USING OBSERVATIONAL AND GENETIC EPIDEMIOLOGY TO INVESTIGATE THE RELATIONSHIP BETWEEN BODY MASS INDEX (BMI) AND SLEEP DURATION

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PhD thesis

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I, Ana Victoria Garfield, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed _____

For my Grandpas, Alfredo Peña & Peter Croft.

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ABSTRACT

Background

Evidence indicates that sleep duration predicts increases in BMI over time and thus, may lead to obesity. However, this relationship has not been explored to the same extent in the opposite direction and it is unclear whether there is a causal association between sleep duration and BMI, or vice versa. Bidirectional epidemiological methods can establish the direction of this association and genetic epidemiological methods may shed light on whether variation in BMI causes changes in sleep duration.

Methods

Observational, bidirectional analyses were carried out using the English Longitudinal Study of Ageing (ELSA) (Chapter 3) and a Norwegian community sample of children (Chapter 4). A genome-wide association study (GWAS) of sleep duration was then undertaken, with the aim of replicating previous, as well as identifying novel, loci (Chapter 5). A large-scale Mendelian randomization (MR) study was subsequently conducted, using genetic variants associated with BMI, to investigate the causal association between BMI and sleep duration in ~142,000 individuals; this was followed by polygenic risk score (PRS) analyses to investigate shared genetic aetiology between the two traits (Chapter 6).

Key findings

In older adults, higher BMI was associated with very small decreases in sleep duration, over 4-year follow-up; there was no association in the opposite direction from sleep duration to change in BMI. There was no association in either direction, between BMI and objective sleep duration in the sample of Norwegian children. The GWAS of sleep duration did not have sufficient power to replicate previous, or identify novel genetic variants for sleep duration, yet effects were consistent with those of previous GWA studies. The heritability of self-reported sleep duration was 7%, which is also in line with previous GWAS.

MR findings suggested that it is uncertain whether a causal relationship exists between BMI and sleep duration and thus, triangulation of results is necessary. A polygenic risk score of BMI was negatively associated with sleep duration, showing some evidence of shared genetic aetiology; however, the variance explained was only 0.02%.

Conclusions

This thesis suggests that BMI and sleep duration are weakly associated and that using a combination of approaches is invaluable in helping us understand this relationship. In older adults, it appeared that prospectively, BMI predicted small changes in sleep duration, whilst in children there was no prospective relationship in either direction. Findings from the GWAS suggest that the heritability of self-reported sleep duration was low. Using MR it was not possible to firmly conclude whether BMI causes changes in sleep duration or not and therefore, more research is needed to determine this. Also, BMI and self-reported sleep duration do not appear to have much underlying common genetic aetiology.

IMPACT STATEMENT

The insight gained from this thesis serves to inform future research for investigators interested in how adiposity might affect our sleep and/or vice versa across the life course. In general, this is an important area as obesity has now reached alarming proportions across the world. Also, there is evidence to suggest that at least in Western countries, we are not getting sufficient sleep and the quality of our sleep is decreasing, with the pressures and stress of modern life. There is still a lot of work to be done, in terms of the relationship between other measures of adiposity (besides BMI) and body composition and other sleep parameters (besides duration). This future research is likely to be in the areas of epidemiology, genetic epidemiology, causal inference, psychology and biology, amongst others. In relation to impact outside of academia, findings from this thesis suggest that people should be aware of what the benefits are of getting sufficient sleep, as well as maintaining a healthy weight, due to the negative health outcomes associated with insufficient sleep and with being overweight or obese. However, this work does not advocate that improved sleep is necessarily a guaranteed benefit of weight loss, or that weight loss is likely to occur as a result of getting sufficient sleep.

Throughout my PhD I disseminated findings as they emerged and gave a range of presentations, which are listed later (pages 16-17). I presented at a variety of events, which included conferences, workshops and seminars. Although most of my presentations were national, I also disseminated my work internationally, with presentations in Norway and Uruguay. Also, the work carried out in Chapter 3 is published in a peer-reviewed journal, the work in Chapter 4 will be submitted for publication and the work in Chapters 5 and 6 contributed to a scientific paper which is currently under review in a scientific journal.

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PUBLICATIONS

PUBLICATIONS BASED ON WORK IN THIS THESIS

- **Garfield V**, Llewellyn CH, Steptoe A, Kumari M. Investigating the Bidirectional Associations of Adiposity with Sleep Duration in Older Adults: The English Longitudinal Study of Ageing (ELSA). *Scientific Reports*. 2017 Jan 9;7:40250. (Based on work in Chapter 3).

- **Garfield, V.**, Fatemifar, G., Dale, C., Smart, M., Bao, Y., Llewellyn, C., Steptoe, A., Zabaneh, D. & Kumari, M. Assessing Potential Shared Genetic Aetiology between Body Mass Index (BMI) and Self-reported Sleep duration in 142,209 Individuals. *Resubmitted to Genetic Epidemiology* (Based on work from Chapters 5 & 6).

PUBLICATIONS ARISING FROM COLLABORATIVE WORK RELATED TO THIS THESIS

These are other relevant publications that I have contributed to substantially during my PhD:

- Okbay, A., Baselmans, B.M., De Neve, J.E., Turley, P., Nivard, M.G., …**Garfield, V.**….Cesarini, D. Genetic variants associated with subjective well-being, depressive symptoms, and neuroticism identified through genome-wide analyses. *Nature genetics*. 2016 Apr 18. (Additional project I was involved in to learn the necessary skills to perform GWAS analyses).

