Review

Melatonin: a novel strategy for prevention of obesity and fat accumulation in peripheral organs through the improvements of circadian rhythms and antioxidative capacity

Bohan Rong, Qiong Wu, Chao Sun*

Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China
*Correspondence: sunchao2775@163.com; Tel: +86-29-87092164, Fax: +86-29-87092164

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ABSTRACT

Melatonin is a well-known molecule for its involvement in circadian rhythm regulation and its contribution to protection against oxidative stress in organisms including unicellular alga, animals and plants. Currently, the bio-regulatory effects of melatonin on the physiology of various peripheral tissues have drawn a great attention of scientists. Although melatonin was previously defined as a neurohormone secreted from pineal gland, recently it has been identified that virtually, every cell has the capacity to synthesize melatonin and the locally generated melatonin has multiple pathophysiological functions, including regulations of obesity and metabolic syndromes. Herein, we focus on the effects of melatonin on fat deposition in various peripheral organs/tissues. The two important regulatory mechanisms related to the topic, i.e., the improvements of circadian rhythms and antioxidative capacity will be thoroughly discussed since they are linked to several biomarkers involved in obesity and energy imbalance, including metabolism and immunity. Furthermore, several other functions of melatonin which may serve to prevent or promote obesity and energy dysmetabolism-induced pathological states are also addressed. The organs of special interest include liver, pancreas, skeletal muscle, adipose tissue and the gut microbiota.

Key words: melatonin, obesity, gastrointestinal tract, circadian rhythms, antioxidant, microbiome.

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1. INTRODUCTION

Historically, melatonin has been known to regulate mammalian circadian rhythms as determined by the day-night cycle. Melatonin was initially identified in bovine pineal gland (1). The primary function of melatonin is to mediate the rhythmically physiological activities (2-4), but many other activities have also been found (5, 6), for example, melatonin has been subsequently identified synthesis and function in immune cells (7). Studies have continuously shown that melatonin also involves in many other physiological processes, including sleep, mood, and especially metabolism (8-14). Since obesity is a serious public health issue which predisposes to various metabolic diseases, to identify the effective remedy for

prevention/treatment of the overweight individuals becomes the urgent requirement for medical researchers (15). Melatonin is emerged as such a molecule due to its potentially rhythmic control on fat metabolism in multiple peripheral tissues/organs (3, 16). Based on published reports, studies related to melatonin-mediated peripheral metabolism have increased markedly over the last two decades, and these are summarized in Figure 1.

Obesity-induced metabolic syndrome renders individuals more susceptible to serious disorders including type 2 diabetes *mellitus*, fatty liver, angiocardiopathy and obesity-related glomerulopathy (17, 18). However, the pathogenic factors remain unclear due to complicated nature of obesity (17, 18). Although the data from experimental animal models are not entirely representative in human cases, the importance of these findings is still significant as the references to prevention of human obesity. Recently, an increase in number of melatonin studies have focused on its physiological events occurring in peripheral tissues/organs mainly related to its antioxidative and anti-dysrhythmia actions (4, 19-21). Notably, obesity which often includes energy dysmetabolism, is a prominent feature of many melatonin studies (13, 22-26). Thus, in this review, melatonin-mediated obesity control and its regulation on energy metabolism will be addressed and then its functional mechanisms and the potential relevance of various physiological aspects will be discussed.

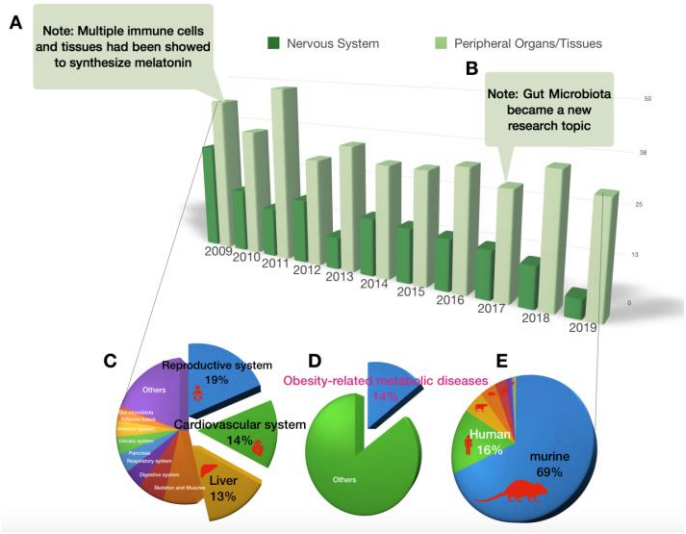

Fig. 1. Distribution of the literature on the melatonin and energy metabolism.

