Circadian Rhythms

All organisms are subjected to changes in photoperiod because of the Earth's rotation around its own axis. As the sun sets, nocturnal animals begin to forage and hunt while diurnal animals undergo a sleeping phase. This light and dark cycle is responsible for physical, biological and resting activity of various animals and all living organisms are governed by a molecular circadian clock (Mazzoccoli et al., 2012; Vitaterna et al., 2001). The word circadian is Latin in origin, meaning 'about a day', hence, oscillations of ~24 hours are referred to as a circadian rhythm. These rhythms are generated by the Earth's rotation, which drives the light-dark cycle(Vitaterna et al., 2001). The human body has a master circadian clock in a control center of the brain known as the suprachiasmatic nucleus (SCN). The suprachiasmatic nuclei are paired structures of the ventral hypothalamus, that contain about 10,000 neurons in mice and about 50,000 neurons in humans(Edgar et al., 1993; Welsh et al., 2010). The dorsal neurons of the SCN and their dorsal reaching efferents straddle the ventral floor of the third ventricle, and the ventral neurons border the optic chiasma. Information on photoperiodic variation reach the SCN from melanopsin-containing retinal ganglion cells (also called "intrinsically photosensitive retinal ganglion cells") via the retinohypothalamic tract (RHT)(Göz et al., 2008). This internal clock regulates the timing of such body rhythms as temperature and hormone levels(Buijs et al., 2019). The primary circadian rhythm that circadian clock controls is the sleep-wake cycle. It functions in a cycle that lasts a little longer than 24 hours(X. Li et al., 2018). In mammals, the circadian clock is "set" primarily by visual cues of light and darkness that are communicated along a pathway from the eyes to the SCN(Lewy et al., 1980). This keeps the clock synchronized to the 24-hour day. Other time cues, known as zeitgebers, also can influence the clock's timing(Aschoff, 1960)(Husse et al., 2015). These clues include exercise schedule,

working schedule, time allocated for meal consumption etc(Damiola et al., 2000; Hara et al., 2001). In mammals, circadian rhythms regulate major physiological activities, including sleep/wake cycles, feeding/fasting cycles, endocrine rhythms, and metabolic rhythms (Dibner et al., 2010). Thus, it is evident that modern lifestyle changes such as rotating shift work, transcontinental travel and jetlag can probably play a major role in global health issues.

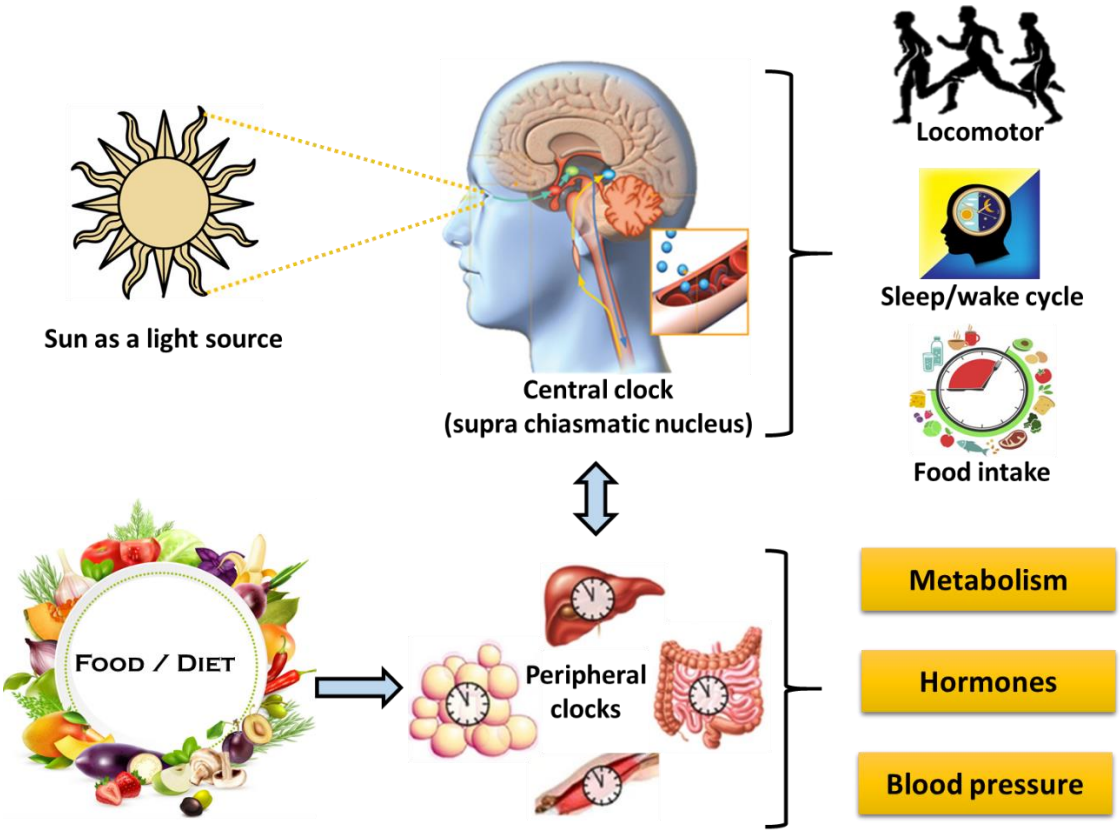

Figure 1: Coordination between central and peripheral clock and its physiological activity.

Molecular Clock

Circadian rhythms are endogenously synchronised by components at the molecular level. These components interact to produce cyclic changes with a periodicity of about 24h. The molecular clock is self-sustained, autonomous and present in every cell type (Reppert & Weaver, 2002). The molecular clock mechanism in mammals is best understood as a transcriptional feedback loop involving primarily ten genes. The genes Clock and Bmal1 encode bHLH-PAS proteins that form the positive limb of the feedback circuit. The CLOCK: BMAL1 heterodimer initiates the transcription by binding to specific DNA elements, E-boxes (5′-CACGTG-3′) and E′-boxes (5′-CACGTT-3′) in the promoter region of target genes. The Per (Per1 and, Per2) and Cry (Cry1 and Cry2) genes forms negative limb of the feedback loop (Rudic et al., 2004)(Partch et al., 2014). The resulting PER and CRY proteins dimerize and inhibit further CLOCK: BMAL1 transcriptional leading to repeated cycles from a level of low transcriptional activity(Husse et al., 2015). Thus, cellular metabolism may prove to play an important role in regulating the transcriptional state, and therefore the phase of the clock. Degradation of the negative limb proteins PER and CRY is required to terminate the repression phase and restart a new cycle of transcription. The paralogs of the Per genes (Per1 and Per2) and the Cry genes (Cry1 and Cry2) have nonredundant roles in metabolic processes(Buhr & Takahashi, 2013).