- **Garfield V.**, Llewellyn C.H., & Kumari, M. The relationship between physical activity, sleep duration and depressive symptoms in older adults: The English Longitudinal Study of Ageing (ELSA). *Preventive Medicine Reports.* 2016 Dec 31;4:512-6. (Additional project related to the topic of this thesis).

- Henry, A., Masi, S., Fatemifar, G., Denaxas, S., Acosta, D., **Garfield, V.** & Dale, C. Investigating the causal association between sleep duration and cognitive outcomes in UK Biobank: a Mendelian randomisation study. *Under review in International Journal of Epidemiology.* (MSc supervision project).

PRESENTATIONS

- February 2018 – Invited speaker: External seminar series, Department of Psychology, Middlesex University. The Science of Complex Traits: Can observational and genetic epidemiology help disentangle cause and effect?
- July 2017 – Invited speaker: Understanding Society Scientific Conference: Colchester, UK. BMI and Self-reported Sleep duration: A Mendelian randomization study.
- June 2017 – Oral presentation: Behavior Genetics Association (BGA) Annual Meeting, Oslo, Norway. Assessing Potential Shared Genetic Aetiology between BMI and Self-reported Sleep duration.
- November 2016 – Invited talk (via video conference): 'Neurobiological basis of sleep' (short course), Facultad de Medicina, Universidad de la Republica Oriental del Uruguay. Genetic epidemiology of sleep duration.
- October 2016 – Poster presentation: English Longitudinal Study of Ageing (ELSA) Wave 7 launch: London, UK. Investigating the bidirectional association of Body Mass Index (BMI) with sleep duration in older adults.
- September 2016 – Oral presentation: British Society for Population Studies (BSPS) conference: Winchester, UK. Investigating the Association between Body Mass Index (BMI) and Sleep duration in Older adults: The English Longitudinal Study of Ageing (ELSA).
- January 2016 – Oral presentation: Bloomsbury Centre for Genetic Epidemiology and Statistics (BCGES) PhD symposium: London, UK. Using Mendelian randomization to examine the Relationship between BMI and Sleep Duration.
- September 2015 – Oral presentation: UK Congress on Obesity (UKCO), Glasgow, UK. Garfield, V., Llewellyn, C., Steptoe, A. & Kumari, M. The Bidirectional Association between Body Mass Index (BMI) and Sleep Duration in Older Adults: The English Longitudinal Study of Ageing (ELSA).

- July 2015 – Oral presentation: Understanding Society Scientific Conference: Genetics Workshop: Colchester, UK. Using Mendelian Randomization to Examine the Association between Body Mass Index and Sleep duration.
- June 2015 – Poster presentation: 'Genetics & the Brain' conference, Bloomsbury Centre for Genetic Epidemiology & Statistics (BCGES): London, UK. Using Genome-wide Data to Examine the Association between Body Mass Index (BMI) & Sleep duration.
- March 2015 – Oral presentation: London ESRC DTC Conference, London School of Economics (LSE), London, UK. Using Genome-Wide Data to Examine the Association between Body Mass Index & Sleep Duration.
- December 2014 – Invited Speaker: UK Household Longitudinal Study (UKHLS) Biomarker & Genetic Data Launch: London, UK. How we can use genetic data to examine the association between Body Mass Index (BMI) & sleep duration.
- October 2014 – Poster presentation: English Longitudinal Study of Ageing (ELSA) Wave 6 Launch, London, UK. A genome-wide association study of sleep duration.

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ABBREVIATIONS

ALSPAC	Avon Longitudinal Study of Parents and Children
BiB-1000	Born in Bradford-1000
BMI	Body Mass Index
CES-D	Centre for Epidemiologic Studies – Depression Scale
CHD	Coronary heart disease
CI	Confidence Interval
CTM	Classical Twin Method
CVD	Cardio-vascular disease
DAG	Directed acyclic graph
ELSA	English Longitudinal Study of Ageing
eQTL	Expression quantitative trait loci
FE	Fixed effects
GCTA	Genome-Wide Complex Trait Analysis
GEE	General estimating equation
GIANT	Genetic Investigation of ANthropometric Traits
GRS	Genetic Risk Score
GWAS	Genome Wide Association Study
IADL	Instrumental activity of daily living
IOTF	International Obesity Task Force
IV	Instrumental Variable
IVW	Inverse Variance Weighted
LD	Linkage disequilibrium
LDSC	LD Score Regression
MAF	Minor Allele Frequency
MCS	Millenium Cohort Study
MR	Mendelian randomisation
MVPA	Moderate to vigorous physical activity
MZ	Monozygotic
OR	Odds Ratio
OSA	Obstructive sleep apnoea
PA	Physical activity
PRS	Polygenic risk score
Q-Q	Quantile-quantile
RCT	Randomised Control Trial
SES	Socioeconomic status
SHHS	Sleep Heart and Health Study
SNP	Single Nucleotide Polymorphism
T2D	Type-2 diabetes
TESS	Trondheim Early Secure Study
UKB	UK Biobank
UKHLS	UK Household Longitudinal Study
WC	Waist circumference
WHO	World Health Organization

1 OBESITY: INTRODUCTION, ASSOCIATION WITH SLEEP DURATION AND AIMS OF THE THESIS

1.1 BRIEF INTRODUCTION AND OVERVIEW OF CHAPTER CONTENTS

Research published within the last ten to fifteen years suggests that adiposity is largely associated with shorter sleep duration. However, little is known about whether greater adiposity causes changes in sleep duration, or whether insufficient/prolonged sleep might cause changes in weight. It may be possible to use genetic epidemiological methods to investigate the causal nature of this relationship. The work undertaken in this thesis used a combination of observational and genetic epidemiology to examine the complex relationship between BMI and sleep duration. However, before this literature is reviewed, it is necessary to describe the genetics of both BMI and sleep duration, with emphasis on recent findings from genome-wide association studies (GWAS).

