

Disruption of circadian rhythm increases the risk of cancer, metabolic syndrome and cardiovascular disease

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Abstract

Incidents of non-communicable diseases (NCD) like cardiovascular diseases, cancer, diabetes, and chronic respiratory disease have increased dramatically and are currently the leading causes of death worldwide. Their rising incidents coincide with the dramatic changes in industrialization and development of societies over the past few hundred years. Therefore, current lifestyle practices should be further explored to uncover novel risk factors for certain cancers (i.e. colon, prostate, and breast cancer), metabolic syndrome (i.e. diabetes and obesity), and cardiovascular disease (i.e. coronary artery disease). This review discusses how a disruption of the “biological clock” or circadian rhythms could be involved in the development of these diseases as circadian rhythms control multiple physiological processes such as wake/sleep cycles, hormonal levels, body temperature, metabolism, and immune system.

Several environmental factors that disrupt circadian rhythms can be identified including exposure to artificial light and electromagnetic (EM) waves, unbalanced diet and night shift work. The mechanisms of how these “chronodisruptors” are associated with NCDs will be discussed. Furthermore, the involvement of genetic factors in the disturbance of circadian rhythms and predisposition to NCDs will be highlighted.

Overall there is strong evidence from animal models and epidemiological studies underlining that circadian disruption is a significant player in several diseases particularly the multifactorial diseases that pose a significant public health challenge in contemporary society. A circadian disruption-based model of cancer, metabolic syndrome and cardiovascular disease etiology can be proposed. But, to fully understand the complex interactions of the different components in the network of disease development due to disruption of circadian rhythms, more investigations are needed to unravel the causal relationship between modern lifestyle, circadian rhythm disruption and complex disease. This summary will help to better understand the mechanisms and aid the development of new methods and policies to lower incidence/death rates.

Keywords: Circadian rhythm, modern society, core clock genes, light at night, shift work, diet, electromagnetic waves, cancer, metabolic syndrome, cardiovascular disease

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1. Introduction

Non-communicable diseases (NCD) are the leading causes of death since 2008, accounting for almost 65% of all causes of death worldwide (World Health Organization WHO, 2011). NCDs include, but are not limited to, cardiovascular diseases, cancer, diabetes, and chronic respiratory disease. The number of new cases and deaths due to NCDs is increasing and around 36 million deaths occurred in 2008 alone (WHO, 2011). Interestingly, urban dwellers are more prone to NCDs compared to rural dwellers in developed and developing countries and the modern changes in lifestyle could account for such differences (WHO, 2011). Despite the difference in nutrition quality between poor and rich economies, nutrition transition is usually accompanied with urbanization (WHO, 2003). Urban dwellers consume more processed and convenient foods, and have less physical activity (WHO, 2003). As a result, obesity as well as diabetes and hypertension are more prevalent in urban dwellers due to the imbalance of energy intake and expenditure (Sobngwi et al., 2002). In addition, urbanization is associated with night shift work and links between metabolic syndrome (a multifactorial NCD) and night shift work have been established (Pietrojusti et al., 2010). As more people are moving towards urban areas (United Nations (UN), 2012), incidents of NCDs are reaching levels that are higher than ever (WHO, 2011). Understanding the prevalence of NCDs is essential to tackling this epidemic and reducing incidents and deaths.

The focus of this review will revolve around modern changes in lifestyle, the effects on our biological clock, the functions of our circadian rhythms and predisposition to complex NCDs such as cancer and metabolic syndrome.

Circadian rhythms synchronize the body functions with the environment, help to optimize energy use, and, therefore promote survival (Foster and Wulff, 2005). Energy consumption has to be balanced over the fluctuating environment and cycles of day and night to ensure efficient performance of the biological system (Foster and Wulff, 2005). This daily cyclical variation in behavior improves reproductive/survival fitness and over millions of years of evolution, a system to regulate daily rhythms was established (Loudon, 2012). It involves an internal “master clock”, entrainable by external stimuli that orchestrate circadian rhythms and the oscillatory function of key peripheral organs (Foster and Wulff, 2005). This system is vital for regulation of sleep/wake cycles, metabolism and energy consumption in humans (Foster and Wulff, 2005).

As circadian rhythms modulate vital processes, it is not surprising that several health problems can be associated with the disruption of these rhythms in humans (Srinivasan et al., 2010). Acute disturbances include gastrointestinal, menstrual irregularities, and sleep disorders (Srinivasan et al., 2010). In addition, these problems are most likely due to a misalignment of the circadian rhythms with the external environment (Srinivasan et al., 2010). Since the industrial revolution, a significant change in lifestyle has occurred. As societies become more industrialized, the demand for day/night activities and services increased (Parliament Office of Science and Technology (POST), 2005). The fixation on growth and productivity increased working hours, creating the “24-hour society” phenomenon (POST, 2005). The implications of such a societal structure can be deleterious to human physical and mental health (POST, 2005).

The trend to more evening and night work has been influenced by several factors including consumer demands, commercial competitiveness, governmental drive for economic growth, and social night activities (POST, 2005). In 2005, approximately 28% (4.1 million of the 14.6 million) of employed Canadians worked non-regular shift hours; the majority (72%), however, worked full time morning hours (30+ hours per week) (Ciarleglio et al., 2008). Around 17% of the American workforce is involved in working evening, night or rotating shifts, while more men than women work evening and night shifts (McMenamin et al., 2007). Within the US population, African-Americans (23.2%) tend to occupy alternating-shift jobs more often than their white (16.7%) or Hispanic (18.1%) counterparts (McMenamin et al., 2007). In Europe, night shift work affects about one employee in five (Le Bihan et al., 2004). However, strict laws were enforced to regulate night

shifts and protect workers from the potentially harmful effects of circadian rhythm disruption (POST, 2005).

The invention of artificial light has revolutionized ways of living by altering behavioral and social attitudes, including sleep patterns (Vollmer et al., 2012). Even though artificial light is not as efficient as natural light in circadian rhythm entrainment (Kohyama et al., 2011), it can contribute to circadian disruption (Czeisler and Gooley 2007).

Additionally, circadian rhythms may be disrupted by low frequency electromagnetic (EM) waves emitted from power lines and electrical appliances. Regular exposure to variable intensities of EM waves can affect melatonin rhythms by interfering with its production and secretion (Brainard et al., 1999).

Circadian rhythms are controlled by a cyclical expression of circadian genes and mutations in these genes, which result in a modification/disruption of the circadian oscillator. Therefore, it is important to analyze genetic factors that may contribute to circadian disruption (Rosato et al., 2006). Despite the advances in treatment of diseases such as cancer, metabolic syndrome, and cardiovascular disease, relevant genetic factors have yet to be identified (Almon, 2012). Polymorphisms in circadian genes can possibly account for the heritability in these diseases (Alhopuro et al., 2010; Turek et al., 2005; Wang et al., 2008). The discovery of novel genes involved in circadian rhythm-related disease will open up new opportunities for therapy and can serve as markers for improved diagnosis/prognosis (Fig. 1).

For a better understanding of the relevance of the circadian clock, the following chapters will address the function and disruption of circadian rhythms.

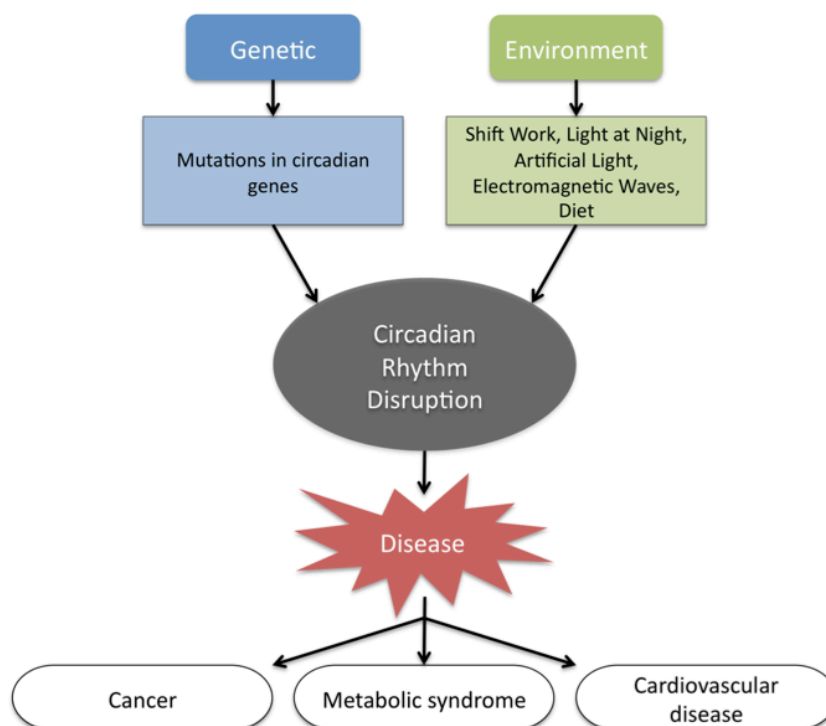


Figure 1: Basic Mechanism of Circadian Rhythm Disruption; several environmental and genetic factors can cause disruption of the circadian rhythms. This disruption could contribute to multifactorial disease such as cancer, cardiovascular disease and metabolic syndrome (adapted from Rüger and Scheer (2009)).

1.1 Definition of Circadian Rhythms

A circadian rhythm is an oscillating biological rhythm that resets approximately every 24 hours (hence the Latin term “circa” (around), “diem” (day)). This 24-hour system exists in many forms of life on earth and is conserved throughout evolution (Loudon, 2012). In humans, circadian rhythms pace the body functions with the level of diurnal and nocturnal activity, by regulating hormones, core body temperature, cell metabolism, cell growth and division (Vitaterna et al., 2001).

The classic phase markers of the biological clock are core body temperature and plasma levels of cortisol and melatonin (Czeisler and Gooley 2007). Two key features of circadian rhythms are that they are entrainable and can be reset accordingly by external stimuli such as light. Yet, in the absence of these external stimuli almost 24-hour rhythms are maintained (Czeisler and Gooley 2007).

1.2 The Circadian Clock System

1.2.1 Central Oscillators

The master clock, which orchestrates and synchronizes circadian rhythms in mammals, is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus. The SCN is mainly entrained by light. The signals are received from retinal ganglion cells, which contain melanopsin, and passed through the retinohypothalamic tract to the SCN. Two clusters of approximately 20,000 neurons in the SCN, consisting of several cell types and yielding neurotransmitters and peptides, are the core of the master clock (Vitaterna et al., 2001). During daytime, the light information transmitted to the SCN suppresses melatonin release and therefore its circulating levels. On the contrary, melatonin's blood-concentration peaks during the night, manifesting the various effects of this hormone (Arendt et al., 2010). Several landmark papers by Lewy (1987, 1999), Czeisler (1990, 1996), Beersma & Daan (2003) and others developed the idea that the melatonin rhythm is entrained by light at the level of amplitude and phase. Khalsa et al. (2005) developed a phase response curve (PRC) that describes the phase shift induced in the endogenous circadian rhythm by light as a function of time of exposure (for review see Duffy et al., 2005). Further, circadian rhythms of digestion and metabolism can also be entrained by food intake in mice (Martinez-Merlos et al., 2004). However such an entrainment has not been confirmed to exist in humans.