Furthermore, the CLOCK: BMAL1 dimers also initiate the transcription of a second feedback loop which acts in coordination with the loop described above. The positive and negative limbs BMAL1/CLOCK initiates E-box mediated transcription of the orphan nuclear-receptor genes RORα/β and Rev-Erbα/β which subsequently can recognize a retinoic acid receptor-related orphan receptor element in the promoter of *BMAL1* thus reducing or enhancing its transcription, respectively(Preitner et al.,

2002). Sirt1 acts as a histone deacetylase and couples with Clock: Bmal1 heterodimer and supresses Bmal1 via its deacetylation activity. Additionally, Sirt1 deacetylates Per1 and resulting in decreased stability and degradation. Clock binds to NAMPT and increases its expression, leading to an increased NAD+ and Sirt1 activity(Tong et al., 2015; Zhou et al., 2014). This suggests that core circadian clock components are closely related to metabolism at the molecular level.

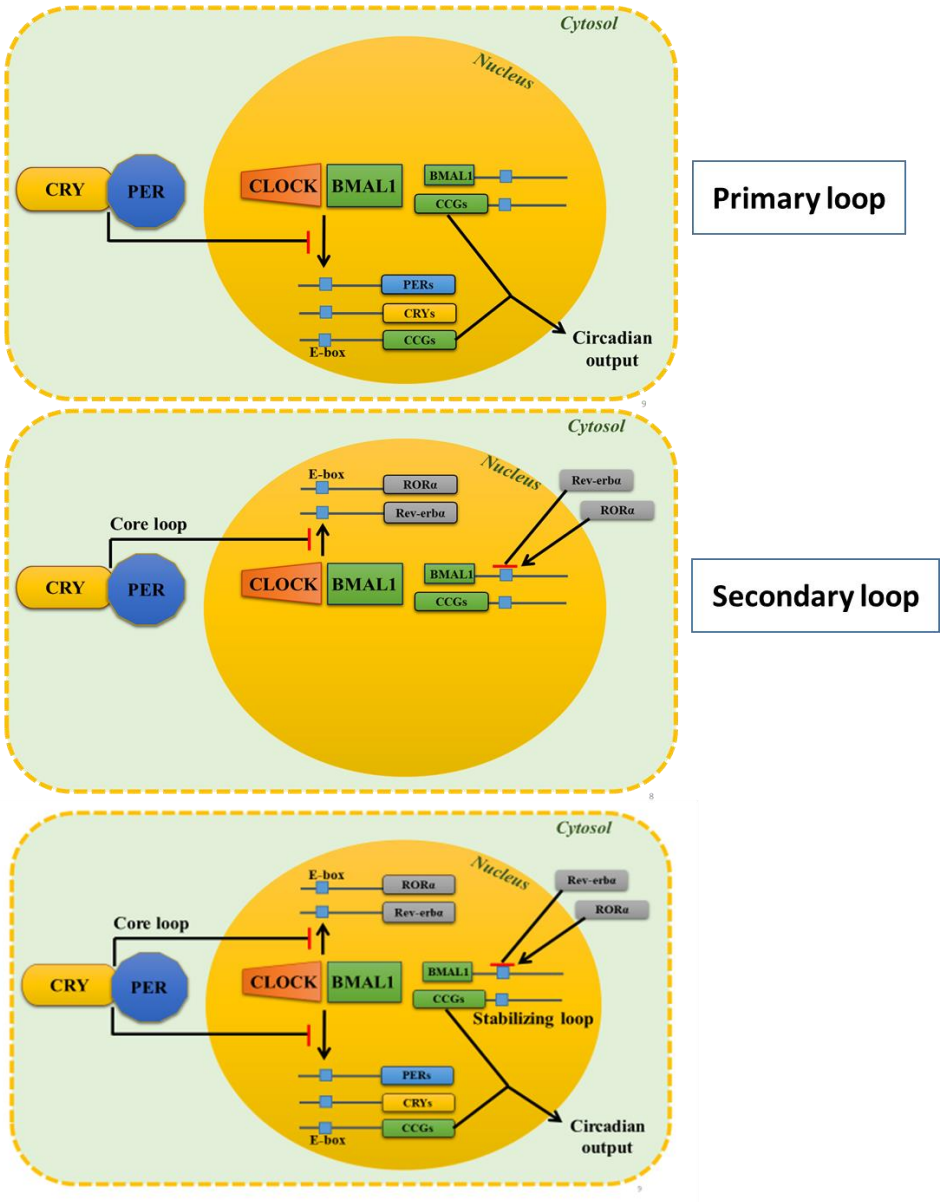

Figure 2: Trancriptional-translational feedback loop of mammalian circadian molecular clock.