Obesity is a medical condition characterised by an excess of adiposity, often leading to decreased quality of life, morbidity and/or mortality, due to related physical and psychological complications. It is a complex trait, influenced by both genetic and environmental factors. Obesity is a major public health concern, as the global epidemic of obesity ('globesity') has now reached alarming rates across both the developed and developing world.

In this chapter, there is a brief introduction to obesity with current prevalence estimates. Next there is an overview of heritability using twin studies, followed by GWAS studies. The subsequent two sections describe the main approaches to measuring both adiposity and sleep duration, outlining pros and cons of each method, with a focus on the measures used in this thesis. This is followed by a brief synopsis of the key environmental determinants of obesity, in which the link with sleep duration is introduced. The next section summarises the literature on the molecular genetic determinants of sleep duration, centred on findings from GWAS. The subsequent section provides a detailed review of the extant literature

on the association of adiposity with sleep duration, separately for children and adults. This chapter ends with the rationale for this research and a description of the aims and objectives of the thesis, followed by a chapter summary.

1.2 BACKGROUND

Being obese is associated with an elevated risk of developing type II diabetes mellitus (T2DM), cardiovascular disease (CVD), hypertension, stroke, certain types of cancer, metabolic syndrome[1], dyslipidemia and osteoarthritis[2] and an elevated risk of Vitamin D deficiency[3]. More recently, evidence suggests an association between obesity and psychological outcomes, specifically psychological distress[4] and common mental disorders (CMDs) such as depression[5–7] and anxiety[5–9], as well as poorer cognitive function[10].

The prevalence of obesity has been rising steadily in the developed and non-developed world and was formally acknowledged as an epidemic by a WHO consultation in 1997[11]. The most recent systematic analysis of 128.9 million children, adolescents and adults, across 200 countries, estimated that the number of obese girls increased from 5 million to 50 million, whilst the number of obese boys increased from 6 million to 74 million, between 1975 and 2016[12]. During this 41-year period, the worldwide number of obese women increased from 69 million to 390 million, and the number of men with obesity increased from 31 million to 281 million. Additionally, 213 million children and adolescents, as well as 1.30 billion adult men and women, were in the overweight but not obese range.

Obesity rates in the UK have increased drastically over the last four decades, such that in 1975 1.7 million men and 2.1 million women, were obese, compared with 6.8 million men and 7.7 million women in 2014[13]. Today the UK is ranked as the second country in Western Europe with the most excess adiposity, after Germany[13].

Obese individuals were once described as gluttonous and lacking in self-control; therefore, treatment and prevention of obesity aimed to target individual

behaviour[11]. However, obesity arises, not only due to environmental factors, but also to genetic ones.

1.3 HERITABILITY

Heritability is the extent to which phenotypic variation in a trait can be accounted for by genetic variation in a specific population, at a particular point in time[14]. It is important to differentiate between narrow and broad sense heritability; the former refers to the proportion of a trait that is accounted for by additive genetics, whilst the latter refers to the proportion of a trait that is due to *all* genetic variance. Heritability is commonly estimated by comparing the resemblance between monozygotic (MZ - identical twins, who share 100% of their genes) and dizygotic (DZ - non-identical twins, who share 50% of their genes) twins[15].

1.3.1 Twin studies and heritability

Twin studies have proved invaluable in uncovering the genetic basis of complex traits. Their main aim is to unravel and quantify the contributions of genetic, shared environmental (factors shared completely by twin pairs, that contribute to their similarity, such as maternal gestational weight gain or family socioeconomic status) and non-shared environmental factors (factors unique to each twin in a pair that contribute to differences between twins) to such traits. The twin method compares the similarity of MZ and DZ twins, who usually grow up in very similar environments[16]. Similarity is measured statistically using a correlation coefficient and as such, if the correlation for MZ twin pairs is larger than the correlation for DZ twin pairs, it is taken as evidence that individual differences in the trait of interest (for example BMI) have some genetic basis[16]. A systematic review and meta-analysis of 88 published twin studies suggests that heritability estimates of BMI are high, ranging between 47% to 90%, and that heritability of BMI is higher at younger ages[17].

1.3.2 Molecular genetic approaches and heritability

With the advent of GWAS (summarised below in section 1.4) in the last ten to fifteen years, researchers discovered that it was possible to also estimate genomic heritability, from the aggregated effects of all measured genetic variation

primarily in the form of single nucleotide polymorphisms (SNPs). SNPs represent single base pair changes in a DNA sequence[18]; thus two individuals may differ in one nucleotide, which could lead to phenotypic variation. For example, these differences could mean that person A has different susceptibility to a particular disease from person B. However, variance explained by single SNPs identified from GWAS appear to explain only a small proportion of the heritability for the majority of traits, even when added together to create genetic risk scores including all identified risk variants.