In humans, the pineal gland in the brain is the main source of melatonin, which upon secretion exerts various effects ranging from energy balance to circadian entrainment by activating melatonin receptors (MT₁, MT₂). These G-protein coupled receptors are expressed in the central nervous system (CNS) as well as several peripheral organs such as the liver, ovary and prostate (Pandi-Perumal et al., 2008). Cortisol is another hormone that is secreted by the adrenal gland in a diurnal fashion and it produces a range of effects including immune system suppression and carbohydrate and fat metabolism. Apart from these two hormones, there are several other signaling molecules like estrogen, testosterone, and inflammatory cytokines that are regulated by circadian rhythms either directly or indirectly (Harmer et al., 2001). These hormones will be discussed in the context of disease in chapters 3.1 to 3.3 of this review.

1.2.2 Peripheral Oscillators

In addition to the central clock each organ has its own internal “clock”. The SCN synchronizes the oscillations of gene transcripts in peripheral organs like the liver and lung, thus maintaining synchrony between organs, external environment, and central clock. In the absence of an entraining stimulus (originally introduced by the German word “Zeitgeber”), the central and peripheral clocks maintain rhythmicity but with an increased risk of de-synchrony to each other and with the external environment. The mechanisms by which central and peripheral oscillations are synchronized are not fully understood.

Hormonal and neuronal connections have been hypothesized to facilitate this synchrony (Stokkan et al., 2001).

The molecular system that controls the circadian rhythmicity of “clock-controlled genes” in peripheral tissues is composed of the following genes - CLOCK, BMAL-1-2, Cryptochrome1, and 2 (CRY1-2), Period1, 2, and 3 (PER1-3), and NPAS2. These genes inhibit or facilitate the transcription of each other and several other genes. The CLOCK and BMAL-1 genes comprise the positive limb while PER and CRY comprise the negative limb of the molecular circadian system. CLOCK and BMAL-1 are transcription factors that activate transcription of PER and CRY, which in turn result in proteins that heterodimerize to form the PER-CRY complex. In addition, CLOCK-BMAL-1 heterodimers trigger the transcription of an array of “clock-controlled genes” (CCGs), which constitute about 8-10% of the transcriptome. The accumulation of PER-CRY heterodimer inhibits CLOCK-BMAL-1 transcriptional activity while the degradation of PER-CRY triggers the activation of CLOCK and BMAL-1 again. This primary feedback loop of core circadian genes cannot maintain 24-hour rhythms on their own. Other associated clock genes form a secondary auto-regulatory feedback loop to maintain the 24-hour cycle. The effects of these genes are mediated by phosphorylation/de-phosphorylation, degradation of proteins, or inhibition of expression of core clock genes (Bechtold et al., 2010).

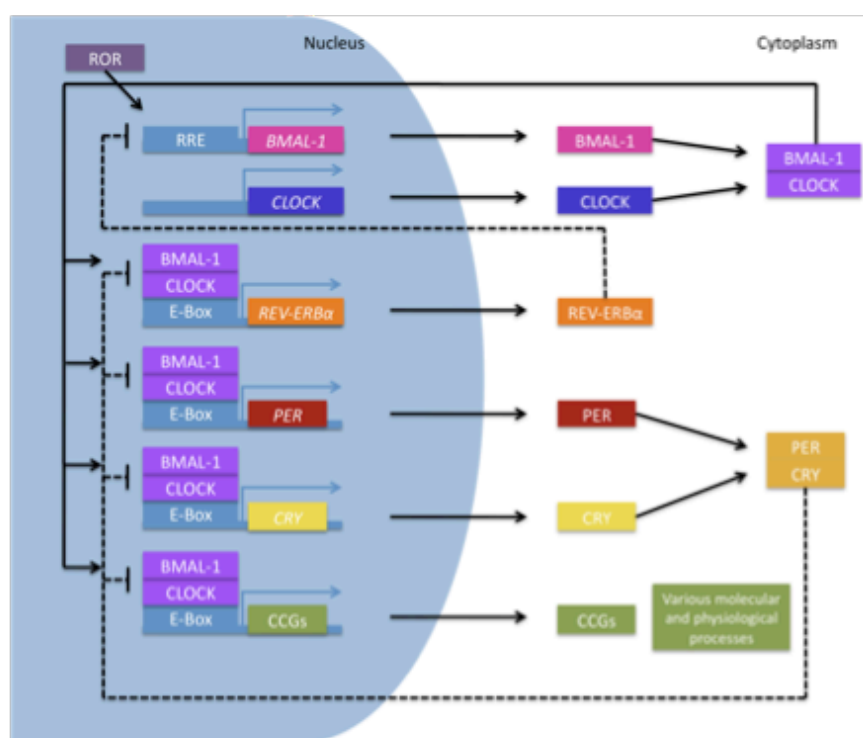


Figure 2: Molecular machinery of core clock genes: CLOCK and BMAL-1 transcription occur in the nucleus, then they are translocated to the cytoplasm where they heterodimerize. The CLOCK/BMAL-1 complex moves to the nucleus and activates the transcription of other core clock genes (REV-ERB α , Period; PER, Cryptochrome; CRY, and Circadian Controlled Genes; CCGs). The PER/CRY heterodimer accumulation cause the inhibition of CLOCK/BMAL-1 activated genes while REV-ERB α inhibits transcription of BMAL-1.

1.3 Disruption of Circadian Rhythms

Circadian rhythms can be maintained in the absence of a source of entrainment, but circadian rhythm de-synchronization occurs when internal oscillations are misaligned

with the external environment. This may also result in a disturbance of hormonal release. As melatonin is entrained by cues of light, light exposure at inappropriate times results in suppression of melatonin at times when it is normally released (Arendt et al., 2010). The internal misalignment of circadian-regulated organs occurs due to differential rates of re-entrainment (Stokkan et al., 2001). This internal de-synchrony results in reduced functionality of circadian-regulated systems, possibly predisposing the entire body to various diseases.

2. Factors Involved in the Disruption of the Circadian Rhythm

Possible mechanisms of circadian disruption will be discussed in chapter 2 before highlighting how a disruption of circadian rhythm may predispose to cancer, metabolic syndrome and cardiovascular disease (chapters 3.1, 3.2 and 3.3 respectively). Both environmental (such as “light at night” or artificial light) and genetic factors (polymorphisms in core clock genes) could result in disruption of the circadian rhythm.

2.1 Environmental Factors Modifying Circadian Rhythms

2.1.1 Occupational - Night Shift Work

The “Light at Night” theory proposes that exposure to light at night leads to a suppression of circulating melatonin (due to a phase shift of the melatonin rhythm) and altered levels of cortisol, estrogen and some other androgens (Reiter et al., 1980; Cohen et al., 1978). The circadian regulation of plasma melatonin levels is important for a wide range of physiological processes such as free radical scavenging, modulation of hormonal circuits and energy balance (Reiter et al., 1991). Therefore, it is not surprising that disruption of melatonin rhythms is associated with several diseases, particularly cancer.

Microarray studies reveal that some enzymes of the liver and the SCN exhibit robust circadian rhythms (Panda et al., 2002; Akhtar et al., 2002; Duffield et al., 2002; Oishi et al., 2003) and thereby regulate the metabolic state of the organism (see also chapter 3.2.1). At night, the metabolic rate slows down and anabolism is favored as crucial enzymes involved in respiration are regulated with the energy production peak during daytime. However, during night shift work, the body is challenged with high-energy requirements. If food intake is involved the body has the additional challenge of digestion and assimilation. Such a misalignment may lead to inefficient metabolism, leading to accumulation of toxic or waste products and resulting in significant stress to the body. While there is no direct evidence to support this theory, recent data from microarray studies highlight the importance of the regulation of downstream clock controlled genes. In addition, there is abundant evidence from population and in vivo studies supporting the link between night shift work and elevated risk for a variety of diseases including cardio-metabolic diseases (chapters 3.2.2 and 3.3.1.1) and cancers (chapters 3.1.1.1, 3.1.2.1 and 3.1.3.1).

2.1.2 Geographic Factors: Seasonal and Latitudinal Variations of Day Length and Melatonin Release

As melatonin has important oncostatic effects on breast cancer (see chapter 3.1.1.1), a reduction of this hormone increases the risk of developing cancer. Since light is the main factor synchronizing the internal and external rhythm, the amplitude/duration of the serum melatonin rhythm depends on day length; i.e. when volunteers were exposed to a “summer” photoperiod of 16 hours light and 8 hours dark for a week followed by a “winter” photoperiod of 10 hours light and 14 hours dark for 4 weeks, the duration of melatonin release was significantly longer (Wehr et al., 1991). Other studies using various model organisms confirmed this finding (Francis et al., 1988; Bittman et al., 1983).

Up to now there is only indirect evidence (discussed in 3.1.1.1) from epidemiological studies and animal models to verify whether polar nights influence circadian rhythms in regions of

extreme latitudes. Moreover, meaningful studies from human populations are still missing possibly due to confounding variables (light exposure and diseases risk).

It also remains unclear whether extreme seasonal changes in day length result in more adaptive or “flexible” circadian rhythms and whether the resulting mismatch of the changing day length to internal circadian rhythm is associated with an elevation of rhythm-linked diseases.

2.1.3 Contribution of Artificial Light to the Entrainment of Circadian Rhythm

Evolutionarily, the daily change of night and day was the primary circadian entrainer (Zeitgeber). The spectral and intensity characteristics of sunlight are unique and are most efficient in stimulating retinal ganglion cells, which entrain the circadian clock in a wavelength and intensity dependent manner (Duffy et al., Khalsa et al., Rueger et al., Berson et al., 2002). However, in the last 100 years artificial light was introduced and became an additional trigger. This is a significant change, especially considering that even during daytime most people are exposed to artificial light rather than sunlight. On average, young adults experience bright light only for a period of about 90 minutes per day (Savides et al., 1986). Therefore, several possible health aspects have to be considered:

1. Indoor lighting is not as effective as sunlight in entraining the circadian oscillator because of its significantly lower intensity and perhaps because of its spectral power distribution. Some studies demonstrated that artificial light is adequate for visual acuity but may be insufficient for efficient circadian entrainment, resulting in chronic circadian misalignment (Schulmeister et al., 2004; Duffy et al., 1996). Given that nowadays exposure to natural sunlight is quite sporadic and possibly inadequate health consequences of indoor lighting as an inefficient circadian entrainer have to be taken in consideration. However, Czeisler et al. (1996) reported that the human circadian system might be more sensitive to low intensity light (~150 lux) than generally expected.
2. Light exposure at night could be another pertinent issue as evolutionarily, nights were dark, but nowadays, artificial lights are used to prolong the day. There is considerable evidence on the effect of “light at night” and deregulation of hormonal rhythms (discussed in sections 3.1.1.1, 3.1.2.1, 3.1.3.1 and 3.3.1).
3. The release of melatonin is most efficiently suppressed with light at a wavelength between 464-484 nm (Berson et al., 2002; Hattar et al., 2003; Gooley et al., 2003, 2010). The emission of computer screens has a high intensity at these wavelengths, therefore contributing to the suppression of melatonin. Therefore, excessive computer use, particularly at “non-circadian” times could be another factor in circadian disruption.
4. Artificial light is used extensively in developed and industrialized countries while it is less used in developing and underdeveloped countries. A high risk of the major complex diseases like metabolic syndrome and cardiovascular diseases in developed countries clearly supports this possible link (WHO, 2011).

2.1.4 Effect of Low Frequency Electromagnetic (EM) Waves

EM waves can directly affect the electrical activity of the SCN. Several studies demonstrate an effect of EM waves from various sources, including cable lines and mobile phones on brain electrical activity (Hossmann et al., 2003; Tattersall et al., 2001; Eulitz et al., 1998). In several animal models, exposure to low frequency EM radiation suppresses nocturnal melatonin levels (for review see: Brainard et al., 1999). As melatonin has a crucial role in radical scavenging and energy balance, an increased risk for cancers and metabolic diseases might occur. However, no obvious effects on melatonin levels were found in humans (Brainard et al., 1999). Overall, the link between EM waves and circadian disruption remains controversial.