Histogram and pie charts display the typical distributions of literature on the effects of melatonin on animal energy metabolism among various sets of experimental modes during last decade. A. Besides pineal tissue, multiple immune cells/tissues have been reported to synthesize and secrete melatonin since 2009. B. Gut microbiota has been first reported to have a close association with melatonin-mediated anti-obesity in rodent since 2017. C. Based on the preliminary statistical analysis, the published literature among the three major sites, including reproductive system, cardiovascular system, and liver account for 19%, 14%, and 13%, respectively. The rest literature is distributed into other physiological sites of skeleton, muscles, digestive system, gut microbiota, respiratory system, pancreas, urinary system, immune system and adipose tissue. D. The literature related to melatonin, obesity and metabolic diseases accounts for approximately 14% of the total publications of melatonin related articles. E. The murine is the most used animal model in the melatonin research, which accounts approximately 69% of the total melatonin-related animal studies. The second popular category is the clinical studies which account with approximately 16% of the total melatonin researches.

2. IMPACTS OF MELATONIN ON LIVER AND PANCREAS.

For several decades, the actions of melatonin on peripheral energy metabolism have been extensively investigated. Most of the studies are carried out in liver because of its importance in energy metabolism (Figure 1). In 2009, Tahan *et al.* and Shieh *et al.* demonstrated that melatonin ameliorated hepatic dysmetabolism [nonalcoholic steatohepatitis and that due to typed diabetes (T2D)] in rodent model (13, 14). The authors concluded that melatonin exerted beneficial effects via very different signaling pathways, indicating that melatonin acts via multiple mechanisms to alleviate energy dysmetabolism in liver (5, 14, 27, 28). Subsequent investigations gradually identified a variety of novel regulatory pathways, including mitochondria, endoplasmic reticulum (ER), and multiple signaling cascades in hepatocytes (22, 29, 30). As a major hepatic disorder related to obesity, nonalcoholic steatohepatitis (NASH) impacts the health of approximately one-third of adult population globally (31). The development of NASH is a long-term course, requiring to convert hepatic steatosis to cirrhosis (31). After excessive lipid accumulation, oxidative stress and apoptosis contribute to accelerating the pathological development of NASH. These two processes are major obstacles in alleviating/treating this syndrome (31). During the past decade, the beneficial effects of melatonin on hepatic physiology have been frequently reported with novel regulatory mechanisms at various pathological stages. Melatonin limits hepatic lipid accumulation and adipogenesis by inhibiting oxidative stress and hepatocyte autophagy. Chen *et al.* using a murine model of hepatic oxidative stress induced by lipopolysaccharide (LPS), has identified that melatonin inhibits the excessive fat accumulation in the liver (19). The results indicated that LPS-induced reactive oxygen species (ROS) contributed to hepatic oxidative stress and triglyceride (TG) accumulation by activating sterol regulatory element-binding protein (SREBP)-1c and up-regulating its target genes (19). Notably, melatonin supplementation significantly interrupted ROS-mediated elevated expression of genes associated with fatty acid synthesis. The authors, however, did not measure the improvement on hepatic oxidative damage with melatonin treatment, and thus melatonin-mediated anti-oxidative activity was not definitively confirmed during this study (19). A study by de Luxán-Delgado *et al.* provided novel evidence to show that melatonin also targeted autophagy in the damaged liver (20). By evaluating catalase activity on a daily basis after melatonin treatment in an obesity model (ob/ob mice), it was found that hepatic oxidative status was obviously inhibited by melatonin treatment and was accompanied by improvement of ROS-triggered hepatocyte autophagy (20). The importance of melatonin's protection in hepatocytes is highlighted at the stage of lipid accumulation.

Melatonin also attenuates hepatic steatosis via modulating oxidative stress including ER stress (29, 32). The prevailing view is that oxidative stress is essential for hepatic steatosis, which contributes to promoting the development of hepatic sterile inflammation, with high level of proton leakage from the inner mitochondrial membrane (33, 34). *In vitro*, melatonin protects isolated rat liver from oxidative stress (reduced levels of hepatic malondialdehyde and superoxide anion) and inflammation (lower production of tumor necrosis factor-α and adiponectin), preserving liver function (35). A growing number of reports have demonstrated that melatonin alleviates mitochondrial dysfunction through inhibition of oxidative stress, which balances membrane potential and inhibits ROS formation. Additionally, melatonin improves mitochondrial respiratory chain activity (5, 36-38). Finally, ROS-induced high levels of hepatic asymmetric dimethylarginine, a metabolic derivative which is considered as an important inducer for many systemic diseases, is inhibited by melatonin-mediated up-regulation of

dimethylarginine dimethyaminohydrolase-1 and -2 in hepatocytes (39).