To address this and enable the estimation of variance explained by all SNPs (SNP heritability) on a chromosome, or genome-wide SNPs, a set of tools called *genome-wide complex trait analysis* (GCTA) was created by a group of researchers[19] (explained in more detail in the Methods section of Chapter 5). GCTA can also be used to estimate the SNP linkage disequilibrium (LD) structure (the non-random association of alleles at a particular locus, i.e. the correlation between SNPs), genetic relationships (using all of the autosomal SNPs, on chromosomes 1 to 22, GCTA can estimate genetic relationships between all of the individuals in a given sample), perform GWAS simulation, and estimate genetic correlations between traits (the extent to which the same SNPs are associated with variation in two traits, i.e. shared genetic aetiology). However, as GCTA requires individual-level genotype data to perform analyses, LD Score regression (LDSC) was developed recently to overcome this issue, allowing SNP heritability estimation using GWAS summary statistics[20].

1.4 GENOME-WIDE ASSOCIATION STUDIES (GWAS)

A GWAS is a hypothesis-free approach which aims to find common genetic variants that are associated with variation in a particular trait. In GWAS, SNPs are used as regional genomic markers. A tag SNP is a variant whose alleles (different forms of a gene) 'tag' other SNPs that are in the surrounding LD region. SNPs included in GWAS usually have a minor allele frequency (MAF – the extent to which the less common allele occurs in the population) of at least 1%, as the aim is to analyse common genetic variants and those with a MAF of <1% are considered to be rare variants.

GWAS flourished with the completion of the Human Genome Project in 2003 and the International HapMap Project in 2005, as researchers developed new technologies that made it possible to carry out this type of research. The GWAS method advocates a 'common disease, common variant' hypothesis, that common genes are likely to influence common disorders[21], and has become increasingly popular since it first emerged more than a decade ago. There are now dozens of GWAS published in major journals every month, as methods have become more accessible, costs of genotyping have reduced, and the field of Genetic Epidemiology has grown into a largely collaborative one.

The first published GWAS by Klein and colleagues[22] aimed to identify polymorphisms associated with age-related macular degeneration (AMD), one of the major causes of blindness in older adults and only one SNP was found to be associated with AMD. This was, at least, partly related to the narrow coverage of SNPs genotyped, which only contained 116,204 genome-wide tagging SNPs. However, researchers are now able to include many more millions of SNPs in GWAS, due to better genotyping arrays. Genotype imputation is the statistical inference of unobserved genotypes, which is done using known haplotypes in the population of interest[23] (more details are included in Chapter 2).

Researchers predicted that the GWAS approach would elucidate causal genetic variants associated with complex traits. However, this quickly proved not to be the case for most of these traits[24] and the term 'missing heritability' was thus adopted to refer to this mismatch between the high variance explained from genetic variation in twin studies, and the low variance explained from the SNPs identified through GWAS[24]. One of the main issues is that as GWAS began to emerge it was apparent that individual SNPs had, for the most part, very small effects. This is directly related to statistical power in GWAS; the conventional p-value threshold used in GWAS is $p<5x10^{-8}$, which is particularly stringent because millions of associations are tested to examine whether each SNP has an effect on the trait of interest and thus, this multiple test correction is required ($p<5x10^{-8}$ applies a Bonferroni correction for millions of tests at an alpha level of 0.05). Therefore, it is sometimes difficult to detect SNPs of larger effects, even with

sizeable samples. For a GWAS to successfully uncover common variants of small effect, even larger sample sizes are required. Further important challenges proposed were: insufficient power to detect gene-gene and gene-environment interactions, and poorly detected rarer variants with potentially larger effects[25].

These issues in GWAS are applicable to both BMI and sleep duration, as illustrated below in the sections entitled: *GWAS of obesity-related traits* and *GWAS of sleep duration*.

1.5 GENETIC DETERMINANTS OF OBESITY

1.5.1 Heritability of obesity-related traits using twin studies

Nearly 30 years ago a study showed that the BMI within-pair correlations for MZ twins were 0.70 for males and 0.66 for females[26], while correlations within DZ were considerably lower (0.30 for males and 0.50 for females), indicating that genetic variation contributes importantly to individual difference in BMI. Further evidence comes from studies of adopted children, whose weight correlate more highly with that of their biological, as opposed to adoptive, parents[27]. This suggests that human body weight has an important genetic basis.

1.5.2 Genome-wide Association Studies (GWAS) of obesity-related traits

GWAS use contemporary molecular genetic methods to estimate the heritability of complex traits, for example, GCTA[19] and more recently, LDSC regression[28]. GCTA and LDSC regression estimate heritability by considering the contribution of each single nucleotide polymorphism (SNP) measured in the study. Therefore, GWAS find substantially lower heritability estimates than twin studies for obesity-related traits. For example, the largest and most recent GWAS estimate of the heritability of BMI was 21%[29].