2.1.5 Dietary Factors

Excessive intake of fats leads to an elevated risk for cardiovascular and metabolic diseases and cancer. Several mechanisms have been discussed to explain this correlation. However, the list of possible mechanisms proposed in the literature is by no means exhaustive. High fat diets could contribute to the development of complex diseases by leading to disruption of circadian oscillation of core clock genes and downstream clock controlled genes. Most of the evidence for this hypothesis comes from studies on rodents which showed that cyclic expression of genes in crucial metabolically active organs like the liver, adipose tissue and hypothalamus was affected by high fat diets (see review by Froy et al., 2007). Rats fed with a high fat diet had disrupted levels of several important hormones including melatonin (Cano et al., 2008). Furthermore, a population study (discussed in 3.2.4.1) demonstrated a gene-diet interaction between CLOCK polymorphisms and lipid intake, suggesting that dietary habits modulate the expression of metabolic syndrome and are linked to certain CLOCK polymorphisms (Garaulet et al., 2009). Identification of the precise mechanisms of this interaction could open an opportunity to minimize the risk associated with circadian disruption and complex diseases.

2.2 Genetic Factors Modifying Circadian Rhythms

2.2.1 Melatonin-Pathway Mutations

2.2.1.1 Receptors

Melatonin receptors are part of the G-protein coupled receptor super family. The two main melatonin receptors are MT₁ and MT₂ (Reppert et al., 1996). MT₁ melatonin receptors are expressed in the pituitary gland, SCN, hypothalamus, cerebral cortex, and cerebellum (Mazzucchille et al., 1996). Activation of MT₁ receptors is associated with the inhibition of adenylate cyclase, and the hydrolysis of phosphatidylinositol. MT₂ melatonin receptors are expressed mainly in the retina, and their expression inhibits cyclic AMP synthesis (Klein et al., 2009). Mutations of the melatonin receptor genes can predispose to metabolic syndrome (3.2.3.1) and breast cancer (3.1.1.2).

2.2.1.2 Enzymes in Synthesis Pathways

Mutations in the enzymes involved in melatonin biosynthesis result in suppressed pineal melatonin levels, and subsequently cause circadian disruption. Mice with defects in enzymatic activity of arylalkylamine N-acetyltransferase (AANAT) and N-acetylserotonin O-methyl transferase (ASMT), which are essential precursors for melatonin biosynthesis, suffer from significantly low levels of melatonin (Shimomura et al., 2010). In addition, rs4446909, a single nucleotide polymorphism (SNP) in the promoter region of (ASMT), influences the expression of the ASMT enzyme. ASMT is involved in the conversion of N-acetylserotonin to melatonin, thus mutation in ASMT can lead to low melatonin levels since N-acetylserotonin will not be transformed into melatonin (Kripke et al., 2010).

2.2.2 Core Clock Genes

As mentioned earlier, clock genes are essential for the maintenance of the circadian rhythm and mutations of these genes can potentially lead to circadian rhythm disruption. The following sections will describe how mutations in PER, CLOCK, BMAL or CRY genes affect the circadian rhythm.

2.2.2.1 PER Gene Family

The PER gene family consists of three genes: PER1 gene acts as a crucial link between circadian rhythm and cell cycle through interacting with cell cycle checkpoint proteins like Ataxia telangiectasia mutated (ATM) and Checkpoint kinase 2 (CHK2; a Serine/Threonine protein kinase) (Gery et al., 2006). PER2 is the positive regulator of Bmal-1 loop in the circadian cycle (Shearman et al., 2000). PER2 gene polymorphism can lead to circadian disruption as well. Familial advanced sleep phase syndrome (FASPS) can be

caused by serine to glycine substitution (S662G) in PER2 gene as shown by some FASPS pedigrees (Toh et al., 2001). This mutation occurs due to hyperphosphorylation of PER2 polypeptide by casein kinase 1 (CSNK1) (Toh et al., 2001). CSNK1 regulates the activity of PER2 by phosphorylating the S662 position (Xu et al., 2007). In a population study, coding exons of PER3 gene were screened. Six sequence variations with amino acid changes were identified in the Per3 loci with four haplotypes of the PER3 gene. One of these four haplotypes was associated with delayed sleep phase disorder (Ebisawa et al., 2001). Consequently, mutations in PER genes might lead to circadian disruption as seen by their relation to phase shifting sleep disorders.

2.2.2.2 CLOCK gene

Two polymorphisms in the CLOCK gene T3111C and T257G, were linked to diurnal preference (Pedrazzoli et al., 2007). Furthermore, Desan and colleagues (2000) analyzed the mouse and drosophila genome and found that homozygotes or heterozygotes for the 3111C allele have higher mean scores on a measure of evening preference than subjects homozygous for the 3111T allele.

2.2.2.3 CRY Gene Family

Mammalian cryptochrome-1 and 2 are part of the negative limb in the circadian feedback loop, since they are negative regulators of the PER and CRY cycles (Shearman et al., 2000). Double mutant mice for mCRY gene lost circadian rhythm, as the biological clock was not functional. PER1 and PER2 circadian regulation is lost in both SCN and peripheral oscillators of the double mutant CRY mice having high levels of PER1 and PER2 mRNAs all the time (Okamura et al., 1999). This indicates the significance of mCRY1 and mCRY2 in circadian regulation.

2.2.2.4 BMAL Gene

BMAL-1 forms CLOCK:BMAL-1 and NPAS2:BMAL-1 heterodimers, which act as transcription factors regulating the transcription of a wide range of genes including CRY genes (Kondratov et al., 2006). The BMAL gene is involved in seasonal variation in humans as demonstrated for a population study that carriers of the allele rs6290035 TT experienced less seasonal variation than individuals who did not carry this polymorphism (Kovanen et al., 2010). The seasonal variation was categorized into sleep length, social activity, mood, weight, appetite and variation in energy level.

3. Circadian Rhythm Disruption Predisposes to Complex (Multifactorial) Diseases

3.1 Cancer

Despite rapid advancements in cancer prevention and treatment, incidence rates of most cancers show a worldwide net increase since 1975. In fact, in the US, some cancers (skin, liver, kidney and thyroid) are on a steady rise. Breast cancer incidence increased from a rate of 103/100,000 in 1975 to 126/100,000 in 2007 despite a small decrease in the previous decade (Parkin et al., 2009; National Cancer Institute, NCI, 2010). The question arises whether modern lifestyle changes associated with a “24-hour society” have a significant role to play in the increase of breast cancer incidents/deaths.

Parallel to this, an increased incidence of colon and breast cancer in developed countries is found, despite advanced screening and prevention (Parkin et al, 2009). These cancers are also linked to circadian disruption, and their epidemiology correlates with regions where circadian disruption is prevalent, as in sub-populations like night shift workers. This evidence supports a circadian disruption-based model for carcinogenesis, particularly breast and colon cancers.

3.1.1 Environmental Factors

Several studies (Davis et al., 2001; Schernhammer et al., 2001; Hansen et al., 2001; Megdal et al., 2005; Hansen and Lassen, 2012) revealed a clear association between night shift work and increased breast cancer incidence. These studies turned the attention towards “Light at Night” (LAN) as a potential “carcinogen”. This theory was further supported by a series of studies linking “Light at Night” to a change of melatonin secretion while the resulting melatonin concentrations were associated with the development of breast cancer (reviewed by: Grant et al., 2009; Stevens, 2009).

As highlighted earlier, LAN can reduce melatonin levels at night (Revell et al., 2007). Animal models, in addition to population studies on night shift work and breast cancer risk (mentioned earlier), support the hypothesis that a suppression of melatonin at night is associated with an increased incidence of cancers (reviewed by: Grant et al., 2009; Stevens, 2009). Constant illumination on mice revealed an increased incidence of breast cancer, among other cancers (Blask et al., 2003, 2005), while, induced cancers in mice are also aggravated under constant light conditions (Anderson et al., 2000; van Den et al., 1999). Pineal-ectomy increases the growth and proliferation of tumor cells in vivo (Blask et al., 1984). Further, blind people who were insensitive to light-induced melatonin suppression showed significantly lower cancer risk, particularly breast cancer (Hahn, 1991; Kliukiene et al., 2001; Flynn-Evans et al., 2009). Therefore, the question is how reduced melatonin production increases the risk for breast cancer. Studies support a direct oncostatic role and a secondary hormonal mechanism for these observations.

In in vitro studies (using breast cancer cells) the general consensus is that melatonin has an anti-proliferative effect, particularly in estrogen receptor ER+ breast cancer cells. Two main mechanisms are discussed: 1) inhibition of estrogen receptor (Kiefer et al., 2002; Molis et al., 1995; del Rio et al., 2004) and 2) inhibition of linoleic acid uptake (Blask et al., 1999). However, there is a debate whether melatonin causes growth inhibition, as some studies have shown the contrary (Annie et al., 1998; Ram et al., 1998). These results may be attributed to the existence of a melatonin-resistant subtype of MCF-7 cells, and the results may also depend on the culture conditions imposed by the laboratory, as these may lead to selection of cell subtypes and/or alteration of cell signaling. Currently, more evidence points towards an oncostatic role for melatonin, and it seems that the circadian-controlled secretion of melatonin is important for keeping growth of mammary tissues/cancers controlled.

Furthermore, melatonin has an inverse effect on estrogen and other circulating reproductive hormones in the blood (Reiter et al., 1980; Cohen et al., 1978). Therefore, a decreased concentration of melatonin in the blood is associated with an increased concentration of androgens like estrogen and progesterone. It can be hypothesized that estrogen’s stimulatory effect on the growth of mammary cells increases the turnover of these cells, thereby increasing the chance for replication errors.

Another mechanism for melatonin’s protective role is its well-described anti-oxidant effect. Melatonin is a potent free radical scavenger and has secondary effects by increasing the expression level of antioxidant enzymes. A decreased level of melatonin will result in an accumulation of free radicals leading to increased risk for cellular damage and somatic mutations.

Schernhammer and colleagues (2003) demonstrated a direct link between night shift work and increased risk of colorectal cancer. An oncostatic effect of melatonin on colon cancer was suggested using colon cancer cell lines and in-vivo induced tumor models (Farriol et al., 2000; Anisimov et al., 1997, 1999, 2000).

Two studies investigating occupational health risks among pilots showed an increased risk for cancer, particularly prostate cancer (Pukkala et al., 2003; Band et al., 1996). The routine of pilots mimics a state of chronic circadian disruption as the internal clock is desynchronized with the environmental cues and re-entrainment, resulting in further disruption. This also entails the earlier conclusion that exposure to “Light at Night” leads to melatonin suppression. Three other studies showed a correlation between prostate cancer risk and night shift work (Conlon et al., 2007; Kubo et al., 2007; Kakizaki et al., 2008). The melatonin/prostate cancer link arises from two main observations – first, circulating melatonin levels are lower in men with prostate cancer compared with those without the disease (Bartsch et al., 1992) and second, melatonin has oncostatic properties via androgen receptors on prostate cancer cell lines (Zisapel et al., 2001).

Further (see also chapter 3.1.1), melatonin has an inverse effect on circulating levels of other androgens that play an important role in the development of prostate cancer. Therefore, environmental influences that affect melatonin or androgen levels can contribute to the progress of prostate cancer (for review see: Zhu, 2006).

Endometrial cancer (Viswanathan et al., 2007) and non-Hodgkin’s lymphoma (Lahti et al., 2008) are two other cancers with increased occurrence in night shift workers. However, the mechanism remains unclear.