An elevated ER stress is considered as another causative factor of hepatic fat deposition. ER stress activates SREBP to accelerate the accumulation of hepatic triglycerides and cholesterol in caspase-2 dependent manner (40). Notably, melatonin prevents hepatic ER stress via multiple mechanisms (41). In palmitic acid-evoked HepG2 cells, Heo *et al.* documented that melatonin significantly reduced the expression of ER stress markers (41). Moreover, this study identified similar improvements in high-fat diet (HFD) mice with melatonin treatment (41). The melatonin-mediated hepatic ER homeostasis is not only confined to one hepatic model (23) and it also reduces adipocyte-derived exosomal resistin by Bmal1 transcriptional inhibition and m6A RNA demethylation in adipocytes, which further alleviates ER stress-induced hepatic steatosis through 5' adenosine monophosphate-activated protein kinase α (AMPKα) signaling (23).

The current focus is on whether melatonin also can alleviate/treat advanced NASH, fatty liver, and cirrhosis (13, 42, 43). In hepatocytes, the orphan nuclear receptor subfamily 4 group A member 1 (NR4A1) up-regulation triggers the activation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and p53. This pathway promotes excessive mitochondrial fission and mitophagy, which dramatically induces hepatic mitochondrial dysfunction and oxidative stress in mice with fatty liver. Melatonin supplementation significantly protects hepatic function from fatty liver-induced mitochondrial dysfunction through blockade of the NR4A1/DNA-PKcs/p53 pathway (42). In cirrhosis, the mitochondrial damage due to lipotoxicity-induced sirtuin (SIRT1)/Mitofusin2 disruption is more serious than that of steatosis, this can be alleviated by melatonin via restoration of enzymatic function related to mitochondrial aerobic respiration and inhibition of glycolytic flux in hepatocytes (43). Excessive lipid accumulation-induced chronic liver injury triggers inflammation which contributes to the production of extracellular matrix proteins from myofibroblasts by activating hepatic stellate cells (HSC) (44). Evidence from early intervention of animal studies shows that melatonin inhibits the expression of NASH marks, including oxidative stress, inflammation, and apoptosis in hepatocytes, and ameliorates the development of NASH (13). These findings indicate multiple functions of melatonin to protect liver from damage. In hepatic stellate cells, melatonin suppresses the production of activated HSC-derived particular collagen 1a1 via reducing the HSC proliferation and nuclear melatonin sensor (retinoic acid receptor-related orphan receptor-alpha) and keeps the lower level of *Alox5* expression (45). Conversely, melatonin significantly interferes with cirrhosis-triggered autophagic flux and unfolded protein response (UPR) in carbon tetrachloride-induced hepatic damage in a mouse model, suggesting that melatonin inhibits HSC activation via the ER pathway (46). Likewise, de Luxán-Delgado *et al.* observed that ob/ob mice were susceptible to hepatic ER stress which then triggered an inflammatory response and melatonin supplementation ameliorated hepatic ER stress-induced UPR and protected hepatocytes from autophagy (29).

There is great interest in investigating other regulatory pathways for the prevention of NASH and its related hepatic dysfunctions, including systematic intervention and crosstalk from distal organs/tissues (23, 47). Clearly, the beneficial actions of melatonin are widely identified in various tissues and organs (4-7). Thus, the studies on melatonin-mediated molecular protection have been documented not only in the liver but in multiple organs/tissues.

For many of its effects, melatonin requires specific membrane and/or nuclear receptors (48). Pancreatic islets highly express these receptors indicating the important actions of melatonin on physiology of pancreas, including glucolipid and energy metabolism (49). The identification of the pathways of melatonin receptors has marked a vital advance in the investigation of melatonin’s role in influencing pancreatic islets, enabled detection and assessment of the

melatonin-mediated improvements in T2D. Melatonin membrane receptors include MT1 and MT2 (*MTNR1A/B*), but only one of them has been defined as the key factor for the modulation of T2D (50, 51). By genome-wide association measures and subsequent verification tests, the *MTNR1B* is considered to be closely associated with high incidence of T2D (51-54). T2D individuals have a reduced expression of melatonin receptors in some cases; however, there are contradictory observations between clinical results and studies in rodents (50-55). Several lines of evidence reveal that melatonin alleviates glycolipid metabolism disorder in diabetes-prone rats, whereas a high level of melatonin and over-expression of *MTNR1B* may decrease insulin secretion and aggravate the risk of T2D in humans (51-55). These findings complicate interpretation of melatonin-mediated regulation in T2D. A ready evaluation for these potentially divergent findings is not yet apparent.