Circadian Desynchrony and lifestyle disorders

Reports on perturbations of internal clock system had shown increased risk for disorders such as obesity, diabetes mellitus, cardiovascular disease, thrombosis and inflammation(Froy, 2007; Keller et al., 2009; Scott, 2015; Takeda & Maemura, 2011; Parsons et al., 2015; Parsons et al., 2015). Strong positive correlation exists between aforesaid metabolic syndromes and Non-alcoholic fatty liver disease (NAFLD). The circadian clock system in the liver plays an important role in regulating metabolism and energy homeostasis. Bmal1: Clock heterodimer can act with SREBP-1c, FASN, HMGCR, LDLr, ACS to control the daily lipid metabolism in the liver (Grimaldi et al., 2010b; Le Martelot et al., 2009; Zhong & Liu, 2018). Clock gene mutant animals display impaired glucose and lipid metabolism and are susceptible to diet-induced obesity and metabolic dysfunction, implies towards the connection between the circadian clock and metabolic homeostasis (Turek et al., 2005). There are reports suggesting that $Bmal1^{-/-}$ knock-out mice results in glucose intolerance, hypoinsulinemia, reduced fat storage, increased circulating fatty acids, increased ectopic fat formation in the liver and muscle, and hepatic steatosis with regular chow-feeding (Shi et al., 2019). Apart from binding to other core clock regulators, Per2 is known to regulate lipid metabolism by directly inactivating the transcriptional activity of PPARα and PPARγ (Grimaldi et al., 2010a). On the other hand, Cry regulate STAT5B phosphorylation and is linked with JAK-STAT pathway and in liver, it is known to regulate gluconeogenesis by blocking cAMP accumulation and by activating gluconeogenic genes (Chaudhari et al., 2017; Narasimamurthy et al., 2012). Mice fed with HFD is known to cause degradation of Cry1 that results in hyperglycemia (Hsieh et al., 2010; Kohsaka et al., 2007). In humans, the clinical conditions of obesity,

NAFLD and metabolic syndrome have been associated with polymorphism in Clock gene(Sookoian et al., 2007).

Lifestyle disorders

Lifestyle disorders are non-communicable diseases associated with the way a person or group of people lives. In recent decade, developed countries has led to urban living style that is closely linked to chronic health problems (Sharma & Majumdar, 2009). Lifestyle disorders include obesity, cardiovascular diseases like atherosclerosis, heart disease, stroke, certain forms of cancer, type 2 diabetes, alcoholic and non-alcoholic fatty liver disease. Lifestyle disorders are known to cause around 70% deaths each year globally (Fuhrman, 2018). Consumption of western diet causes nutrition problems like obesity and cardiovascular disease.

The following factors increase the risk of lifestyle disorders (Tabish, 2017):

i. Modifiable behavioural risk factors: Excessive use of alcohol, bad food habits, eating and smoking tobacco, physical inactivity, wrong body posture and disturbed biological clock.
ii. Non-modifiable risk factors: Age, Race, Gender, genetics are the risk factors that cannot be controlled or modified.
iii. Metabolic risk factors: Obesity, increased blood pressure, increased blood glucose or hyperglycemia, increased levels of fat in the blood or hyperlipidemia are the four major changes in metabolic system that can cause lifestyle disorders.

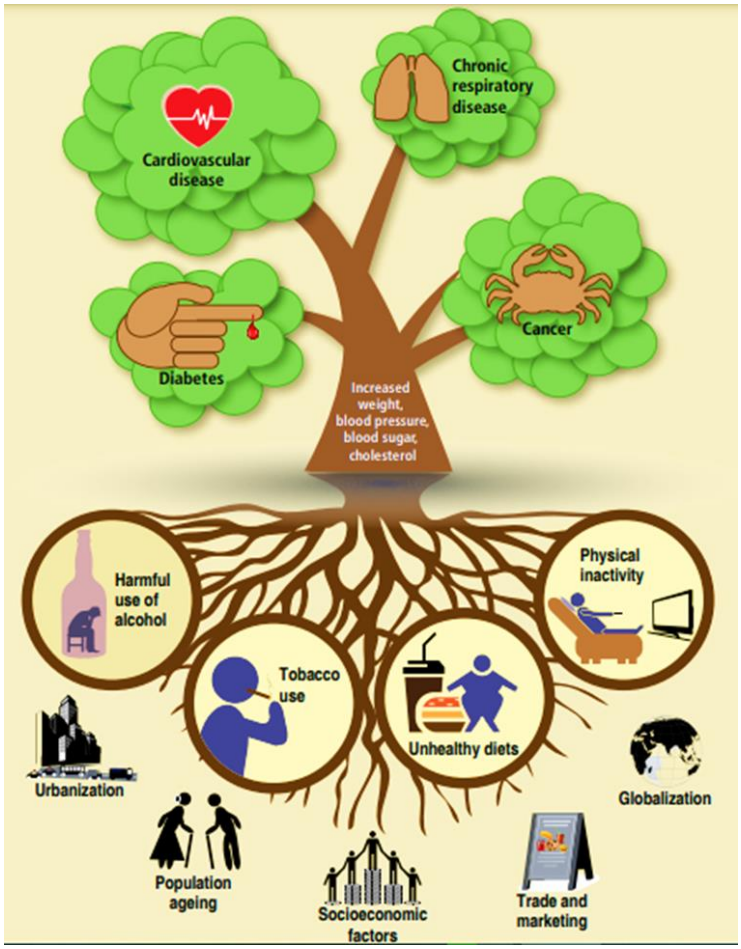

Figure 3: Representation of risk factors associated with lifestyle disorders. (WHO report 2011)

About 25% of the world population suffers from metabolic syndrome. The patients suffering from type 2 diabetes and obesity are in the high-risk category of heart attacks or strokes and metabolic disorders(Fauchier et al., 2021). According to WHO, 422 million adults are estimated to have type 2 diabetes worldwide and it is predicted that diabetic deaths will double by 2030(Ogbu, 2021). A World Economic Forum report says that India will suffer a loss of more than $200 billion due to unhealthy diet and lifestyle (national health portal of India, 2019). Nearly 80% of the deaths are occurring in nations like India where a majority of the people are in the category of low-income and middle-income groups. Also, in these countries, there is a growing burden of

nutritional deficiencies, infectious diseases, poor maternal and prenatal conditions. Lifestyle diseases are reversible and can be prevented from causing serious damage to the overall health. Hence, addressing the issue of unhealthy lifestyle practices in all aspects is essential to reduce the mortality caused.

Causes of lifestyle disorders

The most common familial causes of these lifestyle disorders are related to diet — consuming unhealthy foods, uncontrolled eating, overindulgence on artificial sweeteners, processed foods, and junk foods (Fuhrman, 2018). In addition, addictive habits like tobacco smoking, consumption of alcohol, irregular sleeping habits, very limited exposure to sun, stress and modern-day urbanization have made matters worse. The most frequently observed lifestyle disorders are diabetes, obesity, hypothyroidism, cancer, hypertension, cardiovascular diseases, chronic obstructive pulmonary disease (COPD), etc.