Considering the evidence that light reduces circulating melatonin levels, therefore increasing the risk for breast, colon and prostate cancer, EM waves have to be considered as another pertinent modern environmental influence (see chapter 2.1.4). Though controversial, it is conceivable that exposure to EM fields also suppresses melatonin levels as breast cancer incidence correlates with the increasing exposure to EM waves (Kliukiene et al., 2003; Tynes et al., 1996). Exposure to low frequency EM fields has also been found to increase the risk for colon cancer (Orbach-Arbouys et al., 1999). But, EM waves may also directly interact with colon tumor cells and affect proliferation (Cleary et al., 1993). There is also epidemiological evidence for a link between occupational exposure to EM waves and prostate cancer mortality (Charles et al., 2003).

Other potential risk factors for cancers are seasonal and latitudinal variations in day length. As discussed in chapter 2.1.2, day length significantly affects endocrine secretions including melatonin and androgens leading to changes in behavior and physiology. However, a direct link to cancer epidemiology remains difficult given confounding variables such as light exposure and vitamin D synthesis, as vitamin D has been shown to have a protective effect on the carcinogenesis of several cancers (Garland et al., 2009). Porojnicu and co-workers (2007) demonstrated a latitudinal and seasonal stratification in breast cancer risk and survival. Mason and his group (1990) showed that the season of tumor diagnosis influences factors predicting survival of patients with breast cancer. Some studies have also looked at geographic clustering of colon cancer risk based on light exposure (Garland et al., 1990; Lim et al., 2006). Such studies suggest that day length may be an important factor to consider while assessing the risk for breast cancer. However, the data are conflicting and new approaches are needed to establish a possible link. Studies on animal models and human populations are needed to test the effect of day length and light exposure on diseases risk while normalizing for several other factors like vitamin D synthesis and protection.

3.1.2 Genetic Factors

Cancer is often a complex polygenic disease. It has been estimated that genetic effects could account for 30% of the variability of propensity to breast cancer (Locatelli et al., 2004). Given the clustering of metabolic diseases, cardiovascular diseases and cancer, one might expect a common genetic basis that bridges these diseases. Clock gene disruption may

be the common ground as deregulation of these genes may predispose to any of these diseases. However, the disease phenotype depends on the clock gene affected.

Two population studies have demonstrated a link between polymorphisms of circadian genes and breast cancer. Zhu and his group (2005) showed that a variant PER3 genotype (heterozygous and homozygous 5-repeat alleles) was associated with an increased risk for breast cancer among premenopausal women, while among postmenopausal women the difference was not significant. The results suggest that mPER3 may be involved in the regulation of hormonal secretions, as the menopausal status seems to be an important factor in the relation between PER3 polymorphisms and breast cancer risk. Following this, the same group (Zhu et al., 2008) demonstrated a second genetic link between circadian genes and breast cancer: Women with the heterozygous (Ala394Thr) genotype at the NPAS2 gene had a significantly higher risk for breast cancer compared to those with the common homozygous genotype (Ala394Ala).

Two population-based publications described a link between clock gene deregulation and prostate cancer. In a study conducted in China, Chu and co-workers (2008) found that men with the variant CRY2 allele had a 1.7 fold increased risk for prostate cancer (95% confidence interval (CI), 1.1–2.7). The risk increased to 4.1 fold in prostate cancer (95% CI, 2.2–8.0) in men with insulin resistance. This suggests an additional link between diabetes, prostate cancer and the CRY2 variant C allele. Zhu and colleagues (2009) carried out a population-based case-control study of Caucasian men. At least one of the 9 core clock genes was significantly associated with an increased risk for prostate cancer.

Cancer cells also show aberrant expression levels of clock genes, particularly the PER genes. Several studies have demonstrated a down-regulation of PER1 in breast (Winter et al., 2007), colon (Krugluger et al., 2007; Gery et al., 2006; Mostafaie et al., 2009), prostate (Cao et al., 2009), lung (NSCLC) (Gery et al., 2007), and hematologic (CML) (Ming-Yu et al., 2006) cancers relative to normal tissue controls. In the breast cancer study, decreased PER1 and PER2 levels were also observed in familial as compared to sporadic primary breast tumor tissue. Another study of 55 cases of breast cancer by Chen and co-workers (2005) showed that PER1, PER2 and PER3 expression is disturbed in almost 95% of the women. Furthermore, in vitro experiments suggest that PER genes have an important role in tumor suppression and down-regulation of PER is associated with breast carcinogenesis (Gery et al., 2007; Fu et al., 2002; Hua et al., 2006). Overexpression of PER1 in colon cancer cell lines sensitizes them to DNA damage-induced apoptosis, and inhibition of PER1 causes blunted apoptosis (Gery et al., 2006). In prostate cancer cell lines (overexpressing androgen receptor - 293T and LNCaP cells), PER1 inhibited transactivation of the androgen receptor. In addition, an overexpression of PER1 in prostate cancer cells resulted in significant growth inhibition and apoptosis (Cao et al., 2009). Overexpression of PER1 in NSCLC cell lines led to a significant growth reduction and loss of clonogenic survival. The same authors could also demonstrate that DNA hypermethylation and acetylation are potential mechanisms for down-regulation of PER1 (Gery et al., 2007).

Further Jung-Hynes and colleagues (2010) recently showed that CLOCK and PER2 protein levels were down-regulated whereas BMAL-1 levels were up-regulated in prostate cancer cells compared to normal prostate cells. Further, overexpression of PER2 generally resulted in a significant loss of cell growth and viability. One interesting observation was that melatonin treatment caused an increase in PER2 and CLOCK and decrease in BMAL-1. This supports the hypothesis that melatonin suppression can increase the risk for prostate cancer, possibly by deregulation of clock genes.

Work by Kuo and co-workers (2009) shed light on the possible epigenetic mechanisms of clock gene deregulation and carcinogenesis as 37 of 53 breast cancer cell lines had hypermethylation on the promoters of PER1, PER2, CRY1 or BMAL-1. Further, expression levels of hPER1, hPER2, hPER3, hCRY1, hCRY2 and hBMAL1 were significantly impaired in both chronic phase and blastic crisis of chronic myeloid leukemia (CML) (Ming-Yu et al., 2006) and methylation analysis revealed that the CpG sites of the hPER3 gene were methylated in all of the CML patients. These results support an epigenetic mechanism of clock gene disruption.

Given the above data, clock genes seem to have crucial roles in cell cycle regulation and tumor suppression (for review see Okamura, 2004). However, it is difficult to differentiate between the effects of a loss of rhythmicity and a loss of function of clock genes as they may have functions other than circadian regulation.

A recent population-based study focused on the relation between “other” genes (non-core clock genes) that are involved in circadian rhythm maintenance i.e. melatonin pathway genes and breast cancer risk (Sandra et al., 2012), investigating associations between common polymorphisms in melatonin receptors (1a and 1b) and arylalkylamine N-acetyltransferase (enzyme involved in melatonin synthesis) and breast cancer risk. Two SNPs, one of MTNR1a and one of MTNR1b were associated with elevated breast cancer risk. Further, the effect of the MTNR1b SNP associated with breast cancer depended on menopausal status.

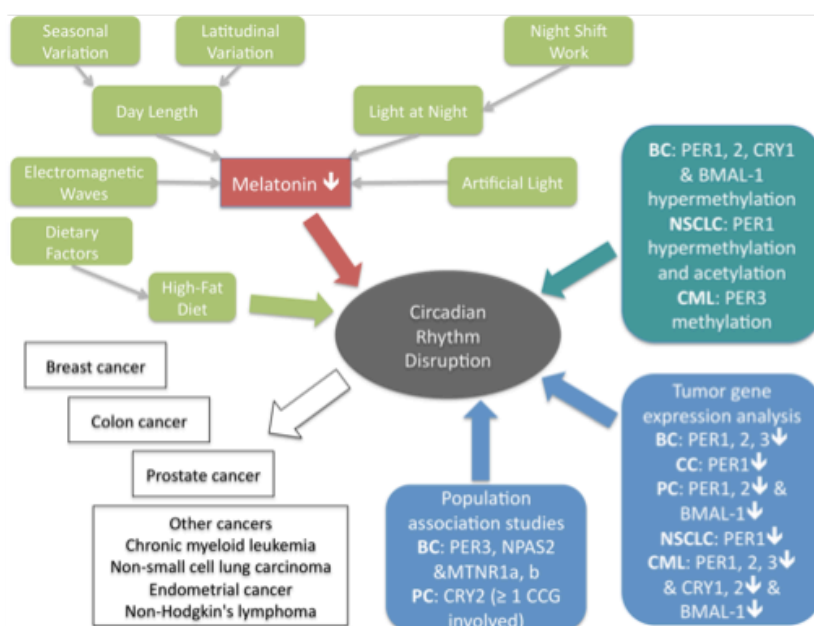


Figure 3: A Circadian disruption based model for the development of cancer; A complex interplay of environmental, genetic and epigenetic influences on the circadian system can contribute to the development of cancer. Environmental factors like artificial light use, exposure to light at night, variation in day length and electromagnetic wave exposure can contribute to circadian disruption by affecting melatonin secretion, which is important carcinogenesis, particularly breast cancer. Additionally, high fat diet intake also lead to molecular circadian disruption. Population based association studies have shown that polymorphisms in several circadian-related genes have been associated with elevated risks for certain cancers. Gene expression analysis on several tumor samples has also revealed deregulation of key genes involved in circadian rhythm maintenance. This deregulation may also be attributed to epigenetic factors such as methylation and acetylation in some cases.

3.2 Metabolic Syndrome and Obesity

It is estimated that about 25% of the world population suffers from metabolic syndrome. These patients have a five-fold increased risk of developing type 2 diabetes and are three times more likely to have heart attacks or strokes (International Diabetes Foundation, IDF, 2006). According to the World Health Organization (2011), 346 million people are estimated to have type 2 diabetes worldwide and it is predicted that diabetes deaths will double between 2005 and 2030.

Obesity has also more than doubled since 1980 and in 2008, globally, 1.5 billion adults, 20 years and older, were overweight. The incidence of obesity and metabolic syndrome is alarmingly high in developed countries and the same tendency arises in developing countries. In the upcoming chapters the evidence supporting a circadian disruption-based model for the development of obesity and metabolic syndrome will be reviewed.

3.2.1 Circadian Control of Metabolism

Metabolism defines life and is key for survival of any organism. From an evolutionary standpoint, metabolism is an important factor in determining the reproductive fitness of an organism. Extending the evolutionary argument, metabolic pathways must be regulated by the environment to optimize efficiency as the environment changes constantly. Entrainment of metabolic pathways of anabolism and catabolism to environmental demands or “stressors” would logically improve survival.

Glucose and lipid homeostasis are under circadian regulation. This concept is supported by the observation that circadian disruption (caused by circadian misalignment between internal clocks and external cues or loss of circadian rhythmicity due to deregulation of clock genes) is associated with increased risk for obesity, diabetes and metabolic syndrome (discussed in chapters 3.2 and 3.3).

The potential impact of circadian regulation of metabolism was confirmed by microarray studies of the mouse liver transcriptome (Panda et al., 2002; Akhtar et al., 2002; Duffield et al., 2002; Oishi et al., 2003). Coordinated circadian expression of glucose transporters, glucagon receptor and key enzymes that catalyze rate-limiting steps of hexose sugar metabolism was observed with peak expression at the early evening, right before the active period of mice (Panda et al., 2002). An important example is the bifunctional enzyme PFK-2, a key control point in glycolysis and gluconeogenesis. PFK-2 showed a robust and high-amplitude cycling. The enzyme PEP carboxykinase, intimately involved in gluconeogenesis also shows circadian oscillation. Additionally, insulin secretion is under circadian control and insulin and glucose responses depend on the time of the meal in a day (Kalsbeek et al., 1998, Van Cauter). These results showed that the glucose response to a meal is significantly higher when the meal is taken in the evening rather than the morning.

