112-114). Studies related to the protective effects of melatonin on liver diseases are listed in the Table 1.

Table 1: List of the studies related to melatonin on HFD induced liver disease.

AUTHORS	KEY FINDINGS
Saha *et al.*, 2022 (115)	Melatonin binds with TL4 and P2X7R consequently deactivating NF- κB and calcium influx as well as Nrf2/NOX/ROS pathways, respectively; hence, inhibits NLRP3 inflammasome. Amelioration of liver fibrosis by enhanced TIMP and restrained MMP-2 and MMP-9.
Joshi *et al.*, 2021 (116)	Melatonin ameliorates oscillatory pattern of clock genes and improves profile of lipid regulatory genes.
Yu *et al.*, 2021 (113)	Melatonin reduces oxidative stress and controls the activation of NLRP3 inflammasome by down regulating IL-1β, IL-18, caspase-1 and related mRNA.
Mansoori *et al.*, 2020(117)	Melatonin decreases AST, ALP and has limited effect on ALT.
Stacchioti *et al.*, 2019 (118)	Melatonin decreases the expression of microRNA-34 a-5p and SREBP1 in presence of SIRT1; minimizing autophagy and repairing mitochondrial damage. Hepatic metabolism and steatosis are enhanced and ER stress is alleviated.
Li *et al.*, 2019 (119)	Melatonin inhibits the expression of total ASK1, phosphor-MKK3/6, phosphor-p38, phosphor-MKK4/7 and phosphor-JNK. β-arrestin activation to block the interaction between ASK1 and TRAFS; thereby, decreases the deubiquitination of ASK1 and its protein stabilization.
Zhou *et al.*, 2018 (120)	Melatonin administration inhibits NR4A1/DNA-PKCs/p^{53} pathway and thus, ceases mitochondrial fission, repairs mitophagy and enhances mitochondrial and liver function.
Mi *et al.*, 2018 (121)	Melatonin activates AMPK by phosphorylating it and deactivates ACC by dephosphorylation. Melatonin upregulates the expression of lipolytic PPARα and CPT1 whereas it down regulates lipogenic mRNA level of SREBP-1c FAS.
Chitrat *et al.*, 2017 (122)	Melatonin subsides the expression of AIAT, FBG, PLG, MBP-A, complement C4 and complement factor B associated with liver pathology due to HFD and scavenges free radicals by lowering plasma serotransferrin.
Sun *et al.*, 2016 (123)	Melatonin attenuates the mRNA expression of pro-inflammatory cytokines such as TNF-α, IL-β, IL-6 and MAPK. Reduced phosphorylation of p38 and JNK1/2 decrease body weight and declines FPG, ALT, AST and LDL.
Ruiz *et al.*, 2014 (124)	Melatonin, MnTBAP or Uric acid reduce the over expression of NASH genes (TNF-α, IFN-γ, MCP-1, caspase-3, 3-tyrosine nitrated proteins). Furthermore, antioxidants and antiperoxynitrites increases OXPHOS subunits encoded by mitochondrial DNA. Nitroso-oxidative stress is the underlying mechanism for the pathogenesis caused by HFD induced NASH.
Celinski *et al.*, 2014 (125)	Pro-inflammatory cytokines (IL-1, IL-6 and TNF-α), Gamma-Glutamyl Transferase (GGPT) activity and Triglycerides and LDL-cholesterol are notably reduced in NFALD patients treated with melatonin and tryptophan.
Hatzis *et al.*, 2013 (112)	Melatonin significantly reduces the MDA, AST and ALT and increases GSH. However, melatonin has no significant effect on lipid markers in fatty liver in contrast to previous findings.
Zaitone *et al.*, 2011 (126)	Alone or mixture of pioglitazone, pentoxifylline, melatonin decreases IR index activities enzymes and malondialdehyde in hepatic tissues whereas the glutathione level is elevated. Serum TNF-α is diminished by pentoxifylline pioglitazone and melatonin with the reduced serum total cholesterol and triglycerides.
Lugo *et al.*, 2010 (127)	Melatonin decreases body weight without compromising the food intake; circulating insulin, glucose triglyceride, cholesterol, leptin mean levels, and the daily pattern of adiponectin as compared to normal diet fed rats.
Hussein *et al.*,	The changes of GSH-PX, HDL fatty acid level in hepatic and renal tissue and