Further, a person may be genetically susceptible to disease such as diabetes, cancer, obesity, high blood pressure and heart stroke based on a person's genetic makeup. A genetic predisposition results from specific variations that are inherited from either parent(Blazer & Hernandez, 2006). External environmental factors are of pivotal importance in entraining internal clock that negatively impacts various physiological activities. Hence, jetlag induced by transcontinental travel, shift job and long work schedules result in erroneous circadian oscillations and; with its long term persistence can cause lifestyle disorders (Parsons et al., 2015).

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is excessive triglyceride (TG) accumulation without a history of alcohol intake. The histological classification discriminates a range of conditions within NAFLD that vary from hepatic steatosis to non-alcoholic steatohepatitis (NASH), which might evolve to many subsequent conditions that include fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma(Anstee et al., 2013; Friedman et al., 2018). Due to increased incidence of metabolic disorders, the global prevalence of NAFLD has dramatically increased during the past three decades. The current prevalence rate of NAFLD varies from 17 to 51% in western countries and about 25% in Asian countries(Amarapurkar et al., 2007). The rising prevalence during the last decade has made NAFLD the second most common cause of liver transplantation in the United States.

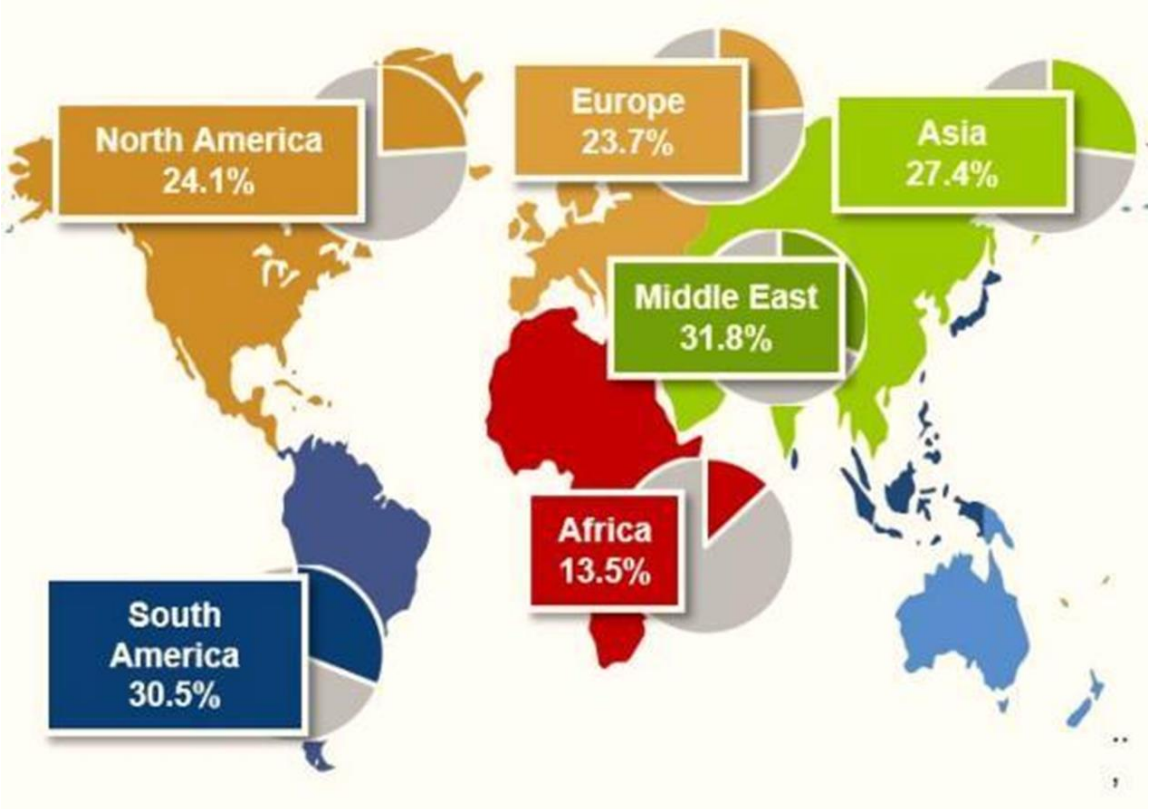

Figure 4: Prevalence of NAFLD across the globe. (Hepatology)

Liver is major regulator of lipid homeostasis and is responsible for orchestrating the synthesis of new fatty acids, their export and subsequent redistribution to other tissues. These processes are closely regulated by complex interactions between hormones, nuclear receptors, and transcription factors, thus keeping hepatic lipid homeostasis under tight control (Gaggini et al., 2013). Perturbation in these pathways may resulting in accumulation of fat in the liver and the subsequent development of NAFLD. Hepatic fat accumulation results from an imbalance in four major pathways viz. uptake of circulating lipids, de novo lipogenesis (DNL), fatty acid oxidation (FAO), and export of lipids as very low-density lipoproteins (VLDL)(Angulo, 2002). These pathways are instrumental in the development and progression of hepatic steatosis. Mechanism of development of NAFLD was earlier explained by a "two hit hypothesis" wherein; fat accumulation in hepatocytes and heightened hepatic ROS caused the first hit. The second hit was characterized by inflammation and extensive fatty degeneration and tissue damage (Buzzetti et al., 2016; Day & James, 1998; Takaki et al., 2013). However, it is now obsolete, as this hypothesis fails to explain a series of metabolic changes taking place in NAFLD. According to multiple hit hypothesis, NAFLD is progressive and multifactorial disease that results in progression of NAFLD to fibrosis, cirrhosis and hepatocellular carcinoma (Michelotti et al., 2013). The series of events associated with NAFLD progression include fat accumulation, increased ROS, mitochondrial dysfunction, ER stress, inflammation etc.