Lipid metabolism is also under circadian control. Microarray studies of the oscillating transcriptome of mouse liver indicate that several enzymes involved in lipid homeostasis have circadian rhythms; i.e. cycling of lipin1 was observed and is thought to be involved in sugar and lipid metabolism. The circadian oscillator enables the usage of short and medium chain fatty acids during night time feeding and long chain fatty acids at night (Peterfy et al., 2001). Transcript levels of membrane-bound Niemann-Pick C1 (NPC1) as well as the enzymes of the cholesterol biosynthetic pathway show circadian regulation. For instance, the conversion of HMGCoA to mevalonate by HMGCoA reductase, a rate-limiting step in the de-novo synthesis of cholesterol expresses a circadian rhythm (Shapiro et al., 1969).

Proteins involved in vesicular trafficking, cytoskeletal dynamics and exo-/endocytosis like synaptojanin-2 (SYNJ2) and disabled-2 (DAB2) also exhibit circadian rhythms. DAB2 is linked to endocytosis of low-density lipoprotein receptors (LDLRs). Components of the adaptor

protein-1 complex (AP-1), which are important for vesicle trafficking, also express circadian rhythms, suggesting that lipoprotein levels in the blood may be under circadian control. Therefore, circadian disruption can lead to loss of lipid homeostasis in the blood resulting in an increased risk for metabolic and cardiovascular diseases.

Circadian disruption can be effected by both genetic and environmental factors, increasing the risk for metabolic syndrome. Fundamentally, it might be the discrepancy between the oscillating transcriptome of organs involved in metabolism (liver, adipose tissue, muscle) and the environmental demands leading to loss of coupling between environmental and internal rhythm, consequently leading to sub-optimal performance.

In the upcoming chapters support for each of the potential environmental circadian disruptors will be reviewed. Further, genetic influences of polymorphisms in clock genes and other circadian controlled genes will be evaluated by reviewing population-based studies, animal models and in vitro studies.

3.2.2 Environmental Factors

The global increase in the prevalence of obesity and metabolic disorders coincides with the increase of shift work. Numerous recent population studies highlight that shift work increases the risk for metabolic syndrome (Karlsson et al., 2001, 2003; Esquirol et al., 2009; Sookoian et al., 2007; De Bacquer et al., 2009; Lin et al., 2009; De Lorenzo et al., 2003; Pietroiusti et al., 2009; Morikawa et al., 2005, 2007; Scheer et al., 2009; Suwazono et al., 2006, 2008; Nagaya et al., 2002). Given the strong epidemiological support for its link with metabolic syndrome, night shift work has to be recognized as an occupational health hazard. The effect of rotating shift work is actually double-edged as an initial circadian misalignment is followed by re-entrainment only to be disrupted again. This leads to chronic misalignment between the environmental demands and the circadian transcriptome of metabolically active organs leading to inefficient metabolism perhaps leading to deleterious changes like hyperglycemia, dyslipidemia and altered LDL/HDL ratio. These changes can promote the development of multifactorial diseases such as diabetes mellitus and cardiovascular diseases.

Pioneer work was done by Karlsson and colleagues (2001) who established this link with a cross sectional study. They presented that obesity, high triglycerides and low concentration of HDL cholesterol occur together in shift workers but not in day workers. A study by De Bacquer and colleagues (2009) analyzed the risk of metabolic syndrome in workers from several large Belgian companies. Again, a higher incidence rate of metabolic syndrome was present among shift workers in comparison to day workers. The issue of night shift work is clearly most important in certain occupations, i.e. for people employed in the healthcare and aviation industries. This was confirmed by a study of Pietroiusti and colleagues (2009), who demonstrated that the development of metabolic syndrome was strongly associated with night shift work in nurses.

However, these results do not confirm that circadian disruption is the main culprit here. An important piece of evidence to support this link is, that glucose and lipid homeostasis is under circadian control and shows a clear circadian rhythm (see reviews by: Kalsbeek et al., 2007; Eckel-Mahan et al., 2009; Van Cauter et al., 1997). Lesioning the SCN leads to chronic circadian misalignment resulting in a loss of diurnal variation in glucose and lipid metabolism (La Fleur et al., 2001). The constant re-entrainment that is experienced in rotating shift work leads to differential re-entrainment among peripheral tissues (Yamazaki et al., 2000). This state of internal de-synchrony as a result of different organs entrained differently may account for the abnormal metabolism in night shift workers. A study on rodents indicated that work during the inactive phase is sufficient to cause a shift in some

rhythms leading to metabolic disturbances such as loss of glucose rhythmicity, inverted triglyceride rhythm and increase in body weight (Salgado-Delgado et al., 2008). Two other studies on rodents strongly support the idea of circadian misalignment leading to metabolic syndrome: 1), when healthy, wild type mice were fed a high fat diet and placed on a 6-hour day (3L:3D), body weight and blood glucose levels were significantly elevated compared to control mice (Oishi et al., 2009). 2), heterozygous tau mutant hamsters with a 22-hour circadian period expressed renal and cardiac disease besides a reduced lifespan. An artificial 22-hour day to match the endogenous circadian period of the mice was protective. This was the first evidence suggesting a causal link between circadian misalignment and health (Martino et al., 2008). Further, in humans, when behavioral rhythms were 12 hours out of phase with the endogenous circadian rhythm, subjects exhibited the most profound alterations in metabolism, including increased glucose and insulin levels, decreased leptin levels and elevated arterial pressure (Scheer et al., 2009).

As discussed in chapter 3.1.1, the circulating melatonin level (associated with circadian disruption) is an important indicator for the pathogenesis of cancer. Therefore, the question arises, whether altered melatonin levels are also related to the metabolic syndrome. A study by Robeva and co-workers (2008) supports such a link. They reported a positive correlation between nighttime melatonin and insulin levels and this relation was more pronounced in metabolic syndrome patients. This suggests a possible coupling between melatonin and insulin levels. Disruption of the melatonin rhythm by any of the described mechanisms can potentially contribute to an increased risk of metabolic syndrome and even the phenotype of such patients. Further, this study reports that the night melatonin-insulin ratio was negatively correlated with serum LDL levels and positively correlated with serum HDL levels. While these results are promising to establish the link, more studies with larger case-control populations have to be done in order to understand the possible involvement of melatonin in metabolic syndrome.

High fat diets disrupt the biological clock in animal models (Kohsaka et al., 2007). These researchers used mice to show that such a diet altered the period of loco-motor activity and also affected the expression of canonical clock genes, nuclear receptors and downstream clock controlled genes in metabolically relevant tissue like the hypothalamus, liver and adipose tissue. Recently, Hsieh et al. (2010) demonstrated that high fat diet in mice leads to altered expression of circadian clock genes and circadian clock controlled genes including PER1-3, CRY1-2, BMAL-1, DBP, E4BP4, CK1 ϵ , PEPCK, PDK4 and NHE3 in kidney and/or liver, while changing the circadian clock at the hormonal level (Froy et al., 2007). Additionally, the circadian clock is delayed by disrupting adiponectin signaling in mouse livers (Barnea et al., 2009). The study also showed that fasting affects circadian entrainment but in the reverse sense, i.e. it advanced circadian expression. Corticosterone levels increased significantly in high-fat diet fed rats, and their 24-hour variation became blunted. Further, nocturnal level of melatonin was significantly decreased in high-fat diet fed mice (Cano et al., 2008).

Several other studies, in different animal models – including primates - (Suter et al., 2011; Cano et al., 2009; Mendoza et al., 2008) underlined that a high fat diet could contribute to metabolic syndrome by causing circadian disruption. However, no studies have been conducted on humans yet and more work should be undertaken to understand the potential role of diet in circadian health. Such insights could be used in local settings to improve circadian health by effecting simple lifestyle changes.

3.2.3 Genetic Links to Circadian Disruption and Metabolic Syndrome

The heritability of metabolic syndrome is about 40%, i.e. genetic factors account for 40% of the observed disease variability. Genes involved in diverse pathways have been linked to metabolic syndrome to explain the observed heritability of the disease. However, the

level of heritability suggests that there might be other unidentified genes involved in the etiology of metabolic syndrome.

Gene defects of the clock genes are risk factors for metabolic syndrome possibly due to a loss of circadian rhythmicity of metabolism. There is a limited, but consistent, set of population based studies which show associations between clock gene and non-clock gene polymorphisms and metabolic syndrome risk (see following chapter). However, the major evidence for the link between circadian disruption and metabolic syndrome comes from clock gene mutant animal models. Evidence from both of these approaches shall be evaluated in the following sections.

3.2.3.1 Population-Based Association Studies

Recently, four population studies investigated associations between clock gene polymorphisms and metabolic syndrome risk (Garaulet et al., 2009; Sookoian et al., 2007, 2008; Englund et al., 2009). Three of them found an association between CLOCK gene polymorphisms and metabolic syndrome risk while one identified NPAS2 and PER2 polymorphisms as risk factors. A study conducted in the United States (white population) by Garaulet and co-workers (2009) revealed that carriers of the CGA haplotype of the CLOCK transcription factor had lower BMI, waist circumference, blood pressure and insulin resistance. There was no association between clock gene polymorphisms and fasting state lipids – triglycerides, HDL, VLDL and total cholesterol. Further, the investigators demonstrated gene/environment interactions. For a certain SNP, (rs4580704) improved insulin sensitivity associated with the minor allele was present only at high mono-unsaturated fatty acid (MUFA) intake. Also, the deleterious effect of a certain gene variant on visceral fat was present only in association with high intake of saturated fatty acids. Other gene variants showed interesting phenotypes such as decreased plasma cytokine levels (IL-6) suggesting either alternative roles for CLOCK or effects of loss of circadian rhythmicity. The observed gene/environment interaction might be due to altered membrane composition of the pineal gland/suprachiasmatic nucleus affecting circadian regulation.

In a sample (n=1106) of unrelated men of European ancestry, obesity was associated with gene variants and haplotypes of the CLOCK transcription factor (Sookoian et al., 2008). Four out of six SNPs were associated with overweight/obesity and two haplotypes were associated with a 1.5 fold risk for overweight/obesity. Further, a remarkable heterogeneity in SNP distribution suggests that local distribution of clock gene SNPs might be important in assessing disease risk.

Another study by the same investigators (Sookoian et al., 2007) linked CLOCK polymorphisms to increased incidence of non-alcoholic fatty liver disease (NAFLD). Two SNPs showed significant correlation with NAFLD. Given that there exists considerable evidence linking CLOCK polymorphisms to metabolic syndrome, its usefulness as a prognostic and diagnostic indicator should be evaluated.

In addition, the association of other CLOCK gene polymorphisms with metabolic syndrome should be investigated. In fact, Englund et al. (2009) reported that circadian gene variants of NPAS2 and PER2 were linked to risk factors of metabolic syndrome like hypertension and high blood glucose.

Besides clock genes, polymorphisms of other genes involved in circadian physiology may also affect the risk for metabolic syndrome. Polymorphisms in melatonin receptor genes (MTNR1B) influence fasting glucose levels (Prokopenko et al., 2009) and a common variant of MTNR1B is associated with an increased risk for type 2 diabetes (Lyssenko et al., 2009).