2007 (128)	atheromatous in blood vessels are preserved by melatonin treatment.
Pan *et al.*, 2006 (12)	The elevated serum alanine aminotransferase, aspartate aminotransferase, total cholesterol, triglycerides and MDA levels, SOD and GSH-Px activities were reduced by melatonin treatment.
Bongiorno *et al.*, 2005 (129)	The competition of melatonin and cholesterol for the hydrophilic binding sites in the reversed micelles modifies Lecithin molar ratio.
Sener *et al*, 2004 (130)	Plasma, liver cholesterol and diene conjugate (DC), liver triglycerides, aortic cholesterol and DC levels are improved by melatonin treatment.
Marcassus *et al.*, 2003 (131)	Melatonin reduces body weight, feeding efficiency, plasma insulin, glucose, leptin and triglyceride levels.
Pita *et al.*, 2002 (132)	Melatonin reduces plasma palmitoleic acid and increases n-6 and n-3 PUFA in hypercholesterolemic rats; therefore, efficiently improves fatty infiltration in arteries due to cholesterol deposition.
Nieminen *et al.*, 2001 (133)	Melatonin shows sexual dimorphism in minks by lowering lipase esterase activity in male while enhancing glucose-6-phosphatase activity and reducing liver cholesterol and plasma polar lipids in female.
Hoyos *et al.*, 2000 (134)	Melatonin decreases total cholesterol and LDL-cholesterol in cholesterol rich diet fed rats whereas reduces HDL-cholesterol, serum uric and bilirubin, LPO and elevated serum glucose levels.

4.1. Melatonin on fat metabolism.

Melatonin is closely associated with fat metabolism including lipolysis, fat deposition, BAT (brown adipose tissue) development, beige adipogenesis. BAT and beige adipogenesis affect energy expenditure (135). Recently, Pan *et al*. (136) have reviewed the potential mechanisms of melatonin signaling on lipolysis and adipogenesis. Melatonin increases adipocyte lipolysis and lipolytic gene expression including hormone-sensitive lipase (HSL), adipocyte triglyceride lipase (ATGL), and perilipin 1 (PLIN1) (137, 138). Melatonin reduces cholesterol absorption and mitigates plasma lipid profiles in high cholesterol diet fed rats (139). The hypocholesterolemic action of melatonin enhances endogenous cholesterol clearance via synthesis of bile acid and inhibition of LDL receptor activation, rather to modify fatty acid synthesis (140, 141). Melatonin modulates the rate of mitochondrial respiratory activity in beige and white adipose tissues (WAT) (142). Irisin, a myokine derived from fibronectin type III domain containing 5 (FNDC5) in muscle and released into the circulatory system, has the ability to promote uncoupling protein 1 (UCP1) expression in WATs and cause WATs to perform non-shivering thermogenesis similar to BATs (143). Melatonin administration for a long period lowered FNDC5 mRNA and restored irisin sensitivity in WATs. The reduced FNDC5 by melatonin increases circulating irisin to stimulate adipocyte browning with enhanced lipid oxidation and thermogenesis (144).

4.2. Melatonin on obesity and insulin resistance.

Numerous medical conditions, including diabetes *mellitus*, cardiovascular diseases, and metabolic syndrome, are directly or indirectly correlated with obesity (145, 146). Males typically have adipose tissues around 8–18% of the body weight while females are around 14–28%. However, adipose tissues can reach to 60–70% of body weight in obese individuals (147). Numerous studies have investigated the regulatory effects of melatonin on body weight and adipogenesis. Tan *et al*. (146) have presented an elaborate and concise review to outline these effects. In middle-aged rats, daily oral melatonin for 12 weeks (0.4 to 4g mL-1) significantly

