Exploration of sleep as a specific risk factor for poor cardiometabolic and mental health & the comparison of subjective and objective assessments of sleep

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Abstract

Numerous studies have attempted to evaluate the impact of sleep on cardiometabolic and mental health, although most of the population-based studies utilised self-reported sleep assessment and health status. Therefore, the main aim of this project is to explore the relationship between accelerometer measured sleep and cardiometabolic and mental health amongst the UK Biobank participants.

The UK Biobank collected extensive information of the general UK population. They have also collected accelerometry data allowing the extraction of sleep duration and quality. Disease status was obtained from their primary care record. Out of the 84,411 participants with available processable accelerometry data, 17.3% slept <5 hours/night, 25.9% slept 5-6 hours/night, 33.5% slept 6-7 hours/night, 18.7% slept 7-8 hours and 4.6% slept >8 hours/night. Short sleep duration was significantly associated with the male gender, older age, high body mass index, social deprivation and ethnic minority group ($p<0.001$). A significant 'U-shaped' association was found between sleep duration and metabolic disease status. Both short and long sleep durations were also associated with negative mood and worse cognitive performances including slower reaction time and worse visual memory ($p<0.001$). These findings showed the importance of sleep in maintaining health. However, sleep misperception was found to be common leading to a discrepancy between subjective and objective measurements of sleep.

A cross-sectional analysis was conducted on 28 human participants (11 controls and 17 patients). Sleep was assessed using a paper sleep diary, wrist-worn tri-axial accelerometer and laboratory-based polysomnography. The level of cortisol, melatonin, mitochondrial DNA damage and gene expression was measured using saliva, urine, skin swab and hair samples, respectively. An overestimation of sleep duration was observed in this study which is consistent with the UK Biobank analysis. Patients were found to have a longer sleep duration, but a lower sleep efficiency. Moreover, patients were found to have a lower level of melatonin and cortisol. A 'U-shaped' association was found between sleep duration and mitochondrial DNA damage level. Finally, circadian rhythm and mitochondria-related pathways have been identified in the gene expression analysis. However, these associations were not found to be statistically significant. Therefore, it is proposed that larger sample size should be considered in future studies informed in part by further power calculations based upon the findings presented in the current thesis.

Declaration

This thesis is submitted for the degree of Doctor of Philosophy. The research for this submission was performed in the Dermatological Sciences at the Translational and Clinical Research Institute, under the supervision of Professor Mark Birch-Machin, Professor Michael Catt, Dr Kirstie Anderson and Dr Gunn. All work is original and performed personally unless acknowledged via reference. None of the material has been submitted previously for a degree or any other qualification at this university or any other institution.

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List of Abbreviations

AASM	American Academy of Sleep Medicine
BMI	Body mass index
BMK1	ERK5/Big MAP kinase 1
cDNA	Complementary DNA
CI	Confidence interval
DLMO	Dim light melatonin onset
ECG	Electrocardiogram
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyogram
EOG	Electrooculogram
ERKs	Extracellular signal-regulated kinases
GABA	Gamma-aminobutyric acid
GP	General practice
IL-6	Interleukin-6
IPA	Ingenuity pathway analysis
JNK/SAPK	c-jun N-terminal or stress-activated protein kinases
L5 value	Activity level of the least active five hours
MAPK	Mitogen-activated protein kinase
mg	Milli-g
MITF	Melanocyte inducing transcription factor
mtDNA	Mitochondrial DNA
MTC	Multiple testing correction
NBlock	Number of episodes of movement
NPV	Negative predictive value
NREM	Non-rapid eye movement
OR	Odds ratio
OSA	Obstructive sleep apnoea
PCA	Principle component analysis
PPV	Positive predictive value
PSG	Polysomnography

qPCR	Real-time polymerase chain reaction
REM	Rapid eye movement
RLS	Restless leg syndrome
ROS	Reactive oxygen species
SD	Standard deviation
SWS	Slow-wave sleep
TNF- α	Tumour necrosis factor-alpha
TRYP	Tyrosinase-related protein
WASO	Wake after sleep onset
6-SMT	6-sulphatoxymelatonin
ΔM5L5	Difference between the most and least active five hours of the same day

Chapter 1. Introduction

1.1 Circadian rhythm

The regulation of the sleep/wake cycle is controlled by the circadian rhythm (Figure 1-1) which is the 24-hour internal clock within an organism. It has a pivotal role in controlling alertness and sleepiness depending on light changes in the external environment which allow organisms to do the appropriate thing at the right time (Reddy S, 2020, Vitaterna et al., 2001). For example, a nocturnal rodent would become easy prey for other animals if it has decided to leave its burrow in the broad daylight. In mammals, the central circadian clock can be found in the suprachiasmatic nuclei which are located in the anterior hypothalamus (Vitaterna et al., 2001). Peripheral clocks, on the other hand, are found in various organs including the heart, lungs, liver and skeletal muscles (Vitaterna et al., 2001, Yamazaki et al., 2000, Carley and Farabi, 2016). The development of the mammalian circadian system occurs postnatally, especially during the first four months of life (Rivkees, 2007, Reddy S, 2020). The circadian rhythm is essential for helping humans adapt to environmental changes such as light, temperature and food availability, as well as optimising energy expenditure. Irregular circadian rhythm due to both voluntary (such as jet lag and shift work) and involuntary (such as age and illness) circumstances can result in various physical and mental health conditions including obesity, diabetes, depression and sleep disorders (Reddy S, 2020, Vitaterna et al., 2001).

1.2 Sleep architecture

Sleep is fundamental for many functions including energy conservation, neuronal remodelling, body healing, memory consolidation and metabolic regulation (Reddy S, 2020). There are two types of sleep: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. REM sleep is especially associated with dreaming (Carley and Farabi, 2016). Switching between these two types of sleep is controlled by the monoaminergic neurons (including noradrenergic and serotonergic neurons) and the cholinergic neurons located in the brainstem (Carley and Farabi, 2016, Hobson et al., 1975). During REM sleep, cholinergic neurons are highly active while monoaminergic neurons are virtually silent (Carley and Farabi, 2016). The current clinical guidelines further divided NREM sleep into three stages: N1, N2 and N3, each with its own characteristics and electroencephalography (EEG) patterns. Under normal circumstances, descent to the deep N3 stage should happen within the first hour of sleep onset. NREM and REM sleep stages typically alternate cyclically every 60-90 minutes throughout the night (Carley and Farabi, 2016).

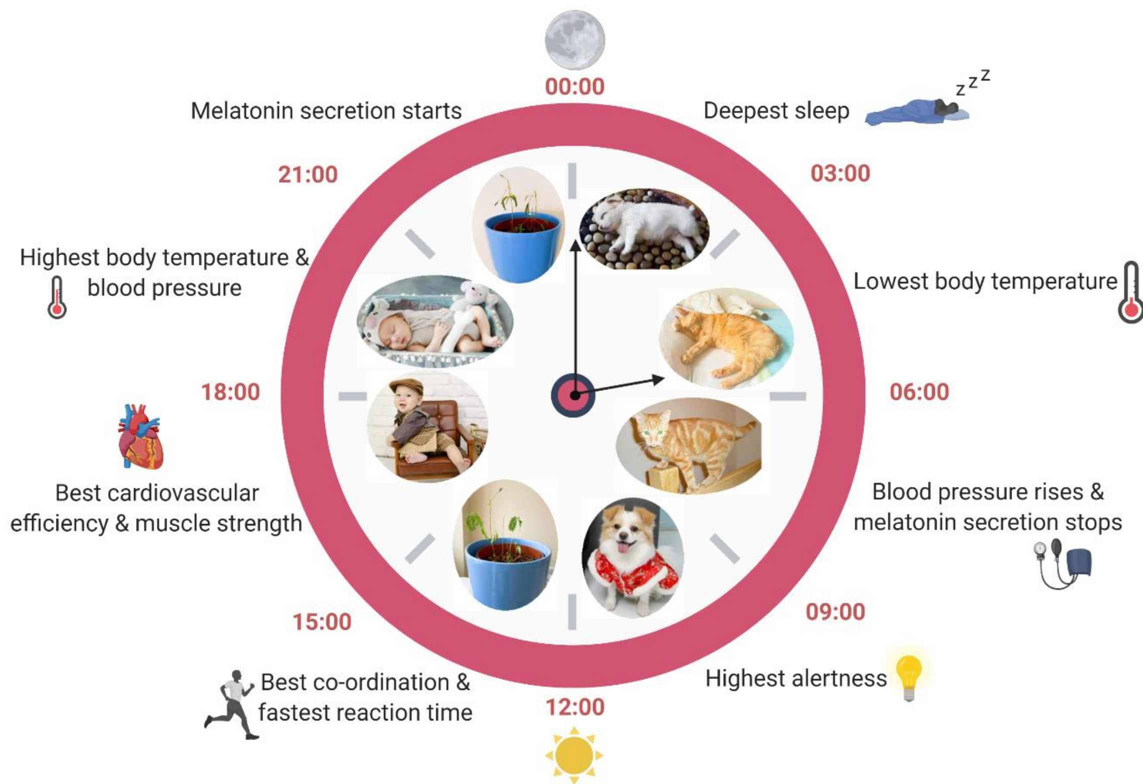


Figure 1-1. Circadian rhythm of various physiological functioning. The sleep/wake cycle is maintained by the circadian rhythm in all organisms including plants, animals and humans. Image adapted from Bjelajac et al. (Bjelajac and Djercan, 2019).

1.2.1 NREM sleep

1.2.1.1 N1 stage

The N1 stage is a transition from wake to sleep, and therefore it is the lightest stage of NREM sleep. This stage lasts approximately 5-10 minutes (Bush and Hudson, 2010). It is characterised by the presence of theta waves with a frequency of 4-7 Hz and the loss of alpha waves (Carley and Farabi, 2016).

1.2.1.2 N2 stage

The N2 stage lasts approximately 20 minutes and is characterised by bursts of waves with a frequency of 11-16 Hz and a duration of >0.5 seconds. It is also marked by the expression of K-complexes which are biphasic waves with a duration of >0.5 seconds (Carley and Farabi, 2016, Bush and Hudson, 2010). During this stage, muscles start to relax, heart rate slows down and body temperature reduces (Eugene and Masiak, 2015). The percentage of the N2 stage increases with age (Ohayon et al., 2004).

1.2.1.3 N3 stage

The N3 stage is the deepest stage of NREM sleep. A person may feel disoriented for a few minutes if woken during this stage (Eugene and Masiak, 2015). It is characterised by the presence of delta waves which are large (>75 µV) and slow with a frequency of 0.5-3 Hz (Carley and Farabi, 2016, Dijk, 2009). It is also associated with the secretion of growth hormone and the falling of thyroid-stimulating hormone (Gronfier and Brandenberger, 1998).

1.2.2 REM sleep

The REM sleep stage accounts for about 18-25% of total sleep time (Dijk, 2009). It has a characteristic of sharp theta waves or wake-like EEG patterns. It is also linked with the lowest skeletal muscle tone (Carley and Farabi, 2016). The amount of REM sleep a person experiences each day changes with age (Ohayon et al., 2004). It is the longest at birth which is about eight hours, then at 20 years of age it becomes approximately two hours, and finally, it is only about 45 minutes by the age of 70 years (Purves D, 2001).

1.3 Variation in sleep architecture

1.3.1 Age

Ageing is a complex biological process. It has a vital impact on various physiological systems and rhythmic behaviours including the circadian sleep/wake rhythm (Kondratova and Kondratov, 2012, Giebultowicz and Long, 2015). The recommended sleep duration for ≥65-year-olds by the National Sleep Foundation is 7-8 hours. However, many older adults may not be able to achieve this as increasing age is associated with a reduction in the duration, quality, depth, intensity and continuity of sleep, even in the absence of sleep disorders (Schmidt et al., 2012, Miner and Kryger, 2017). Studies have found that the total sleep time decreases by approximately ten minutes for every decade in healthy females and eight minutes per decade in healthy males. It tends to plateau after 60 years of age (Li et al., 2018, Dorffner et al., 2015, Ohayon et al., 2004). An increase in the proportion of the N1 and N2 sleep stages, but a decline in the N3 and REM sleep stages with age has also been observed (Ohayon et al., 2004, Redline et al., 2004). This may be due to reduced circadian signals that oppose the homeostatic drive for sleep (Dijk et al., 1999). The amplitude of circadian rhythmicity in melatonin and cortisol productions and endogenous core body temperature was also found to be reduced in older adults leading to a weaker circadian regulation (Schmidt et al., 2012).

The chronotype of an individual may change with age. Older adults tend to prefer an earlier bedtime and an earlier awakening in the morning compared to younger people (Carrier et al.,

1997, Duffy and Czeisler, 2002, Viola et al., 2012, Mander et al., 2017). They also commonly experience longer sleep latency, more sleep fragmentations, shorter or fewer sleep cycles and have more fragile sleep (Mander et al., 2017). It has previously been observed that people ≥65 years old often report insomnia symptoms and drowsiness. This is especially common in people with comorbidity or polypharmacy. This may be due to the fact that the secretion of melatonin can be blocked by using certain beta-blockers and therefore increase sleep fragmentations (Carley and Farabi, 2016). Additionally, the pain associated with various diseases including heart diseases, lung diseases and arthritis can also contribute to sleep disturbances observed in the elderly (Carley and Farabi, 2016). Finally, the frequency of daytime napping also increases with age which may lead to reduced sleep pressure at night-time (Carley and Farabi, 2016). While only 10% of 55–64-year-olds reported regular daytime naps, this has increased to 25% in 75–84-year-olds and >50% of naps are unplanned (Foley et al., 2007). However, it is also important to note that in a proportion of older individuals, subjective daytime sleepiness is reduced as they transit from midlife into older adulthood (Dijk et al., 2010). Therefore, the impact of age on the circadian rhythm and sleep physiology varies between individuals and this could be due to a combination of factors, for instance, gender, genetic polymorphism, lifestyle habits and co-morbid conditions (Viola et al., 2012, Schmidt et al., 2012, Mander et al., 2017).

1.3.2 Gender

The combination of environmental, cultural and social impacts contributes to gender differences which cause males and females to sleep differently (Mallampalli and Carter, 2014). Females experience unique hormonal and physical changes throughout life including menstrual cycle, pregnancy, lactation and menopause which can have a vital impact on the sleep health of females and increase their risk of sleep disorders at specific time points such as restless leg syndrome (RLS), obstructive sleep apnoea (OSA) and insomnia (Lee and Kryger, 2008). For example, with an increasing number of pregnancies, the risk of RLS increases (Berger et al., 2004), and the prevalence of insomnia is higher in postmenopausal women than premenopausal women (Mallampalli and Carter, 2014). Moreover, psychosocial factors have also played a vital role in sleep health. More midlife females have the role of caregivers compared to males and they commonly express stress which then leads to a higher risk of depression and sleep disturbances (Mallampalli and Carter, 2014).

Although, it is important to note that there appears to be a discrepancy between subjective and objective assessments of sleep. Even though females were more likely to self-report sleep complaints and insomnia (Zhang and Wing, 2006), polysomnographic evidence did not support this (Baker et al., 2007). Existing studies have found that older males tend to experience more disturbances during NREM sleep than age-matched females (Mander et al., 2017, Ohayon et al., 2004). The percentages of N1 and N2 sleep stages are also significantly higher in males. As a result, they tend to experience lighter sleep (Redline et al., 2004). On average, older males experience a 3-fold deficit in slow-wave sleep (SWS) than older females (Ohayon et al., 2004). Additionally, females recover better from sleep deprivation than males by having higher sleep efficiency and a greater percentage of the N3 sleep stage (Reynolds et al., 1986). A study involving 34 age-matched males and females has reported that on non-working free days males have significantly later bedtime and wake time than females (Santhi et al., 2016), although it is not yet clear when the gender difference in sleep emerges, some evidence suggests that the gender difference in N3 sleep emerges between 30 and 40 years old (Ehlers and Kupfer, 1997).

Understanding the difference in sleep health between males and females is critical in sleep research as it will potentially help to develop prevention strategies, better diagnosis methods and more targeted treatment for sleep problems.

1.3.3 Ethnicity

Sleep architecture is significantly affected by ethnicity. American Indians were found to have a higher percentage of the N1 and N2 sleep stages, but a lower percentage of the N3 sleep stage compared to white and black ethnicities, although REM sleep did not appear to vary between different ethnicities (Redline et al., 2004). On the contrary, a polysomnographic study reported that the black ethnicity spent more time in REM sleep, as well as more total sleep time than the white ethnicity, but the black ethnicity spent significantly less time in deep sleep (Profant et al., 2002). A similar finding was reported in other studies in which African-Americans were found to have a higher proportion of the N2 sleep stage and less SWS than European-Americans and Caucasians (Egan et al., 2017, Tomfohr et al., 2012). A cross-sectional analysis carried out amongst the UK Biobank population reported that the black ethnicity was more likely to experience inadequate sleep and have a morning chronotype compared to other ethnicities based on self-reported sleep (Malone et al., 2016). Their morning chronotype could be explained by the fact that the black ethnicity is more responsive

to morning light than the white ethnicity (Smith et al., 2009, Malone et al., 2016). Additional to the difference in habitual sleeping habits between different ethnicities, a study also reported a difference in response to jet lag and night shifts (Eastman et al., 2016). Participants had a delayed sleep/wake and meal schedule by nine hours in a controlled sleep laboratory and after three days, the European-Americans showed significantly more phase delay than the African-Americans suggesting that the circadian misalignment caused by jet lag or night-shifts will last longer in African-Americans. Consistent with the UK Biobank study mentioned earlier by Malone et al. (Malone et al., 2016) this study also found that African-Americans scored significantly higher on morningness compared to European-Americans. A possible explanation for this could be due to a greater change in seasonal photoperiod experienced by European-Americans (Eastman et al., 2016).

The exact mechanism for the racial difference in sleep architecture is not entirely clear yet. A possible explanation is their choice of residential areas. The black ethnicity is more likely to work in a job involving shift work and as a result, they are more likely to live in the inner city than in rural areas. Living in the inner city was found to be associated with shorter sleep duration as it is associated with a higher noise level and more light pollution (Hale and Do, 2007). Additionally, they are more likely to face chronic stress which is associated with dysfunction of the hypothalamic–pituitary–adrenal axis and hyperactivation of the sympathetic nervous system. This in turn could cause a decrease in SWS (Tomfohr et al., 2012, Buckley and Schatzberg, 2005).

1.3.4 Occupation and social status

Nowadays, shift work is very common as society becomes increasingly dependent on around the clock operations in many occupations such as healthcare, military and manufacturing. These shift workers are required to modify their sleep/wake pattern according to their assigned working hours which could cause circadian misalignment in those individuals. Additionally, they are chronically exposed to bright light at night-time which is the wrong biological time for it. The combination of these factors can have various detrimental effects on shift workers including metabolic disorders, cancers, cardiovascular diseases and mental illnesses (James et al., 2017, Doljansky et al., 2005, Islam et al., 2020). Apart from circadian patterns, sleep duration is also closely linked with occupations. A cross-sectional epidemiologic survey conducted amongst US residents reported that the prevalence of having a short sleep duration (<6 hours/night) was the highest amongst workers in enterprises,

followed by transportation, manufacturing and public administration (Luckhaupt et al., 2010). The link between occupation and sleep was also supported by a similar study conducted amongst the Chinese population which found that the sleep duration was the lowest for civil servants and the sleep quality was the lowest for professional workers (Sun et al., 2015). Occupation is closely related to the education level and household income, and therefore it is an important component in determining the socioeconomic status of an individual.

A considerable amount of literature has been published on the association between socioeconomic status and sleep. Lower status occupations often involve shift works which as mentioned previously, could cause disruptions to sleeping patterns. Individuals with a lower socioeconomic status were found to have a longer objectively measured sleep latency and wake after sleep onset (WASO), as well as poorer self-reported sleep quality (Mezick et al., 2008). The socioeconomically disadvantaged groups are also more likely to have sleep complaints and experience chronic insomnia symptoms (Grandner et al., 2010b, Green et al., 2014). Various environmental and emotional factors could act as a mediator for this association as individuals with lower socioeconomic status are more likely to have complaints about the noise outside, room temperature and worries regarding their health (Mezick et al., 2008). Lower socioeconomic status may also be associated with medical conditions which could have adverse effects on sleep (Marco et al., 2011). Additionally, not only adults' sleeping patterns are affected by socioeconomic status, but adolescents can also be affected. Adolescents from a low socioeconomic status household were more likely to have a shorter actigraphy-measured sleep duration and later bedtime, as well as more inconsistent sleeping patterns on weekends compared to weekdays. Possible explanations could be that they have less supervision from their parents regarding their sleep habits or parents with less education may not be aware of good sleep hygiene practices (Marco et al., 2011). However, a reversed association was reported in school children. Sleep duration was found to be shorted amongst schoolchildren from a higher socioeconomic status household. This could be due to a combination of factors such as more access to television, video games and the internet which delays their bedtime, and earlier waking times due to both parents going to work in the morning (Arman et al., 2011).

1.3.5 Lifestyle factors

1.3.5.1 Cigarette smoking

There is a large volume of published studies describing the link between sleep habits and various lifestyle factors such as alcohol consumption, smoking and physical activity. Current smokers have previously been reported to commonly experience longer sleep latency, shorter sleep duration, lower sleep efficiency and lighter sleep as indicated by a higher percentage of N1 and N2 sleep stages and lower SWS (Lauderdale et al., 2006, Zhang et al., 2006, Kaneita et al., 2005, Riedel et al., 2004).

Possible mechanisms proposed include the direct activating effect of nicotine on nicotine-acetylcholine receptors leading to the release of neurotransmitters that adversely affect the central nervous system. This in turn affects the sleep/wake cycle (Kaneita et al., 2005). It may also be due to the indirect effects of nicotine on physical health. For example, adverse consequences of smoking include medical conditions such as respiratory diseases which may affect the sleep architecture (Zhang et al., 2006, Kaneita et al., 2005). It is possible that the interaction between smoking and other lifestyle factors contributed to disturbed sleep. Heavy smokers were also found to have higher caffeine intake compared to light smokers and non-smokers (Riedel et al., 2004, Phillips and Danner, 1995).

1.3.5.2 Caffeine

Caffeine is a psychoactive substance found in many dietary sources including coffee, tea and cocoa. Coffee is widely consumed by many people to combat sleepiness and restore alertness (Landolt et al., 2004). Adenosine is a neurotransmitter that promotes sleep by inhibiting the cholinergic neurons in the basal forebrain. The primary mode of action of caffeine is adenosine receptor blockade and therefore, blocks its sleep-promoting effect and increases wakefulness (Roehrs and Roth, 2008). A polysomnographic study found that consuming one cup of regular coffee 30 minutes prior to bedtime has very little effect on sleep measurements, while two cups of coffee are associated with longer sleep latency, shorter sleep duration, lower sleep efficiency and less SWS in healthy young adults (Karacan et al., 1976). This is supported by the findings of Okuma et al. which reported the observation of longer sleep latency, shorter sleep duration and reduced REM sleep following caffeine administration 30 minutes prior to bedtime. Overall, it was found that the effect of caffeine consumption before sleep mimics the symptoms of insomnia (Okuma et al., 1982).

1.3.5.3 Alcohol

Alcohol is known to have a sedative effect that induces sleepiness after consumption. However, it appears that alcohol drinkers have lower sleep quality than non-drinkers as they have shorter sleep duration, increased REM sleep and more fragmented sleep (Kaneita et al., 2005). According to polysomnography (PSG) measurements, following alcohol consumption in the evening, participants experienced shorter sleep latency, lower sleep efficiency, more awakening episodes and more slow-wave sleep. Additionally, they observed a gender difference in the response which showed that females were affected more than males. However, contrary to the previous study mentioned, this study found that alcohol reduced REM sleep (Arnedt et al., 2011). A reduced level of REM sleep was also reported in a study assessing the impact of heavy drinking (Rohsenow et al., 2010). The effect of alcohol consumption could continue into the next day when people experienced increased next-day sleepiness and worse performance in the sustained attention and speed test. This could have serious results such as road accidents or accidents at work for people in safety-sensitive occupations (Rohsenow et al., 2010).

A possible mechanism explaining the acute effect of alcohol on sleep is through its effect on the gamma-aminobutyric acid (GABA). Alcohol consumption causes the release of GABA in the brainstem and spinal cord, as well as enhances the function of $GABA_A$ receptors leading to a decreased level of REM sleep. However, the effect of chronic alcoholism showed the opposite effect. The function of $GABA_A$ receptors is reduced in alcohol dependence leading to an increase in REM sleep (Colrain et al., 2014). This could be the reason why in the first study mentioned in this section, alcohol drinkers showed more REM sleep than non-drinkers.

1.3.5.4 Physical activity

The well-known function of sleep includes energy conservation and restoration. Therefore, it is not a surprise that the need for sleep increases after exercise. The beneficial effects of physical activity on health are widely accepted. Its positive impact on sleep has previously been published in an epidemiology survey where participants reported that after light or moderate exercise in the early evening, they are falling asleep more easily, experiencing deeper sleep and higher alertness in the following morning (Vuori et al., 1988). Total sleep time and SWS are also increased after exercise (Kubitz et al., 1996). Apart from these acute effects of exercise on sleep, the effect of chronic exercise on sleep has also been documented. Athletes were found to have more SWS, longer sleep duration and shorter sleep latency, even

when they are not training (Paxton et al., 1983). Physical exercise has also been found to improve sleep quality and depression measures amongst elderly adults with depression (Singh et al., 1997).

A potential mechanism linking exercise and sleep is through the cytokine response. Strenuous exercise leads to an elevated level of inflammatory cytokines which is associated with sleep onset and SWS as the peripheral cytokine levels could directly modulate the central nervous system or indirectly via the vagal nerves (Dickstein and Moldofsky, 1999). However, the association between sleep and physical activity may be bi-directional. It is possible that those who had enough sleep have more energy to exercise compared to those who did not get enough sleep.

1.4 Relationship between sleep and cardiometabolic health

1.4.1 Weight gain and obesity

Numerous studies have attempted to evaluate the impact of short sleep duration on cardiometabolic health. A meta-analysis involving 45 cross-sectional epidemiological studies from around the world has shown that adults with short sleep duration (<5 hours/night) have 55% higher odds for obesity, while children with short sleep duration (<10 hours/night) have 89% higher odds. Reducing the sleep duration by only one hour per day was associated with a 0.35 kg/m^2 increase in body mass index (BMI) in adults (Cappuccio et al., 2008). This is consistent with a 16-years longitudinal study conducted on 68,183 women which demonstrated that having a self-reported habitual short sleep duration (<5 hours/night) leads to a 1.14 kg weight gain and they have 15% higher odds for developing incident obesity compared to those who have a habitual sleep duration of 7-8 hours/night (Patel et al., 2006). Apart from habitual sleep deprivation, acute partial sleep restriction also showed similar results. Energy intake, body weight and leptin all showed an increase after a reduction of self-reported habitual sleep duration from >8 hours/night to 4 hours/night in 14 healthy women (Bosy-Westphal et al., 2008). This is consistent with the findings of a 6-years longitudinal study conducted in 276 adults aged 21-64 years. This study not only reported an increased risk of weight gain, higher abdominal circumference and obesity amongst subjectively measured short sleepers (5-6 hours/night), but they have also found that longer sleepers (9-10 hours/night) have a similar risk for them as well. The significance of these findings remained even after adjustment for energy intake and physical activity level (Chaput et al., 2008).

However, it is important to note that the impact of sleep duration on BMI may not be consistent throughout life. Amongst younger adults, a negative association was observed between sleep duration and BMI, whereas in middle-aged adults, a 'U-shaped' relationship was observed and finally, no obvious relationship was observed amongst the elderly adults (Grandner et al., 2015b). A longitudinal study carried out on the Japanese adult population also reported a gender effect of sleep on the risk of obesity. They have found that males (n= 31,477) with a self-reported sleep duration of <6 hours/night had a higher risk of weight gain and obesity compared to those who slept 7-8 hours/night. However, no significant association was found for females (n= 3,770) (Watanabe et al., 2010). It is also important to note that there are some contradicting results reported by cross-sectional and longitudinal studies. A cross-sectional analysis (n= 5,021) carried out using the Whitehall II study found that ≤5 hours of self-reported sleep per night is associated with significantly higher BMI and waist circumference, as well as a higher risk for obesity compared to a sleep duration of 7 hours/night. However, no such relationship was observed in their prospective study conducted in 6,592 participants (Stranges et al., 2008a).

A potential mechanism explaining the association between short sleep and obesity is through the change in the level of the hunger-suppressing hormone leptin and hunger-promoting hormone ghrelin. It is thought that chronic partial sleep deprivation leads to increased activation of the sympathetic nervous system causing inhibition of leptin release (Spiegel et al., 2004a). When sleep is restricted in healthy young participants, an increase in ghrelin level and a decrease in leptin level were observed which increased hunger and stimulates their appetite for calorie-dense foods (Spiegel et al., 2004b, Spiegel et al., 2004a). A negative association has also been found between sleep duration and the intake of fats (Grandner et al., 2010a). Additionally, it is possible that by having a shorter sleep duration, people have more opportunities for food intake which eventually leads to weight gain and obesity. Another possible explanation is that sleep-deprived individuals are more likely to experience fatigue which leads to a reduction in physical activity levels (Patel et al., 2006). Therefore, the association observed between sleep duration and obesity could be explained by a change in both energy intake and energy expenditure. Moreover, it is also possible that sleep deprivation is the result of other health problems caused by obesity. The relationship between sleep and obesity could be bi-directional.

1.4.2 Type 2 diabetes

The dramatic increase in obesity and type 2 diabetes incidents seems to have occurred around the same time as the progressive decrease in sleep duration. Many prior studies have evaluated the role of sleep in the regulation of glucose and insulin. During the first half of the nocturnal sleep period, there is an increase in glucose level due to reduced glucose utilization by the brain during SWS and decreased peripheral uptake by the muscles. Glucose tolerance and insulin sensitivity improve during the latter part of the night when glucose utilization increases during REM sleep. The difference in glucose uptake between the early and late part of the night means that the effect of partial and total sleep deprivation on the risk of type 2 diabetes will be different (Spiegel et al., 2005).

To date, many studies have revealed a correlation between sleep and type 2 diabetes. A systematic review involving 13 independent cohort studies has shown that both short (≤6 hours/night) and long (>8 hours/night) sleep durations are associated with a higher risk of type 2 diabetes. The effect appears to be large in males compared to females. Additionally, not only sleep duration has an impact on it, but sleep quality also plays an important part. Those who experience difficulties initiating and maintaining sleep also have higher odds of diabetes (Cappuccio et al., 2010). A similar finding was reported in the Sleep Heart Healthy Study conducted on 1,486 participants which reported higher odds for impaired glucose tolerance and type 2 diabetes in those who self-reported a sleep duration of <6 hours and >9 hours per night compared to 7-8 hours per night (Gottlieb et al., 2005). This is consistent with other studies in this area which showed that short sleep duration is associated with significantly higher odds of diabetes (Altman et al., 2012, Holliday et al., 2013, Ayas et al., 2003b). A prospective study involving 6,599middle-aged Swedish males revealed a significant association between self-reported difficulties initiating sleep or frequent use of hypnotics and the incidence of diabetes almost 15 years later. This association remained significant even after controlling for common confounders such as age, lifestyle, family history and social class (Nilsson et al., 2004). This association was not found amongst the German population as reported in a 7.5-year prospective study by Meisinger et al. However, they have found that after multivariable adjustment, males (n= 4,140) who frequently experienced difficulties maintaining sleep at baseline have a 60% higher risk of type 2 diabetes and females (n= 4,129) with experience of difficulties maintaining sleep have 98% higher risk (Meisinger et al., 2005).

There are a few potential causative mechanisms explaining the relationship observed between sleep and the risk of type 2 diabetes. Acute and chronic sleep deprivation may increase the risk of type 2 diabetes by directly increasing insulin resistance and glucose intolerance or indirectly by stimulating appetite through the change in leptin and ghrelin levels as mentioned in the previous section. This could promote weight gain leading to insulin resistance and type 2 diabetes (Spiegel et al., 2005). Another potential mechanism linking sleep deprivation and type 2 diabetes is via the inflammatory pathway. Partial sleep deprivation (4 hours/night) was found to be associated with increased production of proinflammatory cytokines such as interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α). This was observed after even just a single night (Irwin et al., 2006). There is evidence suggesting a strong positive association between inflammation and insulin resistance (Festa et al., 2000, Vgontzas et al., 2004).

1.4.3 Cardiovascular diseases

As mentioned previously, a significant association between sleep duration and the sympathetic nervous system have been reported by several studies. Blood pressure varies depending on the autonomic nervous system activity. When the sleep duration of young adults was reduced from eight hours to 3.6 hours, an increase in the sympathetic nervous system and blood pressure was observed (Tochikubo et al., 1996). Therefore, it is not a surprise that sleep duration plays an important role in cardiovascular health.

A meta-analysis conducted by Cappuccio et al. which included 15 studies showed that both self-reported short and long sleep durations are related to a greater risk of stroke and coronary heart disease compared to a sleep duration of 7-8 hours/night amongst different populations including Europe, the USA and East Asia. However, when looking at the total cardiovascular disease, the significant association only remained amongst people with a longer sleep duration (Cappuccio et al., 2011). This is consistent with other studies in this area. Both subjectively measured short (<5 hours/night) and long (≥10 hours/night) sleep durations were associated with a higher risk of myocardial infarction and stroke in 30,934 participants (Altman et al., 2012). A US prospective study conducted on 71,617 participants found that those who self-reported a sleep duration of <5 hours/night have a 45% higher risk of coronary heart disease 10 years later, while >9 hours/night is associated with a 38% higher risk (Ayas et al., 2003b). Apart from sleep duration, sleep quality also appears to have a major impact on coronary heart disease risk. People (n= 10,308) who reported sleep disturbances had a

significantly higher risk of coronary heart disease, while those who did not report restless or disturbed nights did not show such an association, despite having a short sleep duration (Chandola et al., 2010). Moreover, insomnia symptoms were also associated with an increased risk of acute myocardial infarction. People (n= 52,610) who have complained about having difficulties initiating and maintaining sleep almost every night had a 45% and 30% higher risk of acute myocardial infarction, respectively (Laugsand et al., 2011). The risk of ischaemic heart disease mortality was also higher amongst those who self-reported sleep duration of <6 hours/night compared to 6-7 hours/night (n= 5,249). However, this association only existed in those using hypnotics. In those who have never used hypnotics, no association between sleep and ischaemic heart disease was observed (Garde et al., 2013).

The relationship between short sleep duration and cardiovascular health may partly be explained by an overactivity of the sympathetic nervous system and increased blood pressure. On the other hand, the association observed between long sleep duration and cardiovascular health may be mediated by the presence of other co-morbidities such as sleep apnoea (Ayas et al., 2003b). Sleep apnoea patients commonly experience fragmented sleep which increased their need for sleep. Those with sleep-disordered breathing were found to have higher odds of cardiovascular diseases including stroke, coronary heart disease and heart failure (Shahar et al., 2001). There are, however, other possible explanations. The change in leptin and ghrelin levels caused by sleep restriction leads to an increased appetite. This in turn facilitates the development of impaired glucose tolerance and obesity which increases the cardiovascular risk (Cappuccio et al., 2011). The possible interference of lifestyle habits and social class cannot be ruled out. The Whitehall II study has found that both long and short sleepers were more likely to have lower socioeconomic status, be a current smoker and have a lower physical activity level. The combination of these factors could increase their risk of cardiovascular diseases, as well as mortality (Stranges et al., 2008b). Similar findings were also reported in a US population-based study (Krueger and Friedman, 2009).

1.5 Relationship between sleep and mental health

1.5.1 Cognitive function

Many studies have tried to investigate whether there is an ideal amount of sleep needed to achieve the best cognitive performance. A cross-sectional study carried out 12 well-established cognitive tests on >10,000 participants observed a “U-shaped” curve between cognitive function and sleep duration. They have illustrated that participants who sleep both

less than and more than 7-8 hours/night performed worse in those cognitive tests regardless of age. Even though sleep duration tends to go down with increasing age. Participants who have slept more than usual on the night prior to the cognitive tests had better performance. Participants with extreme short sleep duration (<4 hours/night) added approximately eight years to their age. Sleep was measured subjectively in this study (Wild et al., 2018). A cross-sectional analysis carried out by Kyle et al. using data from the UK Biobank showed that individuals with a self-reported sleep duration of <7 hours/night or >9 hours/night were more likely to have cognitive impairments (n= 477,529). They also had poorer performance on the reasoning test, slower reaction time and worse numeric, visual and prospective memory. Surprisingly, they have found that people with frequent insomnia symptoms performed slightly better in cognitive tests, but the effect was small, and therefore unlikely to be clinically meaningful (Kyle et al., 2017b). Similar results were also observed in other longitudinal studies (Scullin and Bliwise, 2015). Self-reported short and fragmented sleep was associated with cognitive complaints 22 (Virta et al., 2013) and 28 (Kulmala et al., 2013) years later (n= 2,336 and 2,994, respectively). The Whitehall study has also reported that any adverse changes in sleep duration, for example, decreasing the subjectively measured sleep duration from 6-8 hours/night or increasing it from 7-8 hours/night were associated with a significant reduction in the cognitive function in five years' time amongst 1,459 females and 3,972 males (Ferrie et al., 2011). A meta-analysis investigating the relationship between sleep duration and cognitive disorders found that the risk of developing cognitive disorders is significantly higher in those who sleep <4 hours or >10 hours per night (Xu et al., 2020). Poor sleep such as longer sleep latency (Branger et al., 2015) and acute sleep deprivation (Lucey et al., 2018), and excessive daytime sleepiness (Carvalho et al., 2018) were all found to be associated with increasing amyloid deposition in non-demented individuals which is a biomarker for Alzheimer's disease. Prolonged subjectively assessed sleep duration (>9 hours/night), on the other hand, was also found to be a predictor of neurodegeneration in a 10-years follow-up study and is associated with an increased risk of all-cause dementia and Alzheimer's disease (n= 2,457) (Westwood et al., 2017). A similar result was reported in the meta-analysis carried out by Xu et al. A 'U-shaped' relationship was found between subjectively determined total daily sleep duration and the risk of cognitive disorders (Xu et al., 2020). Simple reaction time was also found to be reduced in 12 sleep-deprived individuals (Choo et al., 2005) and their performance in working memory

tasks deteriorated significantly after 24 hours of acute total sleep deprivation (Chee and Choo, 2004, Wimmer et al., 1992).

The contribution of age to the effect of sleep on cognitive function remains controversial. Some studies suggested that the effect of sleep deprivation on cognitive function is often underestimated in younger individuals while the opposite happens in older individuals. A cross-sectional study looking at reaction time has found that after 24 hours of sleep deprivation, reaction time only deteriorated in ten younger individuals, but has no significant effect in the other ten older people (Philip et al., 2004). Another study involving 60 15-19 years old students from a high performing high school found that 7-nights of partial sleep restriction measured by wrist-worn actigraphy (5 hours/night) increased their subjective sleepiness and reduced their attention, working memory, executive function and positive mood. The cognitive performance of the sleep restriction group was worse than the control group even after two nights of recovery sleep (Lo et al., 2016). However, some studies did not find a significant association between cognitive dysfunction and subjectively measured sleep in 235 young adults (Zavecz et al., 2020).

Various studies have investigated the effect of both chronic partial and acute total sleep restriction on cognitive function. Participants in both sleep restriction groups performed worse than the control group in psychomotor vigilance and digit symbols tasks. It was found that the effect of four hours of partial sleep restriction for 14 days had a similar effect as two nights of total sleep restriction and six hours of partial sleep restriction for 14 days is equivalent to one night of total sleep restriction (n= 48) (Van Dongen et al., 2003). Acute total sleep restriction is known to have a significant deleterious effect on most cognitive domains including attention, memory and processing speed (Lim and Dinges, 2010). In 1959, Peter Tripp stayed awake for 201 hours in order to raise money for charity. He coped well on the first two days and from the third day he started to have visual hallucinations and it worsened with time. During the last few days, he started to develop mental disorders and cognitive impairment. He also developed auditory hallucinations, his speech became slurred and could not recite the alphabet. This provided evidence supporting that sleep is essential for the normal functioning of the body and mind (A Rolls, 2010). However, it is important to note that older individuals were found not to be affected as much as younger individuals under the effect of sleep restriction (Philip et al., 2004).

Speed and accuracy are two main aspects measured for working memory and attention and they were found to be adversely affected by sleep deprivation (Choo et al., 2005, Chee and Choo, 2004, Smith et al., 2002). Additionally, the speed and accuracy of psychomotor vigilance tasks deteriorate linearly with a decrease in sleep duration (n= 66) (Belenky et al., 2003). Due to the fact that iconic memory has a short duration and limited capacity, visual tasks are particularly vulnerable to sleep deprivation. A reduction in accuracy and slower reaction time was observed following a night of total sleep restriction (n= 11) (Raidy and Scharff, 2005). Studies were carried out to investigate the impact of sleep on decision making and they found that acute total sleep deprivation has a significant impact on an individual's flexible decision making and innovative thinking skills. A study conducted on 10 healthy participants found that sleep-deprived individuals often find it difficult to utilise new information in solving complex innovative decision-making tasks as sleep loss increases rigid thinking (Harrison and Horne, 1999). This becomes more pronounced as age increases (Killgore et al., 2006). Additionally, a study involving logic reasoning tasks showed that people's mental processing speed reduces under the effect of acute total sleep deprivation (Monk and Carrier, 1997).

The association between sleep duration and cognitive function might be explained by frontotemporal grey matter atrophy. A longitudinal study conducted amongst middle-aged and elderly adults found that both short and long sleep durations are associated with thinning of the frontotemporal region (Spira et al., 2016). Grey matter deficit has also been previously reported in people with insomnia symptoms including difficulties initiating and maintaining sleep (Joo et al., 2013). Other neurodegeneration biomarkers related to sleep are β-amyloid and tau. A study conducted in middle-aged and elderly adults showed a greater β-amyloid burden amongst short sleepers (<7 hours/night) compared to people who sleep >7 hours/night and it is also higher in those with lower sleep quality (Spira et al., 2013). Tau is also elevated by approximately 50% in sleep-deprived individuals (Holth et al., 2019). Both biomarkers are related to the neurodegeneration observed in Alzheimer's' disease. Moreover, the relationship between metabolic health and various inflammatory biomarkers such as IL-6 and TNF-α has been discussed in previous sections. The association between these biomarkers and cognitive decline has also been investigated which showed that individuals with systematic inflammation have poorer cognitive performances including processing speed, spatial reasoning, short-term memory and executive function (Lin et al., 2018, Charlton et al.,

2018, Marsland et al., 2015). However, it is also possible that short or long sleep durations are a symptom of neurodegenerative disorders, rather than a cause for them.

1.5.2 Mood

Sleep is not only important for the normal functioning of the human body and health. It is also crucial for the well-being of an individual. Mood deficits are increased by 55% in individuals with less sleep (Short et al., 2020). Sleep is commonly disrupted in depressed individuals. Numerous studies attempted to explore the relationship between sleep and mood. In perimenopausal women, a higher depression score was found to be associated with a higher insomnia score, a lower sleep efficiency and a shorter sleep duration objectively measured by PSG. In younger women, those experiencing depressive emotional symptoms had more SWS and REM awakenings. Finally, in postmenopausal women, guilty feelings were linked with longer SWS latency, lower sleep efficiency and shorter sleep duration (Toffol et al., 2014). The percentage of REM sleep was also found to be elevated in depressed individuals (Orff et al., 2012). Chronic sleep deprivation is very common amongst undergraduate students. a cross-sectional study found that shorter self-reported sleep duration (<7 hours/night) and lower sleep quality were both associated with a higher prevalence of depressive symptoms in 9,515 university students (Li et al., 2020). This is in agreement with the findings of the Japanese student population. They reported a significant association between a bedtime later than 1.30 am, sleep latency ≥30 minutes and poor self-assessed sleep quality and the prevalence of depressive symptoms. Additionally, those with poorer sleep quality were more likely to have suicidal ideation (Supartini et al., 2016). Depression alone added a lot of burden on an individual, but poor sleep further contributes to this burden leading to worse consequences. A study investigated the additive impact of chronic partial sleep deprivation and depressive symptoms on physical and cognitive performances in 287 undergraduate students. They reported that those who have depressive symptoms and sleep deprivation have greater anxiety, poorer cognitive performance and worse physical functioning compared to those with depressive symptoms but not sleep deprived. However, the limitation of this study is that all measurements including sleep were assessed using subjective questionnaires (Nyer et al., 2013).

There are several potential mechanisms linking poor sleep and negative mood. Poor sleep is associated with melatonin dysregulation. Phase shifts in dim light melatonin onset (DLMO) were linked with the depressive mood in new mothers which potentially contributes to the

development of postpartum mood disorders (Sharkey et al., 2013). This is consistent with prior findings in non-perinatal women which showed a positive relationship between the severity of depression and circadian misalignment (Emens et al., 2009). Another possible pathway linking sleep and depression is through the activation of the inflammatory response system. Systemic inflammation is common in sleep-deprived individuals and it was found that the level of proinflammatory cytokines including IL-6 and TNF- α are also elevated in depressed individuals (Dowlati et al., 2010). Lastly, good sleep commonly co-exists with other healthy lifestyles which contributes to better physical and mental health.

1.6 Objective vs Subjective assessments of sleep

1.6.1 Different methods of sleep assessment

As mentioned in the previous sections, sleep is fundamental for both physical and mental health. Poor sleep can be a predictor of future diseases. Therefore, the assessment of sleep is an essential component of health checks. Over the years, various methods of sleep assessment have been developed and new technologies are constantly emerging. Most of these home detection methods give binary results in which the states are classified either as wake or sleep. Some more sophisticated methods can give ternary results in which the states are classified as wake, NREM sleep or REM sleep. Finally, the gold-standard laboratory-based PSG are able to classify states as wake, N1, N2, N3 and REM sleep (Ibáñez et al., 2018a).

1.6.1.1 Sleep diaries

Subjective/self-reported sleep is commonly assessed using sleep diaries. The recording typically lasts one or two weeks which an individual provides information on their wake time activities such as exercise, naps, caffeine intake and alcohol intake, as well as sleep pattern each day including their sleep duration and the number of awakenings (Ibáñez et al., 2018a, Ibáñez et al., 2018b). Some sleep diaries may also contain additional information, for example, the cause of waking up, level of stress before bed, recall of dreams and their perception of sleep quality (Ibáñez et al., 2018b). The Pittsburgh Sleep Diary is the oldest documented sleep diary. It contains separate components which need to be completed at bedtime and waketime. It has been validated in healthy controls and sleep disorder patients to show its sensitivity at detecting sleep duration and quality (Monk et al., 1994, Ibáñez et al., 2018b).

Traditionally, sleep diaries are in paper format, but electronic sleep diaries are emerging which has its unique benefits. Scoring is automated and it avoids the inaccuracy caused by participants retrospectively completing several days of recording at the same time from

memory (Tonetti et al., 2016). The main electronic sleep diary available on Google Play include Sleep diary pro (developer: Froggyware, average review: 4.2 out of 5), Healthy sleep diary (developer: Fruct, average review: 3.9 out of 5) and Sleep diary lite (developer: Froggyware, average review: 3.8 out of 5) (Ibáñez et al., 2018b).

The main advantage of sleep diaries is that it allows relative long-term sleep monitoring in their natural sleeping environment compared to more complicated sleep assessment such as laboratory-based PSG. Moreover, it is easy to use and only requires the participant to spend a few minutes each day to complete it. However, sleep diaries also have their limitations. It is subjective and therefore can be unreliable due to sleep misperception which will be discussed in later sections.

1.6.1.2 Wrist-worn research actigraphy

To overcome the limitation of subjective assessments of sleep, wrist-worn actigraphy is the most popular choice. It consists of a two or three-axis accelerometer that measures the movement of the wearer and then converts this activity metric to estimate sleep and wake patterns using various algorithms (Walsh et al., 2017). Various wrist-worn accelerometers have been utilised in large-scale epidemiological studies and validated against sleep diary and PSG recordings. The Axivity AX3 tri-axial wrist-worn accelerometer was utilised by the UK Biobank which provided valuable data on the physical activity level and sleep/wake pattern of their participants (Nikbakhtian et al., 2021, Doherty et al., 2017). Another commonly used wrist-worn accelerometer is the GENEActiv which was used by the Whitehall II study (van Hees et al., 2018).

Its main advantage is that it’s easy to use and once charged, it can take a few weeks of measurements allowing a continuous monitor of sleep/wake pattern while the wearer is in their natural sleeping environment. Furthermore, it is an objective measurement of sleep and therefore it is more reliable than subjective assessments of sleep. However, one of the limitations of wrist-worn actigraphy is that it cannot distinguish between sleep and quiescent wake. As a result, it cannot detect sleep latency. It often overestimates sleep and underestimates wake leading to an overestimation of sleep duration and efficiency (Paquet et al., 2007). Additionally, it cannot be used to stage sleep.

1.6.1.3 *Sleep tracking mat*

There are several under-mattress bed sensors available on the market, for example, the sleep tracking mat manufactured by Withings. Withings mat's market price in 2021 is $99.95 and it proposed to offer the detection of sleep stages (light, deep and REM sleep), heart rate and snoring (Withings). The under-mattress bed sensor utilises a ballistocardiography-based monitoring system which allows the detection of heart rate, breathing rate and musculoskeletal movement and it has been validated against actigraphy and PSG (Mack et al., 2009, Da Woon et al., 2014). The use of cardiorespiratory and movement signals in sleep staging (light, deep and REM sleep) has been tested against PSG in a healthy population giving an accuracy of 69% (Willemen et al., 2014). Pressure-sensitive bed sheets have also been compared against PSG and it has achieved a precision of 70.3% (Samy et al., 2014). The pressure-sensing mat typically consists of a grid of fibre optic pressure sensors and the relative pressure is calculated by the amount of light passing between the emitter and the receiver. The movement caused by respiration and heart rate can be observed as a small cyclic variation in pressure (Walsh et al., 2017).

The advantage of a sleep tracking mat is that the user does not need to wear anything, so it does not cause any discomfort that may lead to sleep disturbances. It is available for domestic usage, raising the possibility for long-term sleep/wake monitoring in users' natural sleeping environments. This is especially beneficial to vulnerable groups such as children, elderly adults and those with cognitive impairment as it allows minimal change to their sleeping environment. However, as this method is relying on breathing and movement patterns to measure sleep, its accuracy is likely to be reduced amongst people with certain conditions involving abnormal breathing and movement such as sleep apnoea and RLS.