The risk-genotype was associated with impairment of early insulin response and faster deterioration of insulin secretion over time. Overall, these studies raise the question of whether these polymorphisms are important in determining the risk for cardiovascular diseases, certain cancers, and metabolic syndrome, given the epidemiological clustering of these diseases. Further, it will be worthwhile to investigate epigenetic changes at these gene loci in patients to explore possible epigenetic links to metabolic syndrome via circadian disruption.

3.2.3.2 Evidence From Animal Models

In 2005, Turek's group demonstrated that homozygous CLOCK mutant mice, relative to wild type mice, expressed an attenuated diurnal feeding rhythm beside being hyperphagic and obese. The mice developed a metabolic syndrome of hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycemia and hypoinsulinemia. Several hypothalamic peptides involved in energy balance were low. This suggests that CLOCK gene networks play key roles in lipid and glucose homeostasis. This also backs some of the above mentioned population studies showing an association between metabolic syndrome and CLOCK polymorphisms. Infact, these studies were inspired by careful studies previously done in genetic mouse models.

Another study by Oishi and co-workers (2006) on the same homozygous CLOCK mutant mice further strengthened the importance of CLOCK in lipid metabolism. Serum levels of free fatty acids and triglycerides were significantly lower in CLOCK mutant mice relative to wild type mice. They actually pinpointed the reason of the extreme impairment in dietary fat absorption in CLOCK mutant mice.

Kennaway's group (2007) studied peripheral circadian disruption by using the Clock Δ 19 + MEL mouse model. These mice have preserved circadian rhythmicity in SCN and pineal gland but show arrhythmic Clock gene expression in the liver and skeletal muscle. They showed fasting hypoglycemia in young-adult males, fasting hyperglycemia in older females and a substantially impaired glucose tolerance overall and substantially low plasma insulin levels, suggesting an impaired insulin secretion.

A more direct link between CLOCK and BMAL-1 disruption and diabetes was suggested by some other studies. Marcheva's team (2010) presented that CLOCK and BMAL-1 mutant mice showed impaired glucose tolerance, reduced insulin secretion and defects in size and proliferation of pancreatic islets. This suggests that circadian disruption in the pancreas can lead to aberrant secretion of insulin (out of phase with systemic needs) or hypoinsulinemia. Rudic et al. (2004) found an abolishment of gluconeogenesis in BMAL-1 mutants possibly leading to the observed suppression of the diurnal variation of glucose and triglycerides.

Other clock gene mutant models (PER1-3, CRY1-2, REVERB α , etc.) were developed but no obvious signs of metabolic syndrome within the time periods monitored were found (for review see: Ko and Takahashi, 2006). However, all these models showed various degrees of alterations in the circadian period and the diversity of phenotypes observed is perhaps due to the different degrees of circadian disruption induced. It is interesting that metabolic syndrome was the most dominant phenotype in CLOCK and BMAL-1 mutants. This points to alternate functions for BMAL-1 and CLOCK in energy homeostasis. More studies are needed to understand if artificial environmental conditions with matching periods to the mutant animals' circadian rhythms are protective. The results will provide direct evidence to prove whether circadian misalignment due to clock gene disruption is involved in the etiology of the disease.

3.2.3.3 Genetic Background of Clock Genes – Risk Assessment for Metabolic Syndrome

There is abundant evidence supporting the idea that polymorphisms in clock genes can lead to disruption in circadian rhythmicity and thereby significantly increase the risk for metabolic syndrome. Inheritance of clock genes may account for a significant portion of the observed 40% heritability of metabolic syndrome. This results in the question whether knowing the clock gene genotypes or the “chronotype” (genetic information on clock genes) of an individual will have some level of diagnostic and/or prognostic significance.

Variations in the distribution of clock gene alleles among populations has been reported (Ciarleglio et al., 2008). Polymorphisms in BMAL-1, BMAL-2, PER2, PER3, CLOCK and AANAT in five distinct human populations from different latitudes and environments were investigated. Genotypic differences between European Americans and Ghanaians were highly different. There were also sharp differences in clock gene genetic backgrounds in different populations. For example, in BMAL-1, the most common haplotype in a Han Chinese subgroup was exceedingly rare in all other populations except for a group from Papua New Guinea, where it was the second most common. Some of the alleles may encode for a defective version of the protein which may contribute to circadian disruption elevating the risk for metabolic syndrome. Given the strong association between disease phenotype and clock gene polymorphisms, a use of clock genes as predictors of disease risk at the population level has to be considered.

Uncovering a new genetic component of a multifactorial disease will also help health care professionals in prognosis and treatment. As discussed (3.2.4.1), gene/environment interactions of clock genes in the etiology of metabolic syndrome were found. A classic example is the relationship between CLOCK gene polymorphisms, menopausal status, dietary intake and metabolic syndrome risk. It would be interesting to undertake a systematic analysis of multiple clock genes and their interactions with the environment in the pathogenesis of metabolic syndrome. Such insights will lead to pro-active personalized medicine to treat the deleterious effects of circadian disruption on health, particularly metabolic syndrome, using comprehensive personalized genetic information on clock genes.

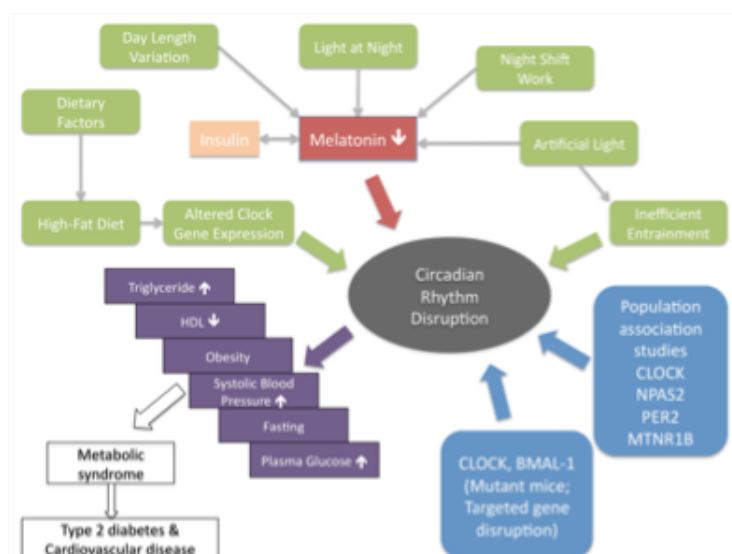


Figure 4: A Circadian disruption based model for the development of Metabolic Syndrome; Artificial light use, exposure to light at night, variation in day length and electromagnetic wave exposure can contribute to circadian disruption by affecting melatonin secretion, which has important roles in energy balance. Further, insulin secretion seems to be coupled with melatonin secretion hence playing an important role in metabolism. High fat diet intake could also lead to circadian disruption at the molecular genetic level and by disrupting circadian regulated hormones important in metabolism. Given metabolic function is under strict circadian control artificial light may be insufficient for circadian entrainment perhaps leading to chronic inefficient metabolism amounting to metabolic syndrome. Population association studies have also demonstrated links between circadian-related genes (CLOCK, NPAS2, PER2 and MTNR1B) and circadian rhythm disruption. Targeted gene disruption (CLOCK, BMAL-1) in animal models also clearly showed the development of metabolic syndrome.

3.3 Cardiovascular Diseases

Cardiovascular disease (CVD) is one of the most significant causes of death in developed countries. Based on the 2005 mortality rates in the United States, around 2400 Americans die every day of CVD, exceeding a rate of 1 death every 37 seconds (Lloyd-Jones et al, 2009). More than 70 million Americans suffer from some form of cardiovascular disease. According to the 2008 edition of the European cardiovascular disease statistics, over 4.3 million deaths are caused by CVD annually; nearly half of all deaths in Europe. These striking facts suggest that more research is needed to understand the contributing factors to cardiovascular disease. It is widely accepted that a disruption of the circadian rhythm could lead to, or at least predispose to cardiovascular disease (Prasai et al, 2008 Ruger et al., 2009; Paschos and Fitzgerald, 2010).

The circadian regulation of the cardiovascular system has been known since the 1960s (Kaneko et al., 1968). The clinical manifestation of cardiovascular disease supports the idea that the cardiovascular system is influenced by circadian oscillators. Myocardial infarction, sudden cardiac arrest, thrombotic stroke, and myocardial ischemia are more likely to occur during the early hours of the day (Ruger et al., 2009; Muller et al, 1985). A possible explanation is that a mismatch of oxygen supply and demand occurs in the morning as the body changes its state from relaxing to an active state. In order to meet the new challenge, blood pressure and heart rate rise causing the oxygen demand by the cardiac myocytes to increase. In addition, the vascular tone of the coronary artery increases, thus, raising the blood pressure, and resulting in a decrease in the coronary blood flow (Maemura et al., 2007, Dominguez-Rodriguez et al., 2010). Moreover, higher blood coagulability is observed in

the morning. This could be attributed to an increase in the platelet function and decrease in fibrinolytic activity due to high plasminogen activator inhibitor-1 (PAI-1) levels and low tissue plasminogen activator (t-PA) levels. Collectively, these changing parameters may contribute to the higher incidence of ischemia and myocardial infarction in the morning (Maemura et al., 2007).

However, there is limited research into how a disruption of the circadian rhythm increases the risk for cardiovascular diseases. The upcoming chapter will summarize the current state of research. The effects of a loss of signaling molecules such as melatonin, epinephrine, norepinephrine, plasminogen activators/inhibitors, and fibrinogen will be emphasized. These molecules control many functions in the body by up-regulating and down-regulating different genes, which in turn have specific functions particularly in the cardiovascular system (Dominguez-Rodriguez et al., 2010).

3.3.1 Environmental Factors

It is known that some environmental factors disrupt circadian rhythm and subsequently predispose to disease, and the cardiovascular system is no exception.

3.3.1.1 Night Shift Work

Multiple reports highlight an increased risk of cardiovascular disease among people who work night shifts (Nakamura et al., 1997; Boggild et al., 1999; McCubbin et al., 2010). Prasai et al., 2008 reviewed the link between night shift work and cardiovascular disease and stated that night shift work increased the risk for cardiovascular disease 3.0 fold in women and 1.6 fold in men aging 45-55 years. Another study of 79,109 female nurses and incidence of coronary heart disease (CAD) reported that there was a 51% increase in CAD incidence rates among night shift nurses (Kawachi et al., 1995). Circadian rhythm disruption is a possible explanation for this trend among shift workers, since the disruption of parameters like melatonin serum levels and inflammatory markers are directly linked to cardiovascular dysfunction (see chapter 3.3.3).

3.3.1.2 EM Waves

EM waves emitted from power supplies and mobile phones can potentially predispose to cardiovascular disease (Hakansson et al., 2003). Savitz and his colleagues (1999) analyzed the medical records of electric utility workers in the United States from 1950 to 1988 and found an association between extremely low frequency EM wave exposure and cardiovascular diseases. These workers had higher risk of developing acute myocardial infarction and sudden cardiac arrest. Also the risk increased proportionally as the total years of exposure increased (Savitz et al., 1999). In a Swedish study, the records of 27,790 subjects from the Twin Registry were analyzed. A slightly increased risk of acute myocardial infarction and sudden cardiac arrest in relation to occupational exposure to EM fields (Hakansson et al., 2003) was described.

However, there is some inconsistency in the literature as other studies did not report a significant relation between exposure to EM fields and cardiovascular disease or other hormonal parameters like blood pressure, heart rate, cortisol, norepinephrine and epinephrine (Braune et al., 2002, Djeridane et al., 2008).

