Sleep and its Association with Cardiometabolic Risk Factors in Young Adults

This thesis is presented for the degree of
Doctor of Philosophy

by

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March 2018
“Home may be perilous and the destination out of reach
But there are no paths without an end, do not grieve”

Hafiz, Persian Poet
Poem, “The lost Joseph”


Declaration

I, Anahita Hamidi, certify that:

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Date: March 2018
Abstract

Cardiovascular disease, encompassing heart disease, stroke and vascular disease, is the leading cause of death in Australia and globally, with the underlying cardiometabolic risk factors including overweight and obesity, hypertension, dyslipidaemia, insulin resistance and inflammation. Known lifestyle factors that influence cardiovascular disease include physical inactivity, diet, alcohol consumption and smoking. Recent evidence suggests that insufficient sleep and/or poor sleep quality are associated with cardiometabolic risk factors. Therefore, inadequate sleep quality and/or duration is potentially a further modifiable lifestyle factor. In particular, studies have shown a positive relationship between insufficient sleep and obesity in children and adolescents. However, evidence in other age groups is inconsistent. In addition to insufficient sleep, a positive association between sleep disordered breathing and cardiometabolic risk factors has been reported in adult studies, however, the results are inconsistent. The influence of sleep and sleep-breathing disorders on cardiometabolic risk factors during young adulthood, prior to establishment of chronic diseases, is still unknown.

This thesis investigated data from young adults that participated in the 22-year follow-up of the Western Australian Pregnancy Cohort (Raine) Study. The aim was to investigate the associations between: (i) objective measurements of sleep and cardiometabolic risk factors; (ii) sleep quality and cardiometabolic factors; and (iii) sleep-disordered breathing (high risk for obstructive sleep apnoea) and cardiometabolic risk factors.

The Raine Study is a population-based pregnancy cohort study established between 1989 and 1991 that included 2900 pregnant women recruited at 18 weeks of gestation. The Raine Study aimed to ascertain the effects of intrauterine, familial, environmental and lifestyle factors on the health of the 2868 offspring. The study has detailed maternal and paternal information during pregnancy, as well as sociodemographic, developmental, anthropometric, clinical and biochemical data collected on the offspring at birth and during follow-up assessments at 1, 2, 3, 5, 8, 10, 14, 17, 18, 20 and 22 years of age. There were 1234 participants at the 22-year follow-up which was conducted from 2012-2014. Sociodemographic and lifestyle behaviours (smoking, alcohol intake, physical activity and depression and anxiety scores) were evaluated from questionnaires. Anthropometric, blood pressure and fasting blood samples (glucose,
insulin, high sensitivity C - reactive protein [hs-CRP] and lipids) were assessed in 975 of the participants. Sleep characteristics were measured objectively by actigraphy with a wrist accelerometer worn for 7 days in the home environment. Accelerometer data were collected from 581 participants and sleep variables including sleep duration, sleep efficiency, sleep latency and Wake after Sleep Onset (WASO) were analysed. Sleep quality was measured subjectively using the Pittsburgh Sleep Quality Index (PSQI) Questionnaire and high risk for sleep apnoea (OSA) was evaluated using the Berlin Questionnaire. Data were analysed using linear and logistic regression models adjusting for gender and lifestyle factors.

Mean sleep duration was 6.60 hours and the prevalence of short sleepers (< 6 hours/night) was 25.5% in this population. There were no associations between any of the actigraphy-derived sleep variables and body mass index, blood pressure, or fasting blood glucose, insulin, lipids and hs-CRP levels before or after adjustment for gender and lifestyle factors.

Based on the PSQI Questionnaire 28% of the individuals had poor sleep quality. Poor sleep quality was not associated with any cardiometabolic risk factors measured although participants with poor sleep quality were more likely to be smokers and have higher depression and anxiety scores.

Using the Berlin Questionnaire, the prevalence of high risk for OSA was 14.75% with similar gender distribution. Smoking was the only lifestyle variable associated with high risk for OSA. Those at high risk for OSA were more likely to be overweight/obese or have central obesity. High risk for OSA was positively associated with systolic blood pressure, triglycerides, hs-CRP and inversely associated with high density lipoprotein cholesterol (HDL-C), before and after adjustment for gender and lifestyle factors. However, these associations were no longer apparent when sensitivity analyses were performed that accounted for BMI.

In summary, this study has shown that sleep characteristics are not related to cardiometabolic risk factors in a young adult population. Further, while being at high risk for OSA associated with obesity and related cardiometabolic risk factors, the relationship is likely due to the underlying presence of obesity in these individuals. In conclusion, data from the Raine Study do not show any evidence for a relationship between sleep characteristics, high risk for OSA and cardiometabolic risk factors (other than obesity) in a young adults.
Acknowledgements

I gratefully acknowledge the University of Western Australia and the Western Australian Pregnancy Cohort (Raine) Study for providing the scholarship and academic support. I would also like to express my gratitude to all of the Raine Study participants, their families and the Raine Study team.

I would like to express my gratitude to my main supervisors, Prof. Trevor Mori and Prof Peter Eastwood for their invaluable advice, tremendous encouragement and support. It has been a great honour to study and work with them. I would also like to thank my co-supervisor A/Prof Rae-Chi Huang and E/Prof Lawrence Beilin for all of their valuable suggestions and support. I am deeply thankful to Mrs. Sally Burrows for her statistical advice and guidance. I would like to thank all of my colleagues at Royal Perth Hospital, the Raine Study House and the Centre for Sleep Sciences at the University of Western Australia.

To my dearest parents and my lovely sister, thank you for your constant support and prayers, you always have given me even though you are thousands miles away from me. Super special thanks go to my precious daughter, Vania for all of her understanding and patience. Thank you for all the cheers and happy moments you created for your sole parent during her PhD study. By completion of this PhD, I hope to model you that you can pursue your dream with your determination and efforts despite the obstacles and challenges. A big special thanks to Ms. Minoo Zand, thank you for all of your support, tremendous encouragement and invaluable advice you have given to me. This thesis is dedicated to my beloved grandmother who passed away while I was doing my PhD.
List of Awards and Presentations

Awards

1. University Postgraduate Award-International student (UPAIS), University Western Australia
2. Scholarship International Research Fee (SIRF), University Western Australia
3. Raine Study Top-Up Scholarship, The Raine Foundation
4. Foundation for High Blood Pressure Research Council Australia Travel Award, Joint Annual Scientific Meeting of the Australian Atherosclerosis Society, the Australian Vascular Biology Society and the High Blood Pressure Research Council of Australia, Hobart, Tasmania, December 2016

Presentations

1. Relationship between sleep and cardiometabolic factors in young adults.

Anahita Hamidi, *The Western Pregnancy Cohort (Raine) Study Annual Scientific Meeting*, Perth, Western Australia, September 2016

2. Relationship between sleep and cardiometabolic factors in young adults.

Anahita Hamidi, *Royal Perth Hospital Medical Research Foundation Young Investigators Day*, Perth, Western Australia, October 2016

3. Relationship between sleep and cardiometabolic factors in young adults: the RAINE Study.

Anahita Hamidi, *Joint Annual Scientific Meeting of the Australian Atherosclerosis Society, the Australian Vascular Biology Society and the High Blood Pressure Research Council of Australia*, Hobart, TAS, December 2016
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# Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AASM</td>
<td>American Academy of Sleep Medicine</td>
</tr>
<tr>
<td>AHI</td>
<td>apnoea-hypopnoea index</td>
</tr>
<tr>
<td>ALP</td>
<td>alkaline phosphatase</td>
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<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
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<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
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<tr>
<td>AusDiab</td>
<td>Australian Diabetes, Obesity and Lifestyle</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>CARDIA</td>
<td>Coronary Artery Risk Development in Young Adults</td>
</tr>
<tr>
<td>CIH</td>
<td>chronic intermittent hypoxia</td>
</tr>
<tr>
<td>CPAP</td>
<td>continuous positive airway pressure</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>DASS</td>
<td>depression anxiety score system</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
</tr>
<tr>
<td>DEXA</td>
<td>dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>DoHAD</td>
<td>Development Origins of Health and Disease</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalographic</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyogram</td>
</tr>
<tr>
<td>EOG</td>
<td>electrooculogram</td>
</tr>
<tr>
<td>FOSQ</td>
<td>functional outcome of sleep questionnaire</td>
</tr>
</tbody>
</table>
GGT gamma-glutamyl transferase
GH growth hormone
g/wk gram/week
HbA1c haemoglobin A1c
HDL-C high density lipoprotein cholesterol
HELIUS multi-ethnic Healthy Life in an Urban Setting
HOMA-IR homoeostasis model assessment for insulin resistance
hs-CRP high sensitivity CRP
IL-6 interleukin 6
IPAQ international physical activity questionnaire
JNC-7 The Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure
LDL-C low density lipoprotein cholesterol
METS metabolic equivalences
min minute
MMAS Massachusetts Male Aging Study
NHANES National Health and Nutrition Examination Survey
NHIS National Health Interview Survey
NO nitric oxide
NPY/AgRP neuropeptide Y/agouti-related protein
NREM non-rapid eye movement
OGTT Oral Glucose Tolerance Test
OSA obstructive sleep apnoea
PA physical activity
POMC/CART propiomelanocortin/cocaine and amphetamine–regulated transcript
PSG polysomnography
PSQI Pittsburgh Sleep Quality Index
Raine Study The Western Australian Pregnancy Cohort Study
REM rapid-eye movement
ROS reactive oxygen species
SBP systolic blood pressure
SBSM Society of Behavioural Sleep Medicine
SDB sleep-disordered breathing
SE sleep efficiency
SOL Sleep Onset Latency
TG triglycerides
TNF-α tumour necrosis factor alpha
TST total sleep time
UA uric acid
WASO wake after sleep onset
WHO World Health Organisation
Chapter 1:
Literature Review & Study Aims
1.1 Cardiometabolic Risk Factors and Role of Sleep

Cardiometabolic risk factors encompass the related conditions of obesity, diabetes, hypertension and cardiovascular disease (1). These conditions share common risk factors and are associated with reduced quality of life, decreased life expectancy and increased economic burden (1). Over the last few decades, the prevalence of these cardiometabolic risk factors has increased worldwide in both developed and developing countries (1). Amongst developed countries, Australia has one of the highest rates of overweight and obesity; the prevalence of overweight and obesity has increased from 56% in 1995 to 63% in 2012 (2). Over the same time period, the proportion of obese Australians increased from 19% to 28% (2). Obesity has been reported to shorten life expectancy by nine years and is associated with chronic diseases and disabilities which bring about significant economic burden (2). Based on the data from the Australian Diabetes, Obesity and Lifestyle (AusDiab) study, the direct and indirect costs of obesity is estimated at $56.6 billion per year (2).

The metabolic syndrome refers to the co-occurrence of several known cardiovascular risk factors including hypertension, dyslipidaemia (elevated triglycerides and lowered high-density lipoprotein cholesterol), insulin resistance, and central obesity (3). These conditions are interrelated and share underlying mediators, mechanisms and pathways. Although there are several definitions of the metabolic syndrome including those by the International Diabetes Federation (4), the World Health Organization (WHO) (5) and the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) (5), a central feature of each is the inclusion of insulin resistance, visceral adiposity, atherogenic dyslipidaemia and high blood pressure. The metabolic syndrome also associates with proinflammatory and prothrombotic states, as well as increased oxidative stress (6). Studies have shown that the metabolic syndrome is associated with a 2-fold increase in cardiovascular mortality and a 1.5-fold increase in all cause mortality, however, it is still unclear whether the prognostic significant of metabolic syndrome exceeds the risk associated with the sum of its individual components (7). There is strong evidence to support the hypothesis that many chronic diseases including cardiovascular disease, begin a long time before their clinical manifestations (8). Autopsy studies of young adults following death from trauma show a significant link between cardiometabolic risk factors in childhood and fatty streaks in coronary arteries (8). Many risk factors related to chronic disease (including cardiometabolic risk factors) are also known to accumulate gradually over time thus contributing to the
development and progression of chronic disease (9). During adolescence and young adulthood a number of behaviour and lifestyle factors can adversely impact on well-being (10). Thus early identification and prevention of these factors may play a significant role in health care policies designed to prevent and diminish the burden of cardiometabolic risk factors.

Recently, the role of sleep in cardiometabolic risk factors has gained attention and the increase in obesity is paralleled with a decrease in nightly sleep duration (11, 12). According to National Health And Nutrition Examination Survey (NHANES) results, adults aged 20-59 have less sleep each night than adults 60 years and over and 37% of adults have less than six hours of sleep per night (13). Online surveys have shown that the average sleep duration in Australia has dropped from 8 hours in 2000 to 7 hours in 2010 (14). Although these data do not provide evidence of a causal relationship between sleep and cardiometabolic risk factors, they highlight the need for studies to better understand the mechanisms underlying the associations between sleep and cardiometabolic risk factors and how these may differ with age.

1.2 Human Sleep

Sleep is defined by the physiological stages of reduced consciousness, lack of activity and reduction in sensory activity (15). The precise role of sleep remains a mystery, although its role in maintaining normal cognitive, motor and metabolic function has been clearly established (16). According to the American Academy of Sleep Medicine (AASM), sleep involves two separate electroencephalograph (EEG) stages: non-rapid eye movement (NREM) and rapid-eye movement (REM) (15). During a sleep episode NREM and REM stages alternate cyclically (17). In a normal night of sleep, there are 3-5 sleep cycles and each sleep cycle (NREM and REM) has a period of 90-120 minutes duration (17). Sleep starts with NREM in all age groups except newborns (17). NREM is further divided into three deeper stages defined as N1, N2 and N3 (Figure 1.1). Increasing depth of NREM sleep is characterized by increases in EEG wave amplitude and decreases in EEG wave frequency. Deeper stages of NREM occur before the first episode of REM. In the average adult, 75-80% of total sleep is spent in the NREM stage. During NREM sleep there is a down-regulation of cardiovascular activity including a decrease in heart rate, stroke volume and arterial blood pressure (17). As a result of the prevalence of parasympathetic over sympathetic activity, metabolic heat
production and body temperature drop at this stage of sleep. These changes in NREM sleep contribute to postural and motor quiescence (17).

In a normal young adult, NREM sleep is followed by REM sleep (17). During the REM stage, low voltage EEG waves occur in addition to rapid movements of the eyes as detected by electrooculogram (EOG). There is also a very low level of muscle tone (except diaphragm, extraocular and sphincter muscles), as detected by electromyogram (EMG);(17) .The most important features of REM are variability in heart rate, arterial blood pressure and irregularities in breathing.

1.3 Objective Sleep Measurements

Studies examining sleep have used both objective and subjective methods to identify sleep patterns and disturbances. Polysomnography and actigraphy are objective methods, most commonly used to assess sleep (18). Self-reported sleep characteristics and validated sleep questionnaires are the most commonly used subjective measures of sleep (18).

Polysomnography (PSG) is the gold standard method used to measure sleep patterns and disturbances (18). It involves the simultaneous gathering, analyzing and interpreting of physiologic signals during sleep, including the electroencephalograph (EEG), electromyogram (EMG), electrooculogram (EOG), electrocardiogram (ECG) and respiratory signals (19). Individuals undergoing PSG are typically required to spend a night in a sleep laboratory, although over the last decade advances in technology have made it possible to monitor sleep by PSG at home (18). Irrespective of whether PSG is
measured in the laboratory or at home, PSG is costly and the recording process may disturb routine sleep patterns (18).

Polysomnography (PSG) is also the gold standard method used to assess obstructive sleep apnoea (20). Obstructive Sleep Apnoea (OSA) is characterised by recurrent decrease and cessation of respiratory airflow during sleep caused by upper airway narrowing and collapse. The apnoea-hypopnoea Index (AHI) is commonly used for estimation of the severity of OSA (20). The AHI is the combined average number of apnoea episodes (cessation of airflow for at least 10 seconds) and hypopnoea episodes (reduction in airflow of at least 50% with a decrease in arterial saturation of at least 4% due to partial airway obstruction) occurring within each hour of sleep (20). The AHI is used to define OSA as mild (5 < AHI < 15 events/hr), moderate (15 ≤ AHI ≤ 30 events/hr) or severe (AHI > 30 events/hr) (20).

Actigraphy is another common objective method of sleep assessment and the American Academy of Sleep Medicine (AASM) considers actigraphy to be a useful and non-invasive method (21). Actigraphy utilises one of the characteristic features of sleep; relative immobility compared to wakefulness. By assessing mobility, actigraphy records periods when an individual is asleep or awake (22). Most simply actigraphy assumes that the wearer is asleep during periods of minimal activity and awake when there is activity. Actigraphy measures limb movement, usually at the wrist, using a small accelerometer embedded in a watch-like device. This device can also be placed on other body parts such as ankles and hips (21).

The clinical diagnostic value of actigraphy is limited as it cannot detect and distinguish sleep disorders such as restless leg syndrome (19). However, actigraphy has a number of advantages over PSG. For example, actigraphy can monitor and save information about an individual's sleep over several days or even weeks (18). Moreover, it is inexpensive and is able to collect sleep pattern information in the participant’s natural sleep environment (18). This is particularly useful for those who do not tolerate sleeping in a laboratory setting, such as some insomnia patients and small children in whom it is often not possible to obtain self-reported behaviour (23). By providing an opportunity for individuals to adhere more closely to their customary sleep and wake time, actigraphy can more accurately estimate typical sleep patterns. Actigraphy is also a practical method for sleep assessment in large-scale population studies (22). High correlations have been reported between PSG and actigraphy-defined sleep, with an
overall agreement of over 90% in normal participants (24, 25). However, actigraphy is known to overestimate the sleep period and underestimate wake time when compared to PSG (18). This discrepancy is due to the different method used to measure sleep onset in PSG and actigraphy. Specifically, sleep onset in PSG is signalled by changes in brain electrical activity patterns, while sleep onset in actigraphy is signalled by immobility of the participant (22) In actigraphy, periods of immobile wakefulness would be scored as sleep, thus overestimating sleep time.

1.4 Self-report Sleep Measurement

Self-report of sleep by questionnaires is a subjective method of sleep measurement often used in epidemiological studies. Previous studies estimating the agreement between subjective and objective measures of sleep duration have reported correlations of 0.31 to 0.63 between the two (26-28). A study of 2080 adults aged 18-74 years found the correlation between self-reported sleep and actigraphy sleep duration was 0.43 (29). Using self-report, the average sleep duration was 7.85 ± 1.12 hours versus 6.74 ± 1.02 hours using actigraphy, with lower correlations in males and those younger than 45 years (29).

A limitation of many previous studies is that correlations are not adequate for estimating agreement, as a correlation only measures the strength of a relationship between two variables and not the agreement between them (30). The few studies that have evaluated sleep duration assessed by self-report and actigraphy using statistical methods other than correlation, have reported a poor agreement between subjective and objective methods of sleep measurement (31, 32).

1.4.1 Pittsburgh Sleep Quality Index Questionnaire

The Pittsburgh Sleep Quality Index (PSQI) Questionnaire is a standardized and validated questionnaire used to assess the quality of sleep (33). The PSQI questionnaire (see Section 2.8.2.1) can reliably categorise individuals as either “good” or “poor” sleepers (33). The questionnaire includes 19 questions which subjectively evaluate sleep. Sleep is then scored for the following categories: sleep quality (individual sleep quality rating), sleep latency (length of time it takes to fully fall asleep), sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medication and daytime dysfunction such as having trouble staying awake while driving. Participants are required to complete the PSQI Questionnaire retrospectively by considering and
recalling their sleep in the past month (33). By adding each single score, the overall score is determined if greater than five the respondent is categorized as being a poor sleeper (33). The PSQI Questionnaire is recognised as a convenient and practical method of assessing sleep quality (34).

Although the PSQI Questionnaire has been suggested as a valid tool for sleep quality assessment, it has poor correlation with the sleep quality index as derived by PSG or by actigraphy. For example, in a sample of 112 adults (53 young adults with a mean age of 23 years and 59 older adults with a mean age of 53 years), Grandner et al (35) showed a poor correlation between PSQI score and actigraphy derived sleep variables. The poor agreement between PSQI and PSG is likely due to the retrospective nature of the PSQI Questionnaire (33). It is also possible that the average of multi-night recordings of PSG, taken over the same period of time as PSQI, might improve the relationship between the two measures (35).

1.4.2 Berlin Questionnaire

The Berlin Questionnaire was designed to identify individuals at risk for OSA (36). The questionnaire has ten questions organised into three categories: snoring, sleepiness/tiredness and obesity/hypertension (see Section 2.8.2.2). Each category is evaluated and scored individually. If at least two of the categories are positive, an individual is considered to be high risk for OSA (36). The Berlin Questionnaire has been validated in adult populations (37, 38).

Previous studies compared the agreement rate between the Berlin Questionnaire score and AHI using PSG. Comparing Berlin scores to mild OSA measured by PSG, the questionnaire was found to have 83% sensitivity and 22% specificity (39). However, when different AHI cut-offs were considered, the questionnaire was found to be limited for mild and moderate OSA (39). Thus it has been suggested that the Berlin Questionnaire may be most effective for predicting severe OSA rather than mild or moderate OSA (40). Despite this, previous studies have recommended the Berlin Questionnaire as a screening tool in the general population in view of its good sensitivity and specificitity. In a study of 240 adults with mean age of 48 years (50% females), the sensitivity and specificity of the Questionnaire were 76.9% and 77.6% to predict AHI $\geq 15$ (40).
1.5 Association Between Short Sleep Duration and Weight Gain

1.5.1 Cross-sectional and Longitudinal Studies in Children and Adolescents

A number of cross-sectional paediatric population studies (Table 1.1) have shown an inverse association between sleep duration and increased risk of obesity (41-51). All of the studies used age-adjusted thresholds of BMI for obesity but they used different cut-offs for average sleep. The cut-off used for average sleep varied by age group according to the sleep requirements of the age group. For example the average sleep duration selected for children aged 5 years and 5-6 years were 11 hours and 11.5 hours respectively. For children aged 7 and 9 years, it was 11 hours and 10 hours.