Moreover, GWAS have proved very successful in identifying specific genetic variants associated with obesity-related traits[1,29–36]. Exactly a decade ago a genome-wide scan for type-2 diabetes-associated genes found that a variant in the intron of the fat mass and obesity associated (*FTO*) gene, on chromosome 16 (rs9939609), predisposes to diabetes through its effect on BMI[1]. This finding was ground-breaking at the time and was subsequently replicated in 38,759

individuals across thirteen cohorts. Notably, rs9939609 is associated with altered BMI and obesity in children by the age of seven, indicating an upward trajectory for fat mass. In humans, the *FTO* gene is predominantly expressed in several nuclei of the hypothalamus, the primary centre for energy homeostasis[30]. FTO spans over 400kb and rs9939609 is located in its first and largest intron[30]. It was recently discovered that variants in the *FTO* gene exert their action on adipocyte function by targeting the Iroquois-class homeobox protein 3 (*IRX-3*) and the Iroquois-class homeobox protein 5 (*IRX-5*)[37]. Evidence from mice, humans and *in vitro* studies indicate that the *IRX3* promoter region interacts with the FTO obesity-associated noncoding region. Obesity SNPs are associated with *IRX3*, but not with expression of *FTO*, as knockout of *IRX3* in mice results in a significant decrease in body weight, greater activation of brown adipose tissue and higher levels of energy expenditure[38]. A study in humans showed that the SNP rs1421085 (an intron in the *FTO* gene) caused activation of *IRX3* and *IRX5* expression and a shift to energy-storing white adipocytes from energy-dissipating beige adipocytes[37]. This suggests that rs1421085 may play a role in genetic predisposition for obesity, but this study was only carried out in lean healthy adults.

Following this large-scale GWAS in 2007, various other independent studies in both adults and children from different ethnic populations, including Europeans [39–44], Asians[45–48] and Africans[49–53] found associations between *FTO* SNPs and obesity-related traits, such as larger hip circumference, body weight and waist to hip ratio[30]. A GWAS carried out in >4,000 Sardinians found that for example, the rs9930506 SNP, within the *FTO* gene, showed strong associations with BMI, hip circumference and weight[54]. The association between rs9930506 and BMI was replicated both in a European American population (n=1,496) and in a Hispanic American population (n=839), with very modest sample sizes. At the time, these authors estimated that approximately another 250 unknown loci of similar effect size to those they described, alongside a larger number of loci with smaller effects on BMI, had yet to be identified.

Common variants in the region near the melanocortin-4 receptor (*MC4R*) gene were also found to be associated with fat mass, weight and increased risk of obesity when analysing GWAS from 16,876 individuals of European ancestry[31]. Specifically, the strongest reported signal for BMI was located near *MC4R*, which was confirmed in 60,352 adults. This finding is important as it implicates the *MC4R* gene, mutations of which result in monogenic severe childhood obesity[31].

Associations between BMI and variants within or near the *MC4R* and *FTO* genes were further confirmed, alongside 6 novel loci near the following genes: *TMEM18, KCTD15, GNPDA2, SH2B1, MTCH2* and *NEGR1*[55]. Another genome-wide scan of >30,000 individuals (predominantly Icelandic, N=25,000) also replicated previously associated BMI variants, and found an additional 7 SNPs on undiscovered pathways[32]. These novel BMI variants are located within or near the brain derived neurotrophic factor (*BDNF*) gene, of which rs6265 had been previously implicated in eating behaviour[33] and BMI[34].

The Genetic Investigation of ANthropometric Traits (GIANT) Consortium subsequently performed a much larger two-stage meta-analysis of 249,796 European individuals and identified 18 novel loci associated with BMI[35], bringing the total number to 32. Particularly motivating was the novel association between BMI and 7a copy number variant (CNV) near the G protein-coupled receptor, class C, group 5, member B (*GPRC5B*) gene, which is thought to modulate insulin secretion, and greater protein expression is associated with type-2 diabetes[36]. Of the remaining 17 novel loci, interestingly, some mapped near important hypothalamic energy balance regulatory genes, including: *MC4R*, proopiomelanocortin (*POMC*), SH2B adapter protein 1 (*SH2B1*) and *BDNF* genes. Another of these novel loci is located nearby the gastric inhibitory polypeptide receptor (*GIPR*) gene, suggesting a potential link between incretins (metabolic hormones), insulin secretion and human body weight regulation.

A more recent GWAS and Metabochip meta-analysis of BMI in 339,224 individuals revealed a total of 97 genetic loci, of which 56 were novel[29]. Taken together, these loci account for 2.7% of the variation in BMI. These findings provide further support for the roles of key obesity-related molecules including

BDNF and *MC4R*. Moreover, there appears to be overlap between loci associated with BMI and genes and pathways involved in neurodevelopment.

1.6 INTRODUCTION TO SLEEP AND CONSEQUENCES OF SHORT SLEEP

Human adults spend on average one third of their lives asleep. Sleep is defined as the shift in consciousness which leads to involuntary processes and is required by humans on a daily basis. Sleep is crucial for preserving psychological and physical well-being and is not simply a lifestyle choice; like eating and breathing, it is a necessity. Important sleep dimensions include: duration, defined as the number of hours slept per night, timing or chronotype, which refers to individuals' sleep schedule, and sleep quality, which refers to an individual's satisfaction with their sleep.

Similarly to obesity, sleep is a particularly complex phenotype, not solely because of how it manifests itself but also because of how it is regulated[56]. Hence it is crucial to understand the mechanisms by which sleep is regulated, which are twofold: homeostatic and circadian[57]. This is also known as Borbely's two-process model[58], and it is the dominant model of sleep regulation.

The homeostatic process increases as a function of hours of wakefulness and decreases as we sleep. Therefore, the longer we are awake, sleep pressure or the 'homeostatic drive' increases and the desire to sleep becomes greater. Once we fall asleep, this pressure is relieved and because it is a cyclic process it repeats itself, with sleep pressure increasing again during wakefulness and then decreasing once we fall asleep again, and so on. Although the specific biochemical process that controls homeostasis is less well understood than the circadian process, research suggests that sleep pressure intensifies due to a build of the molecule adenosine. Adenosine is a nucleoside (an organic compound, which is released during the breakdown of nucleic acids) and it is currently the only known substance that contributes to sleep regulation during homeostasis. Adenosine accumulates in the brain for as long as we stay awake and makes us feel sleepy, as it binds to cells in the forebrain and inhibits their activity. During

sleep adenosine decreases and glycogen energy stores take its place. However, stimulants such as caffeine block the effects of adenosine and thus keep us awake.