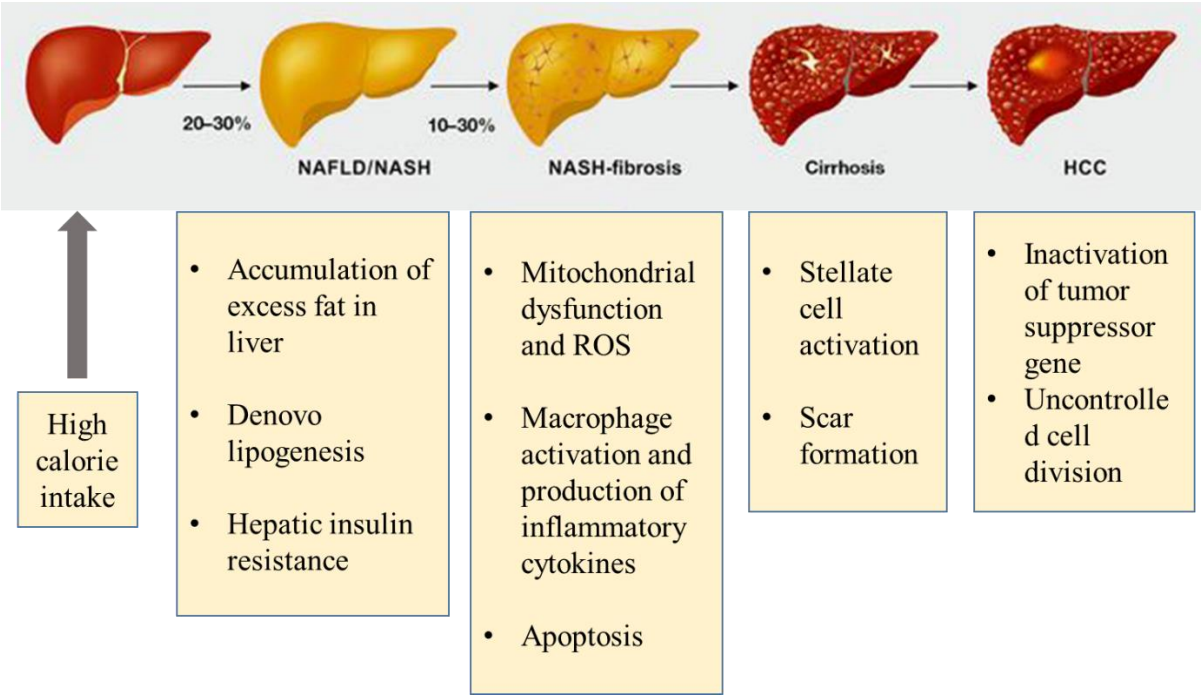

Figure 5: Representation of different pathological stages and physiological changes in liver (Normal, Steatosis, NASH and Cirrhosis)

Crosstalk between lipid transporters, lipogenic and lipolytic genes in Fat metabolism

Transport and uptake of fatty acids circulating in blood is predominately mediated by fatty acid transport proteins (FATP), cluster of differentiation 36 (CD36), and caveolins located in the plasma membrane of hepatocytes(Alves-Bezerra & Cohen, 2017). Various research groups had shown that knockdown of these transporter proteins reverses and improves the condition of steatosis in mice fed with high fat diet (Doege et al., 2008). CD36 is responsible for the transport of long-chain fatty acids and is regulated by peroxisome proliferator-activated receptor (PPAR) γ, pregnane X receptor, and liver X receptor. Mice fed with high fat diet develops hepatic steatosis wherein, increased mRNA and protein expression of CD36 is also observed(Yang et

al., 2020). After the uptake of these hydrophobic fatty acids, they bind to specific fatty acid binding proteins (FABP) of which FABP1 is the predominantly found in liver (Bass & Manning, 1986). On the other hand, FABP1 also affects the expression of PPARα and PPARγ by mediating the transport of PPAR ligands to the nucleus of hepatocytes, and intracellular FABP1 therefore concentrations are correlated with the activity indices PPARα and PPARγ (Hardwick et al., 2009).

Denovo lipogenesis (DNL) enables liver to synthesize new fatty acids from acetyl-CoA. Initially, acetyl-CoA is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC) and malonyl-CoA is then converted to palmitate by fatty acid synthase (FAS) (Lambert et al., 2014). Thus, increased DNL can cause hepatic steatosis and/or hypertriglyceridemia, but may also induce steatohepatitis, as saturated fatty acids, such as palmitate, can cause inflammation and apoptosis (Lambert et al., 2014). In a healthy liver, sterol regulatory element-binding protein 1c (SREBP1c) is activated by insulin and liver X receptor likewise, the carbohydrates activate carbohydrate regulatory element-binding protein (ChREBP). Transcriptome activity of lipogenesis denovo, is regulated by SREBP1c and chREBP (Tian et al., 2016). Reports have shown that patients with NAFLD have enhanced SREBP1c expression that is in agreement with its lipogenic role. There are reports that suggest that ACC and FASN are upregulated as evidenced by their mRNA levels in patients and animal models with NAFLD (Kohjima et al., 2007; Zhu et al., 2011).

Oxidation of fatty acids is a major metabolic pathway by which energy is released from fatty acids. It is transcriptionally regulated by PPARα and occurs mainly in the mitochondria. Translocation of these long chain fatty acids into the mitochondria matrix relies on carnitine palmitoyl transferase 1 (CPT1). B-oxidation of fatty acids results in

reducing products such as NADH and FADH that are integral components of electron transport chain. As a result of lipid overload, this process is increased and generates reactive oxygen species (ROS), oxidative stress, and toxic dicarboxylic acids, that promotes inflammation and disease progression. Increased fatty acid oxidation and ROS results in mitochondrial damage that further results in diminished mitochondrial function (Rao & Reddy, 2001).