The previous epidemiological links of cardiovascular diseases to night shift work and EM field exposure can be explained by the role of melatonin in the cardiovascular system. Blood pressure and heart rate increase as the serum levels of melatonin decline, while myocardial infarction incidence rate increases as melatonin declines (Dominguez-Rodriguez et al., 2010). The melatonin surge in the blood at night is essential for the cardiovascular system. Melatonin lowers serum cholesterol levels by 38% and decreases

low density lipoprotein accumulation by 42% while increasing levels of high density lipoprotein (Dominguez-Rodriguez et al., 2010). In addition, melatonin enhances the activity of nitric oxide synthase, increasing the levels of nitric oxide, which is involved in vasodilatation of blood vessels. As mentioned earlier, melatonin also has a well-described anti-oxidant and ROS scavenger role (Dominguez-Rodriguez et al., 2010). Therefore, when EM fields disrupt pineal gland function (Wilson et al., 1989) or when night shift workers are exposed to “light at night” resulting in reduced secretion, all of the previously mentioned cardiovascular functions will be affected, resulting in increased stress to this system and predisposition to cardiovascular diseases such as acute myocardial infarction and sudden cardiac arrest.

3.3.1.3 High-Fat Diet

High-fat diet causes CVD in patients who suffer from obesity and other metabolic diseases, and many of the well-understood pathways that can lead to cardiovascular disease have been extensively reviewed (Cuevas et al., 2000; Hooper et al., 2001; O’Keefe et al., 2004). As discussed in sections 2.1.5, 3.2.2 and 3.2.3, high-fat diet causes circadian disruption at least in rodent models. High-fat diet also leads to atherosclerosis, endothelial dysfunction, and hypertension (Wassmann et al., 2004; Khan et al., 2005; Samuelsson et al., 2008). These effects could result indirectly from circadian disruption caused by high-fat diet intake. In addition, high-fat diet can cause metabolic dysfunction (sections 3.2.2 and 3.2.3), which is linked to cardiovascular disease.

3.3.1.4 Metabolic Syndrome and Cardiovascular Disease

Metabolic syndrome is linked to CVD. Higher mortality rates of CVD occur in patients who suffer from metabolic syndrome (Malik et al., 2004; Wilson et al., 2005). In a population study of 3323 middle-aged subjects, metabolic syndrome accounted for one third of the developing CVD (Wilson et al., 2005). Metabolic syndrome leads to obesity, disruption of lipid homeostasis, and hyperglycemia. All these symptoms affect the cardiovascular system, causing hypertension, affecting endothelial function, and raising cholesterol levels in the blood (Grundey, 2004). This is an indirect link between circadian rhythm disruption and cardiovascular disease, since circadian disruption can predispose to metabolic syndrome.

3.3.2 Genetic Factors

The role of clock genes in the regulation of cardiovascular function has been extensively investigated. Almost all clock genes are expressed in the heart and their expression in the cardiovascular system exhibit diurnal rhythms (Rudic, 2005; Maemura et al., 2007). For instance, PER1-2 and CRY1-2 reach their highest expression rate during night, while BMAL-1 and NPAS2 peak early in the day and vascular function phenotypes are associated with circadian clock genes dysfunction (Paschos and FitzGerald, 2010). Multiple studies demonstrated a link between circadian clock genes and vascular functions. For instance, the plasminogen activator inhibitor (PAI-1) gene contains two consensus E-boxes in its promoter region, which are controlled by the heterodimers complex, BMAL-2/CLOCK. The BMAL-2/CLOCK complex binds directly to the E-box of the thrombomodulin promoter region and causes its expression to oscillate rhythmically in the vascular endothelial cells in a mouse model (Takeda et al., 2007).

The orphan nuclear receptor REVERB α is part of the circadian transcriptional machinery and it is involved in metabolic regulation pathways (Fontaine and Staels, 2007). Polymorphism in REVERB α can lead to metabolic syndrome, and therefore contribute to CVD (Fontaine and Staels, 2007). This further supports the idea that metabolic syndrome is tightly linked to CVD and circadian rhythm disruption.

3.3.2.1 Population Studies

Clock genes and angiotensin II regulate the expression of the serine protease inhibitor PAI1 as PAI1 levels in the heart are high during the night, while high levels are observed in the aorta during the day (Naito et al., 2003). A-G-4G PAI1 haplotype of the PAI1 gene was found to be associated with high risk of small vessel disease stroke in a population study of 390 subjects, 121 of them suffered from ischemic stroke due to small vessel disease (Adamski et al., 2009).

3.3.2.2 Animal Models

PER2 mutant mice express low mean arterial pressure independent of the central clock time, and have significantly lower levels of norepinephrine and epinephrine in plasma throughout the day (Viswambharan et al., 2007). PER2 mutant mice also suffered from increased vascular senescence. Further, PER2 inhibits Akt signaling pathway, explaining the high levels of Akt signaling in the cells of these mutant mice. High levels of AKT are associated with increased levels of ROS and decreased nitric oxide bioavailability in the mutant mice (Wang et al., 2008). Furthermore, CRY deficient mice have suppressed catecholamine-mediated vasoconstriction response to α -1-adrenergic receptor antagonist (Masuki et al., 2005). These were examples in which the endogenous influence of the gene expression overrides the exogenous regulation through the diurnal fluctuations of the hormonal levels in the body. A study of Nonaka and co-workers (2001) showed that angiotensin II significantly increased the expression of mPer2 in vascular smooth muscle cells in mice, and this increase was diminished after the application of CV11947, an antagonist to angiotensin type I receptor (Nonaka et al., 2001). Thus, the peripheral cardiovascular clock can be entrained through hormones.

This concludes the description of literature investigating how a disruption of the circadian rhythm changes cardiovascular function. The following chapter will highlight how disruptions to circadian rhythm can lead to coronary artery disease.

3.3.3 Coronary Artery Disease

Coronary artery disease (CAD) is a multifactorial disorder. Smoking, unhealthy diet, and lack of exercise are some environmental factors that can lead to coronary artery disease (Lloyd-Jones et al., 2009). CAD has the highest incidents of death in most of the developed countries, despite the increased awareness regarding the importance of exercise and healthy diet. According to the British Heart Foundation (2010), CAD was the first cause of death in the UK in 2008 among respiratory and circulatory diseases, and the second overall, exceeded only by cancer (British Heart Foundation, BHF, 2010). In the US, one in every five deaths is due to coronary artery disease, with a total number of 451,326 deaths in 2004 alone (Ostchega et al., 2007). Furthermore, the actual number of deaths due to CAD declined by only 18% between 1994 and 2004 (Ostchega et al., 2007). In addition, Walker and Sareli (1997) demonstrated a great difference in the number of deaths attributed to CAD in African developing countries as compared to Europe and the US. Only 0.2% of the total deaths in 1994 in Soweto, an urban area in Gauteng in South Africa, were due to CAD. This proportion increases significantly in developed countries like the US and some northern European countries where the deaths incidents caused by CAD are 20.3% and 16% respectively (Verschuren et al., 1995). In addition, developing countries currently demonstrate increased incidents of non-communicable diseases, especially in the urban regions where electricity is available and people are living a westernized lifestyle, while this observation was not true for rural areas of the same geographical and racial background (Walker and Sareli, 1997). This suggests that the modern 24-hour life style, which can potentially disrupt circadian rhythm, might be a reason among others behind the high incidents of CAD.

CAD has a significant genetic component. Subjects who were exposed to extremely low frequency EM fields for prolonged periods of time were found to have higher risk of dying from CAD if they were genetically susceptible to the disease (Hakansson et al., 2003). A study that examined 3,298 monozygotic and 5,964 dizygotic male twins, and 4,012 monozygotic and 7,730 dizygotic female twins, found that the risk of one of the twins dying from CAD - when the other sibling has died from this disease before the age of 55 -, was 8.1 fold and 3.8 fold higher, compared to the risk when none of the twins has died from CAD in monozygotic twins and dizygotic twins, respectively (Marenberg et al., 1994). This study illustrates that genetic factors influence the risk to develop CAD.

In this review, CAD is discussed from a perspective, which might have been clinically underestimated for the past years. Disruption of the circadian rhythm is a factor that contributes to the occurrence of CAD in night shift workers (Martino et al., 2007; Dominguez-Rodriguez et al., 2010). Night shift workers had 40% increased risk of CAD when compared to day workers of the same job (Tenkanen et al., 1997). Given the trend of high CAD risk among night shift workers, and the circadian disruption caused by night shift work, as well as the involvement of clock genes in regulation of the cardiovascular system, it is logical to assume a link between circadian disruption and coronary heart disease.

Circadian disruption might lead to coronary artery disease through the disruption of the melatonin diurnal cycle accompanied with circadian rhythm disruption. As mentioned in section 3.3.1.2, "light at night" suppresses melatonin levels and interferes with melatonin cycle, which is crucial in regulating cardiovascular functions, like heart rate, blood pressure, and endothelial regulation (Dominguez-Rodriguez et al., 2010). People who have elevated levels of LDL cholesterol regularly also have low levels of melatonin. This observation was supported by studies that linked melatonin to LDL oxidation, low cholesterol levels, and increased HDL levels (Wakatsuki et al., 2000; Tamura et al., 2008; Dominguez-Rodriguez et al., 2012).

The relation between CAD and LDL/HDL cholesterol is well understood. An inverse relationship between the risk of coronary artery disease and levels of HDL cholesterol was established (Castelli et al., 1986) as well as a direct relationship between the total cholesterol and LDL levels, and coronary artery disease risk (Castelli et al., 1977). Therefore, a protective effect of melatonin can be lost via circadian rhythm disruption, and thus exposing the cardiovascular system to stressors that predispose to CAD.

Inflammatory markers link circadian rhythm disruption with CAD, as night shift work is associated with high levels of these markers (Irwin et al., 2006; Khorso et al., 2011). For example, on-call physicians had high levels of high tumor necrosis factor α (TNF- α) and an overall increased diastolic blood pressure (Steptoe et al., 2009). When serum markers levels were measured after a sleepless night, high levels of monocyte production of interleukin 6 (IL-6) and TNF- α were found (Irwin et al., 2006). Additionally, IL-6, C-reactive protein, white blood cells, neutrophils, lymphocytes, platelets, and TNF- α were elevated following night shift work, compared to serum levels from day workers. However, the TNF- α increase was not significantly different according to the same study (Khorso et al., 2011).

Nonetheless, TNF- α has a role in insulin signaling, vascular wound repair, and lipid metabolism, which suggests the involvement of this inflammatory marker in cardiovascular disease (Mellick, 2007). In a population study, TNF- α polymorphisms were linked to low PAI levels. The TNF diplotype accounted for 10% of the variance in PAI-1 plasma levels, which significantly exceeds the influence of SERPINE1. SERPINE1 is the gene that codes for the PAI-1 protein, and it accounts for 2% of the variance in PAI-1 levels (Hong et al., 2007). In some experimental human studies, TNF- α was found to increase the levels of t-PA antigen

in the blood. In addition, TNF- α pre-treatment inhibited acetylcholine and nitroprusside induced vasodilation, and increased bradykinin-induced release of t-PA. Elevation of local t-PA levels might deteriorate the state of CAD patients, as it can stimulate degradation of extracellular matrix and increase plaque instability (Lowe et al., 2006). Overall, altered levels of TNF- α are associated with night shift work or gene defects and could be a potential factor contributing to the manifestation of CAD in some patients who suffer from circadian rhythm disruption.

While the link between circadian rhythm disruption and CAD is strong, future work should investigate the link between the high incidence of CAD and night shift workers. The elevation of inflammatory markers and the suppression of melatonin levels, and their effect on the cardiovascular system should be tested in order to verify whether these factors significantly contribute to the disease.