Attention to the *MTNR1B* has instigated the rapid development of research related to melatonin-mediated amelioration on T2D. Melatonin improves insulin sensitivity in obese animals and to control body weight. These positive effects of melatonin on pancreas have been well documented (56, 57). For example, melatonin has been shown to improve the survival of rat insulinoma INS-1 cells and enhance insulin secretion under experimental ER stress conditions (thapsigargin or tunicamycin) *in vitro* (58). Interestingly, in another *in vitro* study, high-level glucose stimulated pancreatic β-cell apoptosis, melatonin also greatly reduced apoptosis via inhibition of ROS, but with no improvement of insulin secretion and this might be due to glucotoxicity-triggered oxidative stress being independent of ER stress (59). The similar result was observed in 4-phenylbutyric acid supplement study in which the recovered insulin secretion through suppressing ER stress, but not cellular apoptosis (59). Thus, a caveat is that the existence of the differences may originate from various conditions of culture which trigger diverse regulatory pathways. However, most clinical results and *in vivo* experiments conclusively show that melatonin reduces blood oxidative stress parameters and ameliorates T2D-induced obesity and the associated metabolic syndrome (60, 61); these benefits have been reported in both young and old individuals (56, 62, 63). Collectively, the findings indicate major differences in the observations related to pancreatic actions of melatonin in various T2D models (mainly including human and murine). Both endogenous and exogenous melatonin in the early stage of pancreatitis in the clinical sections and animal studies similarly show the melatonin reduces the inflammatory response via its antioxidative activity (64, 65). During cerulean-induced rat pancreatitis, melatonin inhibits oxidative stress via nuclear factor erthroid 2-relatived factor 2 and suppresses the inflammatory response through blockade of nuclear factor κB (NF-κB) pathways (64). While similar phenotypes have been found in melatonin-mediated improvement of hepatic steatosis (30), the results have not further detected on association or causality between oxidative stress and inflammation.

Briefly, the beneficial effects of melatonin on liver and pancreas are primarily due to 1). amelioration of hepatic lipid accumulation and hepatocyte autophagy by reducing oxidative stress and adipogenesis; 2). suppression of hepatic steatosis, NASH, fatty liver, and cirrhosis via alleviating mitochondrial and ER stress; 3). induction of insulin secretion via reducing ER or oxidative stress, therefore, to lower pancreatic β-cell inflammation and apoptosis. Overall, these results demonstrate that melatonin can remarkably improve energy metabolism in hepatic diseases and pancreas dysfunction by improving glucolipid metabolism, hyperglycemia and dyslipidemia which is summarized in Figure 2.

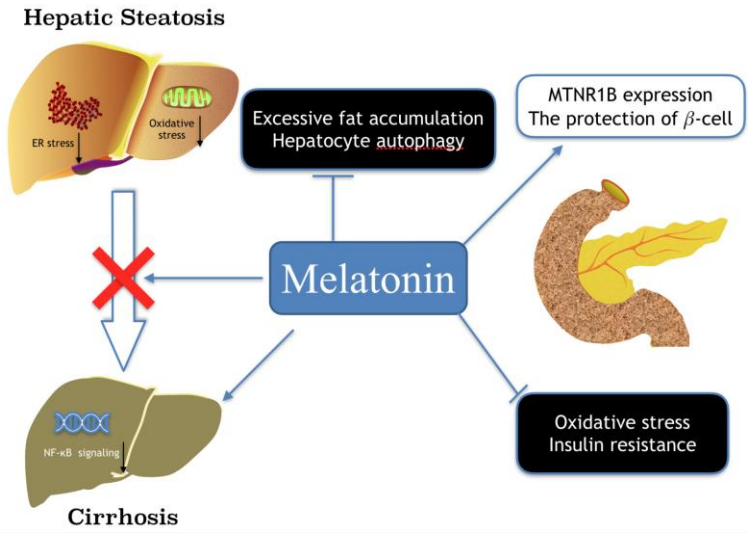

Fig. 2. A summary of the mechanisms by which melatonin influences pancreatic and hepatic metabolism.

Diagram represents multiple melatonin-mediated protections on pancreatic and hepatic metabolism. The blue arrow represents positive effects. The blue T line and X represent blocking activities. The black arrow represents decreasing expression of these phenotypes.