1.6.1.4 *Sleep tracking ring*

Another smart sleep tracker is the ŌURA ring which is a multisensory device claiming to be able to distinguish sleep stages (light, deep and REM sleep) and detect naps. It also monitors the heart rate and body temperature throughout the day and night (ring). The ŌURA ring consists of a tri-axial accelerometer, gyroscope, two infrared LED photoplethysmographs and a temperature sensor (Chee et al., 2021). Several studies have validated the ŌURA ring against PSG and research actigraphy which demonstrated its sensitivity at capturing sleep patterns, although it was found that the ŌURA ring significantly underestimates deep sleep and REM sleep, while significantly overestimating WASO, but it is consistent with measurements taken

by the research actigraphy (de Zambotti et al., 2019, Chee et al., 2021, Asgari Mehrabadi et al., 2020). It is important to note that a study found that the discrepancies in sleep staging were greater when the wearer chose to wear it on their ring finger. This is possibly due to different blood supplies giving different optical sensor outputs (de Zambotti et al., 2019).

The ŌURA ring is small in size which means that it causes a minimal disturbance during sleep. It also allows long-term domestic sleep monitoring while the wearer sleeps in their familiar environment. However, there is still room for improvement regarding the sleep staging algorithm adopted by the ŌURA ring.

1.6.1.5 Polysomnography

PSG is a medical procedure commonly performed in a sleep laboratory that involves several measurements. Sleep stages are identified by brainwave activity measured by electroencephalogram (EEG) and eye moments are recorded by electrooculogram (EOG) which is important for staging REM sleep. The differentiation of REM sleep from wakefulness is based on muscle activity recorded by chin electromyogram (EMG), while periodic limb movement is identified by limb EMG. Other measurements include an electrocardiogram (ECG) which monitors the heart rate, pulse oximetry which measures the oxygen saturation and a nasal cannula which detects the airflow. Additionally, microphones and video cameras are placed in the room to record sounds and movements (Ibáñez et al., 2018a).

The main advantage of PSG is its accuracy in detecting and staging sleep. However, it also has some disadvantages. Individuals undertaking PSG will need to sleep in an unfamiliar environment which could induce stress and affect their sleep. Additionally, the sleep laboratory may not be as quiet as their home and with the numerous wires connect to their body, it may lead to sleep disturbances throughout the night. Finally, laboratory-based polysomnography is quite time-consuming and expensive as the set-up is completed carried out by specialist sleep physiologists. Therefore, this method is typically carried out for only one night and it cannot be used for long term sleep monitoring.

1.6.2 Sleep misperception

Sleep duration is known to be a predictor of the development of cardiometabolic disorders. Most of the population studies utilitied self-reported sleep. Moreover, the diagnosis of some sleep disorders such as insomnia is commonly based on subjective discomfort caused by difficulties initiating and maintaining sleep. However, it has been found that subjective and

objective measures of sleep quantity and quality do not always agree with each other. The discrepancy between subjective and objective assessments of sleep is known as sleep misperception (Park et al., 2020).

Poor correlation between sleep questionnaires and actigraphy has been previously reported (Girschik et al., 2012). This is consistent with other studies in this area. A study comparing recordings of single-channel EEG, actigraphy and sleep diary found that the difference between the two objective measurements did not differ from each other significantly. However, both methods were significantly different from the sleep diary in terms of total sleep time and sleep efficiency, while no clinically significant difference was found regarding sleep latency and WASO (Chou et al., 2020). Sleep durations reported by retrospective sleep diary and sleep questionnaire were found to be longer compared to that measured by actigraphy and PSG (Matthews et al., 2018).

Several factors have been associated with sleep misperception such as gender and psychosocial factors. For instance, males are less likely to underestimate their sleep duration compared to their spouses (Park et al., 2020). Satisfaction with socioeconomic status was positively associated with an overestimation of sleep. However, this association was only observed in females. Overall, females are more prone to sleep misperception (Park et al., 2020). Additionally, Unemployment and self-perceived stress are both associated with a higher risk of reporting poor sleep, while no impact was observed on objectively measured sleep (Tworoger et al., 2005, Buysse Daniel et al., 2008). Subjective perception of sleep can have an impact on daytime functioning. Self-reported poor sleep was associated with negative feelings and sleepiness on the following day (Semler and Harvey, 2005).

Sleep misperception is also present amongst people with disorders including insomnia, depression and bipolar disorders (Perlis et al., 1997). The association between subjective and objective assessed sleep duration can be modified by insomnia and depressive symptoms, as well as the perception of health (Matthews et al., 2018). When comparing sleep diary, actigraphy and PSG, no significant difference was detected between the latter two, while sleep latency, WASO and sleep duration detected by sleep diary were significantly different to PSG (McCall and McCall, 2012). A study involving 122 participants with insomnia complaints found that their self-reported sleep assessment underestimated their sleep duration and overestimated their sleep latency compared to the sleep laboratory recordings (Carskadon et al., 1976). This is consistent with other studies in this area. Insomniacs often underestimate

their sleep duration, while the opposite occurs in healthy controls. On average, insomniacs identify approximately 73% of sleep as wakefulness compared to PSG. Healthy controls, on the other hand, commonly overestimate their sleep duration. The discrepancy observed in insomniacs could be due to their altered memory function around sleep onset (Perlis et al., 1997). Similar to insomniacs, bipolar disorder patients also tend to underestimate their sleep duration compared to healthy controls. However, on the contrary to insomniacs, they do not overestimate their sleep latency (Ritter et al., 2016).

Another factor affecting the perception of sleep is the length of sleep opportunity and the time of the day. When a group of healthy adults were given 12 hours of night-time sleep opportunity which is longer than their habitual sleep duration of 7-8 hours/night, they significantly underestimate their sleep duration. This mimicked the characteristics of insomnia which an individual spent a lot of time being awake in bed. The opposite was observed when the same participants were given four hours of napping opportunity which they overestimated their sleep (Bianchi et al., 2012).

Sleep efficiency was also found to be a significant factor affecting self-perception of sleep measures in children and their parents. A study comparing sleep diary and actigraphy recordings in children and their parents showed that amongst children with lower sleep efficiency, the discrepancy was greater between objective and subjective measures. Both the children themselves and their parents overestimated their sleep time by more than 30 minutes and underestimate the duration of WASO, although their estimation of bed-time and wake time were relatively accurate. The source of discrepancy observed in children could be due to several factors, such as the general level of cognitive functioning and their ability to estimate time (Mazza et al., 2020).

Understanding sleep misperception amongst insomniacs and bipolar disorders has important clinical implications for the management of these disorders. Results from previously published studies suggested that subjective sleep measurements should be evaluated carefully and encourage the use of validated actigraphy for the assessment of sleep-related measures to improve the reliability of sleep detection. In order to capture reliable measures of sleep, it was found that at least five or six nights of recordings are needed (Aili et al.).

1.7 Association between sleep and melatonin, cortisol and gene expression

1.7.1 Melatonin and sleep

1.7.1.1 What is melatonin?

N-acetyl-5-methoxytryptamine, also known as melatonin, is the main hormone produced by the pineal gland. The release of melatonin is under the control of suprachiasmatic nuclei. The secretion is maximal during the dark phase of the day and halted during the light phase in all species, regardless of whether they are nocturnal or diurnal (Van Someren, 2000, Arendt and Skene, 2005). The nocturnal production of melatonin is regulated by the pineal serotonin N-acetyltransferase activity and protein level. An increase in the protein level shows a parallel change in the activity level which increases the level of circulating melatonin (Gastel et al., 1998). Prior studies have measured melatonin in saliva and plasma, or as 6-sulphatoxymelatonin (6-SMT) in urine (Arendt and Skene, 2005).

Sleep deprivation under a bright-light environment leads to a suppression of melatonin secretion (Strassman et al., 1991). This is mediated by a reduced pineal serotonin N-acetyltransferase activity caused by light exposure (Gastel et al., 1998). It has been shown that DLMO can be used to reliably assess the circadian phase position and it is useful for assessing phase-shifting caused by bright light and chronobiologic disorders, for example, winter depression (Lewy and Sack, 1989).

1.7.1.2 Role of melatonin in sleep regulation

The role of melatonin in the regulation of the sleep/wake cycle is well-documented. The nocturnal secretion of melatonin allows a smooth transition from the wake stage to sleep. The circadian rhythm of melatonin release closely correlates to the nocturnal hours of sleep. Any alteration in the melatonin synchronisation, for instance, jet lag or night shifts, is associated with sleep disturbances (Shochat et al., 1998). Monitoring of the circadian rhythm secretion of urinary 6-SMT showed that the increase in sleep propensity significantly correlates to the onset of melatonin secretion (Tzischinsky et al., 1993). The same pattern was also observed in blind people (Nakagawa et al., 1992).

Melatonin has been associated with increased subjective sleepiness and lower body temperature when provided during daylight hours. similarly, bright light exposure at night-time suppresses melatonin secretion and leads to reduced sleepiness and increased body temperature. Administration of >5mg of melatonin prior to nocturnal sleep can lead to an

increase in REM sleep (Arendt and Skene, 2005, Dijk and Cajochen, 1997). This is consistent with the findings of other studies. Exogenous melatonin administered prior to a 2-hours evening nap leads to both shorter sleep latency and longer sleep duration (Nave et al., 1995), although it is important to note that the hypnotic effect of melatonin supplement may be time-dependent suggesting an interactive effect of exogenous melatonin with endogenous melatonin. It was found that when 5mg of melatonin was provided at noon, sleep propensity occurs about 3.7 hours later. However, the latency to sleep propensity was reduced to only one hour when melatonin is administered at 9 pm (Tzischinsky and Lavie, 1994).

The hypnotic effect of melatonin raises the possibility of treating melatonin-deficient insomniacs with exogenous melatonin administration. Fast-release melatonin was found to be beneficial in terms of sleep initiation, while slow-release melatonin was found to improve sleep maintenance amongst elderly insomniacs (Haimov et al., 1995).

1.7.1.3 Therapeutic benefits of melatonin in age-related changes, Jet lag and shift work

The inevitable age-related changes in circadian rhythm are well-documented. Daytime serum level of melatonin progressively decreases with age, regardless of gender. The same pattern was also observed regarding the nocturnal melatonin level. It was low during the first six months of life and it peaks at 1-3 years of age then it progressively declines with age. The total 24-hour 6-SMT secrete also shows an age-related decline for both males and females. The sharp rise in nocturnal melatonin secretion after dark observed in the younger group diminishes in the older group (Iguchi et al., 1982, Waldhauser et al., 1988, Bojkowski and Arendt, 1990).

Benzodiazepines are commonly used to improve sleep initiation amongst the elderly population but it has also been associated with poor sleep maintenance as it inhibits melatonin production (Garfinkel et al., 1997). Therefore, the pharmacological effects of melatonin supplements in the elderly with sleep complaints have been evaluated in various placebo-controlled studies (Olde Rikkert and Rigaud, 2001). Three weeks of treatment with 2 mg melatonin per night was found to significantly improve sleep efficiency while no significant impact was observed on sleep duration. This increase in sleep efficiency was mainly due to shorter sleep latency and WASO (Garfinkel et al., 1995). The beneficial impact of melatonin replacement therapy on sleep initiation and maintenance has also been reported (Haimov et al., 1995). Amongst those who have been taking benzodiazepines, taking 2 mg of melatonin each night for three weeks has significantly increased their sleep duration and decreased their

WASO, sleep latency and sleep fragmentation. As a result, they have higher sleep efficiency (Garfinkel et al., 1997). Melatonin was taken two hours before bedtime in these studies. Reduced sleep latency was observed even at a lower dose of 0.5 mg suggesting its role in promoting sleep. However, no improvement in sleep duration, sleep efficiency and WASO was observed at this dosage (Hughes et al., 1998).

Other than the age-related events experienced by everyone, some lifestyle events can also lead to a change in melatonin production and circadian misalignment. For instance, when travelling across different time zones, a desynchronisation of circadian rhythm occurs which requires a resynchronisation of body rhythm to the local time. This is known as jet lag. It is still debatable whether melatonin should be administered to combat the negative symptoms of jet lag. A study that investigated the use of exogenous melatonin in alleviating jet lag found that by taking 5mg of melatonin three days prior to their flight and continuing for another four days after their arrival in the new time zone (eight time zones west), participants did not report self-perceived jet lag, whereas the placebo groups did report jet lag (Arendt et al., 1986). Even a lower dose of 3 mg of melatonin appears to be sufficient for the alleviation of jet lag symptoms caused by flying 11 time zones east (Takahashi et al., 2002), although not all studies proved the beneficial effects of melatonin on jet lag. Edward et al. compared those who took 5 mg of melatonin to those who did not when travelling 10 time zones east and they all reported fatigue on the first day after arrival which improved after that. No significant effect of melatonin on sleep disturbances, sleep latency and night-time awakening episodes was found when comparing the two groups. A possible explanation could be that all the participants in this study were highly motivated to adapt to the new environment (Edwards et al., 2000). Suggesting that amongst those with high motivation, the effects of self-perceived jet lag are not significant even in the absence of exogenous melatonin administration.

Apart from jet lag, individuals may experience circadian misalignment due to shift work which is required by many professions. There are various negative outcomes associated with shift work which include disturbed sleep, decreased alertness and cognitive deficits, as well as a higher risk for diseases. In particular, those who work in a job involving night shifts are required to adapt to daytime sleep and work when their body is ready for sleep (Burgess et al., 2002). Night shift workers adapt differently to their light/dark profile. A study measured 6-MST in night nurses to investigate their phase position found that some people experienced phase advance and a few people experienced phase delay, while the majority of them have

the same melatonin profile as non-shift workers. Those who did not have a phase shift in their melatonin profile had shorter main sleep duration compared to the other groups but they were more likely to take evening naps and therefore, overall sleep duration was the same for all three groups (Dumont et al., 2001). The intensity of light exposure during the night shift appears to play an important role in phase shifting. Higher light intensity at night is associated with phase delay in melatonin secretion (Boudreau et al., 2013). It was found that those who have adapted well to the night shift (i.e. their salivary melatonin level peaks during their sleep period at day-time) have higher subjective mood and alertness, as well as longer daytime sleep duration and higher sleep efficiency (Boudreau et al., 2013). Numerous studies have also evaluated the impact of exogenous melatonin in improving shift workers' tolerance to night shifts. It was found that 5 mg of melatonin taken prior to sleep has significantly increased sleep duration and sleep quality. It is also associated with better mood and higher alertness (Folkard et al., 1993). A lower dose of 1.8 mg of slow-releasing showed similar results. When taken half an hour before daytime sleep, sleep duration increased significantly amongst shift workers (Sharkey et al., 2001). These findings suggest that melatonin supplements could potentially improve sleep in shift-workers and in turn have beneficial effects on their work performances.

1.7.2 Cortisol and sleep

1.7.2.1 What is cortisol?

Cortisol is released into the blood when the corticotropin-releasing hormone is secreted by the paraventricular nucleus which acts on its receptors in the anterior pituitary leading to the release of adrenocorticotropic hormone. The adrenocorticotropic hormone then acts on the adrenal cortex and leads to the release of cortisol. Cortisol has feedback inhibition on the paraventricular nucleus and anterior pituitary leading to a decreased level of corticotropin-releasing hormone and adrenocorticotropic hormone (Bush and Hudson, 2010). On the contrary to the melatonin profile, cortisol level is at the lowest at about midnight and a rise in cortisol level typically begins 2-3 hours after sleep onset. The level of cortisol peaks at approximately 9 am, then it progressively decreases throughout the day to the nadir (Buckley and Schatzberg, 2005).

1.7.2.2 Role of cortisol in sleep regulation

In healthy individuals, wakefulness and N1 sleep stage are both associated with a higher cortisol level, while the plasma cortisol level decreases in SWS sleep (Nicolas C. Nicolaides, 2000). The level of urinary cortisol secretion was found to be significantly associated with the

amount of REM sleep in healthy individuals with no sleep problems (Vgontzas et al., 1997). Higher evening cortisol level has been associated with less REM sleep and more awakening episodes (Vgontzas et al., 2003). The integrity of sleep progressively diminishes with increasing age. This could be mediated through the age-related changes in the cortisol profile. The plasma cortisol profile of 18-83 years old adults showed a progressive increase of nocturnal nadir with age in both males and females, although the diurnal rhythmicity of cortisol secretion was preserved in the elderly, the relative amplitude appears to be dampened and there is a phase advance change in the circadian elevation (Van Cauter et al., 1996). This is consistent with the findings of other studies. Moreover, the concentration of plasma cortisol is associated positively with the total wake time and this association is strong in older adults compared to younger adults (Vgontzas et al., 2003).

Other than the inevitable changes in cortisol levels associated with ageing, an elevated level of cortisol caused by depression or other stress-related disorders are associated with sleep disturbances characterised by lighter sleep and frequent awakenings (Bush and Hudson, 2010). Evening cortisol level was also found to be higher amongst chronic insomnia patients and it correlates significantly with the number of night-time awakening episodes (Rodenbeck et al., 2002). Amongst insomniacs, the level of cortisol is dependent on their sleep duration. Those with a shorter objectively measured sleep duration (<5 hours/night) showed a higher cortisol level (D'Aurea et al., 2015). A positive association between self-perceived sleep problems and cortisol levels has also been reported. Those reporting more sleep complaints have a higher cortisol level throughout the day compared to those with fewer sleep problems and the difference was greater regarding the evening cortisol level (Hackett et al., 2020). Additionally, sleep deprivation is known to be associated with increased cortisol level and when given an additional stressor, sleep-deprived individuals shows an amplified response (Minkel et al., 2014). Prior study has found that both partial (4 hours/night) and total sleep deprivation leads to a significant increase (37% and 45%, respectively) in cortisol level compared to a normal sleep schedule of 8 hours/night (Leproult et al., 1997). Another study has also reported a reduced amplitude of cortisol rhythm under the sleep-restricted condition (<4 hours/night). It also took 1.5 hours longer to reach nocturnal nadir from morning acrophase. Additionally, cortisol levels measured in the afternoon and evening are both higher when the participants are sleep-restricted (Spiegel et al., 2004a). A reduced rhythm amplitude of cortisol was also reported amongst shift workers with a fast rotating shift system (Touitou et al., 1990).

The elevated cortisol level could be a marker for corticotropin-releasing hormone hyperactivity, therefore any interventions to decrease corticotropin-releasing hormone activity and cortisol level will potentially be beneficial to insomniac and other sleep disorders patients (Bush and Hudson, 2010). A study assessed the impact of two hours mid-afternoon nap on the cortisol level in sleep-deprived individuals and they reported a beneficial effect. After napping, participants showed improved alertness, less self-perceived sleepiness and a significant decrease in the cortisol level, although the cortisol level did not remain decreased, it progressively increased with time suggesting that sleep has an inhibiting effect on the secretion of cortisol, while wake and alertness can result in an increase in cortisol (Vgontzas et al., 2007). The use of an antidepressant in insomnia patients has been evaluated and the results illustrated a positive impact on the cortisol level. After administration, the mean cortisol level significantly reduced and improvement in sleep was observed (Rodenbeck et al., 2003).

1.7.3 Gene expression and sleep

The circadian clock gene network oscillates with a 24-hour cycle which controls the rhythms in physiological and behavioural processes. The first circadian mutant in Drosophila was identified by Konopka and Benzer in 1971 which was *period*, then the mammalian circadian gene, *Clock*, was identified in mice and cloned (Rijo-Ferreira and Takahashi, 2019, Konopka and Benzer, 1971, King et al., 1997). Over the past decades, many more circadian genes have been identified. For instance, *Cry1*, *Cry2* (van der Horst et al., 1999) and *Bmal1* (Bunger et al., 2000) have all been associated with the maintenance of circadian rhythmicity using mouse models. Additionally, *mPer1* and *mPer2* deficient mice showed disrupted locomotor activity rhythms (Bae et al., 2001). Mutations in those genes could lead to the development of various sleep disorders by altering the sleep phase (Patke et al., 2017), contributing to tumour progression through enhanced cell proliferation and metabolism dysregulation (Papagiannakopoulos et al., 2016) and increasing the susceptibility to viral infections via the circadian activity of the metabolic and trafficking pathways (Edgar et al., 2016). The association between circadian genes and ageing has also been reported. The interaction of *Per2* and *Cry1* is regulated by the level of polyamines and its level decreases with age (Zwighaft et al., 2015). Additionally, studies have found that *Bmal1*-deficient mice show symptoms of premature ageing such as sarcopenia, cataracts and organ shrinkage. This indicates that it plays an important role in ageing (Kondratov et al., 2006).

1.7.3.1 Circadian control of sleep

Individuals with circadian gene mutations are at risk of developing circadian rhythm sleep disorders. For instance, *hPer2*, *CKIdelta* and *Cer2* missense mutations have all been associated with the familial advanced sleep phase disorder which is characterised as having an earlier habitual sleep time compared to the societal norm, i.e., “morning larks”. They also have a four-hour advance in melatonin rhythm (Toh et al., 2001, Xu et al., 2005, Hirano et al., 2016). On the contrary to the familial advanced sleep phase disorder, delayed sleep phase disorder is a form of insomnia characterised by a shift to a later habitual sleep time compared to the societal norm, i.e., “night owls”. It has been associated with a mutation of the *Cry1* gene (Patke et al., 2017). With the increasing popularity of genetic testing services such as 23andMe and biobanks such as the UK Biobank, researchers are able to utilise a large amount of genetic information to investigate the relationship between genes and chronotypes, as well as their relationships with mental and physical disorders. Using a genome-wide association study, 351 loci have been identified to have an association with chronotype in the UK Biobank and 23andMe participants. Some of the genes identified include *Per1*, *Per2*, *Per3* and *Cry1*. As mentioned above the *Per2* gene has been associated with the familial advanced sleep phase disorder, therefore it is not a surprise that it has been associated with the morning chronotype. An interesting finding of this genome-wide association study was that morning chronotype was positively associated with subjective well-being and negatively associated with intelligence and psychiatric problems such as schizophrenia and depressive symptom (Jones et al., 2019).

1.7.3.2 Circadian control of metabolism

A constant change in the sleep/wake cycle, such as shift workers, has been linked to a higher risk of metabolic syndromes (Karlsson et al., 2001). It is therefore not a surprise that disruptions of the circadian genes lead to a higher risk of metabolic diseases. The role of *Bmal1* and *Clock* in glucose homeostasis regulation has been identified. As a result, mutations in these two genes can trigger glucose intolerance, insulin insensitivity and eventually the onset of diabetes (Rudic et al., 2004, Marcheva et al., 2010). *Clock* has also been associated with the development of obesity. Animal models showed that *Clock* mutant mice are obese and developed hyperleptinemia, hyperlipidemia and hepatic steatosis (Turek et al., 2005). Moreover, *Per2* is known to be essential in the normal metabolism of lipids. Lack of *Per2* could therefore lead to enhanced adipocyte differentiation (Grimaldi et al., 2010).

1.7.3.3 Circadian control of the immune system

Aside from the circadian control of the metabolic system, the role of circadian genes in controlling the immune system has also been addressed in many studies. For example, *Bmal1* has been identified as an important gene in the control of inflammatory monocytes. Depletion of *Bmal1* is associated with the development of acute and chronic inflammation (Nguyen et al., 2013). It was also found that *Bmal1* plays an important role in the innate immune system. *Bmal1* deficiency could result in a blunted antioxidant response and increased production of proinflammatory cytokines (Early et al., 2018). The circulation of lymphocytes also shows circadian regulation. The migration of lymphocytes to lymph nodes peaks at night and the reverse occurs during the day (Druzd et al., 2017). These findings show the importance of circadian genes in response to infections and could potentially be useful in developing therapeutic interventions for infections.

1.7.3.4 Circadian control of the cardiovascular system

Many cardiovascular complications such as myocardial infarction and stroke have a higher incidence in the morning. This could be medicated by the diurnal rhythmicity in the vascular tone and thrombus formation (Paschos and FitzGerald, 2010). When circadian genes such as *Bmal1*, *Clock* and *Npas2* are either mutated to deleted, disruption of the circadian variation in blood pressure and heart rate was observed (Curtis et al., 2007). The deficiency of *Bmal1* in myeloid cells was found to have a negative impact on atherosclerosis as it enhances the recruitment of monocytes to atherosclerotic lesions and causes an increase in the lesion size (Huo et al., 2017). Even the time of operation on the heart seems to play an important role in the post-operation outcome. It was found that those who had an operation in the afternoon had a lower incidence of perioperative myocardial injury compared to those who had an operation in the morning. This could be due to the higher expression of *Rev-Erbα* in the morning (Montaigne et al., 2018). Understanding the circadian rhythm in the cardiovascular system helps to provide better perioperative myocardial protection.

1.8 Literature searches

The above literature review was conducted using the PubMed and UK Biobank publication databases. No pre-defined eligibility criteria were used as it was not a systematic review. Keywords in each heading and subheading in this chapter were input into the PubMed database in turn. Publications (including both research and review papers) were then reviewed and relevant publications were included in the literature review. Prior UK Biobank

studies mentioned in this chapter were found by typing 'sleep' and 'accelerometer' as keywords into the UK Biobank publication database. All publications were reviewed and relevant publications were included in this chapter.

1.9 Aims and hypothesis

1.9.1 UK Biobank analysis

- Investigate the association between objectively assessed sleep duration and both self-reported health and GP records of illnesses
- Examine the relationship between sleep and sociodemographic factors, anthropometry measurements, lifestyle habits, cognitive function and mood

1.9.2 Subjective and objective assessments of sleep

- Compare sleep duration and efficiency detected by three methods:
  - paper sleep diary
  - Axivity AX3 tri-axial accelerometer
  - Laboratory-based video-polysomnography (PSG)
- Investigate the association between sleep duration and quality and various biomarkers:
  - Cortisol
  - Melatonin
  - Mitochondrial DNA damage
  - Gene expression

1.9.3 Hypothesis

The general hypothesis is that those with short or long sleep duration, as well as fragmented sleep, are at a higher risk of metabolic and mental disorders. It is also hypothesised that the sleep detected by the accelerometer will be more similar to PSG compared to the sleep diary and different levels of circadian clock gene, cortisol and melatonin will be detected between the control and patient group. By monitoring those with abnormal circadian rhythms, mental disorders and metabolic health risks can be identified and interventions can be provided at an early stage.

Chapter 2. Methods and Materials

Part of this chapter has been published within a research paper in PlosOne (Zhu et al., 2019) and another research paper in Nature and Science of sleep (Zhu G, 2021).

2.1 UK Biobank

2.1.1 UK Biobank population and study design

A cross-sectional analysis was carried out using data from the UK Biobank (Project ID: 43537). The UK Biobank received ethics approval from the Northwest Multi-centre Research Ethics Committee (Reference: 16/NW/0274). The UK Biobank also obtained generic Research Tissue Bank approval which covers most research using the resource. Therefore, each applicant does not need to obtain separate ethics approval.

UK Biobank recruited more than 500,000 volunteers. They were aged between 37-73 years and recruited from the general public all around the UK. This age group was recruited because it allows the comparison of normal and abnormal ageing to understand what psychosocial biometric data predicted subsequent long-term health and disease. Full-scale recruitment took place between 2007 and 2010. Each participant was invited to a baseline visit which lasted approximately 90 minutes where informed consent was taken (Figure 2-1). Sociodemographic, lifestyle, occupation and health-related information were collected using machine-assisted touch-screen questionnaires (>300 questions in total). Biological samples (blood and urine) and physical measurements (including height, hip and waist measurements, heel bone ultrasound and spirometry) were taken by trained nurses, healthcare technicians and clerical staff.

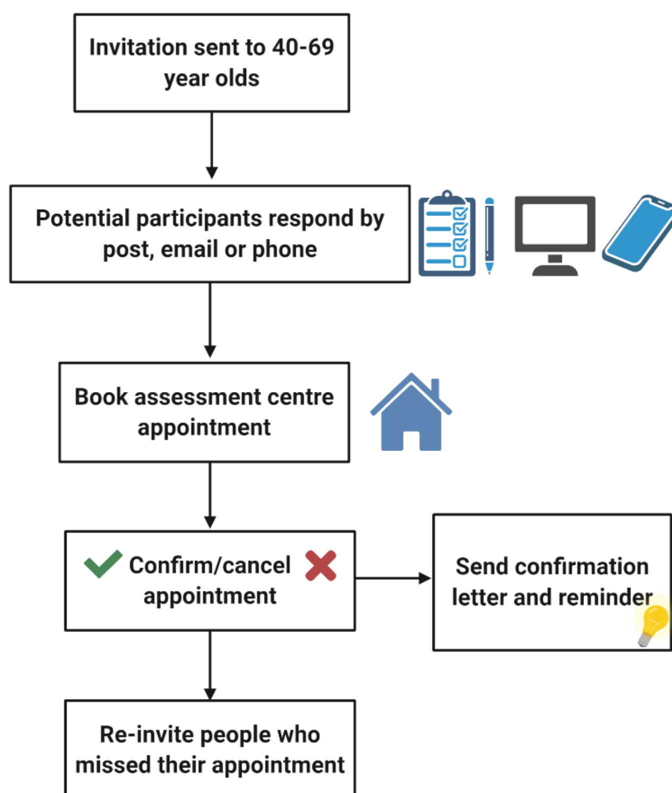


Figure 2-1. UK Biobank recruitment and baseline assessment procedure. Image created with BioRender.com

2.1.2 Baseline assessments

A detailed description of all measurements collected by the UK Biobank can be found on the UK Biobank data showcase webpage (Biobank). Sociodemographic information (Category ID: 100062) such as age, date of birth and ethnic background was recorded, and the Townsend deprivation index was calculated immediately prior to participants joining UK Biobank based on the location of their postcode and preceding national census output areas. Townsend deprivation index takes the following four variables into account: employment status, car ownership, home ownership and household overcrowding (Foster et al., 2018).

Manual body measurements (Category ID: 100008) including waist and hip circumferences and whole-body bioimpedance measurements including fat percentage and fat mass were performed.

Various health and medical conditions and participants' age at diagnosis (Category ID: 100044) were recorded in the touch-screen questionnaire including vascular/heart problems, cancer, diabetes and high blood pressure. If the participants reported a specific disease, there was a

follow-up question asking “what was your age when x was first diagnosed?” where x is the disease of interest.

Lifestyle and environmental data (Category ID: 100050) were taken as part of the touch-screen questionnaire which includes self-reported sleep, smoking status, alcohol consumption, type and duration of various physical activities and time spent in sedentary activities. Sleep-related measurements include average sleep duration, chronotype, whether they nap during the day and if they had any experience of insomnia and narcolepsy. Self-reported sleep duration was measured by asking the question “About how many hours sleep do you get in every 24 hours? (please include naps)". Insomnia was measured by the question "Do you have trouble falling asleep at night or do you wake up in the middle of the night?", while narcolepsy was measured through the question "How likely are you to doze off or fall asleep during the daytime when you don't mean to? (e.g. when working, reading or driving)". If participants were unsure of any questions, they have the option of providing an estimate for the past four weeks or selecting “Do not know”.

Cognitive function tests (Category ID: 100026) collected data regarding participants’ cognition including reaction time, numeric and visual memory, reasoning and word production. Reaction time was assessed by presenting rounds of two cards on the screen, participants were asked to press the snap-button as quickly as possible when two cards are the same. The mean duration to first press of the snap button was used to give a crude measure of the reaction speed. To measure the numeric memory of each participant, they were shown a 2-digits number on the screen which will disappear and the participants were asked to recall and enter the number on the screen. If the number were recalled correctly, the lengths of the number will increase by one digit up to a maximum of 12 digits. The maximum number of digits a participant remembered was recorded. Visual memory, on the other hand, was recorded by displaying a series of pictures of houses with certain windows lit, participants had to recall which window was lit after a 10-seconds delay. Reasoning ability was assessed through the fluid intelligence test which was designed to investigate each participant’s capability to solve logic questions. Participants were asked to answer as many questions as possible for two minutes. Finally, participants were asked to state as many words as possible that begin with the letter ‘S’ to assess their word production ability.

For the analysis of various mood and mental health-related questions (Category ID: 100060), participants were asked to consider if they had any specific mood problems such as mood

swings, irritability, anxious and nervous feelings…etc. They were also asked to evaluate their mood during the two weeks prior to taking the questionnaire such as how frequently they experience depressed mood, disinterest, and lethargy…etc. The duration of some negative feelings was also recorded. If a participant is unsure how to answer a particular question, a help button is available where the following statement will appear on the screen: "Work through these questions quickly and do not think about the exact meaning of the question".

As part of the questionnaire, information regarding participants' happiness and subjective well-being were also collected (Category ID: 147). Participants were asked to consider how happy they feel in general ("In general how happy are you?") and regarding their own health ("In general how happy are you with your HEALTH?"), and whether they consider their life to be meaningful ("To what extent do you feel your life to be meaningful?").

2.1.3 Accelerometry data

After appropriate consent, some participants were invited to wear an Axivity AX3 triaxial accelerometer (Open Lab, Newcastle University, UK) on their dominant wrist for seven consecutive days which objectively monitors their sleep-wake cycle and activity level. Recruitment took place a few years after the baseline assessment appointment, between 2013 and 2015. Approximately 103,720 volunteers were included in the accelerometry study with a response rate of 44% (Biobank, 27 Jan 2016).

Raw data from accelerometers were processed using R Package GGIR version 1.7-1 by van Hees VT et al (van Hees et al., 2015). Various studies have assessed the general algorithm and its accuracy in detecting sleep period windows (Doherty et al., 2017, van Hees et al., 2018, Sundararajan et al., 2021).

2.1.3.1 Sleep categories

Only participants with available processable accelerometry data were included in the current study. Participants were categorised into five groups based on their average sleep duration per night using previously published self-report thresholds (Shan et al., 2015): (1) <5 hours; (2) 5-6 hours; (3) 6-7 hours; (4) 7-8 hours; (5) >8 hours. Groups 1 and 2 were used to investigate the impact of extremely short objective sleep durations.

2.1.3.2 Physical activity

Acceleration levels (measured in milli-g (mg)) of each participant were extracted from the accelerometry data which is a measurement of physical activity level. This method of assessing

physical activity has previously been validated and published in detail using the biobank cohort which has also been compared to other accelerometry devices (Doherty et al., 2017).

2.1.4 Primary care data

In collaboration with GP systems suppliers, the UK Biobank subsequently extracted primary care data related to UK Biobank participants. These data included participants' health, all coded disease diagnoses, laboratory test results and immunisation records, as well as their interactions with their primary health care provider such as appointment dates. Data exchanged were in an encrypted format and via secure transfer. These data were first released in 2019 on approximately 230,000 (45%) UK Biobank participants. The hospital inpatient records cover diagnoses made since 1992 (Biobank, September 2019). ICD-10 codes were used when analysing diagnosis-related clinical information in the current study.

2.1.5 Optimal VS less optimal sleep

Sleep fragmentation-related measurements were also extracted from the accelerometry data that include WASO, the activity level of the least active five hours (L5 value) and the number of episodes of movement (NBlock). Analysis was carried out to compare optimal and less optimal sleep where less optimal sleep is characterised as meeting all the following criteria: sleep duration <5 or >8 hours/night, WASO ≥95.98 mins (75th percentile), L5 value ≥4.24 milli-g (75th percentile) and NBlock ≥39.63 (mid-point between its bimodal distribution).

2.1.6 Clinical performance specifications

The prevalence of diseases amongst the UK Biobank population included in the current study was calculated and clinical performance specifications are defined below(Altman and Bland, 1994, Loh et al., 2020) :

- $Postive\ predictive\ value\ (PPV) = \frac{Sensitivity \times prevalence}{Sensitivity \times prevalence + (1 - specificity) \times (1 - prevalence)}$
- $Negative\ predictive\ value\ (NPV) = \frac{Specificity \times (1 - prevalence)}{(1 - sensitivity) \times prevalence + specifcity \times (1 - prevalence)}$
- $Specificity = \frac{True\ negative}{False\ negative + True\ negative}$
- $Sensitivity = \frac{True\ positive}{True\ positive + False\ negative}$

2.1.7 Statistical analysis

UK Biobank baseline, accelerometry and primary care data were all analysed using IBM SPSS Statistics version 24 (Armonk, New York, USA).

2.1.7.1 Categorical variables

As guided by a university statistician, Dr Kim Pearce, the Chi-square test was used to investigate the association between sleep groups and categorical variables. Once a significant difference is detected between any sleep groups, the z-test was used to compare column proportions of each variable and p-values were adjusted using the Bonferroni method. If columns in the same row have been assigned the same letter, then their column proportions do not differ from each other significantly. On the contrary, if columns in the same row have been assigned different letters, then their column proportions are significantly different from each other. The Monte Carlo method with a 99% confidence level was used to estimate the exact significant level.

2.1.7.2 Continuous variables

A normality test was first performed to check the distribution of sleep data. Non-parametric Kruskal-Wallis H and Dunn-Bonferroni post-hoc tests were used to analyse continuous variables. A p-value <0.05 was accepted as statistically significant.

2.1.7.3 Binary regression model

Binary logistic regression was used to investigate the odds ratio of some key variables. Adjusted odds ratios (OR), with 95% confidence intervals (CI) were reported. Participants were categorised into four age groups (43-49 years, 50-59 years, 60-69 years and 70-79 years) and the Townsend deprivation index was divided into quintiles (from zero to four where zero represents the least deprived individuals). Logistic regression models were adjusted for age (reference = '43–49'); gender (reference = 'Female') and Townsend deprivation index (reference = 'Least deprived). Significance for all statistical tests was set at $p<0.05$.

2.2 Objective and subjective assessment of sleep

2.2.1 Study design

Ethical approval has been obtained from London- Chelsea Research Ethics Committee (IRAS ID: 249392, R&D number: 08965). The inclusion criteria for selecting the participants were as follows:

1) Aged 18-60 years

2) Able to provide informed consent
3) Fluent in English (due to the involvement of questionnaire assessments)
4) Those without any known circadian rhythm disorder (for healthy control group only)

This study intended to recruit 50 participants who were either recruited from the Regional Sleep Service (Newcastle upon Tyne Hospitals NHS Foundation Trust) from the cohort of patients attending the sleep clinic for investigation of sleep disorders, or from a healthy control group from university students and employees. Due to the fact that this is a pilot study, no power calculation was conducted prior to the study. Participants in the patient group were selected from patients with PSG appointments scheduled and they have a range of sleep problems. Some patients have complaints about short and fragmented sleep, while others have complaints about excessive daytime sleepiness and long sleep duration. Healthy volunteer recruitment posters were produced and advertised within the university campus. Recruitment emails were also sent to all research institutes within the Faculty of Medical sciences. One-to-one consent meetings were arranged with each potential participant that showed interest in joining the study. During the meeting, potential participants were shown all equipment involved in this study such as an accelerometer, a diagram of PSG equipment, sample collection tubes and a paper sleep diary. The timeline of the study was also explained (Figure 2-2) and information sheets and consent forms were provided for each potential participant prior to attending the sleep study and they were all given at least 24 hours to consider and discuss with family members if they wish. However, due to the Covid-19 global pandemic, only 28 participants (11 healthy controls and 17 patients) were recruited. This was due to the closure of the sleep laboratory during the national lockdown in 2020 which no participants could attend the overnight PSG appointment. Even after the lockdown was lifted, initially, each participant had to self-isolate for two weeks and carry out a covid test prior to attending their PSG appointment which some participants were not able to complete or if they tested positive for covid, their appointment had to be cancelled. All 11 healthy controls were recruited prior to the lockdown in March 2020, while all 17 patients were recruited after the lockdown eased in July 2020 which allowed the restart of clinical research projects.

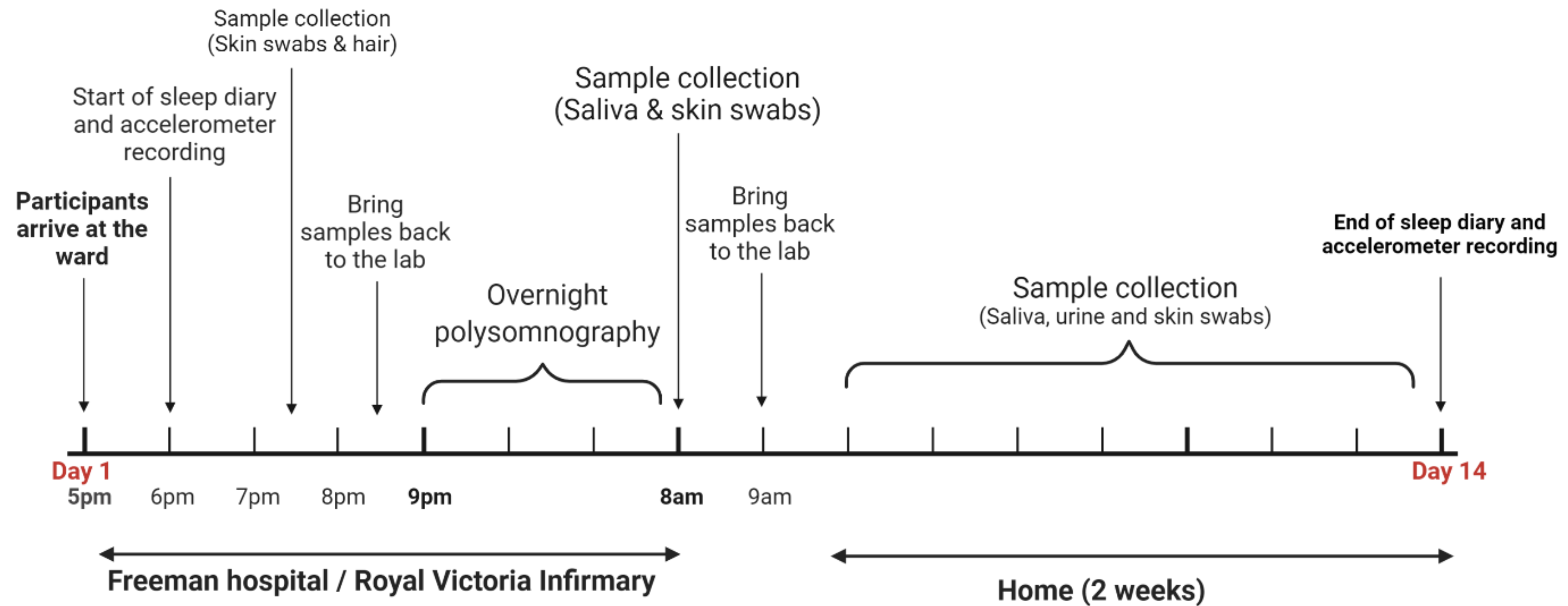


Figure 2-2. Timeline of the study for each participant. It takes two weeks to complete the study. Image created with BioRender.com

2.2.2 Methods of sleep detection

2.2.2.1 Paper sleep diary

A Paper sleep diary (Figure 2-3) and pen were provided to all participants. Participants were asked to record their daytime activities for two weeks including consumption of alcohol, tea and coffee, exercise and meal. They were also asked to record their bedtime and sleep duration during this period. Finally, they were asked if they are taking any sleep aid medication.

REGIONAL SLEEP SERVICE

SLEEP DIARY

Name

Date started Day of Week

Do you ever, at any time, take any medication (prescribed or purchased over the counter) to aid your sleep? Yes ☐ No ☐

If yes, please detail name of medication, dose & time on back page.

Instructions:

- Please leave diary near your bedside
- It is important that you fill out this chart each morning
- Mark your diary in the following way:

ACTIVITIES
A - each alcoholic drink
C - each caffeinated drink includes coffee, tea, chocolate, cola
P - every time you take a sleeping pill or tranquilliser
M - meals
S - snacks
X - exercise
T - use of toilet during sleep-time
N - noise that disturbs your sleep
W - time of wake-up alarm (if any)

SLEEP TIME (including naps)
↓ - mark with a "down" arrow each time you got into bed
↑ - mark with an "up" arrow each time you got out of bed
| - mark with a line the time you began and the time you ended your sleep; Then join the line to indicate sleep periods
| - mark with a line the time you began and the time you ended any naps, either in the chair or in bed; then join up lines with a broken line to indicate nap periods

Please describe (on the back page) any events that influenced your sleep

Example:

Week 1

Week 2

COMMENTS

Day

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14

Regional Sleep Service
Newcastle upon Tyne Hospitals
Newcastle

NUTH would like to thank the Centre of Sleep and Chronobiology, University of Toronto For the original concept of the Sleep Disorder Patient Chart originated by Moldofsky/MacFarlane©1990

Figure 2-3. Paper sleep diary used by the regional sleep service, Newcastle upon Tyne. Participants record their daytime activities including meals, alcoholic drinks and exercise, as well as bedtime and wake time.

2.2.2.2 Axivity AX3 accelerometer

Each participant was provided with a wrist-worn accelerometer (Axivity AX3 3-Axis Logging accelerometer, Axivity, UK) to wear on the dominant wrist for 14 consecutive 24-hour periods. This period aligns with the sleep diary recording to allow valid comparisons between subjective and objective sleep measurements. Participants were encouraged to wear their accelerometer at all times as the watch is robust and fully waterproof. However, participants were given the option of removing it during shower or bath, but they were informed that they should place the accelerometer back on as soon as possible. At the end of 14 days, accelerometers were collected from the participants and data were transferred to the computer for further analysis.

Raw data from accelerometers were downloaded as .cwa files and visualised through the AX3 OMGUI Configuration and Analysis Tool (Axivity, UK). Downloaded accelerometer files were processed into .csv files using R studio version 3.5.1 (Boston, USA) and open-source R package GGIR (van Hees et al., 2015). R scripts were provided by Professor Michael Catt (Appendix B).

2.2.2.3 Video-polysomnography

Standard PSG was carried out in the sleep laboratory (Ward 29, Freeman hospital and Ward 15, Royal Victoria Infirmary, Newcastle upon Tyne). Participants were asked to attend their sleep appointment at 6.45 pm at the Freeman hospital or 5 pm at the Royal Victoria Infirmary. Sleep laboratory physiologists set up the PSG (Figure 2-4) and participants have the option to do anything they wish in the evening and choose the time they wish to sleep. However, they could not leave the room due to the limited length of various wires. On the following morning, participants can leave at a time that they chose. The physiological data recorded are listed below:

1) EEG: monitors brain electrical activity
2) EOG: records eye movements and blink
3) EMG: records muscle tone and leg movements from both chin and both lower limbs
4) Nasal flow, chest and abdominal belts: assess breathing in detail such as detecting snoring, leg movements and abnormal breathing patterns including hypopnoea and apnoea
5) Oximetry: measures blood oxygen levels both baseline and desaturations
6) Overnight video recording: records sound and body movement

Precise percentages of time spent within each sleep stage were calculated by the clinical physiologists based on the version 2.2 (released in 2015) of the American Academy of Sleep Medicine (AASM) sleep scoring. In addition, any abnormal behaviours were also noted.

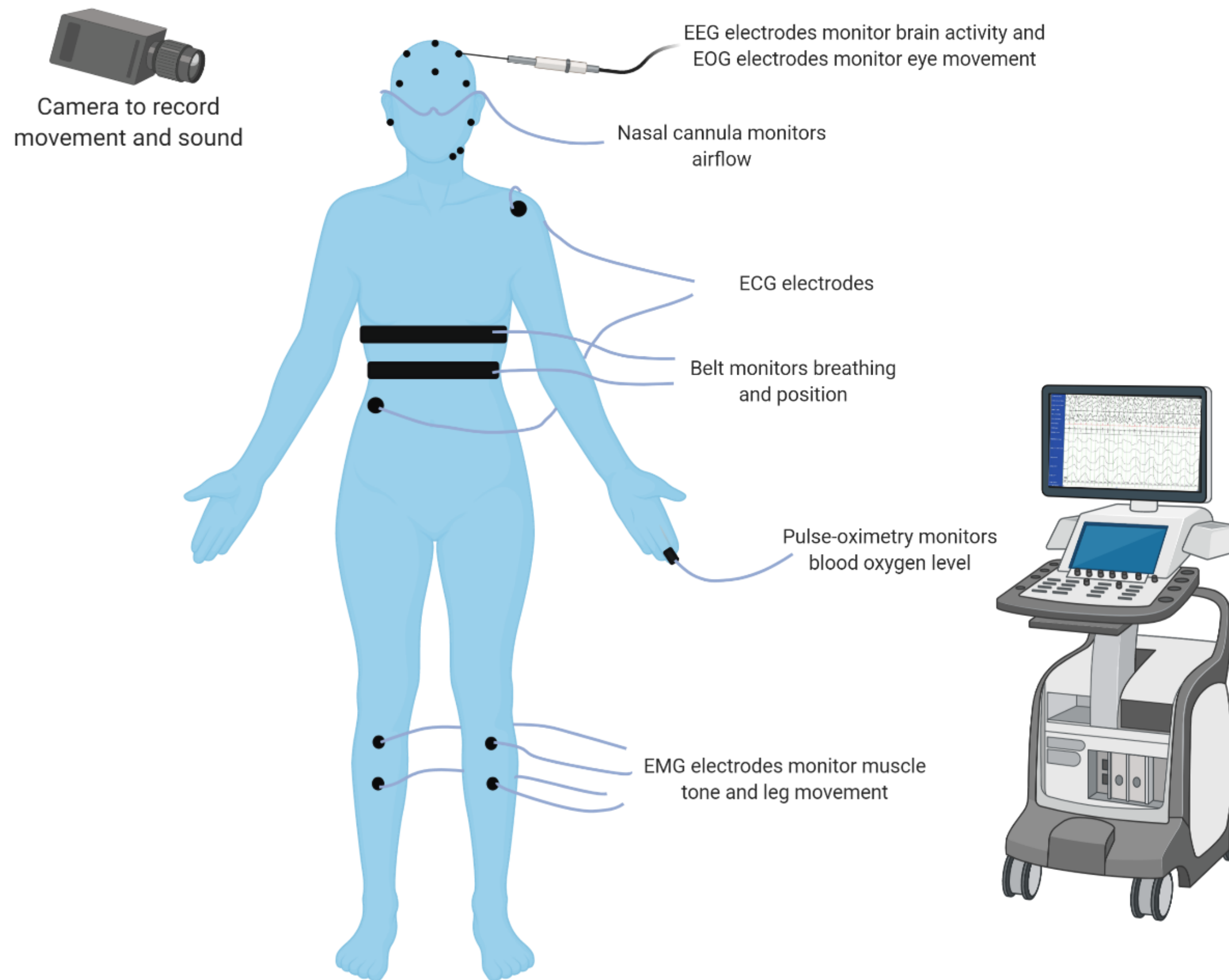


Figure 2-4. Diagram demonstrating video polysomnography equipment by staff at the sleep laboratory. Image created with BioRender.com

2.2.3 Sleep categories

Due to the small sample size, participants were categorised into three groups based on their accelerometer sleep duration per night: (1) <7 hours; (2) 7-8 hours; (3) >8 hours.

2.2.4 Biological samples

All sample collection and analysis were performed by Gewei Zhu unless specified otherwise.

2.2.4.1 Urinary melatonin assay

Each participant was asked to collect urine samples for 48-hours (Figure 2-5). They were instructed to collect a sample approximately every four hours when awake and every eight hours during the sleep period. Participants were asked to urinate into a 1 L urine collection pot and note down the total volume of each void, then transfer approximately 10 mL into a sample collection tube. All samples were kept at -20°C until analysis.