Although the definition of average sleep duration differed, the findings were similar. For example, Locard et al (41) assessed 1031 five-year olds and found the odds ratio for obesity was 1.4 for sleep duration less than 11 hours, compared to those with longer sleep duration. Von Kries et al (42) showed in a study of 68625 children aged 5-6 years, that the prevalence of obesity was 5.4% (95% CI: 4.1, 7.0) in those sleeping equal or less than 10 hours, while this rate was 2.1% (95% CI: 1.5, 2.9) in those sleeping equal or more than 11.5 hours. By using the wide range of confounders, the study concluded that the effects of sleep duration on obesity are not influenced by confounders. In a sample of 8274 children aged 6-7, Sekine et al (46) reported a 2.89 fold (95% CI: 1.61, 5.05) higher propensity for obesity for those sleeping less than 8 hours after adjusting for confounders including age, gender and parental obesity. Padez et al (47) reported a decreased risk of overweight (OR 0.44, 95% CI: 0.38, 0.49) and obesity (OR 0.39, 95% CI: 0.35, 0.42) with an increase in the number of hour of sleep in 4511 children aged 7-9 years. It is noteworthy that most of the studies used one parental question to estimate the sleep duration of the children and as such the estimated average sleep duration might not be accurate. Additionally, the parental sleep question varied among the studies; while some studies asked about sleep both during weekdays/weekend (42) (47),other studies collected sleep duration irrespective of weekdays/weekends (41). Lastly very few of these studies took into account important potential confounding factors such as physical activity and some did not adjust for age and gender.
A number of cross-sectional studies have evaluated the association between sleep duration and weight in adolescence. Two of these studies used either wrist actigraphy or hip accelerometer. By using wrist actigraphy in 383 adolescents, Gupta et al (48) reported that for every hour reduction in sleep duration, the odds of obesity increased by 5-fold. While other studies in adolescents reported an association between short sleep duration and obesity risk (49, 50), one study on 4486 American teens reported no association in girls but a significant association between short sleep duration and the incidence of being overweight in boys (51). Other adolescent studies used a single self-report question, which is a poor measure of sleep duration. The confounding effect of puberty, which may influence obesity risk was not evaluated in any of the adolescent studies (52).
Table 1.1. Cross-sectional Studies of Sleep Duration and Weight Gain in Children and Adolescents [modified (53)]

<table>
<thead>
<tr>
<th>First Author (Year)</th>
<th>Sample Size</th>
<th>Age range or Mean</th>
<th>Subjective Sleep Measure/Potential confounders</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locard (1992)⁴¹</td>
<td>1,031</td>
<td>5</td>
<td>One question to parent/age, ethnicity, breastfeeding, TV viewing, maternal age, socioeconomic, single parent, parental obesity</td>
<td>Obesity odds of 1.4 for sleep duration &lt; 11 hrs.</td>
</tr>
<tr>
<td>Von Kries (2002)⁴²</td>
<td>6,862</td>
<td>5-6</td>
<td>Parental questions/age, breastfeeding, diet, snacking, TV viewing, socioeconomic, single parent, PA</td>
<td>Obesity odds of 2.2 for sleep duration &lt; 10.5 hrs.</td>
</tr>
<tr>
<td>Chaput (2006)⁴³</td>
<td>422</td>
<td>5-10</td>
<td>One question to parent/ age, sex, TV viewing breastfeeding, PA, regular breakfast, parental obesity</td>
<td>Obesity odds of 3.4 for sleep duration ≤ 10 hrs.</td>
</tr>
<tr>
<td>Ben Slama (2002)⁴⁴</td>
<td>167</td>
<td>6-10</td>
<td>One question to parent/ None</td>
<td>Positive association between short sleep duration and obesity risk (58% obese participants had sleep duration less than 8 hours).</td>
</tr>
<tr>
<td>Giugliano (2004)⁴⁵</td>
<td>165</td>
<td>6-10</td>
<td>One question to parent/None</td>
<td>Obese children had 30 minutes shorter sleep compared to normal-weight children.</td>
</tr>
<tr>
<td>Sekine (2002)⁴⁶</td>
<td>8274</td>
<td>6-7</td>
<td>One question to parent/age, sex, TV viewing, PA</td>
<td>Obesity ORs, 1.49 and 1.89 for sleep durations 9-10 hrs and 8-9 hrs respectively.</td>
</tr>
<tr>
<td>Padez (2005)⁴⁷</td>
<td>4,511</td>
<td>7-9</td>
<td>Parental questions/age, sex</td>
<td>Positive association between short sleep duration and obesity risk.</td>
</tr>
<tr>
<td>First Author (Year)</td>
<td>Sample Size</td>
<td>Age range or Mean</td>
<td>Subjective Sleep Measure/Potential confounders</td>
<td>Findings</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------</td>
<td>-------------------</td>
<td>------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Chen (2006)</td>
<td>656</td>
<td>13-18</td>
<td>One question to child/age, sex</td>
<td>Inverse association between obesity risk and having sleep duration of at least 6-8 hours.</td>
</tr>
<tr>
<td>Knutson (2005)</td>
<td>4,486</td>
<td>17</td>
<td>One question to child/age, sex, ethnicity, PA, socioeconomic</td>
<td>Association between short sleep duration and higher BMI z-score and overweight risk in boys only.</td>
</tr>
<tr>
<td>Gupta (2002)</td>
<td>383</td>
<td>11-16</td>
<td>Actigraphy (24 hours)/ age, sex, puberty status</td>
<td>5-fold increase in obesity OR for every hour reduction in sleep duration.</td>
</tr>
<tr>
<td>Benefice Senegal (2004)</td>
<td>40</td>
<td>13-14</td>
<td>Accelometer (72-96 hours)/ age, sex, PA, height</td>
<td>Inverse association between short sleep duration and BMI.</td>
</tr>
</tbody>
</table>

PA: Physical Activity
Consistent with cross-sectional studies, longitudinal studies have also found an inverse association between sleep duration and obesity in children (54-57). Taveras et al (54) found that at age 3, the odds of being overweight were 2.04 (95%CI: 1.07, 3.91) in those sleeping less than 12 hours during infancy. Their study adjusted for a wide range of infant confounders (birth weight, breast feeding duration and daily physical activity) and maternal confounders (education, income, pre pregnancy BMI). However, a major limitation of the study was that sleep duration was estimated using a maternal question (asking about infant’s sleep duration during the last month) instead of using objective method of sleep measurement or diaries (54). In a study of 1441 children aged 3-12, Snell et al found that the association between sleep and overweight was moderated by age (55). According to this study, at 3-8 years, an additional hour of sleep was associated with reduced probability of being overweight ($\beta = - 0.061, P < 0.01$) but this association did not continue in older children (55). The use of parental reports and lack of adjustment for confounders are both limitations of most of the previous longitudinal studies undertaken in children and adolescents and some did not assess weight at the time of sleep measurement (56, 57). As growth rate is not constant during childhood, it is important to measure weight at the time of sleep assessment in order to have a better estimation of the role of sleep on body weight.

1.5.2 Cross-sectional and Longitudinal Studies in Adults

In contrast to the link observed between sleep and obesity in children, the results of cross-sectional studies of sleep and weight gain in adults are varied (Table 1.2). Studies that have used questionnaires to ascertain sleep have reported a positive association between short sleep duration and weight gain, a U-shaped relationship or no association.

A limitation of all of these large studies is that they were designed to evaluate the effects of different behaviours on health outcome and not to only assess sleep. Importantly, while these studies adjusted for gender, the effects of other important sleep confounders were generally ignored. Lastly, these studies have used different cut offs to define sleep duration categories.

One of the largest cross-sectional studies was conducted by the American Cancer Society (58). The study included over 1 million adults with cancer, ranging in age from 33-102 years. They found that women had a BMI that was 1.39 kg/m$^2$ higher if they had 4 hours of sleep compared to 7 hours (58). In men the BMI was 0.57 kg/m$^2$ higher if
they had 4 hours of sleep compared to 7 hours (58). Similarly, in a study of 13742 adults aged ≥ 20 years, short sleepers (≤ 6 hours) were more likely to be obese (OR 1.10, 95%CI: 1.03, 1.16) compared to normal/long sleepers (≥ 7 hours) after adjusting for a wide range of confounders (including age, gender, employment, smoking and alcohol use) (59). In contrast, in a study of 104,000 adults aged 40-79 years, Tamakoshi et al (60) reported a significant association between less sleep duration and reduced BMI. For those with a sleep duration of ≤ 4, 5 and 7 hours the mean BMI were 22.2, 22.6 and 22.7 kg/m² respectively (60). This is the only study reporting an association between short sleep duration and reduced BMI.

There are several reports of a U-shaped association between sleep duration and BMI in adults. The FIN-D2D Study of 2770 adults aged 45-74 reported higher prevalence of obesity in men with either short sleep duration (≤ 6 hours) or long sleep duration (≥ 8 hours) (61). Similarly, Liu et al found a higher rate of obesity (OR 1.32, 95%CI: 1.21, 1.43) in short sleeper (≤ 6 hours) and those sleeping more than ten hours (OR 1.60, 95%CI: 1.34, 1.93) (62). Interestingly, in a study of 1486 adults with mean age of 70 years, Gottlieb et al (63) showed no association between sleep duration and BMI. In a study of 191 women aged 80-92 years using actigraphy for sleep estimation, no association was found between sleep duration and BMI (64)
Table 1.2. Cross-sectional Studies of Associations Between Sleep Duration and Weight in Adults [modified (65)]

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Sample Size</th>
<th>Age range or mean</th>
<th>Sleep measure</th>
<th>Association between sleep duration and BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kripke et al (2002) (58)</td>
<td>1,116,936</td>
<td>33-102</td>
<td>One sleep question</td>
<td>Inverse association</td>
</tr>
<tr>
<td>Ford et al (2014) (59)</td>
<td>13742</td>
<td>≥ 20</td>
<td>One sleep question</td>
<td>Inverse association</td>
</tr>
<tr>
<td>Gottlieb et al (2005) (63)</td>
<td>1486</td>
<td>53-93</td>
<td>One sleep question</td>
<td>No association</td>
</tr>
<tr>
<td>Chaput et al (2007) (66)</td>
<td>90</td>
<td>50-70</td>
<td>One sleep question</td>
<td>No association</td>
</tr>
<tr>
<td>Tuomilehto et al (2008) (61)</td>
<td>2770</td>
<td>45-74</td>
<td>One sleep question</td>
<td>U-shaped association</td>
</tr>
<tr>
<td>Kim et al (2015) (64)</td>
<td>669</td>
<td>35-49</td>
<td>5 days actigraphy</td>
<td>No</td>
</tr>
</tbody>
</table>

The results of longitudinal adult studies have also provided inconsistent data, with reports of negative associations, no associations or U-shaped associations between sleep duration and weight gain. According to the Nurses’ Health Study of 68,183 women, the relative risk of a 1.5 kg weight gain over 16 years in those sleeping ≤ 5 hours and 6 hours was 1.28 (95%CI: 1.15, 1.42) and 1.10 (95%CI: 1.04, 1.17) respectively (67). In another longitudinal study of 3803 male adults aged 40-59 years, a significant increase in BMI was reported in those sleeping less than 5 hours (β = 0.015 kg/m²) over 4 years (68).

There have also been reports of longitudinal studies showing no association between sleep duration and BMI (69, 70). In NHANES I, 3,208 adults aged 32-49 years showed no significant association (β = 0.053, P = 0.27) after 10 years of follow-up (69). The only longitudinal study that has used actigraphy to monitor sleep included 612 adults aged 33-45 years and reported no association between sleep and BMI after 5 years (70).

Similar to the findings in cross-sectional studies, some longitudinal studies have shown a U–shaped association between sleep duration and BMI (71) and the association varies...
by sex and age (72, 73). The Quebec family study of 276 adults aged 21-64 years over 6 years showed those who slept < 6 hours or > 9 hours gained 1.84 and 1.49 kg more weight respectively, however other studies with U-shaped associations found this association to be evident only in females (72) or those aged < 40 (73).

To summarize, there is no consistent evidence of an association between short sleep duration and weight gain in adults. This may be due, in part, to lack of a standard definition for short and long sleepers. Previous research studies have also used a single self-reported question or sleep questionnaire to assess sleep duration estimation instead of using actigraphy over several days. Another reason may be related to differences in study populations. For example, some studies recruited participants from the general population, whilst others recruited nurses or cancer survivors to evaluate the association between sleep and obesity. Other potentially important confounders including racial/ethnic influences which are often not taken into consideration.

1.5.3 Physiological Basis for an Association Between Sleep Restriction and Obesity

Several mechanisms could underlie a possible association between sleep restriction, body weight and obesity. Experimental physiological studies suggest the changes in appetite-regulating hormones (leptin and ghrelin), sympathetic nervous activity and energy intake that occur as a result of sleep restriction may induce obesity (74).

Leptin and ghrelin are appetite-regulating hormones which can be influenced by sleep restriction (75). Leptin is secreted by adipose tissue and leptin levels increase following a meal and during the night. Ghrelin is produced in the stomach. Both hormones exert their effects in the arcuate nucleus of the hypothalamus through propiomelanocortin/cocaine and amphetamine–regulated transcript (POMC/CART) and Neuropeptide Y/agouti-related protein (NPY/AgRP) neurons. Leptin decreases hunger and induces satiety (75). In contrast, ghrelin stimulates appetite, prolonging postprandial glucose responses while stimulating growth hormone (GH) release.

Experimental sleep studies have shown changes in these hormones following sleep restriction (75, 76). Following a short period of sleep restriction (2 consecutive nights of 4 hours in bed) in 12 healthy young men, leptin decreased by 18% while ghrelin increased by 28% with an increase in appetite with a preference for salty or high carbohydrate foods (74). Another study of eight young adult males undergoing sleep
restriction of 4 hours sleep per night for seven days showed a reduction in leptin level by 33% after sleep restriction (76). In contrast, other studies have shown no change in leptin and ghrelin levels (77, 78). A study of 11 healthy young adults (six males with a mean age of 39 ± 5 years) that completed two 14-day periods (with three months interval) in sleep laboratory found no change in leptin or ghrelin levels following sleep restriction (5.5 hours) period compared to normal sleep duration (8.5 hours) period (77). Additionally, no changes were found in the levels of leptin and ghrelin in a sample of 17 healthy young adults following sleep restriction of two-thirds of normal sleep time for 8 nights (78). All of these studies are limited by a small sample size and the inclusion of a young healthy adult population. As the body metabolism varies through different stages of life, it is unclear if similar associations exist in other age groups. Another possible factor is differences in the duration of sleep restriction (short period of sleep restriction/long period of sleep restriction), as it has an impact on leptin and ghrelin levels and weight gain (77).

Another possible mechanism linking sleep restriction to weight gain is through the sympathetic nervous activity (79). The sympathetic system produces epinephrine and norepinephrine. During sleep, vagal tone increases and sympathetic nervous activity decreases. Experimental sleep studies show an increase in urinary epinephrine and norepinephrine indicating increased sympathetic activity following a 24-h period of continuous wakefulness in 14 adult males (79).

Sleep restriction can also potentially contribute to weight gain by influencing energy intake and/or food preference. Previous studies have shown an increase in energy intake following experimental sleep restriction from 180 to 559 kcal/d (78, 80-82). A similar association between habitual sleep duration and energy intake has been reported in observational studies (83). The results of the NANES Study of 5587 adults with a mean age of 46 years showed the highest energy intake (2201 kcal) in short sleepers (5-6 hours) with higher intake of sugars and total fat compared to normal sleepers (all P < 0.005)(83). Grander et al reported an inverse association between actigraphy sleep duration and total energy intake and fat intake in 459 post-menopausal women monitoring their sleep duration using one week of actigraphy (84). Overall, if the observed dietary changes, particularly an increase in total energy and fat intake, are sustained over long-term and are not accompanied by an equivalent increase in energy expenditure, it could explain the association between short sleep duration and obesity.
1.6 Sleep Duration and Glucose Metabolism

1.6.1 Sleep Duration and Glucose Metabolism

During normal sleep, physiological changes occur in glucose metabolism in order to maintain glucose level at the same level as wakefulness. A number of studies have reported changes in sleep duration can alter glucose and insulin levels which may lead to increased risk of diabetes (76, 77).

Blood glucose levels must be tightly regulated in the human body to prevent hypoglycaemia and hyperglycaemia. During nocturnal sleep and overnight fasting, a number of mechanisms keep glucose levels stable. During the first half of the sleep period, glucose metabolism slows, as cerebral glucose uptake rates decrease by 40% and peripheral glucose utilization rates decrease (85, 86). This reduction in glucose metabolism during sleep occurs in brain regions such as the frontal, temporal and occipital cortex and the anterior/dorsomedial thalamus (87).

Two hormones that regulate glucose during sleep are growth hormone and cortisol. Growth hormone levels begin to rise at the onset of sleep and peak during slow wave sleep (88). In contrast, cortisol levels rise during the second half of the sleep, predominantly in REM sleep.

During the NREM phase of sleep, a reduction in brain glucose metabolism, reduced muscle tone and anti-insulin effects of growth hormone are responsible for a drop in glucose utilisation (75). During REM sleep, glucose utilization increases although it is lower compared to the wakeful state.

1.6.2 Sleep Duration, Glucose Dysregulation and Risk of Diabetes

While a large number of studies have assessed the impact of sleep on glucose regulation and metabolic dysregulation, the focus of these studies has shifted recently. With the increase in the prevalence of obesity, studies have shifted towards examining the impact of sleep duration, particularly the impact of sleep restriction on metabolic regulation (12). The results of experimental, cross-sectional and longitudinal studies suggest that sleep restriction plays a role in altering glucose metabolism (89-91).
Most of the recent experimental studies are focused on changes in glucose metabolism in response to acute sleep restriction (92, 93). Generally these studies have shown increased insulin levels and insulin resistance (lowered levels of response to insulin in the body tissue) following acute sleep restriction (89, 92, 93). A small intervention trial of 7 healthy adults (6 males, 1 female) with a mean age of 23 years showed sleep restriction of four hours of sleep per night for four nights led to insulin resistance and increased insulin concentration (measured by an intravenous glucose tolerance test and cellular insulin sensitivity index in adipocyte subcutaneous fat biopsy) of up to 300% (92). Another study of 21 healthy male adolescents with a mean age of 16 years showed sleep restriction of four hours of sleep per night for three nights led to 59% increase in fasting insulin without any changes in glucose (94).

Although the majority of these acute sleep restriction studies reported changes in insulin sensitivity, these changes might be due to a physiologic response to acute intensive temporary sleep restriction (four hours of sleep for 3-4 nights). Therefore further sleep studies of chronic sleep loss that are more clinically relevant are required. Furthermore, the majority of these studies have included only young male participants that is another potential limitation of previous studies.

A number of cross-sectional and longitudinal studies have evaluated the association between sleep duration and risk of diabetes. A study of 740 adults aged 21-64 years who undertook 75 g Oral Glucose Tolerance Test (OGTT) found a 2-fold increased odds ratio for diabetes in those who slept 5-6 hours, after adjusting for potential confounders including BMI, waist circumference and physical activity (95). The NHANES longitudinal study of 8,992 adults aged 32-86 years showed those adults who only slept five hours a night had a 1.4 greater odds ratio (95%CI: 1.03, 2.09) for diabetes (estimated by self-reported or physician-diagnosed diabetes) over a 10 year follow-up period (96). This study also reported that 9 hours or more of sleep increased the odds ratio to 1.5 (95%CI: 1.06, 2.18)(96). Similarly, a cohort study of 70,000 nurses indicated an association between both short (≤ 5 hours) and long (≥ 9 hours) sleep duration with type 2 diabetes. The relative risk of type 2 diabetes (assessed through self-questionnaire) was 1.34 (95%CI: 1.04, 1.72) for short sleepers and 1.35 for long sleepers (95%CI: 1.04, 1.75) (97). According to the Massachusetts Male Aging Study (MMAS) which monitored men with no history of diabetes for 15 years, the risk of diabetes (assessed through self-questionnaire) increased 2-fold (95%CI, 1.05, 3.48) in those sleeping less than 6 hours while this risk increased 3-fold in those sleeping more
than 8 hours (95%CI: 1.83, 7.46) (98). A Swedish study following 2,000 people for over 10 years showed a positive association between short duration of sleep (< 5 hours) and higher incidence of diabetes in men (99). All of these studies used a sleep question for assessing sleep. Additionally, study participants limited to overweight participants (95) or females only (97). The confounding effects of abdominal obesity is not considered in some of these studies (97).

There are several meta-analyses evaluating the association between short sleep duration and diabetes (100). The meta-analysis of Cappuccio et al (100) was based on seven studies and showed a relative risk of 1.28 in short sleepers with larger effect in male short sleepers. In contrast, the meta-analysis based of Holliday et al (101) including 10 studies showed an increased risk of diabetes in short sleepers irrespective of gender.

1.7 Sleep Duration and Other Cardiometabolic Risk Factors

Although most studies have investigated the role of sleep duration on obesity and glucose metabolism, some studies have also evaluated the role of sleep duration on other cardiovascular factors such as hypertension, dyslipidaemia and C-Reactive Protein (CRP).

Studies examining short sleep duration and hypertension suggest the two are linked. A study of 3,068 men and women aged 50 years evaluated sleep duration by self-report questionnaire over four years showed that increased risk of incident hypertension in those having short sleep duration (≤ 5 hours) (102). The participants had no previous history of hypertension and were not taking any antihypertensive medication. The risk of hypertension was 1.73 (95%CI: 1.08, 2.76) in men and 1.44 (95%CI: 1.00, 2.07) in women after adjusting for potential confounders including age, BMI, physical activity and smoking (102). The US National Health Interview Survey (NHIS) of 56,507 adults aged 18-85 years showed both short (< 7 hours) and long sleep (> 8 hours) associated with increased risk of hypertension (103). In a study of 578 adults aged 33-45 years, Knutson et al (104) reported the odds of hypertension increased by 1.37 (95%CI: 1.05, 1.75) in those with shorter sleep duration after adjusting for age, race and sex confounders. This is the only study that has evaluated the association between sleep duration and hypertension in the later period of young adulthood to middle age. All of these studies are limited as sleep duration was assessed using self-reported responses. Furthermore, sleep-disordered breathing was not assessed in these studies. As sleep-disordered breathing might contribute to hypertension better than sleep duration, it is
necessary to consider the confounding role of sleep-disordered breathing on the association between sleep duration and hypertension.

It has been suggested that increased levels of CRP, IL-6 and TNF-α are important risk factors for atherosclerosis, stroke and cardiovascular diseases. CRP a 120 kDa pentamer is an important inflammatory marker predominately synthetised in the liver (105). CRP has a significant role in the pathogenesis of atherosclerosis and a moderate elevation of CRP in healthy adults is a strong predictor of cardiovascular risk (105). The studies that have evaluated sleep duration and CRP have shown inconsistent results. The Wisconsin Study of 907 males with a mean age of 52 years showed no association between sleep duration and CRP levels after adjustment for potential confounders including age, sex and BMI (106). In contrast, a study of 4,642 adults with a mean age of 50 years by Miller et al (107) found a U shape association between sleep duration and CRP levels in women only. Women with sleep duration < 5 hours or sleep duration > 9 hours had higher CRP levels compared to those sleeping 7 hours (107).

Dyslipidaemia is an increase in total cholesterol or triglyceride levels with or without a reduction in high density lipoprotein cholesterol (HDL-C) levels (108). Very limited studies have evaluated the association between sleep duration and dyslipidaemia with inconsistent results (109-111). One of these studies was the large epidemiological Korean National Health and Nutrition Examination Survey (109). This study of 13,609 adults aged 20 and over that adjusted for covariates including age and sex found no association between short sleep duration (≤ 5 hours) and high low density lipoprotein cholesterol (LDL-C) or high triglycerides (109). However, a significant association was found between long sleep duration (≥ 9 hours) and low HDL-C. In a study of 1600 women and 2,300 men aged 20 and over that adjusted for a wide range of confounders, both short (< 5 hours) and long (> 8 hours) sleep was associated with both high triglyceride and low HDL-C levels in women. In men, a link between long sleep duration and high LDL-C was the only observed association (110).

To summarize, the results of previous studies suggest the role of sleep duration on cardiometabolic risk factors is inconsistent. It is noteworthy that the majority of these studies have been carried out in middle-aged individuals and have used self-reported sleep duration questionnaires instead of an objective sleep measure. Residual confounding due to unmeasured variables may also contribute to the inconsistent findings of previous studies.
1.8 Sleep Quality and Cardiometabolic Risk Factors

The majority of previous studies have analyzed sleep by duration and its effect on cardiometabolic risk factors. However, as sleep has both quantitative and qualitative aspects, to better understand the link between sleep and cardiometabolic risk factors the impact of both quantity and quality of sleep needs to be investigated (112). In this regard, a number of studies have evaluated the impact of sleep quality on cardiometabolic risk factors particularly on body weight and hypertension (110-119). However, according to our current knowledge there is little epidemiological data on sleep quality and other cardiometabolic risk factors.

Studies examining the association between sleep quality and increased body weight show varied results (112-114). The BiDirect Study of 753 adults aged 35-65 years found an association between poor sleep quality (assessed by PSQI Questionnaire), obesity and fat mass after adjustment for sociodemographic and lifestyle factors (115). Rahe et al (115) also showed an association between poor sleep quality and general obesity and fat mass after adjustment for sociodemographic and lifestyle factors including education, job status, smoking and physical activity. However, further adjustment for depression and comorbidities (including hypertension, diabetes and myocardial infarction) attenuated the association (115). Despite the association between poor sleep quality and obesity, the results of other studies suggest there is no relationship between sleep quality and other cardiometabolic risk factors (10, 116, 118).