decreased abdominal adiposity and body weight (148, 149). Melatonin administration for 16 weeks (4 mg kg-1 day-1) reduced weight gain and other obesity-associated metabolic changes including high visceral fat deposition, increased serum TG, insulin, leptin, and HDL-C levels (149, 150). Both animal and human studies have demonstrated the important contribution of melatonin in regulating blood glucose. In pinealectomized animals, the expression of the glucose transporter type 4 (GLUT4) gene is decreased, which induces glucose intolerance and melatonin supplement can correct these abnormalities (151, 152). An eight-week course of co-treatment with insulin (NPH, 1.5 U/100gr/day) and melatonin (0.2 mg/kg/day in drinking water) in streptozotocin (STZ) induced diabetic rats improves glucose homeostasis and insulin sensitivity of white adipose tissue (153). Melatonin production is decreased in diabetic rats with the lower level of arylalkylamine N-acetyl transferase (AANAT) activity which is the rate-limiting enzyme of melatonin synthesis. Insulin therapy has been shown to increase melatonin levels (154). Melatonin treatment at the dosages of 5 or 10 mg/kg for 4 or 8 weeks ameliorates the progression of NAFLD in rats. Melatonin showed a strong ability to reduce hepatic steatosis, serum aminotransferases, portal vein pressure, and liver weight. Long-term HFD feeding increases parameters like liver weight, liver weight over body weight ratio, portal vein pressure, serum aminotransferases, TC, TG, LDL, and HDL levels in the rats while melatonin treatment improves all these parameters (112). These findings are consistent with earlier data shown melatonin's hepatoprotective effect in rat models of diet-induced NAFLD (12). In db/db mice, melatonin administration significantly decreased body weight, liver weight, serum lipids, blood sugar, serum insulin, and hepatic enzymes (113). Melatonin also lowers NAFLD-related pathogenesis, such as lipid storage, steatohepatitis, fibrosis, and levels of oxidative stress (113). Srinivasan *et al*. (149) have elaborated on ameliorating effect of melatonin on HFD-induced weight gain and inflammatory responses. As melatonin has the anti-inflammatory and anti-obesity properties, melatonin has been considered to have a therapeutic role in NAFLD.

4.3. Anti-inflammatory function of melatonin on NAFLD.

Melatonin has the anti-inflammatory property. Dysregulation of lipid metabolism is frequently linked to an inflammatory state during the onset and development of hepatic steatosis (155). The chronic inflammatory response in the body induces insulin resistance, which leads to ectopic fat accumulation in the liver (123, 156). In the mice model of alcohol-induced hepatic injury, Hu *et al*. (157) have discovered that melatonin treatment significantly reduces the severity of hepatic cell damage, steatosis, and the immigration of inflammatory cells, decreases serum and tissue inflammatory cytokines levels, tissue lipid peroxidation, neutrophil infiltration, and inhibits hepatocyte apoptosis. Moreover, melatonin also stimulates T-cell proliferation in a dose-dependent manner. Additionally, melatonin inhibits the synthesis of IFN-γ at concentrations of 0.1 to 1 mM (158, 159). Oral melatonin decreases levels of pro-inflammatory cytokines of IL-6, TNF-α, and CRP, oxidative stress, or minimize inflammation in young Zucker rats, which is an experimental model of the metabolic syndrome and type 2 diabetes (160). Ozkanlar *et al*. (161) have observed that type 1 diabetes rats have lower serum levels of IL-1β after receiving melatonin. A recent study shows that melatonin significantly declines NF-κB levels in diabetic rats. NF-κB is a transcription factor that regulates the synthesis of the pro-inflammatory cytokines of TNF-α, IL-1β, and IL-6. In addition, melatonin can lower the transcriptional activation of TNF-α and IL-1β (162) by preventing NF-κB from binding to DNA (163).

Melatonin significantly reduces MMP, serum IL-1β and IL-18 levels and minimizes the hepatic tissue's NLRP3 inflammasome levels. The downregulation of the NLRP3, caspase-1, IL-1β, and IL-18 proteins inhibits NLRP3 inflammasome cascade inactivation (113). These findings ensure the anti-inflammatory activity of melatonin in NAFLD.