Urine samples were analysed using 6-Sulfatoxy Melatonin Enzyme-linked immunosorbent assay (ELISA) from urine BÜHLMANN 96 wells (Product code：EK-M6S, Alpha Laboratories, UK) following the suggested protocol. All urinary samples were diluted 1 in 200 with Incubation buffer. The microtiter plate was washed with wash buffer. Calibrators and samples were then pipetted into the appropriate wells in duplicate. M6S-Biotin conjugate solution and anti-serum solution were in turn added to all wells. The plate was then mixed at 900 rpm for one minute and incubated at 4°C for three hours. The plate was then washed with wash buffer. Enzyme labels were added to each well and incubated at 4°C for 30 minutes. Each well was washed with the wash buffer. TMB buffer was then added to all wells and mixed at 900 rpm briefly and incubated at room temperature for 15 minutes. Stop solution was finally to all wells to stop the reaction. Absorbance was read at 450 nm within 30 minutes of adding the stop solution.

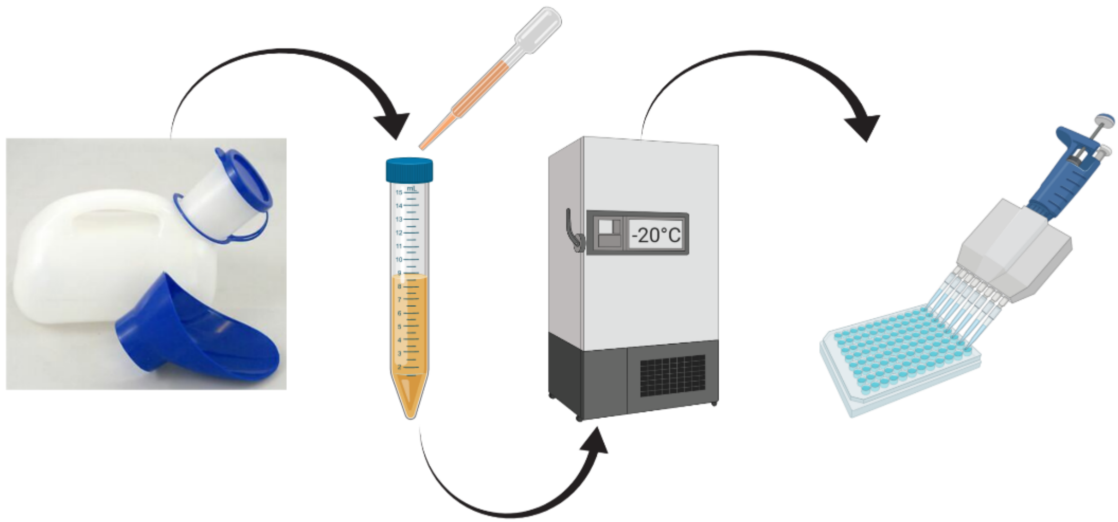


Figure 2-5. Procedure for collecting urine samples. All collection tubes and pots were provided for each participant at the beginning of the study. They could choose any 48-hours that was convenient for them to collect the samples. Samples were stored at -20 ℃ until enzyme-linked immunosorbent assay (ELISA) analysis. Image created with BioRender.com

2.2.4.2 Salivary cortisol ELISA assay

Each participant was asked to collect two morning saliva samples first thing when they woke up – one sample at home and one sample in the sleep laboratory during the morning following PSG (Figure 2-6). To minimise contamination, participants were asked to rinse their mouths thoroughly with water for 10 minutes before sample collection. Saliva was collected by unstimulated passive drool using 2 mL Salimetrics Cryovial (Product code: 5004.06, Stratech, UK) and Salimetrics Saliva Collection Aid (Product code: 5016.02, Stratech, UK). All samples were stored at -20°C until analysis.

Saliva samples were analysed using Salimetrics salivary Cortisol ELISA Kit (Product code: 1-3002, Stratech, UK) following the manufacturer's suggested protocol. Samples were thawed thoroughly on the day of analysis and centrifuged at 1500 x g for 15 minutes. Assay standards, controls and saliva samples were first added to the appropriate wells in duplicate. Enzyme conjugate was then added to all wells and mixed at 500 rpm for five minutes, then incubated at room temperature for one hour. Plates were then washed thoroughly with the wash buffer. Substrate solution was then added to each well and mixed at 500 rpm for five minutes, then incubated in the dark at room temperature for 25 minutes. Stop solution was finally added to all wells and mixed at 500 rpm for three minutes. Absorbance was read at 450 nm within ten minutes of adding the stop solution.

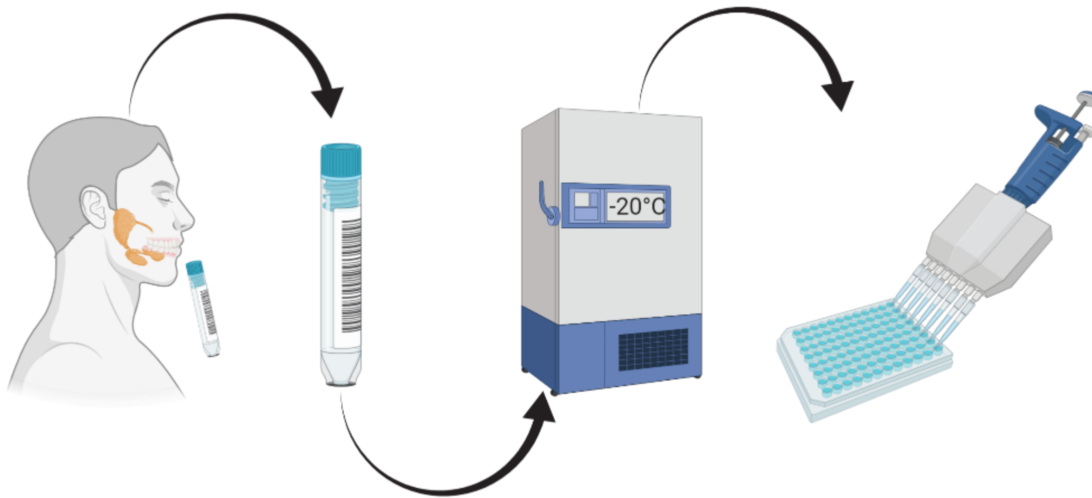


Figure 2-6. Diagram explaining the procedure of collecting and analysing saliva samples. All collection aids and tubes were provided to participants at the beginning of the study. Samples were stored at -20℃ until enzyme-linked immunosorbent assay (ELISA) analysis. Image created with BioRender.com.

2.2.4.3 Mitochondria DNA damage assay from skin swabs

Three sets of skin swabs were collected from each participant to look at the level of mitochondrial DNA (mtDNA) damage in the skin (Figure 2-7). Skin swabs were taken in the evening of PSG, the morning after PSG and two weeks after PSG. Swabs were taken from the inner arm of each participant which is a photo-protected site and both sides of the cheek were also swabbed which are photo-exposed sites. Photo-exposed sites are more exposed to the external environment and therefore can be affected by both internal and external stressors leading to intrinsic and extrinsic ageing. On the other hand, photo-protected sites are less affected by external stressors, therefore it was used to mainly investigate intrinsic ageing. The area was sterilised by wiping alcohol wipes up and down four times. The skin swabs were then taken by rubbing a sterile cotton swab up and down 40 times on the required area. The cotton swabs were stored at room temperature until DNA extraction. Purification of DNA from the skin swabs was carried out using the Isohelix BuccalPrep Plus DNA Isolation Kit (Product code: BPP-50, Isohelix, UK) following the manufacturer's protocol with a few amendments. Each swab was cut into individual Eppendorf tubes and mixed thoroughly with 500 µL Lysis and Stabilisation buffer. 20 µL proteinase K solution was then added to all tubes and incubated at 60°C for one hour. 500 µL DNA Precipitation buffer was added and centrifuged at 12.9K rpm for ten minutes. The supernatant was discarded and pellets were dissolved in 60 µL TE buffer. After 10 minutes of incubation at room temperature, all samples were centrifuged at 12.9K rpm for 15 minutes. The supernatant containing DNA was finally removed for further analysis.

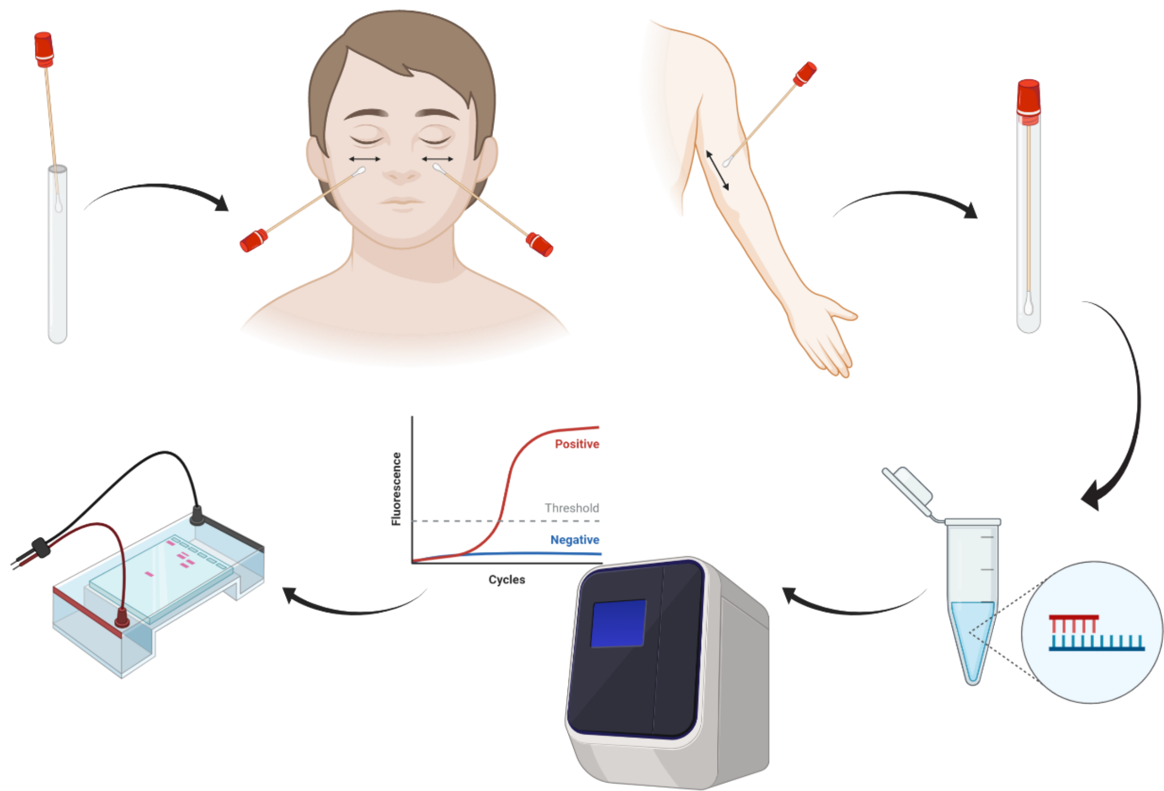


Figure 2-7. Procedure for the collection and analysis of skin swab samples. Ethanol wipes and cotton swabs were provided for participants at the beginning of the study. Samples were taken from both sides of the cheek and inner upper arm. Real-time polymerase chain reaction (qPCR) was performed on samples to determine mitochondrial DNA damage. Gel electrophoresis was finally performed to check that the correct product was produced by qPCR. Image created with BioRender.com

2.2.4.3.1 83bp housekeeping real-time polymerase chain reaction (qPCR)

DNA samples extracted from skin swabs were analysed on QuantStudio™ 3 0.2 mL Real-Time PCR System (Product code: A28137, ThermoFisher Scientific, UK).

Short 83bp mtDNA sections were amplified using the JumpStart SYBR Green Kit (Sigma-Aldrich, UK). This allows the determination of mtDNA copy number. Components listed in

Table 2-1 were added to each well of a 0.2 mL MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Product code: 4483354, ThermoFisher Scientific, UK) to a final volume of 25 µl and sealed with MicroAmp™ Clear Adhesive Film (Product code: 4306311, ThermoFisher Scientific, UK). Run conditions are shown in Table 2-2.

Table 2-1. Reagents for 83bp real-time PCR using previously established protocol (Koch et al., 2001).

Component	**Volume (µl)**
UltraPure™ DNase/RNase-Free Distilled Water (Product code: 10977035, ThermoFisher Scientific, UK)	8.25
1x SYBR® Green JumpStart™ Taq ReadyMix™ (Product code: S4438-500RXN, Sigma-Aldrich, UK)	12.50
100x ROX Reference dye (Product code: S4438-500RXN, Sigma-Aldrich, UK)	0.25
10 µM of forward primer (Eurofins genomics, Germany)	1.00
10 µM of reverse primer (Eurofins genomics, Germany)	1.00
DNA template	2.00
Total	**25.00**

The template DNA was amplified according to previously established settings:

Table 2-2. Previously established amplification settings for 83bp real-time PCR.

Stage	**Temperature (°C)**	**Time (minutes)**	**Cycles**
Initial denaturation	94	2	1
Denaturation	94	0.25	35
Annealing	60	0.75	
Extension	72	0.75	
Final extension	72	2	1
Melt curve was produced with the same condition			

2.2.4.3.2 1kb strand break qPCR

After the copy number had been determined, longer mtDNA sections of 1kb were amplified. This allows the detection of mtDNA damage. Components listed in

Table 2-3 were added to each well of a 0.2 mL MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Product code: 4483354, ThermoFisher Scientific, UK) to a final volume of 20 µl and sealed with MicroAmp™ Clear Adhesive Film (Product code: 4306311, ThermoFisher Scientific, UK). Run conditions are shown in Table 2-4.

Table 2-3. Reagents for 1kb real-time PCR using a previously established protocol (Rothfuss et al., 2010).

Component	**Volume (µl)**
UltraPure™ DNase/RNase-Free Distilled Water (Product code: 10977035, ThermoFisher Scientific, UK)	Confidential
2x Bioline SensiMix SYBR Low-ROX (Product code: QT62505, Scientific laboratory supplies, UK)	Confidential
10 µM of forward primer (Eurofins genomics, Germany)	Confidential
10 µM of reverse primer (Eurofins genomics, Germany)	Confidential
DNA template	Confidential
Total	**20.0**

The template DNA was amplified according to previously established settings:

Table 2-4. Previously established amplification settings for 1kb real-time PCR.

Stage	**Temperature (°C)**	**Time (minutes)**	**Cycles**
Initial denaturation	95	10	1
Denaturation	95	0.25	40
Annealing	60	0.25	
Extension	72	1.25	
Final extension	72	7	1
Melt curve was produced with the same condition			

2.2.4.3.3 Gel electrophoresis

Gel electrophoresis was used to confirm the correct length of the product had been amplified. Samples were selected randomly from each plate and all samples with a cycle threshold greater than 30 were also selected for gel electrophoresis. Amplified qPCR products were analysed on Gel-Red (Product code: SCT122, Merck) stained 3% w/v (for 83bp products) and 0.8% w/v (for 1kb products) agarose gels. Molecular weight markers used were the Low Molecular Weight DNA Ladder (Product code: N3233L, New England Biolabs) and GelPilot High Range Ladder (Product code: 239145, Qiagen) for 83bp and 1kb, respectively. All gels were visualised using the Li-Cor Odyssey Fc system.

2.2.4.4 Hair collection and RNA extraction

Hair follicle collection was performed by Gewei Zhu, while RNA extraction and sequencing was performed by Lexogen GmbH (Vienna, Austria). Finally, statistical analysis was performed by Gewei Zhu with guidance provided by Dr David Gunn.

2.2.4.4.1 Hair follicle sample collection

Approximately 25 anagen hair follicles were collected per participant. Individual or groups of 2-3 pigmented anagen hairs were plucked at a time using ethanol decontaminated forceps, all surfaces were wiped with RNaseZap™ RNase Decontamination Solution (Product code: AM9780, ThermoFisher Scientific, UK). The site of collection varied across the scalp. Anagen hair follicles were placed into the labelled sterile tube containing 1.5 mL RNAlater™ Stabilization Solution (product code: AM7020, ThermoFisher Scientific) and sealed with parafilm, then stored in a -20^0 C freezer before shipping to Lexogen GmbH (Vienna, Austria) on dry ice.

2.2.4.4.2 RNA-sequencing library construction, sequencing and alignment

Lexogen performed RNA extraction on 10-15 hair follicles from each sample and were of high quality; therefore, double-stranded complementary (cDNA) libraries were prepared. All cDNA samples were processed as per manufacturer's instructions and sequencing was conducted on an Illumina NextSeq 2000 Sequencing System.

2.2.4.4.3 Gene expression analyses and statistics

Read counts provided by Lexogen were statistically analysed using GeneSpring (14.9.1-GX, Aligent, Santa Clara, CA, USA). During the pre-processing step, the raw signals threshold was set to 1.0 and a quantile normalisation algorithm was used. Baseline transformation to median was applied to all samples. Two-way ANOVA was used to investigate the interaction between the gender and the patient and healthy control groups. Genes with $p<0.05$ were considered statistically significant. Moderated t-test was used to compare patients and controls in males and females separately. Individual genes with Westfall Young Permeative adjusted p-value <0.2 were considered statistically significant. In addition, a list of genes with unadjusted $P<0.05$ were used for pathway analyses.

2.2.4.4.4 Pathway analyses and statistics

Pathway analysis was carried out with the significantly altered genes using QIAGEN's Ingenuity pathway analysis (IPA) (http://www.ingenuity.com). Log expression ratio was used with a 1.2-

fold change threshold and $p<0.02$ was considered statistically significant. IPA canonical analysis identified the pathways that were most significantly enriched in the patients versus controls gene lists. There were three ways of measuring the significance of the association between the dataset and the canonical pathway: (1) a ratio of the number of molecules from the dataset that map to the pathway divided by the total number of molecules that map to the canonical pathway is displayed, (2) a Z-score displaying the upregulation or downregulation of genes in that respective canonical pathway, and (3) a P-value calculated using the Fisher's exact test was used to determine the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone. IPA upstream regulator analysis is based on prior knowledge of expected effect using the Ingenuity Knowledge Base. It examines how many known targets of each transcription regulator were present in the dataset. It then compares their direction of change to what is expected from the literature which predicts the likely relevant transcriptional regulators. For each potential transcriptional regulator, two statistical measures were computed: (1) an overlap p-value calculated using the Fisher's exact test which identifies transcriptional regulators that are able to explain observed gene expression changes, and (2) an activation z-score which infers the activation states of predicted transcriptional regulators (systems).

2.2.5 Statistical analysis:

Statistical analysis was carried out using IBM SPSS Statistics version 27 (Armonk, New York, USA). Sleep durations detected by the three methods of sleep detection were compared using the Kruskal-Wallis H test. This method was also used for comparing cortisol and melatonin levels between participants in different sleep groups. Mann-Whitney U test was used to make comparisons between two methods of sleep detection, as well as comparing the patient groups to the control group. Wilcoxon signed-rank test was used when comparing cortisol levels in samples taken at home to those taken at the hospital. Spearman rank correlation test was used to investigate the correlation between melatonin, cortisol and mtDNA damage levels. In all analyses, the Monte Carlo method with a 99% confidence level was used to estimate the exact significant level. P values less than 0.05 were considered statistically significant.

Chapter 3. Exploration of sleep as a specific risk factor for poor health: a UK Biobank study of >84,000 participants

3.1 Chapter overview & aims

The sleep/wake cycle is controlled by the daily circadian rhythm. Sleep is known to have many crucial functions including energy conservation, metabolic pathway regulation and memory consolidation. The recommended sleep duration is typically 7-9 hours/night for an adult but it changes significantly with age and there is much individual variation. However, chronic partial sleep deprivation is very common in modern society due to longer working hours and more entertainment and social events. Chronic partial sleep deprivation has many adverse effects on health, for example, it is a strong risk factor for cardiovascular and metabolic diseases including type 2 diabetes. It is also associated with worse cognitive performance and mood. Many studies have demonstrated the beneficial effects of sleep extension in sleep-deprived individuals.

The UK Biobank collected extensive information regarding sociodemographics, health, lifestyle, mood and cognition amongst participants recruited from the general UK population. Both self-reported and accelerometer assessed sleep data are available. Primary care health records were released on approximately 45% of the participants in 2019. Prior studies carried out using the UK Biobank were mostly based on self-reported sleep duration. However, it is subject to bias due to sleep state misperception where people with sleep disorders tend to underestimate their sleep duration while people without sleep disorders tend to overestimate their sleep duration. Therefore, to overcome this limitation, this study utilised sleep data extracted from wrist-worn accelerometers.

The main aim of this chapter is to investigate the association between objectively assessed sleep duration and health-related characteristics including cognitive function and both self-reported physical health and GP records of diseases. Additionally, it also examines the relationship between sleep and lifestyle habits, cognition and mood. Finally, this study aims to evaluate whether objective measures of poor sleep is a valid predictor for subsequent worse health using sensitivity, specificity and predictive values.

Portions of this chapter have already been published in PlosOne (Zhu et al., 2019) and Nature and Science of sleep (Zhu G, 2021).

3.2 Results

Data were received on 502,459 participants- 54% females and 46% males; the age of these participants was 56.5 (±8.10) years (Figure 3-1A). After excluding those with un-processable accelerometer data, 84,411 participants were included in the analysis- 56% female and 44% male, the age of these participants was 62.4 (±7.8) years (Figure 3-1B).

Out of these participants, 14,635 (17.34%) slept <5 hours/night, 21,889 (25.93%) slept 5-6 hours/night, 28,236 (33.45%) slept 6-7 hours/night, 15,757 (18.67%) slept 7-8 hours/night and 3,894 (4.61%) slept >8 hours/night. The average sleep efficiency of these participants was 82.13% (±11.47%). Finally, 76,603 (90.8%) participants were White/British, while the remaining 9.2% participants belong to other ethnicity groups.

3.2.1 Sociodemographic characteristics

Sociodemographic characteristics across the sleep groups are summarized in Table 3-1. It is apparent from this table that the percentage of males with extremely short sleep duration (<5 hours/night) was significantly higher compared to other sleep groups, while females were more likely to sleep ≥7 hours/night. A greater percentage of participants were found in the '60-69 years' age group. Amongst the oldest age group, a significantly higher percentage of participants slept <5 hours/night. A closer inspection of Table 3-1 shows that the majority of participants had a BMI between 25 and 29.9 kg/m^2. This is slightly higher than the ideal range and those with a higher BMI tend to have shorter sleep duration. Additionally, according to the Townsend deprivation index, socioeconomic status increased across the sleep groups up to the second quintile then it decreased across the groups. Finally, as Table 3-1 shows, most of the participants in this study were White/British and they were more likely to have a sleep duration of ≥7 hours/night. For other ethnicities, a significantly higher percentage of participants were found in the '<5 hours' sleep group.

Those with the shortest sleep duration (<5 hours/night) were 115%, 15%, 100%, 221% and 89% more likely to be male, over the age of 70 years, have a high BMI, lived in the most deprived area or belongs to an ethnic minority group respectively, compared with the '7-8 hours' sleep group (Table 3-2).

The relationship between sleep efficiency and various sociodemographic characteristics is illustrated in Figure 3-2. Females appeared to have significantly higher sleep efficiency compared to males ($p < 0.001$). However, it was very similar across the different age groups

(p= 0.005). When comparing each age group, the difference between most of them was not found to be statistically significant, except that those in the ’60-69 years’ age group had slightly higher sleep efficiency than participants in the ’50-59 years’ age group. Moreover, sleep efficiency seemed to decrease as the BMI increased (p< 0.001). Participants with a BMI of >30 kg/m^2 had significantly lower sleep efficiency than those with a BMI of 18.5-24.9 kg/m^2 (p< 0.001) and 25-29.9 kg/m^2 (p= 0.001). Additionally, sleep efficiency steadily decreased across the Townsend deprivation index groups (p= 0.068). Surprisingly, after Bonferroni correction of p-values, those who were least deprived had significantly higher sleep efficiency than other groups, except for when compared to the most deprived group. Finally, when investigating sleep efficiency amongst different ethnicity groups (p= 0.002), participants in the ‘White/British’ group had significantly higher sleep efficiency when compared to the ‘Black/African’ and ‘Chinese’ ethnicity groups (p= 0.006 and 0.010, respectively).

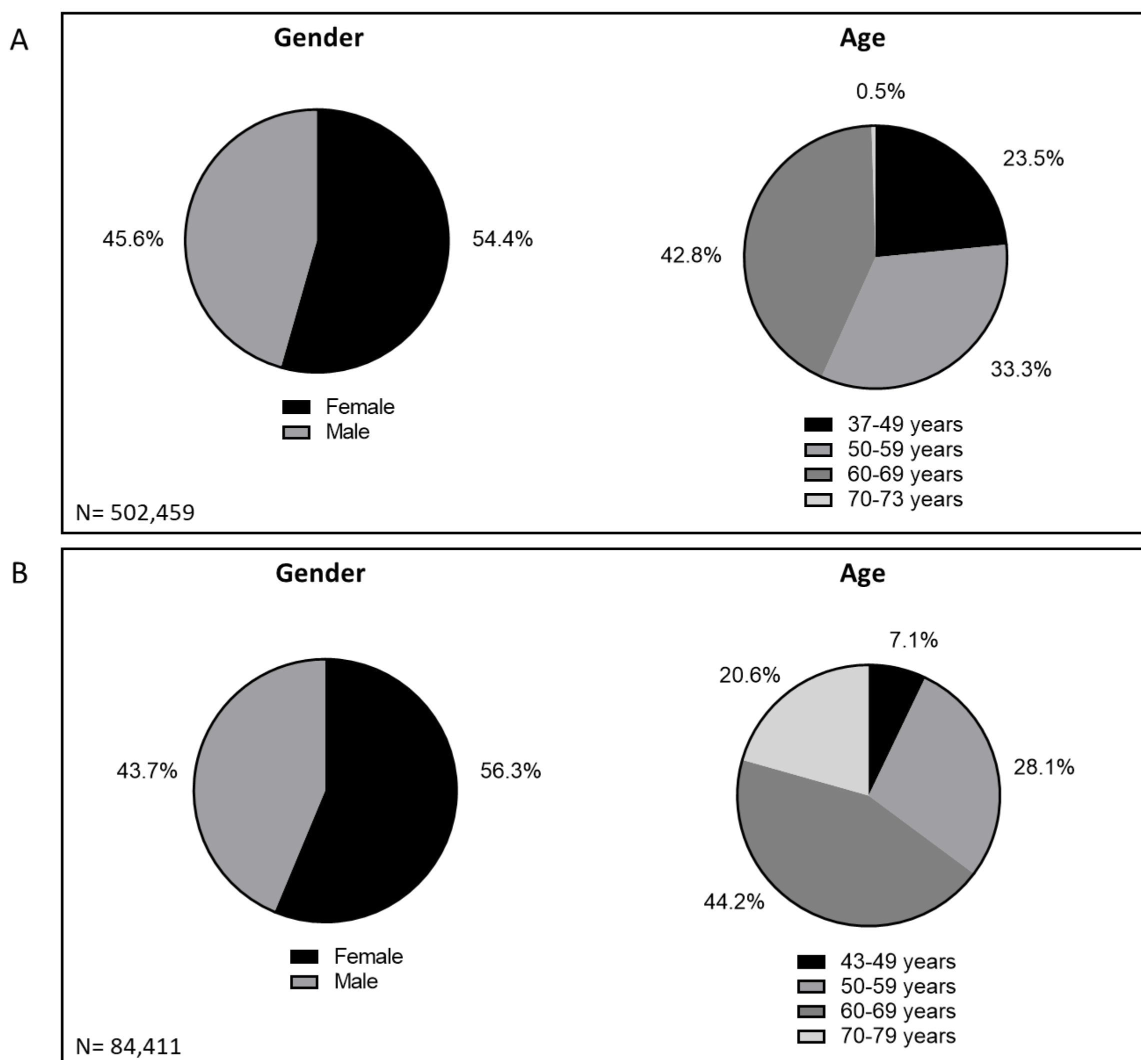


Figure 3-1. Distribution of the total UK biobank participants (A) and participants with available, processable accelerometer data (B) across gender and age groups.

Table 3-1. Summary of sociodemographic characteristics across the five sleep groups (n= 84,411).

	<5 hours (n=14,635)	**5-6 hours (n=21,889)**	**6-7 hours (n=28,236)**	**7-8 hours (n=15,757)**	**>8 hours (n=3,894)**
Gender (n)	**14,635**	**21,889**	**28,236**	**15,757**	**3,894**
% Female	45.2 a	52.7 b	59.6 c	63.9 d	64.6 d
% Male	54.8 a	47.3 b	40.4 c	36.1 d	35.4 d
Age (n), years	**14,635**	**21,889**	**28,236**	**15,757**	**3,894**
43-49 (%)	6.1 a	7.7 b	7.5 b	7.0 b,c	6.1 a,c
50-59 (%)	26.9 a	29.8 b	28.7 c	26.3 a	25.7 a
60-69 (%)	43.3 a,b	42.5 b	44.5 a,c	45.9 c,d	47.0 d
70-79 (%)	23.7 a	19.9 b,c	19.3 c	20.8 b	21.2 b,c
BMI (n), kg/m^2	**1,593**	**21,838**	**28,182**	**15,728**	**3,885**
<18.5 (%)	0.5 a	0.5 a	0.7 a	0.6 a	0.7 a
18.5-24.9 (%)	29.8 a	37.1 b	41.4 c	43.5 d	43.5 c,d
25-29.9 (%)	42.0 a,b	42.1 b	40.6 a,c	40.4 c	39.9 a,b,c
>30 (%)	27.8 a	20.4 b	17.3 c	15.5 d	15.9 c,d
Townsend deprivation quintile (n)	**14,624**	**21,857**	**28,209**	**15,738**	**3,890**
0 (Least deprived) (%)	38.6 a	40.6 b	43.7 c	44.8 c	45.1 c
1 (%)	37.3 a	37.8 a	37.0 a	37.6 a	36.0 a
2 (%)	16.5 a	15.9 a,b	14.6 c	13.4 d	14.2 b,c,d
3 (%)	6.8 a	5.3 b	4.4 c	4.0 c	4.3 b,c
4 (Most deprived) (%)	0.8 a	0.4 b	0.3 b	0.3 b	0.4 b
Ethnicity (n)	**14,635**	**21,889**	**28,236**	**15,757**	**3,894**
White/British (%)	91.8 a	93.6 b	94.9 c	95.8 d	96.2 d
Mixed (%)	0.3 a	0.2 b	0.2 a,b	0.1 b	0.1 b
Asian (%)	0.3 a	0.2 a,b	0.1 c	0.1 b,c	0.2 a,b,c
Black/African (%)	0.8 a	0.4 b	0.2 c	0.2 c	0.2 b,c
Chinese (%)	0.4 a	0.3 a,b	0.2 b,c	0.1 c	0.1 b,c
Other (%)	6.4 a	5.3 b	4.3 c	3.6 d	3.4 c,d

Percentages expressed are within each sleep group. Column proportions were compared using the z-test and p-values were corrected using the Bonferroni method. Therefore, if two columns were assigned the same letter then the difference between them was not statistically significant (p>0.05), but if two columns were assigned different letters then the difference between them was statistically significant (p<0.05). BMI= body mass index.

Table 3-2. OR (95% CI) of being male, aged over 70 years, high BMI, living in the most deprived area and being an ethnic minority across the different sleep groups (n= 84,411).

	Male		**Age >70 years**		**High BMI (>30kg/m^2)**		**Social deprivation**		**Ethnic minority**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
7-8 hours	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
<5 hours	2.15 (2.06 to 2.26)	<0.001	1.15 (1.08 to 1.21)	<0.001	2.00 (1.89 to 2.12)	<0.001	3.21 (2.24 to 4.61)	<0.001	1.89 (1.71 to 2.09)	<0.001
5-6 hours	1.62 (1.55 to 1.68)	<0.001	0.93 (0.88 to 0.98)	0.004	1.36 (1.29 to 1.44)	<0.001	1.58 (1.09 to 2.30)	0.016	1.44 (1.31 to 1.59)	<0.001
6-7 hours	1.21 (1.17 to 1.26)	<0.001	0.90 (0.86 to 0.95)	<0.001	1.13 (1.07 to 1.19)	<0.001	1.29 (0.89 to 1.87)	0.181	1.17 (1.06 to 1.29)	0.001
>8 hours	0.97 (0.90 to 1.04)	0.965	1.03 (0.95 to 1.12)	0.489	1.02 (0.93 to 1.13)	0.632	1.55 (0.86 to 2.81)	0.149	0.90 (0.75 to 1.08)	0.261

All logistic regression models were adjusted for age (reference = '43–49'); gender (reference = 'Female') and Townsend deprivation index (reference = 'Least deprived). Townsend deprivation index was divided into quintiles (from zero to four where zero represents the least deprived individuals). Ethnic minority is considered to be all ethnicity apart from White/British. The '7-8 hours' sleep group was used as the reference group. OR= odds ratio, CI= confidence interval, BMI= body mass index

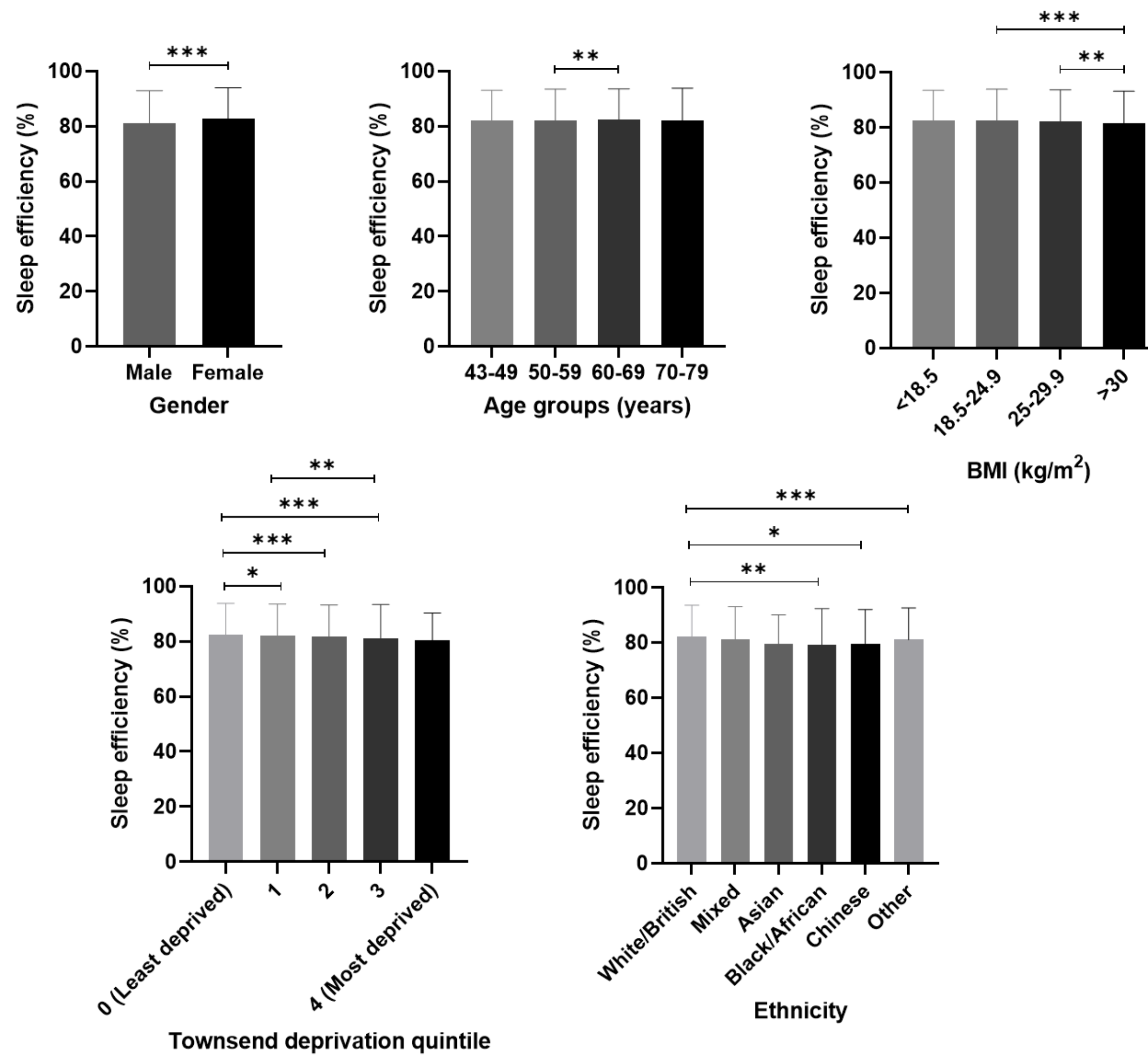


*Figure 3-2. Relationship between sleep efficiency and various sociodemographic characteristics. *P<0.05, **P<0.01, ***P<0.001. BMI= Body mass index.*

3.2.2 Anthropometric measurements

Table 3-3 compares various anthropometric measurements between the sleep groups. Waist circumference was significantly lower in those who slept ≥6 hours/night, regardless of their gender. The percentage of males with a waist circumference >102cm was the highest in the ‘<5 hours’ sleep group. A similar pattern was also observed amongst females, those in the ‘<5 hours’ sleep group were more likely to have a waist circumference >88cm. In both genders, no significant difference was detected between participants who slept ≥6 hours/night. Additionally, when looking at the waist to hip ratio as illustrated in Table 3-3, those in the ‘<5 hours’ sleep group had a significantly higher percentage of participants with a high waist to hip ratio. The same pattern was observed in both genders.

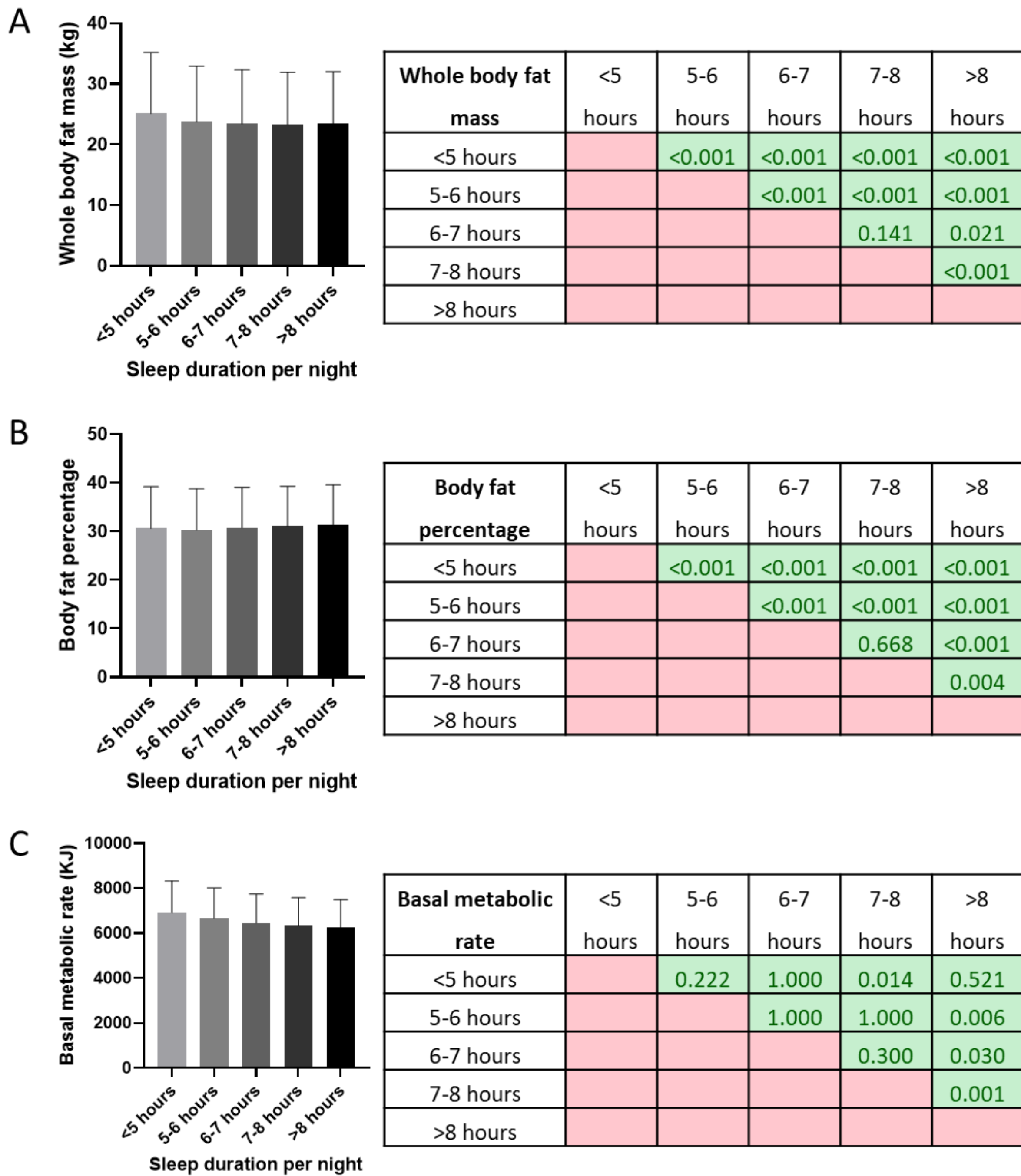
It can be seen from the data in Figure 3-3 that the whole-body fat mass was the highest in those who slept <5 hours/night ($p< 0.001$), and the body fat percentage increased slightly across the sleep groups ($p< 0.001$). However, data in Figure 3-3A and Figure 3-3B shows that the difference between those who slept 6-8 hours was not statistically significant. Finally, the basal metabolic rate decreased steadily across the sleep groups ($p< 0.001$). However, post-hoc results presented in Figure 3-3C show that only the difference between ‘<5 hours’ and ‘7-8 hours’ sleep groups was statistically significant ($p= 0.014$), as well as the difference between those who slept >8 hours and those who slept 5-6 hours, 6-7 hours and 7-8 hours ($p= 0.006$, 0.030 and 0.001, respectively). The difference between other groups was not found to be statistically significant.

Pairwise comparison results illustrated in Table 3-4 show that males in the ‘<5 hours’ sleep group were 95% and 62% more likely to have a high waist circumference (>102cm) and high waist to hip ratio (>0.9) respectively, compared to males who slept 7-8 hours/night. Similarly, females who slept <5 hours/night were 66% and 55% more likely to have a high waist circumference (>88cm) and high waist to hip ratio (>0.85) respectively, compared to females who sleep 7-8 hours/night (Table 3-4).

Table 3-3. Summary of various anthropometric measurements across the five sleep groups (n= 84,411).

	<5 hours (n=14,635)	**5-6 hours (n=21,889)**	**6-7 hours (n=28,236)**	**7-8 hours (n=15,757)**	**>8 hours (n=3,894)**
Waist circumference groups (males) (n)	**8,009**	**10,346**	**11,414**	**5,683**	**1,376**
<94cm (%)	37.8 a	45.4 b	48.4 c	49.4 c	49.8 c
94-102cm (%)	31.3 a	31.5 a	30.9 a	31.0 a	30.6 a
>102cm (%)	30.9 a	23.1 b	20.7 c	19.5 c	19.6 c
Waist circumference groups (females) (n)	**6,599**	**11,513**	**16,789**	**10,052**	**2,515**
<80cm (%)	36.6 a	43.4 b	47.0 c	48.2 c	48.4 c
80-88cm (%)	26.6 a	27.1 a	28.0 a	27.5 a	27.7 a
>88cm (%)	36.8 a	29.5 b	25.1 c	24.3 c	23.9 c
Waist-hip ratio groups (males) (n)	**14,605**	**21,856**	**28,200**	**15,734**	**3,891**
<0.899 (%)	54.1 a	64.0 b	69.1 c	72.1 d	71.0 c,d
>0.9 (%)	45.9 a	36.0 b	30.9 c	27.9 d	29.0 c,d
Waist-hip ratio groups (females) (n)	**14,605**	**21,856**	**28,200**	**15,734**	**3,891**
<0.8499 (%)	34.9 a	44.0 b	50.1 c	53.4 d	52.8 d
>0.85 (%)	65.1 a	56.0 b	49.9 c	46.6 d	47.2 d

Percentages expressed are within each sleep group. Column proportions were compared using the z-test and p-values were corrected using the Bonferroni method. Therefore, if two columns were assigned the same letter then the difference between them was not statistically significant (p>0.05), but if two columns were assigned different letters then the difference between them was statistically significant (p<0.05).



Whole body fat mass	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		<0.001	<0.001	<0.001	<0.001
5-6 hours			<0.001	<0.001	<0.001
6-7 hours				0.141	0.021
7-8 hours					<0.001
>8 hours					

Body fat percentage	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		<0.001	<0.001	<0.001	<0.001
5-6 hours			<0.001	<0.001	<0.001
6-7 hours				0.668	<0.001
7-8 hours					0.004
>8 hours					

Basal metabolic rate	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		0.222	1.000	0.014	0.521
5-6 hours			1.000	1.000	0.006
6-7 hours				0.300	0.030
7-8 hours					0.001
>8 hours					

Figure 3-3. Pairwise comparisons between each sleep group regarding (A) whole body fat mass, (B) body fat percentage and (C) basal metabolic rate. The analysis was controlled for age, gender and BMI.

Table 3-4. OR (95% CI) of having high waist circumference and waist to hip ratio across sleep groups in both males and females (n= 84,411).

	High waist circumference in males (>102cm)		**High waist circumference in females (>88cm)**		**High waist to hip ratio in males (>0.9)**		**High waist to hip ratio in females (>0.85)**	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
7-8 hours	1.00	-	1.00	-	1.00	-	1.00	-
<5 hours	1.95 (1.83 to 2.09)	<0.001	1.66 (1.57 to 1.74)	<0.001	1.62 (1.53 to 1.72)	<0.001	1.55 (1.46 to 1.65)	<0.001
5-6 hours	1.31 (1.23 to 1.40)	<0.001	1.25 (1.20 to 1.31)	<0.001	1.17 (1.11 to 1.24)	<0.001	1.17 (1.11 to 1.23)	<0.001
6-7 hours	1.09 (1.02 to 1.16)	0.012	1.05 (1.00 to 1.09)	0.049	1.07 (1.01 to 1.13)	0.017	1.05 (1.00 to 1.10)	0.074
>8 hours	0.98 (0.87 to 1.10)	0.741	0.98 (0.91 to 1.06)	0.625	1.10 (1.00 to 1.21)	0.057	1.07 (0.98 to 1.17)	0.147

The binary regression model was adjusted for age, gender and Townsend deprivation index. The '7-8 hours' sleep group was used as the reference group. OR= odds ratio, CI= confidence interval

3.2.3 Lifestyle characteristics

This study considered various lifestyle habits and results are summarised in Table 3-5. The majority of the participants considered themselves more as a morning than an evening person. Significantly more people in the ‘<5 hours’ sleep group found getting up in the morning very easy, while more people with a sleep duration of >8 hours/night found getting up in the morning not at all easy. In addition, participants with shorter sleep duration were more likely to take naps during the day. On the contrary, those with a sleep duration of ≥6 hours/night never or rarely nap during the day. More than half of the participants don’t have shift work jobs or jobs involving night shift work. The shorter the sleep duration, the higher the percentage of participants who reported snoring. As can be seen from Table 3-5, significantly more participants in the ‘<5 hours’ sleep group were either a current smoker or have been a smoker previously. On the other hand, significantly fewer participants in the ‘<5 hours’ sleep groups were current alcohol drinkers. However, they were more likely to drink daily or almost daily basis than those who slept >6 hours/night.

Finally, those who slept <5 hours/night were 117%, 26% and 47% significantly more likely to take naps during the day, experience snoring and be a current smoker respectively, compared to those in the ‘7-8 hours’ sleep group (Table 3-6).

Table 3-5. Percentage of participants with various lifestyle characteristics across the different sleep groups (n= 84,411).

	<5 hours (n=14635)	5-6 hours (n=21889)	6-7 hours (n=28236)	7-8 hours (n=15757)	>8 hours (n=3894)
Morning/evening person (chronotype) (n)	**14533**	**21776**	**28071**	**15665**	**3874**
Definitely a morning person (%)	24.9 a	24.0 a,b	22.9 b	21.5 c	19.3 d
More a morning than evening person (%)	30.9 a	32.4 b	34.7 c	35.3 c	40.9 c
More an evening than morning person (%)	24.5 a,b	24.4 b	24.5 a,b	25.4 a,b	26.5 a
Definitely an evening person (%)	9.6 a	9.1 a	7.5 b	7.6 b	7.2 b
Getting up in the morning (n)	**14533**	**21776**	**28071**	**15665**	**3874**
Not at all easy (%)	3.4 a,b	3.0 b	3.1 b	3.7 a	5.0 c
Not very easy (%)	12.8 a	13.2 a	13.8 a	14.9 b	16.7 b
Fairly easy (%)	48.7 a	50.5 b	52.0 c	52.3 c	51.0 a,b,c
Very easy (%)	34.9 a	33.3 b	31.1 c	29.0 d	27.3 d
Nap during the day (n)	**14315**	**21543**	**27772**	**15495**	**3815**
Never/ rarely (%)	51.4 a	59.5 b	62.8 c	64.1 c	62.8 c
Sometimes (%)	41.3 a	35.9 b	33.7 c	33.0 c	33.7 b,c
Usually (%)	7.3 a	4.6 b	3.5 c	3.0 d	3.5 c,d
Job involves shift work (n)	**8795**	**14041**	**17669**	**9297**	**2131**
Never/rarely (%)	83.1 a	86.6 b	87.9 c	88.2 c	86.0 b,c
Sometimes (%)	7.0 a	6.2 a,b	5.6 b	5.6 b	6.1 a,b
Usually (%)	2.2 a	1.5 b	1.4 b	1.3 b	1.5 a,b
Always (%)	7.4 a	5.3 b,c	5.0 b,c	4.8 c	6.3 a,b
Job involving night shift work (n)	**1486**	**1851**	**2131**	**1100**	**298**
Never/rarely (%)	46.0 a	50.7 a,b	53.8 b,c	57.9 c	53.4 a,b,c
Sometimes (%)	28.9 a	29.8 a	27.8 a	25.5 a	27.2 a
Usually (%)	9.2 a	7.3 a	7.1 a	6.5 a	6.8 a

	<5 hours (n=14635)	5-6 hours (n=21889)	6-7 hours (n=28236)	7-8 hours (n=15757)	>8 hours (n=3894)
Always (%)	15.4 a	11.8 b	10.9 b	9.8 b	12.4 a,b
Snoring (%)	**38.8 a**	**34.6 b**	**32.3 c**	**30.4 d**	**31.0 c,d**
Smoking status (n)	**14625**	**21879**	**28226**	**15751**	**3893**
Never (%)	51.9 a	56.0 b	58.4 c	59.7 c	58.8 c
Previous (%)	38.6 a	36.3 b	35.3 b,c	34.4 c	35.2 b,c
Current (%)	9.3 a	7.5 b	6.1 c	5.8 c	5.8 c
Alcohol drinker status (n)	**14624**	**21879**	**28226**	**15751**	**3893**
Never (%)	3.6 a	2.7 b	2.7 b	2.9 b	3.0 a,b
Previous (%)	3.4 a	2.8 b,c	2.5 c	2.4 c	3.3 a,b
Current (%)	92.9 a	94.4 b,c	94.8 c	94.7 b,c	93.6 a,b
Alcohol intake frequency (n)	**14624**	**21879**	**28226**	**15751**	**3893**
Daily or almost daily (%)	23.9 a	23.5 a	22.4 b	21.8 b	22.0 a,b
3/4 times a week (%)	24.4 a	25.3 a	26.7 b	26.7 b	24.7 a,b
1/2 times a week (%)	23.4 a	25.2 b	25.6 b	25.8 b	25.2 a,b
1-3 times a month (%)	10.9 a	11.1 a	10.9 a	10.7 a	11.7 a
special occasions only (%)	10.3 a	9.4 b	9.2 b	9.7 a,b	10.1 a,b
Never (%)	7.0 a	5.5 b,c	5.2 c	5.3 b,c	6.3 a,b

Percentages expressed are within each sleep group. Column proportions were compared using the z-test and p-values were corrected using the Bonferroni method. Therefore, if two columns were assigned the same letter then the difference between them was not statistically significant (p>0.05), but if two columns were assigned different letters then the difference between them was statistically significant (p<0.05).