3. MELATONIN-MEDIATED IMPROVEMENT OF SKELETAL MUSCLE AND ADIPOSE TISSUE

As a major energy metabolic tissue, skeletal muscle is undoubtedly considered as an important physiological target for melatonin. The first investigation on this aspect was carried out in 1987 (66). This study was initiated because of the reported different half-lives of melatonin in various sites and the observation that melatonin increased the proliferation of chick myocytes (67). The result indicated that melatonin indeed impacted the skeletal muscle function. The subsequent studies confirmed the observation that melatonin protected skeletal muscle cells from oxidative stress, inflammation and apoptosis (68, 69). These data show that the antioxidant actions of melatonin, as expected, occur in muscle tissues as well. While melatonin-mediated skeletal myogenesis has been demonstrated in human subjects and rodents; however, in-depth understanding of the regulatory mechanisms has yet to be identified (68, 69). Ochoa *et al*. and Borges *et al*. have both detected strenuous exercise-induced muscular oxidative stress and inflammation which can be inhibited by melatonin in adult male volunteers and rats (68, 69). To better understand the regulatory pathways requires more data from metabolomics and single-cell investigations which can accurately identify targeting genes and molecular signaling pathways. As to myocytes, melatonin also protects cardiomyocytes from the intake of an HFD (70).

Similar to liver and pancreas, skeletal muscle shares a common feature such that they exhibit oxidative stress triggering mitochondrial dysfunction and subsequent cell death (71). In a rodent model, ROS induced mitochondrial depolarization and increased membrane permeability with insulin resistance. All of these are counteracted by melatonin via activation of AKT signaling (72). Furthermore, via this signaling pathway, melatonin prevented myoblast cells from ROS-induced apoptosis and autophagy (73). Interestingly, this pathway seems to elevate the beneficial effects on mitochondria. Hong *et al*. showed that the suppression of ER stress may be another mechanism of melatonin to inhibit autophagy of skeletal myoblasts (74). Recently, the potential

association between mitochondrial disorders and ER stress was uncovered by Tezze *et al*. (75). They demonstrated that oxidative stress-triggered mitochondrial dysfunction and activation of ER stress worsened the metabolism of skeletal myoblasts, suggesting an additional mechanism by which melatonin regulates myocyte metabolism. In addition to the oxidative stress, intramuscular energy dysmetabolism also causes inflammation and promotes cell death. Suppression of proinflammatory cytokines [such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6] is an obvious beneficial effect for melatonin-mediated anti-inflammatory activity. To date, there is limited information regarding the regulatory effects of melatonin on cellular energy dysmetabolism. Due to the nature that effects of melatonin on cellular energy metabolism may be organ specific, this is an important area for further research.

In mammals, adipose tissue is highly sensitive to the changes in energy requirement and possesses significant capacity to remodel energy homeostasis via various cellular processes (76). Subsets of mammalian adipose exist in the forms of energy-storing white adipose tissue (WAT), and calorifacient brown adipose tissue (BAT) and beige adipocytes (77). Among these adipocytes, homeostasis is vital for energy metabolic balance which controls obesity and obesity associated diseases (78). Rodent BAT usually exists in scapular area throughout the entire lifecycle, whereas it had been considered as an irrelevant tissue in adult humans, with negligible amounts at approximately 0.5% of total body fat (79). However, this viewpoint was challenged by three high-impact clinical reports that appeared in the *New England Journal of Medicine* in 2009 (79-81). These studies repositioned BAT as a potentially significant factor in obesity and energy metabolism. The interrelationship of BAT to beige adipocytes (WAT browning) has gradually subverted the conservative view that adipose tissue is only physiologically important for energy storage. These are energy-sensitive tissues and the metabolic physiology of various adipocytes is closely associated with obesity and the related metabolic syndrome.

Considering that melatonin regulates hepatic lipometabolism and further affects body weight (20), investigations have focused on whether this modulative effects of melatonin also apply to adipose tissues. The evidence has shown that melatonin indeed alters the physiological activities of various adipocytes by targeting their differentiation, proliferation, apoptosis, pyroptosis and adipocytokines secretion (4, 23, 82-84). Mice are most frequently used animal model to examine metabolic factors which influence weight gain/loss. The Swiss mouse originated 3T3-L1 fibroblast cell line usually serves as a differentiated pre-adipocyte model (85-87) and is used in the melatonin studies. In the 3T3-L1 cell studies, two reports claimed that melatonin (1mM) promoted adipogenesis via increasing the expression of peroxisome proliferator-activated receptor γ, a nuclear receptor for which lipids and their metabolic products are known ligands (82, 88). In contrast, Alonso-Vale *et al.* reported the adipogenesis of 3T3-L1 cells is inhibited by melatonin-mediated transcriptional suppression of CCAAT/enhancer-binding protein β (C/EBPβ) (12). This inconsistent may be a consequence of the definition of various differentiation stages of the adipogenesis.