A study of 927 women of reproductive age (16-40 years) found no relationship between poor sleep quality (assessed by PSQI Questionnaire) and BMI after adjusting for age, race and depressive symptoms (117). Another study of 660 adults aged over 90 years found 22% of adults had poor sleep quality (119). Using the World Health Organization (WHO) recommendation for Asian populations and classifying participants into underweight (BMI < 18.5), normal weight (18.5 ≤ BMI < 23.0), overweight (23.0 ≤ BMI ≤ 27.5) and obese (BMI > 27.5 kg/m²), this study found no significant difference among BMI categories and prevalence of poor sleep quality (using PSQI Questionnaire) after adjusting for age, gender and lifestyle factors including smoking, alcohol drinking and exercise habits (119). The discrepancies between studies may be due to differences in study populations including age, limitation of criteria used to define poor sleep quality.
quality (PSQI Questionnaire instead of using objective method of sleep quality assessment) or use of different covariates.

Most of the studies have investigated the role of sleep quality on hypertension. In a study of 133 adults with a mean age of 43 years, Erden et al (120) showed reducing blood pressure at night was inversely correlated with sleep quality. Moreover, previous studies reported an association between poor sleep quality and hypertension (121, 122). In a study of 250 normal weight adults, a significant difference in the prevalence of hypertension among poor and good sleep quality groups (87.1% vs 35.1%) was found (121). Similarly, a study of 9,404 adults with a mean age of 52 years showed a 2.3-fold greater risk of hypertension in those with poor sleep quality (122).

1.9 Sleep-disordered Breathing and Cardiometabolic Risk Factors in Adults

In addition to sleep duration and quality, sleep disorders such as Sleep-Disordered Breathing (SDB) are also thought to impact on cardiometabolic health (123). It is important to note that the causes of sleep-disordered breathing in children are different compared to adults. Adenotonsillar hypertrophy and anatomical or neuromuscular factors play a significant role in SDB in children in contrast to adults (124). Neurocognitive dysfunction and abnormal behaviours are the most common and important complications of SDB, meanwhile metabolic complications of SDB are less common in SDB children compared to adults (125). Therefore, the focus of the current section is restricted to associations between SDB and cardiometabolic risk factors in adults.

1.9.1 Sleep-disordered Breathing in the General Population

SDB refers to respiratory disorders characterized by abnormalities in respiration during sleep (126). This comprises a wide spectrum of sleep-related breathing disorders ranging from the mild form of snoring to the most severe form of obstructive sleep apnoea (OSA) where upper airway respiratory collapse results in decreased oxygen saturation in the blood (126, 127). The Apnoea Hypopnea Index (AHI, see Section 1.3) is used to indicate the severity of obstruction. These repetitive hypoxic events result in
arousal from sleep, sleep fragmentation and cause excessive daytime sleepiness and fatigue.

SDB has been recognised as a common problem in the general population, with a prevalence of 56% in the United States, 31% in Western Europe and 23% in Japan (128). A study of 741 adult men suggests the prevalence of SDB increases with age from 3.2% in participants aged 20-44 to 18.1% in those aged >61 years (129).

By using the minimal diagnostic criteria for the sleep apnoea syndrome (an apnoea-hypopnea score of 5 or higher and daytime hypersonmolence), 4% of middle-aged work force males and 2% of females were estimated to have sleep apnoea in the developed world in 1993 (130). Recent data have shown the prevalence rate over the following two decades has increased to 23% in females and 50% in males in the middle-aged to elderly (131). In males and females aged 30-49 years, the prevalence of OSA is estimated at 10% and 3% respectively (132). Although OSA is known to be the disorder of middle and old-age groups, recent studies suggest OSA is also evident in young populations (133, 134). A study of 916 Chilean college students reported that approximately 8% had OSA (135). The prevalence of OSA in reproductive aged women is 0.6-15%, with most undiagnosed (136). A study of a Chinese population with mean age of 20 years reported OSA at a rate of 13% (137). In an Australian longitudinal cohort study on 13423 adult men (aged 10-55 years), the prevalence of self-reported professional-diagnosed OSA was 2.2% in those aged 18-25 years (138).

1.9.2 Sleep-disordered Breathing and Obesity

Obesity is an important risk factor for OSA (139). Obesity induces fat deposits in upper airway muscles and lumen and reduces tracheal traction contributing to air flow obstruction and subsequent OSA (139). While most of the previous studies suggest a one-way cause and effect relationship, with obesity contributing to the pathogenesis of OSA, current evidence indicates that OSA itself can contribute to or exacerbate obesity, thus creating a bidirectional link between OSA and adiposity (139, 140;Wosu, 2014 #175)

There are two main mechanisms responsible for increased body weight in OSA. One of these is sleep restriction (139). In OSA, disturbed respiratory gas exchange due to the upper airway collapse contributes to sleep fragmentation and eventually sleep restriction. This results in daytime tiredness and reduced physical activity leading to
weight gain (Figure 1.2). The other mechanism responsible for obesity in OSA is variation in appetite regulatory hormones including leptin and ghrelin (139). Previous studies have shown increased leptin levels in OSA participants, but others have reported a reduction in leptin and increase in ghrelin levels (141-143). The changes in leptin and ghrelin contribute to an increase in appetite and caloric intake and exacerbate obesity in OSA participants (139). As oxidative stress and Reactive Oxygen Species (ROS) can change metabolism in adipose tissue, they also can contribute to obesity in OSA (144).

![Figure 1.2. Cycle of obesity and OSA and its potential mechanisms](image)

**Figure 1.2. Cycle of obesity and OSA and its potential mechanisms**

UAW, Upper Air Way (139).

A number of studies have shown a positive association between obesity and OSA (139). The Wisconsin Sleep Cohort Study of 690 US adults (mean age of 46 years, 56% males) followed up during 4 years showed weight gain was associated with increased prevalence of OSA, with a 10% weight gain as the severity of OSA increased to 32% (95%CI, 20, 45) after adjustment for sex, baseline age and BMI and smoking habit(145). As 27% of the participants in this study had incomplete follow-up, the results can be due to participant bias. Furthermore, the average weight change was within ± 20%, thus the effect of large weight change in OSA cannot be determined. Lastly as the participants were initially overweight, the results of the study cannot be generalised to normal-weight participants. In a study of 704 participants attending primary health care clinic (aged 14 to 81 years, 58% males) Mahboub et al (146) showed 70% of those at high risk of OSA were obese. Additionally, a cross-sectional study of 916 Chilean college students showed a 9.9-fold (95%CI: 4.42, 22.45) higher risk of obesity in those with OSA (135). A major limitation of the latter study is it used
the Berlin questionnaire instead of performing an overnight sleep study to assess OSA. An Australian longitudinal cohort study of 13423 adult men (aged 10-55 years) also found a significant association between OSA and higher BMI; the risk of obesity and overweight was 3.6-fold and 1.7-fold higher respectively, in OSA participants (138). A recent meta-analysis study of 2966 participants from 19 studies showed that weight and BMI has no correlation with OSA after checking for publication bias (by removing small studies)(147).

Continuous Positive Airway Pressure (CPAP) is the standard treatment for OSA. Therefore, it theoretically could reduce BMI (148). This is supported according to the results of two non-randomised studies (149, 150). In a study of 31 obese participants (29 males, mean age of 52.2 ±3.3 years), visceral fat accumulation and subcutaneous fat accumulation decreased significantly following six months treatment with CPAP without any change in body weight, fasting insulin and cortisol levels(149). Another retrospective cohort study of 32 overweight/obese participants (70% males, mean age of 59 years) showed a facilitating role of CPAP on weight loss (BMI change = −1.6 ± 0.7, P < 0.05) in the CPAP group. (150). However, both of these studies have limitations including a non-randomised design and small sample size. Additionally, it is unclear whether CPAP can contribute to weight loss in severe obese or extreme obese participants. Interestingly, these findings were not replicated by more recent studies (151-153). The results of a retrospective study of 228 middle-aged adults (64% males) showed the BMI of the CPAP and control groups did not differ following one year of CPAP treatment (P = 0.32) (151). The limitations of the study were small sample size and its retrospective design. In a study of 20 obese males with a mean age of 60, Garcia et al(153) showed an increase in body weight (109.6 ± 5.4 0.04 vs. 108 ± 5.3 kg, P = 0.04), BMI (37.1 ± 1.80 vs. 36.5 ± 1.8, P = 0.06) and HOMA-IR index (5.9 ± 1 vs 7.5 ± 1.2, P = 0.04) following CPAP treatment. However, the study was initially designed to evaluate the effect of CPAP on insulin and not BMI. The study is also limited in comparing the BMI changes in the CPAP group and the non-interventional control group due to the lack of a control group. In a recent meta-analysis of 25 randomised trials of 3181 middle-aged participants (83.9% males) using CPAP at least for a month (median three months), there was an increase in BMI with CPAP treatment group (154). The calculated BMI post-intervention minus pre-intervention in the control group was −0.018 ± 0.243 kg/m² and 0.134 ± 0.27 kg/m² in CPAP group (154). The increased BMI
in the CPAP treatment group was not influenced by age, gender, baseline BMI, OSA severity and CPAP compliance.

In summary, the results of previous studies were inconsistent regarding the role of CPAP on body weight. This is due to the study design (retrospective), variation in CPAP therapy duration or the lack of having control group.

1.9.3 Sleep-disordered Breathing and Glucose Metabolism

A number of studies have shown the presence and/or severity of OSA is associated with glucose metabolism and increased risk of diabetes or poor diabetic control (155, 156). Studies of snoring and impaired glucose tolerance suggest the two are linked (157, 158). The Nurses' Health Study cohort of 69,852 healthy female nurses aged 40-65 years with a history of snoring, found the risk of diabetes was increased 2.02-fold (95%CI: 1.91, 2.66) over a 10 year follow up following adjustment for age and BMI (157). There are several limitations of this study including the fact it only included females and it did not account for the confounding role of abdominal obesity in the analyses. Similarly, Elmasry et al in a study of 2504 men aged 30-69 showed that 5.4 % of snorers developed diabetes over 10 years compared to 2.4% of those without snoring (P < 0.001) (158). Limitations of this study were that information regarding snoring and diabetes was obtained through self- questionnaires and the study did not account for the confounding role of abdominal obesity. As abdominal obesity is a component of the metabolic syndrome and plays a vital role in the pathogenesis of insulin resistance, it is important to consider the role of abdominal obesity when examining the associations between diabetes and OSA (159).

As obesity plays an important role in both SDB and glucose metabolism, it is important to consider obesity as a confounder when evaluating the association (155). The studies controlling for obesity also reported an association between snoring and risk of diabetes (155, 157, 158).

A number of studies have shown a positive association between OSA and diabetes (155, 156, 160). A study of 544 non-diabetic adults (85% males with a mean age of 60 years) showed an independent association between OSA and incident diabetes (hazard ratio per quartile 1.43; 95%CI, 1.10, 1.86) after adjusting for age, gender, baseline glucose level, BMI and waist circumference over a 2.7 year follow up (160). The Sleep Heart Health Study of 2,656 with a mean age of 68 years showed that the odds of glucose
intolerance (using oral glucose tolerance test) were 1.27 (95% CI, 0.98, 1.64) in moderate OSA and 1.46 (95% CI, 1.09, 1.97) in severe OSA (155). Marshal et al showed higher incidence of diabetes in 399 adults (aged 40-65 years) with moderate to severe OSA (OR 13.45, 95% CI: 1.59, 114.11) after adjustment for confounders (age, sex, BMI and BP) and within four years of follow-up (161). However, the results of these studies are limited by several factors. Firstly, the populations comprised mainly obese males. Secondly, the effect of residual confounding such as, for example, the role of family history of diabetes has not been considered in all of these studies.

It has been suggested that insulin resistance can be influenced by SDB (155, 156, 162). In this regard, a study of 270 non-diabetic subjects attending sleep clinic for OSA evaluation showed a higher incidence of insulin resistance in OSA participants, suggesting an independent role of OSA on insulin resistance (162, 163). A study of 400 non-diabetic Swedish women aged 20-70 also showed a relationship between OSA and insulin resistance suggesting OSA severity can influence insulin resistance (163). The study showed an association between AHI and increased fasting and 2-h insulin levels (95% CI: 0.14, 0.99 and 95% CI: 0.28, 6.47) respectively) after adjusting for confounders (163). The study used insulin sensitivity index derived from oral glucose tolerance test instead of using gold standard method of euglycaemic insulin clamp technique for insulin sensitivity evaluation.

Although the exact physiological mechanisms of the association between SDB and glucose metabolism remain unknown, previous studies has suggested the roles of sleep fragmentation, recurrent hypoxia, oxidative stress and sympathetic over activation (Figure 1.3) on insulin resistance (144, 163). Insulin sensitivity is reduced during hypoxia as a consequence of oxidative stress and cytokines release (164, 165). Additionally, sympathetic overactivation can increase glycogen breakdown and higher levels of gluconeogenesis. These changes contribute to impairment in glucose homeostasis, resulting in higher glucose levels (166).

Despite the relationship between SDB and glucose metabolism, treatment of OSA with CPAP has shown inconsistent results in improving insulin sensitivity and glucose metabolism. A study of 50 diabetic participants (60% males with a mean age of 61 years) showed a 0.4% reduction in HbA1c levels (95% CI: 20.7, 20.04, P < 0.05) after adjustment for age, sex and AHI following 6-months open-label CPAP intervention (167). Loachimescu et al showed a decreasing trend in the level of HbA1c only in
individuals with excellent CPAP compliance (using CPAP for \( \geq 90\% \) of nights and \( \geq 8 \) h per night) in a non-randomized cohort study of 928 overweight/obese participants (86% males, with mean age of 54 years) (168). Conversely, the results of a study of 298 type 2 diabetic patients (60% male, mean age of 60 years) randomised to CPAP treatment during a 6 months period showed no significant difference in HbA1c among the groups (169). The CPAP studies to date had several limitations including small sample size, non randomized design /open label designs and heterogeneity in patient selection (male predominance, lack of normal weight group, lack of control group). The possibility of the effects of residual confounders (including lack of control for family history of diabetes, abdominal/visceral obesity and change in physical activity and dietary pattern) remain in all of these studies.

![Obstructive Sleep Apnoea Diagram](image)

**Figure 1.3.** Obstructive sleep apnoea and cardiometabolic risk factors.
1.9.4 Sleep-disordered Breathing and Hypertension

A number of studies have investigated the relationship between hypertension and SDB, particularly OSA and reported an association between OSA and hypertension (170, 171).

Several mechanisms may be responsible for the relationship between hypertension in SDB, though hypoxia and sympathetic activation are considered as the main factors (128, 171-173). Generally blood pressure and heart rate fall by 25% or more during uninterrupted sleep, due to diminished sympathetic overflow and amplification of vagal tone (171). However, in patients with OSA, nocturnal dipping in blood pressure is absent or diminished due to hypoxia and sympathetic over activation (171). Gradually the cumulative effects of sympathetic over activation together with the release of reactive oxygen species and reduced levels of nitric oxide (NO), an endothelial molecule controlling vascular tone, induce constant daytime hypertension (171).

Large epidemiological studies have shown an association between OSA and hypertension based on measures of office blood pressure ≥ 140/90 or using antihypertensive medication (173, 174). The results of a cross-sectional study of 11911 participants (70% male, mean age of 52) suspected of having OSA underwent overnight sleep studies showed that OSA was independently associated with hypertension after adjustment for various confounders including age, sex, obesity and diabetes (173). Additionally the severity of OSA can positively influence the odds of hypertension (175). A study of 2677 adults aged 20-85 years referred to a sleep clinic with suspected OSA, showed that OSA associated with hypertension after adjusting for potential confounders including age, sex and BMI (175). This study also showed that the incidence of hypertension increased by OSA severity; 46% in moderate and 53% in severe OSA participants, suggesting for every unit increase in AHI (severity of OSA), the odds of hypertension increased by 1% (95%CI: 0.75, 1.5) (175). Similarly in a study of 1,741 participants (42% males, with mean age of 47 years), Bixler et al (129) showed a positive association between OSA and hypertension which was stronger as the severity of OSA increased. Another notable community based study was the Wisconsin Sleep Cohort Study that evaluated 1,060 participants aged 30-60 years and showed a linear increase in blood pressure with increasing AHI index after adjusting for age, sex and BMI (176). According to the study, the odds of hypertension for mild OSA was 1.42 (95%CI, 1.13, 1.78), meanwhile in moderate OSA and severe OSA these figures
were 2.03 (95%CI: 1.29, 3.17) and 2.89 (95%CI: 1.46, 5.64) respectively. The majority of these studies included middle-aged adults selected from sleep clinics and thus were not representative of general population. The effects of other confounders including abdominal obesity were not evaluated in these studies.

Despite the role of SDB in hypertension, there are studies that have suggested a role of age on this relationship. These studies have shown inconsistent results, with some showing the association in young and middle-aged while others reporting an association in the elderly. The Sleep Heart Health Study of 6,120 adults stratified into middle-aged (< 60 years) and older aged (≥ 60 years) showed a relationship between SDB and hypertension only in middle-aged adults (177). However, another longitudinal study of 2,148 adults aged 30-70 years, found no association after adjusting for age, sex, BMI and lifestyle factors including smoking and alcohol intake (178).

Despite the current evidence of an association between SDB and hypertension mainly in middle-aged and older participants, the relationship between SDB and hypertension has not completely established in other age groups.

1.9.5 Sleep-disordered Breathing and Inflammation

A number of studies have shown an association between OSA and inflammatory markers such as CRP. A cross-sectional study of 245 adults with a mean age of 48 years found increased levels of high sensitivity CRP (hs-CRP) in severe OSA participants (β = 0.53, P = 0.005) after adjusting for BMI and metabolic syndrome (179). In a case-control study of 76 middle-aged adults (mean age 52, mainly males), Guven et al (180) reported higher hs-CRP levels in participants with OSA. The study also showed a relationship between hs-CRP and AHI independent of obesity (180). In 1,835 middle-aged Korean adults those with moderate-to-severe OSA had 1.78 higher odds of being in the highest tertile of CRP after BMI adjustment (181). Despite the positive association between SDB and increased CRP levels, the results of other studies have shown no association between OSA and CRP levels (106, 182). In 231 habitual snorers who underwent PSG, the association between OSA severity (AHI) with hs-CRP was not significant (r = 0.10, P = 0.33) after adjusting for age and BMI (182). In Wisconsin Sleep study of 907 adults (50% males. Mean age of 52) Taheri et al (106) found no independent association between OSA and CRP, suggesting the positive association between OSA and CRP is driven by obesity.
Although the association between inflammation in OSA is not fully understood, hypoxia, oxidative stress and synthesis of reactive oxygen species (ROS) are in part responsible for the endothelial dysfunction that associates with OSA (183).

To summarize, the results of previous studies that have examined the association between OSA and CRP are conflicting. Limitations of those studies that have shown an association between OSA and CRP include small study sample size and lack of control for abdominal obesity.

1.9.6 Sleep-disordered Breathing and Dyslipidaemia

The role of SDB on dyslipidaemia has been investigated mainly in clinical studies and suggests a possible association between dyslipidaemia and OSA (184). In this regard, a study of 2,081 patients (638 women) undergoing nocturnal recording for clinical suspicion of OSA found higher triglyceride and lower HDL-C levels in OSA participants (185). A systematic review study based on 64 studies also showed a positive correlation between dyslipidaemia and OSA (186). Other studies have shown an improvement in dyslipidaemia with CPAP therapy (187) and a 6% increase in HDL-C levels after 6 months treatment with CPAP (187). A study of 6,440 females aged 65 and over with moderate to severe OSA showed similar findings (188). However, all randomised controlled trials have shown no improvements in dyslipidaemia with CPAP therapy (148, 189). A randomised study of 24 adults with a mean age of 46 years reported no significant changes in lipid levels after CPAP treatment for four months despite a significant reduction in carotid intima thickness (148). Another crossover study on 34 adults aged 49 years found no difference in lipid levels following CPAP treatment for six weeks (189).

Some studies have shown OSA associates with changes in lipoproteins (190, 191). For example, higher levels of oxidized LDL-C, which is more atherogenic, have been reported in patients with OSA (190). Furthermore, HDL-C from OSA participants is less able to prevent oxidation of LDL-C leading to HDL-C dysfunction in OSA patients (160, 161).

Overall, current data has suggested chronic Intermittent Hypoxia (CIH) is likely one of the main factors linking endothelia dysfunction with OSA (180).
1.10 Summary

The prevalence of overweight and obesity has increased dramatically over the past few decades, escalating the burden of cardiovascular disease. While an imbalance between food intake and physical activity is known to be the key factor in increasing weight gain, recent evidence suggests other factors, such as insufficient sleep may also be important in weight gain and its cardiometabolic complications (10). Insufficient sleep includes both sleeping for less than the recommended period or having poor sleep quality (10). Previously published reviews suggest there is a relationship between insufficient sleep, obesity and cardiometabolic risk factors; however, this relationship is not seen in all age groups. While this association has been shown to be stronger in children and adolescents, the association is inconsistent in other age groups.

To plan cardiometabolic prevention strategies to minimize the negative impact of obesity and its cardiometabolic consequences, it is important to clarify potential risk factors (other than physical inactivity and increased caloric intake) in different age groups including young adults. Few studies have explored the association between insufficient sleep, obesity and cardiometabolic risk factors in young adults, and rarely have they used objective methods of sleep measurement. Similarly, only a few studies have examined sleep-disordered breathing and cardiometabolic risk factors in a young adult population. In a study of 558 adolescents and young adults (aged 14-28), those with a high risk for OSA had higher BMI, waist circumference and neck circumference (P = 0.001), and were more likely to have features of the metabolic syndrome (38.9% vs 7.0%, P = 0.001) (137). Young adults access the health service less often, the under-representation of this age group in clinical setting contributes to under-representation of this age group in clinical research studies (192, 193). Moreover, in non-clinical research settings, random sampling is rarely used in this age group, thus the validity and generalizability of the findings are limited (194).

1.11 The Western Australia Pregnancy Cohort (Raine) Study

The Western Australian Pregnancy Cohort (Raine) Study (see further details in the Methods Chapter) commenced in 1989-1992 (195). This unique longitudinal study has gathered an extensive range of general health, clinical, biochemical, behavioural and genetic data of the parents during pregnancy, and the offspring at birth and at ages
1,2,3,5,14,17,18,20 and 22 years. Some of the key cardiometabolic findings from the Raine study are summarised below.

Based on the results of cohort at age 8, overweight and obese children had adverse cardiometabolic risk factors such as lower HDL-C and higher triglycerides in addition to higher blood pressure compared to their peers (196). BMI at age 8 was predicted positively by birth weight, maternal BMI and paternal BMI (196). There was a U- shape association between birth weight and a high-risk cardiometabolic cluster that included higher body mass index, high blood pressure, an adverse lipid profile and high serum glucose levels (197).

At 14 years of age, a two-step cluster analysis was used to derive a distinct high-risk group with features consistent with the metabolic syndrome (197). The results showed 29% of participants were classified as being in the high-risk cardiometabolic cluster. Compared to those in the low-risk cluster, these individuals had higher BMI, (95% CI: 24.5, 25.4 vs 19.5, 19.8), waist circumference (95% CI: 83.4, 85.8 vs 71.0-71.8), insulin (95% CI: 3.5, 3.9 vs 1.7, 1.8 vs.), systolic blood pressure (95% CI, 116.7 - 118.9 vs 110.8, 112.1) and triglycerides (95% CI: 1.25, 1.35 vs 0.780.80.), and lower HDL cholesterol (95% CI: 1.20, 1.26 vs 1.44, 1.48) (197). Those in the high-risk cluster also had increased levels of CRP, ALT, GGT and UA irrespective of gender (198) and central obesity appeared to play a central role (199).