The circadian process influences the duration, quality and timing of sleep onset and offset, and is governed by the 'biological clock', whose cycle lasts approximately 24 hours. The biological clock acts as an internal pacemaker and regulates the timing of sleep as well as other physiological rhythms that play a role in sleep, such as body temperature and secretion of melatonin and cortisol. It is located in the suprachiasmatic nucleus (SCN), which is a very small region of the hypothalamus and comprises approximately 20,000 nerve cells that respond to light through the retina and optic nerve. The 24-hour light-dark cycle is the most important external cue that synchronises the biological clock to the timing of the natural rotation of the Earth, and therefore this cycle helps regulate the homeostatic and circadian processes, whereby decreased sleep pressure corresponds to the circadian process that promotes wakefulness. External factors such as temperature, physical activity and dietary intake can also affect the timing of the biological clock.

Human infants and children spend a large amount of their time asleep, which then tends to plateau in adulthood. Young adults and adults require between seven and nine hours of sleep for optimal health, whilst older adults should sleep between seven to eight hours per night. However, sleep curtailment has increased in the UK[59] and has coincided with the obesity epidemic. Evidence suggests that the proportion of people sleeping for less than the recommended amount is increasing and that due to insufficient sleep the UK economy loses 200,000 working days per year at a cost of £40 billion[59]. Germany, another large European economy, also loses 200,000 working days per year, at a cost of $60 billion, due to insufficient sleep[59].

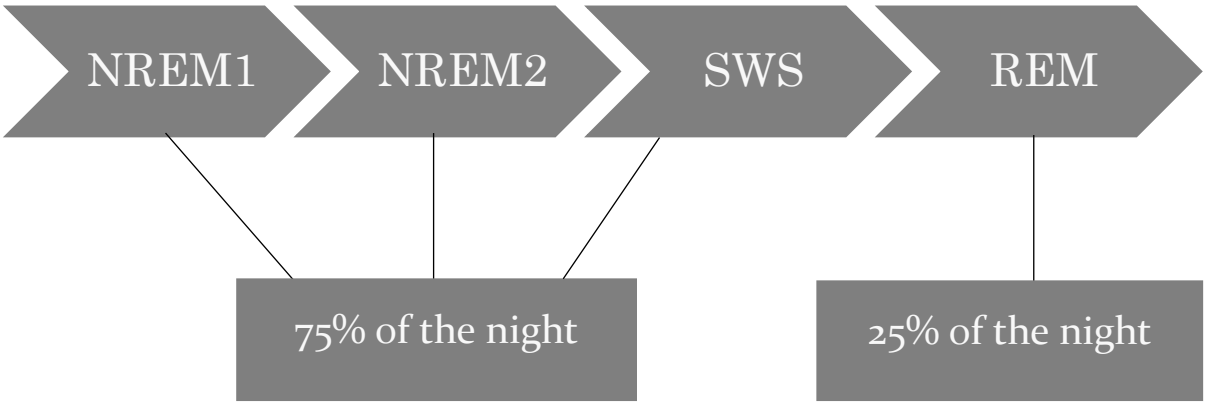
Important predictors of reduced sleep are smoking and alcohol consumption, whilst physical inactivity predicts short sleep duration[60]. Furthermore, meta-analyses of published studies find that short sleep duration (usually defined as less than or equal to five hours, or in some studies less than six hours per night)

is predictive of diagnosis or death from CHD and stroke[61], as well as greater risk of T2D[62,63], hypertension[64] and all-cause mortality[65,66]. On the other hand, although less prevalent than short sleep, long sleep (usually defined as more than eight or nine hours per night) is also associated with greater risk of CHD, stroke, total CVD[61], T2D[62,63].

1.6.1 The Sleep Cycle

Mammalian sleep is divided into two broad categories: Non-Rapid Eye Movement (NREM) sleep and Rapid Eye Movement (REM) sleep. The sleep cycle is made up of four sequential stages (Figure 1.1), which alternate between NREM and REM sleep in such a way that throughout a typical night the cycle is repeated approximately every ninety minutes. The initial stage NREM stage is characterised by light sleep and drifting between wakefulness and sleep. The onset of sleep occurs during the second stage, whereby we become disengaged from our surroundings, and body temperature decreases. Stage 3, or slow-wave sleep (SWS) is regarded as the stage when the most restorative and deep sleep is experienced. As blood pressure decreases, breathing becomes slower, muscle tone is reduced, there is an increase of blood supply to muscles, growth and repair of tissue occurs, energy is restored, and hormones are released (for example, Growth hormone). REM sleep is characteristic of the fourth stage and happens for the first time approximately ninety minutes after falling asleep, recurring every ninety minutes thereafter. This type of sleep supplies energy to the brain and body, which allows the brain to be active for dreaming, and the body is immobilised and relaxed, as muscle tone is reduced even further. It is crucial to experience all four stages of sleep in order to wake up feeling rested.