In addition to adipogenesis, obesity is also associated with adipocyte proliferation. Circadian rhythms are commonly known involving in cellular proliferation (89-91). Given the role of melatonin on circadian rhythms, an impact of melatonin on proliferation of adipocytes is more than likely. In mice study, melatonin supplement drives the formation of circadian locomotor output cycles kaput/histone deacetylase 3/c-Myc complex and further shapes the rhythmic amplitudes of proliferation-related genes, resulting in adipocyte proliferation (4). This finding clearly reveals a novel regulatory pathway of melatonin in adipocytes, which may also link melatonin to sleep deprivation-mediated energy dysmetabolism.

Programmed cell death is another regulated form in cellular development, including, apoptosis, necroptosis, ferroptosis and pyroptosis (92). Instead of structural damage-triggered cell death caused by environmental factors such as diseases, the programmed cell death is considered to underpin homeostasis, including the inhibition of cancer and the improvement of auto-immunity (93). Mitochondrial pathway is known to play a central role in apoptosis (92). After mitochondrial outer membrane permeabilization, downstream apoptotic signaling is activated, which triggers cytochrome c release and high-level expression of caspases (92). Following the reports that melatonin-mediated anti-apoptosis in myocytes and pancreatic *β*-cells (59, 68), adipocytes gradually become a new focus of attention in this field, as it exerts equally vital modulation in obesity and energy dysmetabolism (94). In WAT, melatonin supplementation alleviates obesity and metabolic disorder via improving mitochondrial function, which are similar to that found in liver and pancreas (94). Jimenéz-Aranda A *et al.* has demonstrated that melatonin inhibited proton leak from mitochondria and oxidative stress with the elevated respiratory control ratio and thus suppressed adipocyte apoptosis in inguinal WAT of Zucker diabetic fatty rats, but they failed to detect the apoptosis markers of caspase family (94).

Among various forms of regulated cell death, pyroptosis is a recent discovery that is characterized by cytosolic inflammasome activation (95). Traditionally, the induction of pyroptosis has been considered as a special apoptosis in immune cells in response to pathogenic microbiota infection (96). But increasing findings determine it to be a widespread innate immune mechanism in multiple types of cells (97). Inflammatory reactions are strongly responsible for cellular pyroptosis, and hypertrophic adipocytes generally have a high-level expression of pro-inflammatory cytokines (84, 98). This has been detected in HFD-induced obese mice and ameliorated by melatonin treatment. Liu *et al.* reported that melatonin blocked NF-κB signaling and further inhibited the expression of downstream gasdermin D which is the key manipulator for pyroptosis (84). Moreover, melatonin has been shown to directly suppress the transcriptional activity of gasdermin D, suggesting anti-inflammation is a novel pathway of melatonin to prevent obesity (84).

Compared to all mechanisms mentioned above, melatonin-mediated mitochondrial thermogenesis appears more directly to alleviate obesity (99). The excessive energy uptake contributes to occurrence of obesity; however, the reduced energy expenditure also associates to obesity. As a result, the heat expenditure has a strongly positive association with weight loss. Activated BAT up-regulates the expression of energy-stimulatory molecule, uncouple protein 1 (UCP1), which uncouples oxidative phosphorylation and ATP synthesis to dissipate the energy to heat, resulting in non-shivering thermogenesis and weight loss (25, 99). Thus, BAT is considered as a key manipulator for energy metabolism and a potential target for obesity.

Based on the published data, the mechanisms of melatonin on thermogenesis in BAT are categorized into two regulatory pathways. One is via promoting the activity of citrate synthase and respiratory chain to activate UCP1 in which a large quantity of energy is converted to form of heat without ATP synthesis (25, 99). Another is that melatonin increases the proliferation of mitochondria (100). In porcine intramuscular adipocytes, Liu *et al*. detected that melatonin treatment significantly elevated the mitochondrial copy number and the expression of thermogenesis gene (UCP3) in BAT (100). By using multiple inhibitors, it is found that protein kinase A (PKA) and ERK 1/2 signaling are responsible for activating mitochondrial thermogenesis, suggesting that melatonin-mediated the high-level of lipolysis triggers mitochondrial heat expenditure in BAT (100). Likely, Fernández Vázquez *et al.* have also observed that melatonin increased the thermogenesis of BAT in response to cold exposure via

activating citrate synthase and respiratory chain complexes I and IV in Zücker diabetic fatty rats (25). These findings indicate that melatonin up-regulates mitochondrial energy expenditure by enhancing the generation of heat in BAT. In addition, melatonin promotes trans differentiation of WAT into the beige fat which has the similar function as BAT. This is another potential strategy for the prevention of obesity (101). Since energy expenditure is significantly increased without storage in WAT, the loss of weight becomes a remarkably benefit by melatonin treatment (101).