Results from Raine participants at the age 17 years showed the prevalence of prehypertension or hypertension in overweight and obese individuals was 34% and 38% respectively (200). The study also examined the association between smoking status and hs-CRP using a three-level variable, girls not using oral contraceptives, girls using oral contraceptives, and boys (201). Smoking was significantly associated with higher hs-CRP levels in girls not using oral contraceptives, but not in girls using oral contraceptives or in boys. Oral contraceptives use in non-smoking girls was the strongest factor associated with higher hs-CRP levels (P < 0.001). Further, girls taking oral contraceptives had 3.27 and 1.74 mmHg higher systolic and diastolic blood pressure compared with non-users (200).

At the age of 20, the prevalence of prehypertension was 33.4% while for hypertension; the figure was 3.6% (202). There was a significant sex difference in mean systolic blood pressure: males had on average 11.3 mmHg higher systolic blood pressure compared to females (202). Longitudinal analysis of the association between pregnancy life stress
events (e.g. pregnancy problems, death of close relatives or friends, marital problems, problems with children, loss of job in the family, money problem and residential move) and systolic blood pressure from age 14 through age 20, showed an inverse relationship which was highly significant (202).

1.12 Study Aims

Considering the current evidence of the Raine Study findings suggesting the presence of cardiometabolic risk factors at young age (variation of cardiometabolic factors among genders) and knowledge from the literature of a possible association between sleep characteristics/disorder (including sleep duration, quality and high risk of sleep apnoea disorder) and cardiometabolic risk factors, the current research hypothesized that sleep insufficiency/sleep disorder would associate with adverse cardiometabolic risk factors in a young adult population.

Therefore, the objectives of this thesis were to investigate the association between:

1. Objective measurements of sleep and cardiometabolic risk factors;
2. Sleep quality and cardiometabolic risk factors;
3. Sleep-disordered breathing (high risk for obstructive sleep apnoea) and cardiometabolic risk factors; and
4. Whether the associations differed between males and females

in the Raine Study participants at 22 years.
Chapter 2:
Methods
2.1 Background of the Raine Study

A complete description of the Western Australian Pregnancy Cohort (Raine) Study has been previously published (195). In brief, the study was commenced as a randomised controlled trial that aimed to evaluate the effects of ultrasound scans on pregnancy outcomes. The study was conducted between May 1989 and November 1991 (195). Pregnant women attending the public antenatal clinic at King Edward Memorial Hospital were enrolled in the project that formed the basis of the Raine Study. The inclusion criteria were gestational age between 16 and 20 weeks, an intention to remain in Western Australia in the coming years, an expectation to deliver at the King Edward Hospital and sufficient proficiency in English (195). As this hospital was at the time the sole tertiary referral centre for obstetrics in Western Australia, these mothers could be considered as representative of the antenatal population of the Perth metropolitan area. The initial written informed consent sought approval for long term follow up of the cohort with the view that the study would have a significant potential for investigating the developmental origins of health and disease.

A total of 2,900 pregnant women were recruited into the study. They were asked to complete questionnaires that asked about their socio-economic status, lifestyle factors, environmental exposure and past medical history. The partners were also asked to complete a questionnaire containing information regarding their height, weight, occupation, education and environmental exposures. The women were then allocated to either an intensive group who had 5 ultrasounds during their pregnancy (n = 1,415) at 18, 24, 28, 34 and 38 weeks gestation or a ‘regular’ group who had one ultrasound examination at 18 weeks. Any further ultrasound examinations were conducted only if requested by clinician (n = 1,419). Allocation was done using computer-generated random numbers. There were 66 enrolled women with multiple pregnancies who were not allocated to either intensive or regular groups.

Of those recruited to the study there were 2868 live births from 2,826 pregnant mothers. There were 2,801 (99%) women with single pregnancies, while 66 women had multiple pregnancies during the recruitment phase. The outcomes of the initial study indicated a higher intrauterine growth retardation (birthweight < 10th centile and birthweight < 3rd centile) in the intensive ultrasound group, suggesting that frequent exposure to ultrasound may influence fetal growth (195). However, by the age of 1 and thereafter no significant difference observed in physical size among the ultrasound groups (203).
Subsequent recalls of the cohort have examined and documented a wide range of health and behavioural data variables at 1, 2, 3, 5, 14, 17, 18, 20 and 22 years of age (Figure 2.1). Currently over 85,000 phenotypic and behavioural variables have been collected in addition to establishment of a genetic database (Table 2.1). Biological samples including blood and urine have been collected at many of these follow-up assessments (204).

**Figure 2.1. The Raine Study: Retention of the cohort from 1 to 22 years of age.**

The Raine Study currently incorporates 25 broad areas of research including asthma & allergy, anaesthetic, Development Origins of Health and Disease (DoHAD), cardiovascular, cognitive neuroscience, dental health, nutrition, eating disorders, endocrinology, infectious diseases, language & social development, mental health, risky behaviour, musculoskeletal, ophthalmology, physical activity, reproductive health (204).

The overarching aim of the Raine study has been to evaluate the interrelationship between health, diet, lifestyle, behavioural, psychosocial and genetic factors from infancy through childhood into adolescents and young adulthood. The adolescent/young adulthood period is considered a critical period during which lifestyle behaviours that are likely to substantially influence adult cardiovascular risk tend to become established.

At all recalls there have been core data collected including questionnaires relating to psychosocial, lifestyle and medical information, in addition to anthropometry (height,
weight, waist circumference, skinfolds) and clinical (blood pressure, vascular function) measures (Table 2.1).

Furthermore, blood samples have been collected for biochemical variables including fasting lipids, glucose, insulin, liver function and iron metabolism. The cohort recall at 22 years of age had a specific focus on aspects of sleep, cardiovascular measures, spinal pain and work productivity (204).
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</table>
2.2 Follow-up of the Raine Study Participants at 22 Years

Participants were contacted using an established confidential contact database of the Raine study by telephone around the time of their 22nd birthday. The follow-up procedure was explained to them by the recruitment team members and an appointment was arranged. All of the participant information, consent forms and core questionnaires (general and medical questionnaires) were mailed to those who accepted to participate in the follow-up study at 22 years of age. On arrival, participants provided written informed consent. The trained research assistants explained protocols to the participants. The physical assessments were completed over a 3 hour appointment and included asthma, skin, musculoskeletal and tissue sensitivity. The asthma assessments included spirometry, mannitol challenge test and induced sputum. The skin assessments included skin prick testing and mole counting. The musculoskeletal assessments included back muscle endurance (in which the participants completed prone trunk hold) and hip accelerometer (which used to capture movement during wakefulness). Tissue sensitivity examinations included pressure pain threshold and cold pain threshold (204).

The participants were asked to complete food frequency, sleep specific and work attendance questionnaires. Once these questionnaires were completed they were asked to stay overnight at the Centre for Sleep Science, at the University of Western Australia for an overnight sleep study (including polysomnography and wrist accelerometer which used to capture movement data during sleep period). Participants went home wearing the hip and wrist accelerometers for the following 7 days (see section Objective Sleep Measurement for details). The recruitment for the study was commenced in March 2012 and completed in July 2014. The total number of participants in this follow up was 1,234.

All aspects of this study were approved by the University of Western Australia Human Research Ethics Committee (RA/4/1/5002) (204). The participants provided written informed consent for data collection and the study was conducted in accordance with the Declaration of Helsinki (204).

2.3 Anthropometric Measurements

Body weight was measured to the nearest 100 g using a Wedderburn Chair Scale (Wedderburn, AUS). Height was measured without shoes to the nearest 0.1 cm using a
Holtain Stadiometer (Crosswell, UK). All anthropometric measurements were performed with participants wearing light clothing. Body Mass Index (BMI) was calculated as weight/height\(^2\) (kg/m\(^2\)) \((205)\). The BMI was categorised as normal-weight \((< 25 \text{ kg/m}^2)\), over-weight \((\geq 25 < 30 \text{ kg/m}^2)\) and obese \((\geq 30 \text{ kg/m}^2)\) based on WHO recommendation \((205)\). As the majority of the Raine Study population (90\%) is Caucasian, the Caucasian-based BMI cutoffs were used in the current study \((206)\).

Waist circumference was measured using a measuring tape at the halfway point of the lowest rib and iliac crest. Hip circumference was measured at the level of maximum extension of the buttocks, to the nearest 0.1 cm and hip-to-waist ratio calculated.

### 2.4 Blood Pressure Measurement

Systolic and diastolic blood pressures (BP) were recorded using an oscillometric sphygmomanometer (Dinamap ProCare 100; Soma Technology, Bloomfield, Connecticut, USA) with an appropriate cuff size in a sitting position. Following resting for 5 minutes, BP and heart rate recordings were repeated every 2 minutes until at least 5 separate measurements were obtained. BP was calculated from the average of the last 5 readings. Systolic and diastolic prehypertension were defined as SBP 120-139 mmHg and DBP 80-89 mmHg. Systolic BP \(\geq 140\) and diastolic BP \(\geq 90\) were defined as systolic and diastolic hypertension using the adult The Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-7) criteria \((207)\).

### 2.5 Blood Collection

Participants were advised not to eat or drink after 10 pm in order to fulfil eight hours of fasting. Blood samples were collected in the morning after an overnight fast by standard phlebotomy from an antecubital vein. The total volume of collected blood was 100 ml. Blood samples were processed within 2 hours of collection by centrifugation at 3,500 rpm for 5 minutes at 4 \(^\circ\)C. Serum and plasma were stored at -80\(^\circ\)C until analyses were performed. All blood biochemical measurements were carried out at the Path West Laboratory at Royal Perth Hospital.

Fasting blood glucose was measured by a standard spectrophotometric assay (Abbott Diagnostics, Abbott Laboratories, USA). Serum insulin was assayed by the immunoassay technique (Abbott Diagnostics, Abbott Laboratories, USA). Inter-assay
coefficients of variation for glucose and insulin were recorded as 0.54%-1.76% and 1.78%-2.59%, respectively. The homeostasis model assessment for insulin resistance (HOMA-IR) score was calculated based on fasting insulin (\(\mu\text{U/ml}\)) × fasting glucose (mmol/l) /22·5 (208).

Total cholesterol and triglycerides (TG) were measured with enzymatic colorimetric assays (Abbott Diagnostics, Abbott Laboratories, USA). The inter-assay CVs of cholesterol and TG ranged from 0.51% to 0.87% and 1.02% to 1.92%, respectively. The LDL cholesterol (LDL-C) was calculated according to the Friedewald equation (209). The HDL cholesterol (HDL-C) was measured with an enzymatic, colorimetric assay (Abbott Diagnostics, Abbott Laboratories, USA). The inter-assay CV of HDL-C ranged between 1.94%-2%.

High sensitivity CRP (hs-CRP) was measured by CRP Vario Reagent (Abbott Diagnostics, Abbott Laboratories, USA); the inter-assay CV was 2.07%-2.08%.

Liver function tests included measurements of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase. ALT was measured using an activated alanine aminotransferase reagent (Abbott Diagnostics, Abbott Laboratories, USA); the inter-assay CV ranged between 1.37%-3.68%. AST was measured using an activated aspartate aminotransferase reagent (Abbott Diagnostics, Abbott Laboratories, USA); the inter-assay CV ranged between 0.77% to 2.87%. GGT was measured using a GGT reagent (Abbott Diagnostics, Abbott Laboratories, USA); the inter-assay CV ranged between 0.64% to 1.44%. ALP was assayed using an Alkaline Phosphatase Reagent (Abbott Diagnostics, Abbott Laboratories, USA) with an inter-assay CV of 1.96% to 2.18%.

2.6 Questionnaires

The questionnaires included general, medical, food frequency and sleep questionnaires. All of the questionnaires were delivered in a paper-based format. The general questionnaire focused on 4 main categories including:

a. Socio-economic factors (e.g. income, employment and education).

b. Risk taking behaviours (e.g. smoking and alcohol).

c. Family life (marital status, having child).

d. Lifestyle (e.g. physical activity, hormonal contraceptive use in females only).
A medical questionnaire evaluated the presence of any medical condition in addition to past medical history and prescription medication of individuals. The sleep questionnaires included Pittsburgh Sleep Quality Index, Berlin and Epworth Sleepiness Scale Questionnaires. The details of all sleep questionnaires are provided in the next sections.

2.7 Selected Risk Taking and Lifestyle Variables

Smoking and alcohol consumption were used as the main indicators of risk taking behaviour in the final analyses. The dichotomous response (yes/no) to the questions about smoking was used in this study. Alcohol intake was estimated based on the self-report questionnaire. For each type of alcohol beverage the number of drinks consumed on one occasion and the frequency of drinking were asked. Based on the average alcohol content in different types of beverages, the average of alcohol consumption per week (gram/week) was calculated and used in subsequent analyses.

In females, current use of any hormonal contraceptive pill, implant, intrauterine device and injection in females was classified as hormonal contraceptive usage.

The physical activity level of participants was estimated from self-report questionnaires based on the International Physical Activity Questionnaire (IPAQ). The IPAQ questionnaires assessed 3 different types of activity including walking, moderate intensity activities and vigorous intensity (210). For each of the activities the frequency (as days per week) and duration (time per day) were measured. Time spent in each activity was converted to Metabolic Equivalences (METS) defined by weighting each type of activity by its energy requirements. Met-minutes were calculated by multiplying the MET score by the minutes performed.

The median MET-minutes were used as a continuous variable for assessing physical activity in participants based on the following formula:

Walking MET-minutes/week = 3.3 * walking minutes * walking .days.

Moderate MET-minutes/week = 4.0 * moderate-intensity activity minutes * moderate days.

Vigorous MET-minutes/week = 8.0 * vigorous-intensity activity minutes * vigorous-intensity.
Total physical activity in MET-min/week was calculated as the sum of walking + moderate + vigorous MET-min/week scores.

2.8 Subjective Sleep Measurements

2.8.1 Sleep Diary

During the study at the Centre for Sleep Sciences, University of Western Australia, a sleep diary was given to each participant in order to collect sleep hours and time to bed and time to rise. All of the participants received information on how to fill in their sleep diary in the evening and morning. They were required to note the time they went to bed and the time they got up each day for 7 days. The sleep diary was given to the participants in the morning after the overnight sleep study at the Centre for Sleep Sciences. Sleep diary reminders were added to the calendar system and telephone calls, text messages or Facebook messages were used to remind participants to fill in their diaries.

2.8.2 Sleep Questionnaires

A number of sleep-related questionnaires were given to the participants in the evening of overnight sleep study at the Centre for Sleep Sciences. These included: the Pittsburgh Sleep Quality Index, the Berlin questionnaire (to assess high risk for OSA), the Epworth Sleepiness Questionnaire and the Short Functional Outcome of Sleep Questionnaire (FOSQ).

2.8.2.1 Pittsburgh Sleep Quality Index Questionnaire

The Pittsburgh Sleep Quality Index (PSQI) questionnaire is a self-administrated questionnaire that measures sleep quality and sleep disturbance during the previous month (33). It has seven components including subjective sleep quality, sleep latency (the length of time it takes to achieve transmission from full wakefulness to sleep), sleep duration, habitual sleep efficiency (time spent in bed divided by actual asleep time), sleep disturbance, usage of sleep medication and daytime dysfunction. The total score is calculated by adding the seven components scores together. A high total score (≥ 5) indicates of poor sleep quality (Figure 2.1) (33).
2.8.2.2 Berlin Questionnaire

The Berlin questionnaire is a self-administrated questionnaire that identifies individuals at risk of having sleep apnoea (36). It has 3 main categories. The first category consists of 5 questions that aim to evaluate the presence and frequency of snoring. The second category consists of 3 questions regarding daytime sleepiness and fatigue and the last (third) category evaluates history of obesity or hypertension. The first and second
categories are considered a positive response when there are 2 or more positive responses to the questions. The last category is considered positive in the presence of BMI > 30 kg/m² or a history of hypertension (Figure 2.2). Two or more positive categories indicate a high likelihood of sleep apnoea (36). Previous studies have recommended the Berlin Questionnaire as a screening tool in the general population for its good sensitivity and specificity (40; Senaratna, 2017 #458). In the Raine Study the last category of the Berlin questionnaire was omitted because these aspects were addressed elsewhere during the 22-year assessment. The modified Berlin Questionnaire used in the Raine Study is presented in Appendix 1.
2.8.2.3 *Epworth Sleepiness Scale Questionnaire*

This questionnaire aims to evaluate daytime sleepiness and is composed of 8 questions which are scored equally. The questions ask the probability of falling asleep in different conditions (212). A higher total score indicates higher probability of falling asleep (212).

![Image of the Berlin Questionnaire](image-url)

### Berlin Questionnaire Scoring:

**Scoring Questions:**
Any answer within box outline is a positive response

**Scoring Categories:**
- Category 1 is positive with 2 or more positive responses to questions 2-6
- Category 2 is positive with 2 or more positive responses to questions 7-9
- Category 3 is positive with 1 or more positive response and/or a BMI > 30

**Final Results:**
- 2 or more positive categories indicates a high risk of obstructive sleep apnea

---

*Figure 2.3. Berlin Questionnaire (211).*
2.8.2.4 Short Functional Outcome of Sleep Questionnaire

This self-administered questionnaire is designed to evaluate the impact of excessive sleepiness on various activities relating to an individual’s daily living including relationships, productivity, social and physical activity, and vigilance (213).

2.9 Objective Sleep Measurement

2.9.1 Wrist Accelerometer

Sleep actigraphy has been used as an objective measurement of sleep (214). This method is based on monitoring the amount of movement a person makes. As minimal movement occurs during sleep compared to wakefulness, sleep actigraphy can be used to distinguish between sleep and wakefulness (214). By using sleep actigraphy overall sleep measures can be obtained. The overall sleep measurements included:

1. Total Sleep Time (TST): duration of sleep which reported as hours or minutes (215).
2. Sleep Onset Latency (SOL): time period between reported bed time and actigraphy scored sleep onset time (215).
3. Wake After Sleep Onset (WASO): the minutes awake during the sleep period after sleep onset (215).
4. Sleep Efficiency (SE): time spent in bed divided by actual asleep time (215).

In order to calculate all mentioned sleep measurements, time into bed (lights out) and time out of bed (lights on) are also needed (215). According to the Society of Behavioral Sleep Medicine (SBSM) actigraphy monitoring guideline, it is recommended to identify lights out and lights on time by individual-recorded method such as individual sleep diary or actigraphy log (215). According to the guideline, participants are required to keep and fill out their actigraphy log or sleep diaries while they are wearing a wrist accelerometer. This method uses a device called an accelerometer that is equipped with a light sensor and is placed on the wrist. The common location for placing an accelerometer is the non-dominant wrist although it can also be placed on the leg, shoulder or hip (215, 216).
The reliability and validity of sleep actigraphy has been evaluated in many studies (22, 25, 217, 218). The range of agreement between actigraphy and Polysomnography (PSG) has been shown to be between 91-93% (219).

In the Raine Study we used a GT3X+ activity monitor (Actigraph, FL, USA; Figure 2.3) which is a small (4.6 x 3.3 x 1.5 cm), lightweight (19 g) tri-axial solid state water-resistance accelerometer.

![Figure 2.4. The Wrist Accelerometer used in the Raine Study participants.](image)

### 2.9.2 Wrist Accelerometry (Actigraphy) Data Collection

During the overnight sleep study at Centre for Sleep Science, at the University of Western Australia, the participants received an information sheet and verbal instructions on how to wear and use the device. They were required to wear the device for at least 8 consecutive nights (including the overnight study). They were advised to continue their
normal lifestyle and sleep-wake pattern in their home environment. They were provided with an instruction sheet and a self-addressed Express post bag in order to return the device when the measurements were completed.

2.9.3 Actigraphy Software & Analysing Sleep Actigraphy Data

The raw data were collected at 30 Hz and recorded in one-minute period (epoch). All accelerometer files were downloaded on a computer and checked by a Raine study research assistant for any technical problems. The Actilife software (Actigraph 2012, Actilife 6.8) was used to download and analyse the data. As the device used in this study was not equipped with an event marker button to record significant events such as “lights-out” and “lights-on” time, these variables were extracted from the sleep diaries as recommended by the SBSM Actigraphy Monitoring Guidelines (215).

The Actilife software uses the Sadeh algorithm (219) that is applied to a one minute period (epoch) of vertical axis movement data. The algorithm then transforms that epoch of movement data, based on an 11 minute window, with that epoch at centre, and also considers the previous and the following five minutes of data (219). Any missing epochs are considered as zero. The vertical axis data are placed in the Sadeh algorithm.

By using this algorithm the software considers an epoch as asleep if the result of the algorithm is greater than $-4$. The software calculates all four overall sleep measurements including total sleep time (TST), sleep latency, sleep efficiency and wake after sleep onset (WASO) (see Wrist Accelometer Section in Chapter 2-Methods, page 48).

According to the Society of Behavioral Sleep Medicine (SBSM) actigraphy monitoring guideline, participants are required to keep and fill out their actigraphy log or sleep diaries while they are wearing a wrist accelerometer (215).

During the Raine study, actigraphy data were considered invalid if the wrist accelerometer malfunctioned (220). The sleep diary data were also considered invalid if the participant did not enter the diary variables correctly (wrong date and time) or if there was more than one hour discrepancy between the sleep diary and actigraphy (220).

Sleep diaries were used to identify when the lights in the room were turned off (time of going to bed with intention to sleep) and when they were turned on (time out of bed and awake) in those participants that had sleep diary data. After checking the actigraphy data, sleep times (lights out and lights on) were entered manually into the sleep analysis
tool of Actilife software by clicking on the “Add Bed Time “button. By confirming the
time, the selected time periods were highlighted on the graph window. The final sleep
report for each individual was exported to an excel sheet.

Prior to entering information relating to lights out and lights on, the activity counts were
evaluated in order to check for periods of low and moderate activity. If the actigraphy
data of low and moderate activity were in accordance with the sleep diary data, the sleep
times (lights out and lights on) were extracted from the sleep diary and entered into the
software. In total there were 497 participants in which their sleep diaries were valid and
therefore used to identify “lights on” and “lights out”.

Data loss from either the wrist accelerometer, actigraphy log or sleep diary is a common
issue in sleep studies (215). As a solution, an alternative method adapted from the
SBSM guide to actigraphy monitoring was applied to missing data in the Raine sleep
study. According to the SBSM actigraphy monitoring guideline, the following
alternative methods are recommended in order to identify lights out and lights on time
in those whose their sleep diaries are incomplete, inaccurate or disagreeing with
actigraphy data (215). These included:

- Visual identification of time based on movement data.
- Excluding a night with unreliable or missing diary data.
- Using lights out and lights on time from previous night if having regular sleep
  habits.
- Additional device feature such as light measure and non-wear detection.
- Proprietary device and software features such as automated bed period detection
  algorithms.

Visual manual identification of sleep periods whenever sleep diary data are incomplete,
inaccurate or missing is the most common approach used in the literature (221). This
approach was used in the Raine study.

All actigraphy files with sleep diaries were analysed using The Actilife software. The
actigraphy files without sleep diaries were scored by visual manual identification, and
also by a second qualified sleep technician who was blinded to the first scorer’s values.
After recognising light out and light on time using the visual manual identification
method, results were compared. Following visual manual scoring all 84 files were
added to the rest of the data.
2.10 Statistical Methods

Quality and efficiency of sleep was assessed using the four actigraphy derived variables: total sleep time, sleep efficiency, sleep latency and Wake after Sleep Onset (WASO) as well as PSQI derived sleep quality. In addition, a classification of Obstructive Sleep Apnoea (OSA) was determined from the Berlin Questionnaire.

As BMI was included in the Berlin Questionnaire, obesity-adjusted estimates may be subject to over adjustment. By using the Berlin Questionnaire a modified classification of OSA, excluding category three (in which BMI is located) was undertaken. In the Berlin Questionnaire, OSA is defined as 2 positive classifications from any of the three categories (snoring, fatigue/sleepiness and BMI/hypertension). A modified, 3 level classification was generated using the number of categories in which a positive score was obtained from only the snoring and fatigue/sleepiness categories. A resulting score of 2 equated to a diagnosis of OSA on the full Berlin as the third category would be unnecessary for a positive determination. A score of 1 remains dependent on the result of the third omitted category to form a definite diagnosis so these were classified as possible OSA. A score of 0 identified negatives as the result of the third category would not alter the diagnosis.