Table 3-6. OR (95% CI) of having various lifestyle habits (n= 84,411).

	Morning person chronotype		**Always nap during the day**		**Snoring**		**Current smoker**		**Consume alcohol on a daily basis**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
7-8 hours	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
<5 hours	1.00 (0.95 to 1.04)	0.840	2.17 (1.94 to 2.43)	<0.001	1.26 (1.20 to 1.32)	<0.001	1.47 (1.35 to 1.61)	<0.001	1.03 (0.97 to 1.09)	0.33
5-6 hours	1.02 (0.98 to 1.06)	0.333	1.47 (1.32 to 1.65)	<0.001	1.12 (1.07 to 1.17)	<0.001	1.02 (1.10 to 1.31)	<0.001	1.07 (1.02 to 1.13)	0.007
6-7 hours	1.05 (1.01 to 1.09)	0.014	1.17 (1.04 to 1.31)	0.008	1.06 (1.02 to 1.11)	0.008	1.01 (0.93 to 1.09)	0.88	1.03 (0.98 to 1.08)	0.214
>8 hours	0.96 (0.90 to 1.03)	0.279	1.18 (0.97 to 1.43)	0.103	1.03 (0.96 to 1.12)	0.423	1.00 (0.86 to 1.16)	0.991	1.00 (0.92 to 1.09)	0.922

The binary regression model was adjusted for age, gender and Townsend deprivation index. The '7-8 hours' sleep group was used as the reference group. OR= odds ratio, CI= confidence interval

Using the accelerometer, the physical activity levels of those participants were monitored continuously throughout the day. This allows the determination of the most active and least active five hours of each day using the detected acceleration level measured in milli-g (Table 3-7). An inverse ‘U-shaped’ association between activity level and sleep duration was observed. The average acceleration level during the most active five hours of the day (M5) was found to be the highest in those who slept 6-7 hours and the lowest in participants who slept >8 hours/night (Figure 3-4A). On the other hand, the average acceleration level during the least active five hours of the day (L5) decreased across the sleep groups. It was the highest in those who slept <5 hours/night, while the difference between the other four sleep groups was relatively small. As a biomarker for healthy ageing, the difference between the most and least active five hours of the same day (ΔM5L5) was calculated from the M5 and L5 readings (Anderson et al., 2014). It was the highest in the ‘6-7 hours’ sleep group (Figure 3-4B) and significantly lower in those who slept <5 hours/night and >8 hours/night ($p < 0.001$).

Table 3-7. Association between the activity level and sleep duration (p<0.001) (n= 84,411).

	<5 hours	**5-6 hours**	**6-7 hours**	**7-8 hours**	**>8 hours**
Average acceleration during M5 (milli-g)	55.56 ± 22.76	58.21 ± 20.94	58.39± 20.70	57.39 ± 19.70	54.23 ± 18.98
Average acceleration during L5 (milli-g)	4.58 ± 3.11	3.76 ± 1.86	3.44 ± 1.94	3.27 ± 1.48	3.24 ± 2.11
ΔM5L5 (milli-g)	50.98 ± 22.32	54.45 ± 20.87	54.95± 20.59	54.11 ± 19.63	50.99 ± 18.44

Results are expressed as mean ± standard deviation

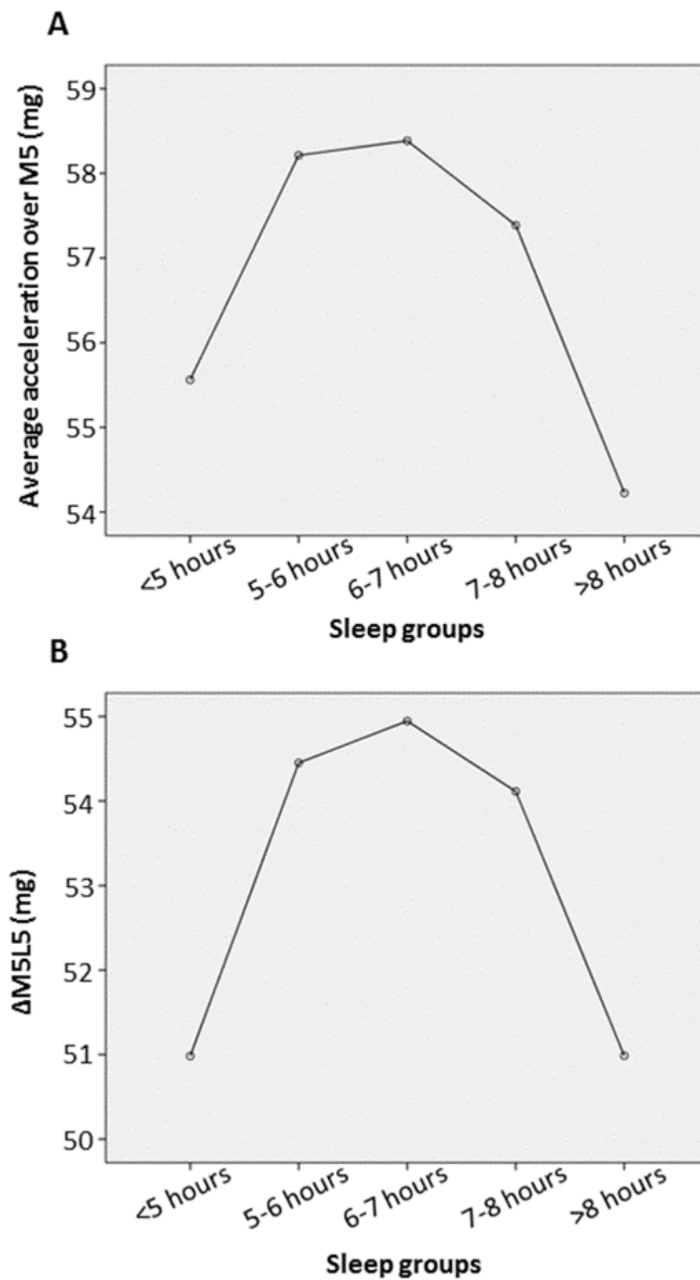


Figure 3-4. Distribution of acceleration measured in milli-g (mg) across the five sleep groups (p<0.001). (A) average acceleration over the most active five hours of the day (M5). (B) Difference in acceleration between the most active five hours and least active five hours (ΔM5L5).

The relationship between sleep efficiency and various lifestyle habits is illustrated in Figure 3-5. Sleep efficiency was very similar across the different chronotypes (p= 0.026). When comparing each group, the difference between the most of them was not found to be statistically significant, except that those who were 'definitely a morning person' had slightly lower sleep efficiency than participants who were 'more of a morning than evening person' (p= 0.019). Regardless of how participants rated how easy they were getting up in the morning, no statistically significant difference was detected between each group. Additionally, a significant association was detected between sleep efficiency and whether participants take naps during the day (p< 0.001). Those who never or rarely napped during the day had significantly higher sleep efficiency than those who sometimes or usually nap during the day. Finally, as illustrated in Figure 3-5 those who never or rarely have a job involving shift work had significantly higher sleep efficiency than those who usually have a job involving shift work (p= 0.002).

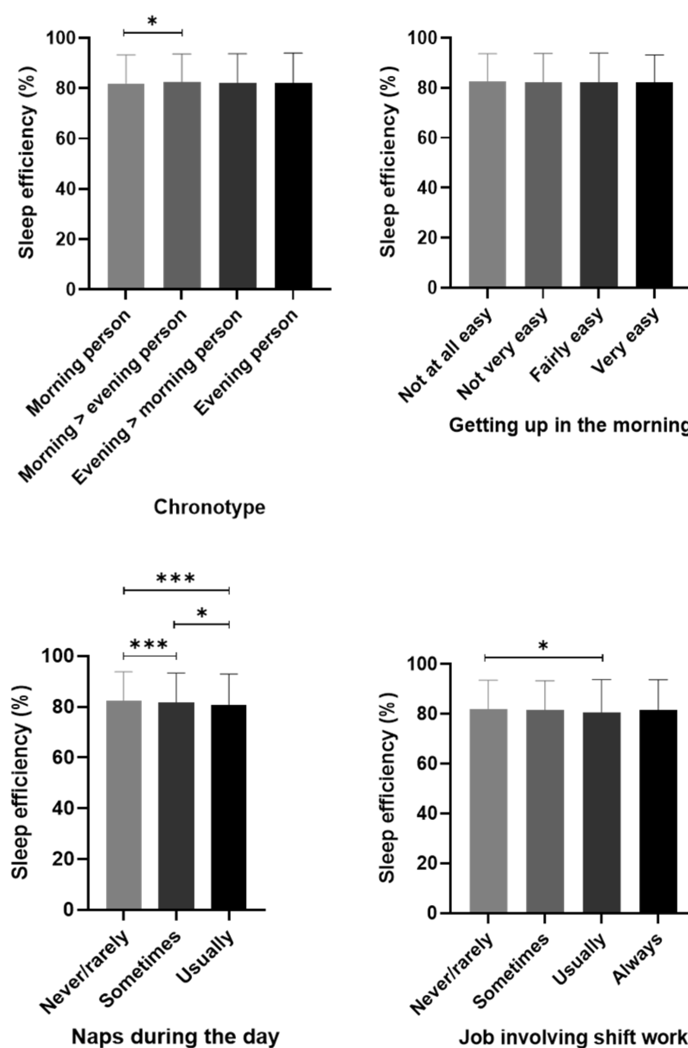


*Figure 3-5. Relationship between sleep efficiency and various lifestyle measurements. *P<0.05, **P<0.01, ***P<0.001*

3.2.4 Physical health

3.2.4.1 Self-reported disease characteristics

As shown in Table 3-8, a significantly higher percentage of participants who slept ≥7 hours/night never/rarely experience daytime dozing/sleepiness while those who slept <5 hours/night were more likely to experience it often. Sleeplessness/insomnia, on the other hand, was significantly higher in both short (<5 hours/night) and long (>8 hours/night) sleepers. When participants were asked to rate their overall health, the majority of participants who slept 5-8 hours rated their health as "excellent" and "good", while both short and long sleepers were more likely to select "fair" and "poor". Additionally, the percentage of participants with vascular/heart problems was significantly lower in those who slept 6-8 hours (Table 3-8). Finally, the bottom half of Table 3-8 compares the mean age that various metabolic diseases were diagnosed by a doctor. They were relatively similar across the different sleep groups and the difference between each group was not found to be statistically significant.

A 'U-shaped' relationship between sleep duration and various metabolic diseases is illustrated in Figure 3-6. Significantly more participants who slept <5 hours/night self-reported sleep apnoea. In addition, those who slept <5 hours/night were significantly more likely to report long-standing illness, cardiometabolic, cardiovascular diseases, diabetes and hypertension. However, the difference between those in the '6-7 hours' and '7-8 hours' sleep groups was not found to be statistically significant for all diseases.

As Table 3-9 shows, those in the '<5 hours' sleep group were 102%, 44%, 42%, 80% and 42% more likely to self-report sleep apnoea, cardiometabolic diseases, cardiovascular disease, diabetes and hypertension respectively, compared to those in the '7-8 hours' sleep group.

Table 3-8. Summary of health-related characteristics amongst different sleep groups (n= 84,411).

	<5 hours (n=14,635)	5-6 hours (n=21,889)	6-7 hours (n=28,236)	7-8 hours (n=15,757)	>8 hours (n=3,894)	P-value
Daytime dozing/sleeping (narcolepsy) (n)	**14,625**	**21,879**	**28,226**	**15,751**	**3,893**	
Never/rarely (%)	71.6 a	76.9 b	80.2 c	82.1 d	83.5 d	
Sometimes (%)	24.2 a	20.7 b	17.7 c	16.2 d	15.1 d	
Often (%)	3.9 a	2.3 b	1.9 c	1.6 c,d	1.2 d	
Sleeplessness/insomnia (n)	**14,625**	**21,879**	**28,226**	**15,751**	**3,893**	
Never/rarely (%)	24.6 a	27.0 b	25.6 a	24.4 a	22.1 c	
Sometimes (%)	45.4 a	47.3 b	48.1 b,c	48.9 c	49.0 b,c	
Usually (%)	29.9 a	25.7 b	26.3 b	26.6 b,c	28.8 a,c	
Overall health rating (n)	**14,284**	**21,512**	**27,723**	**15,466**	**3,809**	
Excellent (%)	18.3 a	22.1 b,c	22.9 c	22.3 b,c	20.3 a,b	
Good (%)	56.9 a	59.8 b	61.0 b,c	61.4 c	60.0 b,c	
Fair (%)	20.6 a	15.8 b	14.1 c	14.1 c	16.1 b	
Poor (%)	4.2 a	2.4 b	2.0 b	2.1 b	3.5 a	
Vascular/heart problems (n)	**14,304**	**21,526**	**27,747**	**15,486**	**3,808**	
Heart attack (%)	2.3 a	1.6 b	1.3 b,c	1.1 c	1.6 a,b,c	
Angina (%)	2.1 a	1.6 b	1.3 b	1.4 b	1.6 a,b	
Stroke (%)	1.1 a	0.9 a,b	0.8 b	0.8 b	1.0 a,b	
High blood pressure (%)	26.0 a	20.6 b	19.6 b	19.6 b	21.3 b	
None of the above (%)	68.3 a	75.2 b	76.9 c	77.0 c	74.3 b	

	<5 hours (n=14,635)	5-6 hours (n=21,889)	6-7 hours (n=28,236)	7-8 hours (n=15,757)	>8 hours (n=3,894)	P-value
Age heart attack diagnosed (mean±SD)	52.43 ± 7.922	53.47 ± 7.793	53.05± 8.168	54.04 ± 7.536	51.98 ± 8.321	0.388
Age stroke diagnosed (mean±SD)	50.90 ± 7.232	54.50± 7.422	54.54 ± 6.415	52.89 ± 10.493	51.50± 3.536	0.321
Age deep vein thrombosis (blood clot in leg) diagnosed (mean±SD)	43.46 ± 14.266	46.08 ± 12.672	44.75 ± 12.317	51.93 ± 14.638	50.31 ± 13.155	0.654
Age pulmonary embolism (blood clot in lung) diagnosed (mean±SD)	46.38 ± 12.777	45.53 ± 12.611	45.11 ± 13.141	45.27 ± 13.934	45.94 ± 15.585	0.999
Age high blood pressure diagnosed (mean±SD)	50.66 ± 9.655	50.23 ± 9.868	50.40 ± 9.970	50.55 ± 10.010	50.70 ± 9.741	0.254
Age diabetes diagnosed (mean±SD)	51.01 ± 11.924	51.23 ± 12.564	50.94 ± 12.792	50.61 ± 12.346	53.05± 9.556	0.533

Percentages expressed are within each sleep group. Column proportions were compared using the z-test and p-values were corrected using the Bonferroni method. Therefore, if two columns were assigned the same letter then the difference between them was not statistically significant (p>0.05), but if two columns were assigned different letters then the difference between them was statistically significant (p<0.05).SD= Standard deviation.

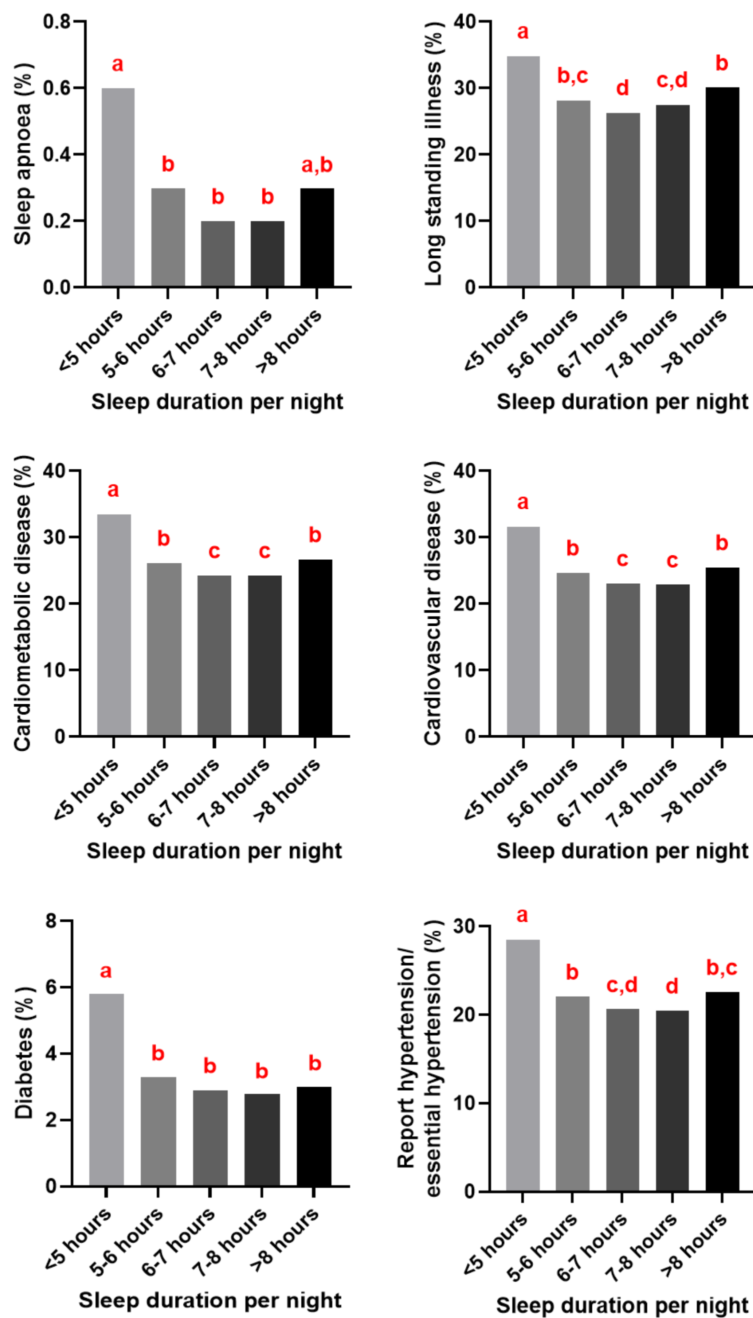


Figure 3-6. 'U-shaped' graphs demonstrating the relationship between sleep duration and metabolic health. Percentages expressed are within each sleep group. If two bars are assigned the same letter then the difference between them was not statistically significant (p>0.05), but if two bars are assigned different letters then the difference between them was statistically significant (p<0.05).

Table 3-9. OR (95% CI) of having sleep apnoea, long-standing illness, cardiometabolic disease, cardiovascular disease, diabetes and hypertension (n= 84,411).

	Sleep apnoea		**Long-standing illness**		**Cardiometabolic disease**		**Cardiovascular disease**		**Diabetes**		**Hypertension**	
	OR (95% CI)	**P-values**	**OR (95% CI)**	**P-values**	**OR (95% CI)**	**P-values**	**OR (95% CI)**	**P-values**	**OR (95% CI)**	**P-values**	**OR (95% CI)**	**P-values**
7-8 hours	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
<5 hours	2.02 (1.37 to 3.02)	0.001	1.18 (0.66 to 2.13)	0.572	1.44 (1.37 to 1.52)	<0.001	1.42 (1.35 to 1.50)	<0.001	1.80 (1.60 to 2.03)	<0.001	1.42 (1.35 to 1.50)	<0.001
5-6 hours	1.27 (0.84 to 1.91)	0.264	0.83 (0.47 to 1.49)	0.536	1.09 (1.04 to 1.15)	<0.001	1.09 (1.04 to 1.14)	<0.001	1.11 (0.98 to 1.25)	0.102	1.09 (1.04 to 1.15)	0.001
6-7 hours	0.99 (0.65 to 1.50)	0.948	1.19 (0.71 to 2.00)	0.515	1.02 (0.97 to 1.07)	0.491	1.02 (0.97 to 1.07)	0.447	1.01 (0.90 to 1.14)	0.817	1.02 (0.97 to 1.08)	0.371
>8 hours	1.20 (0.59 to 2.42)	0.619	1.72 (0.79 to 3.76)	0.174	1.13 (1.04 to 1.23)	0.003	1.15 (1.05 to 1.25)	0.001	1.06 (0.86 to 1.30)	0.598	1.12 (1.03 to 1.22)	0.010

The binary regression model was adjusted for age, gender and Townsend deprivation index. The '7-8 hours' sleep group was used as the reference group. OR= odds ratio, CI= confidence interval

3.2.4.2 Primary care disease data

The prevalence of diseases expressed as percentages are presented in Table 3-10. It is apparent that the prevalence rate is relatively low for many diseases. The least prevalent disease group was "Schizophrenia/schizotypal/delusional diseases", which has a prevalence rate of 0.11% (n= 96), while the most prevalent disease was "Hypertensive diseases", which has a prevalence rate of 17.63% (n= 14,880).

Table 3-11 summarises the number of participants with certain diseases in each sleep group. A "U-Shaped" curve was observed in most diseases as illustrated in Figure 3-7. Those who slept <5 hours/night were significantly more likely to have diabetes, disorders of glucose regulation, obesity, mental disorder, and various heart diseases. On the other hand, those who slept >8 hours/night were more likely to have schizophrenia, mood disorders, pulmonary heart disease and cerebrovascular heart disease. However, closer inspection of the table showed no significant difference was detected between the '6-7 hours' and '7-8 hours' sleep groups in all diseases.

It can be seen from the data in Table 3-12 that compared to those who slept 7-8 hours/night, participants who slept <5 hours/night were 58%, 48%, 106% and 44%, more likely to have diabetes, obesity and other hyperalimentation, organic mental disorder and mood disorders, respectively. When assessing cardiovascular health, those who slept <5 hours/night were 36%, 25%, 33%, 27%, 37% and 36% more likely to have hypertensive diseases, ischaemic heart diseases, pulmonary heart diseases, cerebrovascular heart diseases, other forms of heart diseases, and diseases of arteries, arterioles and capillaries, respectively.

On the other hand, those with longer sleep durations (>8 hours/night) were 116%, 461% and 55% more likely to have an organic mental disorder, schizophrenia/schizotypal/delusional disorders and mood disorders respectively, when compared to those who slept 7-8 hours/night. They were also 11%, 17%, 72%, 22%, 58% and 32% more likely to have hypertensive diseases, ischaemic heart diseases, pulmonary heart diseases, cerebrovascular heart diseases, other forms of heart diseases, and diseases of arteries, arterioles and capillaries respectively, compared with those in the '7-8 hours' sleep group (Table 3-12).

Table 3-10. Number of participants with various mental and metabolic diseases extracted from primary care records.

ICD Disease categories	Frequency (n)	Prevalence (%)
Diabetes	3,322	3.94
Disorders of glucose regulation and pancreatic internal secretion	120	0.14
Obesity and other hyperalimentation	2,314	2.74
Organic mental disorder	173	0.20
Schizophrenia/schizotypal/delusional disorders	96	0.11
Mood disorder	2,175	2.58
Hypertensive diseases	14,880	17.63
Ischaemic heart diseases	5,297	6.28
Pulmonary heart diseases	762	0.90
Other form of heart disease	4,173	4.94
Cerebrovascular disease	1,207	1.43
Diseases of arteries, arterioles and capillaries	1,233	1.46

Table 3-11. Percentage of participants with various diseases in each sleep group (n= 84,411).

	<5 hours (n=14,632)	**5-6 hours (n=21,889)**	**6-7 hours (n=28,233)**	**7-8 hours (n=15,757)**	**>8 hours (n=3,893)**
Diabetes (%)	6.6 a	3.7 b	3.3 b,c	3.1 c	3.2 b,c
Disorders of glucose regulation and pancreatic internal secretion (%)	0.2 a	0.1 a,b	0.1 b	0.1 a,b	0.2 a,b
Obesity and other hyperalimentation (%)	4.5 a	2.8 b	2.3 c	2.1 c	2.0 c
Organic mental disorder (%)	0.3 a	0.1 b	0.2 b	0.2 b	0.3 a,b
Schizophrenia/schizotypal/delusional disorders (%)	0.1 a	0.1 a	0.1 a	0.1 a	0.5 b
Mood disorder (%)	3.6 a	2.3 b	2.1 b	2.5 b	3.9 a
Hypertensive diseases (%)	23.5 a	17.2 b	16.0 c	15.7 c	17.2 b,c
Ischaemic heart diseases (%)	8.4 a	6.5 b	5.5 c	5.4 c	6.3 b,c
Pulmonary heart diseases (%)	1.2 a	0.9 b	0.8 b	0.8 b	1.4 a
Other form of heart disease (%)	6.6 a	4.9 b	4.6 b,c	4.1 c	5.0 b,c
Cerebrovascular disease (%)	1.8 a	1.5 a,b	1.3 b	1.2 b	1.9 a
Diseases of arteries, arterioles and capillaries (%)	1.9 a	1.5 a,b	1.3 b,c	1.2 c	1.6 a,b,c

ercentage expressed are within each sleep group. Column proportion was compared using a z-test and p-values were corrected using the Bonferroni method. Therefore, if two columns were assigned the same letter, then the difference between them was not statistically significant (p>0.05). However, if two columns were assigned different letters, then the difference between them was statistically significant (p<0.05).

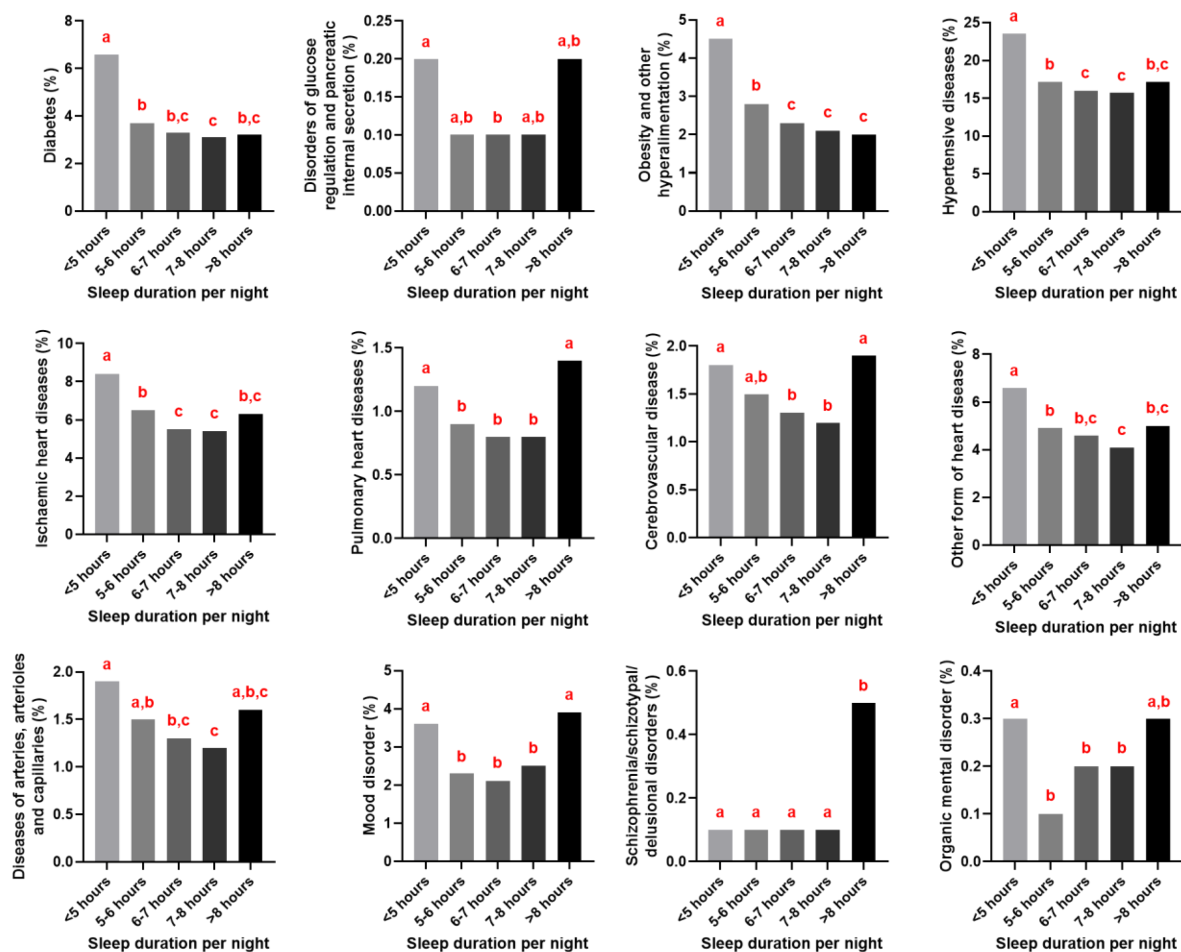


Figure 3-7. A "U-shaped" relationship between disease characteristics and sleep duration. Percentages expressed are within each sleep group. If two bars were assigned the same letter then the difference between them was not statistically significant (p>0.05), but if two bars were assigned different letters then the difference between them was statistically significant (p<0.05).

Table 3-12. OR (95%CI) of having various diseases diagnosed by doctors (n= 84,411).

	7-8 hours	<5 hours		5-6 hours		6-7 hours		>8 hours	
	Odds Ratio (95%CI)	**Odds Ratio (95%CI)**	**P-value**	**Odds Ratio (95%CI)**	**P-value**	**Odds Ratio (95%CI)**	**P-value**	**Odds Ratio (95%CI)**	**P-value**
Diabetes	1.00	1.58 (1.40 to 1.77)	<0.001	1.05 (0.93 to 1.18)	0.436	1.02 (0.91 to 1.15)	0.679	1.02 (0.83 t0 1.25)	0.883
Disorders of glucose regulation and pancreatic internal secretion	1.00	1.72 (0.97 to 3.07)	0.064	1.18 (0.66 to 2.11)	0.575	0.82 (0.45 to 1.48)	0.505	1.80 (0.78 to 4.14)	0.169
Obesity and other hyperalimentation	1.00	1.48 (1.28 to 1.70)	<0.001	1.12 (0.97 to 1.29)	0.115	1.04 (0.90 to 1.19)	0.613	0.92 (0.71 to 1.19)	0.524
Organic mental disorder	1.00	2.06 (1.26 to 3.37)	0.004	0.91 (0.53 to 1.56)	0.733	1.32 (0.82 to 2.14)	0.256	2.16 (1.10 to 4.25)	0.026
Schizophrenia/schizotypal/delusional disorders	1.00	1.36 (0.68 to 2.72)	0.383	0.84 (0.42 to 1.70)	0.627	0.95 (0.49 to 1.83)	0.877	5.61 (2.81 to 11.21)	<0.001
Mood disorder	1.00	1.44 (1.26 to 1.65)	<0.001	0.89 (0.78 to 1.02)	0.093	0.84 (0.74 to 0.95)	0.001	1.55 (1.28 to 1.87)	<0.001
Hypertensive diseases	1.00	1.36 (1.28 to 1.44)	<0.001	1.07 (1.01 to 1.13)	0.025	1.03 (0.97 to 1.09)	0.312	1.11 (1.01 to 1.22)	0.039
Ischaemic heart diseases	1.00	1.25 (1.14 to 1.37)	<0.001	1.13 (1.04 to 1.24)	0.007	1.01 (0.93 to 1.10)	0.819	1.17 (1.01 to 1.36)	0.040
Pulmonary heart diseases	1.00	1.33 (1.05 to 1.68)	0.017	1.04 (0.83 to 1.31)	0.712	0.97 (0.78 to 1.22)	0.818	1.72 (1.25 to 2.38)	0.001

	7-8 hours	<5 hours		5-6 hours		6-7 hours		>8 hours	
	Odds Ratio (95%CI)	**Odds Ratio (95%CI)**	**P-value**	**Odds Ratio (95%CI)**	**P-value**	**Odds Ratio (95%CI)**	**P-value**	**Odds Ratio (95%CI)**	**P-value**
Other form of heart disease	1.00	1.37 (1.24 to 1.52)	<0.001	1.16 (1.05 to 1.28)	0.004	1.11 (1.01 to 1.23)	0.032	1.22 (1.03 to 1.44)	0.020
Cerebrovascular disease	1.00	1.27 (1.05 to 1.54)	0.013	1.17 (0.98 to 1.41)	0.086	1.05 (0.88 to 1.25)	0.609	1.58 (1.20 to 2.07)	0.001
Diseases of arteries, arterioles and capillaries	1.00	1.36 (1.13 to 1.65)	0.001	1.26 (1.05 to 1.51)	0.014	1.14 (0.96 to 1.36)	0.144	1.32 (0.98 to 1.76)	0.065

The binary regression model was adjusted for age, gender and Townsend deprivation index. The '7-8 hours' sleep group was used as the reference group. OR= odds ratio, CI= confidence interval

Those with less optimal sleep were 42%, 73% and 40% more likely to have diabetes, glucose dysregulation and obesity, as well as 77%, 110% and 68% more likely to have an organic mental disorder, schizophrenia/schizotypal/delusional disorders and mood disorders respectively, when compared to those with optimal sleep. They were also 22%, 12%, 37% and 13% more likely to have hypertensive diseases, ischaemic heart diseases, pulmonary heart diseases, and other forms of heart diseases respectively, compared with those with optimal sleep (Table 3-13).

Table 3-13. Binary regression model showing odds ratio of various diseases between those with optimal/good sleep and those with less optimal/poor sleep (n= 84,411).

Primary care data code	Optimal sleep (OR)	Less optimal sleep (OR, 95% CI)	P-value
Diabetes	1.00	1.42 (1.32 to 1.53)	<0.001
Disorders of glucose regulation and pancreatic internal secretion	1.00	1.73 (1.21 to 2.48)	0.003
Obesity and other hyperalimentation	1.00	1.40 (1.28 to 1.52)	<0.001
Organic mental disorder	1.00	1.77 (1.31 to 2.40)	<0.001
Schizophrenia/schizotypal/delusional disorders	1.00	2.10 (1.41 to 3.14)	<0.001
Mood disorder	1.00	1.68 (1.54 to 1.83)	<0.001
Hypertensive diseases	1.00	1.22 (1.18 to 1.27)	<0.001
Ischaemic heart diseases	1.00	1.12 (1.05 to 1.19)	<0.001
Pulmonary heart diseases	1.00	1.37 (1.18 to 1.59)	<0.001
Other form of heart disease	1.00	1.13 (1.06 to 1.21)	<0.001
Cerebrovascular disease	1.00	1.10 (0.98 to 1.24)	0.114
Diseases of arteries, arterioles and capillaries	1.00	1.10 (0.98 to 1.24)	0.106

The binary regression model was adjusted for age, gender and Townsend deprivation index. Optimal sleep was used as the reference group. Less optimal sleep is characterised as meeting all the following criteria: sleep duration <5 or >8 hours/night, WASO ⩾95.98 mins (75th percentile), L5 value ⩾4.24 milli-g (75th percentile) and NBlock ⩾39.63 (mid-point between its bimodal distribution). OR = odds ratio, CI = confidence interval

Predictive values, sensitivity and specificity for each disease characteristic are compared in Table 3-14. Positive predictive values (PPV) were relatively low and they ranged between 0.21% (Schizophrenia/schizotypal/delusional disorders) and 22.18% (Hypertensive diseases). Negative predictive value (NPV), on the other hand, ranged between 83.65% (Hypertensive

diseases) and 99.97% (Organic mental disorders). Sensitivity was the lowest for 'Mood disorder' which was only 2.55% and highest for 'Organic mental disorder' which was 87.10%. On the contrary, specificity was very similar for all diseases, ranging between 78.07% and 79.27%.

The relationship between sleep efficiency and various disease characteristics is illustrated in Figure 3-8. For all diseases, except Schizophrenia/schizotypal/delusional disorders, those who have been diagnosed had a lower sleep efficiency compared to those who have not been diagnosed with those diseases. However, statistical analysis results showed that the relationship was only significant for Diabetes ($p = 0.006$), Obesity and other hyperalimentation ($p = 0.044$), mood disorder ($p = 0.011$) and hypertensive diseases ($p < 0.001$) (Figure 3-8).

Table 3-14. Clinical performance specification (n= 84,411).

	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Diabetes	5.91	96.62	32.93	78.50
Disorders of glucose regulation and pancreatic internal secretion	0.23	99.88	35.83	78.07
Obesity and other hyperalimentation	3.97	97.60	31.81	78.33
Organic mental disorder	0.81	99.97	87.10	78.10
Schizophrenia/schizotypal/delusional disorders	0.21	99.91	40.63	78.07
Mood disorder	0.31	96.81	2.55	78.30
Hypertensive diseases	22.18	83.65	27.62	79.27
Ischaemic heart diseases	7.97	94.20	27.88	78.45
Pulmonary heart diseases	1.25	99.20	30.45	78.13
Other form of heart disease	6.25	95.42	27.73	78.35
Cerebrovascular disease	1.83	98.68	28.09	78.14
Diseases of arteries, arterioles and capillaries	1.80	98.63	27.01	78.13

PPV= positive predictive value; NPV= negative predictive value

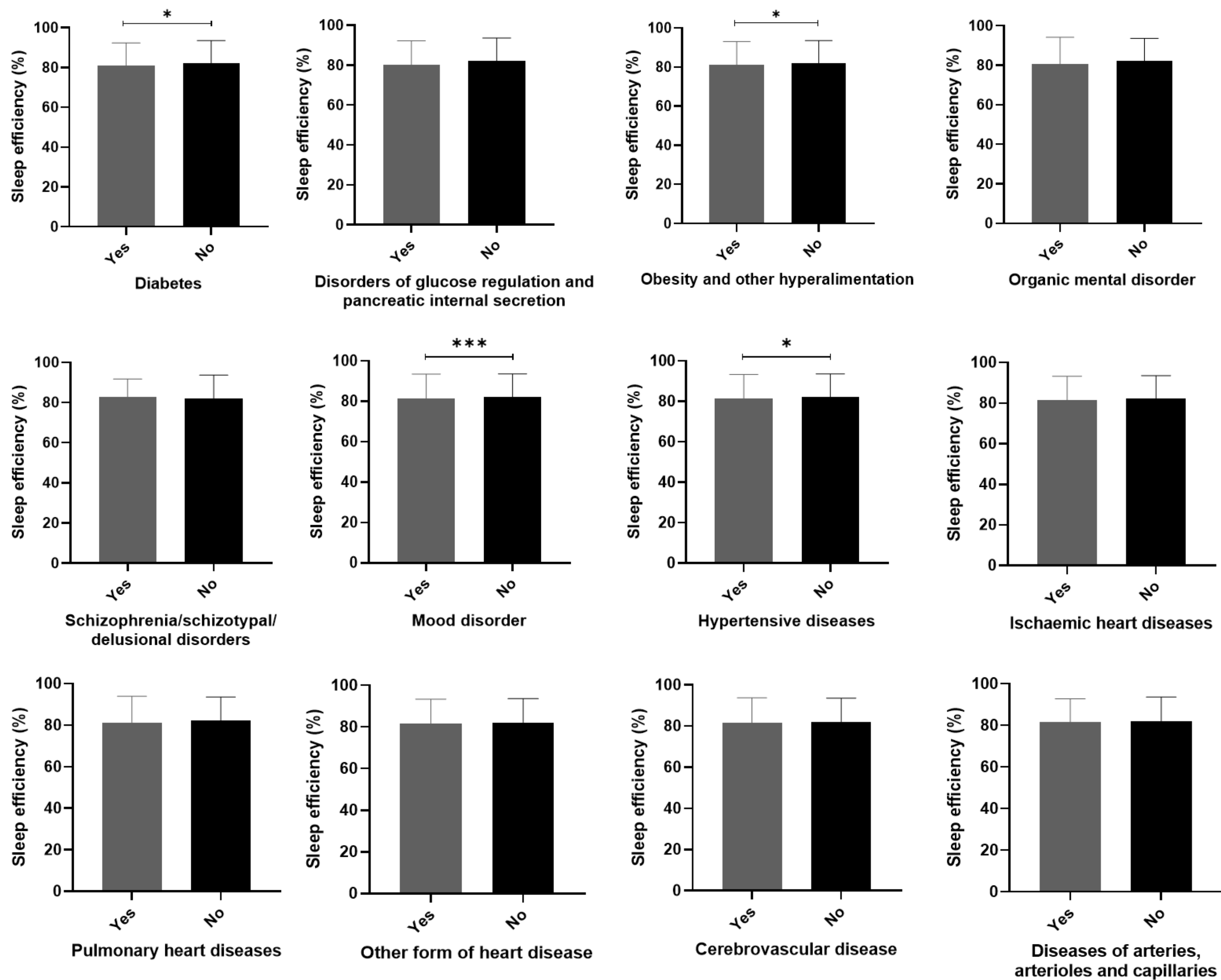


*Figure 3-8. Relationship between sleep efficiency and various disease characteristics. *P<0.05, **P<0.01, ***P<0.001*

3.2.5 Mental health

3.2.5.1 Cognitive function

It seems that those who slept >8 hours/night had the best numeric memory as shown in Table 3-15. However, no significant difference was detected between each sleep group. Those in the '<5 hours' sleep group were less likely to recall correctly in the prospective memory test. When looking at the number of puzzles solved correctly, no significant difference was detected between the five sleep groups (Table 3-15).

A 'U-shaped' association between reaction time and sleep duration was observed. Participants with both short and long sleep duration had a significantly slower reaction ($p< 0.001$). However, only the difference between the '<5 hours' sleep group and '5-6 hours', '6-7 hours' and '7-8 hours' sleep groups were statistically significant. As well as the difference between '>8 hours' and '5-6 hours', '6-7 hours' and '7-8 hours' sleep groups. The difference between other sleep groups was not found to be statistically significant (Figure 3-9A). Moreover, a reversed 'U-shaped' association was observed between sleep duration and the fluid intelligence score ($p< 0.001$). Closer inspection of Figure 3-9B shows that only the difference between the '<5 hours' sleep group and '5-6 hours', '6-7 hours' and '7-8 hours' sleep groups, and the difference between '>8 hours' and '5-6 hours' and '6-7 hours were statistically significant. Finally, a reversed 'U-shaped' association was also observed between sleep duration and visual memory as illustrated in Figure 3-9C ($p< 0.001$), although only the difference between the '<5 hours' sleep group and '5-6 hours' and '6-7 hours', as well as the difference between '>8 hours' and '5-6 hours' and '6-7 hours were statistically significant. Comparisons between other groups were not found to be statistically significant.

Finally, participants with both short and long sleep duration took longer to complete the numeric ($p< 0.001$) and alphanumeric ($p= 0.004$) paths. However, closer inspection of Figure 3-10A and Figure 3-10B shows that only the difference between the '<5 hours' sleep group and '5-6 hours', '6-7 hours' and '7-8 hours' sleep groups were statistically significant in both cases. The difference between other groups was not found to be statistically significant. Lastly, those who slept 6-7 hours/night matched more symbol to digit correctly ($p= 0.001$), but the difference was only statistically significant when comparing the '<5 hours' sleep group to both '5-6 hours' and '6-7 hours' sleep groups.

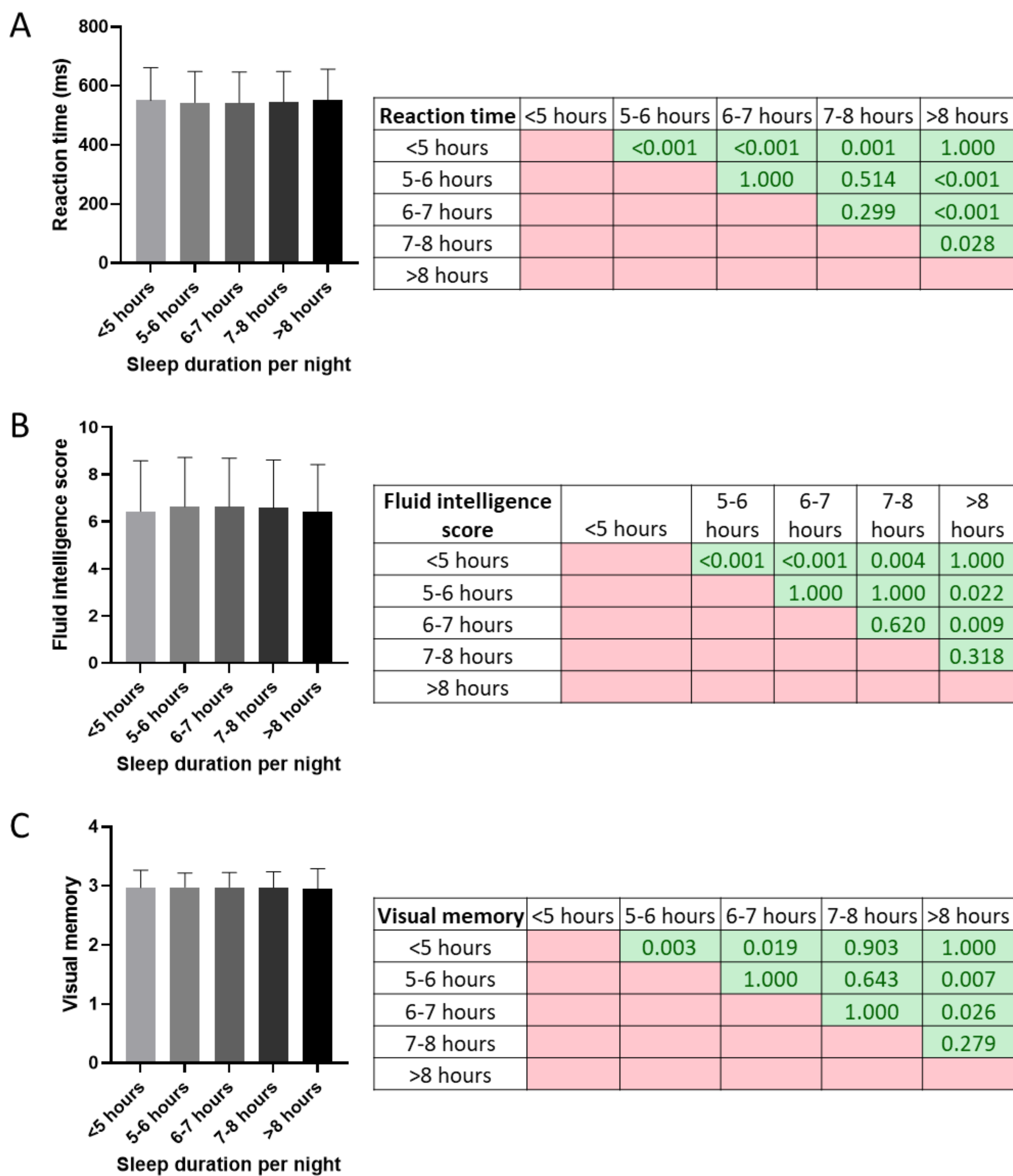
Spearman's rank-order correlation analysis showed a very weak positive correlation between the numeric memory and the sleep efficiency and the correlation was not found to be

statistically significant (rs= 0.005, p= 0.594). Similarly, a very weak positive correlation was found between sleep efficiency and reaction time (rs= 0.002, p= 0.595). When investigating the correlation between fluid intelligence score and sleep efficiency, a significant correlation was detected, but it is a very weak positive correlation (rs= 0.021, p< 0.001). Finally, a very weak correlation was detected between sleep efficiency and visual memory (rs= 0.005, p= 0.148). The univariate regression model revealed that participants who do better in the prospective memory test had higher sleep efficiency. However, the difference between them was not found to be statistically significant (p= 0.638).

Table 3-15. Summary of cognitive function test results across the five sleep groups (n= 84,411).

	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours	P-value
Numeric memory (mean ± SD)	6.94 ± 1.24	6.95 ± 1.25	6.91 ± 1.24	6.87 ± 1.27	7.00 ± 1.19	0.063
Prospective memory (n)	5759	8360	10559	5919	1456	
Correct recall on first attempt (%)	82.2 a	84.7 b	85.0 b	83.7 a,b	83.1 a,b	
Correct recall on second attempt (%)	15.0 a	13.1 b	12.9 b	14.3 a,b	14.2 a,b	
Skipped or incorrect recall (%)	2.8 a	2.2 a	2.2 a	2.1 a	2.7 a	
Number of puzzles correct (mean ± SD)	9.94 ± 3.29	10.13 ± 3.17	10.12 ± 3.20	9.93 ± 3.21	9.74 ± 3.24	0.182

Percentages expressed are within each sleep group. Column proportions were compared using the z-test and p-values were corrected using the Bonferroni method. Therefore, if two columns were assigned the same letter then the difference between them was not statistically significant (p>0.05), but if two columns were assigned different letters then the difference between them was statistically significant (p<0.05). SD= standard deviation.



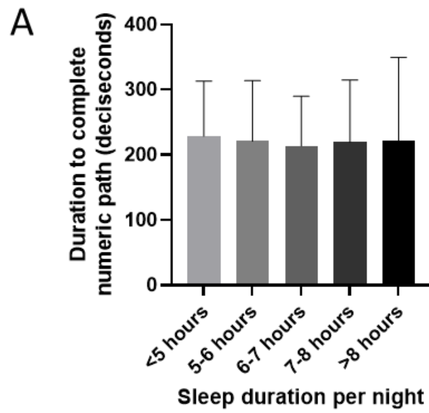
Reaction time	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		<0.001	<0.001	0.001	1.000
5-6 hours			1.000	0.514	<0.001
6-7 hours				0.299	<0.001
7-8 hours					0.028
>8 hours					

Fluid intelligence score	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		<0.001	<0.001	0.004	1.000
5-6 hours			1.000	1.000	0.022
6-7 hours				0.620	0.009
7-8 hours					0.318
>8 hours					

Visual memory	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		0.003	0.019	0.903	1.000
5-6 hours			1.000	0.643	0.007
6-7 hours				1.000	0.026
7-8 hours					0.279
>8 hours					

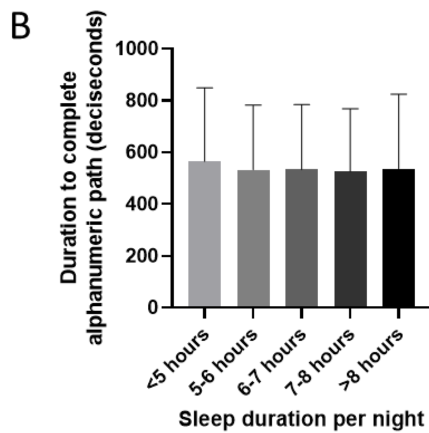
Figure 3-9. Pairwise comparisons between each sleep group regarding (A) reaction time, (B) fluid intelligence score and (C) visual memory. Analysis was controlled for age, gender and BMI.