4. MELATONIN-MEDIATED IMPROVEMENTS IN THE GUT MICROBIOTA

As an effective strategy of energy regulation, melatonin makes noteworthy contributions to the physiology of gut microbiota (24, 102-104). In mammals, gut microbiota is markedly sensitive to the changes of energy requirements and possesses a high capacity to remodel energy homeostasis (105, 106). Based on this physiological property and the applicability to real life situations, Yin *et al.* investigated the alterative patterns of gut microbiota in lipid dysmetabolic mice (24). In HFD-treated mice, the authors have showed that reprogramming gut microbiota is a key regulatory factor in HFD-mediated energy imbalance via the generation of short chain fat acids (24). Due to the limitation of the methodology, this work did not determine whether the modulatory actions were tissue-specific. Thus, the development of relative tissue-specific gene knockout mice should be a direction for further research in this field. The mucin-degrading bacterium, *Akkermansia muciniphila*, is a novel signaling marker that inhibits nutrient uptake and WAT expansion (104). Interestingly, HFD-induced inflammation is also down-graded by *Akkermansia* supplementation, suggesting that this bacterium plays an essential role of the inhibition for energy uptake during the positive energy state (104). Moreover, Xu *et al.* have evaluated the obesity-derived gut microbial community and the positive effects of melatonin (102).

Among various studies, an important finding is that gut microbiota are the potential manipulators for energy metabolism in mice (78). In regards to the gut microbiota, evidence for host energy administration has been widely reported, but its role in melatonin-mediated energy optimization has not been investigated. Under a HFD background, Xu *et al.* successfully detected the reversion of metabolic homeostasis and gut microbial depletion-induced WAT beiging inhibition by using the classical fecal microbiota transplant (FMT) in germ-free mice. The results indicated that gut microbial remodeling by melatonin was required for the maintenance of host energy metabolic homeostasis (102). The shortcomings for this investigation are failed to perform total flora analysis, determine the functional effects of single or several bacterial strains and definitely establish an integrated regulatory circuit originating from these symbionts to target organs/tissues (102). In addition to promoting energy expenditure in mouse adipose tissue, melatonin exerts potent immunomodulatory actions and contribute to modulating adipokine secretion partly through gut microbial pathways (103). In line with this, simultaneous testing technology in a mouse model has confirmed this possibility, and subsequent FMT further demonstrated gut microbiota to involve in this regulation (102). Overall, gut microbiota should be considered as an important link in melatonin-mediated regulatory circuit from host gut to adipose tissues. Indeed, the gut is of special interest because of its physical position for intestinal flora. The results of several studies show that gut microbiota play multiple roles in intermittent fasting-mediated energy metabolic optimization via enterohepatic pathways, including intestinal barrier protection, hepatic metabolism reprogramming, and lifespan extension (107-109). A study by Gao *et al.* have determined that gut microbial abundance is associated with the improved intestinal barrier integrity after melatonin treatment (103). Compared with the control,

Aeromonas-plantarum, a highly abundant bacterium and a commensal negatively associated with gut barrier function, is significantly inhibited in mice treated with melatonin, suggesting that melatonin may improve intestinal health via reducing the gut microbiota-mediated defensive load (103). Meanwhile, this research also offers evidence to imply the melatonin-mediated positive effects on mice at different growth phases, including enhancements in energy metabolism and lifespan extension (103). While there have been no subsequent studies related to these findings, it is presumed that there are still some novel linkages which need to be explored.

Inflammatory bowel disease (IBD), a chronic intestinal illness including Crohn's disease and ulcerative colitis, is believed to be a result of multiple causes (110, 111). Of note, recent clinical data show that incidence of IBD is increasing in parallel with the rising number of obese patients, indicating that IBD may be related to or a consequence of obesity (111). Beyond the intimate association and immune interactions between gut microbiota and the intestines, the elevated prevalence of obesity in IBD patients implies that there may be significant energy dysmetabolism. In view of this, it is possible that energy control (such as intermittent fasting) may be a new effective strategy to combat inflammation and IBD. Gao *et al.* has reported the close association between gut microbiota and host intestinal immunity (103). For this study, dextran sodium sulfate-induced IBD pathological model (mouse) was performed to induce IBD. Melatonin treatment provided significant protection against this intestinal inflammation (103). Furthermore, melatonin treatment also increased gut repair capacity through promoting the proliferation of colonic stem cells (103). Of note, these improvements might be represented by FMT suppression from wild mice to a pathological model, whereas restoring FMT resulted in inflammatory occurrence (103). These findings indicate that the special pattern of gut microbiota not only represents a corresponding physiological state but also enables it to be inherited/delivered. Notably, exogenous melatonin is an orally administered substance, implying that it may reach higher concentrations in digestive tract than in the serum. Thus, gut microbiota may be a key link and important target for melatonin in its regulation of anti-obesity (Figure 3).