Overall, current evidence underlines the urgency to discuss the issue of shift work and health, and whether night shift workers need regular check-ups or regulations that limit the number of shifts they take per week.

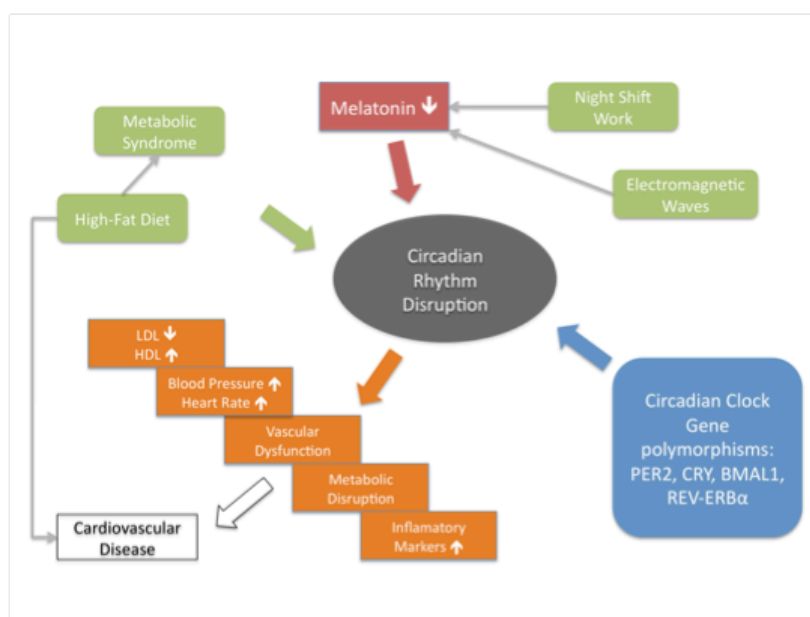


Figure 5: A Circadian disruption based model for the development of cardiovascular disease; Night shift work, exposure to light at night (LAN), and electromagnetic fields are factors that lower serum melatonin levels. Both low melatonin levels and high fat diet intake along with polymorphisms in clock genes can lead to circadian disruption. Circadian rhythm disruption can affect various cardiovascular parameters leading to high LDL, low HDL levels, high bp and heart rate, vascular dysfunction, metabolic disruption, and high levels of inflammatory markers. All these combined or separately can predispose to cardiovascular disease. In addition, high fat diet can directly lead to CVD.

4. Conclusion

An emerging public health issue is the increased incidence of metabolic syndrome, cardiovascular diseases and certain cancers like breast cancer. While there is clear evidence that a disruption of the circadian rhythm is a potential explanation for this trend, more studies are needed to fully establish this link and open innovative new avenues for prevention and therapeutic intervention. After further validation of these links the mechanism needs to be explored. Future research should consider the following issues:

Regarding night shift work, despite convincing epidemiological evidence associating night shift work with several diseases, the mechanisms through which night shift work leads to or at least predisposes to diseases is not fully understood.

To explain an elevated cancer risk, the major mechanism is disruption of hormonal rhythms (particularly melatonin) through exposure to “light at night”. But, this viewpoint is incomplete and fails to explain other pieces of epidemiological evidence such as the strong link between metabolic syndrome and night shift work. As discussed in chapter 3.2.1, microarray studies of oscillating transcriptomes of metabolically active organs showed cycling of important genes involved in several key pathways can offer a possible unifying answer to the question. Whenever the body is challenged with environmental demands at the “wrong” time (i.e. when the cellular transcriptomes are not “prepared” to deal with the external demand) stress results, and the likelihood for disease increases.

An integrated systems approach is needed to model the macroscopic physiological effects of oscillating downstream clock control genes in different tissues/organs to understand how the cycling of genes results in circadian regulation of physiological parameters. Furthermore, the physiological basis of the damage/stress caused by challenging the body at the “wrong” circadian time should be carefully investigated and the list of oscillating genes should be narrowed to those that could be important in disease development.

Another focus area is the long term effect of exposure to artificial light on circadian health. Direct evidence is missing to understand the differences, if any, between the effects of natural and artificial light on circadian rhythm. Some studies demonstrated that artificial light is less effective at circadian entrainment, which implies that artificial light could be associated with circadian disruption. However, no population-based studies are done with artificial light exposure to confirm the link between circadian health and diseases like the metabolic syndrome. While animal studies may not be as conclusive they still give some hints to understand the long-term effects of exposure to sunlight versus artificial light.

Given that circadian entrainment by light is important, possible public health implications of extremes in seasonal and latitudinal variations of day length should be investigated. It remains unsolved whether polar nights/days in regions like Norway, Sweden, Iceland or Canada increase the risk for cancers or metabolic syndrome or whether special adaptations in circadian genes are present in these populations to cope with the extreme light-dark cycle.

It also remains uncertain whether a disturbance of the circadian rhythm could contribute to the metabolic syndrome and cardiovascular disease through high fat diet intake. Recently the possibility that high-fat diets lead to circadian disruption at the cellular level was discussed in rodents where the presence of a food-entrainable oscillator has been predicted. However, more studies need to be done to explore if this applies to humans as well.

In regard to genetic influences on circadian disruption, several genes, mostly core clock genes, have been associated with diseases. These genes play a predisposing role rather than being a direct influence. Nevertheless, the effect of clock gene polymorphisms on disease is highly significant and important. For instance, CLOCK and PER3 polymorphisms are linked to metabolic syndrome and breast cancer respectively. Therefore, prognostic/diagnostic applications of these genes should be investigated. Further, these genes may not just predispose to a particular disease but also affect the disease phenotype. As discussed, the distribution of alleles of clock genes in a population may be used to estimate disease risk.

Depending on the precise role of the gene in the cellular transcription/translation feedback loop, ablation or loss of function of that gene can result in chronic circadian disruption. There

are several genes involved in this intricate molecular network (chapter 1.2) and disruption of any of these genes could potentially lead to circadian disruption. Therefore, other genes that are important in the feedback loop should be studied for disease association. Such search should not be restricted to genes involved in the cellular feedback loop i.e. not only the core clock genes but also may include genes involved in melatonin biosynthesis (AANAT) and signaling (Melatonin receptor 1a & 1b).

Again, the association studies do not prove that the reason behind elevated disease risk is loss of circadian rhythmicity per se. In fact, some of the results suggest alternative functions for core clock genes like CLOCK and BMAL-1; therefore, organ-specific knockout models with verification of loss of circadian rhythmicity should be used. A critical look at other crucial functions of core clock genes in metabolism and cell cycle control will also help to understand the possible interactions.

Some authors demonstrated gene-environment interactions in their population association studies. For instance, the link between CLOCK polymorphisms and metabolic syndrome risk was mediated through dietary intake of mono-unsaturated fatty acids. PER3 polymorphisms and its association with breast cancer depend on menopausal status to mention another example. The physiological bases of these interactions are important questions and solving them may give important answers in regard to the functions of circadian genes and circadian physiology in general. Investigations in this area may open up opportunities for therapeutic intervention i.e. modifying disease phenotype by making appropriate lifestyle change in diet for instance. These promising results will motivate epidemiologists to look for gene-environment interactions.

Until a better understanding of the mechanisms and interactions has been achieved the following thoughts might be helpful to reduce the health risks:

Increased work demands in the twenty-first resulted in the formation of a work-driven on-demand “24-hour society” in which regular sleep-wake cycles are not considered a necessity. Therefore, night shift work is a real health hazard. Clearly, the risks are underestimated and serious steps should be taken to maintain the health of workers and therefore, long-term productivity.

For instance, less frequent rotations should be considered, and to ease the transition between shift changes, at least 48 hours should be given between shift changes. Furthermore, developing a regular schedule, avoiding caffeine or physical activity before sleep, and wearing eye masks/ear plugs while sleeping should ensure good sleep quality. Night shift workers should also have regular health checks. In addition, light and melatonin therapy have also been suggested for treatment of circadian rhythm disruption and re-entrainment of central/peripheral oscillators with the external environment.

The hazards of artificial light are not limited to disturbance of entrainment. Artificial light has made light omnipresent. This factor, coupled with disrupted sleep-wake schedules leads to constant light exposure at night that can result in severe disruption to melatonin rhythms. In this regard, the use of computers, particularly at night, may be another aspect of modern life that leads to circadian disruption and should be reduced.

In conclusion, circadian disruption is caused by a variety of mechanisms. Environmental and genetic factors may help to explain the trends for some of the major multifactorial (complex) diseases plaguing contemporary society. Despite the tremendous advances in knowledge about circadian rhythms, many important questions still remain unanswered. Tackling these questions could open up new opportunities for diagnosis, prevention and therapy, contributing to the battle against non-communicable diseases in the twenty-first century.

Glossary

- AANAT - Arylalkylamine N-acetyltransferase
- AMI - acute myocardial infarction
- AP-1 - Adaptor Protein -1
- ASMT - N-acetyl-serotonin O-methyl transferase
- ATM - Ataxia telangiectasia mutated
- BMAL - Brain and Muscle Aryl hydrocarbon receptor nuclear translocator (ARNT)-like
- CAD - Coronary Artery Disease
- CCGs - Clock Controlled Genes
- CHK2 - Checkpoint kinase 2
- CK1 ϵ - Casein Kinase 1 ϵ
- CLOCK - Circadian Locomotor Output Cycles Kaput
- CML - Chronic Myeloid Leukemia
- CNS - Central Nervous System
- CpG - Cytosine - phosphate - Guanine dinucleotide
- CRY - Cryptochrome
- CSNK1 - Casein Kinase 1
- CVD - Cardiovascular Disease
- DAB2 - Disabled-2
- DBP - D site of albumin promoter (albumin D-box) binding protein
- E4BP4/NFIL3 - Nuclear factor, interleukin 3 regulated
- EM - Electro-magnetic
- ER - Estrogen Receptor
- FASPS - Familial Advanced Sleep Phase Syndrome
- HDL - High Density Lipoprotein
- HMGCoA - 3-hydroxy-3-methyl-glutaryl-CoA
- IDF - International Diabetes Foundation
- IL-6 - Interleukin - 6
- LAN - Light at Night
- LDL - Low Density Lipoprotein
- LDLRs - Low Density Lipoprotein receptors
- MCF-7 - Michigan Cancer Foundation - 7 cell line
- MEL - Melatonin
- MT1 - Melatonin Receptor subtype 1
- MT2 - Melatonin Receptor subtype 2
- MTNR1B - Melatonin Receptor 1B
- MUFA - Monounsaturated Fatty Acid
- NAFLD - Non-alcoholic Fatty Liver Diseases
- NCD - Non-communicable disease
- NHE3 - Sodium-hydrogen exchanger 3
- NPAS - Neuronal PAS domain-containing protein
- NPC1 - membrane bound Niemann Pick C1
- NSCLC - Non-Small Cell Lung Carcinoma
- PAI-1 - plasminogen activator inhibitor-1
- PDK4 - Pyruvate dehydrogenase lipoamide kinase isozyme 4, mitochondrial
- PEPCCK - Phosphoenolpyruvate carboxykinase
- PER - Period
- PFK - 2 - Phosphofructokinase-2
- POST - Parliament Office of Science and Technology
- REVERB α - rev-erbA-alpha
- ROS - Reactive Oxygen Species
- SCA - sudden cardiac arrest

- SCN – Suprachiasmatic Nucleus
- SNP – Single Nucleotide Polymorphisms
- SYNJ2 – Synaptojanin - 2
- t-PA - tissue plasminogen activator
- TNF- α – Tumor Necrosis Factor alpha
- US – United States
- VLDL – Very Low Density Lipoprotein
- WHO – World Health Organization

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