All continuous variables were tested for normality and where departures were found, log transformed. Subject characteristics were summarised using mean (95% CI) or median (1st, 3rd quartiles) as appropriate for continuous variables and proportion (95% CI) for categorical variables. For log transformed variables, data is presented as geometric means (95% CI).

Comparisons of those who attended follow-up at age 22 with those who did not attend were performed to identify potential bias in the participating sample. Similar comparisons were made within the 22 year participants between those who participated in the actigraphy sleep study and those who did not. Comparisons were also made between male and female participants in the 22 year age cohort to identify gender differences.

BMI, waist circumference, systolic and diastolic blood pressure, biochemical variables and HOMA-IR as an index of insulin resistance (see Sections 2.3, 2.4 and 2.5 in Chapter 2-Methods, pages 40-41) were analysed as continuous variables. Differences in
continuous variables were investigated using linear regression and logistic regression for categorical variables.

The following method was used to assess the association between each of the independent sleep variables and cardiometabolic risk factors. Firstly scatterplots, Locally Weighted Scatter plot Smoothing (lowess), Multiple Variable Rank Sum multivariable regression splines (mvrs) were used to assess the linearity of associations with continuous independent variables (only for actigraphy derived sleep variables), prior to analysis using univariate linear regression. Univariate regressions were followed by models adjusted for gender then gender and BMI (excluding models where BMI was outcome) and finally gender, BMI and lifestyle factors. The lifestyle confounders included in each model were selected based on their associations with the particular cardiometabolic risk factors identified through univariate regression models (P < 0.2).

There was a large amount of missing data for physical activity variable. Therefore, it was necessary to separate the effect on the sleep variable coefficient of accounting for physical activity and the loss of sample its inclusion generated. The regression was run using the sample with physical activity data, without including the physical activity variable. The same model was then run again including the physical activity variable. Comparison of the sleep coefficient from these two models allowed the effect of physical activity to be assessed. If the coefficient was unchanged, physical activity was excluded to retain the larger sample in the analysis.

Examination of scatterplots identified some outlier values of BMI whose influence warranted further investigation. Where statistical significance was identified, a sensitivity analysis was undertaken to assess the results for robustness to the removal of these outliers. Both analyses, with and without the outliers, are reported.

The Raine cohort is known to contain siblings whose measures may not be independent due to familial genetics or environmental background. A family identifier variable was used to apply a per family cluster variance adjustment to all regressions to account for the potential correlation between siblings.

Statistical significance was set at P < 0.05. The data was analysed using STATA (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).
Chapter 3:
Results
3.1 Follow-up Response of the Raine Study Participants at 22 Years of Age

Figure 3.1 shows the retention rates of the Raine study cohort from birth to 22 years of age. Of the original cohort of 2,868 live births there are 41 deceased, with an additional 561 that have withdrawn and 1,029 who are lost and/or have deferred. There were 1,234 participants from the original 2,868 live-born children who participated at the 22 year follow-up, representing a 43% response which is similar to previous cohort recalls. Of the 1,234 that participated at 22 years, 975 healthy young men and women as ascertained from medical history questionnaires, provided full anthropometry and biochemical data.

A logistic regression model determined potential predictors for participating at age 22. These included maternal (age at conception, BMI before pregnancy, education), socioeconomic and demographic (family income) variables, in addition to anthropometric variables collected at birth. Table 3.1 shows maternal age, maternal higher educational level and high family income were significantly positively associated with participation at age 22.
Number of Participants at birth $N = 2868$

| Follow-up: Year 1 | $N = 2441$ | Deferred=204 Lost=174 Total Withdrawn=21 Deceased=28 |
| Follow-up: Year 2 | $N = 1988$ | Deferred=381 Lost=418 Total Withdrawn=51 Deceased=30 |
| Follow-up: Year 3 | $N = 2280$ | Deferred=321 Lost=158 Total Withdrawn=79 Deceased=30 |
| Follow-up: Year 5 | $N = 2237$ | Deferred=339 Lost=135 Total Withdrawn=127 Deceased=30 |
| Follow-up: Year 8 | $N = 2140$ | Deferred=376 Lost=124 Total Withdrawn=198 Deceased=30 |
| Follow-up: Year 10 | $N = 2047$ | Deferred=281 Lost=162 Total Withdrawn=348 Deceased=30 |
| Follow-up: Year 17 | $N = 1771$ | Deferred=379 Lost=206 Total Withdrawn=447 Deceased=35 |
| Follow-up: Year 20 | $N = 1342$ | Deferred/lost =840 Total Withdrawn=485 Deceased=36 |
| Follow-up: Year 22 | $N = 1234$ | Deferred/lost =1029 Total Withdrawn=561 Deceased=41 |

Figure 3.1. The Raine Cohort Study Retention from birth to 22 years.
Table 3.1. Univariate Regression Analyses Evaluating Factors Predicting the Likelihood of Participating at the 22 Year Follow Up

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Participants attending follow up (n=1234)</th>
<th>Participants not attending follow up (n=1634)</th>
<th>OR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>29.45 (29.1, 29.7)</td>
<td>27.02 (26.7, 27.3)</td>
<td>1.03 (1.02, 1.05)</td>
<td>0.016</td>
</tr>
<tr>
<td>Maternal BMI (kg/m²)</td>
<td>22.17 (22.0, 22.40)</td>
<td>22.50 (22.30, 22.70)</td>
<td>0.99 (0.95, 1.03)</td>
<td>0.73</td>
</tr>
<tr>
<td>Offspring birth weight (kg)</td>
<td>3.3 (3.2, 3.3)</td>
<td>3.2 (3.2, 3.3)</td>
<td>1.00 (0.99, 1.00)</td>
<td>0.19</td>
</tr>
<tr>
<td>Income*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Income %</td>
<td>38 (35, 40)</td>
<td>56 (53, 58)</td>
<td>1.03 (0.89, 1.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>High Income %</td>
<td>61 (59, 64)</td>
<td>43 (41, 46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal post-school higher education (%)</td>
<td>57.4 (54.5, 60.1)</td>
<td>41.5 (39.1, 44.0)</td>
<td>1.42 (1.2, 1.6)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean (95%CI) for continuous variables and proportion (95%CI) for categorical variables.

*Low and high income is defined by the 1989 poverty line cut-offs (AUD$ 325.8 /week).
3.2 Socioeconomic and Lifestyle Characteristics of the Raine Study Participants at 22 Years

Socioeconomic and lifestyle characteristics are presented in Table 3.2. There was no significant difference in the distribution of higher education (post school higher education) between males and females (Table 3.2). Although there was no difference in the proportion of females and males in full/part time employment (P = 0.23), a significantly greater proportion of males earned more than $604 per week (P = 0.002).

Smoking, alcohol intake, usage of hormonal contraceptive, physical activity and depression, anxiety and stress score (DASS) were evaluated as lifestyle behaviours of the study population. There was a significant difference in the rate of smoking among gender (P = 0.02, Table 3.2). The percentage of alcohol drinkers in females was 76.4% (95%CI: 72.8, 79.7) compared with 82% (95%CI: 78.4, 85.2) in males (P = 0.024). Males consumed more alcoholic drinks per week (g/wk) compared to females (P < 0.001). Hormonal contraceptives were used by 50.6% of females. Males undertook almost twice as much physical activity per week than females (P < 0.001). Evaluating the adverse emotional states of DASS score questionnaires showed that females had a significantly higher total DASS score (P < 0.001).
### Table 3.2. Socioeconomic Characteristics of the Participants at 22 Years

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N (Females/Males)</th>
<th>Females</th>
<th>Males</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-school higher education, %</td>
<td>1101 (593/508)</td>
<td>52.6 (47.9, 57.2)</td>
<td>48.4 (43.6, 53.2)</td>
<td>0.22</td>
</tr>
<tr>
<td>Total usual pay/wk after tax &gt;$ 604</td>
<td>1026 (557/469)</td>
<td>41.8 (37.1, 46.7)</td>
<td>52.9 (47.9, 57.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Employed, %</td>
<td>1139 (604/535)</td>
<td>82.9 (79.6, 85.7)</td>
<td>80.5 (76.9, 83.6)</td>
<td>0.23</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>1139 (603/536)</td>
<td>14.0 (11.5, 17.1)</td>
<td>19.6 (15.8, 22.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total weekly alcohol consumption (g/wk)*</td>
<td>1146 (607/539)</td>
<td>88 (73.6, 101.84)</td>
<td>194.4 (170.3, 218.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Physical activity (MET-min/wk)*</td>
<td>962 (515/447)</td>
<td>2392.50 (2133.90, 2682.42)</td>
<td>4003.57 (3551.10, 4513.68)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total DASS score*</td>
<td>1078 (583/495)</td>
<td>18.00 (16.48, 19.62)</td>
<td>12.70 (11.50, 14.00)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean (95%CI) for continuous variables or proportion (95%CI) for categorical variable.

*Not normally distributed variables presented as geometric mean (95%CI).

Standard error adjusted for 20 families who had two children in the study.

#### 3.3 Anthropometric and Biochemical Characteristics of the Raine Study Participants at 22 Years

There was no significant difference in BMI between males and females (Table 3.3). According to the adult criteria of BMI categorization, 61.5% of participants were normal weight (BMI < 25), 24% were overweight (25 ≥ BMI < 30) and 15% were obese (BMI ≥ 30). More males were overweight (28.6%) compared with 18.4% of females (OR 1.70, 95% CI: 1.2, 2.2, P < 0.001) but there was no significant difference in the proportion of obesity between genders (OR 0.79, 95% CI: 0.5, 1.1, P = 0.18).

Using adult criteria of waist circumference, 37% of females and 20% of males (P < 0.001) had central obesity according to the criteria of the World Health Organization (males ≥ 102 cm and female ≥ 88 cm) (205).

Mean Systolic blood pressure (SBP) was higher in males compared to females (123 mmHg vs 113.8 mmHg, P < 0.001). There was no significant difference in DBP between genders. Using criteria for pre-hypertension as defined by 120 mmHg < SBP ≤ 139 mmHg or 80 mmHg < DBP ≤ 89 mmHg, 125 (23%) females and 289 (53%) of
males were pre-hypertensive (222). Hypertension defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, showed 5 females and 34 (6%) males were categorized as hypertensive (222). Males had a higher prevalence of pre hypertension and hypertension compared to females (P < 0.001).

Table 3.3 illustrates the anthropometric, blood pressure and biochemical characteristics of female and male participants. Mean glucose levels were significantly higher in males (P < 0.001), whereas females had higher levels of insulin (P < 0.001) and HOMA-IR (P < 0.001). Using HOMA-IR ≥ 2.73, as a measure of insulin resistance, showed 15.8% of females and 11.3% of males were insulin resistant (P = 0.04) (223).

Females had higher levels of total cholesterol (P = 0.001), HDL-C (P < 0.001) and hs-CRP (P < 0.001) compared to males. In contrast males had higher levels of the liver enzymes AST, ALT, ALP and GGT, compared with females (P < 0.001). Females using hormonal contraceptive had lower BMI and waist circumference, while having higher total cholesterol, HDL-C and triglycerides (Table 3.4).
Table 3.3. Characteristics of the Raine Participants at 22 Years Stratified by Gender

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (975)</th>
<th>Females (480)</th>
<th>Males (495)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>25.22</td>
<td>25.25</td>
<td>25.28</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>(24.94, 25.59)</td>
<td>(24.73, 25.78)</td>
<td>(24.89, 25.67)</td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>83.27</td>
<td>80.54</td>
<td>85.95</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(82.45, 84.10)</td>
<td>(79.27, 81.81)</td>
<td>(84.96, 86.94)</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>118.6</td>
<td>113.8</td>
<td>123.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(117.9, 119.3)</td>
<td>(112.9, 114.7)</td>
<td>(122.3, 124.2)</td>
<td></td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>67.0</td>
<td>67.2</td>
<td>66.7</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>(66.5, 67.4)</td>
<td>(66.6, 67.8)</td>
<td>(66.1, 67.4)</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.97</td>
<td>4.86</td>
<td>5.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(4.95, 5.00)</td>
<td>(4.82, 4.89)</td>
<td>(5.05, 5.12)</td>
<td></td>
</tr>
<tr>
<td>Insulin (*)</td>
<td>7.36</td>
<td>8.0</td>
<td>6.74</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(7.14, 7.59)</td>
<td>(7.72, 8.41)</td>
<td>(6.46, 7.04)</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR(*)</td>
<td>1.62</td>
<td>1.73</td>
<td>1.52</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(1.57, 1.67)</td>
<td>(1.65, 1.81)</td>
<td>(1.45, 1.59)</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>4.62</td>
<td>4.71</td>
<td>4.53</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(4.57, 4.67)</td>
<td>(4.64, 4.78)</td>
<td>(4.46, 4.60)</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.35</td>
<td>1.47</td>
<td>1.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(1.33, 1.37)</td>
<td>(1.44, 1.50)</td>
<td>(1.21, 1.25)</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.76</td>
<td>2.74</td>
<td>2.77</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>(2.71, 2.80)</td>
<td>(2.69, 2.80)</td>
<td>(2.70, 2.83)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.09</td>
<td>1.06</td>
<td>1.12</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>(1.06, 1.13)</td>
<td>(1.02, 1.11)</td>
<td>(1.08, 1.17)</td>
<td></td>
</tr>
<tr>
<td>hs-CRP (*) (mg/L)</td>
<td>1.06</td>
<td>0.75</td>
<td>0.45</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(0.98, 1.15)</td>
<td>(0.67, 0.84)</td>
<td>(0.38, 0.53)</td>
<td></td>
</tr>
<tr>
<td>ALT (*) (U/L)</td>
<td>25.26</td>
<td>22.81</td>
<td>34.60</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(24.49, 26.04 )</td>
<td>(21.65, 23.97)</td>
<td>(32.77, 36.43)</td>
<td></td>
</tr>
<tr>
<td>AST (*) (U/L)</td>
<td>26.48</td>
<td>23.85</td>
<td>29.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(25.85, 27.12)</td>
<td>(23.0, 24.6)</td>
<td>(28.0, 30.0)</td>
<td></td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>69.49</td>
<td>61.4</td>
<td>77.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(68.22, 70.75)</td>
<td>(59.8, 62.9)</td>
<td>(75.64, 79.09)</td>
<td></td>
</tr>
<tr>
<td>GGT (*) (U/L)</td>
<td>17.62</td>
<td>15.27</td>
<td>20.24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(17.10, 18.15 )</td>
<td>(14.66, 15.91)</td>
<td>(19.47, 21.05 )</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean (95%CI). Linear regression models for continuous variables.

(*) log transformed was used for in regression model.
Table 3.4. Characteristics of females stratified by hormonal contraceptive use.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Females not using contraceptive</th>
<th>Females using contraceptive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>26.38 (25.30, 27.46)</td>
<td>24.70 (24.06, 25.35)</td>
<td>0.009</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>83.77 (81.26, 86.28)</td>
<td>79.12 (77.50, 80.74)</td>
<td>0.002</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>4.58 (4.46, 4.70)</td>
<td>4.82 (4.73, 4.92)</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.39 (1.33, 1.46)</td>
<td>1.51 (1.47, 1.55)</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.98 (0.89, 1.03)</td>
<td>1.14 (1.08, 1.19)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>1.08 (0.88, 1.33)</td>
<td>1.87 (1.61, 2.17)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>66.30 (63.52, 69.07)</td>
<td>58.45 (56.51, 60.39)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean (95% CI).

(*) log transformed was used for in regression model.

3.4 Actigraphy Data of the Raine Study Participants at 22 Years

Figure 3.2 summarises the sleep actigraphy data. In total, 587 participants had at least one night of actigraphy data. Of these, 6 participants were excluded from further analyses as their actigraphy variables were in outlier range [sleep efficiency < 50%, Wake After Sleep Onset (WASO) >120 minutes, sleep latency is less than 5 minutes or more than 60 minutes]. Therefore a total of 581 participants (303 females and 278 males) were used for data analysis of actigraphy data (ranging from one night to 8 nights per participant). There were 497 actigraphy files with sleep diaries and 84 files without any sleep diary data.

A total of 111 participants had less than 3 nights of actigraphy data, while 470 had ≥ 3 nights. The mean values of the actigraphy sleep variables [total sleep time, sleep efficiency, sleep latency and Wake After Sleep Onset (WASO)] were compared among these groups. Because there were no significant differences among those having actigraphy sleep variables for less than 3 nights compared to those with ≥ 3 nights for any sleep variable, data from all participants were pooled (Table 3.5).
Figure 3.2. Flowchart of sleep actigraphy data.

Table 3.5. Comparison of Actigraphy Sleep Parameters Stratified by Total Number of Nights

<table>
<thead>
<tr>
<th>Sleep parameter</th>
<th>Participants having ≥3 nights (Mean ± SD)</th>
<th>Participants having &lt;3 nights data (Mean ± SD)</th>
<th>Difference among groups (Mean ± SE)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time (min)</td>
<td>398.3 ± 52.5</td>
<td>386.6 ± 79.9</td>
<td>11.6 ± 6.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>81.6 ± 8.1</td>
<td>81.2 ± 8.1</td>
<td>0.36 ± 0.6</td>
<td>0.59</td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>11.6 ± 8.4</td>
<td>10.2 ± 10.8</td>
<td>1.3 ± 0.9</td>
<td>0.138</td>
</tr>
<tr>
<td>Wake After Sleep Onset (min)</td>
<td>78.6 ± 32.1</td>
<td>78.1 ± 41.8</td>
<td>0.43 ± 3.3</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Logistic regression was used to compare characteristics between those participating (n = 529) and those not participating (n = 415) in the actigraphy study. These characteristics included variables collected at birth (birth weight and mother’s weight) and at 22 years (age, BMI, waist circumference, smoking, self-reported sleep duration, Table 3.6). There were no significant differences observed in any birth variables or gender distribution among those who participated or did not participate in the actigraphy study, although those who participated in the actigraphy study were 10 months younger, less likely to smoke and less physically active. There was no significant difference in blood pressure between the groups, although those that participated in the actigraphy study tended towards being less obese (P = 0.07).

Table 3.6. Comparative Characteristics of the Raine Study Participants Stratified by Attendance in the Actigraphy Study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total Participants with full data (n)</th>
<th>Participants who did the sleep actigraphy study(n)</th>
<th>Participants who did not do sleep the actigraphy study(n)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (g)</td>
<td>3319 (944)</td>
<td>3318.4 (529)</td>
<td>3320 (415)</td>
<td>0.954</td>
</tr>
<tr>
<td>Mother weight (kg)</td>
<td>59.52 (923)</td>
<td>59.52 (523)</td>
<td>59.51 (400)</td>
<td>0.99</td>
</tr>
<tr>
<td>Age</td>
<td>22.16 (945)</td>
<td>22.11 (530)</td>
<td>22.21 (415)</td>
<td>0.024</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>43/56 (945)</td>
<td>47/53</td>
<td>41/59</td>
<td>0.054</td>
</tr>
<tr>
<td>BMI at age 22 (kg/m²)</td>
<td>25.28 (945)</td>
<td>24.99 (530)</td>
<td>25.64 (415)</td>
<td>0.07</td>
</tr>
<tr>
<td>Waist circumference at age 22 (cm)</td>
<td>83.5 (944)</td>
<td>82.8 (530)</td>
<td>84.5 (414)</td>
<td>0.059</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>118.90 (945)</td>
<td>118.44 (530)</td>
<td>119.48 (415)</td>
<td>0.173</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>67.04 (945)</td>
<td>66.76 (530)</td>
<td>66.96 (415)</td>
<td>0.74</td>
</tr>
<tr>
<td>Smoker at age 22, n [ %]</td>
<td>134 (892) [15.0]</td>
<td>66 (518) [12.7]</td>
<td>69 (384) [18.1]</td>
<td>0.023</td>
</tr>
<tr>
<td>Self-reported duration of sleep (mins)</td>
<td>480.3 (873)</td>
<td>481 (508)</td>
<td>478 (365)</td>
<td>0.599</td>
</tr>
<tr>
<td>Total weekly alcohol consumption (g/wk)</td>
<td>137.20 (896)</td>
<td>126.68 (520)</td>
<td>151.73 (376)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total DAS score</td>
<td>20.28 (845)</td>
<td>20.40 (495)</td>
<td>20.12 (350)</td>
<td>0.83</td>
</tr>
<tr>
<td>Physical Activity (MET-min/wk)</td>
<td>5565 (759)</td>
<td>4700 (457)</td>
<td>6874 (302)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean. Linear regression models for continuous variables.
3.4.1 Actigraphy-derived Sleep Variables

There was no significant difference in the number of males and females with actigraphy data. The average sleep duration (minutes) for males and females combined was 395.6 mins (SD 59.6) and the average sleep efficiency was 81 % (SD 6.2). Mean sleep latency and wake after sleep onset (WASO) were 14.4 mins (SD 8.4 mins) and 71.2 mins (SD 23.7 mins) respectively. Males had a significantly lower sleep duration compared to females (P = 0.023, Table 3.7). Sleep efficiency, latency and WASO were not different between males and females.

Table 3.7. Actigraphy Sleep Characteristics of the Raine Participants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total</th>
<th>Females (n = 303)</th>
<th>Males (n = 278)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sleep Time (min)</td>
<td>395.70 ± 59.51</td>
<td>402.29 ± 59.53</td>
<td>388.47 ± 58.75</td>
<td>0.02</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>81.62 ± 6.22</td>
<td>82.06 ± 6.05</td>
<td>81.13 ± 6.37</td>
<td>0.21</td>
</tr>
<tr>
<td>Sleep Latency (min)</td>
<td>14.40 ± 8.42</td>
<td>13.45 ± 7.6</td>
<td>9.19 ± 13.29</td>
<td>0.56</td>
</tr>
<tr>
<td>Wake After Sleep Onset (min)</td>
<td>71.23 ± 23.70</td>
<td>70.52 ± 22.80</td>
<td>72.09 ± 22.09</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Linear regression models for continuous variables.

3.4.2 Association between Actigraphy Sleep Variables and Cardiometabolic Profile

3.4.2.1 BMI and Sleep Variables

There was no evidence of any linear association between sleep variables and BMI as determined by scatterplot and multivariable spline models (Figures 3.3 - 3.6). In addition based on the results of multivariable spline models, there was no evidence of any nonlinear relationship observed between BMI and total sleep time, sleep efficiency, sleep latency and WASO.

Table 3.8 shows the results of linear regression models examining the association between actigraphy sleep variables and BMI. There was no association between total sleep time (min) in univariate analysis (model 1) and no gender interaction. The association remained non-significant after adjusting for gender (Model 2) and additional inclusion of potential confounders (gender, current smoker, physical activity, alcohol
intake and DASS; Model 3). Similar models indicated no association between BMI and sleep efficiency, latency and WASO.

**Figure 3.3.** Scatter plot association between BMI and total sleep time (min).

Y axis: BMI (kg/m²). Lowess: Locally Weighted Scatter plot Smoothing. Fitted values: Ordinary least square regression fit.

**Figure 3.4.** Scatter plot association between BMI and sleep efficiency (%).

Y axis: BMI (kg/m²). Lowess: Locally Weighted Scatter plot Smoothing. Fitted values: Ordinary least square regression fit.
Y axis: BMI (kg/m²). Lowess: Locally Weighted Scatter plot Smoothing. Fitted values: Ordinary least square regression fit.

Figure 3.5 Scatter plot association between BMI and sleep latency (min).

Y axis: BMI (kg/m²) Lowess: Locally Weighted Scatter plot Smoothing. Fitted values: Ordinary least square regression fit.