4.4. Regulatory function of melatonin on mitochondrial dysfunction.

Melatonin and its metabolites are potent antioxidants that ameliorate liver damage by scavenging free radicals including $O2^{\cdot-}$, $OH^{\cdot}$, H_2O_2 and peroxyl radicals (164, 149). It can also instigate antioxidant enzymes including SOD, CAT, GPX and glutathione reductase (GR) (154). Melatonin rectifies obesity-associated mitochondrial dysfunctions by inducing FFA-mediated downregulation of mitochondrial β-oxidation and decreasing hepatic fat deposition (165). A recent study has reported that melatonin inhibits PA-induced mitochondrial fragmentation by regulating the expression of genes involved in sustaining a healthy mitochondrial morphology. The balance between mitochondrial fission and fusion is regulated by a set of crucial genes that maintains mitochondrial dynamics (166, 167). The FFA-induced mitochondrial fragmentation, followed by a decline in mitochondrial mass, is inhibited by melatonin. Melatonin restores the canonical expression of mitofusin 2 (MFN2), which is involved in mitochondrial fusion and thereby maintained normal mitochondrial mass (168). SIRT1 deacetylates and stabilizes MFN2 in healthy hepatocytes that get disrupted during hepatic injury (169). Higher metabolic flux elevates ROS generation and increases mitochondrial fission (170). Mitochondrial fission triggered by lipids like palmitate decreases proton leakage, hyperpolarization of mitochondrial membrane and enhancing ROS production. Interestingly, melatonin is found to rescue the mitochondrial membrane potential, and reduces oxidative damage to the cells (171, 168).

4.5. Role of melatonin on liver fat accumulation and NAFLD.

The impairment of glucose and lipid metabolism and insulin resistance have all been linked to hepatic steatosis (172). NAFLD is a reversible disease but can cause steatohepatitis if not properly treated. Pan *et al*. (12) shows that high-fat diet-induced NAFLD can be mitigated by melatonin treatment. Rats fed with HFD have high oxidative stress, leading to significant liver steatosis. Melatonin decreases hepatic steatosis and inflammation with low serum AST, ALT, liver total cholesterol, and triglycerides in rats receiving a high-fat diet compared to the controls. Melatonin is also able to reduce hepatocyte apoptosis (173). When methionine- and choline-deficient diet-induced non-alcoholic steatohepatitis rats are treated with melatonin (50 mg/kg/day, intraperitoneally) for one month, their oxidative stress, proinflammatory cytokines and hepatocyte apoptosis level are reduced (123). Melatonin is a hydroxyl and peroxyl radical scavenger and an immunomodulatory agent (174) and it is mainly produced by pinealocytes at night but, a large quantity of melatonin is also generated by entero-endocrine cells of the gastrointestinal tract and liver (175). Several experimental studies show the therapeutic effect of MT on liver injury mediated by its antioxidant action (176, 177). Pan *et al*. (12) have demonstrated that MT exerts a protective effect against fatty liver of rats induced by HFD and is accompanied by decreased plasma ALT and AST activity. Another report shows that a four-week treatment with L-tryptophan, a precursor of MT, in patients with NASH results in a statistically significant reduction in plasma GGT and pro-inflammatory cytokine levels (178).

5. CONCLUSION

Currently, obesity has become a concerning issue throughout the world. Increased high saturated fat consumption is a main factor for obesity and its associated health hazards. Treatment with antioxidants can reverse the pathological complexity of obesity. Among them, melatonin has potent anti-oxidative and anti-inflammatory properties. In several clinical and animal studies, melatonin improves lipid metabolism and lowers hepatic fat accumulation. Melatonin also reduces HFD-induced insulin resistance and diabetic condition. The protective roles of melatonin on HFD induced NAFLD and its associated pathways have been illustrated in Figure 1. As melatonin is safe to use with minimal side effects and inexpensive, it can serve as a therapeutic alternative for the prevention and treatment of dyslipidemia and hepatic steatosis, its use may completely reverse the NAFLD and its pathologies.