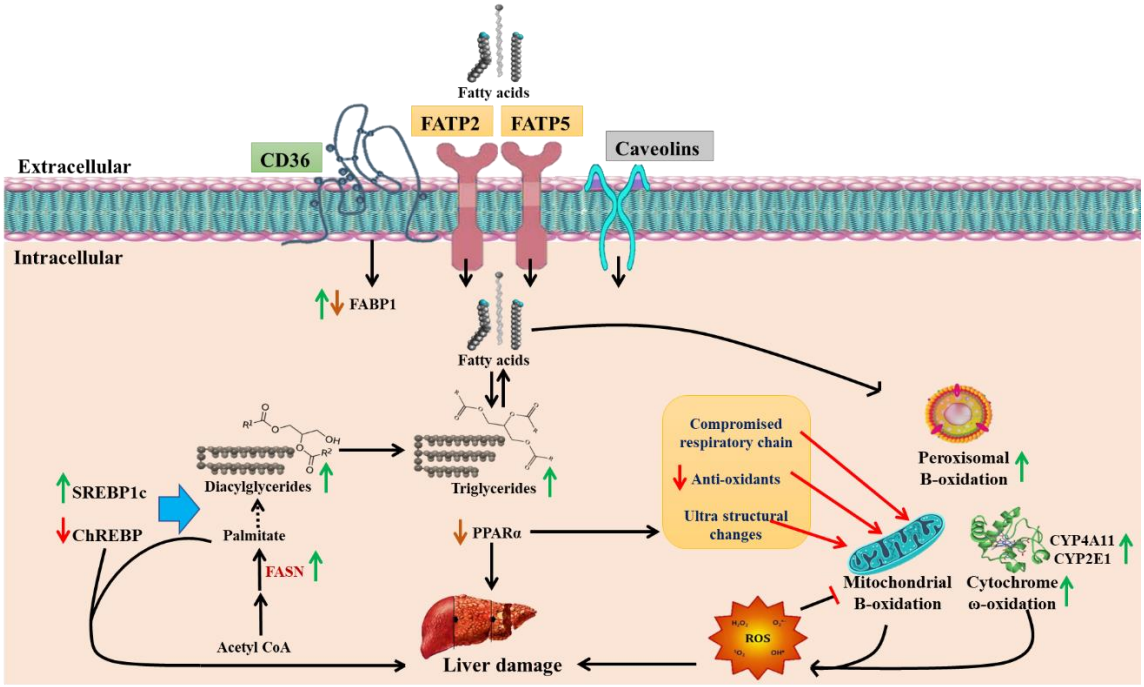

Figure 6: Regulation of fat metabolism by lipid transporters, lipolytic genes and lipogenic genes.

Antioxidant Pathway in NAFLD

Transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is a positive regulator of the expression of a set of genes involved in the protection against oxidative/electrophilic stress (Kaspar et al., 2009). Nrf2 is a basic leucine zipper molecule that regulates transcriptional induction of ARE-containing genes encoding antioxidant enzymes, electrophile-conjugating enzymes, ubiquitin/proteasomes, and chaperone and heat-shock proteins in response to cellular stresses including ROS

(Kaspar et al., 2009). The ARE is a *cis*-acting enhancer sequence that mediates transcriptional activation of genes in response to changes in the cellular redox status, such as an increased production of free radical species. Under normal conditions, Nrf2 is mainly localized in the cytoplasm through an interaction with Kelch ECH associating protein 1 (Keap1). The binding to and regulation of Nrf2 by Keap1 have been explained by a "hinge and latch model". During exposure to electrophiles or oxidative stress, Keap1 becomes oxidized at critical cysteine residues. As a result, Nrf2 escapes Keap1 control and translocates to the nucleus, where it dimerizes with musculoaponeurotic fibrosarcoma (Maf) proteins and promotes the expression of ARE-containing genes (Nguyen et al., 2009). Apart from regulating oxidative stress, Nrf2 is known to participate in hepatic fatty acid metabolism, as a negative regulator of genes that promote hepatosteatosis in rodents (Chambel et al., 2015). Recent reports had suggested that Nrf2 mediates the crosstalk between lipid metabolism and antioxidant defense in experimental models of NAFLD, and the nutritional or pharmacological activation of Nrf2 may provide new strategy for prevention and treatment of NAFLD (Chambel et al., 2015). In summary, Nrf2 manifests a protective role against steatosis by inhibiting lipogenesis and promoting fatty acid oxidation.

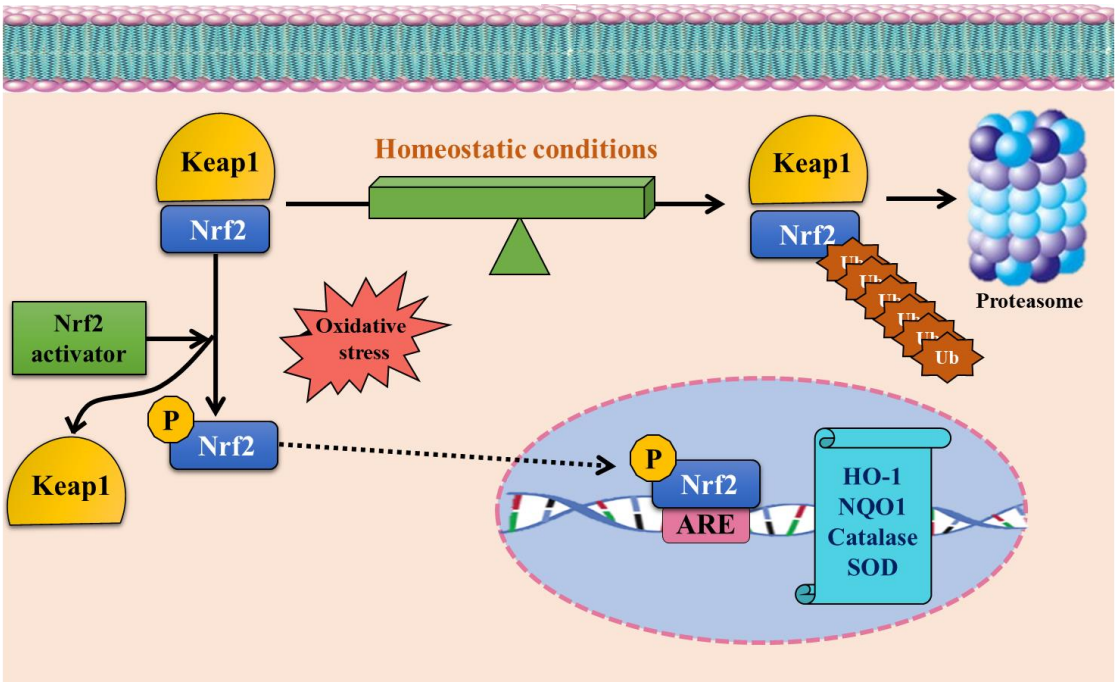

Figure 7: Keap1 dependent Nrf2-ARE signalling pathway.