Figure 3.6. Scatter plot association between BMI and Wake After Sleep Onset [(WASO); (min)]
Table 3.8. Linear Regression Models of BMI and Sleep Indices

<table>
<thead>
<tr>
<th></th>
<th>Model 1*</th>
<th></th>
<th></th>
<th>Model 2**</th>
<th></th>
<th></th>
<th>Model 3***</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>Total Sleep Time (min)</td>
<td>-0.002</td>
<td>-0.007, .006</td>
<td>0.95</td>
<td>-0.007</td>
<td>-0.007, .007</td>
<td>0.95</td>
<td>-0.002</td>
<td>-0.007, .006</td>
<td>0.91</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>-0.011</td>
<td>-0.07, .05</td>
<td>0.74</td>
<td>-0.016</td>
<td>-0.08, .05</td>
<td>0.74</td>
<td>-0.016</td>
<td>-0.090, .056</td>
<td>0.65</td>
</tr>
<tr>
<td>Sleep Latency (min)</td>
<td>0.003</td>
<td>-0.04, .047</td>
<td>0.88</td>
<td>0.003</td>
<td>-0.04, .04</td>
<td>0.88</td>
<td>0.004</td>
<td>-0.04, .04</td>
<td>0.98</td>
</tr>
<tr>
<td>Wake After Sleep Onset (min)</td>
<td>0.003</td>
<td>-0.01, .01</td>
<td>0.62</td>
<td>0.003</td>
<td>-0.01, .01</td>
<td>0.62</td>
<td>0.002</td>
<td>-0.01, .01</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* Model 1: Unadjusted ** Model 2: adjusted for gender.
* **Model 3: Model 2 + physical activity, current smoker, alcohol intake and total depression and anxiety score (DASS). COEFF: Beta correlation coefficient
3.4.2.2 Waist Circumference and Sleep Variables

Scatterplots showed no linear relationship between waist circumference and sleep variables (Figures 3.7 - 3.10). Multivariable spline modes showed no evidence of any nonlinear relationship between waist and total sleep time ($P = 0.97$), sleep efficiency ($P = 0.66$), sleep latency ($P = 0.15$) and WASO ($P = 0.64$). Table 3.9 shows the linear regression models examining the association between waist circumference and sleep variables. All the models showed waist circumference was not associated with any of the sleep variables, in univariate analysis, or after adjustment for gender and potential confounders.

![Graph showing correlation between waist circumference and total sleep time for females and males.](image)

**Y axis:** Waist circumference (cm). **Lowess:** Locally Weighted Scatter plot Smoothing. **Fitted values:** Ordinary least square regression fit.

**Figure 3.7.** Scatter plot association between waist circumference (cm) and total sleep time (min).
Figure 3.8. Scatter plot association between waist circumference (cm) and sleep efficiency (%).

Figure 3.9. Scatter plot association between waist circumference (cm) and sleep latency (min).
Y axis: Waist circumference (cm). Lowess: Locally Weighted Scatter plot Smoothing. Fitted values: Ordinary least square regression fit.

**Figure 3.10** Scatter plot association between waist circumference (cm) and Wake After Sleep Onset (WASO); (min).
Table 3.9. Linear Regression Models of Waist Circumference and Sleep Indices

<table>
<thead>
<tr>
<th></th>
<th>Model 1*</th>
<th></th>
<th>Model 2**</th>
<th></th>
<th>Model 3***</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>Total Sleep Time (min)</td>
<td>.0002</td>
<td>-.01, .02</td>
<td>0.98</td>
<td>.005</td>
<td>-.01, .02</td>
<td>0.61</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>-.049</td>
<td>-.21, .11</td>
<td>0.56</td>
<td>-.019</td>
<td>-.18, .14</td>
<td>0.81</td>
</tr>
<tr>
<td>Sleep Latency (min)</td>
<td>.084</td>
<td>-1.2, 1.4</td>
<td>0.90</td>
<td>-.019</td>
<td>-1.31, 1.27</td>
<td>0.97</td>
</tr>
<tr>
<td>WASO (min)</td>
<td>.011</td>
<td>-.02, .043</td>
<td>0.49</td>
<td>.009</td>
<td>-.02, .04</td>
<td>0.56</td>
</tr>
</tbody>
</table>

* Model 1: Unadjusted.

** Model 2: adjusted for gender.

***Model 3: Model 2 + physical activity, current smoker, alcohol intake and total depression and anxiety score (DASS).
3.4.2.3 Blood Pressure, Biochemical Variables and Sleep Variables

Scatterplot showed no evidence of linear relationship between sleep variables and systolic and diastolic blood pressure or any of the biochemical variables. In addition multivariable spline modes showed no evidence of any nonlinear relationship between sleep variables and blood pressure or biochemical variables.

Regression analyses showed no evidence of an association between total sleep time and systolic or diastolic blood pressure or any of the biochemical variables measured including glucose, insulin, HOMA-IR, total cholesterol and hs-CRP (Table 3.10). Similarly there were no significant associations between systolic or diastolic BP or biochemical variables and sleep efficiency (Table 3.11), sleep latency (Table 3.12) or WASO.
### Table 3.10. Linear Regression Models of Total Sleep Time and Blood Pressure and Biochemical Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Sleep Time (mins)</th>
<th>Model 1*</th>
<th>Model 2**</th>
<th>Model 3***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
<td>COEFF</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>5.45e-06</td>
<td>-.01, .01</td>
<td>0.99</td>
<td>5.45e-06</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>.011</td>
<td>.002, .021</td>
<td>0.12</td>
<td>.011</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-.0004</td>
<td>-.001, .0007</td>
<td>0.08</td>
<td>-.0003</td>
</tr>
<tr>
<td>Insulin* (mU/L)</td>
<td>-.00006</td>
<td>.0007, .0006</td>
<td>0.85</td>
<td>-.0001</td>
</tr>
<tr>
<td>HOMA - IR*</td>
<td>-.0002</td>
<td>-.0009, .0004</td>
<td>0.51</td>
<td>-.0002</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>.0007</td>
<td>-.0004, .001</td>
<td>0.22</td>
<td>.0005</td>
</tr>
<tr>
<td>hs-CRP* (mg/L)</td>
<td>.0001</td>
<td>-.001, .001</td>
<td>0.83</td>
<td>-.001</td>
</tr>
</tbody>
</table>

*Log transformed used

* Model 1: Unadjusted  ** Model 2: adjusted for gender

* **Model 3: Model 2 + physical activity, current smoker, alcohol intake and total depression and anxiety score (DASS)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1*</th>
<th></th>
<th></th>
<th>Model 2**</th>
<th></th>
<th></th>
<th>Model 3***</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
<td>COEFF</td>
<td>95% CI</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>-0.014</td>
<td>-0.14, 0.11</td>
<td>0.82</td>
<td>0.033</td>
<td>-0.08, 0.15</td>
<td>0.59</td>
<td>-0.031</td>
<td>-0.09, 0.15</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>0.072</td>
<td>-0.014, 0.160</td>
<td>0.10</td>
<td>0.069</td>
<td>-0.018, 0.156</td>
<td>0.12</td>
<td>0.064</td>
<td>-0.030, 0.159</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.005</td>
<td>-0.014, 0.003</td>
<td>0.20</td>
<td>0.004</td>
<td>-0.013, 0.003</td>
<td>0.27</td>
<td>-0.006</td>
<td>-0.014, 0.002</td>
</tr>
<tr>
<td>Insulin* (mU/L)</td>
<td>-0.002</td>
<td>-0.001, 0.004</td>
<td>0.47</td>
<td>0.003</td>
<td>-0.010, 0.004</td>
<td>0.40</td>
<td>-0.002</td>
<td>-0.008, 0.004</td>
</tr>
<tr>
<td>HOMA - IR*</td>
<td>-0.002</td>
<td>-0.009, 0.005</td>
<td>0.56</td>
<td>-0.002</td>
<td>-0.009, 0.005</td>
<td>0.52</td>
<td>-0.002</td>
<td>-0.008, 0.004</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>-0.007</td>
<td>-0.01, 0.004</td>
<td>0.56</td>
<td>-0.008</td>
<td>-0.02, 0.003</td>
<td>0.15</td>
<td>-0.01</td>
<td>-0.02, 0.0001</td>
</tr>
<tr>
<td>hs-CRP* (mg/L)</td>
<td>-0.005</td>
<td>-0.02, 0.01</td>
<td>0.49</td>
<td>-0.008</td>
<td>-0.02, 0.007</td>
<td>0.28</td>
<td>-0.007</td>
<td>-0.02, 0.008</td>
</tr>
</tbody>
</table>

*Log transformed used        * Model 1: Unadjusted.  ** Model 2: adjusted for gender.

* **Model 3: Model 2 + physical activity, current smoker, alcohol intake and total depression and anxiety score (DASS).
Table 3.12. Linear Regression Models of Sleep Latency and Blood Pressure and Biochemical Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1*</th>
<th>Sleep Latency (mins)</th>
<th>Model 2**</th>
<th>Sleep Latency (mins)</th>
<th>Model 3***</th>
<th>Sleep Latency (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
<td>COEFF</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>.07</td>
<td>-.02 ,.16</td>
<td>0.13</td>
<td>.04</td>
<td>-.04 ,.12</td>
<td>0.35</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>.01</td>
<td>-.05 ,.07</td>
<td>0.75</td>
<td>.01</td>
<td>-.05 ,.07</td>
<td>0.70</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>.0006</td>
<td>-.004 ,.005</td>
<td>0.79</td>
<td>-.0001</td>
<td>-.004 ,.004</td>
<td>0.96</td>
</tr>
<tr>
<td>Insulin* (mU/L)</td>
<td>.003</td>
<td>-.001 ,.007</td>
<td>0.16</td>
<td>.003</td>
<td>-.0009 ,.007</td>
<td>0.12</td>
</tr>
<tr>
<td>HOMA- IR*</td>
<td>.002</td>
<td>-.001 ,.007</td>
<td>0.22</td>
<td>.003</td>
<td>-.001 ,.007</td>
<td>0.20</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>-.0007</td>
<td>-.008 ,.006</td>
<td>0.83</td>
<td>-.0002</td>
<td>-.007 ,.006</td>
<td>0.94</td>
</tr>
<tr>
<td>hs-CRP* (mg/L)</td>
<td>-.001</td>
<td>-.01 ,.01</td>
<td>0.86</td>
<td>.0007</td>
<td>-.01 ,.01</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*Log transformed   * Model 1: Unadjusted   ** Model 2: adjusted for gender.

***Model 3: Model 2 + physical activity, current smoker, alcohol intake and total depression and anxiety score (DASS).
3.4.3 Sleep Duration Categorization

There were 447 participants with actigraphy data from both a weekday and a weekend. For sleep duration, the difference between weekends and weekdays was 12.00 ± 3.57 minutes (P = 0.001). Adjusting analyses to compensate for weekend vs weekday data had no effect on the associations between sleep variables, BMI, blood pressure other biochemical variables.

The participants were divided according to whether they were short sleepers (< 6 hrs), normal sleepers (≥ 6 hrs) or normal/long sleepers (≥ 8 hrs, Table 3.12) (224). The percentages of short sleepers and normal/long sleepers were 25.6% (95%CI: 22.2, 29.3), 60.7% (95%CI: 62.8, 70.5) and 7.5% (95%CI: 5.6, 10.0), respectively. As there were only few long sleepers in this population, normal and long sleepers were combined and categorised as one group. Males were more likely to be short sleepers (P = 0.012).

Analyses examining associations between socioeconomic variables and sleep categories showed no evidence of a gender interaction. Among sleep duration categories, there were no significant differences in socioeconomic status such as post school education, income or having job (Table 3.13). The odds of unemployment in those that sleeping ≥ 6hrs was 0.56 compared to those that slept < 6 hrs (P = 0.06). Lifestyle behaviours including smoking, physical activity and weekly alcohol consumption were no different according to sleep duration categories across the two genders (Table 3.14). There were no significant differences between sleep categories for BMI, waist circumference and systolic and diastolic BP. There was also no significant difference in distribution of overweight (OR 0.71, 95%CI: 0.45, 1.12, P = 0.15) and obese participants (OR 0.82, 95%CI: 0.46, 1.45, P = 0.51) between the sleep categories (Figure 3.11).
Table 3.13. General and Cardiometabolic Characteristics According to Sleep Categories

<table>
<thead>
<tr>
<th>Sleep Duration Categories</th>
<th>&lt; 6 hrs (149)</th>
<th>≥ 6 hrs (423)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Employed (%)</td>
<td>63 (89.5)</td>
<td>86</td>
</tr>
<tr>
<td>Post school education (%)</td>
<td>30 (55.5)</td>
<td>36</td>
</tr>
<tr>
<td>High income [&gt;605/wk], (%)</td>
<td>17 (27.0)</td>
<td>40</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>9 (14.0)</td>
<td>8</td>
</tr>
<tr>
<td>Total weekly alcohol</td>
<td>41.5</td>
<td>79.0</td>
</tr>
<tr>
<td>consumption (g/wk)*</td>
<td>(11.1, 97.1)</td>
<td>(5.9, 206.8)</td>
</tr>
<tr>
<td>Physical activity (MET-min/wk)*</td>
<td>1575</td>
<td>3791</td>
</tr>
<tr>
<td>DASS score*</td>
<td>20 (10, 44)</td>
<td>14 (6, 28)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5 ± 7.2</td>
<td>24.7 ± 4.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82.4 ± 16.5</td>
<td>83.8 ± 10.3</td>
</tr>
<tr>
<td>BMI 25-29.9 (kg/m²)</td>
<td>13 (20.6)</td>
<td>28 (32.5)</td>
</tr>
<tr>
<td>BMI &gt;30 (kg²/m)</td>
<td>13 (20.6)</td>
<td>8 (9.3)</td>
</tr>
<tr>
<td>Abdominal obesity, (%)</td>
<td>19 (74.6)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>113.5 ± 8.7</td>
<td>122.1 ± 10.2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>65.8 ± 6.0</td>
<td>66.17 ± 6.3</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD for continuous variables and number (proportion) for categorical variables.

*Not-normally distributed variables presented as median (Q1, Q3).
### Table 3.14. Socioeconomic Status According to Sleep Categories

<table>
<thead>
<tr>
<th></th>
<th>Sleep duration &lt;6 hrs (n=149)</th>
<th>Sleep duration ≥6 hrs (n=432)</th>
<th>OR (95% CI) / Coeff (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post school education (%)</td>
<td>23.83</td>
<td>76.17</td>
<td>1.03 (0.69, 1.52)</td>
<td>0.87</td>
</tr>
<tr>
<td>Income &gt;AU$ 605/week (%)</td>
<td>15.74</td>
<td>84.26</td>
<td>0.96 (0.64, 1.44)</td>
<td>0.85</td>
</tr>
<tr>
<td>Employed (%)</td>
<td>20.16</td>
<td>79.84</td>
<td>0.56 (0.30, 1.03)</td>
<td>0.06</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>12.23</td>
<td>14.46</td>
<td>1.22 (0.68, 2.22)</td>
<td>0.49</td>
</tr>
<tr>
<td>Total weekly alcohol consumption (g/wk)*</td>
<td>56.60</td>
<td>68.00</td>
<td>6.36 (-22.89, 35.61)</td>
<td>0.66</td>
</tr>
<tr>
<td>Physical activity (MET-min/wk)*</td>
<td>3052</td>
<td>2784</td>
<td>223 (-1120.18, 674.18)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Data presented as proportion for categorical variables.

*Median is presented for non-normally distributed variable.

![Figure 3.11. BMI distributions according to sleep duration categories.](image)

3.5 Associations between the Pittsburgh Sleep Quality Index (PSQI) and Socioeconomic, Risk Taking Behaviour and Cardiometabolic Factors in the Raine Study Participants at 22 Years

Overall there were 801 study participants (51.5% females) with completed PSQI questionnaire. Using the global PSQI score of >5, there were 223 (27.8%) identified as having poor sleep quality. Amongst those with poor sleep quality, 57.8% was female
and 42.15% was males (P = 0.01). Within gender 31.5% of females had poor sleep quality while within male gender 24.0% had poor sleep quality.

The socioeconomic, lifestyle, anthropometric and blood pressure characteristics of the study population stratified by sleep quality are presented in Table 3.15. The characteristics of study population stratified by sleep quality and sex are also presented in Supplementary Table S1. Participants with good sleep quality were more likely to be employed (P = 0.05) and to have a higher income (50.2% vs. 40.5%, P = 0.01). There was no significant difference in post-school higher education between the high and poor sleep quality groups (P = 0.43).

With respect to lifestyle behaviours, there was a significantly higher percentage of smoking in the group with poor sleep quality (P = 0.002). Low sleep quality was also associated with a higher DASS score compared to high sleep quality (P < 0.001). No significant difference was observed in the level of alcohol consumption or physical activity according to sleep quality. Low sleep quality was associated with higher regular intake of sleeping pill (P < 0.001).

There was a significant difference in mean BMI among sleep quality subgroups (P = 0.01) with more individuals likely to be overweight/obese if they had poor sleep quality (P = 0.02). There were 129 (22.3% ) overweight and 74 (12.8% ) obese participants in the high sleep quality group, while in the poor sleep quality group these figures were 59 (26.4% ) and 39 (17.4% ), respectively. Participants with poor sleep quality were more likely to have central obesity (P = 0.01). There were no significant differences in mean systolic or diastolic blood pressure or the proportions of individuals with prehypertension and hypertension between the groups.

Those differences in characteristics that differed between the poor sleep quality compared with good sleep quality groups were evident in both females and males (Supplementary Table S1).
Table 3.15. Characteristics of the Raine Study Participants According to PSQI Sleep Quality

<table>
<thead>
<tr>
<th></th>
<th>PSQI ≤ 5 Good Sleep Quality (n = 578)</th>
<th>PSQI &gt; 5 Poor Sleep Quality (n = 223)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females to males ratio, (Female %)</td>
<td>280/298 (48.4)</td>
<td>129/94 (57.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Employed (%)</td>
<td>322 (85.8)</td>
<td>145 (80.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Post school education (%)</td>
<td>297 (51.7)</td>
<td>108 (48.6)</td>
<td>0.43</td>
</tr>
<tr>
<td>High income (%)</td>
<td>273 (50.2)</td>
<td>83 (40.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>67 (11.6)</td>
<td>45 (20.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Total weekly alcohol consumption (g/wk)*</td>
<td>69.3 (11.1, 165.6)</td>
<td>70.3 (11.6, 187)</td>
<td>0.51</td>
</tr>
<tr>
<td>Physical activity (MET-min/week)*</td>
<td>3093 (1455, 6792)</td>
<td>2796 (1142, 5973)</td>
<td>0.79</td>
</tr>
<tr>
<td>Weekly intake of sleeping pill (%)</td>
<td>2 (0.3)</td>
<td>26 (11.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total DASS score*</td>
<td>12 (4,20)</td>
<td>28 (16, 50)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.8 (21.7, 26.6)</td>
<td>24.3 (21.4, 28.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Overweight and obese (%)</td>
<td>203 (35.1)</td>
<td>98 (43.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>79.5 (74.0, 88.7)</td>
<td>80.1 (72.4, 90.2)</td>
<td>0.45</td>
</tr>
<tr>
<td>Central obesity b (%)</td>
<td>78 (13.5)</td>
<td>45 (20.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117.4 (110.8, 125.0)</td>
<td>117.2 (109.6, 123.8)</td>
<td>0.48</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>66.6 (62.2, 71.2)</td>
<td>67.2 (62.2, 71.8)</td>
<td>0.87</td>
</tr>
<tr>
<td>Prehypertension &amp; hypertension c (%)</td>
<td>242 (41.8)</td>
<td>88 (39.4)</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Data are presented as number of participants (%) for categorical variables or median (1rd, 3rd quartiles) for continuous variables.

- Variable not normally distributed
- Overweight was defined as (25 kg/m² > BMI < 30 kg/m²) and obese as BMI > 30 kg/m².
- Central obesity was defined as waist circumference ≥102 cm in males and waist circumference ≥ 84 cm in females.
- Prehypertension was defined as 120 mmHg < SBP ≤ 130 mmHg or 80 mmHg < DBP ≤ 89 mmHg and hypertension was defined as SBP ≥ 140 mmHg or < DBP ≥ 90 mmHg.
Linear regression analyses showed the PSQI score was significantly associated with BMI in univariate analysis (Coeff, 0.23; 95%CI: 0.05, 0.42; P = 0.012) and after adjusting for gender (Coeff, 0.24; 95%CI: 0.06, 0.43; P = 0.008). The association between PSQI score and BMI remained significant (Coeff, 0.19; 95%CI: 0.02, 0.37; P = 0.02) after adjusting for gender and socioeconomic status (i.e. being employed, income) and lifestyle characteristics (i.e. smoking). There were ten morbidly obese participants with a BMI in the range 41 to 55 kg/m², of those four participants with a BMI > 47 kg/m². While an accurate measure of each individual’s BMI, these 4 measurements could be considered as outliers in terms of the statistical distribution of BMI in this particular sample. For this reason, sensitivity analyses were undertaken to examine whether the association between PSQI score and BMI was influenced by these four outliers. Linear regression analysis (unadjusted model) was performed by consecutively removing each measure of BMI in descending order (i.e. between 55 and 47 kg/m²).

By removing BMI > 55 kg/m², the association between BMI and PSQI score became weaker (Coeff, 0.17; 95%CI, 0.02, 0.32; P = 0.02). Sequentially removing each of the four participants with BMI > 47 kg/m², the association between PSQI score and BMI was no longer evident (Coeff, 0.14; 95%CI: -0.004, 0.30; P = 0.05). Removing the four participants with BMI > 47 kg/m² revealed that these morbidly obese subjects were responsible for the association between PSQI and BMI. As there were only ten participants with morbid obesity, there was insufficient power to evaluate this group separately.

Table 3.16 shows the results of unadjusted and adjusted models of linear regression analyses between sleep quality and waist circumference and systolic and diastolic blood pressure. There was no relationship between sleep quality and waist circumference, systolic and diastolic blood pressure with and without adjustment for gender and BMI.