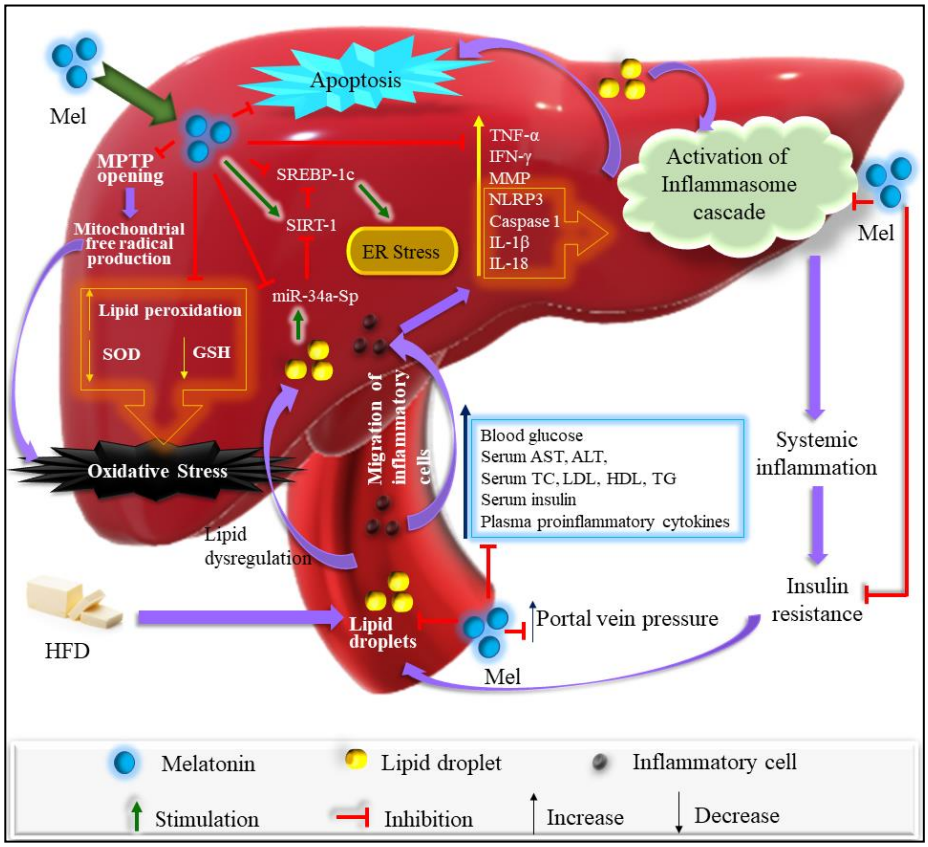

Figure 1: Schematic representation of protective roles of melatonin on HFD induced NAFLD and its associated pathways.

MPTP: Mitochondrial Permeability Transition Pore, AST: Aspartate Transaminase, ALT: Alanine Transaminase, TC: Total Cholesterol, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, TG: Triglyceride, HFD: High Fat Diet, SOD: Superoxide Dismutase, GSH: Glutathione, SREBP1: Sterol Regulatory Element-Binding Transcription Factor 1, SIRT1: Sirtuin 1, miR-34a-Sp: Micro RNA 34a-Sp, TNF-α: Tumor Necrosis Factor-α, IFN-γ: Interferon-γ, MMP: Mitochondrial Membrane Potential, NLRP3: Nod Like Receptor Protein 3, IL-1β: Interleukin-1β, IL-18: Interleukin-18, ER stress: Endoplasmic Reticulum stress.

Abbreviations

NAFLD- Non-alcoholic fatty liver disease
NASH- Non-alcoholic steatohepatitis
NLRP3- NLR family pyrin domain containing 3
HFD-High fat diet
TG-Triglycerides
DNL-De novo lipogenesis
FFA- Free fatty acid
LPL- Lipoprotein lipase
IR- Insulin resistance
SFA- Saturated fatty acid
PUFA- Poly unsaturated fatty acid
T2DM- Type 2 Diabetes Mellitus
VLDL- Very low-density lipoprotein
TLR- Toll like receptor
LPS- Lipo-polysaccharides
DAMP- Damage-associated molecular patterns
PAMP- Pathogen-associated molecular patterns
SOD- Superoxide dismutase
GPX- Glutathione peroxidase
ROS- Reactive oxygen species
MT- Melatonin
ALT- Alanine transaminase
AST- Aspartate aminotransferase
GGT- Gamma glutamyl transferase
BAT- Brown adipose tissue
WAT- White adipose tissue

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AUTHORSHIP

DB conceptualised and revised the manuscript critically and approved it. SG developed the conception, drafted the manuscript, and edited it. RK constructed the table and edited the manuscript. SS prepared the figure and edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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