Circadian regulation of antioxidant pathway

Emerging body of evidence suggests that circadian clock regulates processes that keep reactive oxygen species (ROS) at physiological levels and protect organisms from oxidative stress(Xu et al., 2012). Several studies have shown that alterations in circadian rhythm at various time points in a day may result in DNA damage, lipid peroxidation and protein oxidation thus implying towards a circadian basis of oxidative stress responses(Fanjul-Moles & López-Riquelme, 2016). Expression levels of various antioxidants and enzymes are known to show circadian oscillations wherein, glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase are known to peak in the morning hours (Wilking et al., 2013). Xu et al. (2012) had investigated the expression patterns for antioxidant genes in mice liver and they found that nuclear factor erythroid-2-related factor 2 (Nrf2) expression was highest during daytime and showed a peak at 18:00, thus proving evidence on the circadian regulation of cellular antioxidant defense system (Xu et al., 2012). Circadian-clock-dependent

regulation of redox status, ROS homeostasis, and antioxidant defense is studied by various research groups wherein; they had shown preliminary evidence on Bmal1 as a transcriptional regulator of Nrf2(Pekovic-Vaughan et al., 2014). In macrophages treated with LPS, Bmal1 has been shown to activate Nrf2-mediated antioxidant pathway and reduce the levels of pro-inflammatory cytokine and interleulin-1 beta (IL-1β) (Early et al., 2018).

Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) is a lipophilic molecule synthesized primarily in the pineal gland, but also is produced in the retina, extraorbital lacrimal gland, Harderian gland, gastrointestinal tract, blood platelets, and bone marrow cells(do Amaral & Cipolla-Neto, 2018; Huether, 1993). It acts through melatonin receptors, and regulates numerous physiological processes including circadian entrainment, blood pressure (BP), oncogenesis, retinal physiology, seasonal reproduction, ovarian physiology, immune function and osteoblast differentiation(Claustrat et al., 2005). Also, melatonin was proved to be a potent-free radical scavenger and a broad-spectrum antioxidan(Colares et al., 2016)mt. Melatonin has been extensively reported to entrain the circadian rhythms with external environmental conditions(Afeche et al., 2008). The multiple mechanism of actions of melatonin include:

(i) signaling through G-protein coupled receptors (MT1 and MT2) to decrease the linoelic acid uptake,

(ii) inducing QR2 (a detoxifying enzyme)

(iii) functioning as a scavenger of reactive oxygen and reactive nitrogen species.

(iv) increasing calmodulin degradation.

(v) binding to nuclear receptors (RZR/RORα and RZRβ) to alter transcription of target genes, and

(vi) as a modulator of hemopoiesis and immune cell production and function

Melatonin is principally secreted at night and is represented as a hormone of darkness, specialized photoreceptive cells in the retina detect light and suppress its production. SCN play as important role in regulating its synthesis in circadian pattern that is generated by a primary circadian clock of the brain(Welsh et al., 2010). Specifically, melatonin is synthesized from tryptophan under the control of various enzymes that are inhibited by light and stimulated by the dark.