In summary, the prevalence of poor sleep quality according to PSQI in Raine Study participants at 22 years of age was 27.8%, with higher prevalence estimates in females. Participants in the poor sleep quality group were more likely to be smokers, unemployed and with higher DASS scores. These differences were consistent in both males and females. No associations were found between sleep quality and BMI, waist circumference and blood pressure in this study sample.
Table 3.16. Linear Regression Analyses: Association Between Sleep Quality and Waist Circumference, and Systolic and Diastolic Blood Pressure

<table>
<thead>
<tr>
<th>Model</th>
<th>Waist Circumference (cm) Coef (SD) P value</th>
<th>Systolic BP (mmHg) Coef (SD) P value</th>
<th>Diastolic BP(mmHg) Coef (SD) P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: Unadjusted</td>
<td>0.92 (1.13) 0.45</td>
<td>-0.60 (0.85) 0.48</td>
<td>0.08 (0.54) 0.87</td>
</tr>
<tr>
<td>Model 2: Adjusted for gender</td>
<td>1.45 (1.11) 0.19</td>
<td>0.46 (0.77) 0.55</td>
<td>0.05 (0.55) 0.92</td>
</tr>
<tr>
<td>Model 3: Adjusted for gender + BMI</td>
<td>-0.26 (0.39) 0.50</td>
<td>-0.13 (0.71) 0.84</td>
<td>-0.01 (0.54) 0.97</td>
</tr>
</tbody>
</table>
Table 3.17. Characteristics of the Raine Study Participants Stratified by Sex and PSQI Sleep Quality

<table>
<thead>
<tr>
<th></th>
<th>Females (n = 409)</th>
<th>Males (n = 392)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSQI ≤ 5</td>
<td>PSQI &gt; 5</td>
</tr>
<tr>
<td></td>
<td>(n = 280)</td>
<td>(n = 129)</td>
</tr>
<tr>
<td>Employed, %</td>
<td>89.2 (85.0, 92.4)</td>
<td>80.6 (72.7, 86.6)</td>
</tr>
<tr>
<td>Post school higher education, %</td>
<td>54.1 (48.2, 60.0)</td>
<td>51.1 (42.4, 59.8)</td>
</tr>
<tr>
<td>High income, %</td>
<td>46.6 (41.4, 51.8)</td>
<td>34.5 (27.6, 42.0)</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>10.0 (7.3, 13.5)</td>
<td>19.4 (14.2, 25.9)</td>
</tr>
<tr>
<td>Total weekly alcohol consumption (g/wk)</td>
<td>41.7 (6.5, 103.6)</td>
<td>52.7 (11.1, 122.1)</td>
</tr>
<tr>
<td>Physical activity (MET-min/wk)*</td>
<td>2453 (996, 5172)</td>
<td>2238 (924, 4644)</td>
</tr>
<tr>
<td>Weekly intake of sleeping pill, %</td>
<td>0</td>
<td>12.4 (7.6, 19.4)</td>
</tr>
<tr>
<td>Total DASS score*</td>
<td>14 (6, 24)</td>
<td>35 (18, 54)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 (24.0, 25.1)</td>
<td>26.2 (25.0, 27.4)</td>
</tr>
<tr>
<td>Overweight and obese, % a</td>
<td>31.9 (26.8, 37.4)</td>
<td>43.4 (35.6, 51.4)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>79.3 (77.8, 80.8)</td>
<td>81.9 (79.2, 84.5)</td>
</tr>
<tr>
<td>Central Obesity, % b</td>
<td>18.3 (14.3, 23.1)</td>
<td>25.5 (19.1, 33.2)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113.6 (112.6, 114.6)</td>
<td>113.7 (112.1, 115.3)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>67.2 (66.4, 68.0)</td>
<td>66.9 (65.7, 68.0)</td>
</tr>
<tr>
<td>Prehypertension &amp; hypertension, % c</td>
<td>22.6 (18.1, 27.6)</td>
<td>26.0 (19.5, 33.6)</td>
</tr>
</tbody>
</table>

Data are presented as mean (95%CI) for continuous variables and proportion (95%CI) for categorical variables.

- Variable not normally distributed presented as median (1rd, 3rd quartiles).
- a Overweight was defined as (25 kg/m² > BMI < 30 kg/m²) and obese as BMI > 30 kg/m².
- b Central obesity was defined as waist circumference ≥102 cm in males and waist circumference ≥ 84 cm in females.
- c Prehypertension was defined as 120 mmHg < SBP ≤ 130 mmHg or 80 mmHg < DBP < 89 mmHg and hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg mmHg.
3.6 Relationships Between Obstructive Sleep Apnoea Risk and Cardiometabolic Risk in the Raine Study Participants at 22 Years

The Berlin Questionnaire is a validated tool used to identify participants with high risk for Obstructive Sleep Apnea (OSA) (189, 190). It contains three main categories: Category 1 evaluates sleep and snoring behaviour; Category 2 determines the presence of daytime sleepiness and Category 3 assesses the patient's history of obesity (BMI > 30 kg/m²) and/or self-reported hypertension. High risk for OSA is defined when two or more categories are positive (see Method Chapter).

Overall there were 850 study participants (48.5% females) who completed the Berlin Questionnaire. According to the criteria defined by the Berlin Questionnaire, there were 125 (14.7%) participants with high risk for OSA (i.e. positive for two or three categories, Table 3.18). There were 332 (39.1%) participants who were positive for either Category 1 (snoring) or Category 2 (fatigue/sleepiness), and 67 (7.9%) were positive for both categories (snoring and fatigue/sleepiness). Amongst those with high risk for OSA, 45.6% were female and 54.4% were male (P = 0.46). Within each gender, 13.8% of females and 15.5% of males were identified as having high risk for OSA.

Table 3.18. Number and Proportion of Individuals with Positive Berlin Scores for (i) Overall Score, (ii) Each Category and (iii) Combinations of Individual Categories.

<table>
<thead>
<tr>
<th>Categories</th>
<th>n (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1 positive</td>
<td>186 (21.8)</td>
<td>19.2, 24.8</td>
</tr>
<tr>
<td>Category 2 positive</td>
<td>280 (32.9)</td>
<td>29.8, 36.1</td>
</tr>
<tr>
<td>Category 3 positive</td>
<td>160 (18.8)</td>
<td>16.3, 21.6</td>
</tr>
<tr>
<td>Categories 1 and 2 positive</td>
<td>67 (7.9)</td>
<td>6.2, 9.9</td>
</tr>
<tr>
<td>Categories 1 or 2 positive</td>
<td>332 (39.1)</td>
<td>35.8, 42.4</td>
</tr>
<tr>
<td>Total Berlin Score positive</td>
<td>125 (14.7)</td>
<td>12.5, 17.2</td>
</tr>
</tbody>
</table>

Data are presented as number of individuals, N (%) and 95% Confidence Interval (95% CI).

Table 3.19 shows the characteristics of study participants stratified by high risk for OSA. There were no differences between participants at high- compared with low risk for OSA with respect to gender, social factors (employment, education and income), alcohol consumption or physical activity. However, high risk for OSA was associated with a higher BMI, waist circumference and systolic blood pressure. Participants with
high risk for OSA were more likely to be smoker, overweight/obese, hypertensive and having central obesity.

Table 3.19. Characteristics of the Raine Study Participants According to High Risk for Obstructive Sleep Apnoea

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>High Risk for OSA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 850)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No (n = 725)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes (n = 125)</td>
<td></td>
</tr>
</tbody>
</table>

| Female to male ratio, (females %)      | 413/437 (48.5)    | 0.46    |
|                                        | 356/369 (49.1)    |         |
|                                        | 57/68 (45.6)      |         |
| Currently employed, %                  | 83.2 (80.4, 85.7) | 0.32    |
|                                        | 84.5 (81.4, 87.1) |         |
|                                        | 78.1 (68.5, 85.3) |         |
| Post school education, %               | 51 (47.4, 54.5)   | 0.11    |
|                                        | 52 (47.9, 55.8)   |         |
|                                        | 43.1 (33.0, 53.8) |         |
| High income, %                         | 47.4 (43.8, 51.1) | 0.05    |
|                                        | 47.2 (43.1, 51.3) |         |
|                                        | 57.8 (46.7, 68.1) |         |
| Current smoker, %                      | 17.5 (15.0, 20.3) | < 0.001 |
|                                        | 14.4 (11.8, 17.4) |         |
|                                        | 28.8 (20.6, 38.8) |         |
| Total weekly alcohol consumption (g/wk)* | 71.4 (14.3, 185) | 0.24    |
|                                        | 71.3 (16.6, 178)  |         |
|                                        | 73.6 (0, 230)     |         |
| Physical activity (MET-min/wk)*        | 3279 (1386, 660)  | 0.25    |
|                                        | 3292 (1386, 6742) |         |
|                                        | 3262 (1485, 6102) |         |
| Physical activity (MET-min/wk)*        | 3279 (1386, 660)  | 0.25    |
|                                        | 3292 (1386, 6742) |         |
|                                      | 3262 (1485, 6102) |         |
| BMI (kg/m²), mean                     | 25.5 (25.1, 25.9) | < 0.001 |
|                                      | 24.5 (24.1, 24.8) |         |
|                                      | 31.2 (30.0, 32.4) |         |
| Normal weight [BMI <25 kg/m²], %       | 59.7 (56.3, 62.9) | < 0.001 |
|                                      | 66.5 (62.8, 70.0) |         |
|                                      | 20.8 (14.4, 28.9) |         |
| Overweight & obese [BMI≥25 kg/m²], %   | 40.2 (37.0, 43.6) | < 0.001 |
|                                      | 33.4 (29.9, 37.1) |         |
|                                      | 79.2 (71.0, 85.5) |         |
| Waist circumference (cm)              | 83.8 (82.9, 84.7) | < 0.001 |
|                                      | 81.7 (80.8, 82.6) |         |
|                                      | 96.9 (94.0, 99.8) |         |
| Central obesity *, %                  | 17.4 (15.0, 20.1) | < 0.001 |
|                                      | 11.3 (9.1, 14.0)  |         |
|                                      | 53.2 (43.6, 62.6) |         |
| Systolic BP ( mmHg)                   | 118.8 (118.0, 119.5) | < 0.001 |
|                                      | 117.9 (117.1, 118.7) |     |
|                                      | 123.6 (121.2, 126.0) |   |
| Diastolic BP (mmHg)                   | 67.0 (66.5, 67.5) | 0.05    |
|                                      | 66.7 (66.1, 67.2) |         |
|                                      | 68.3 (66.7, 70.0) |         |
| Prehypertension & Hypertension *, %    | 43.8 (40.5, 47.2) | 0.01    |
|                                      | 42.2 (38.5, 46.0) |         |
|                                      | 55.5 (45.9, 64.7) |         |

*Data are presented as mean (95%CI) for continuous variables or proportion (95%CI).
* Variable not normally distributed. Data presented as median (1st, 3rd quartiles) for continuous variables.
* Central obesity was defined as waist circumference ≥102 cm in males and ≥ 84 cm in females.
* Prehypertension was defined as 120 mmHg < SBP ≤ 130 mmHg or 80 mmHg < DBP ≤ 89 mmHg and hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg.
Table 3.20. Metabolic Variables of the Raine Study Participants According to High Risk for Obstructive Sleep Apnoea (OSA)

<table>
<thead>
<tr>
<th></th>
<th>High Risk for OSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 780)</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>4.6 (0.8)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.1 (0.5)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.7 (0.7)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 (0.6)</td>
</tr>
<tr>
<td>Insulin * (mU/L)</td>
<td>7.0 (5.4, 9.6)</td>
</tr>
<tr>
<td>HOMA,IR *</td>
<td>1.5 (1.1, 2.1)</td>
</tr>
<tr>
<td>hs-CRP * (mg/L)</td>
<td>1.0 (0.4, 2.3)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD).

- Variable not normally distributed. Data presented as median (1rd, 3rd quartiles).

Table 3.20 shows the metabolic variables of the participants stratified by high risk for OSA. Univariate regression analyses showed that participants with high risk for OSA had higher triglycerides (P < 0.001) and lower HDL-C levels (P < 0.001). High risk for OSA was also associated with significantly higher insulin (P < 0.001), HOMA-IR (P < 0.001), and hs-CRP levels (P < 0.001).

Using linear regression analyses, high risk for OSA was significantly positively associated with systolic blood pressure (P < 0.001), triglycerides (P < 0.001), HOMA-IR (P < 0.001) and hs-CRP (P < 0.001), and inversely associated with HDL-C (P < 0.001), before and after adjustment for gender and lifestyle factors (Table 3.21).

As blood pressure and metabolic variables can be influenced by BMI (i.e. Category 3 measures in the Berlin Questionnaire), univariate and multivariate analyses were further adjusted for BMI and sensitivity analyses were undertaken.

Tables 3.22 and 3.23 show the results of sensitivity analyses (linear regression models with and without adjustments for BMI) in participants with one positive category (either
snoring or fatigue/sleepiness) and both positive categories (both snoring and fatigue/sleepiness). Such analyses were not performed for Category 3 due to the focus of this category on blood pressure and obesity.

Those with only one positive category showed no association with systolic blood pressure and metabolic variables before or after adjustment for BMI (Table 3.22). In those with two positive categories, there was a significant inverse association with HDL-C, and a positive association with HOMA-IR and hs-CRP levels, before adjustment for BMI. These associations were no longer statistically significant after adjustment for BMI (Table 3.23).
Table 3.21 Linear Regression Analyses Between High Risk for OSA (Total Berlin Questionnaire) and Systolic and Diastolic Blood Pressure and Metabolic Variables

<table>
<thead>
<tr>
<th>Model</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>Triglycerides (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>HOMA-IR *</th>
<th>hs-CRP* (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: unadjusted</td>
<td>5.3</td>
<td>1.5</td>
<td>0.2</td>
<td>-0.1</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>(3.0, 7.7)</td>
<td>(0.01, 3.1)</td>
<td>(0.1, 0.3)</td>
<td>(-0.2, -0.1)</td>
<td>(0.2, 0.4)</td>
<td>(0.5, 0.9)</td>
</tr>
<tr>
<td>Model 2: adjusted for gender</td>
<td>5.0</td>
<td>1.5</td>
<td>0.2</td>
<td>-0.1</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>(2.9, 7.1)</td>
<td>(0.02, 3.1)</td>
<td>(0.1, 0.3)</td>
<td>(-0.2, -0.1)</td>
<td>(0.2, 0.4)</td>
<td>(0.6, 1.0)</td>
</tr>
<tr>
<td>Model 3: adjusted for gender + lifestyle factors</td>
<td>4.9</td>
<td>1.7</td>
<td>0.2</td>
<td>-0.16</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>(2.7, 7.7)</td>
<td>(-0.1, 3.6)</td>
<td>(0.1, 0.3)</td>
<td>(-0.2, -1.0)</td>
<td>(0.2, 0.4)</td>
<td>(0.6, 1.0)</td>
</tr>
</tbody>
</table>

Data presented as Coeff (95% CI).

*log transformed.
Table 3.22. Linear Regression Analyses Between One Positive Category (Snoring or Sleepiness) and Systolic Blood Pressure and Metabolic Variables

<table>
<thead>
<tr>
<th>Model</th>
<th>SBP (mmHg)</th>
<th>Triglycerides (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>HOMA-IR *</th>
<th>hs-CRP* (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff (95% CI)</td>
<td>P value</td>
<td>Coeff (95% CI)</td>
<td>P value</td>
<td>Coeff (95% CI)</td>
</tr>
<tr>
<td>Model 1: unadjusted</td>
<td>0.9 (-0.6, 2.5)</td>
<td>0.2</td>
<td>-0.005 (-0.07, 0.06)</td>
<td>0.8</td>
<td>0.007 (-0.04, 0.06)</td>
</tr>
<tr>
<td>Model 2: adjusted for gender</td>
<td>0.38 (-1.0, 1.8)</td>
<td>0.6</td>
<td>-0.01 (-0.07, 0.06)</td>
<td>0.8</td>
<td>0.02 (-0.02, 0.07)</td>
</tr>
<tr>
<td>Model 3: adjusted for gender + BMI</td>
<td>0.08 (-1.2, 1.4)</td>
<td>0.9</td>
<td>-0.02 (-0.08, 0.04)</td>
<td>0.5</td>
<td>0.02 (-0.02, 0.07)</td>
</tr>
</tbody>
</table>

Data presented as Coeff (95% CI). *Log transformed.
Table 3.23. Linear Regression Analyses Between Both Positive Categories (Snoring or Sleepiness) and Systolic Blood Pressure and Metabolic Variables

<table>
<thead>
<tr>
<th>Model</th>
<th>SBP (mmHg)</th>
<th>Triglycerides (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>HOMA-IR *</th>
<th>hs-CRP* (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff (95% CI)</td>
<td>P value</td>
<td>Coeff (95% CI)</td>
<td>P value</td>
<td>Coeff (95% CI)</td>
</tr>
<tr>
<td>Model 1: unadjusted</td>
<td>2.1 (0.7, 2.5)</td>
<td>0.2</td>
<td>0.07 (-0.07, 0.2)</td>
<td>0.3</td>
<td>-0.11 (-0.2, -0.4)</td>
</tr>
<tr>
<td>Model 2: adjusted for gender</td>
<td>2.3 (0.2, 5.0)</td>
<td>0.7</td>
<td>0.06 (-0.08, 0.2)</td>
<td>0.8</td>
<td>-0.11 (-0.2, -0.04)</td>
</tr>
<tr>
<td>Model 3: adjusted for gender + BMI</td>
<td>0.6 (2.0, 3.1)</td>
<td>0.6</td>
<td>-0.04 (-0.1, 0.01)</td>
<td>0.5</td>
<td>-0.05 (-0.1, 0.008)</td>
</tr>
</tbody>
</table>

Data presented as Coeff (95% CI). \*Log transformed.
Chapter 4:
Discussion and Conclusions
The work presented in this thesis represents one of the largest studies evaluating the associations between various aspects of sleep and cardiometabolic risk factors in young adults from the general population. It addresses a gap in our understanding of these relationships in young adults, as the majority of previous studies have focused on older populations. All measurements were undertaken in the Western Australian Pregnancy Cohort (Raine) Study, one of the largest successful prospective cohorts of pregnancy, childhood, adolescence and now early adulthood to be carried out anywhere in the world.

The Raine Study provided an excellent resource with which to study the associations between sleep and cardiometabolic factors in young adults, as it has collected a wealth of data on familial, socioeconomic, lifestyle, anthropometric, biochemical and clinical factors when the participants were approximately 22 years old. These factors and other variables were used as potential confounders, strengthening the analyses of the presence or absence of any cardiometabolic associations by adjusting for many potential covariates. A unique aspect of the study is the use of objective measures of sleep through actigraphy in addition to subjective measures, including the PSQI and Berlin Questionnaires.

4.1 Sleep Duration

Compared with previous epidemiological studies of young adults, the mean sleep duration was lower in the current study. The average sleep duration in the Raine Study participants was less than the National Sleep Foundation’s recommendation for 7–9 hours of sleep in those aged 18–25 years (225).

The mean sleep duration of participants was 6.6 hours (396.10 ± 59.5 minutes), with 25.5% classified as short sleepers (< 6 hours). In a study of 658 adults with a mean age of 24 years, mean sleep duration was 7.7 hours (226). According to the US National Longitudinal Study of Adolescent Health that evaluated 15,700 individuals aged 13–32 years, the average sleep duration was 8.5 hours (SD 2.6) in those aged 22 years (227). This latter study also reported short sleep duration (< 6 hours) at a rate of 8.9% in those aged 19–22 (227). Another study of 4714 participants with a mean age of 27 years reported mean sleep duration of 6.9 hours (228).
There is strong evidence showing a decline in sleep duration in adolescents (participants aged 13–18 years) caused by puberty onset sleep phase delay and social factors, such as early school start time (229, 230).

However, it is not clear if sleep duration curtailment continues during early young adulthood (19–22 years). Two studies have suggested that there is an increase in sleep duration beginning at age 19, a result of the typical transition from high school, followed by a decrease in sleep duration at age 22 (226, 227). It has also been suggested that early young adulthood is a period when individuals possess a greater ability to choose their own sleep schedule compared with other age periods (227).

Large epidemiological studies have shown variable rates of short sleep duration in young adult populations. The National Health and Nutrition Examination Survey 2005–2008 examined 2,391 young adults (aged 20–39) and reported that 14% of individuals experienced short sleep duration (< 6 hours) (231). Although a study of 17,465 university students aged 17 to 30 years reported that short sleepers comprised 6% of the cohort (232).

In the current study, the percentage of short sleepers was higher than in previous reports (227, 229, 232). However, it should be emphasised that all the previous epidemiological studies in young adults measured sleep using sleep questions and / or questionnaires, which can be strongly influenced by recall bias.

4.2 Association between Actigraphy-derived Sleep Variables and Cardiometabolic Risk Factors

The results of the current study showed no association between actigraphy-derived sleep variables (sleep duration, sleep efficiency, sleep latency and Wake after Sleep Onset [WASO]) and cardiometabolic risk factors in this young population.

This study is one of only a few to evaluate actigraphy-derived sleep variables in a healthy young adult population. Generally previous association studies using actigraphy have not reported sleep latency and WASO, as these measures are known to be accompanied by large errors in their estimations (215, 216, 233). However, as sleep latency and WASO are considered as two of the four main variables measured by actigraphy, both are included in analyses in this study. For this reason and in consistent
with previous studies, the Discussion chapter of this thesis focuses mainly on the association between sleep duration and cardiometabolic factors.

4.2.1 Association between Sleep Duration and Efficiency and Body Weight

In this study, there was no association between sleep duration and body weight. Further, there was no significant difference in the distribution of overweight and obese participants among sleep categories (short vs normal/long sleepers).

Previous systematic reviews examining the association between sleep duration and body weight in children and adolescents have consistently shown short sleep duration to be associated with increased body weight (53, 234). In contrast, the association between sleep duration and body weight in adults has been less consistent, and appears to differ throughout the different stages of adulthood (53, 234, 235). For example, studies with larger sample sizes drawn from general populations have shown a negative linear association in young adults (235-237), a U-shape association in the middle-aged (235, 236, 238) and an absence of any association in the elderly (235, 239-241). The results of the National Health and Examination Survey (NHANES) of 5607 adults aged 16 and over showed a linear negative association between sleep duration and higher BMI only in those aged 18–49, a U-shaped association in middle-aged adults and no evidence of a linear relationship among older age groups (235). The results of the present study are contrary to those previous studies reporting a negative linear association between sleep duration and body weight in young adults.

A potential reason for these discrepant findings is the lack of objective measures of sleep in most previous studies (234, 242). Only a few small studies have used objective measures of sleep in a young population (243, 244). One such study of 132 young adults (mean age of 23 years) measured sleep by actigraphy for 1–2 weeks and found no association between sleep duration and body weight or adiposity (fat mass and fat-free mass measured by bioelectrical impedance analysis) (243). Another study of 330 young women (mean age of 20 years) measured sleep by actigraphy for 7 nights and reported no association between sleep duration and weight or adiposity (244). Therefore, the results of the current study, with a substantially larger sample size, agree with these studies.
Several studies have reported that weight gain was less likely to occur if short sleepers at the time of the study had reported that they had been short sleepers for many years prior to study commencement (245, 246). Such findings have suggested that as the body physiologically adjusts itself to the effects of chronically short sleep, such individuals do not continue to gain weight linearly throughout the period of being short sleepers (245, 246). Thus, it has been suggested that in order to detect any physiological change, the start point of the study needs to be around the acute phase of having short sleep (i.e., start of the short sleep period). In the current study, this was not accounted for as initial measurements were obtained when the participants were 22 years of age. In addition, it has been suggested that having extra longer sleep (recovery) on non-working days can modify sleep debt (sleep deficiency) and might be an important factor in body weight regulation (247).

The present study found no relationship between sleep efficiency and body weight. Currently, only a few studies have investigated the role of actigraphy-derived sleep efficiency on body weight in young adult populations. The two above-mentioned studies of young adults reported no association between sleep efficiency and body weight; however, both studies found an inverse association between sleep efficiency and fat mass (243, 244). No association between sleep efficiency and any anthropometric measurement was found in the present study. Such inconsistent results may be due to the way in which body composition is measured. Specifically, the present study used BMI as an overall measure of body composition, whereas other studies have used air displacement plethysmography (244) or bioelectrical impedance (243), both of which can purportedly distinguish between fat and fat-free mass.

Considering the absence of any association between sleep and body weight in the current study, and consistent with the findings of the two other studies that have used objective sleep measures in this age range (243, 244), it seems unlikely that sleep duration and efficiency are related to body weight in young adults.

**4.2.2 Association Between Sleep Duration and Glucose and Insulin Resistance**

This study found no evidence of any associations between actigraphy-based sleep duration and fasting glucose or insulin resistance in this young adult population.
These results contrast with cross-sectional studies that have shown higher levels of fasting plasma glucose, insulin and insulin resistance (HOMA-IR) among short sleepers in middle-aged adults (95, 248). In the Quebec Family Study of 740 adults aged 21–64 years, the odds of type 2 diabetes/impaired glucose tolerance was 2.09 (1.34–2.98) for those with 5–6 hours of sleep, after adjustment for potential confounding variables (95). In a study of 161 type 2 diabetic participants (119 women, 42 men) with a mean age of 57, sleep duration was a significant predictor of haemoglobin A1c (HbA1c), a key marker of long term glycaemic control (249). The absence of any association between sleep and glucose metabolism in this study contrasts with the studies of middle-aged adults showing an association between sleep duration and increased risk of developing type 2 diabetes. According to the results of a meta-analysis on seven studies involving 10307 adults, there was no significant difference in the degree of insulin resistance measured by HOMA-IR between short and long sleepers in those participants without Obstructive Sleep Apnoea (OSA) or diabetes (250). The majority of studies with positive relationships between short sleep duration and poor glycaemic control were carried out in either diabetic patients or individuals with OSA. In addition it has suggested that sleep recovery during non-working days can improve insulin sensitivity (251). Consistent with the results of this meta-analysis mentioned, our results also indicated that there is no association between insulin resistance and sleep duration in a healthy young population.