A



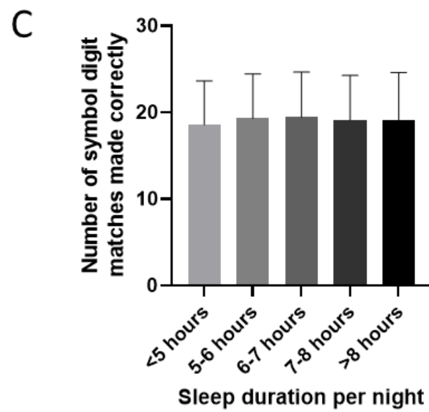
Duration to complete numberic path	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		0.019	<0.001	0.007	0.467
5-6 hours			0.246	1.000	1.000
6-7 hours				1.000	1.000
7-8 hours					1.000
>8 hours					

B



Duration to complete alphanumberic path	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		0.003	0.008	0.019	1.000
5-6 hours			1.000	1.000	1.000
6-7 hours				1.000	1.000
7-8 hours					1.000
>8 hours					

C



Number of symbol digit matches made correctly	<5 hours	5-6 hours	6-7 hours	7-8 hours	>8 hours
<5 hours		0.016	<0.001	0.129	0.353
5-6 hours			1.000	1.000	1.000
6-7 hours				0.961	1.000
7-8 hours					1.000
>8 hours					

Figure 3-10. Pairwise comparisons between each sleep group regarding (A) duration to complete numeric path, (B) duration to complete alphanumeric path and (C) number of symbol digit matches made correctly. The analysis was controlled for age, gender and BMI.

3.2.5.2 Mood

The relationship between sleep duration and various moods is summarised in Figure 3-11. Those with a shorter sleep duration were less happy in general and regarding their financial situation and health, whereas those with a sleep duration >8 hours/night had a lower work satisfaction level. People with a shorter sleep duration (<5 hours/night) were more likely to self-report mood swings, irritability, loneliness and risk-taking behaviours. People with longer sleep duration (>8 hours/night), on the other hand, were more likely to self-report mood swings, miserableness, anxious feelings and loneliness. However, closer inspection of Figure 3-11 showed that when comparing different sleep groups, the difference between some groups was not statistically significant.

Figure 3-12 illustrates the relationship between sleep efficiency and self-rated happiness and moods. It revealed that sleep efficiency was very similar between positive and negative feelings. Participants who reported better moods seemed to have a slightly higher sleep efficiency. However, the difference between them was not found to be statistically significant, except for risk-taking behaviours ($p < 0.001$) as shown in Figure 3-12.

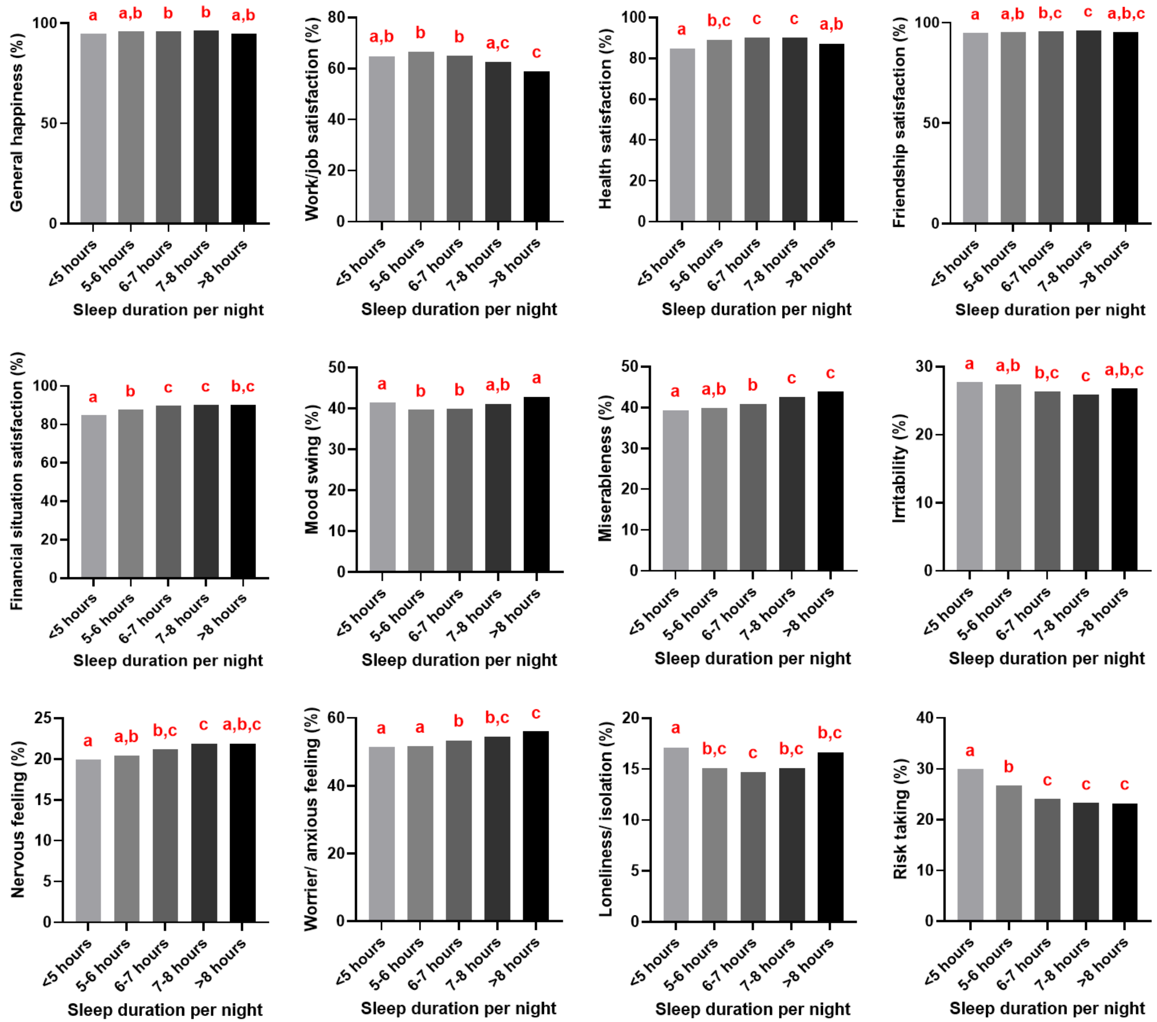


Figure 3-11. Association between sleep duration and various moods. Percentages expressed are within each sleep group. If two bars were assigned the same letter then the difference between them was not statistically significant (p>0.05), but if two bars were assigned different letters then the difference between them was statistically significant (p<0.05).

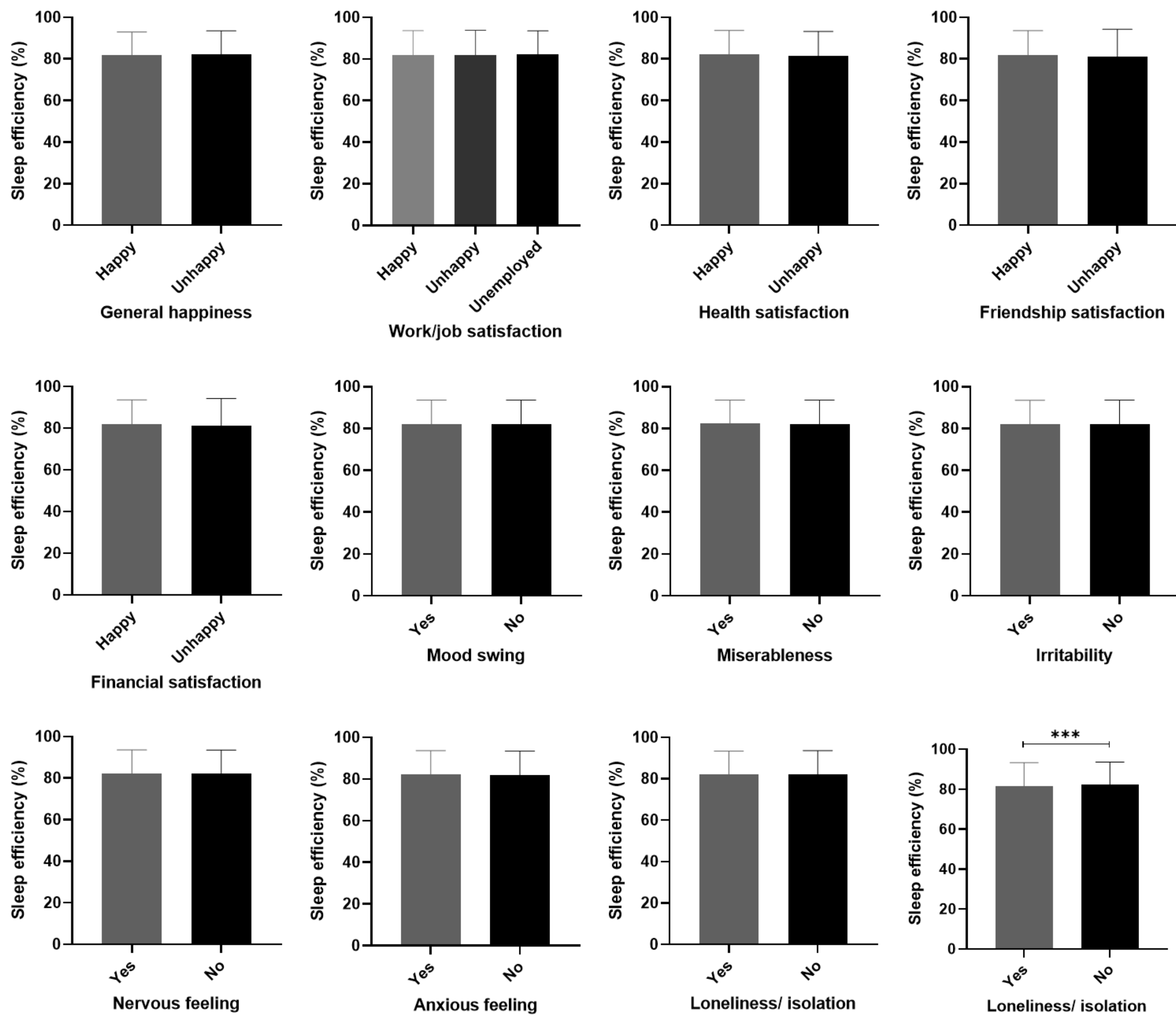


*Figure 3-12. Relationship between sleep efficiency and self-rated mood/happiness. *P<0.05, **P<0.01, ***P<0.001*

3.3 Discussion

3.3.1 Sociodemographic and anthropometric measurements

Using the largest accelerometer cohort to date, this study found that short objectively measured sleep duration is significantly associated with the male gender, older age, high BMI, social deprivation and ethnic minority group. All prior UK biobank sleep studies have used self-reported sleep data. An association between worse cardiometabolic health and impaired task performance with sleep duration of <7 hours or >9 hours per night was reported (Kyle et al., 2017b, Sophie Cassidy, 2016). Previously published large cohort studies have also utilised wrist-worn actigraphy to assess sleep objectively (Zeitzer et al., 2018, Spira et al., 2017, Smagula et al., 2016, Maglione et al., 2013, Fung et al., 2013). However, no one has to date assessed the objective sleep-wake patterns and physical activity using accelerometry in the Biobank cohort.

We found that 43.3% of the UK Biobank cohort had an objective sleep duration of <6 hours/night. The American Academy of Sleep Medicine and Sleep Research Society recommended a sleep duration of >7 hours/night for adults aged 18-60 years for good health and reduced mortality (Wrede et al., 2015). Only 23.3% of the UK Biobank participants reached this recommended sleep duration, this may mean that in an older population the ideal sleep duration may not be 7-8 hours but instead somewhat shorter when assessed using this accelerometer-based objective method rather than self-report. Many studies have found both macro and micro sleep changes are related to increasing age. With ageing, people tend to experience longer sleep latency, more fragmented sleep, earlier bedtime and wake times, as well as shorter and lighter sleep (Mander et al., 2017). In this study, we found that with increasing age, there was a decrease in sleep duration and sleep efficiency. This is consistent with the results published by Redline et al. which showed that older adults have lower sleep efficiency, as well as reduced percentage of REM sleep when comparing those who were aged >61 years to younger adults (Redline et al., 2004). Age-adjusted norms remain debated and are key when considering recommendations and advice for an ageing population. In fact, a study reported that careful assessment of healthy older adults excluding sleep disorders has shown a clear decrease in sleep need with age without affecting daytime alertness (Dijk et al., 2010). Social jet lag was also assessed in the UK Biobank cohort as a possible marker of societal sleep restriction and therefore one reason for objective short sleep time. Participants' sleep duration was slightly longer on Fridays and Saturdays. However, the difference was not

statistically significant. This could be due to many of the participants having reached retirement age and therefore, their sleep duration was not significantly affected by longer working hours during the week. However, it could also suggest that their shorter sleep times were not indicative of restricted or inadequate sleep and support 6-7 hours being the likely optimum sleep duration for older adults. Additionally, this study did not further explore the relationship between sleep and health outcomes amongst the older age (>70 years) subgroup as the association is likely to be confounded by co-morbidity and polypharmacy. Due to time limitations, medication information was not available in this study which means that polypharmacy could not be investigated. Medication usage could be included in future studies.

Even though there were many studies that provide evidence of age-related sleep changes, not all elderly adults experience the same degree of disturbances during sleep. Therefore, this suggested that other factors apart from age are contributing to this inter-individual variability. One of the interacting factors for age is gender, although it is yet unclear at what age the gender effect of sleep emerges (Mander et al., 2017). In this study, we found that females had a significantly longer objective sleep duration and higher sleep efficiency than males. This seems to contradict a prior UK Biobank study which found that females are more likely to self-report insomnia symptoms and shorter sleep duration (Kyle et al., 2017b). However, evidence from polysomnographic measures and quantitative electroencephalographic analysis does not support this (Baker et al., 2007). Males were found to have lighter sleep than females as males tend to experience more stages 1 and 2 NREM sleep and significantly less REM sleep (Redline et al., 2004). Our results suggested that sleep state misperception may be more common amongst females.

Based on the analysis of the Townsend deprivation index, those who lived in the most deprived area were found to have the shortest sleep duration and lowest sleep efficiency. This accords with an earlier study carried out on the Finnish adult population. This study showed a 'U-shaped' association between socioeconomic circumstances and sleep duration. Those with a disadvantaged socioeconomic position such as low income, unemployment and low education reported poorer sleep as indicated by shorter sleep duration and frequent insomnia-related symptoms (Lallukka et al., 2012). This result may be explained by the fact that people who are socially deprived may have to work multiple jobs or work in a job that involves night shifts which could potentially cause abnormal circadian rhythm. Furthermore, if they live in an unsafe neighbourhood, they might be forced to sleep with a light on for extra

security which can have an adverse impact on their sleep duration and efficiency. A study that also utilised the data obtained from the UK Biobank cohort found that those who live in the most deprived area reported the lowest prevalence of healthy lifestyle behaviours compared to others and they are more likely to be underweight or obese. Social deprivation is also associated with higher odds of mortality. This might be due to a combination of reasons such as increased psychosocial stress, lack of social support and limited access to health care (Foster et al., 2018).

Amongst the UK Biobank population, White/British participants had significantly longer sleep duration and higher sleep efficiency. This result broadly supports the work of other studies in this area linking sleep habits to ethnicity. Many aspects of the sleep architecture including the percentage of REM sleep seem to vary significantly between different ethnicities (Redline et al., 2004). Subjectively measured short sleep duration was found to be more common amongst ethnic minority groups as illustrated in a 34-years longitudinal study conducted on 6,928 participants (Stamatakis et al., 2007). Hale et al. reported that the White ethnicity was the least likely to self-report a sleep duration of ≤6 hours/night than other ethnicities including Black, Mexican American, Hispanic and Non-Hispanic ethnicities, while the Black ethnicity was more likely to self-report a shorter sleep duration (n= 32,749). This was not a surprising finding as this cross-sectional study also found that the White ethnicity reported a higher education level, better family income, more likely to be employed and have a lower stress level (Hale and Do, 2007). Therefore, they had better socioeconomic circumstances. Apart from sleep duration, sleep quality was also found to be lower amongst the Black ethnicity compared to the White ethnicity in a cross-sectional multi-ethnic study conducted on 2,230 participants (Chen et al., 2015). As discussed previously, those with better socioeconomic positions tend to have better sleep. Most of the studies discussed so far are based on self-reported sleep duration. A cross-sectional study was done by Curtis et al. which included 426 participants and utilised wrist actigraphy to measure sleep. They compared both sleep duration and efficiency between Black/African Americans and White/European Americans and reported shorter sleep duration and lower sleep efficiency amongst Black/African American participants (Curtis et al., 2017). A possible explanation for this racial difference in sleep duration may be due to the language barrier and immigration status meaning that ethnic minority groups were more likely to work multiple jobs or have longer commuting time and as a result, they may not have many

opportunities for sleeping in. Additionally, higher stress levels can make initiating and maintaining sleep more difficult (Hale and Do, 2007).

Age, gender, ethnicity and sociodemographic factor are known to influence a person's choice of diet and lifestyle (Park et al., 2005). An unhealthy diet and a sedentary lifestyle are two common risk factors for overweight and obesity worldwide. Extensive research has evaluated the association between sleep and obesity. Results of the current study show that higher BMI was associated with shorter sleep duration and lower sleep efficiency. This is consistent with findings in other literature. Those with a BMI >30.7 kg/m^2 had significantly lower sleep efficiency (Redline et al., 2004). Most prior studies used '7-8 hours/night' as the reference category. They found that both shorter and longer sleep duration was associated with increased odds of obesity in adults as indicated by a BMI >30 kg/m^2 (Buxton and Marcelli, 2010, Magee et al., 2010, Anic et al., 2010). However, a study only observed this 'U-shaped' association amongst middle-aged adults (Grandner et al., 2015a). In some studies, a negative association between sleep duration and BMI was observed indicating that only short sleep duration was significantly associated with a higher BMI (Potter et al., 2017). Results reported by Grandner et al. showed a linear negative relationship between sleep duration and BMI in young adults, this relationship became 'U-shaped' amongst middle-aged adults, while an unclear relationship was observed in older adults. This indicates that age has a significant impact on the relationship between sleep and BMI (Grandner et al., 2015a). Additionally, a Romanian study found that perceived stress is a potential moderator for the relationship between sleep duration and BMI. They reported a 'U-shaped' association between sleep duration and BMI in participants over the age of 40 years, but only in participants who reported self-perceived stress. In those without self-perceived stress, the association was not statistically significant. This may be due to higher levels of cortisol production caused by stress which in turn activates the release of adrenaline and noradrenaline from the sympathetic system which eventually leads to emotional eating and weight gain (Rusu et al., 2019, Geiker et al., 2018), although it is important to note that most of the studies mentioned utilised self-reported sleep duration and analysis were cross-sectional, therefore results need to be interpreted carefully and a causal relationship cannot be determined from these results.

Prior literature investigating the link between obesity and sleep mainly used measurements of overall obesity such as BMI as mentioned in the previous section, but now some studies have demonstrated that central adiposity indicated by waist circumference may be a better

predictor of health, for example, cardiovascular risk (Zhu et al., 2002). Consistent with the literature, the current study found that people with a shorter sleep duration had significantly higher odds for high waist circumference and a high waist-hip ratio compared to those who slept 7-8 hours/night. A meta-analysis carried out by Sperry et al. reported a significant negative relationship between sleep duration and waist circumference. Shorter sleep duration was associated with an increased waist circumference (Sperry et al., 2015). A similar trend was observed in many other studies carried out amongst the UK, Korean and Iran adult populations (Potter et al., 2017, Najafian et al., 2010, Nam et al., 2017). Those who slept <6 hours/night can have as much as 3.4 cm higher waist circumference than those who slept >10 hours/night (Ford et al., 2014). Apart from waist circumference, studies also found that lower sleep efficiency and shorter sleep duration are associated with higher waist-hip ratio, body fat mass and mean percentage of body fat (Acar Tek N, 2020). Habitual long sleepers were also associated with a higher fat mass (Tan et al., 2019). Results reported in the current study are consistent with prior research which found that <6 hours/night of sleep is associated with higher waist circumference and higher waist to hip ratio regardless of gender. Additionally, <5 hours of sleep per night was associated with a higher whole-body fat mass, while sleep duration >8 hours/night was associated with a higher fat percentage. An underlying mechanism might be that short sleepers commonly have increased levels of the appetite-stimulating hormone ghrelin and decreased levels of the appetite-suppressing hormone leptin. The combination of these hormones contributes to increased hunger and therefore stimulates weight gain through more food intake (Spiegel et al., 2004b, Taheri et al., 2004). By staying up later, short sleepers also have more opportunities for food intake and may also have an unhealthier eating pattern involving more snacks and therefore increase their energy intakes. Due to the fact that most of the studies mentioned are cross-sectional analyses, therefore, a causal relationship between sleep duration and waist circumference cannot be determined. It is possible that central obesity indicated by the increased waist circumference increases the risk of diseases such as sleep apnoea which in turn leads to poor sleep (Nam et al., 2017). Additionally, it is worth noting that weekday and weekend sleep duration may not be the same for many people. A cross-sectional analysis carried out amongst Black women found that those who sleep more than two hours longer during weekends compared to weekdays had significantly higher BMI and waist circumference. This may be due to a shift in circadian rhythm leading to an imbalance in energy homeostasis and a change in physical activity, as well as timing and quantity of food intake (Ash et al., 2020, Rutters et al., 2014).

3.3.2 Lifestyle

Mid-day naps (known as a siesta in some countries) are normal in young children but less common in young and middle-aged adults (Mantua and Spencer, 2017). However, with increasing age, the prevalence of daytime napping appears to increase, along with excessive daytime sleepiness. A telephone interview with >1,500 American adults found that the prevalence of napping increased from 15% in 55–64-year-olds to 25% in 75–84-year-olds (Foley et al., 2007). This might be caused by the common age-related sleep changes mentioned in the previous section such as lighter and fragmented sleep. The current study found that those with shorter sleep duration had a higher odds of napping during the day. Additionally, those who do not nap during the day appear to have higher sleep efficiency than those who do nap. This is consistent with previously published data. Amongst healthy adolescents, daytime naps were found to be a predictor of nocturnal sleep – those who nap during the day are found to have short sleep duration and lower sleep efficiency at night. This may be because that napping reduces sleep pressure at night time which in turn leads to later bedtime (Jakubowski et al., 2017). However, shorter nocturnal sleep duration may then induce daytime sleepiness and then create a need for naps. In partially sleep-deprived adolescents, napping was found to be beneficial as it was associated with enhanced memory (Lo and Chee, 2020). In both sleep-deprived and non-sleep deprived individuals, sleepiness tends to increase while cognitive ability decrease throughout the day, but mid-day nap has shown a recovery ability (Mantua and Spencer, 2017). Even when 6.5 hours of total sleep duration was split between five hours of nocturnal sleep and 1.5 hours of daytime nap, people had better cognitive performance than those who slept 6.5 hours nocturnally (Cousins et al., 2019). A possible mechanism is that those who napped were found to have increased density of stage 2 sleep spindles which was associated with the reactivation and transfer of newly learned information between brain networks leading to better performance on various memory tasks (McDevitt et al., 2018). Additionally, increased REM theta power was observed amongst those who nap and the theta oscillation was found to be closely related to memory processing (Lisman and Jensen, 2013). However, those with sleep split between nocturnal sleep and daytime nap showed higher blood glucose and insulin levels, as well as increased levels of pro-inflammatory markers exposing them to a higher risk of type 2 diabetes (Lo et al., 2019, Leng et al., 2016).

This study also found that those with shorter sleep duration had significantly higher odds of snoring, although due to the cross-sectional nature of this study, the causal relationship between snoring and sleep duration could not be determined. A possible mechanism is that habitual snoring often co-exists with sleep apnoea or hypopnoea which is characterised by an intermittent collapse of the airway during sleep. This could result in repeated arousal from sleep which contributes to the short sleep duration and fragmented sleep (Xie et al., 2014). Snoring is known to have many adverse effects on health. Short sleep duration and habitual snoring were found to be associated with major chronic diseases 15 years later (Shi H, 2021). A questionnaire carried out amongst middle-aged adults (40-69 years) found that those who are habitual snorers had a significantly higher risk of diseases including hypertension and ischaemic heart disease (Koskenvuo et al., 1985). This is similar to the finding of another prospective study which found that those who frequently snore had a 2.5 times higher risk of cardiovascular events including acute myocardial infarction six years later when compared to those who do not snore (Xie et al., 2014). This may be due to acute haemodynamic changes related to snoring including enhanced cardiac arrhythmia and increased intracranial pressure which contributes to the development of cardiovascular diseases (Franklin, 2002). As previously mentioned, heavy snoring may lead to fragmented sleep and intermittent hypoxemia, this could then cause an increase in sympathetic activity and promote tumour growth which in turn increases the odds for various cardiometabolic diseases and cancers (Shi H, 2021, Zhang et al., 2013).

In the current study, comparing sleep duration against smoking status showed that current smokers had a shorter sleep duration when compared to past smokers and those who have never smoked previously. This finding broadly supports the work of other studies in this area. Smokers were found to have significantly worse Pittsburgh Sleep Quality Index scores in terms of sleep duration, sleep latency and sleep efficiency, as well as experiencing more sleep disturbance. This is regardless of their education level, living regions and marital status (Liao et al., 2019, Cohrs et al., 2014). Smokers were also more likely to report daytime sleepiness, minor accidents and depression (Phillips and Danner, 1995). There are multiple potential mechanisms explaining the link between smoking and sleep duration. However, it is worth noting that the association between sleep and smoking habits could be bi-directional. Nicotine in cigarettes could increase the risk of sleep restriction, but sleep restriction may induce stress and in turn lead to a higher chance of smoking which is illustrated in various previously

published literature. Nicotine addiction may directly reduce sleep duration via a physical urge to smoke at night-time leading to a later bedtime (Liao et al., 2019). Additionally, nicotine was also able to significantly increase alertness as measured by EEG (Griesar et al., 2002). If nicotine usage was close to bedtime, this may explain the longer sleep latency and worse sleep efficiency experienced in smokers which in combination contribute to shorter sleep duration. Finally, exposure to cigarette smoke could cause a decreased expression of clock genes including *Bmal1* and *Sirtuin1* which then leads to dysregulated central and peripheral clocks and eventually causes disrupted circadian rhythm. This in turn leads to unhealthy sleep habits and insufficient sleep (Hwang et al., 2014). As mentioned previously, the relationship between sleep and smoking may be bi-directional. Sleeping problems at a younger age could predict subsequent smoking years later. This could be due to the stress related to having trouble sleeping which causes adolescents to seek the stimulant effects of nicotine to improve their mood (Bellatorre et al., 2017). Therefore, this creates a feedback loop – poor sleep leads to smoking and smoking leads to poor sleep.

An inverse ‘U-shaped’ curve was observed when comparing physical activity levels of the five sleep groups, those who slept 6-7 hours/night were most active and a decreased activity in very short and long sleepers. Apart from healthy sleeping habits, the beneficial effects of an active lifestyle on health are well known. The ability of objectively measured physical activity in improving sleep has been shown. Those who have met the recommended World Health Organisation physical activity guidelines (>150 minutes of moderate-intensity or >75 minutes of vigorous-intensity per week) self-reported shorter sleep latency, fewer early awakenings and fewer leg cramps during sleep and less usage of sleeping pills (Loprinzi and Cardinal, 2011). Our findings may suggest that physical activity can promote healthy sleep duration, but it may also be that healthy sleeping habits promoted a more active lifestyle. Strong evidence suggests that inadequate moderate-to-vigorous physical activity was closely associated with increased risks of metabolic diseases and all-cause mortality after controlling for age, gender, race and weight (Committee, 2008). Physical inactivity could also lead to direct and indirect costs which result in considerable financial burdens on society (Allender et al., 2007). However, it is important to note that due to the nature of the cross-sectional study, this study is unable to prove the direction of the relationship between physical activity and sleep.

3.3.3 Primary care record of disease status

Using the largest accelerometry cohort to date, this study demonstrated a 'U-shaped' relationship between objectively measured sleep duration and primary care records of disease status indicating that both short and long sleep durations, as well as fragmented sleep, are associated with worse physical and mental health. This is in agreement with Li et al's findings, which found that compared to seven hours of sleep, a sleep duration of <6 or >8 hours/night was linked with a higher risk of metabolic syndrome and hyperglycaemia four years later (Li et al., 2015). Among the middle-aged and older adult population, sleep <6 hours at night-time was also associated with depressive symptoms, but the symptoms can be improved with longer daytime naps (Li et al., 2017). Previous UK Biobank sleep studies commonly used self-reported sleep duration. A cross-sectional study design involving 477,529 UK Biobank participants found that cognitive performance is impaired in those with short (<7 hours/night) and long (>9 hours/night) self-reported sleep durations (Kyle et al., 2017a). Another study evaluated the importance of sleep duration on cardiometabolic health in 233,110 UK Biobank participants and results indicated that those with cardiometabolic disease profiles including diabetes and cardiovascular diseases were more likely to report poor sleep (<7 or >8 hours/night) (Sophie Cassidy, 2016). Several accelerometry studies have assessed sleep objectively, but the sample sizes were relatively smaller (Smagula et al., 2016, Spira et al., 2017, Zeitzer et al., 2018). Additionally, many prior UK Biobank studies utilised self-reported disease status and self-completed mental health questionnaire data rather than data extracted from primary care health records (Kyle et al., 2017a, Davis et al., 2020, Fung et al., 2013). In reviewing the literature, no one has to date assessed both the primary care and accelerometry data in the UK Biobank cohort in a longitudinal fashion.

The overall 'U-shaped' relationship between sleep duration and disease status is consistent with previous studies, which showed a higher prevalence of poor health among those who slept <6 hours/night (Lauderdale et al., 2016) and sleeping for approximately 7 hours/night was linked with a lower risk of cardiovascular disease, coronary heart disease and stroke (Yin et al., 2017). On closer inspection, even though the percentage of participants diagnosed with diseases including disorders of glucose regulation, organic mental disorder and hypertensive diseases was higher in long sleepers (>8 hours/night) compared to those who slept 5-8 hours/night, the difference between them was not found to be statistically significant. It remains controversial whether long sleep duration has any direct adverse effects on health as

many studies attempted to identify the underlying mechanism of long sleep that causes morbidity and mortality. It is thought that depressive symptoms and low socioeconomic status might be potential confounders, so it is possible that the two peaks of a 'U-shaped' association do not mean the same thing (Knutson and Turek, 2006).

In the current study, extreme short sleep duration (<5 hours/night) increased the risk of diabetes by 58% and obesity by 48%, in comparison to 7-8 hours of sleep. This is in agreement with earlier studies that found that partial sleep restriction from eight hours to 4-5 hours of sleep per night increased pre-diabetic symptoms such as glucose intolerance and insulin resistance, as well as other changes in the endocrine function (Ayas et al., 2003a, Spiegel et al., 1999). Additionally, apart from the direct effects of sleep restriction on glucose and insulin level, it also has an indirect effect on the risk of diabetes. Sleep deprivation can cause an elevation in the ghrelin level, which then promotes hunger and weight gain (Schmid et al., 2008). This in turn increased the risk of diabetes and other metabolic syndromes. This suggests that partial sleep deprivation might accelerate the severity of age-related chronic disorders (Ayas et al., 2003a, Spiegel et al., 1999). Risks of organic mental disorders and mood disorders were increased by 106% and 44% in short sleepers who slept <5 hours/night compared to those who slept 7-8 hours, respectively. Mood disorders are common among patients with chronic sleep problems and are strongly associated with sleep complaints; however, the causal relationship is often thought to be bidirectional (Benca et al., 1997). An underlying mechanism is that sleep deprivation can induce neuro-inflammation including the activation of leucine-rich repeat protein-3 inflammasome, which then induces depressive-like behaviours, mood disorders and cognitive diseases (Verkhratsky et al., 2020, Xia et al., 2018, Alcocer-Gómez and Cordero, 2014). The risk of hypertensive diseases increased by 36% among the extreme short sleepers. This supports the Sleep Heart Health Study, which showed that <6 hours of sleep increased the prevalence of hypertension by 66% (Gottlieb et al., 2006). During sleep, blood pressure reduces by 10-20%. When this reduction is absent, the risk of resistant hypertension and cardiovascular mortality increased. The odds of incident hypertension was found to increase by 37% with each hour of reduced sleep duration (Calhoun and Harding, 2010). Therefore, it is not surprising that cardiovascular risk is also elevated by sleep deprivation as found in the current study. The risk of various heart diseases was 25-37% higher in participants who slept <5 hours/night compared to those who slept 7-8 hours/night. These results supported other previously published studies, which have

established that sleeping for 4-6 hours/night compared to 7-8 hours/night leads to a greater risk of coronary heart disease and acute myocardial infarction (Ayas et al., 2003b, Kripke et al., 1979, Liu and Tanaka, 2002). Various studies have shown that the lack of sleep could cause an increase in the activity of the sympathetic nervous system and a decreased level of parasympathetic cardiovascular modulation, leading to an increase in blood pressure (Zhong et al., 2005, Tochikubo et al., 1996). Sleep deprivation was also associated with elevated nocturnal catecholamine and C-reactive protein levels, which in turn contributes to cardiovascular diseases through inflammation in the vascular wall, as well as vasoconstrictive and prothrombotic processes (Irwin et al., 1999, Meier-Ewert et al., 2004).

Another important finding was that when combining short and long sleep durations with sleep fragmentation to compare optimal and less optimal sleep, the current study found that those with less optimal sleep had significantly higher odds for diabetes, obesity, and various heart diseases. These results matched those observed in earlier studies. Those with a sleep duration shorter or longer than 7-8 hours/night and highly fragmented sleep were associated with significantly higher odds of high BMI and obesity. This relationship may partly be due to dysregulated glucose metabolism caused by frequent arousals (van den Berg et al., 2008a, Zhao et al., 2021). Inflammatory-related white blood cells including monocytes and neutrophils were also increased by fragmented sleep, which leads to atherosclerosis and contributes to the development of hypertension and various heart diseases (Vallat et al., 2020). However, those with poorer health such as metabolic diseases and heart problems may experience shorter and more disturbed sleep. Therefore, it is possible that the relationship between sleep fragmentation and diseases is bi-directional.

The combination of these findings raised the possibility that poor sleep can be a predictor of poor cardiometabolic and mental health. However, the results on predictive values, specificity and sensitivity did not suggest sufficient sensitivity and specificity for sleep can be used as a stand-alone predictor of poor health. The low positive predictive values indicated that poor sleep (<5 or >8 hours/night) is not a useful screening tool for diseases alone, although the high negative predictive values suggested that good sleep (5-8 hours/night) could potentially be useful to identify people at lower risk of disease. The relatively low specificity and sensitivity showed that a significant association in a large epidemiology study does not necessarily translate to a clinically useful screening tool for predicting health status. These findings may be limited by the fact that the UK Biobank population may have a lower prevalence of diseases

than the general population creating a selection bias and this may have an impact on PPV and NPV and consequently clinical utility (Fry et al., 2017). Incorporation of objective sleep measures into risk equations alongside other measures may still be useful. Future studies will need to be undertaken and these should take other factors as well as sleep duration into account.

3.3.4 Mental health

Another important finding of this study is that both short (<5 hours/night) and long (>8 hours/night) sleep duration was associated with worse cognitive performances including slower reaction time, lower fluid intelligence score and worse memory. These results supported evidence from previously published literature. Henry et al. (Henry et al., 2019a) performed a Mendelian randomisation study to investigate the relationship between self-reported sleep duration and cognitive functions in 395,803 UK Biobank participants. A 'U-shaped' or reversed 'U-shaped' curve was observed in most variables. Kyle et al. (Kyle et al., 2017b) also carried out a cross-sectional analysis in 477,529 UK Biobank participants using self-reported sleep duration and cognitive function data. Both short (<7 hours/night) and long (>9 hours/night) sleep durations were associated with impaired cognitive performance such as poorer memory and slower reaction time, although it is worth noting that a study involving >10,000 participants found that a sleep duration less or more than 7-8 hours only affects higher-order cognitive processes such as reasoning and verbal skills while having no significant effects on the short term memory domain (Wild et al., 2018). A 25% reduction in total sleep time can significantly increase sleepiness, cause attention lapses and increased reaction time. This could have severe outcomes including automobile accidents caused by drowsiness (Queiroz et al., 2020). Apart from partial chronic sleep deprivation, acute sleep deprivation caused by night shifts could also increase reaction time considerably (Saadat et al., 2017). There are two main theories behind the general adverse impact of partial sleep deprivation on cognition – (1) through the effects on attention and alertness and (2) by altering certain brain structures and therefore affecting brain functions (Alhola and Polo-Kantola, 2007). Under the sleep deprivation state, there is a reduction in the activation of both the left thalamus and superior parietal regions which are key areas involved in working memory operations (Chee et al., 2006). Apart from reaction time and memory, prior studies have also reported a decrease in fluid intelligence amongst habitual short sleepers (≤6 hours/night) compared to those who slept 7-9 hours/night (Curtis et al., 2018). However, a

study carried out amongst adolescents did not find a significant association between sleep duration and fluid intelligence. Instead, an association was found between self-reported sleep difficulty and fluid intelligence suggesting that sleep quality also plays an important role in fluid intelligence (Johnston et al., 2010). It is worth noting that it could be a bi-directional relationship between sleep and cognitive function. As mentioned previously, certain brain structures are altered by sleep restriction. However, brain structures also show deterioration in patients with cognitive impairment such as dementia which have a negative impact on an individual's rest-activity cycles leading to sleep disturbances (Wennberg et al., 2017). Additionally, the risk of polypharmacy increases with age which could contribute to sleep disturbances. Cholinesterase inhibitors such as donepezil are commonly used to improve symptoms of cognitive disorders including Alzheimer's disease and it has been associated with an increased incidence of insomnia. Therefore, it is often recommended by doctors that these medications should not be administered at a time later than the evening meal which reduces the risk of insomnia (clinic, 2021).

Another important finding regarding the mental health of UK Biobank participants was that both short (<5 hours/night) and long (>8 hours/night) sleep durations are associated with higher odds of negative moods. Those with a short sleep duration felt unhappy, experienced mood swings, and felt irritable and lonely. On the other hand, those with long sleep duration reported miserableness, anxious feelings and loneliness. In accordance with the present results, previous studies have demonstrated that those who slept <7 hours/night reported more emotional problems such as depression, anxiety and anger (Bauducco et al., 2016). Another study involving UK Biobank participants also showed that those who slept 7-9 hours/night showed fewer depressive symptoms (Sarris et al., 2020). Those psychopathological symptoms were also found to be the best predictors for sleep difficulties such as difficulty falling and staying asleep which are closely related to short sleep duration (Tafoya et al., 2013). The negative impact of sleep deprivation on mood became significant even after a single night of total sleep restriction (Short and Louca, 2015). A negative association was also observed between circadian disruption and mood. Those with lower circadian amplitude were found to report poorer subjective well-being and have a higher risk of mood disorders. They were also more likely to self-report loneliness and lower health satisfaction (Lyall et al., 2018). The relationship between sleep deficit and negative mood may be explained by the down-regulation of the functional connectivity between the amygdala

and the ventral anterior cingulate cortex and medial prefrontal cortex during sleep debt leading to mood deterioration and enhanced response to negative emotional stimuli (Motomura et al., 2013, Motomura et al., 2017). Whilst research mentioned so far has been carried out to investigate the link between sleep deprivation and mood, there are very limited studies existed which found the link between having too much sleep and its impact on mood. The current study found a 'U-shaped' association between sleep duration and mood where longer sleep duration is also associated with negative moods. This relationship may partly be explained by the fact that longer sleep duration could be an indication of other underlying illnesses and pains which limits their activities and therefore contributes to having negative feelings such as miserableness and loneliness. A meta-analysis involving >25,000 participants found that those with longer sleep duration were 42% more likely to have depression (Zhai et al., 2015). Amongst those who have already been diagnosed with depression and anxiety, having a sleep duration of ≥10 hours/night was associated with the persistence of depression and anxiety (van Mill et al., 2014). However, it is possible that the relationship is bi-directional. Those with negative moods might use sleep as a method of escaping from reality and therefore have a longer sleep duration. Additionally, antidepressant drugs with antihistaminergic action such as mirtazapine are known to have a sedative effect which leads to better sleep quality and longer sleep duration (Wichniak et al., 2017). However, due to the cross-sectional nature of this study. The causal relationship between sleep duration and mood cannot be determined.

3.4 Conclusion

Overall, males, older adults, those with a high BMI, socially deprived individuals and those in ethnic minority groups are more likely to have a shorter sleep duration. Those with a shorter sleep duration tend to have higher waist circumference regardless of gender. They are also more likely to nap during the day, be a current smoker and be less physically active. Moreover, a significant 'U-shaped' association between sleep duration and disease status was found suggesting that 6-8 hours of sleep each night is associated with better long-term health. Both short and long sleep durations increase the odds of metabolic and mental diseases. Apart from diagnosed diseases, those with a shorter sleep duration had a slower reaction time, lower fluid intelligence score and worse memory, as well as experiencing more negative moods. However, this association cannot be translated into clinical utility on its own as a valid screening tool for

predicting health. Other factors, for example, diet and physical activity, may need to be taken into consideration as well as sleep in future studies.

The main strength of the current UK Biobank study is the extensive information collected on a large sample size tracked over time. The accelerometry data allowed the possibility of measuring sleep duration objectively using open-access algorithms. This is likely to be more accurate than self-reported sleep duration previously reported in this cohort. Additionally, other sleep-related variables were also provided by the accelerometer such as WASO and episodes of movements which self-reported methods of sleep assessments cannot accurately provide. Similarly, a comprehensive and complete primary care dataset is likely to provide reliable health-related measurements compared to short questions of self-reported health as previously collected. This is by far the largest UK accelerometry cohort to study sleep duration as a specific risk factor of metabolic and mental health. One of the limitations is that analysis could not be controlled for obstructive sleep apnoea due to a small number of cases within the UK biobank. The lack of specific assessment for conditions such as obstructive sleep apnoea remains underdiagnosed in the general population over the age of 50 and the specific population studied within the UK Biobank. The medication record was not available in this study meaning that analysis also could not be controlled for medication. Additionally, those in better health are more likely to attend assessment visits over time. Moreover, there was a few years' time gap between the baseline assessment and accelerometer data collection which means that participants' sleeping patterns may have changed during this time. Lastly, due to the cross-sectional nature of this study, a causal relationship could not be determined.

The current study found a discrepancy between self-reported and accelerometry assessments of sleep. Self-reported sleep duration of the Biobank cohort showed that 78% of participants had a self-reported sleep duration of >7 hours/night, but accelerometry data showed that only 23% of participants slept >7 hours/night. Therefore, participants overestimated their sleep duration. The objective and subjective assessments of sleep will therefore be explored further in the next chapter.

Chapter 4. Subjective and objective assessments of sleep

4.1 Chapter overview & aims

As discussed in the previous chapter, sleep plays a fundamental role in both physical and mental health. Therefore, the assessment of sleep is an essential component of health checks. A wide range of subjective and objective sleep detection methods are available on the market for general home use or research purposes. Subjective assessments include paper and digital sleep diaries which give binary results as wake or sleep. However, it is subject to sleep misperception. To overcome this limitation, various devices are designed to monitor sleep/wake status objectively. Objective assessments such as research accelerometers and sleep tracking mats and rings are better at detecting sleep fragmentation and avoiding sleep misperception. Finally, the gold standard laboratory-based PSG are capable of staging sleep. Each method has its advantages, as well as disadvantages.

The main aim of this chapter is to compare sleep duration and efficiency detected by three methods: paper sleep diary, Axivity AX3 tri-axial accelerometer and PSG. Participants were recruited from the local sleep service (the patient group) and the university (the healthy control group). Each participant completed two weeks of sleep diary and accelerometer recordings, as well as one-night PSG in the sleep laboratory. The level of sleep-related biomarkers such as cortisol, melatonin, mitochondrial DNA damage and gene expression were compared between the patient and control groups through the collection and analysis of saliva, urine, skin swabs and hair follicle samples, respectively. Moreover, participants were divided into three groups according to their sleep duration and the level of those biomarkers were also compared between each sleep group.

4.2 Results

4.2.1 Recruitment

The participant information sheet was sent to 94 individuals who showed interest in the study. After completing a one-to-one informed consent meeting, 58 individuals agreed to participate. However, due to limited PSG slots available and national lockdowns during the COVID-19 pandemic, only 30 participants were able to complete the PSG. Two participants failed to return the accelerometer, sleep diary and biological samples. Therefore, 28 participants were included in the analysis (Figure 4-1) which includes 20 females (71.4%) and 8 males (28.6%). The age of these participants ranged between 21 and 60 years and the mean age was 36.5 (±14.4) years (Figure 4-2).

Unless stated otherwise, all biological samples were received back from participants. Out of the 28 accelerometers received, one accelerometer was not able to be processed due to unknown technical errors. Therefore, 27 accelerometer data were included in the analysis. One participant did not return urine samples, two participants did not provide enough samples and three participants did not provide information on the volume of void for the calculation of the melatonin level per 24 hours. As a result, urinary melatonin level is only available for 22 participants.

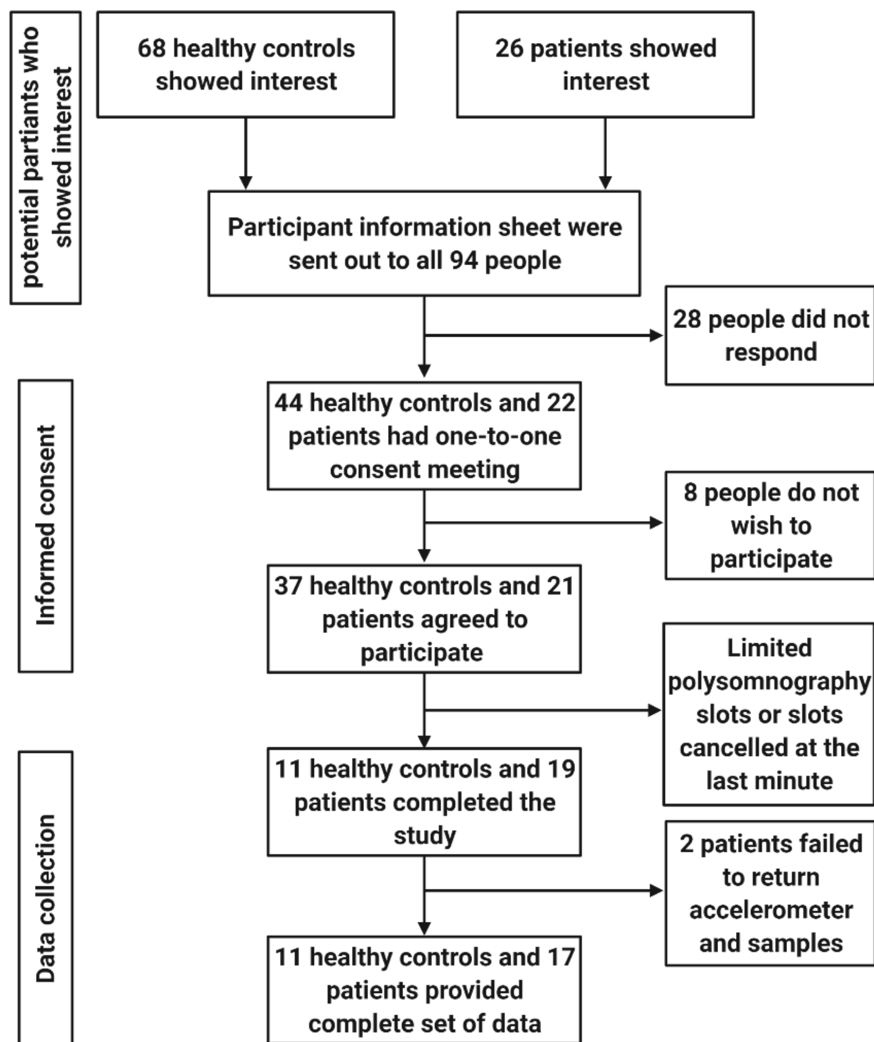


Figure 4-1. Flow chart demonstrating the participant recruitment process and the number of people involved at each stage.

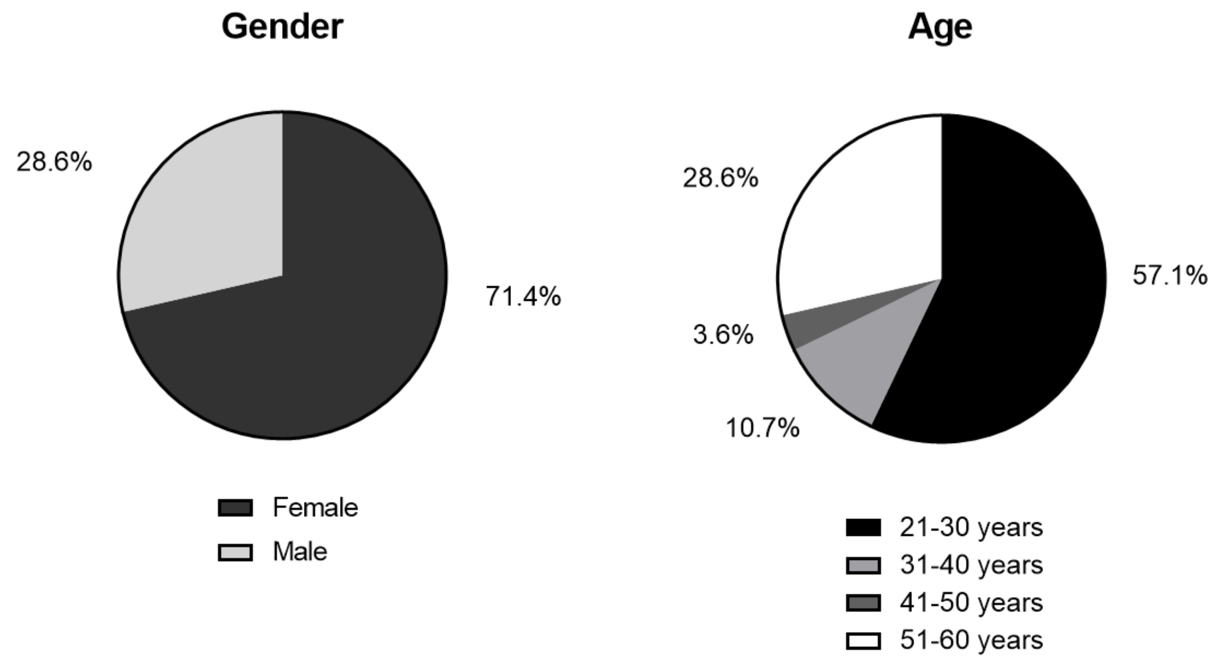


Figure 4-2. Distribution of study participants across gender and age groups (n= 28).