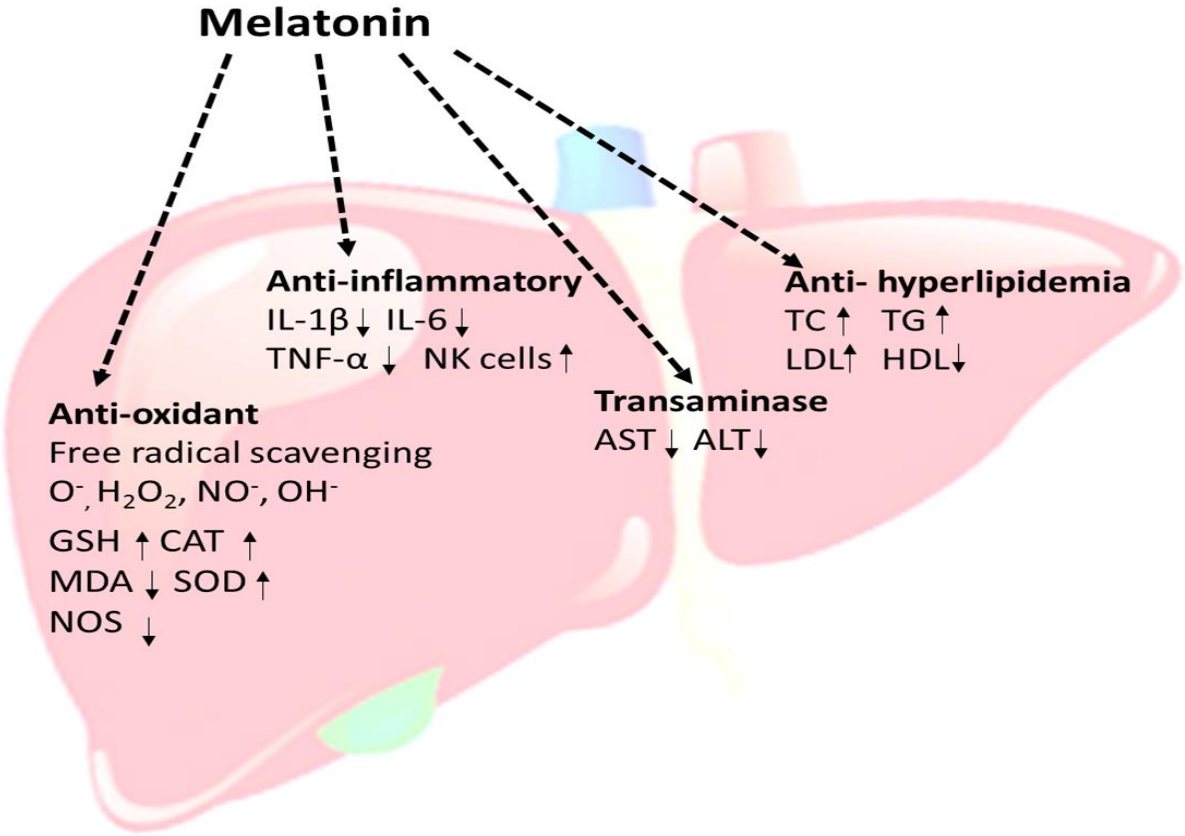

Figure 8: Multifactorial role of melatonin

Melatonin and diabetes

Diabetes is the most common metabolic disorder characterized by hyperglycemia resulting due to either insulin resistance or decreased insulin secretion. There are reports that suggest decreased melatonin production in animal models of diabetes and with exogenous melatonin supplementation leading to an improved glucose tolerance(Metwally et al., 2018). In rats treated with streptozotocin (STZ)-induced diabetes, melatonin has been reported to improve glucose homeostatsis and insulin sensitivity. Also, melatonin attenuates various complication associated with diabetes via its antioxidant potential, ability to neutralize ROS in beta cells, diabetes-induced harmful effects on the heart (diabetic cardiomyopathy), etc(Pourhanifeh et al., 2020). Further, melatonin activates PGC1α-SIRT3 signalling pathway and exerts a cardioprotective action in streptozotocin treated SD rats (Zhai et al., 2017). Melatonin ameliorates diabetic neuropathy through inhibition of oxidative stress by improving the activity levels of CAT, SOD and GPx and GSH and by activating Nrf-2-HO-1 pathway in streptozotocin treated SD rats(Negi et al., 2011).

Melatonin and obesity

Obesity is recognized as a major medical and public health problem and is strongly associated with many serious medical complications including metabolic syndrome and Type 2 diabetes (T2DM). Melatonin plays an important role in modulation of white adipose tissue, lipid metabolism and mitochondrial function(Genario et al., 2020). Additionally, there are evidence on beneficial effects of oral consumption of melatonin that cause increment in brown adipose tissue volume and improves blood lipid levels in patients with melatonin deficiency, suggesting that melatonin is a possible BAT activator(Halpern et al., 2019). Natural production of melatonin by pineal or exogenous

melatonin supplementation is instrumental in reduction of body weight gain and visceral fat deposit in young and aging in SD rats(Prunet-Marcassus et al., 2003).

Melatonin and Cancer

Several epidemiologic studies had revealed that the disruption of normal circadian rhythm may increase the risk of developing cancer(Shafi & Knudsen, 2019). In the recent years, several research groups had explored oncostatic properties of melatonin(Jung & Ahmad, 2006). Both in invitro model and pharmacological or physiological doses of melatonin are known to decrease growth of malignant cells(Blask et al., 2002). Most commonly known mechanism of the oncostatic property of melatonin is via its antimitotic, antioxidant and cell cycle regulation potential. However, other studies had revealed that melatonin showed protective effect on tumor growth and angiogenesis in xenograft model of breast cancer by reducing tumor growth, cell proliferation and angiogenesis via decreasing the expression of VEGF receptor 2(Jardim-Perassi et al., 2014). Melatonin is known to effectively augment activation of Natural Killer cells and induces cytokine production (IL-2 and IL-6)(Garcia-Mauriño et al., 1998). Also, it protects hematopoietic precursors from the toxic effect of chemotherapy and radiotherapy.

Protective Effects of Melatonin in Non-alcoholic Fatty Liver Disease (NAFLD)

In liver, melatonin shows beneficial effects against fatty liver by binding to membrane bound receptor 1 (MT1). Various studies had showed that melatonin was protective against fatty liver primarily because of its ability to lower oxidative stress(de Almeida Chuffa et al., 2013; Y. Li et al., 2019; Mi et al., 2018). Melatonin is known improve the activity status of antioxidant enzymes (SOD and GSH-Px) activities and decreased the MDA level in fatty liver(Pan et al., 2006). Additionally, melatonin also decreased hepatic steatosis and inflammation by lowering serum ALT, AST, liver total

cholesterol, and TGs in the fatty liver. The TG, MDA, and conjugate dienes (DC) are also reported to be lowered and GSH-Px activity was found to be higher after treatment with melatonin. This suggested that hepatic oxidative stress in NAFLD was reduced by melatonin(Pan et al., 2006). In addition, melatonin lowered the manifesting fatty liver by decreasing the level of pro-inflammatory cytokines and by improving biochemical indices of fat metabolism in patients with NAFLD(Stacchiotti et al., 2019). Melatonin supplementation is also known to improve hepatic morphological, ultrastructural and metabolic damage to ob/*ob* mice(Stacchiotti et al., 2016). Recent report had demonstrated that melatonin treatment improves the physiology of diet induced NAFLD/NASH in mice by reducing expression of microRNA-34a-5p in wild type mice but the same was not recorded in HET mice(Stacchiotti et al., 2019). Melatonin is also known to abrogate the release of proinflammatory cytokines and ROS that initiate mitochondrial dysfunction and thus prevent stellate cells activation and fibrosis progression in free fatty acids treated HepG2 cells(Das et al., 2017).

Rationale

Though a growing body of evidence suggests a correlation between circadian clock and NAFLD (in clock gene ablation models), these experimental models often fail to mimic the complexity of a lifestyle disorder. Hence, a Jetlag and/or High fat high fructose model was used herein; that truly mimics chronodisruption in shift workers and transcontinental travellers. This experimental model is appropriate for investigation of subtle alterations in circadian oscillations of clock genes and its association with antioxidant defence system with its implications in experimentally induced NAFLD. The role of clock genes and melatonin in metabolic rewiring under conditions of HFHF and/or JL models is a lacuna in the available scientific information. On the other hand, role of melatonin in modulating circadian rhythm is well established but its role in re-entrainment of altered circadian cycle by HFHF and/or JL in NAFLD is not known. This study is the first to investigate the shift in clock gene oscillations and Nrf2-HO-1 in HFHF and/or JL induced NAFLD, wherein merits of exogenous melatonin in making corrective changes has been contemplated.