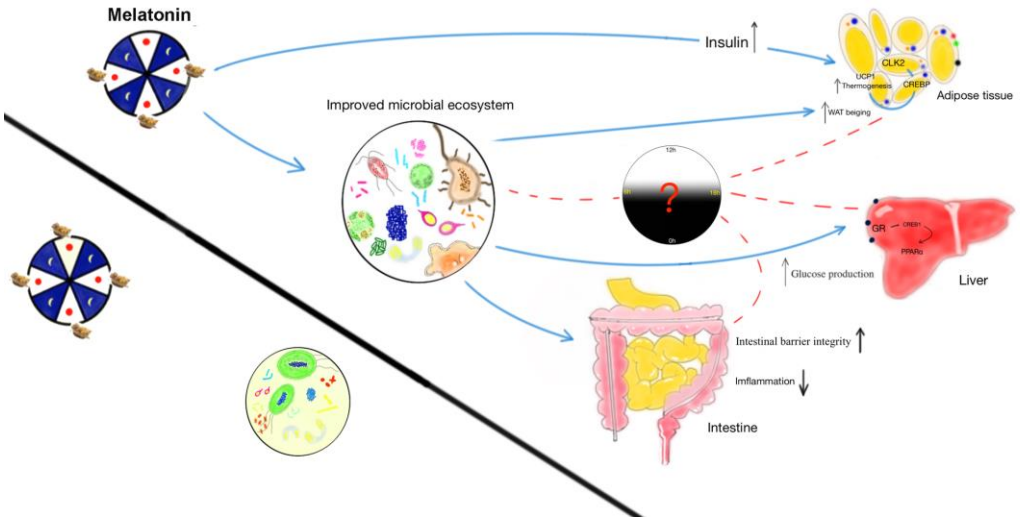

Fig. 3. Interactions of melatonin with gut microbiome and liver under different conditions.

Melatonin promotes WAT beiging via improving gut microbial ecosystem, insulin sensitivity and glucose tolerance. The optimized microbial community is also responsible for the protection of host intestinal barrier. Melatonin mediated-regulatory circuit is from gut microbiota to liver. Thus, the role of host bio-clock is a valuable target for further investigations.

5. SUMMARY AND FUTURE PERSPECTIVES

As a significant neurohormone and antioxidant in vertebrates, research within the last two decades has revealed that the melatonin also plays an essential role in maintaining energy metabolism and immunity via various signaling pathways. Based on the available information, the molecular pathways of melatonin on protections of multiple organs/tissue can be classified into following categories: 1). amelioration of oxidative stress and preservation of mitochondrial function, as a result to maintain energy metabolism and inhibit cellular apoptosis; 2). improvement of lipo-metabolism and cytokine secretion by alleviating ER stress; 3). inhibition of the activation of inflammatory pathways via reducing ER or oxidative stress, thus, to protect dysmetabolism-triggered low-grade inflammation in a variety of cells; 4). promotion of mitochondrial thermogenesis to reduce the extra energy storage; 5). modulation of cellular proliferation via shaping the rhythmic amplitude of circadian genes; 6). recovery of obesity-induced pattern of gut microbiota and host metabolic homeostasis.

Since mechanisms of melatonin on some mammalian physiological functions have been well elucidated, these investigations provide clear directions for subsequent studies in the possible use of melatonin to overcome obesity and also as a treatment for chronic inflammatory diseases. In recent years, investigations have focused on the interaction between melatonin and gut microbiota, which further expands the opportunity to deepen our understanding of why some individuals are susceptible to developing obesity which others are not. However, many unknowns still exist, requiring further explorations including 1). the interactions of melatonin and genetics in the context of metabolism via omics technology; 2). additional local regulatory mechanisms of melatonin in reference to fat accumulation; 3). identification of unknown regulatory factors from peripheral tissues that influence lipid metabolism and storage; 4). additional mechanistic studies regarding how melatonin influences metabolism generally and 5). microbiota-based actions which contribute to or resist obesity and related chronic metabolic diseases.

The research related the effects of melatonin in patients with dysregulated metabolism such as in T2D or with gastrointestinal diseases including IBD are certainly worthy of further extensive investigations. Based on numerous positive findings and the increasing number of non-invasive technologies, it is hopeful that research related to melatonin as summarized in the current report will soon be applied at the clinical level. The results of such studies will provide new treatment options which would aid in improving human health. Evidence is strong that melatonin is both beneficial and safe for clinical use.

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AUTHORSHIP

B.R. and Q.W. conceived the idea and were involved in data collection. B.R. had the primary responsibility for the manuscript writing. R.R. aided in the discussion of data and edited the manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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