4.2.2 Subjective and objective assessments of sleep

4.2.2.1 Accelerometer

Figure 4-3 illustrates a 7-day accelerometer data which showed that it was possible to detect night-time sleep and daytime naps, as well as different intensities of daytime activities. Activity labelling was completed using a sleep diary of the same person.

On average, participants spent 9.70 (±1.02) hours/night in bed (median= 9.30 hours/night, interquartile range= 1.88 hours/night). The sleep duration of the 27 participants with processable accelerometer data ranged between 5.01 hours/night and 9.35 hours/night and the mean sleep duration was 7.08 (±1.01) hours/night. Both the time spent in bed and sleep duration were slightly higher in the patient group compared to the control group (p= 0.393 and p=0.880, respectively) (Table 4-1). The distribution of participants in each sleep group amongst the control and patient groups is shown in Figure 4-4A and Figure 4-4B, respectively. Overall, 14 participants (51.9%) slept <7 hours/night, eight participants (29.6%) slept 7-8 hours/night and five participants (18.5%) slept >8 hours/night.

Apart from sleep duration, accelerometers were also able to provide data on sleep quality and fragmentation including sleep efficiency, wake after sleep onset (WASO) and episodes of movement. These results are shown in Table 4-1. The mean sleep efficiency of these participants was 73.49% (±6.45%). The control group (mean, 75.72%) had higher sleep efficiency compared to the patient group (mean, 72.18%) (p=0.160). On average, participants experienced 87.76 (±29.01) episodes of movement during sleep (median= 83.79, interquartile range= 43.17). The difference was very small between the control (mean, 87.73) and the patient group (mean, 87.77) (p= 0.725). WASO ranged between 1.49 hours/night and 4.21 hours/night. The mean WASO was 2.62 (±0.68) hours/night (median= 2.44 hours/night, interquartile range= 1.14 hours/night) and it was slightly higher in the patient group (mean, 2.78 hours) compared to the control group (mean, 2.36 hours) (p= 0.269). Finally, the level of movement during the least active five hours of the 24 hours period was indicated by the L5 value which was 6.08 (±2.61) milli-g on average. Much like the WASO, the mean L5 value was also found to be higher in the patient group (mean, 6.20 milli-g) compared to the control group (mean, 5.85 milli-g) (p= 0.668). It is worth noting that a higher level of WASO and L5 value indicates more restless/ fragmented sleep.

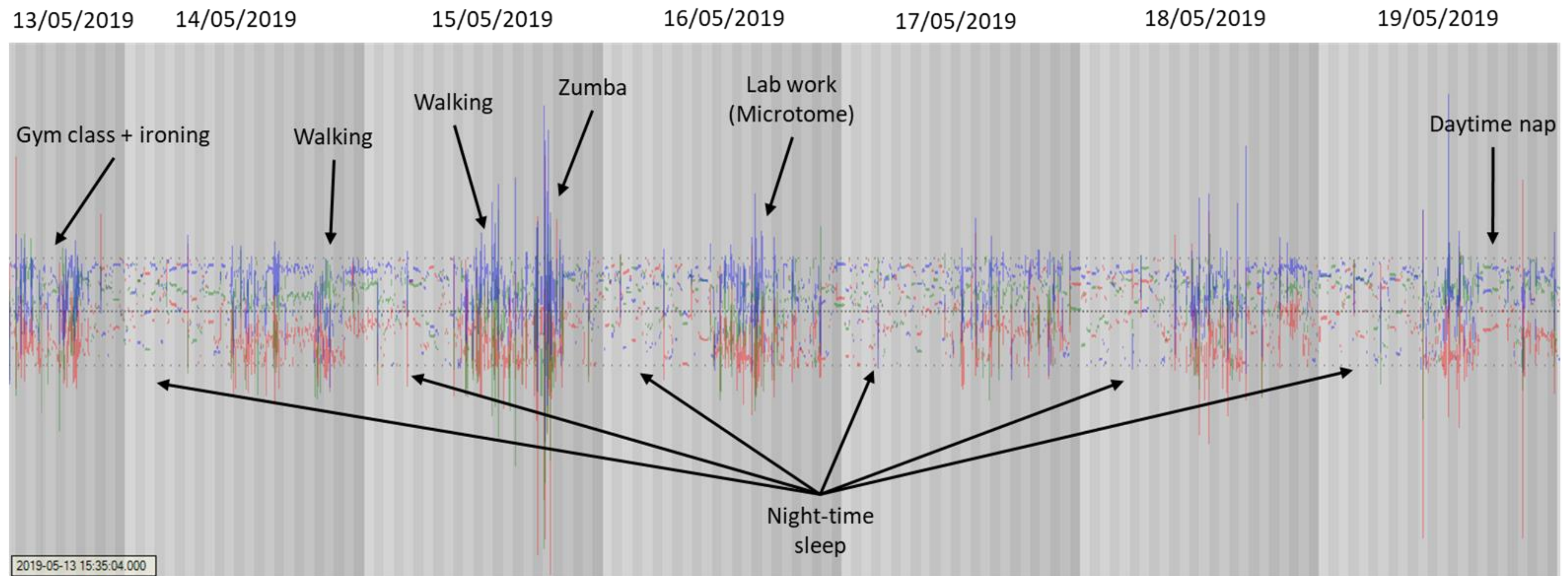


Figure 4-3. A week of accelerometer data showing various daytime activities with different movement intensity and night-time sleep. Activity labelling was completed using the sleep diary of the same person. Visualisation of the image was carried out using the OmGui software.

Table 4-1. Descriptive analysis results of various accelerometer variables (10 healthy controls and 17 patients). Data were obtained from two weeks of accelerometer recording.

	Mean			**Standard Deviation**			**Minimum**			**Maximum**		
	Control	**Patient**	**combined**	**Control**	**Patient**	**combined**	**Control**	**Patient**	**combined**	**Control**	**Patient**	**combined**
Total time in bed (hour)	9.42	9.87	9.70	0.64	1.17	1.02	8.75	7.91	7.91	10.69	11.60	11.60
Sleep duration (hour)	7.03	7.10	7.08	0.67	1.18	1.01	6.03	5.01	5.01	8.26	9.35	9.35
Sleep efficiency (%)	75.72	72.18	73.49	3.87	7.37	6.45	67.52	52.68	52.68	80.36	84.74	84.74
Episodes of movement (n)	87.73	87.77	87.76	11.94	35.88	29.01	74.08	38.17	38.17	106.50	181.75	181.75
WASO (hour)	2.36	2.78	2.62	0.36	0.78	0.68	1.83	1.49	1.49	3.09	4.21	4.21
L5 value (milli-g)	5.85	6.20	6.08	3.11	2.42	2.61	2.61	2.44	2.44	11.40	12.10	12.10

WASO= wake after sleep onset

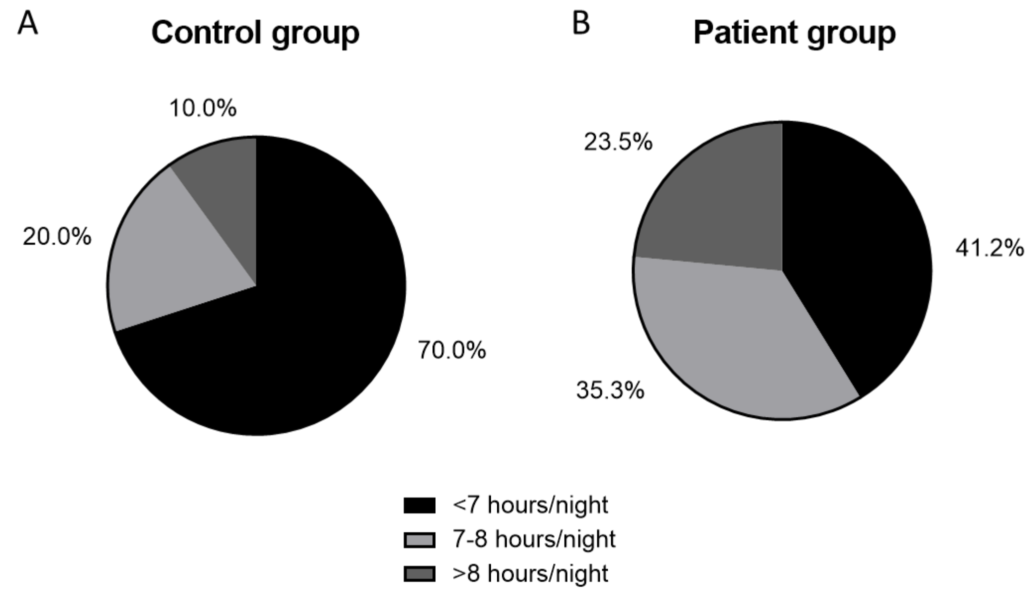


Figure 4-4. Distribution of participants across the three sleep groups. Sleep duration was measured by the two weeks of accelerometer recording. A: Control group (n=10), B: Patient group (n= 17), C: Control and patient groups combined (n= 27).

4.2.2.2 Polysomnography

The results, as shown in Table 4-2, indicates that the sleep duration measured by PSG was slightly higher in the patient group (mean, 6.74 hours) compared to the control group (mean, 6.37 hours) (p= 0.384). Overall, the sleep duration ranged between 4.36 hours/night and 8.83 hours/night. The mean sleep duration was 6.59 (±1.27) hours/night. Sleep efficiency was also higher in the control group (mean, 81.73%) compared to the patient group (mean, 76.40%) (p= 0.180). The mean sleep efficiency of these 28 participants was 78.49% (±10.12%). Both the minimum and maximum sleep efficiency were observed in the patient group which were 52.30% and 92.50%, respectively. WASO ranged between 0.28 hours and 2.89 hours. The mean WASO was 1.42 (±0.70) hours and it was significantly higher in the patient group (mean, 1.82 hours) than in the control group (mean, 1.06 hours) (p= 0.011). Both groups experienced a similar number of awakening episodes throughout the night and it ranged between 8 and 56 episodes (p= 0.672). On average, participants experienced 23.75 (±11.09) awakening episodes. Finally, the bottom half of Table 4-2 shows the percentage spent at each sleep stage from N1 to REM. As expected, more than half of the nights were spent in the N2 stage and the least was spent in the N1 stage.

According to the PSG measured sleep duration, no one in the control group is in the '>8 hours/night' sleep group and most of the participants in the control group slept <7 hours/night (Figure 4-5A). More than half of the patient group also slept <7 hours/night (Figure 4-5B). Overall, 16 participants (57.1%) slept <7 hours/night, eight participants (28.6%) slept 7-8 hours/night and the remaining four participants (14.3%) slept >8 hours/night.

Table 4-2. Descriptive analysis results of various PSG variables (n= 28). Data were obtained from a single night of PSG.

	Mean			Standard Deviation			Minimum			Maximum		
	Control	**Patient**	**combined**	**Control**	**Patient**	**combined**	**Control**	**Patient**	**combined**	**Control**	**Patient**	**combined**
PSG sleep duration (hour)	6.37	6.74	6.59	1.07	1.40	1.27	4.45	4.36	4.36	7.67	8.83	8.83
Sleep efficiency (%)	81.73	76.40	78.49	7.12	11.37	10.12	72.20	52.30	52.30	92.50	92.00	92.50
WASO (hour)	1.06	1.82	1.42	0.51	0.67	0.70	0.28	0.82	0.28	1.88	2.89	2.89
Number of awakening episodes	23.91	23.65	23.75	9.15	12.45	11.09	13.00	8.00	8.00	44.00	56.00	56.00
Percentage spent in each stage												
N1 (%)	3.57	5.55	4.78	2.88	5.10	4.41	0.30	0.00	0.00	9.10	16.60	16.60
N2 (%)	59.13	50.44	53.85	10.96	9.58	10.84	39.80	29.50	29.50	82.60	69.90	82.60
N3 (%)	22.54	24.19	23.54	11.39	11.26	11.13	8.20	8.20	8.20	51.60	50.70	51.60
REM (%)	14.76	19.81	17.83	7.67	9.12	8.80	1.10	3.90	1.10	28.40	35.40	35.40

PSG= Polysomnography, WASO= wake after sleep onset, REM= Rapid eye movement

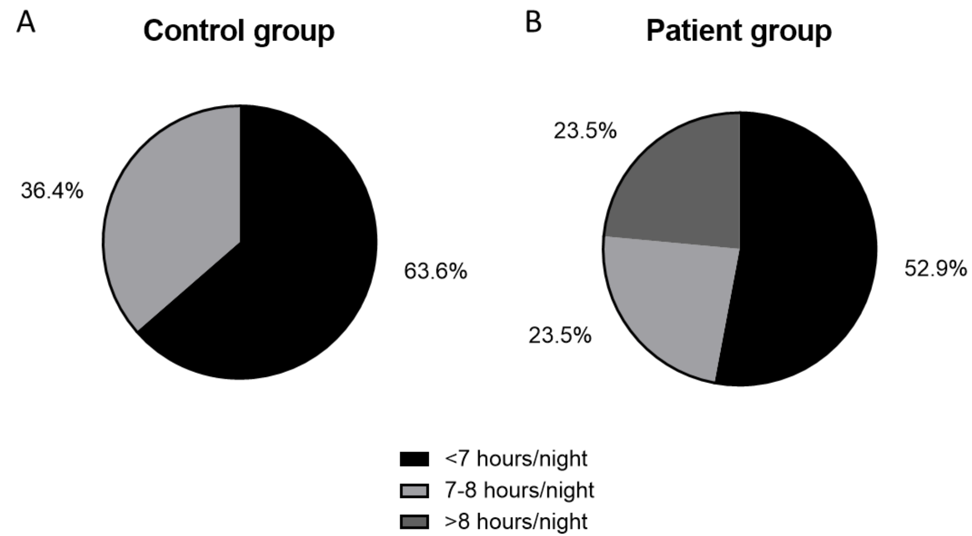


Figure 4-5. Distribution of participants across the three sleep groups. Sleep duration was measured by a single night of polysomnography in the sleep laboratory. A: Control group (n=11), B: Patient group (n= 17), C: Control and patient groups combined (n= 28).

4.2.2.3 Sleep diary

Sleep diary recording showed that participants spent on average 9.05 (±0.96) hours/night in bed (Table 4-3). The minimum amount of time they spent in bed was 6.83 hours/night, while the maximum duration was 11.11 hours/night. When comparing the control group to the patient group, results indicated that the patient group (mean, 9.41 hours) spent significantly more time in bed than the control group (mean, 8.60 hours) (p= 0.013). Sleep duration, on the other hand, ranged between 5.18 hours/night and 10.25 hours/night. The mean sleep duration was 7.51 (±1.24) hours/night. Consistent with prior findings which showed that the patient group spent longer in bed, they also had longer sleep duration (mean, 7.74 hours) when compared to the control group (mean, 7.17 hours). Although the difference detected was not found to be statistically significant (p= 0.138). The sleep efficiency of these participants ranged between 58.20% and 95.97%. Table 4-3 shows that the mean sleep efficiency was 82.19% (±9.96%) and on average the control group (mean, 83.33%) had higher sleep efficiency than the patient group (mean, 81.29%) (p= 1.000).

No one in the control group had a self-reported sleep duration of >8 hours/night, four participants (36.4%) slept <7 hours/night, while the other seven participants (63.6%) slept >8 hours/night (Figure 4-6A). Additionally, when looking at the patient group alone, seven participants (41.2%) slept <7 hours/night, only one participant (5.9%) slept 7-8 hours and the remaining nine participants (52.9%) slept >8 hours/night (Figure 4-6B). Overall, participants distributed quite evenly across the three sleep groups: 11 participants (39.3%) slept <7 hours/night, eight participants (28.6%) slept 7-8 hours/night and the remaining nine participants (32.1%) slept >8 hours/night.

Table 4-3. Descriptive analysis results of various sleep diary variables (n= 28). Data were obtained from two weeks of sleep diary recording.

	Mean			**Standard Deviation**			**Minimum**			**Maximum**		
	Control	**Patient**	**combined**	**Control**	**Patient**	**combined**	**Control**	**Patient**	**combined**	**Control**	**Patient**	**combined**
Total time in bed (hour)	8.60	9.41	9.05	0.46	1.10	0.96	7.75	6.83	6.83	9.30	11.11	11.11
Sleep duration (hour)	7.17	7.74	7.51	0.73	1.46	1.24	6.21	5.18	5.18	7.93	10.25	10.25
Sleep efficiency (%)	83.33	81.29	82.19	6.98	11.99	9.96	71.90	58.20	58.20	92.97	95.97	95.97

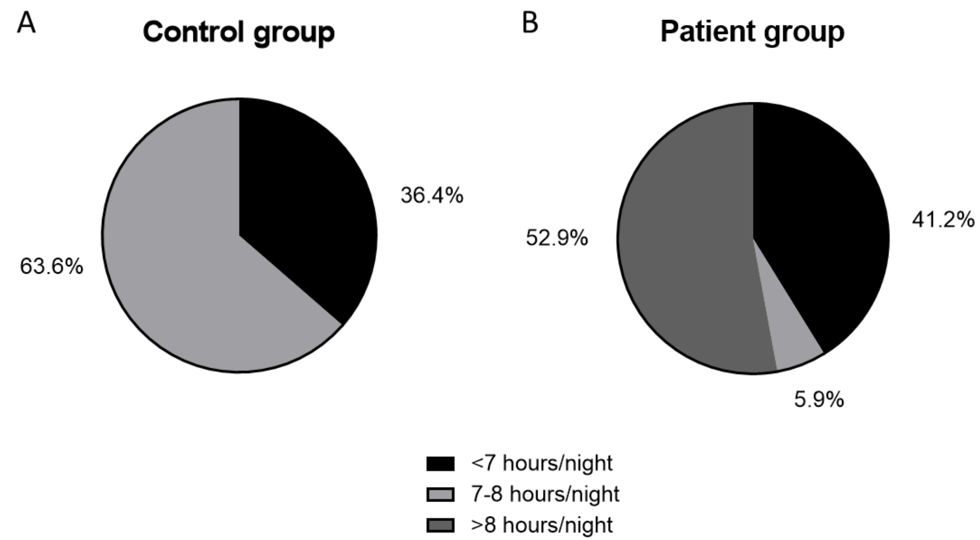


Figure 4-6. Distribution of participants across the three sleep groups. A: Control group (n=11), B: Patient group (n= 17), C: Control and patient groups combined (n=28). Sleep duration was measured by two weeks of sleep diary recording.

4.2.2.4 Comparison between three methods of sleep detection

The sleep duration detected by the sleep diary, accelerometer and PSG during the night in the sleep laboratory were very similar (Figure 4-7A). The mean sleep duration measured by the sleep diary was the shortest which was 6.29 (±2.31) hours/night, followed by the accelerometer recording which was 6.35 (±2.49) hours/night and it was the longest for the PSG measurement which was 6.59 (±1.27) hours/night. However, statistical analysis showed that the difference detected between each method of sleep detection was not statistically significant (p= 0.987).

When analysing the sleep efficiency data, no statistically significant difference was found between the three methods of sleep detection (p= 0.092). The sleep efficiency detected was very similar between the sleep diary and accelerometer. The mean sleep efficiency was the lowest for the accelerometer recording which was 69.59% (±13.03%), followed by the sleep diary recording which was 70.02% (±26.74%) and finally, it is the highest for the PSG recording which was 78.49% (±10.12%) (Figure 4-7B).

Figure 4-7C illustrates the comparison between the three methods of sleep duration detection at an individual level where the recording for each participant is connected by a line. No obvious pattern was observed. Approximately half of the participants showed a 'U-shaped' curve while the rest showed a reversed 'U-shaped' curve. Overall, it seems that sleep durations detected by the sleep diary and polysomnography were relatively similar, while the sleep duration detected by the accelerometer was either longer or shorter than that detected by the other two methods (Figure 4-7C).

Finally, the comparison of sleep efficiency between the three methods at an individual level is shown in Figure 4-7D. Resembling the graph for sleep duration, the graph for sleep efficiency did not show any noticeable patterns. A reversed 'U-shaped' curve was observed in a few participants, while the rest showed a 'U-shaped' curve.

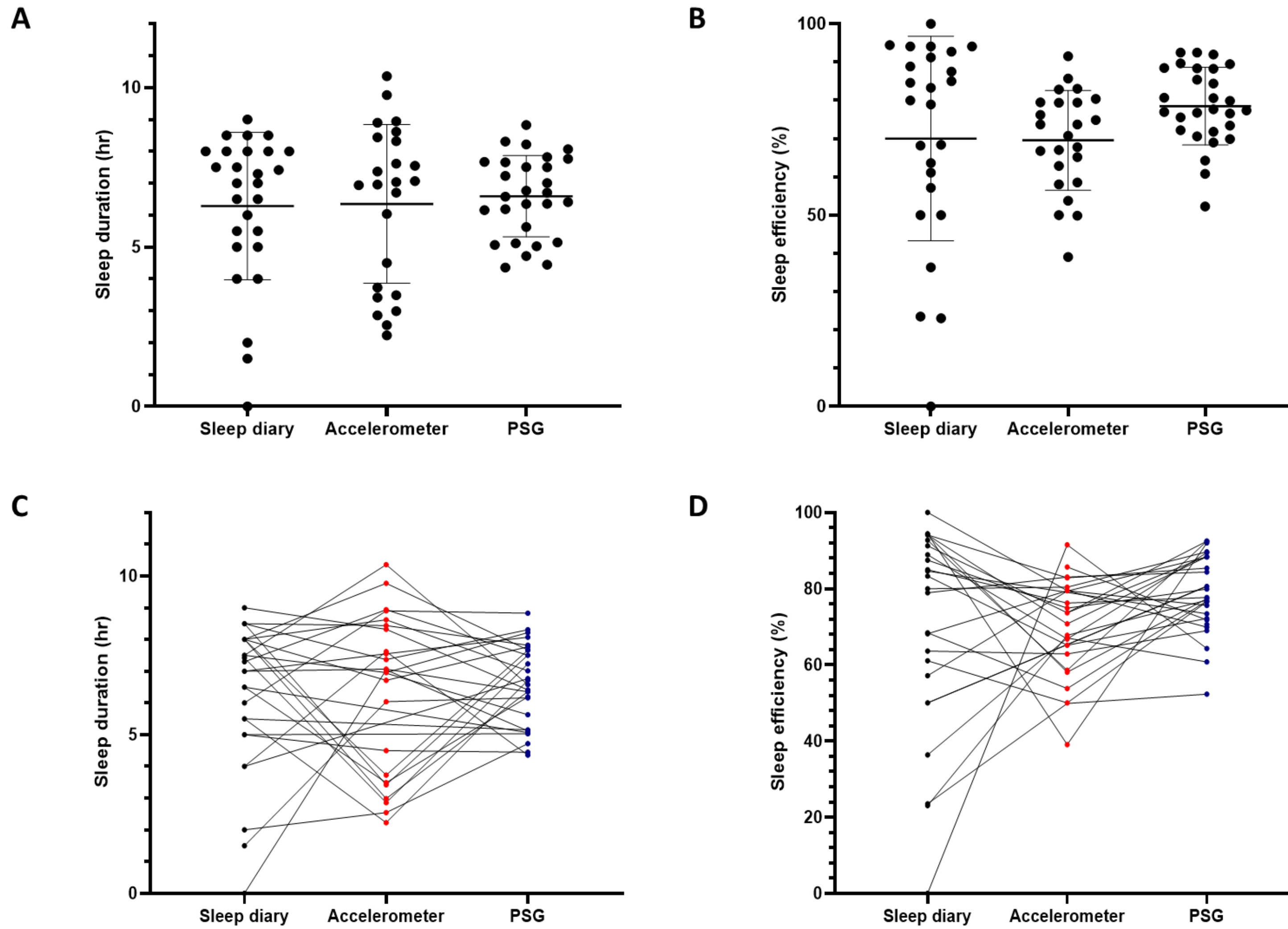


Figure 4-7. Comparison of sleep duration (A) and sleep efficiency (B) between the three methods of sleep detection. C and D compare sleep duration and efficiency at an individual level. Recordings from the same individual is connected by a line. PSG= polysomnography.

4.2.2.5 Comparison between controls and patients

Regardless of the sleep detection method, sleep duration was slightly higher amongst the patient group compared to the control group (Figure 4-8A). However, the difference was not found to be statistically significant (p= 0.138, p= 0.880 and p= 0.384 for sleep diary, accelerometer and PSG, respectively).

The trend observed for sleep efficiency was the opposite. Sleep efficiency appears to be higher amongst the control group compared to the patient group, regardless of the sleep detection method (Figure 4-8B). Once more, the difference detected was not statistically significant (p= 1.000, p= 0.160 and p= 0.180 for sleep diary, accelerometer and PSG, respectively).

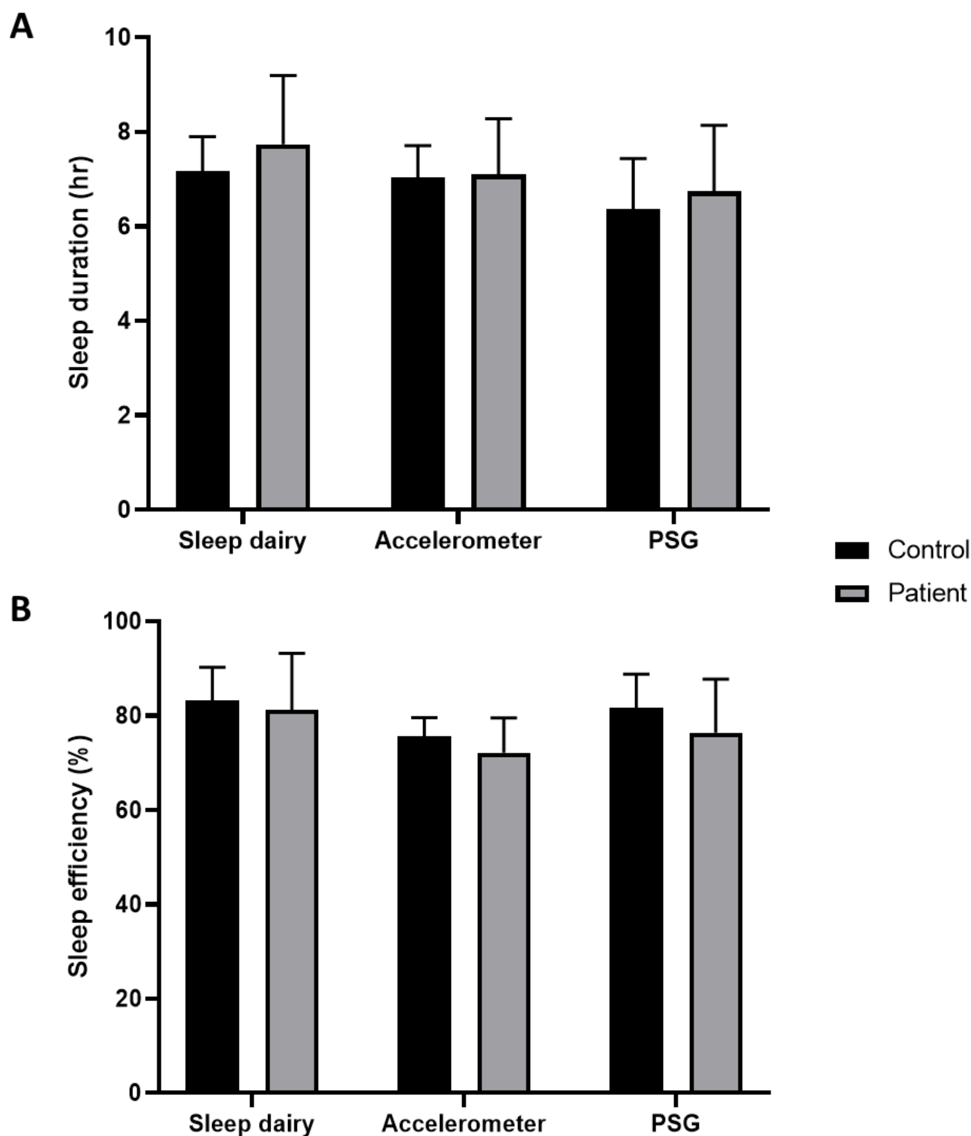


Figure 4-8. Comparison of sleep duration (A) and sleep efficiency (B) between the control and patient group for each method of sleep detection. None of the differences detected was found to be statistically significant. PSG= polysomnography.

4.2.3 Questionnaire

Data related to questionnaire questions are summarised in Table 4-4. When participants were asked “How well did you sleep in the sleep laboratory? (On a scale of 1 to 10, with 10 being very well)”, the mean rating was 4.50 (±2.12). Their selection ranged between 1 and 8. The control group had a higher rating than the patient group, but the difference was not statistically significant (p= 0.563). Unsurprisingly, a positive relationship between the PSG measured sleep duration and their subjective sleep rating was observed. Participants in the “<7 hours/night” sleep group had the lowest rating, while those who slept >8 hours/night had the highest rating. On the other hand, a reversed ‘U-shaped’ relationship was observed between the accelerometer measured sleep duration and their subjective sleep rating. Those who slept 7-8 hours/night had the highest rating, while participants who slept <7 hours/night or >8 hours/night had similar ratings and they were slightly lower. Finally, a ‘U-shaped’ relationship was found between the sleep duration measured by the sleep diary and their subjective sleep rating. Participants in the ‘>8 hours’ sleep group had the highest rating, followed by the ‘<7 hours’ sleep group and then the ‘7-8 hours’ sleep group. However, any differences detected between sleep groups were not statistically significant, regardless of the sleep detection method (p= 0.408, p= 0.701 and p= 0.249 for PSG, sleep diary and accelerometer, respectively).

They were also asked about how comfortable they felt staying overnight in the sleep laboratory and on a scale of 1 to 10 (with 10 being very comfortable), their selection covered the whole range and the mean rating was 5.8 (±2.36) (Table 4-4). A higher rating was found in the control group compared to the patient group, but the difference was not found to be statistically significant (p= 0.268). On the contrary to the previous question, the relationship between the PSG measured sleep duration and the rating had a negative trend. Therefore, surprisingly, those with a shorter sleep duration had a higher rating. A reversed ‘U-shaped’ relationship was found between the accelerometer measured sleep duration and the rating. Those who slept 7-8 hours/night felt the most comfortable with the overnight stay in the sleep laboratory, while those in the ‘>8 hours’ sleep group found it the least comfortable. Finally, a ‘U-shaped’ association between the sleep diary recorded sleep duration and their rating was observed. Those who self-reported 7-8 hours of sleep were the least comfortable staying overnight in the sleep laboratory. Once more, any differences reported so far were not found

to be statistically significant (p= 0.789, p= 0.156 and p= 0.394 for PSG, sleep diary and accelerometer, respectively).

Lastly, out of the few options listed as the main source of discomfort, the most selected option was “PSG wires” (n= 20), followed by “Noise level” (n= 18) and then “Change of environment” (n=12) (Table 4-5).

Table 4-4. Summary of Questionnaire answers (n= 26).

	How well you slept	**How comfortable you felt**
Mean		
Control	4.73	6.36
Patient	4.33	5.36
Combined	4.50	5.80
Standard deviation		
Control	1.90	1.74
Patient	2.32	2.73
Combined	2.12	2.36
Minimum		
Control	2.00	3.00
Patient	1.00	1.00
Combined	1.00	1.00
Maximum		
Control	7.00	9.00
Patient	8.00	10.00
Combined	8.00	10.00
PSG sleep groups		
<7 hours (mean ± SD)	4.06 ± 2.11	6.00 ± 2.78
7-8 hours (mean ± SD)	5.00 ± 2.00	5.71 ± 1.60

	How well you slept	How comfortable you felt
>8 hours (mean ± SD)	5.67 ± 2.52	5.00 ± 2.00
Accelerometer sleep groups		
<7 hours (mean ± SD)	3.91 ± 2.26	5.60 ± 2.63
7-8 hours (mean ± SD)	5.80 ± 1.92	6.80 ± 2.39
>8 hours (mean ± SD)	4.00 ± 1.79	4.83 ± 1.72
Sleep diary sleep groups		
<7 hours (mean ± SD)	4.54 ± 2.15	6.92 ± 2.27
7-8 hours (mean ± SD)	4.38 ± 1.69	5.00 ± 1.69
>8 hours (mean ± SD)	5.50 ± 2.65	5.25 ± 2.06

Participants were asked to rate their overnight stay in the sleep laboratory on a scale of 0-10, where 0= not well at all/not comfortable at all and 10= very well/very comfortable. SD= standard deviation.

Table 4-5. Participants' vote on the main cause of discomfort during their overnight stay in the sleep laboratory (n= 26).

Main cause of discomfort	Number of participants
Change of environment	12
The bed	5
PSG wires	20
Light intensity	1
Noise level	18
Room temperature	8
Other	3

4.2.4 Biological samples

4.2.4.1 Melatonin level in different sleep groups

A 48-hours melatonin profile is shown in Figure 4-9. During the first 24 hours, melatonin concentration began to increase after 10 pm and it peaked at about 10 am before it started to decrease again. This pattern was also observed in the next 24 hours, but with a lower peak at 10 am.

The melatonin level of the participants ranged between 0.07 x 10^7 ng/24 hours and 2.61 x 10^7 ng/24 hours. The mean melatonin level was 0.48 x 10^7 ng/24 hours (standard deviation= 0.56 x 10^7 ng/24 hours, median= 2.94 x 10^6 ng/24 hours, interquartile range= 3.82 x 10^6 ng/24 hours) (Figure 4-10). No significant difference was detected between the control and patient groups

(p= 0.203). Although, the patient group appears to have a lower level of melatonin compared to the control group. The mean melatonin level was 0.65 x 10^7 ng/24 hours (±0.79 x 10^7 ng/24 hours) and 0.36 x 10^7 ng/24 hours (±0.32 x 10^7 ng/24 hours) for the control and patient groups, respectively (Figure 4-11A).

No clear pattern has been observed when comparing melatonin level with sleep duration measured by two weeks of sleep diary and accelerometer recording (Figure 4-11B). When looking at the sleep duration measured by the sleep diary, a reversed 'U-shaped' curve was observed and those with a longer sleep duration had the lowest level of melatonin - <7 hours/night: 0.49 x 10^7 ng/24 hours (±0.34 x 10^7 ng/24 hours), 7-8 hours/night: 0.71 x 10^7 ng/24 hours (±0.91 x 10^7 ng/24 hours) and >8 hours/night: 0.23 x 10^7 ng/24 hours (±0.15 x 10^7 ng/24 hours). However, the difference between each sleep group was not found to be statistically significant (p= 0.222). The opposite trend was observed between melatonin level and sleep duration measured by the accelerometer and those who slept <7 hours/night have the highest level of melatonin - <7 hours/night: 0.54 x 10^7 ng/24 hours (±0.72 x 10^7 ng/24 hours), 7-8 hours/night: 0.38 x 10^7 ng/24 hours (±0.29 x 10^7 ng/24 hours) and >8 hours/night: 0.42 x 10^7 ng/24 hours (±0.42 x 10^7 ng/24 hours). Same as for the sleep diary, the difference between each accelerometer measured sleep group was not statistically significant (p= 0.985).

Finally, no significant correlation has been observed between the level of melatonin and accelerometer measured sleep efficiency (r= 0.116, p= 0.616) (Figure 4-11C). Similarly, no significant correlation has been observed between the level of melatonin and sleep diary measured sleep efficiency (r= -0.135, p= 0.560) (Figure 4-11D).

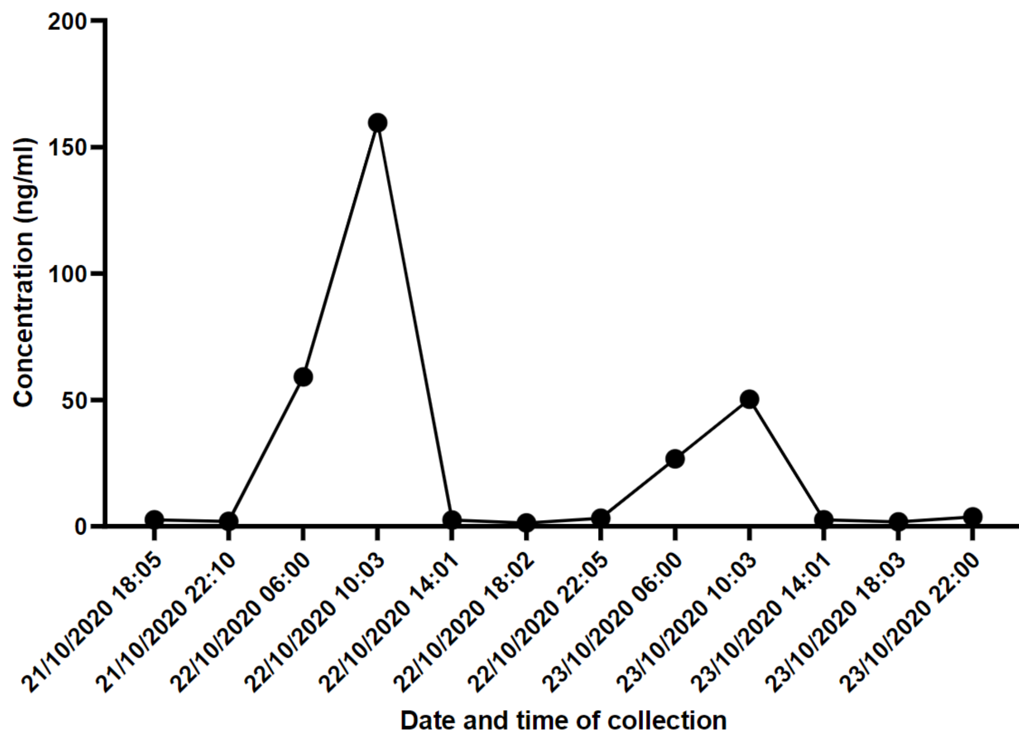
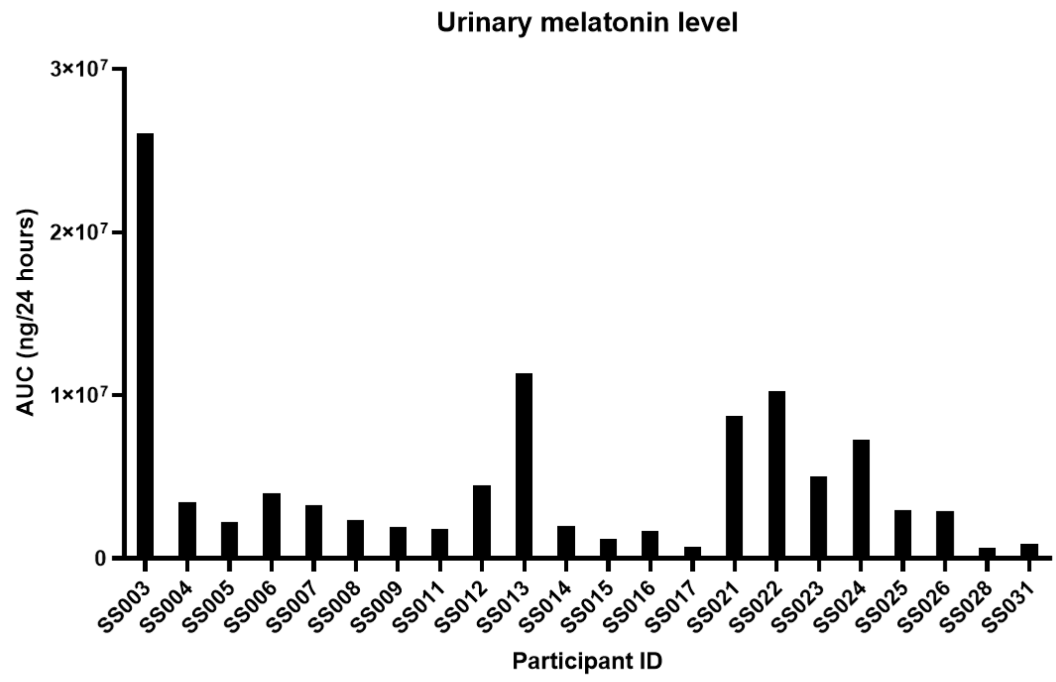


Figure 4-9. 48-hours of urinary melatonin measurements taken every four hours when awake and eight hours when asleep (n= 1).



	Grouping	Melatonin level AUC (x 10^7) (ng/24 hours)
Mean	Control	0.65
	Patient	0.36
	Combined	0.48
Standard Deviation	Control	0.79
	Patient	0.32
	Combined	0.56
Minimum	Control	0.18
	Patient	0.07
	Combined	0.07
Maximum	Control	2.61
	Patient	1.03
	Combined	2.61

Figure 4-10. Melatonin level (ng/ 24 hours) measured from 48-hours urine samples (n=22).

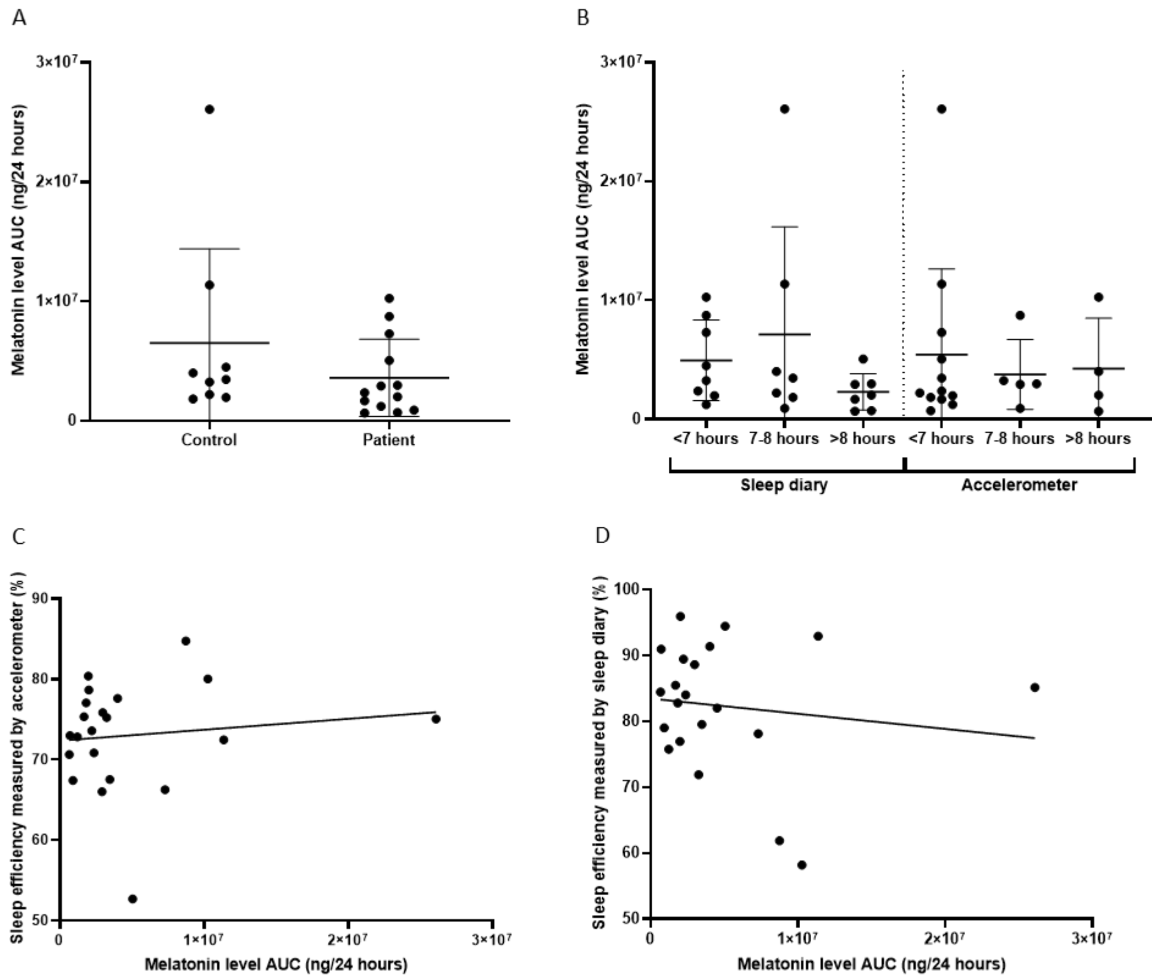


Figure 4-11. Comparison of melatonin level between A: the control and patient group, B: different sleep groups measured by two weeks of sleep diary and accelerometer recordings, C: sleep efficiency measured by accelerometer, and D: sleep efficiency measured by sleep diary.

4.2.4.2 Cortisol level in different sleep groups

A curve demonstrating 24-hours cortisol level is shown in Figure 4-12. Cortisol concentration was the lowest at midnight then it increased throughout the early morning. It peaked at around 8 am and remained stable until noon then it started to decrease again.

The cortisol level of the participants ranged between 0.06 and 1.08 µg/dL at home. The mean cortisol level at home was 0.38 µg/dL (standard deviation= 0.25 µg/dL, median= 0.26 µg/dL, interquartile range= 0.37 µg/dL). On the other hand, cortisol levels at the hospital ranged between 0.02 µg/dL and 1.11 µg/dL. The mean concentration at the hospital was 0.42 µg/dL (standard deviation= 0.26 µg/dL, median= 0.39 µg/dL, interquartile range= 0.28 µg/dL) which was slightly higher than that at home (Figure 4-13).

The cortisol level was slightly higher for the samples taken in the hospital compared to that taken at home. However, the difference between them was not found to be statistically

significant (p= 0.789) (Figure 4-14A). The mean cortisol level at home was slightly higher amongst the control group compared to the patient group. However, the cortisol level at the hospital was very similar between the two groups (Figure 4-14B). Regardless of whether the samples were collected at home or at the hospital, the difference between the control and patient group was also not statistically significant (p= 0.091 and 0.677 for samples collected at home and the hospital, respectively). Figure 4-14C illustrates the percentage change in cortisol levels at the hospital compared to home in both the control and patient groups. The patient group appears to experience a greater change in cortisol level.

Figure 4-15A illustrates the relationship between the cortisol level at home and sleep duration measured by two weeks of accelerometer and sleep diary recordings. In both methods, those who sleep 7-8 hours/night had the highest salivary cortisol concentration. However, the difference detected between each group was not statistically significant (p= 0.340 and p= 0.883 for accelerometer and sleep diary recordings, respectively). The association between the cortisol level at the hospital and sleep duration measured by a single night of accelerometer, sleep diary and PSG recording is shown in Figure 4-15B. A reversed 'U-shaped' association was found between cortisol concentration and accelerometer measured sleep duration (p= 0.528), while a 'U-shaped' association was found between cortisol concentration and sleep diary and PSG measured sleep duration (p= 0.159 and p= 0.252, respectively). As indicated by the p-values, none of the differences detected between sleep groups was statistically significant.

Finally, no significant correlation was observed between accelerometer measured sleep efficiency (based on the average of two weeks of recording) and cortisol level measured at home (r= 0.316, p= 0.109). Similarly, no significant correlation was observed between sleep diary measured sleep efficiency (based on the average of two weeks of recording) and cortisol level measure at home (r= 0.052, p= 0.795). Moreover, no significant correlation was observed between PSG measured sleep efficiency (based on a single night of recording) and cortisol level measure at the hospital (r= 0.145, p= 0.460).

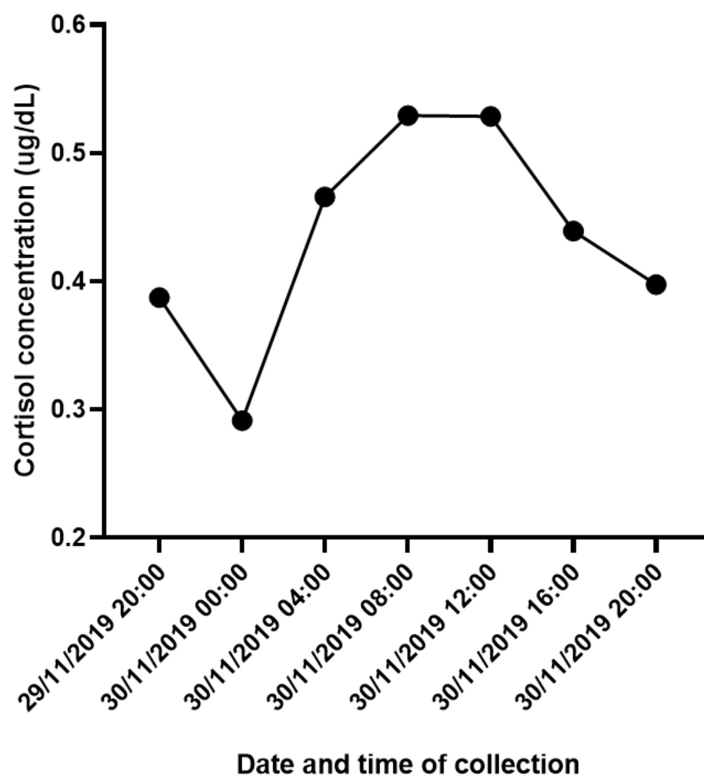
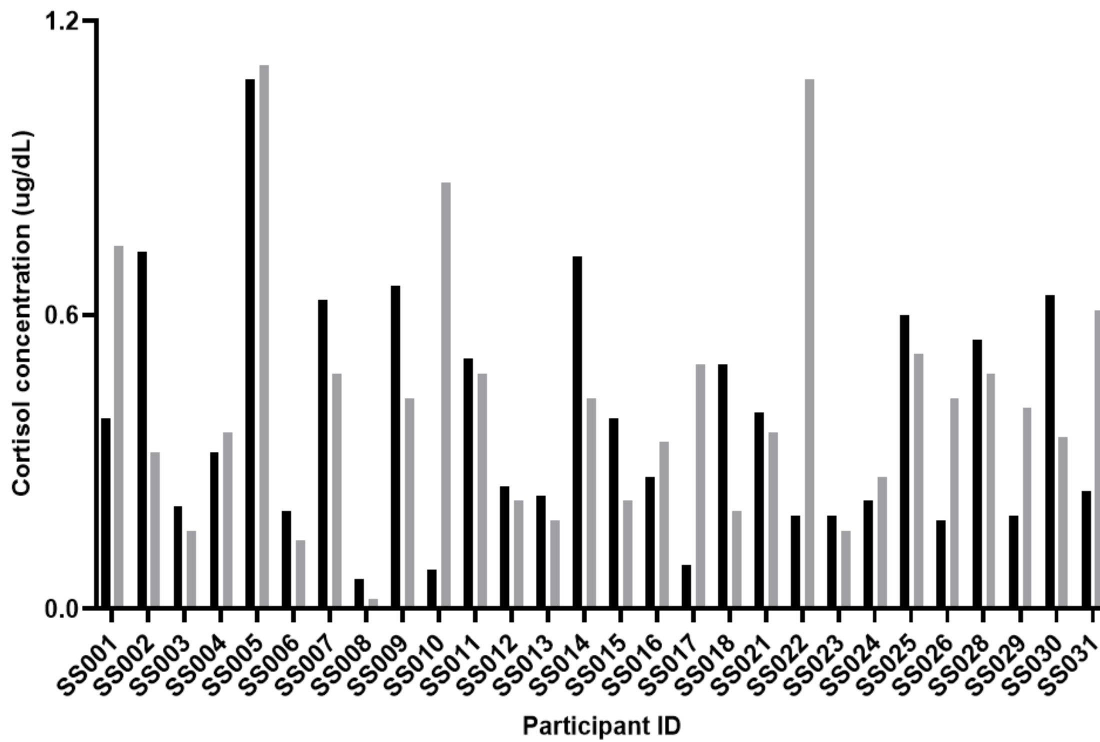


Figure 4-12. Salivary cortisol concentration throughout a 24-hours period. A sample was taken every four hours (n= 1).