Figure 1.1 The Sleep Cycle[67]



1.7 GENETIC DETERMINANTS OF SLEEP DURATION

1.7.1 Heritability of sleep duration using twin studies

Currently, a modest body of research exists in the area of human sleep genetics, which has shown that specific sleep phenotypes (duration, timing, quality) are heritable[68]. Twin studies have estimated that genetic factors account for between 30% to 50% of the variance in the duration, quality and patterns of sleep[69–71].

1.7.2 GWAS of sleep duration

GWAS have described SNPs that are associated with distinct measures of sleep, however they remain largely un-replicated[68,72–74], and the effect sizes of the identified SNPs are very small. However, the variant reported to have the largest effect size to date accounted for approximately 5% of the variation in sleep duration[73], yet this has not been replicated in subsequent studies. A more recent and much larger GWAS of sleep duration found that the maximum variance explained by a single variant was 0.07%[74].

The first GWAS of sleep duration was published in 2007. Seven hundred and forty nine participants were genotyped for 100,000 SNPs, and the analyses examined associations between these SNPs and self-reported usual sleep duration[72]. Only one intergenic SNP on chromosome 13 (rs6599077) was associated with sleep duration at $p=1.4x10^{-7}$. This means that no SNPs were associated with sleep

duration at genome-wide significance, but this SNP was significant at a genome-wide suggestive level of significance.

More recently, three GWAS of sleep phenotypes have been published. The first GWAS to focus on self-reported sleep duration alone had one SNP (rs11046205) reach genome-wide significance [73], which is an intronic variant in the adenosine triphosphate-binding cassette, sub-family C, member 9 (*ABCC9*) gene. This gene is involved in encoding a potassium channel (K_{ATP}), which contributes to energy metabolism; it has also been associated with Cantú syndrome and dilated cardiomyopathy[75,76]. However, neither of these conditions are related to sleep. Another GWAS performed on 2,323 Australian individuals found no genome-wide significant SNPs for self-reported sleep duration, sleep time, latency, quality or depth[68]. Seven SNPs, however, were suggestive of associations and these seven variants are located on different chromosomes and nearby or within distinct genes.

The next GWAS was carried out in 47,180 individuals of European ancestry and found seven loci associated with self-reported sleep duration, 4 of which are on chromosome 2 and 3 on chromosome 6 [74]. A further 11 loci were suggestive of associations with sleep duration but did not reach the genome-wide significant threshold. The strongest is an intergenic variant, located on chromosome 2 near the paired box thyroid-specific transcription factor (*PAX8*). PAX8 encodes a nuclear protein, which is involved in the expression of thyroid-specific genes, as well as thyroid follicular cell development [77], whereas the Cobalamin Synthase W Domain-Containing Protein 2 gene (*CBWD2*) is highly expressed in the brain, but remains poorly characterised [74]. This association was found to be in the same direction in an African-American sample, although it was not genome-wide significant ($p=9.3*10^{-4}$).

The most recent GWAS of self-reported sleep duration was performed in 127,573 UK Biobank participants, from which three genome-wide significant variants emerged[78]. The main distinctions between this study, by Jones et al[78] and that of Gottlieb and colleagues[74] were the following: the sample size was almost three times greater; SNP heritability was estimated and, rather than combining several

studies in a meta-analysis they were able to use a single, very large sample. Only three novel loci were found to be associated with self-reported sleep duration: rs62158211, rs17190618 and rs1380703 on chromosome 2. The effect alleles for each of these two SNPs were associated with a 2-minute decrease in sleep duration, whilst the effect allele for rs1380703 was associated with a 1.5-minute increase in sleep duration. rs62158211 is an intron in the *PAX8* gene and is in high LD with two variants previously reported by Gottlieb and colleagues[74]. Thus, Jones et al. (2016)[78] were the first to replicate an association in the same region as previously reported. rs17190618 and rs1380703 are both intronic variants within the Vaccinia Related Kinase 2 (*VRK2*) gene. GWAS have found this gene to be associated with schizophrenia[79] and epilepsy[80] although not these specific sleep duration variants.

The current GWAS literature suggests that there are three SNPs associated with self-reported sleep duration. The most recent study in the UK Biobank[78] was substantially larger than earlier studies, but still only managed to uncover two novel loci, with one replicated from previous research. Thus, to date there are only three SNPS associated with self-reported sleep duration.

In terms of how these studies have contributed to our understanding of the biology of sleep, the *PAX8* gene (for which SNPs associated with sleep duration were identified in the GWASs by both the CHARGE and the UKB studies) encodes a protein which is involved in the expression of thyroid-related genes. *PAX8* is associated with hypothyroidism and patients that do not receive treatment for this disease are more likely to have obstructive sleep apnoea (OSA) episodes[81]. Mutations in the *PAX8* gene, amongst others, may result in the thyroid stimulating hormone receptor (*TSHR*) gene being only partially activated. Whilst this is important, as the prevalence of hypothyroidism is approximately 2% (UK)[82], the *PAX8* gene is not highly expressed in the hypothalamus, for example, which is responsible for the regulation of sleep in the brain.

Variants in the *VRK2* gene have been associated with schizophrenia, a psychiatric illness which is known to have consequences for patients' sleep[83]. Evidence suggests that sleep disturbances may contribute to the onset of psychosis in

young people[84]. However, similarly to the *PAX8* gene pathways, this may not be so informative when it comes to the average sleep duration of the population, as these pathways relate to specific diseases, such as schizophrenia and hypothyroidism. Thus, more research is needed to uncover common genes that may predispose to increases or decreases in sleep duration.