4.2.3 Association between Sleep Duration and Blood Pressure

Several studies reported an association between short sleep duration and increased blood pressure in different age groups, including adolescents and young and middle-aged adults (104, 252-255), while others report variable associations (256-259). The present study undertaken in young healthy adults showed no association between sleep duration and increased blood pressure.

In a study of 331 healthy young adults with a mean age of 22 years, Fujiyama et al. (254) showed that short sleep duration (5 hours or less) was associated with elevated 24-hour blood pressure. Similarly, a study of 246 adolescents with a mean age of 16 years showed that actigraphy-assessed sleep duration was inversely related to 24-hour systolic and diastolic blood pressure in adolescents (255). The results of the Coronary Artery Risk Development in Young Adults (CARDIA) cohort study of 578 adults aged 33–45 years using 3 days of actigraphy-assessed sleep found that short sleep duration
predicted higher systolic and diastolic blood pressure levels cross-sectionally and over five years follow-up after adjustment for a wide range of covariates (104).

Other studies have reported variable associations between short sleep duration and blood pressure (256-258). For example, the NHANES study of 4810 adults from the general population found that short sleep duration (≤ 5 hours) was associated with increased blood pressure only in those aged between 32 and 59 years (257). In a study of 1214 middle-aged adults (30–54 years), Hall et al. (258) was unable to find any association between short sleep duration and increased blood pressure. Similarly, the multi-ethnic Healthy Life in an Urban Setting (HELIUS) Study of 12805 adults aged 18–70 years showed no relationship between short sleep duration (< 7 hours) and hypertension after adjusting for potential covariates in most ethnic groups, except Turks (259).

The inconsistent findings between studies may be a consequence of the different methods used to measure blood pressure (e.g. 24-hour monitoring vs office measurement) or to the use of different categorisations for short, normal and long sleep duration. In view of the results from the two largest studies to date—NHANES, which showed an association only in those aged 32–59 years, and the HELIUS Study, which showed no relationship—it appears that the participants in current study were too young for any strong relationship between sleep duration and blood pressure to be evident.

4.2.4 Association Between Sleep Duration and Inflammatory Markers and Lipids

In this study of Raine participants at 22 years, actigraphy-derived sleep duration showed no association with hs-CRP and lipid levels.

Previous studies that have examined the association between sleep duration and inflammatory markers such as CRP have reported inconsistent findings (259-263). For example, the Cleveland Family Study of 614 adults, each extra hour of habitual sleep duration (based on self-reported questionnaire) was significantly associated with increased CRP levels, while each hour of reduction in sleep was associated with no change in CRP levels (264). In contrast, in the Wisconsin Sleep Cohort Study of 907 Caucasian participants, which evaluated sleep by both questionnaires and PSG, no association was detected between sleep duration and CRP levels (106). Similarly,
Suarez et al. (265) study on 127 healthy young males and females found no association between sleep duration and CRP levels.

Several studies have shown that CRP levels can be influenced by age, BMI and cardiometabolic risk factors, including hypertension and diabetes (266, 267). In this regard, a study of 5,003 middle-aged adults showed that the association between short sleep duration and CRP levels was attenuated, and disappeared after adjustment for BMI, systolic blood pressure and cholesterol levels (267). The results of the current study agree with previous reports that indicate no association between sleep and CRP levels.

The current study also found no relationship between sleep duration and fasting lipids. These results are consistent with data from the HELIUS Study of 12,805 adults aged 18–70 years, which reported no association between sleep duration and lipids (259). Other epidemiological studies examining the association between sleep duration and lipids have shown inconclusive results. For example, while some studies have reported an association between short sleep duration and increased odds of high triglycerides (110, 258), Aora et al (268) have reported an association between high triglycerides and long sleep duration. Studies examining the relationship between sleep duration and HDL cholesterol have produced similarly inconsistent results. In a longitudinal study of 8,766 healthy males aged 40–55 years, moderate (5–7 hours) to long (> 7 hours) sleep duration was reported to decrease the risk of having low HDL cholesterol after 6 years follow-up (111). In contrast, the Korean National Health and Nutrition Examination Survey of 13609 adults aged 20 and over found no association between short sleep duration and HDL cholesterol (109).

These inconsistencies results are most likely caused by differences in age distribution of study subjects, different cut-offs used to define short sleep duration or incomplete adjustment for important confounding factors, including hypertension, diabetes and stress level. In general, very few cross-sectional studies have been undertaken to evaluate the association between sleep duration and lipid profile and those that have been done have reported inconclusive findings. Further studies are required to better understand the relationship, if any, between sleep duration and the lipid profile.
4.2.5 Summary of Evidence of Association Between Sleep Duration and Cardiometabolic Risk Factors

The current study of 22-year old Raine participants showed no relationship between actigraphy-derived sleep duration and cardiometabolic risk factors. Such a finding is consistent with a study using objective sleep measures in 492 adults aged 35–64 years, which also showed no association between sleep and cardiometabolic risk factors between sleep duration and hypertension, obesity and diabetes (269). Another cross-sectional study of 1524 employees (92% females with a mean age of 36 years) evaluating sleep duration by using actigraphy, showed that different dimensions of work-family conflict adversely affect sleep duration and cardiometabolic health (270).

Previous studies that have reported an association between sleep and cardiometabolic factors have used subjective sleep measures. This is an important consideration as such measures can be influenced by recall bias, which could confound any reported associations (26).

Another significant source of variability between study findings is the definition of short and long sleep duration among studies. For instance, some studies defined normal sleepers as having sleep duration of > 6, ≥ 7, 7–8 and 7–9 hours (271) and others have used different categorisations for short, normal and long sleepers (227, 231, 232). The specific criteria used in the present study for short sleep duration (< 6 hours) or the short period of monitoring (7 days) compared with other studies (2 weeks) may have contributed to the lack of association between sleep duration and cardiometabolic risk factors. Furthermore, the lack of association using sleep duration as a continuous measure supports the lack of associations among categorical sleep groups.

It is notable that some studies have reported associations between cardiovascular function and sleep in young adults. In general these studies have used more specific or more targeted measurements of cardiovascular function. For example, several studies have shown a relationship between sleep duration on coronary calcification and circulation (272, 273). In a study of 26 healthy males with a mean age of 29 years, Sekine et al. (272) showed lower coronary flow circulation after sleep restriction. Similarly, a longitudinal study of 494 participants aged 35–47 years found that each additional hour of sleep was associated with a 33% reduction in coronary calcification (273).
Further, changes in 24-hour blood pressure and sympathetic activity have been reported to occur early with insufficient sleep (274, 275). Thus, it is better to use more sensitive assessment for vascular function, such as 24-hour blood pressure monitoring, to detect blood pressure variation, sympathetic activity and heart rate variability. Alternatively, Doppler ultrasound evaluation could track changes in vascular endothelium.

4.3 Sleep Quality (PSQI Questionnaire) and Socioeconomic, Lifestyle and Cardiometabolic Risk Factors

Using the PSQI Questionnaire to assess sleep quality, our study showed that 28% of the Raine participants experienced poor sleep quality, with females more likely to fall into this category. Participants with poor sleep quality were more likely to be unemployed, smoke and have higher DASS scores. No association was found between sleep quality and anthropometric measures, including BMI and waist circumference.

The prevalence of poor sleep quality in our study is consistent with a study of Mongolian university students that reported 27.8% of the students had poor sleep quality (276). Consistent with our data, previous studies have shown that females have poorer sleep quality than males (115, 277). A study of 26,851 members of the general population living in rural areas (mean age of 38 years) and urban areas (mean age of 42 years) of Hunan, China, showed a higher PSQI score for females than males (277). Similar to our findings, the results of the BiDirect Study, which included 753 participants with a mean age of 53, showed that poor sleepers (35%) were more likely to be females (115).

An association between unemployment and poor sleep quality has been reported in previous studies (278, 279). In a German community sample of 9,284 participants aged 18–80 years, poor sleep quality was twofold higher (OR, 2.11; 95% CI = 1.73 – 2.53; P < 0.001) in unemployed participants (278). Additionally, Wong et al. (279) reported poor sleep quality was three times higher in unemployed participants. The results of our study are consistent with these previous studies that indicate unemployment is associated with poor sleep quality.

An association between lifestyle factors and sleep quality has been reported in previous studies; in particular, among those with poor sleep quality there is a higher prevalence of cigarette smoking (118, 278, 280). Similar to the results of this study, Dugas et al. (280) study of 405 young adults found that cigarette smokers were more likely to have
poor sleep quality. Our results also indicated a higher percentage of smokers in those with poor sleep quality.

Consistent with previous studies, low sleep quality in our study was associated with adverse psychological factors, including higher depressive and anxiety symptoms (115, 280). These results highlight that lifestyle factors in young adults associate with poor sleep quality.

We found no association between poor sleep quality and obesity or a range of cardiometabolic measures. The association between poor sleep quality and overweight/obesity has been reported in studies of young populations (244, 281, 282). However, a study of 702 young college students with a mean age of 21 years found that PSQI scores were not correlated with anthropometric measures (283). The results of the present study are in agreement with this; no association was found between poor sleep quality and anthropometric measures, including BMI and waist.

Our study did not find any association between blood pressure and poor sleep quality. These results contrast with previous studies that found a positive association between blood pressure and poor sleep quality (269, 284, 285). However, these findings are confined to studies with older participants (122, 269, 285). In a study of 5461 Chinese adults stratified into two groups, with the age of 45 years as the cut-off, poor sleep quality was associated with hypertension in male subjects of all ages and in female subjects aged ≤ 45 years (286).

The lack of association in this study could be because of the effects of young age and the lower rate of hypertension in this age group. Mechanistically, this may be caused by sympathetic nervous activation accompanying aging, which could be a consequence of sleep disorders and may contribute to hypertension (287).

4.4 Relationships Between Risk of Obstructive Sleep Apnoea and Socioeconomic, Lifestyle and Cardiometabolic Risk Factors

This study aimed to evaluate high risk for OSA (by using the Berlin Questionnaire) and its relationship with cardiometabolic risk factors. The Berlin Questionnaire has three main categories: sleep/snoring behaviour, daytime sleepiness and obesity or hypertension.
Our data show that 14.7% of the participants were at high risk of OSA, with a similar distribution between genders. Most of the previous studies examining this relationship were undertaken on middle-aged or elderly populations, although there are several studies of young adults (133, 135, 288). The prevalence for high risk of OSA in our study sample (14.7%) is higher than what has generally been reported for young adult populations (133, 135, 289). For example, Wosu et al. (135) and Pensuksan et al. (290) reported that the prevalence of high risk for OSA in young non-Western populations was between 6% and 9%, respectively. Additionally, a National Sleep Foundation Sleep in America 2005 poll with 1,506 respondents reported the rate of high risk for OSA was 18% among those aged 18–29 years (289). The likely explanation for the difference in prevalence rates between studies are the differences in obesity distribution among Western and non-Western studies. For example, in our study the percentage of high risk for OSA decreased to 8% when obesity was excluded from the obesity/hypertension category of the Berlin Questionnaire.

For Raine participants, there was no significant difference in gender distribution among those with or without high risk for OSA. Similar to our results, a study of 916 college students with a mean age of 21 found no gender difference among OSA categories (135). However, current evidence indicates that OSA is more prevalent in males than females (163), which is likely related to males having more fat deposits in the neck, trunk and abdomen, making them more susceptible to OSA (290, 291). It is well known that obesity and central adiposity are strong risk factors for sleep apnoea through mechanisms such as mechanical effects and the effects of adipokines on airway neuromuscular control through the central nervous system (291). In our study, the prevalence of obesity was similar in both genders, and males were less likely to have abdominal obesity compared with females (20% vs 37%, P < 0.001).

Evaluation of lifestyle variables in relation to risk of OSA showed that only smoking was associated with high risk of OSA in this young adult population. This finding is consistent with other studies of similar age populations (135, 290). Wosu et al. (135), in a study of 916 college students with a mean age of 22, found an association between high risk for OSA and age and cigarette smoking. Similarly, a study of 2,911 young adults with a mean age of 20 years reported a relationship between high risk for OSA and age, male sex and cigarette smoking (290).
Cigarette smoking can induce OSA or increase its severity through mechanisms such as increased upper airway inflammation, alternation in sleep architecture and upper airway neuromuscular function (292).

The current study found no associations between high risk for OSA and a range of cardiometabolic risk factors, including blood pressure and biochemical variables. The prevalence of overweight/obesity and central obesity in those at high risk for OSA was high in the present study (79% and 53%, respectively). Such findings are consistent with several previous studies showing a relationship between high risk for OSA and obesity (135, 290, 293). In a study of 916 college students, Wosu et al. (135) reported 10-fold increased odds of general obesity and 2.7-fold increased odds of central obesity. Similarly, Qu et al. (137) found in their study of 559 adolescents and young adults with a mean age of 20 years that those at high risk for OSA had higher BMI and waist circumferences. Mahboub et al. (146) reported that among 1214 adults with a mean age of 36, nearly 70% of those at high risk for OSA had BMI ≥ 30 kg/m², while 70% of those with low risk for OSA had BMI < 30 kg/m².

Obesity and increased fat deposits in the neck and abdomen can contribute to OSA through mechanisms such as reduced pharyngeal lumen size (due to fat deposition in the airway walls). In those with central obesity, due to increased abdominal pressure, end-expiratory lung volumes reduce, which contributes to reducing tracheal traction and increasing collapsibility of the upper airway (294). There is good evidence that OSA can also cause obesity and metabolic dysfunction (140). Insufficient sleep, sleep fragmentation and daytime sleepiness can increase food intake through mechanisms such as alternation in appetite regulatory hormones, physical activity and energy balance (80, 295-297).

We found no significant association between high risk for OSA and blood pressure based on our sensitivity analyses that excluded the obesity/hypertension category from the Berlin Questionnaire and used a classification based on the presence of snoring and sleepiness. The association between OSA and hypertension could be caused by an independent effect of OSA on increased blood pressure or obesity-induced hypertension in OSA (222). However, data from the Sleep Heart Health Study of 6,132 participants (aged ≥ 40 years) indicates that the association between OSA and hypertension was only significant (OR, 1.54; 95% CI= 1.02 - 2.31) in obese participants (298). In support of this hypothesis, longitudinal analyses of the Sleep Heart Health Study with 2,470
participants found no statistically significant association between OSA and hypertension following adjustment for baseline BMI (299). Another study on 2,911 college students found a significant association only in overweight/obese participants or those with central obesity (290).

The current data from the Raine participants supports previous reports suggesting obesity is the main factor contributing to hypertension in individuals with OSA.

Several mechanisms likely explain the association between OSA and increased blood pressure in obesity. These mechanisms include increased sympathetic activity, intermittent hypoxia, elevated oxidative stress and inflammatory markers, and increased angiotensin level, in addition to endothelial and kidney dysfunction (127, 139, 300). As the independence of the relationship between OSA and hypertension is still unclear, further research is required to determine whether the association is dependent on obesity.

The current analysis of the Raine participants at 22 years showed higher levels of insulin and HOMA-IR in those at high risk for OSA. However, sensitivity analyses showed the association was no longer present after accounting for obesity. Previous studies have reported conflicting results regarding the relationship between measures of insulin resistance and OSA. In a study of 52 young, healthy, men of normal weight aged 18–30 years, OSA was independently associated with a 27% decrease in insulin sensitivity and a 37% increase in insulin levels (164). Conversely, Kapsimalis et al. (301), in a study of 67 middle-aged men, showed that after adjustment for obesity there were no significant differences in insulin and glucose levels or the HOMA-IR index between participants with or without OSA. Additionally, a case-control study in which OSA patients and a control group were matched for age, gender and BMI reported no difference in insulin levels in OSA between the two groups (302). In adolescents, many of the positive associations between OSA and insulin level and HOMA-IR disappear after controlling for adiposity and BMI (303). It is likely that these inconsistent results are due to variations in OSA assessment (e.g., using the PSG or Berlin Questionnaire) or study populations (e.g., general population, participants having OSA or type 2 diabetic patients with OSA).

There was no association between high risk for OSA and hs-CRP after adjusting for BMI. Such a finding suggests that the association between OSA and hs-CRP is likely to be a consequence of the coexistence of obesity in high risk OSA participants.
Conversely, several previous reports found an independent relationship between OSA and increased CRP level after controlling for BMI (181, 304, 305). According to a study of 1835 adults with a mean age of 58 years, moderate and severe OSA participants had 1.7 and 2.0 times the risk of having higher hs-CRP after adjustment for obesity (181). Similarly, in a study that compared 22 newly diagnosed OSA patients (mean age of 48 years) with 20 healthy adults (matched for age and BMI), Shamsuzzaman et al. (305) reported higher CRP levels in OSA participants. However, in agreement with the findings from the current study, Ryan et al. (306) showed no association between CRP and OSA in 110 men with a mean age of 40 years. It is likely that any association between CRP and OSA may be related to increased CRP levels in adipose tissue as a consequence of obesity (295). Moreover, it should be noted that a positive relationship between CRP and OSA has, to date, been reported exclusively in older participants.

4.5 Strengths and Limitations

The major strength of this study is the use of data from a large population-based cohort of 22-year-olds. The study population is representative of the Western Australia population, with all participants having the same age and similar gender distribution (307). The large sample size and comprehensive anthropometric, clinical and socioeconomic and lifestyle information allowed for adjustment of association analyses for a wide range of confounders. Objective assessment of sleep using actigraphy is another strength of the study, as most previous studies of large cohorts have used subjective evaluation of sleep through questionnaires.

There are several limitations to the study. Firstly, its cross-sectional design means that causality cannot be inferred. Comparing the Raine Study participants who attended the sleep actigraphy study monitoring with those who did not, showed that those in the sleep actigraphy study were less physically active and had a smaller waist circumference. Thus, the presence of selection bias in the actigraphy study is possible. Secondly, few participants were long sleepers or very short sleepers. A consequence of this is that it was not possible to categorise by the extremes of sleep and independently investigate such groups. Thirdly, as body fat was not directly measured in our study (e.g., via a DEXA scan or impedance), we were unable to assess changes in fat mass independently of BMI. Fourthly, 24-hr blood pressure was not measured in our study, so any such variability over the course of day and night, particularly nocturnal blood pressure dipping, could not be assessed.
The advantage of examining young adults is that these participants are less likely to have co-morbidities that can confound the analyses. However, it is becoming increasingly apparent form previous studies that healthy young individuals might require more sensitive measurements (than the standard measurements) to detect metabolic impacts. For instance, Donga et al showed that by using the hyperinsulinaemic euglycemic clamp in nine healthy lean volunteers, there was impairment in insulin sensitivity (increased endogenous glucose production) following a night of sleep restriction (4 hours of sleep), despite their normal fasting insulin and glucose levels (308). Similarly, Harsch et al showed impaired insulin sensitivity by using the hyperinsulinaemic euglycaemic clamp in forty OSA patients (309). Therefore, fasting insulin, glucose or HOMA measurements as used in the current study, may not be sufficiently sensitive for detecting early changes in glucose metabolism in young healthy adults.

Actigraphy data collected over seven days has been used to evaluate the predicting roles of a number of parameters including circadian rhythm, diurnal activity, napping and sleep fragmentation, on cardiometabolic risk factors (310) However, the present study was unable to collect actigraphy data for seven consecutive days in all participants, thus the effects of these sleep parameters could not be examined. Similarly, the present study was unable to obtain complete sets of sleep diary data which limited our ability to determine correlations between total sleep time derived by sleep diaries with total sleep time derived actigraphy.

Lastly, there are possible risks of under-adjustment and over-adjustment when using the Berlin questionnaire to identify OSA and its association with cardiometabolic risk factors. The current study is limited in diagnosing OSA by the Berlin questionnaire instead of objective OSA diagnosis. Considering the strong effect of obesity on metabolism and OSA, it is likely that adjustment for obesity would remove any potential effect of OSA in statistical models. Therefore, an objective OSA diagnosis is essential for detecting potential interactions between OSA and obesity.

4.6 Conclusions and Future Directions

This study has shown that among a young adult population sleep characteristics including objective (actigraphy) sleep measures and subjective sleep measures (sleep quality questionnaire) and high risk for obstructive sleep disorder are not related to cardiometabolic risk factors. Further, while being at high risk for OSA as a young adult is associated with BMI, any subsequent association with cardiometabolic risk is likely
to be modulated by obesity and is not yet apparent in these 22-year-olds. Considering the lack of association in the present study, it is more likely that mechanisms linking sleep duration to cardiometabolic risk factors have varying effects at different stages of life.

The results of this study lead to several recommendations for future studies. Firstly, we suggest evaluating this association through longitudinal studies. As the exposure to shorter sleep varies across days, months and years, a snapshot view may not be indicative of sleep patterns over time. It has been suggested that metabolic consequences of sleep loss can be resolved with recovery sleep (311). Therefore, targeting habitual short sleepers and evaluating their cardiometabolic risk factors is recommended.

The current study has few participants who are very short sleepers or long sleepers. Targeting and recruiting participants who are long, short or very short sleepers would be helpful to investigate the cardiometabolic effects of sleep duration.

Short sleepers are a heterogeneous group, comprising those who choose to be short sleepers to meet their personal demands, those who are natural short sleepers and those with sleep impairment. As previous studies have not evaluated these characteristics of short sleepers, evaluating such characteristics and their effects on cardiometabolic health is recommended in future studies.

Future studies would also benefit by the inclusion of anthropometric assessments that measure fat mass, such as DEXA scans. Additionally, the Berlin Questionnaire is limited so far as it only estimates the risk of OSA. Future studies should consider using polysomnography, which is the gold standard method for diagnosing OSA. Such polysomnography based measures would provide a better estimate of the association between OSA, its severity and its relation to cardiometabolic risk factors.
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Appendix 1

Berlin Questionnaire

The questions relate to your sleep pattern and sleep disturbance

1. Do you snore? *(please tick one)*
   - [ ] YES  [ ] NO

2. If you snore, your snoring is:
   - [ ] a. Slightly louder than breathing
   - [ ] b. As loud as talking
   - [ ] c. Louder than talking
   - [ ] d. Very loud; can be heard in adjacent rooms

3. How often do you snore?
   - [ ] a. Nearly every day
   - [ ] b. 3 to 4 nights per week
   - [ ] c. 1 to 2 nights per week
   - [ ] d. 1 to 2 nights per month

4. Has your snoring ever bothered other people?
   - [ ] a. Yes
   - [ ] b. No/don’t know

5. Has anyone noticed that you quit breathing during your sleep?
   - [ ] a. Nearly every day
   - [ ] b. 3 to 4 times a week
   - [ ] c. 1 to 2 times a week
   - [ ] d. 1 to 2 times a month

6. How often do you feel tired or fatigued after your sleep?
   - [ ] a. Nearly every day
   - [ ] b. 3 to 4 times a week
   - [ ] c. 1 to 2 times a week
   - [ ] d. 1 to 2 times a month

7. During your wake time, do you feel tired, fatigued, or not up to par?
   - [ ] a. Nearly every day
   - [ ] b. 3 to 4 times a week
   - [ ] c. 1 to 2 times a week
   - [ ] d. 1 to 2 times a month

8. Have you ever nodded off or fallen asleep while driving a vehicle?
   - [ ] a. Yes
   - [ ] b. No/don’t know

9. If yes, how often does it occur?
   - [ ] a. Nearly every day
   - [ ] b. 3 to 4 times a week
   - [ ] c. 1 to 2 times a week
   - [ ] d. 1 to 2 times a month