		Cortisol level at home (ug/dL)	Cortisol level at hospital (ug/dL)
Mean	**Control**	0.47	0.42
	Patient	0.33	0.43
	combined	0.38	0.42
Standard Deviation	**Control**	0.28	0.29
	Patient	0.21	0.26
	combined	0.25	0.26
Minimum	**Control**	0.20	0.14
	Patient	0.06	0.02
	combined	0.06	0.02
Maximum	**Control**	1.08	1.11
	Patient	0.72	1.08
	combined	1.08	1.11

Figure 4-13. Cortisol level was measured from a morning saliva sample taken at home and the hospital after polysomnography (n= 28).

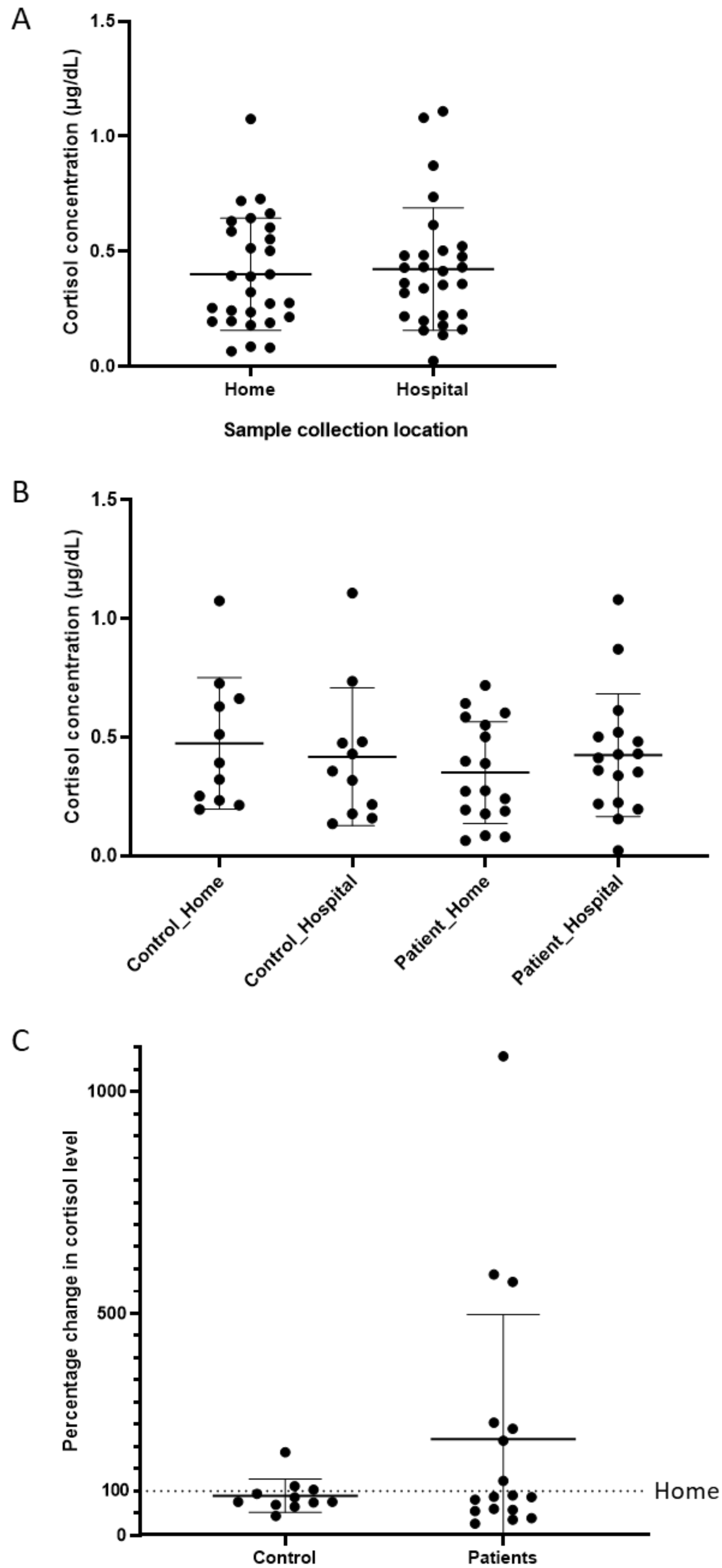


Figure 4-14. Comparison of cortisol level between different locations of samples collection (A), between patient and control group at different locations (B) and percentage change in cortisol level at the hospital compared to home (C).

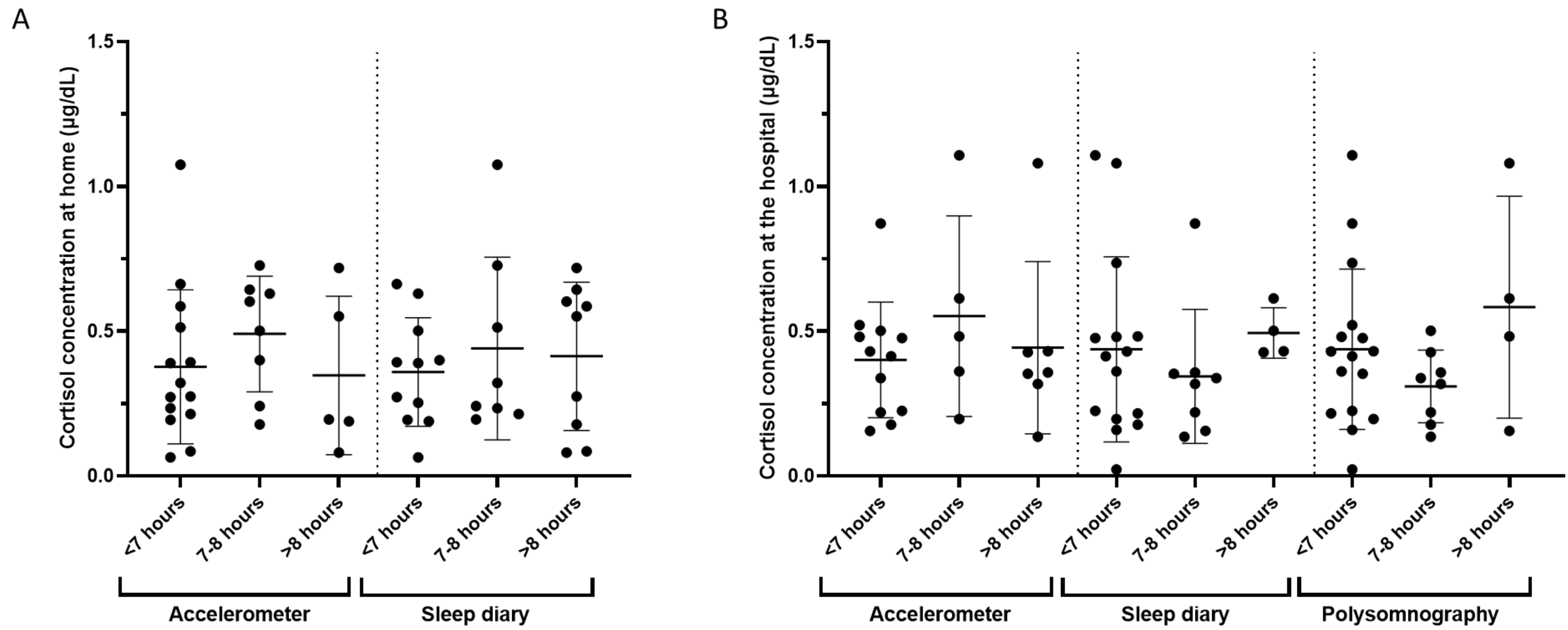
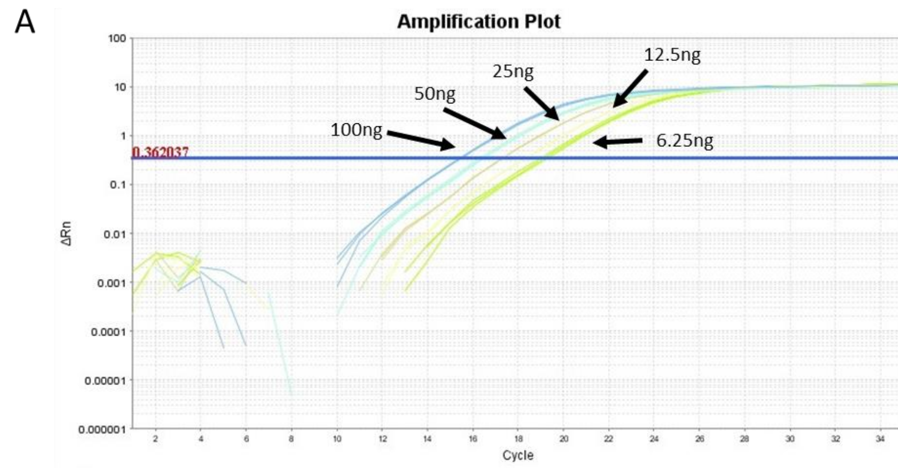


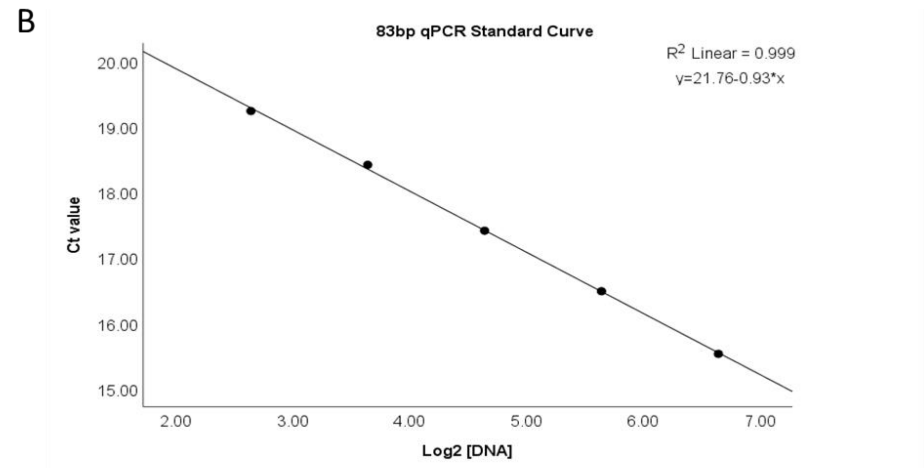
Figure 4-15. Comparison of cortisol levels between different sleep groups. A- comparison of cortisol level at home between sleep groups measured by two weeks of accelerometer and sleep diary recording. B- comparison of cortisol level at the hospital between sleep groups measured by a single night of accelerometer, sleep diary and polysomnography recordings.

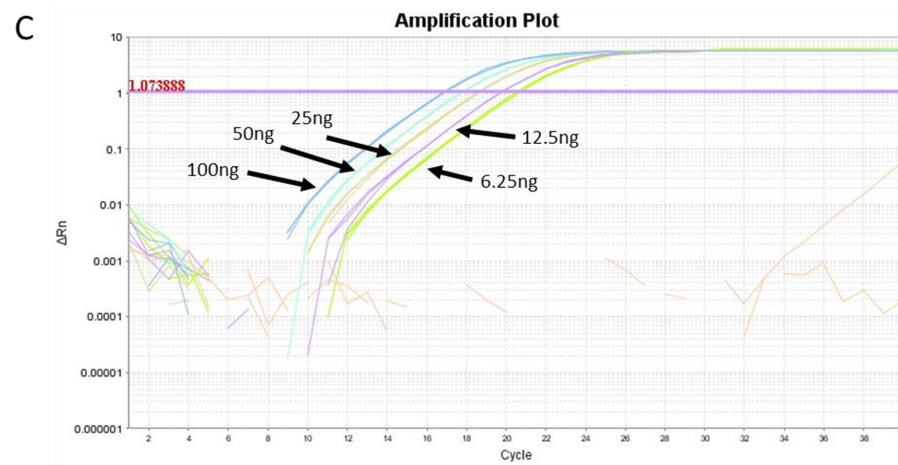
4.2.4.3 Sleep vs mtDNA damage in skin

The standard curves evaluating the dynamic linear range and primer efficiency of 83bp and 1kb qPCR are shown in Figure 4-16. The amplification plot of 83bp qPCR (Figure 4-16A) was produced using a 2-fold serial dilution and the DNA content ranges from 6.25ng and 100ng. As can be seen from the plot, approximately 1 Ct was detected between each dilution as expected. The correlation coefficient R-value was 0.999 which indicates that the efficiency of this assay is almost 100% meaning that for every cycle the mtDNA content doubles (Figure 4-16B). The same serial dilution was used to produce the amplification plot for the 1kb qPCR assay (Figure 4-16C) and the difference in Ct between each dilution was approximately 1. This is supported by the R^2 value calculated from the standard curve which was 0.997 indicating that the efficiency of this assay was also close to 100% (Figure 4-16D).

Positive 83bp bands were determined by the QuantStudio3™ melt curve analysis which showed that each amplicon reached a peak maximum at the same temperature of 80°C (Figure 4-17A). Agarose gel analysis of all standard curve amplicons showed a band at the 83bp position and no evidence of non-specific binding or primer-dimer was found (Figure 4-17B). Similarly, melt curve analysis showed that each 1kb amplicon reached a peak maximum at the same temperature of 84°C (Figure 4-17C). Agarose gel analysis of all standard curve amplicon showed a band at the 1kb position and no evidence of non-specific binding or primer-dimer was observed (Figure 4-17D).







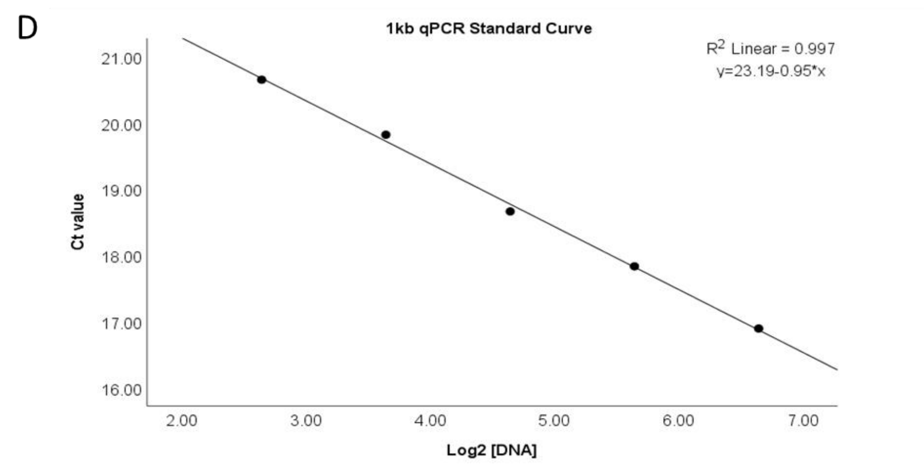


Figure 4-16. A: standard curve for the 83bp qPCR assay with DNA content ranging from 6.25ng and 100ng; B: Efficiency of 83bp qPCR calculated from the standard curve; C: Standard curve for the 1kb qPCR assay with DNA content ranging from 6.25ng and 100ng; D: Efficiency of 1kb qPCR calculated from the standard curve. n= 3 per data point.

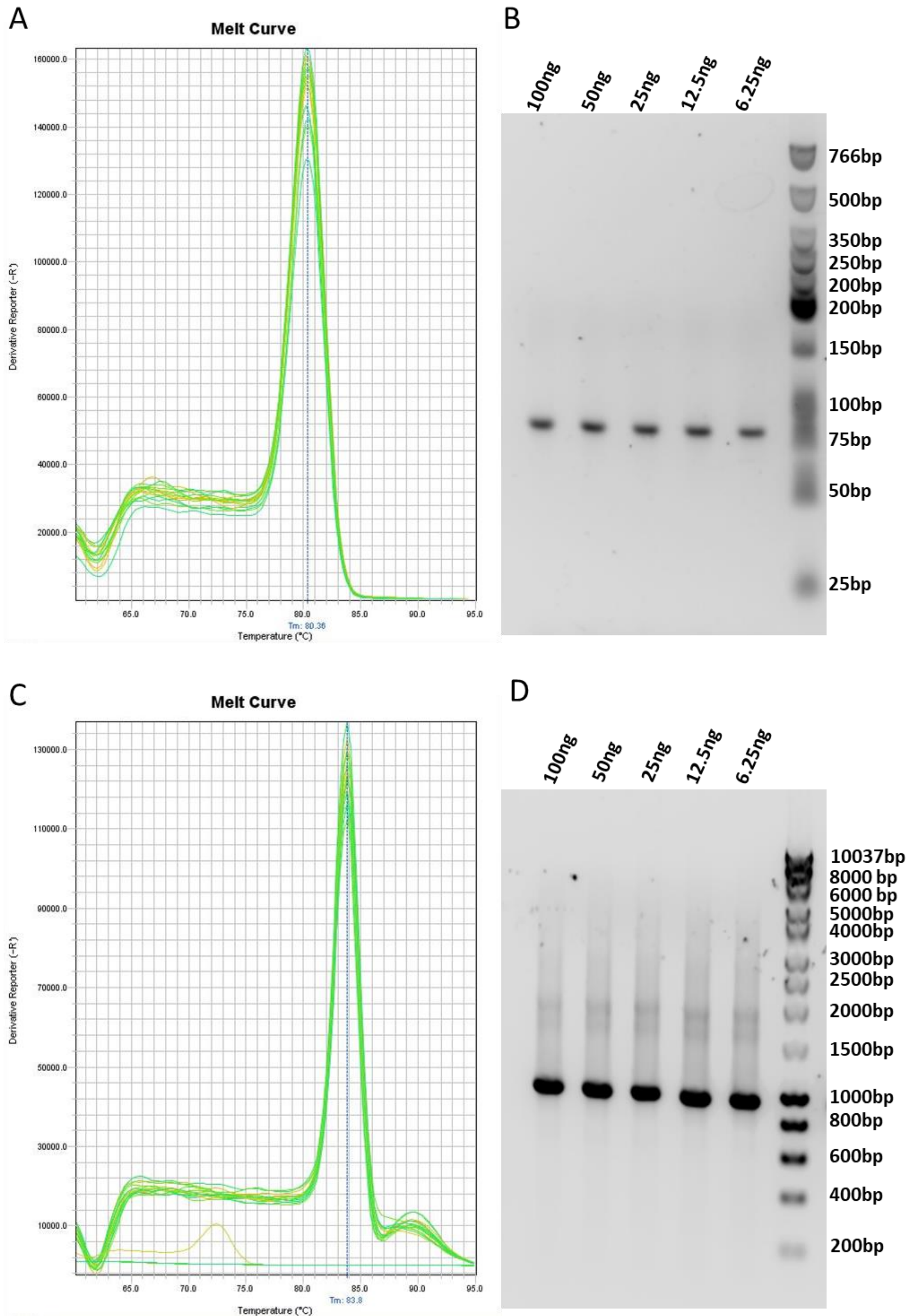


Figure 4-17. A: Melt curve of the 83bp qPCR assay; B: Gel electrophoresis image showing a band at 83bp location indicating that the correct products were amplified; C: Melt curve of the 1kb qPCR assay; D: Gel electrophoresis image showing a band at 1kb location indicating that the correct products were amplified

The level of mtDNA strand-break damage is summarised in Figure 4-18A. A higher level of 1kb/83bp ratio is an indication of a higher level of mtDNA damage. Overall, the mean 1kb/83bp ratio was lower in samples taken in the morning after PSG, regardless of the skin site. When comparing the 1kb/83bp ratio at baseline (i.e., before PSG) between the control and patient groups (Figure 4-18B), the level was slightly higher in the left cheek amongst the control group (1.19 ± 0.08) compared to the patient group (1.17 ± 0.09). The 1kb/83bp ratio was very similar between the control (1.20 ± 0.06) and the patient (1.19 ± 0.12) group for the right cheek. Moreover, the 1kb/83bp ratio was significantly higher in the control group (1.22 ± 0.05) than in the patient group (1.15 ± 0.11) regarding the damage level detected in the inner arm (p= 0.035). The differences detected in the left and right cheek were not statistically significant (p= 0.226 and p= 0.319, respectively). The 1kb/83bp ratios detected in the samples collected in the morning after PSG were significantly higher in the control groups compared to the patient group for the left cheek and inner arm (p= 0.039 and p= 0.02, respectively). The same trend was also observed in the right cheek but the difference was marginally significant (p= 0.051) (Figure 4-18C). Finally, no statistically significant difference was detected between the control and patient groups in samples collected two weeks after PSG (p= 0.943, p= 0.537 and p= 0.150 for the left cheek, right cheek and inner arm, respectively) (Figure 4-18D). The mean 1kb/83bp ratio in the left cheek was slightly lower in the control group (1.15 ± 0.08) than in the patient group (1.16 ± 0.08). The right cheek, on the other hand, showed a slightly higher 1kb/83bp ratio in the control group (1.17 ± 0.07) than in the patient group (1.15 ± 0.09). Finally, a higher 1kb/83bp ratio was observed in the control group (1.22 ± 0.06) than in the patient group (1.18 ± 0.05) for the inner arm.

The change in 1kb/83bp ratio throughout the study for each skin site is illustrated in Figure 4-19 A-C. For most participants, the difference was very small. Generally, the level was slightly lower in the samples collected in the morning after PSG compared to that collected before PSG and two weeks after PSG. Once more, it is important to note that the differences detected were not statistically significant (p= 0.417, p= 0.192 and p= 0.372 for the left cheek, right cheek and inner arm, respectively).

In order to investigate the association between sleep duration and mtDNA damage level, the 1kb/83bp ratio obtained from samples collected two weeks after PSG was compared between sleep groups measured by two weeks of sleep diary and accelerometer recordings (Figure 4-19 D-F). Overall, a 'U-shaped' association was observed between sleep duration and 1kb/83bp

ratios indicating that participants in the '7-8 hour' sleep group had a lower level of mtDNA strand-break damage. However, the difference was only significant when comparing the 1kb/83bp ratio in the right cheek and accelerometer measured sleep duration (p= 0.043). The Post-hoc test results revealed that this was due to the statistically significant difference detected between the '<7 hours' and '7-8 hours' sleep groups (p= 0.033), the difference between other groups was not significant.

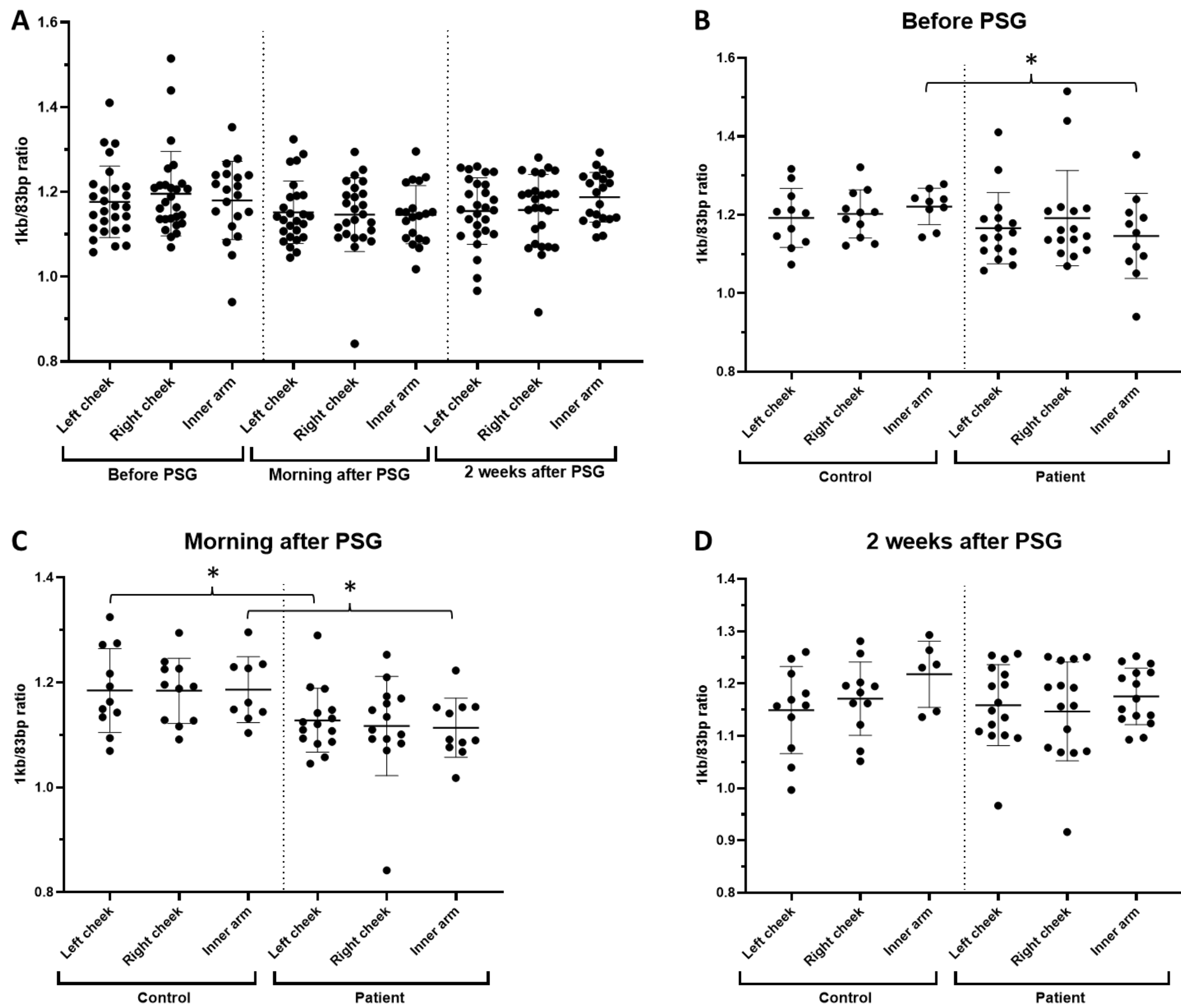


*Figure 4-18. mtDNA strand-break damage levels at various skin sites taken at different time points. A higher level of damage is indicated by a higher 83bp/1kb ratio. A- mtDNA damage level at three different skin sites taken at three different time points in all participants (n= 28). B- Comparison of mtDNA damage level between the control and patient group in samples taken before PSG; C- Comparison of mtDNA damage level between the control and patient group in samples taken in the morning following PSG; D- Comparison of mtDNA damage level between the control and patient group in samples taken two weeks following PSG. *p<0.05*

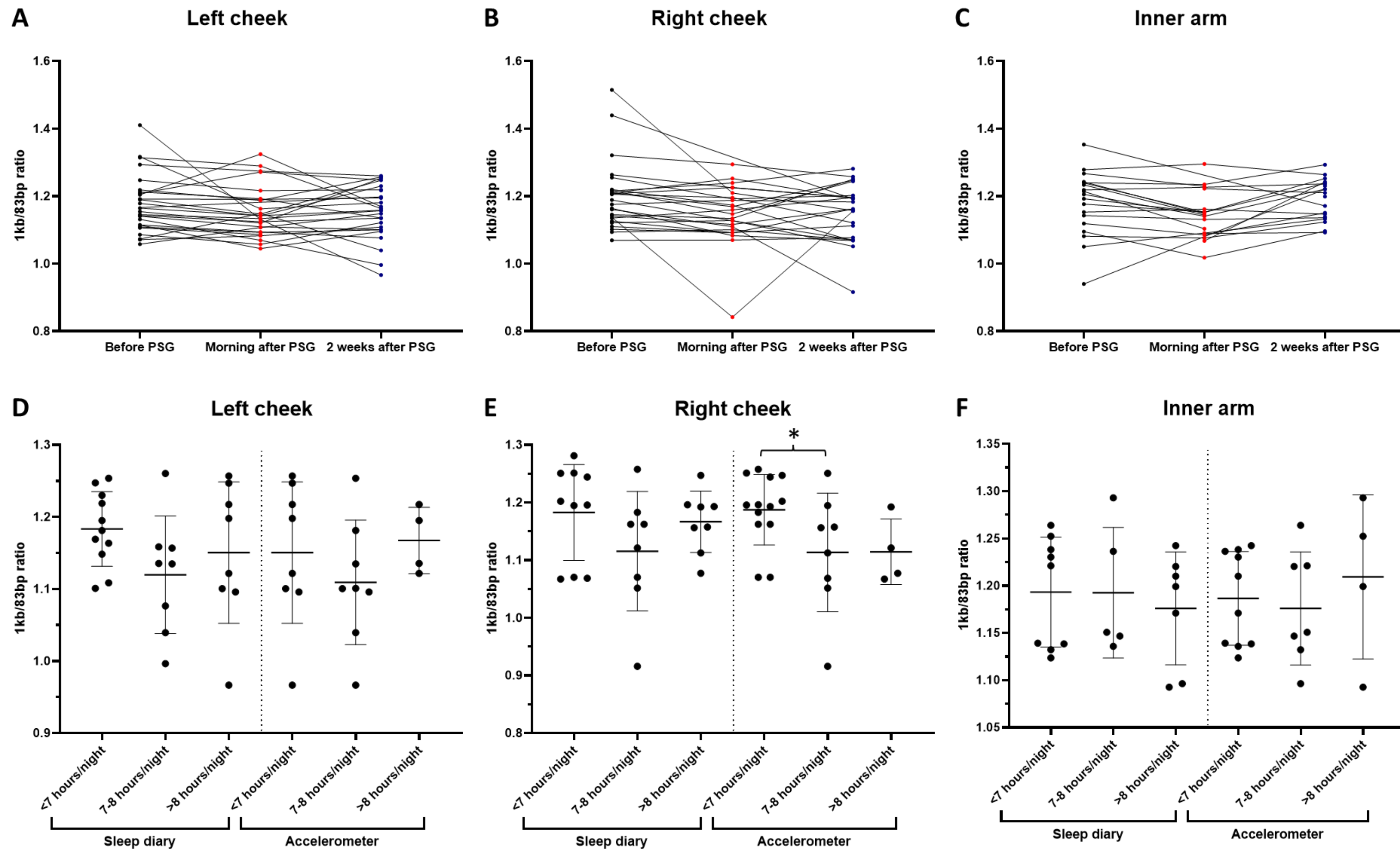


*Figure 4-19. mtDNA damage level of all participants as indicated by the 83bp/1kb ratio (n= 28). A-C: Change in level with time in the left cheek, right cheek and inner arm; D-F: Comparison of mtDNA damage between different sleep groups detected by two weeks of sleep diary and accelerometer recordings in the left cheek, right cheek and inner arm. *p<0.05*

4.2.4.4 Hair

14,504 out of 56,623 genes had read counts of >10 in at least 10% of samples. Principle component analysis (PCA) was carried out on all samples using the sleep group significant gene lit from 2-way ANOVA (see below, n= 555 genes). PCA results (Figure 4-20) showed separation between the two groups and that the difference between male controls and patients was greater than the difference between female controls and patients on the first principal component, which explained 19.2% of the variation.

555 genes were statistically significant between the control and the patient groups in the two-way ANOVA analysis including both genders. In females, 685 genes reached the statistical significance level in the moderated t-test analysis without multiple testing corrections and this number was reduced to 29 genes when the p-value was Westfall Young Permeative adjusted. In males, 717 genes reached the statistical significance level in the moderated t-test analysis without multiple testing correction and this number was reduced to 93 genes when the p-value was adjusted with the Westfall Young Permeative multiple correction test. These gene lists were then brought forward for the pathway analysis using Ingenuity pathway analysis (IPA). A number of mitochondrial genes were present in the female's results and, hence, a list of all 37 mitochondrial genes was included in the IPA to determine whether this represented a significant enrichment of mitochondrial genes or not.

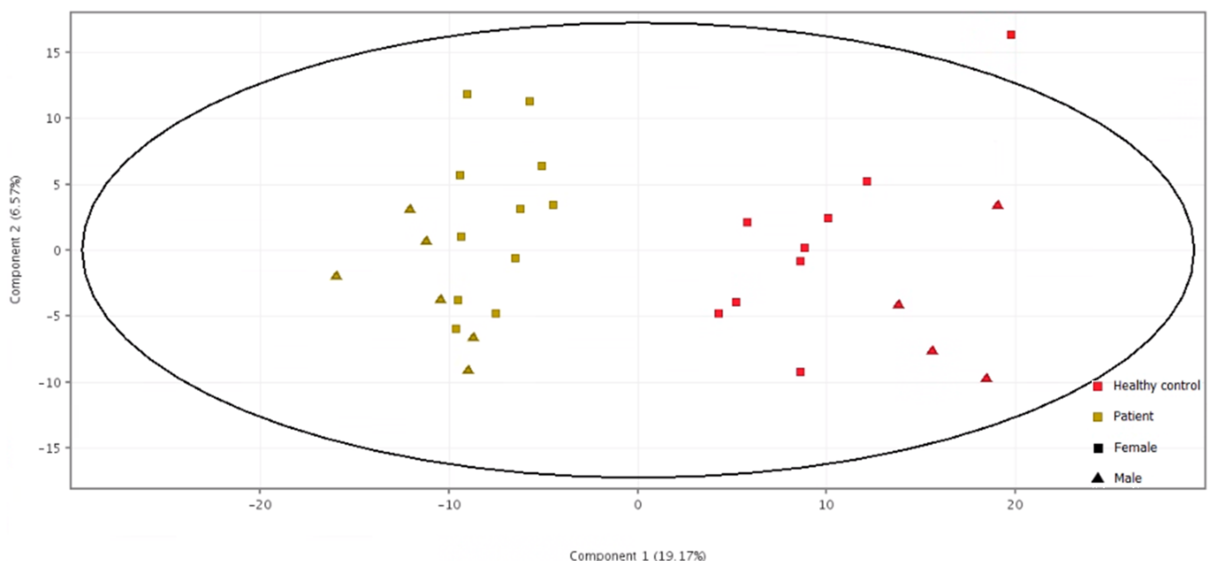


Figure 4-20. Principle component plot (PCA1 and PCA2) illustrating the interaction between gender and control/patient grouping.

Using the gene list created with the multiple testing correction (MTC), n=27 after a 1.2-fold filter, 16 significantly enriched Ingenuity canonical pathways were identified in females. The

most significant pathway was the "eumelanin biosynthesis" pathway (p= 0.003). The significant (ranked using p-values) pathways are shown in Table 4-6. Additionally, 83 upstream regulators were identified in females (Appendix C). For the mitochondrial list, one mitochondrial gene was found within the MTC list and six were present when no MTC was applied. Both were significant enrichments in the IPA analysis, MTC list was p= 0.031 (Table 4-6) and no MTC was p<0.001. The circadian rhythm-related regulator, *CLOCK*, was amongst the list of identified upstream regulators (p= 0.022).

Table 4-6. Significant Ingenuity canonical pathways identified in females

Ingenuity Canonical Pathways	**P-value**	**Ratio**	**z-score**
Eumelanin Biosynthesis	0.003	0.250	NaN
D-myo-inositol (1,4,5,6)-Tetrakisphosphate Biosynthesis	0.010	0.011	NaN
D-myo-inositol (3,4,5,6)-tetrakisphosphate Biosynthesis	0.010	0.011	NaN
NAD Phosphorylation and Dephosphorylation	0.011	0.077	NaN
3-phosphoinositide Degradation	0.011	0.011	NaN
D-myo-inositol-5-phosphate Metabolism	0.011	0.010	NaN
3-phosphoinositide Biosynthesis	0.013	0.010	NaN
Superpathway of Inositol Phosphate Compounds	0.016	0.009	NaN
Glutaryl-CoA Degradation	0.018	0.048	NaN
Tryptophan Degradation III (Eukaryotic)	0.023	0.036	NaN
MIF-mediated Glucocorticoid Regulation	0.030	0.028	NaN
MIF Regulation of Innate Immunity	0.036	0.023	NaN
Ephrin A Signalling	0.039	0.021	NaN
nNOS Signalling in Skeletal Muscle Cells	0.040	0.021	NaN
Oestrogen Receptor Signalling	0.046	0.005	NaN

NaN indicates that an activation the z-score was not achieved.

In males, 214 Ingenuity canonical pathways were identified using the gene list created with the multiple testing correction (n= 89 after a 1.2-fold filter). Twenty-six (ranked using p-values) pathways were significantly enriched in this gene list (Table 4-7). The most significant pathway identified was the "Role of MAPK Signalling in Promoting the Pathogenesis of Influenza" (p= 0.006), although a z-score was not achieved. However, using the gene list created without

multiple testing corrections, a down-regulation of the "Role of MAPK Signalling in Promoting the Pathogenesis of Influenza" pathway was found (p= 0.002, z-score= -1.667). Moreover, 100 upstream regulators were identified in males (Appendix D). However, no circadian-related regulators were found.

Table 4-7. Significant Ingenuity canonical pathways identified in males.

Ingenuity Canonical Pathways	p-value	Ratio	z-score
Role of MAPK Signalling in Promoting the Pathogenesis of Influenza	0.006	0.027	NaN
Endocannabinoid Developing Neuron Pathway	0.009	0.024	NaN
AMPK Signalling	0.009	0.017	NaN
4-aminobutyrate Degradation I	0.010	0.333	NaN
UVC-Induced MAPK Signalling	0.013	0.039	NaN
Ovarian Cancer Signalling	0.016	0.019	NaN
Glutamate Degradation III (via 4-aminobutyrate)	0.017	0.200	NaN
Molecular Mechanisms of Cancer	0.017	0.011	NaN
Human Embryonic Stem Cell Pluripotency	0.018	0.018	NaN
Chondroitin and Dermatan Biosynthesis	0.020	0.167	NaN
Cell Cycle: G1/S Checkpoint Regulation	0.022	0.029	NaN
Ceramide Biosynthesis	0.023	0.143	NaN
Role of MAPK Signalling in Inhibiting the Pathogenesis of Influenza	0.028	0.026	NaN
Pulmonary Healing Signalling Pathway	0.029	0.015	NaN
Coronavirus Pathogenesis Pathway	0.030	0.015	NaN
Cyclins and Cell Cycle Regulation	0.032	0.024	NaN
Calcium Transport I	0.033	0.100	NaN
BMP signalling pathway	0.034	0.023	NaN
Role of NFAT in Cardiac Hypertrophy	0.038	0.014	NaN
Pyroptosis Signalling Pathway	0.039	0.022	NaN
Non-Small Cell Lung Cancer Signalling	0.039	0.021	NaN
Small Cell Lung Cancer Signalling	0.041	0.021	NaN
IL-1 Signalling	0.041	0.021	NaN

Ingenuity Canonical Pathways	p-value	Ratio	z-score
Oleate Biosynthesis II (Animals)	0.043	0.077	NaN
Mouse Embryonic Stem Cell Pluripotency	0.047	0.019	NaN
Breast Cancer Regulation by Stathmin1	0.048	0.008	1

NaN indicates that an activation the z-score was not achieved.

4.2.4.5 Correlation between melatonin, cortisol and mtDNA damage

No significant correlation has been observed between the cortisol and melatonin levels (rs= -0.268, p= 0.227) (Figure 4-21). Additionally, no significant correlation was found between the cortisol level and mtDNA damage level in the left cheek (rs= -0.210, p= 0.292), right cheek (rs= -0.070, p= 0.736) and inner arm (rs= -0.207, p= 0.369) (Figure 4-22A). Finally, once more, no significant correlations were found between the melatonin level and mtDNA damage level in the left cheek (rs= 0.270, p= 0.224), right cheek (rs= 0.155, p= 0.503) and inner arm (rs= 0.224, p= 0.388) (Figure 4-22B).

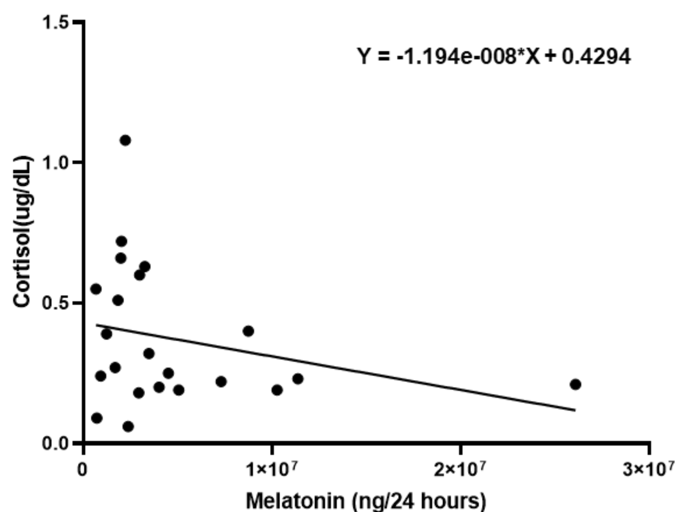


Figure 4-21. Correlation between cortisol level measured in samples taken at home and melatonin level per 24 hours.

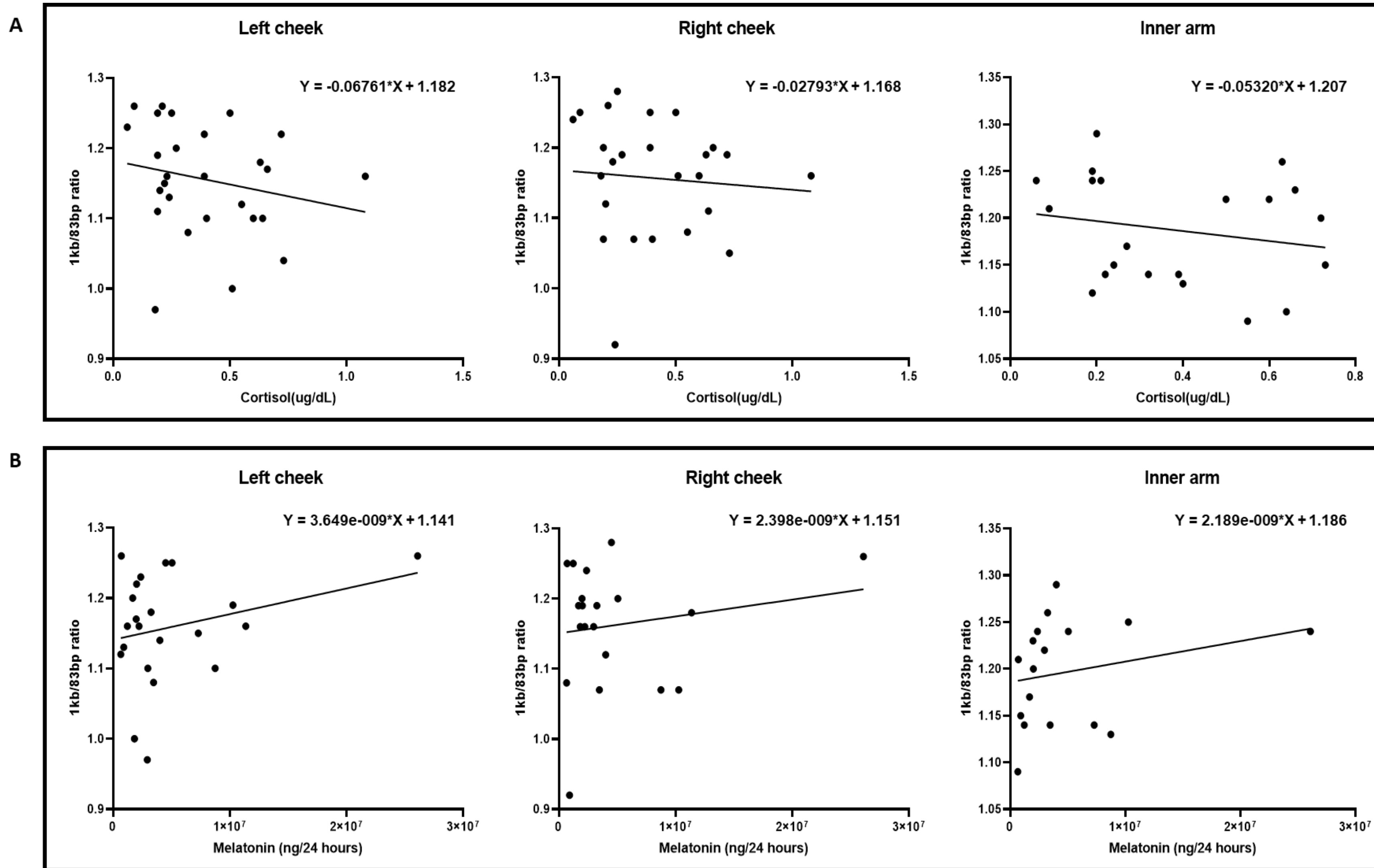


Figure 4-22. Correlation between cortisol level measured in samples taken at home and mtDNA damage level in skin swabs taken two weeks after PSG (A) and melatonin level per 24 hours and mtDNA damage level in skin swabs taken two weeks after PSG (B).

4.2.5 Optimal vs less optimal sleep

Those with optimal sleep appeared to have higher melatonin and cortisol levels. However, the differences between those with optimal and less optimal sleep were not statistically significant (Table 4-8).

Table 4-8. Comparison of melatonin and cortisol levels between those with optimal and less optimal sleep. Less optimal sleep is defined as having short or long sleep duration, as well as more fragmented sleep.

	Optimal sleep (n= 12)		**Less optimal sleep (n= 15)**		**P-value**
	Mean	**Standard deviation**	**Mean**	**Standard deviation**	
Melatonin level (AUC)	84.37	50.38	61.60	55.72	0.166
Cortisol level at home (ug/dL)	0.43	0.24	0.36	0.26	0.456
Cortisol level at hospital (ug/dL)	0.48	0.27	0.39	0.27	0.373

4.3 Discussion

4.3.1 Comparison of sleep measurements between the patient and control groups

The current study found that the patient group has a higher average time in bed and sleep duration, while the control group has a higher sleep efficiency, regardless of the sleep assessment method. This suggests that the patient group may experience longer sleep latency and/or WASO. However, the statistical analysis results were rather disappointing. The difference detected between the patient and control groups was not found to be statistically significant. A possible explanation for it may be the lack of adequate statistical power due to the small sample size. As the medical records are not available for the participants in the patient group, it is therefore not possible to determine what sleep disorder they have. Some participants in the patient group have reported difficulties falling asleep and others have complaints about excessive sleepiness during the day and at night-time.

While the common sleep disorder, insomnia, is associated with short sleep duration, others such as narcolepsy and idiopathic hypersomnia are characterised by long sleep duration and excessive daytime sleepiness. Prior studies conducted in narcolepsy patients found that their sleep duration was approximately 1.5 hours longer than controls, while sleep efficiency was similar between the two groups (Vernet and Arnulf, 2009b). However, contradicting results have been reported which showed higher insomnia scores and shorter self-reported night-

time sleep duration amongst those with the narcoleptic syndrome (Parkes et al., 1998). Narcolepsy patients also tend to experience frequent awakening episodes leading to poorer sleep quality (Roth et al., 2013). This is a possible explanation for the higher daytime sleepiness observed in narcolepsy patients and they are more likely to nap in the afternoon compared to controls (Parkes et al., 1998). Another sleep disorder characterised by daytime sleepiness and prolonged sleep duration is idiopathic hypersomnia which is a chronic neurologic disorder. Its true prevalence and exact pathology are yet unclear (Trotti, 2017). A US cross-sectional study found that 8.4% of the general population reported excessive quantity of sleep (main sleep duration of ≥9 hours/24 hours accompanied by complaints of distress due to excessive sleep) and it is the most common amongst 18-24 year olds (Ohayon et al., 2013). PSG measured sleep duration was found to be longer in hypersomniacs compared to controls, while sleep efficiency appears to be similar between them. Apart from longer nocturnal sleep duration, hypersomniacs also experience longer daytime sleep. As a result, they have a much longer sleep time per 24 hours than controls (Vernet and Arnulf, 2009a). A meta-analysis showed that long sleep duration (most studies considered long sleep duration as ≥8 hours/night) has been associated with a 39% higher risk of mortality, 26% higher risk of incident diabetes, 25% higher risk of cardiovascular disease, 46% higher risk of stroke and 24% higher risk of coronary heart disease (Jike et al., 2018). Short sleep duration (<6 hours/night) has also been associated with a higher risk of metabolic syndromes (Ju and Choi, 2013). Therefore, identifying those with a short or long sleep duration is crucial for early prevention and management of these diseases which would benefit those affected, their carer and society.

4.3.2 Comparisons between subjective and objective measured sleep

A discrepancy has been observed between the sleep duration measured by the two weeks of accelerometer and sleep diary recordings. Although, it is important to note that the difference detected was not statistically significant. Overall, participants overestimated their sleep duration. About 39.3% of the participants in this study self-reported habitual sleep duration of <7 hours/night, while accelerometer results showed that 51.9% of them slept <7 hours/night. On the other hand, 32.1% of participants self-reported a sleep duration >8 hours/night, but only 18.5% of them had an accelerometer recording of >8 hours of sleep per night. Overestimation of sleep duration was observed in both the control and patient groups. This finding broadly supports the work of other studies in this area. A large cohort study demonstrated that approximately 40% of the participants had significantly under- or over-

estimation of sleep duration by at least one hour (Santos et al., 2021). A study compared wrist actigraphy and self-reported sleep in 2,086 Hispanic/Latino participants detected more than one hour of discrepancy between the objective and self-reported sleep duration (Cespedes et al., 2016). A similar finding was reported by Lauderdale et al. which showed an overestimation of subjective sleep duration, especially amongst those with shorter sleep duration (Lauderdale et al., 2008). The Rotterdam study also reported an overestimation of sleep duration between diary-assessment and actigraphy measured sleep duration. It was found that those with shorter sleep duration were more likely to self-report a longer sleep duration compared to those who slept 7-8 hours/night (Van Den Berg et al., 2008b). It was found that overestimation of sleep is associated with a higher BMI, lower anxiety/depression and a higher frequency of co-morbidity (Santos et al., 2021). However, an underestimation of sleep was reported in an Australian study involving only females. 66% of the participants in this study self-reported a sleep duration of <7 hours, while actigraphy showed that only 39% of the participants slept <7 hours (Girschik et al., 2012). This is consistent with the findings reported by Ronaldo et al. which revealed an association between underestimation of sleep and age, gender, race and social status. Those who tend to underestimate their sleep duration were more likely to be older, females, belongs to the black or mixed-race ethnicity and have a lower income (Santos et al., 2021).

When comparing PSG recordings to the sleep diary and accelerometer recording obtained during the single night in the sleep laboratory, both sleep duration and efficiency detected by PSG were slightly higher. Although the difference detected was not significant. A similar trend was also reported in a cross-sectional study involving 223 participants. PSG measured sleep duration was found to be slightly longer than that measured by actigraphy. However, contrary to the current study, they found that PSG measured sleep duration was shorter compared to sleep diary recorded sleep duration. However, the association between the different methods of sleep detection appears to be weakened by various factors such as higher hostility scores, depressive symptoms and poor self-rated health (Matthews et al., 2018).

The discrepancy between subjective and objective assessments of sleep could be due to inaccurate memory recall of sleep onset and WASO, as a result, actual sleep was not distinguished from time in bed. Even though the laboratory-based PSG is the gold-standard method for detecting sleep, it does involve participants sleeping in an unfamiliar environment and therefore, cannot reflect their true habitual sleep duration and quality. It was not a

surprise that in this study when the participants were asked about how well they slept in the sleep laboratory, those with shorter sleep duration reported a lower rating compared to those in the '>8 hours' sleep group. Therefore, whenever possible, multiple sleep detection methods should be utilised when assessing sleep to give a more accurate diagnosis. Out of the total number of main causes of discomfort, some cannot be changed such as PSG wires and the change of environment. However, some are potentially modifiable to improve the experience for sleep laboratory patients. For instance, reduce the noise level by using soundproof doors and remind the staff to keep the noise level low at night-time and make the room temperature adjustable such as providing a fan or heater depending on their need. By improving the comfortableness of those attending PSG, it will encourage a better sleeping environment for a more accurate recording.

4.3.3 Relationship between sleep and melatonin level

A comparison of the melatonin level between the healthy controls and patients showed a reduced level of melatonin amongst the patient group. Additionally, the level of melatonin was higher in those with optimal sleep compared to less optimal sleep. This is not a surprising finding as melatonin plays an important role in circadian control, and therefore those with sleep problems will expect to have a reduced level of it. Nevertheless, the differences detected in the current study were not found to be statistically significant.

Prior studies have reported the difference in melatonin levels between healthy controls and those with sleep disorders. A significantly lower level of plasma melatonin level has been detected in chronic primary insomnia patients compared to healthy controls (Leger et al., 2004). The severity of reduction in plasma melatonin was found to be associated with the duration of their insomnia complaints (Hajak et al., 1995). It was found that those with more than five years of sleep complaints had a significantly reduced plasma melatonin level compared to healthy controls, as well as compared to those with less than five years of sleep complaints (Rodenbeck et al., 1999). Insomnia is common in people with post-traumatic stress disorder which shows symptoms such as difficulties initiating and maintaining sleep. Prior study has found a reduced melatonin production in the affected individuals compared to healthy controls (Paul et al., 2019). The symptoms of insomnia could be elevated through the administration of melatonin supplementation. By increasing the level of melatonin using a 2.5mg supplement, both sleep duration and quality can be significantly improved (Scheer et al., 2012, Buscemi et al., 2005). On the contrary to insomnia, narcoleptic patients showed an

elevated melatonin level during the day while maintaining a normal nocturnal increase in melatonin compared to the control. This could potentially explain the excessive daytime sleepiness observed in patients with narcolepsy (Blazejova et al., 2008). A similar result was also reported by Donjacour et al. which revealed a similar average concentration of melatonin between narcoleptic patients and control, but a significantly higher daytime melatonin level amongst the patients (Donjacour et al., 2012). These results illustrate the importance of melatonin in the regulation of sleep.

It was rather disappointing that no significant association between sleep duration and efficiency and the melatonin level has been found in this study. No obvious trend was found between sleep efficiency and melatonin level. A reversed 'U-shaped' association was found between sleep diary recording of sleep duration and melatonin level, while a 'U-shaped' association was observed between the sleep duration recorded by the accelerometer and the melatonin level. Previously published literature has shown that both objectively measured short sleep duration (<5 hours/night) and lower sleep efficiency are associated with a significantly lower level of melatonin secretion. On the other hand, an inverse relationship was observed between the level of nocturnal melatonin secretion and self-reported daytime sleepiness (Saksvik-Lehouillier et al., 2015). This was also shown in other studies. A reduced level of melatonin has been observed amongst patients with shorter sleep duration, longer sleep latency and lower sleep efficiency (Takaesu et al., 2015). This is not a surprise as the hypnotic effect of melatonin has been illustrated in multiple studies. It, therefore, raises the possibility of using exogenous melatonin to improve sleep in people with various sleep-related disorders.

The beneficial effect of melatonin supplements has been reported. When 3mg of melatonin was administered in people experiencing disturbed REM sleep, their REM sleep percentage increased significantly compared to placebo (Kunz et al., 2004). The sleep onset latency was found to be reduced by exogenous melatonin in people with insomnia and delayed sleep phase syndrome (Buscemi N, 2004). Even in healthy adults, administration of 5mg of melatonin significantly increased sleep propensity and subjective sleepiness. It was also found that the time of administration also has an impact on its hypnotic effect. When administered at noon, it took approximately 3 hours 40 minutes to reach its maximum effect, while the latency to maximum effect was only one hour when administered at 9 pm. This suggests an interactive effect of endogenous and exogenous melatonin in the regulation of sleep

(Tzischinsky and Lavie, 1994). Understanding the association between melatonin and sleep could help to identify individuals at risk of circadian-rhythm-related sleep disorders and provide appropriate interventions to minimise the adverse effects caused by it.

4.3.4 Relationship between sleep and cortisol level

A higher level of cortisol was observed in the samples taken in the hospital compared to that taken at home. This suggests that a change in sleeping environment potentially induced stress in participants. Overall, participants with optimal sleep had a slightly higher cortisol level compared to those with less optimal sleep. However, it is important to note that the difference detected was not statistically significant. Comparisons between the patient group and the control group did not find any significant difference. Moreover, a reversed 'U-shaped' association was found between cortisol level and sleep duration measured by the accelerometer and sleep diary. However, once more, the difference was not statistically significant. Prior studies have shown an inverse relationship between cortisol and melatonin levels as melatonin onset occurs when the level of cortisol secretion is low (Zisapel et al., 2005). Although it is important to note that no obvious correlation was found between cortisol and melatonin levels in the current study.

Cortisol secretion begins to increase after sleep and it peaks at about 30 minutes after awakening (i.e., cortisol awakening response) then it progressively decreases during the day. The Whitehall II study reported an association between self-reported short sleep duration (<5 hours/night) and increased cortisol awakening response, as well as a flatter diurnal cortisol profile. Evening cortisol level was also found to be higher in those with short sleep duration and experience more sleep disturbances (Kumari et al., 2009). A similar finding has been reported by Spiegel et al. which showed that when sleep duration was restricted to 4 hours/night from 8 hours/night in healthy adults, the amplitude of cortisol rhythm was reduced and the evening cortisol level was found to be higher under the sleep-restricted condition. Moreover, it took longer to decline from morning acrophase to nocturnal nadir, but the increase from nocturnal nadir to morning acrophase was faster (Spiegel et al., 2004a). Apart from acute partial (4 hours/night) sleep restriction, chronic sleep deprivation in healthy adults can also lead to an increase in the evening cortisol level compared to normal sleep duration (8 hours/night) (Leproult et al., 1997, Spiegel et al., 1999). However, in disagreement with these studies, some studies in this area did not observe any change in cortisol rhythm in sleep-deprived individuals (Moldofsky et al., 1989, Brun et al., 1998).

The association between sleep disorders such as insomnia and cortisol secretion has also been reported in various studies and the results were inconsistent (Basta et al., 2007). In some studies, insomniacs were found to have an increased 24-hour cortisol secretion compared to controls. Additionally, those with a higher degree of sleep disturbance were found to have a higher level of cortisol secretion compared to those with a lower degree of disturbances (Vgontzas et al., 2001). However, opposite results have been reported by another study which demonstrated that those with primary insomnia were found to have a lower level of cortisol after awakening compared to healthy controls. Although no significant difference was found between insomniacs and controls regarding the evening cortisol level. The low awakening cortisol level was significantly correlated with more awakening episodes and lower sleep quality (Backhaus et al., 2004). In contrast to insomnia, narcolepsy, as characterised by excessive daytime sleepiness and disrupted nocturnal sleep, is associated with reduced plasma cortisol levels compared to healthy controls. Although, Dalmazi et al. only observed this association in males (Arnold, 2017).

The exact mechanism linking sleep and cortisol secretion is not entirely clear. It is thought that short sleep duration and flattened cortisol rhythm may result from common mechanisms associated with stress (Kumari et al., 2009). McEwen reviewed the consequences of chronic sleep deprivation which includes increased appetite, increased level of proinflammatory cytokines and elevated cortisol level. He, therefore, conceptualised chronic sleep debt itself as a stressor (McEwen, 2006). Evidence also suggested that frequent arousals are associated with an increase in cortisol secretion. Compared to undisturbed sleep, when individuals experience continuous arousals, an increased level of cortisol secretion was noted (Späth-Schwalbe et al., 1991). This may explain the higher cortisol level observed in samples taken at the hospital following the night of PSG in the current study. Participants experienced on average 24 awakening episodes as measured by PSG and the change in sleeping environment may be a potential stressor. Therefore, improving the sleep environment for PSG could help to minimise the number of arousals and their cortisol level. Various longitudinal studies have shown the adverse effect of having a flatter cortisol rhythm. Earlier mortality has been observed in breast cancer patients with a relatively flat cortisol rhythm compared to those patients with a normal diurnal variation. This may be mediated by the reduced number and activity of natural killer cells (Sephton et al., 2000). Therefore, understanding the association

of sleep and cortisol rhythm is important as it helps to identify individuals at risk of dampened cortisol rhythm.

4.3.5 Relationship between sleep and mtDNA level

Overall, a ‘U-shaped’ association was found between the level of mtDNA damage in the skin and sleep duration, regardless of the method of sleep detection. Although almost all of the differences observed were not statistically significant.

The skin is considered the largest organ in the body, comprising approximately 16% of the total body weight, and it is the physical barrier separating the internal organs from the external environment (Venus et al., 2010). The skin consists of three layers: the outermost epidermis, the middle dermis and the innermost subcutis (Lai-Cheong and McGrath, 2009). Perceived age is the estimation of a person’s age based on their physical appearance. It was found that people who look younger than their chronological age are better at retaining their functional capabilities. Therefore, perceived age is considered a useful clinical marker for healthy ageing (Coma et al., 2014). Mitochondria are subcellular organelles responsible for the generation of cellular energy, therefore they are commonly known as the powerhouse of the cell. Mitochondria play essential roles in the skin such as cell signalling, wound healing, antimicrobial defence and pigmentation (Stout and Birch-Machin, 2019). The role of mitochondria in skin ageing has been reviewed in various studies. It is thought that intrinsic skin ageing is mainly caused by reduced cell turnover and an accumulation of reactive oxygen species leading to an accumulation of mtDNA damage in the skin. External factors such as smoking, ultraviolet radiation exposure and sleep deprivation could accelerate the rate of skin ageing (Stout and Birch-Machin, 2019). This supports the finding of the current study which showed that participants with short sleep duration had a higher level of mtDNA damage.

The finding of this study is consistent with prior studies. OSA is commonly associated with shorter sleep duration and fragmented sleep. A higher level of oxidative stress-induced mtDNA damage has been seen in patients with OSA. This is possibly mediated by intermittent hypoxia commonly seen in OSA which could trigger systemic inflammation, activation of the sympathetic nervous system and oxidative stress (Lacedonia et al., 2015). A study carried out on monozygotic twins found that having a sleep duration of <7 hours/24 hours reduces their mitochondrial DNA copy number compared to having a longer sleep duration (7-9 hours/24 hours). This might be due to the increased level of oxidative stress caused by chronic sleep

deprivation which reduces mitochondria fission and decreases mtDNA copy number (Wrede et al., 2015).

As mentioned previously, sleep is known to have an important impact on skin ageing. The common expression of “beauty sleep” is supported by various studies in this area. Acute sleep deprived (5 hours/night) individuals are perceived as less healthy, more tired and less attractive compared to when they received a normal night of sleep (8 hours/night) (Axelsson et al., 2010). A similar finding has been reported by Sundelin et al. which demonstrated the association between sleep deprivation (5 hours/night) and facial appearance. Under the sleep-deprived condition, participants were perceived as having darker circles under the eyes, more hanging eyelids and more wrinkles and fine lines under the eyes (Sundelin et al., 2013). Those with chronic short sleep duration (≤5 hours/night) showed increased signs of intrinsic skin ageing, lower skin barrier recovery ability and lower satisfaction with their appearance and physical attractiveness compared to those with a habitual sleep duration of 7-9 hours/night (Oyetakin-White et al., 2015).

The potential mechanism linking poor sleep and accelerated skin ageing could be mediated by psychological stress. Insomniac psychologic stress has been associated with decreased epidermal cell proliferation and differentiation which compromises the barrier function of the skin against exogenous insults (Choi et al., 2005). Additionally, as discussed in the previous chapter, sleep deprivation can lead to glucose intolerance and insulin insensitivity which increases the risk of type 2 diabetes. it was found that exposure to elevated glucose concentration could decrease the proliferation of keratinocytes which is the main cell type of the epidermis (Spravchikov et al., 2001). The role of melatonin in the regulation of skin ageing has also been previously published in the literature. Melatonin receptors are expressed in many cell types within the skin and it possesses a strong antioxidative property. Melatonin is able to counteract reactive oxygen species and reduce the level of mitochondrial damage. Therefore, melatonin has been proposed as an effective anti-skin ageing compound (Kleszczynski and Fischer, 2012). However, in the current study, no obvious correlation was found between melatonin level and mtDNA damage level. Short sleep duration has been associated with a reduced level of melatonin, this could then leads to an increase in oxidative stress and eventually result in both intrinsic and extrinsic skin ageing (Guan L., 2017). Finally, sleep plays an essential role in cellular repair. Murine models have shown that under both total and partial sleep deprivation conditions, there was a significant increase in the level of

oxidative DNA damage (Everson et al., 2014). Both intracellular and extracellular oxidative stresses are associated with accelerated skin ageing (Masaki, 2010).

Overall, short sleep duration has been associated with accelerated skin ageing. Moreover, sleep deprivation leads to fatigue looking facial appearance which may have a social impact on these people's daily life as it will affect the first impression that they leave on other people.

4.3.6 Relationship between sleep and gene expression level

Several hair, circadian and mitochondria-related canonical pathways were identified in this study as being altered by sleep complaints. However, due to the small number of genes identified, after MTC an z-score was not achieved. Therefore, we cannot conclude if there is an up-or down-regulation of these pathways. The eumelanin biosynthesis pathway was the most significant pathway identified in females. Eumelanin is one of the three forms of melanin present in humans, along with pheomelanin and neuromelanin. They are responsible for many functions including pigmentation of skin and hair, as well as photoprotection of skin and eye (Schlessinger et al., 2021). Melanins are synthesised endogenously by melanocytes and this process is known as melanogenesis (D'Mello et al., 2016). Eumelanin biosynthesis occurs in three chromophoric phases: (1) formation of the red pigmentation, L-dopachrome, (2) formation of melanochrome through oxidation and polymerisation, and (3) further polymerisation of melanochrome to form the eumelanin (CYC). Eumelanin biosynthesis is catalysed by a number of specific melanogenic enzymes such as Tyrosinase-related protein (TRYP) 1 and TRYP2. The production of these enzymes is driven by various signalling pathways including protein kinase C, Cyclic adenosine monophosphate, WNT and MEK which act as a regulator for the activity of the melanocyte inducing transcription factor (MITF) which is important in controlling the development of melanocytes (D'Mello et al., 2016). TRYP1 in particular was thought to be associated with increased eumelanin to pheomelanin ratio and offers protection against oxidative stress (Videira et al., 2013). The production of eumelanin in the hair shaft involves the interactions of follicular melanocytes, keratinocytes and dermal papilla fibroblasts in the hair follicle pigmentary unit (Slominski et al., 2005). Melanogenesis occurs at a cellular level in the follicular melanocytes, melanin granules are transferred into cortical and medullary keratinocytes which then forms pigmented hair shaft. Unlike the skin, hair pigmentation is only active during the anagen stage and the melanocytes in hair follicles are more sensitive to the influence of ageing compared to that in the skin leading to a mixture of pigmented and white hair leading to the perception of grey hair (Slominski and Paus, 1993,

Tobin and Paus, 2001). The ageing of melanocytes is thought to be associated with the accumulation of ROS-mediated nuclear DNA and mtDNA damage (Tobin and Paus, 2001).

Mitochondrial dysfunction was also amongst the list of canonical pathways identified by IPA analysis in females, although it was not statistically significant. Murine models have linked mitochondrial dysfunction and reduced mitochondrial gene expression to skin wrinkling and visual hair loss, as well as increased inflammatory responses and dysfunctional hair follicles, interestingly, female mice showed more severe hair loss suggesting a sex-hormone regulation of mitochondrial function (Singh et al., 2018). In humans, mitochondrial dysfunction can arise from defects in the nuclear or mitochondrial genome. Mitochondrial dysfunction has been associated with many age-related chronic diseases; therefore, it is accepted as a hallmark of ageing. Severe genetic defects causing mitochondrial dysfunction can result in mitochondrial diseases. Since mitochondria are maternally inherited, Y-linked mitochondrial diseases have not been reported yet (Bornstein et al., 2020). Mitochondrial dysfunction has been associated with accelerated senescence and one of the theories of ageing is known as the mitochondrial free radical theory of ageing which was first proposed in 1954 (Harman, 2006). This theory suggested that ageing is accelerated by the level of oxidative damage caused by ROS generated by the electron transport chain as a by-product of oxidative phosphorylation. The oxidative damage could cause cellular impairment which includes electron transport chain impairment and this creates a vicious cycle of damage leading to increased production of ROS (Bornstein et al., 2020). Mitochondrial dysfunction has been previously associated with cell apoptosis dysregulation (Li and Zhan, 2019). Mitochondrial dysfunction could lead to a change in mitochondrial membrane potential which causes an alteration in the mitochondria-mediated apoptosis signalling pathway. Dysregulation of this pathway could facilitate tumorigenesis and tumour development (Hou et al., 2018). In addition, mitochondrial dysfunction also contributes to the pathogenesis of neurological disorders. The association between mitochondrial dysfunction and Alzheimer's disease is thought to be mediated by increased aggregation of amyloid-beta, tau phosphorylation and synaptic degeneration (Norat et al., 2020). Pathogenesis of Parkinson's disease, on the other hand, is mediated by a deficiency in mitochondria complex I activity and reduced mitochondria membrane potential (Gu et al., 1998). These findings have demonstrated the importance of mitochondrial function in human ageing and health. Since this pathway has been found to be altered in the patient

group compared to the control group suggests the impact of sleep on ageing and health could potentially be mediated by mitochondrial dysfunction.

It was encouraging to find CLOCK was identified as a significant upstream regulator in the analysis of the female sleep gene list. The *Clock* gene was identified as a mammalian circadian gene in the early 1990s (Marcheva et al., 2009). The protein encoded by *CLOCK* plays an essential role in the regulation of circadian rhythm. The *Clock* encoded protein can form a heterodimer with BMAL1 and activate the transcription of *Per* (*Per1*, *Per2* and *Per3*) and *Cry* (*Cry1* and *Cry2*) genes. On translation, PER and CRY proteins form heterodimers and repress the activity of CLOCK/BMAL1 heterodimers. This creates a feedback loop that produces a 24-hour gene transcription rhythmicity (Database, Bozek et al., 2009, Marcheva et al., 2009). A daily profile of circadian gene expressed showed that *Per1*, *Per2* and *Per3* peak in the early morning, while *Clock* and *Bmal1* peaks at approximately midnight (Takimoto et al., 2005). Polymorphism in *Clock* was associated with diseases including metabolic syndromes, obesity, delayed sleep phase disorder and major depressive disorder (Database, Bozek et al., 2009, Marcheva et al., 2009). Various studies have illustrated the effect of sleep and sleep deprivation on circadian genes. For instance, an increase in PER2 was observed under the partial sleep-deprivation state (Curie et al., 2015). However, a reduction in BMAL1, CRY1 and PER2 has been observed in total sleep restriction (Bolsius et al., 2021). The mechanism linking sleep deprivation and circadian gene expression could be mediated by the TNF-α. TNF-α is enhanced by both acute and chronic sleep deprivation and this could lead to an increase in *Per1* and *Per2*, but a reduction in *Bmal1* and *Cry1* (Yoshida et al., 2013, Cavadini et al., 2007). It has also been found that melatonin, discussed in earlier sections, was able to induce rhythmic expression of *Bmal1*, *Clock*, *Cry1* and *Per1* (Charrier et al., 2017, von Gall et al., 2005). Patients with circadian rhythm sleep disorders showed a different gene expression rhythm (Takimoto et al., 2005). Polymorphisms in *Per2* and *Cry2* were both found to be significantly associated with winter depression. The level of CRY2 was also lower in depressed bipolar patients (Lavebratt et al., 2010). These results illustrated the important association between sleep and the *clock* gene. individuals with different sleep disorders or complaints may experience abnormal expression of these circadian-related genes.

Finally, the “Role of MAPK Signalling in Promoting the Pathogenesis of Influenza” was the top IPA canonical pathway identified in males suggesting an association between sleep and the immune system. Prior study has found that partial sleep deprivation could lead to a

suppression of immunity to influenza virus infection (Brown et al., 1989). The Mitogen-activated protein kinase (MAPK) signalling is an important pathway that converts extracellular stimuli such as cytokines, hormones, growth factors, pathogens and oxidative stress into a wide range of cellular responses (Cargnello and Roux, 2011). The MAPK pathway also plays an important role in the regulation of gene expression, apoptosis, metabolism and cell differentiation (Roux and Blenis, 2004). The MAPK pathway can be activated by several viruses including influenza viruses and leads to a down-regulation of virus replication. However, MAPK signalling is sometimes misused by the viruses to aid their replication (Kumar et al., 2018). There are four subgroups in the MAPK family: (1) Extracellular signal-regulated kinases (ERKs), (2) The p38 MAPK, (3) c-jun N-terminal or stress-activated protein kinases (JNK/SAPK), and (4) ERK5/Big MAP kinase 1 (BMK1). The influenza virus has been shown capable of activating all of them. The activation of the MAPK signalling pathway regulates the expression of RANTES production which is a chemokine that can attract eosinophils leading to a pro-inflammatory response. ERKs in particular are able to increase the expression of proinflammatory interleukins (IL1β, IL-6 and IL-8) and TNF-α (Gaur et al., 2011). Prior studies have reported the association between circadian genes and the MAPK pathway. The MAPK pathway is able to regulate the expression of circadian genes, as well as phosphorylate CLOCK, BMAL1, CRY1 and CRY2 in the circadian system (Wang et al., 2020). Additionally, the ERK signalling pathway could regulate sleep duration through gene expression. The ERK pathway is activated by wakefulness and the phosphorylation of ERK could lead to an increase in sleep duration in a drosophila model (Foltenyi et al., 2007). Another study also showed that ERK loss-of-function and ERK phosphorylation were associated with reduced sleep duration (Mikhail et al., 2017). A down-regulation of the MAPK pathway has been observed in the patient group of the current study which means that they might not be able to down-regulate the virus replication and therefore, have a weaker immune response and increase their susceptibility to the influenza virus.

Results of the current study showed a potential association between sleep and pathways involved in circadian rhythm, mitochondrial function and the immune system. However, as the activation of the z-score was not achieved, we cannot conclude the direction of expression (i.e., up- or down-regulation). Further analysis, such as investigating links between mRNA levels and actual sleep patterns (via the accelerometer or sleep diary data) will need to be

conducted to further explore the association between gene expression and sleep, as well as replication of these findings.

4.4 Conclusion

Overall, an overestimation of sleep duration has been observed in the current study which is consistent with the findings of the UK Biobank analysis. Regardless of the sleep assessment methods, the patient group have a longer sleep duration and time in bed, but they had lower sleep efficiency, compared to the control group. This may be due to longer sleep latency and WASO. Moreover, the level of melatonin secretion per 24 hours was found to be lower in the patient group compared to the control group. Although this difference was not found to be statistically significant. Surprisingly, no significant correlation has been found between the level of cortisol and melatonin. The cortisol level of these participants was higher after their overnight stay in the sleep laboratory compared to when they were at home suggesting that sleeping in an unfamiliar environment may induce stress leading to higher cortisol production. The main source of discomfort reported by the participants includes a change in sleeping environment, having PSG wires connected to their body and the noise level at the sleep laboratory. Finally, a 'U-shaped' association was found between the level of mtDNA damage in the skin and sleep duration. This is consistent with previously published studies that showed accelerated skin ageing in sleep-deprived individuals.

The strength of this study includes that both patients and healthy controls were recruited which covered an age range between 21 and 60 years. Various samples were collected to measure sleep-related biomarkers and the mtDNA damage level was measured by skin swabs which is a non-invasive method compared to skin biopsy commonly seen in other studies. Additionally, the use of next-generation sequencing provided valuable genetic information related to sleep. However, this study also has its limitations. Most of the relationships found in this study were not statistically significant. This is possibly due to the small sample size. Moreover, the medical records of these participants were not available and as a result, it was not possible to determine the diagnosis of the patient group. Finally, hair samples were only collected at the beginning of the study and were not taken again at the end of the study to track any change with time. These factors need to be taken into consideration when optimising future studies.

Chapter 5. Conclusion

Sleep has been proven to have fundamental roles in many biological functions including metabolic regulation and memory consolidation. Sleep duration and quality can be affected by many factors including ageing, gender, ethnicity, lifestyle habits and social status. Many hormones also have a significant impact on sleep including cortisol and melatonin. The recommended sleep duration is typically 7-8 hours for healthy adults. Individuals sleeping less or more than the recommended amount of sleep are at a higher risk of metabolic diseases such as obesity, type 2 diabetes and various heart diseases. Sleep also has a crucial impact on the cognitive function and subjective well-being of an individual. Individuals with habitual short sleep duration tend to perform worse in cognitive function tests and are more likely to report negative moods. Worse cognitive performance could be particularly dangerous in people working in certain jobs in which safety is critical.

Most of the studies investigating the relationship between sleep and health often utilise self-reported methods such as sleep diaries or sleep questionnaires which are both subjective and may not reflect an individual's true sleep pattern. Therefore, the main aim of this study is to explore the relationship between sleep and metabolic health using the accelerometer data in the UK Biobank which is an objective method of assessing sleep duration and sleep efficiency, as well as sleep fragmentation. The secondary aim of this study is to investigate sleep misperception using various subjective (sleep diary) and objective (wrist-worn tri-axial accelerometer and laboratory-based PSG) methods to assess sleep in healthy controls and patients over the age of 18 years. Sleep-related biomarkers were also collected to compare cortisol, melatonin, mtDNA damage and gene expression between healthy controls and patients, as well as between participants with different sleep durations.

5.1 'U-shaped' association between sleep and metabolic health

Using the accelerometer data in over 84,000 UK Biobank participants, this study found that only approximately 23% of these participants have reached the recommended sleep duration of >7 hours/night. Males, ages >70 years, high BMI, social deprivation and ethnic minority groups were all associated with having a short sleep duration. Prior UK Biobank study reported higher self-reported insomnia symptoms amongst females (Kyle et al., 2017b), but our accelerometer data did not support this. This is consistent with PSG data which showed that males tend to experience lighter sleep. Lighter and fragmented sleep are also both age-related changes seen in ageing. With increasing age, there's a progressive decline in sleep duration

and sleep efficiency. Sleep latency and WASO, on the other hand, are prolonged in older adults. There might also be a shift in chronotype, older adults are more likely to have an earlier bedtime and wake time. Additionally, socially deprived and those who belong to ethnic minority groups often have a shorter sleep duration. This is likely to be due to their living environment and work patterns. Disadvantaged groups are more likely to live in urban areas and work in jobs that involve night shifts which means that they could be exposed to more light pollution and exposed to light at the wrong biological time leading to circadian misalignment. Their stress level may also be higher than others which could have an adverse impact on their sleep (Redline et al., 2004). Age, gender, social deprivation and ethnicity could all have an impact on an individual's lifestyle habits. One of the outcomes associated with a poor lifestyle is overweight and obesity which was also found to be associated with poor sleep. A 'U-shaped' association was found between sleep and BMI (Grandner et al., 2015a). This could be mediated by other underlying diseases such as OSA. High BMI increases the risk of OSA which is characterised by short and fragmented sleep.

Similar to the relationship found between sleep and BMI, a 'U-shaped' association was found between various metabolic diseases and sleep suggesting that both short and long sleep durations are linked with a higher risk of obesity, type 2 diabetes, ischaemic heart diseases, pulmonary heart diseases…etc. This was also reported by another UK Biobank study conducted by Cassidy et al. (Cassidy et al., 2018) which showed that those with worse cardiometabolic profiles were more likely to self-report poor sleep. The potential mechanism for this association is through the rise in the level of ghrelin and reduced level of leptin which promotes hunger and stimulates weight gain, predisposing an individual to obesity which is a risk factor for metabolic syndromes (Spiegel et al., 1999). Moreover, the increased risk associated with poor sleep could be mediated by the level of inflammation (Meier-Ewert et al., 2004). Systemic inflammation could contribute to atherosclerosis which eventually leads to cardiovascular diseases if left untreated. Although, the relationship could be bi-directional. It is possible that those with metabolic and cardiovascular diseases might get uncomfortable at night-time which contributes to fragmented and short sleep.

These findings demonstrated the importance of sleep in maintaining health. It is, therefore, important to identify individuals with poor sleep in order to promote healthy sleeping patterns which in turn could help to reduce their risk of metabolic diseases. This will not only be beneficial for the affected individuals but will also relieve the burden on their carer and the

healthcare system. However, it is important to note that the analysis carried out in this study is cross-sectional which means that no causal relationship between sleep and health can be determined.

5.2 Relationship between sleep and mental health

In addition to the 'U-shaped' association observed between sleep duration and metabolic health, a similar trend was also found between sleep and cognitive function indicating that individuals with 5-8 hours of sleep per night have faster reaction time, better memory and higher fluid intelligence score. This is consistent with other UK Biobank studies conducted by Henry et al. (Henry et al., 2019b) and Kyle et al. (Kyle et al., 2017b) which utilised self-reported sleep duration. Reduction in sleep duration was associated with increased daytime sleepiness and slower reaction. This could have severe outcomes such as an increased risk of automobile accidents or accidents at work, especially when heavy pieces of machinery are involved in the job. The relationship between sleep and cognitive performance could be mediated by reduced attention and alertness or through the alteration of brain structures and functions (Alhola and Polo-Kantola, 2007).

Additionally, this study has found that individuals with both short and long sleep durations were more likely to report negative moods. Those with a short sleep duration felt unhappy, experienced mood swings, felt irritable and lonely. Having too much sleep, on the other hand, is associated with a higher incidence of miserableness, loneliness and anxious feelings. This effect became significant even after just a single night of sleep restriction (Short and Louca, 2015). This relationship could be explained by the downregulation of the functional connectivity between the amygdala and the ventral anterior cingulate cortex and medial prefrontal cortex in sleep-deprived individuals leading to mood deterioration (Motomura et al., 2013). However, it is also possible that those with poor sleep may have other co-morbidities which could cause discomfort or pain. This could then contribute to the negative feelings reported by them.

Understanding the relationship between sleep and cognitive function is very important in promoting healthy sleep habits in students as maintaining optimal cognitive performance will be essential during learning and exams. In adults, high cognitive performance is also crucial when driving or operating machinery in order to minimise road accidents, as well as accidents at work.

5.3 Subjective and objective assessment of sleep amongst patients and controls

No significant difference was detected when comparing sleep duration and sleep efficiency measured by the sleep diary, accelerometer and PSG. This is likely due to the small sample size in this study. However, a trend was still noticed which showed a discrepancy between the accelerometer and sleep diary recordings of sleep duration. Similar to the UK Biobank participants, the participants in the sleep study also showed an overestimation of sleep duration. Research in this area showed inconsistent results. Some studies showed an overestimation of sleep, while other studies showed an underestimation of sleep duration. Discrepancies between subjective and objective assessments of sleep are common and this could be due to inaccurate memory recall of sleep onset, as a result, actual sleep was not distinguished from an inactive period spent in bed. Additionally, WASO could also be difficult to estimate, especially if it was a brief period of awakening. The discrepancy is also likely to be mediated by other confounding factors. Overestimation has been associated with higher BMI and co-morbidity, while an underestimation of sleep was associated with older age, females, ethnic minority and lower social status (Santos et al., 2021).

Different types of sleep disorders have different impacts on sleep duration. While insomnia is associated with short sleep duration, individuals affected by narcolepsy and idiopathic hypersomnia commonly have long sleep duration and excessive daytime sleepiness. In this study, comparisons between participants in the control and patient groups showed that time in bed and sleep duration were both higher in the patient group, but sleep efficiency was found to be higher in the control group. This is possibly mediated by shorter sleep latency and WASO in the control group. However, it is important to note that the difference detected was not found to be statistically significant. This is possible due to the small sample size as mentioned previously.

Diagnosis of some sleep disorders, such as insomnia, is commonly based on self-reported sleep complaints. Therefore, understanding the association between subjective and objective measurements of sleep is important. Individuals with sleep complaints may not be affected as much as they have perceived. The discrepancy between different methods of sleep assessment also suggests that multiple assessment methods should be considered when diagnosing individuals with sleep disorders.

5.4 Association between sleep and cortisol, melatonin, mtDNA damage

No significant difference was detected in terms of morning cortisol level, total melatonin level per 24 hours and mtDNA damage in the skin. Although, it was found that healthy controls, as well as those with optimal sleep (7-8 hours of sleep per night and less fragmented sleep), had a higher level of melatonin. This further proves the role of melatonin in promoting sleep. Melatonin onset is known to be associated with an increase in sleep propensity as it causes an increase in subjective sleepiness and lower body temperature which create an urge to sleep (Arendt and Skene, 2005). Therapeutic benefits of exogenous melatonin in elderly adults, those affected by jet lag and shift-workers have been reported. However, inconsistent results were found in some studies, therefore, there is no official recommendation for oral melatonin and while it is available to buy in health food shops or online in some countries, it is currently not available for purchase without a prescription in the UK.

On the contrary to the melatonin profile, cortisol is produced in the early morning and tends to peak approximately 30 minutes after awakening. No significant relationship was found between sleep duration and morning cortisol level in the current study. A reversed 'U-shaped' association was observed. This is not an expected finding as short sleep duration and fragmented sleep have previously been associated with a higher cortisol level in the morning and a flatter cortisol profile which means that the evening cortisol level was also higher in those with short and fragmented sleep (Kumari et al., 2009). Another finding is that the cortisol level was higher in samples taken in the hospital compared to that taken in the participants' home suggesting that sleeping in an unfamiliar environment may cause an increase in stress level. Sleeping in the sleep laboratory may cause a reduction in sleep duration and promote fragmented sleep. The questionnaire used in the current study asked the participants to rate their sleep and suggest their main cause of discomfort. Those with a shorter sleep duration had a lower rating than those with longer sleep duration. Some of the sources of discomfort are inevitable such as change in the environment and PSG wires attached to them. However, other factors are potentially modifiable to promote a better sleeping environment for those attending assessments at the sleep laboratory. For instance, reducing the noise level at night and adjusting the room temperature to suit individuals' needs.

Moreover, a 'U-shaped' association was found between sleep duration and mtDNA damage in the skin. This could be mediated by the increased level of oxidative stress and inflammation caused by short and fragmented sleep (Lacedonia et al., 2015). Accelerated skin ageing has

been associated with poor sleep. Sleep-deprived individuals are often perceived as more tired looking, having darker circles under their eyes and more wrinkles and fine lines under their eyes. Overall, people who are sleep deprived are perceived as less attractive (Oyetakin-White et al., 2015). This could have a social impact on an individual as you never get a second chance to leave the first impression, especially during important events, for example, job interviews.

Additionally, it was encouraging to find that several hair, circadian and mitochondria, as well as immunity-related canonical pathways were identified in this study as being altered in the sleep patient group. Circadian genes such as *Clock*, *Bmal1*, *Per* and *Cry* all play crucial roles in the regulation of circadian rhythm which measures time on a scale of 24 hours (Reppert and Weaver, 2002). Dysregulation of circadian gene expression could therefore have adverse effects on sleep, as well as on mood and hormone secretion (Bunney et al., 2015). Additionally, mitochondrial dysfunction was amongst the list of canonical pathways identified. Mitochondria are known to be an important component in the ageing process. Mitochondrial dysfunction has been found to be associated with skin ageing, poorer defence against infection, diabetic cardiomyopathy and Parkinson's diseases (Haas, 2019). Moreover, influenza suppressing MAPK signally pathway was also amongst the list of altered pathways identified in the current study suggesting that the patient group may be more susceptible to infections. Sleep deprivation has previously been found to have detrimental effects on immune function, as well as immune cell number (Bryant et al., 2004). However, we cannot conclude the direction of gene expression (i.e., up- or down-regulation) due to the fact that the activation of the z-score was not achieved.

These results showed the importance of sleep on cortisol, melatonin, mtDNA damage and gene expression levels. However, due to the cross-sectional nature of the current study, the causal relationship cannot be determined.

5.5 Future work

As shown in the previous chapter, the human sleep research study demonstrated a trend in cortisol, melatonin and mtDNA damage levels. However, due to the Covid-19 pandemic, the sample size was relatively small. Future studies should be conducted with an increase in sample size, ideally with an equal number of participants in each sleep duration group and a balanced number of participants should be included in both the healthy control and the patient groups. Ethical approval should also be applied in order to obtain the clinical records of these participants as this will help to differentiate participants with abnormally short sleep

duration (e.g., insomnia) or long sleep duration (e.g., narcolepsy), as well as those with fragmented sleep (e.g., parasomnia and OSA). In addition to the gene expression analysis conducted using the hair samples, the western blot technique should be used to determine the corresponding protein expression level (Mahmood and Yang, 2012). Lastly, Ingenuity pathway analysis was conducted in the current study which was appropriate for categorical variables such as when comparing healthy controls and patients. However, it was less suitable for continuous variables. Therefore, it cannot be used to investigate the relationship between sleep duration and gene expression level. To overcome this issue, future analysis should be conducted using the Limma package in R. This package has been a popular choice over the past decade for handling complex gene expression experimental designs and it can overcome the problem of small sample size (Ritchie et al., 2015).

Apart from the additional analysis on the current samples collected, additional samples could be incorporated into future studies. Metabolic response measurements could be incorporated into the study which allows the determination of an individual's ability to handle glucose, for instance, the oral glucose tolerance test. Participants should have at least eight hours of fasting prior to the test and a baseline blood sample will be taken. They will then consume an appropriate amount of glucose and further blood samples should be taken every 30 minutes for two hours (Eyth E, 2021). This will be useful for comparing metabolic responses in participants with different habitual sleep durations, as well as a comparison between those with and without a diagnosed sleep disorder. Using the same blood samples, an additional assay could be conducted to investigate the level of reactive oxygen species (ROS). This could complement the skin swab assay with was used to investigate mtDNA damage caused by stressors including ROS. A chemiluminescence assay for ROS detection has been published by Yamazaki et al. (Yamazaki et al., 2011). This assay involves the use of chemiluminescent probes Luminol and phorbol 12-myristate 13-acetate. The luminescence could be visualised using a Tecan Infinite 200 PRO reader which is available at the Newcastle University medical school. Additionally, to investigate the impact of sleep on appearance and wellbeing further, non-invasive molecular and histological analyses could be conducted. The effect of sleep on skin barrier functions could be investigated through the transepidermal water loss analysis. This method has been utilised in many studies (Alexander et al., 2018). Moreover, further analysis of skin ageing could be conducted using the Canfield VISIA Gen 7 which is available in Skin Life Analytics (Catalyst, Newcastle upon Tyne, UK). This equipment will be useful for the

exploration of habitual sleep duration, as well as sleep disorders, on the level of spots, wrinkles, texture, pores, UV spots, brown spots, red areas and porphyrins in the facial skin (Canfield).

Chapter 6. References

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Appendix A

Portions of this work have resulted in two peer-reviewed publication:

- **Zhu G**, Catt M, Cassidy S, Birch-Machin M, Trenell M, et al. (2019) Objective sleep assessment in >80,000 UK mid-life adults: Associations with sociodemographic characteristics, physical activity and caffeine. PLOS ONE 14(12): e0226220.
- **Zhu G**, Cassidy S, Hiden H, Woodman S, Trenell M, Gunn DA, Catt M, Birch-Machin M, Anderson KN. Exploration of Sleep as a Specific Risk Factor for Poor Metabolic and Mental Health: A UK Biobank Study of 84,404 Participants. Nat Sci Sleep. 2021; 13:1903-1912.

Other publications:

- **Zhu G**, Anderson K. Does insomnia worsen cardiometabolic health? ACNR 2018;18(2):20-22.
- **Zhu G**, Catt M, Anderson K, Gunn D, Birch-Machin M. P069 Investigating the impact of insulin resistance on ageing and wellbeing using sleep as a model system 2019.
- Ruddy E, **Zhu G**, Idowu O, Birch-Machin MA. Chapter 9 - Skin aging and mitochondria. In: de Oliveira MR, editor. Mitochondrial Dysfunction and Nanotherapeutics: Academic Press; 2021. p. 237-59.

This work has been presented at several conferences:

- Science of healthy diet, sports & wellbeing conference, Bradford, 2018: oral presentation
- Mitochondria, Nutrition and Health conference, Newcastle, 2018: poster presentation
- 10th Alliance annual healthy ageing conference, Newcastle, 2019: poster presentation
- 25th Congress of the European sleep research society, virtual, 2020: poster presentation
- British sleep society BSS conference, virtual, 2021: poster presentation and lightning talk

This work has been presented at internal and collaborative meetings:

- BBSRC-Unilever ICP studentship workshop, Colworth, 2018: poster presentation
- Institute of Cellular Medicine seminar, Newcastle university, 2018: oral presentation
- Clinical sleep meeting, Freeman hospital Newcastle, 2018: oral presentation,

- Dermatological sciences research in progress seminar, Royal Victoria Infirmary Newcastle, 2018: oral presentation
- BBSRC-Unilever ICP studentship workshop, Liverpool, 2019: oral presentation

Award:

This work has been awarded with the Colin Sullivan Award at the virtual BSS 2021 conference.

Appendix B

```
#args <- commandArgs(trailingOnly = TRUE)

#This is the correct script to prcess sleep data, and physical activity

library(GGIR)

g.shell.GGIR(#=======================================

        # INPUT NEEDED:

        mode=c(1,2,3,4,5),

        overwrite = FALSE,

        #datadir= args[1],

        #outputdir= args[2],

        datadir = "____________",

        outputdir =  "____________",

        #f0=1, f1=2,

        #-------------------------------

        # Part 1:

        #-------------------------------

        # Key functions: reading file, auto-calibration, and eastracting features

        do.enmo = TRUE,         do.anglez=TRUE,

        chunksize=2,            printsummary=TRUE,

        #-------------------------------

        # Part 2:

        #-------------------------------

        strategy = 2,           ndayswindow=16,

        hrs.del.start = 0,      hrs.del.end = 0,
```

```
maxdur = 9,              includedaycrit = 16,
winhr = c(5,10),
qlevels = c(c(1380/1440),c(1410/1440)),
qwindow=c(0,24),
ilevels = c(seq(0,400,by=50),8000),
mvpathreshold =c(100,120),
bout.metric = 4,
closedbout=FALSE,
#-------------------------------
# Part 3:
#-------------------------------
# Key functions: Sleep detection
timethreshold= c(5),      anglethreshold=5,
ignorenonwear = TRUE,
#-------------------------------
# Part 4:
#-------------------------------
# Key functions: Integrating sleep log (if available) with sleep detection
# storing day and person specific summaries of sleep
excludefirstlast = FALSE,
includenightcrit = 16,
def.noc.sleep = c(),
relyonsleeplog=FALSE,
outliers.only = TRUE,
```

```
criterror = 4,

#relyonsleeplog = FALSE,

sleeplogidnum = TRUE,

colid=1,

coln1=2,

do.visual = TRUE,

nnights = 16,

#-------------------------------

# Part 5:

# Key functions: Merging physical activity with sleep analyses

#-------------------------------

threshold.lig = c(50), threshold.mod = c(120),  threshold.vig = c(500),

boutcriter = 0.8,     boutcriter.in = 0.9,     boutcriter.lig = 0.8,

boutcriter.mvpa = 0.8, boutdur.in = c(1,10,30), boutdur.lig = c(1,10),

boutdur.mvpa = c(1),   timewindow = c("WW"),

#-----------------------------------

# Report generation

#-------------------------------

# Key functions: Generating reports based on meta-data

do.report=c(2,4,5),

visualreport=TRUE,     dofirstpage = TRUE,

viewingwindow=1)
```

Appendix C

Upstream regulators identified in females.

Upstream Regulator	p-value
JAG2	0.001
takinib	0.001
SMYD1	0.001
CYP24A1	0.002
RPS19	0.002
PLX5622	0.002
HS-243	0.003
BUB1B	0.003
epicatechin	0.003
5-fluoro-2-hydroxycinnamaldehyde	0.005
5-fluoro-2-benzoyloxycinnamaldehyde	0.005
MALSU1	0.005
EIF3M	0.005
HSD17B12	0.005
MT-TE	0.006
MRPL14	0.006
TCF12	0.007
MRPL12	0.007
SERPINC1	0.007
TERF2	0.007
bicuculline/dalfampridine	0.007
teriflunomide	0.010
LRPPRC	0.010
ALKBH7	0.011
miR-451a (and other miRNAs w/seed AACCGUU)	0.011
Vegf	0.011
trypsin	0.012
DAP3	0.012
miR-135a-5p (and other miRNAs w/seed AUGGCUU)	0.012
F10	0.012
MSI1	0.012

MT-TM	0.013
NAE1	0.013
IFT57	0.014
pCPT-cAMP	0.015
actinonin	0.015
telapristone acetate	0.016
TAL1	0.017
CDK1	0.018
HNRNPAB	0.018
TWNK	0.020
9Z,11E-octadecadienoic acid	0.020
CLOCK	0.022
NRG1	0.024
TLX1	0.025
trans-cinnamaldehyde	0.026
BCAP31	0.026
chrysotile asbestos	0.026
LIN28A	0.028
RAB1B	0.030
FLCN	0.030
glucocorticoid	0.030
RYR1	0.031
TERC	0.031
SYK/ZAP	0.032
CORT	0.032
IKZF2	0.032
nafenopin	0.032
NSUN6	0.034
SET	0.034
discodermolide	0.034
ATP7B	0.035
GTF2IRD1	0.035
EP400	0.037
NFYC	0.038

GBX2	0.038
fasudil	0.038
conjugated linoleic acid	0.039
Hbb-b2	0.040
ABCG1	0.041
AURK	0.043
baicalin	0.043
ANLN	0.043
TAF7L	0.043
TXN	0.043
FAS	0.044
TRPS1	0.044
TFE3	0.044
AGT	0.045
RAC2	0.045
CKAP2L	0.046
CD40LG	0.048
VEGFB	0.049

Appendix D

Upstream regulators identified in males.

Upstream Regulator	p-value
CKAP2L	0.001
mir-27	0.002
hnRNP H	0.003
HJURP	0.003
GABRD	0.003
RGS9	0.003
miR-27b-5p (miRNAs w/seed GAGCUUA)	0.003
miR-3584-5p (and other miRNAs w/seed GGAGGAG)	0.003
RGS11	0.003
RGS7	0.003
ATP6V1B1	0.003
SPINK7	0.007
miR-509-3p (miRNAs w/seed GAUUGGU)	0.007
miR-577 (miRNAs w/seed AGAUAAA)	0.007
PDGF BB	0.007
ELAVL1	0.008
miR-124-3p (and other miRNAs w/seed AAGGCAC)	0.009
ADCYAP1	0.010
GALNT14	0.010
MMS19	0.010
CIAO2B	0.010
Gamma tubulin	0.010
CIAO1	0.010
RPP38	0.010
RGS6	0.010
miR-139-3p (miRNAs w/seed GGAGACG)	0.010
miR-485-3p (and other miRNAs w/seed UCAUACA)	0.010
MIR23A/24-2/27A cluster	0.010
mir23a/24-2/27a	0.010
HR	0.011
USP24	0.014

PHF19	0.014
miR-27a-5p (miRNAs w/seed GGGCUUA)	0.014
miR-625-5p (and other miRNAs w/seed GGGGGAA)	0.014
BICD2	0.014
GABRA1	0.014
GNG13	0.014
Activin	0.014
nandrolone decanoate	0.014
testosterone cypionate	0.014
1,25-dihydroxyvitamin D	0.015
SWI-SNF	0.017
NEUROD4	0.017
PDCL	0.017
HLA-B	0.017
starch	0.017
methyltestosterone	0.017
TSC2	0.019
miR-204-5p (and other miRNAs w/seed UCCCUUU)	0.020
FOXQ1	0.021
TP53RK	0.021
FSTL3	0.021
TRPV6	0.021
UBA3	0.021
alpha-ketoisocaproic acid	0.021
phenelzine	0.021
juglone	0.021
PLN	0.021
ANXA7	0.024
miR-519a-3p (and other miRNAs w/seed AAGUGCA)	0.024
SF3B2	0.024
miR-9-5p (and other miRNAs w/seed CUUUGGU)	0.025
IKZF3	0.025
GnRH-A	0.026
LASP1	0.027

MAD2L2	0.027
UBR1	0.027
ONECUT2	0.027
GnRH analog	0.029
levodopa	0.030
CCND1	0.030
MSGN1	0.031
UBR2	0.031
STK17A	0.031
UBQLN1	0.031
2-oxoadipic acid	0.031
2-aminoadipic acid	0.031
mir-634	0.034
IGBP1	0.034
alprazolam	0.034
phenylephrine	0.035
DHTKD1	0.038
RLIM	0.038
NEUROD2	0.038
SD6	0.038
enoxacin	0.038
RAF1	0.040
L-histidine	0.041
RB1CC1	0.041
pentylenetetrazol	0.041
SAMHD1	0.044
GNB3	0.044
FZR1	0.044
PITX1	0.044
4-nonylphenol	0.048
mir-302	0.048
ACVR1B	0.048
pentobarbital	0.048
sirolimus	0.162

IFNG	0.445