1.8 MEASURING ADIPOSITY AND SLEEP DURATION

1.8.1 Brief overview of adiposity measures

Numerous methods exist for measuring adiposity, yet they vary in accuracy and cost. The most common measurements include: body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), bioelectrical impedance and skinfold thickness[85]. However, magnetic resonance imaging (MRI), computerized topography (CT) and dual energy X-ray absorptiometry (DEXA) are the most widely used "reference measurements" [85]. BMI, WC and WHR are all simple to collect, as well as inexpensive, which is also the main reason these measures are most commonly used in large-scale scientific studies. MRI and CT and DEXA scans are more likely to be employed in validation studies of body measurement methods. It is well accepted that CT and MRI imaging provide the highest accuracy when it comes to measuring organ, tissue, whole-body fat mass, in addition to lean mass and bone mass[85].

In this thesis, the main measurement of adiposity analysed was BMI, the reasons for which are: accessible datasets all had measured BMI (as opposed to BMI calculated from self-reported height and weight); and to date, a total of 97 genetic variants have been associated with BMI[86]. This is important when using genetic epidemiological methods, such as Mendelian Randomisation, to investigate causality between a given exposure and outcome (Chapter 6).

1.8.1.1 Body Mass Index (BMI)

BMI is calculated by dividing weight by height squared (kg/m^2). A BMI equal to or greater than 25 kg/m^2 is in the overweight range, whilst individuals with a BMI equal to or greater than 30 kg/m^2 are classed as obese. BMI is the most commonly employed measure of adiposity because it is easy to calculate, cost-effective, has

standardized cut-off points, is typically highly correlated with body fat levels, and is predictive of chronic diseases and early mortality[85]. BMI also benefits from reliable reference data for children; children's adiposity varies with age and sex so these factors must be taken into account when using paediatric BMI, and this is done by comparing the child's BMI to reference data[87].

However, the reliability of BMI in adults as a measure of adiposity has been questioned due to its lack of ability to differentiate between fat and fat-free mass, for instance, bone and muscle mass[1,88,89]. Other disadvantages of BMI are: it is an indirect measurement of adiposity; it has a reduced ability to predict body fat in older adults compared to younger and middle-aged adults[88,90]; its nonlinear relationship with percentage of body fat, and that it differs for men and women; and its weak sensitivity and specificity[90]. Regarding sensitivity, some research suggests that BMI can lead to false-negative results and in terms of specificity, one study observed that BMI incorrectly classified 8% of all men and 7% of all women as obese, using standard BMI cut-off points[90].

1.8.2 Brief overview of sleep duration measures

Sleep duration can be measured in several ways, which broadly fit into subjective and objective methods. Self-reported sleep duration usually involves asking how many hours individuals sleep per night, whilst objective sleep studies either monitor participants in a laboratory setting or ask them to wear a device to track their sleep duration. The most widely known objective sleep duration methods are: polysomnography (PSG) and actigraphy. PSG is considered to be the gold standard for the diagnosis of some sleep disorders[91]. Actigraphic sleep duration is measured using a wearable wrist or waist device and a questionnaire is usually administered alongside it. This questionnaire aids interpretation of the actigraphic data, as it asks questions about whether individuals are equally as active/inactive and whether they have been ill during the time that they wear the actigraph.

The research carried out in this thesis predominantly used self-reported sleep duration measures, with the exception of actigraphy-measured sleep duration in

the TESS study (Chapter 4). Thus, in this section there is a description of actigraphy and self-reported sleep duration.

1.8.2.1 Self-reported sleep duration

Self-report sleep duration is widely used in epidemiological research. . Other dimensions of sleep such as disturbance and quality are commonly measured by means of reliable and valid scales, for example the Pittsburgh Sleep Quality Index (PSQI)[92], the Epworth Sleepiness Scale (ESS) [93] or the Jenkins Sleep Scale (JSS)[94]. A sleep duration item also forms part of some of these questionnaires.

The main advantages of self-report sleep duration measures are: they are inexpensive and easy to administer, particularly in large studies, as one or two questions can usually be asked as part of a larger questionnaire; they are simple to code and to analyse, as a typical question asks about the number of hours an individual sleeps for, thus researchers can choose to use this or for example, convert to minutes if required. The majority of studies measure sleep using self-report, due to these advantages, thus comparison with other research is always possible. Also, as mentioned earlier, these measures have criterion validity as evidence shows that both self-reported short and long sleep duration are predictive of CHD[61] (short sleep RR=1.48, long sleep RR=1.38), stroke[61] (short sleep RR= 1.15, long sleep RR=1.65), type-2 diabetes mellitus[63] (short sleep RR=1.09, long sleep RR=1.14) and mortality[66] (short sleep RR=1.12, long sleep RR=1.22).

However, there are also limitations of using subjective sleep duration. First, there is potential for measurement error, as people might not accurately report how many hours they sleep for various reasons. For instance, they may not know and may be inclined to guess, they may have irregular sleep patterns making it difficult to estimate, or they may not want to provide a true report if they have atypical sleep durations (short or long sleep, for example). Second, some individuals may report the number of hours they sleep from the time they actually go to bed, rather than the time they fall asleep. This time between full wakefulness and sleep onset is known as sleep latency and is distinct from sleep duration. Notably, though, most normal sleepers, who do not suffer from a sleep

disorder (particularly, insomnia) report similar sleep duration estimates to those observed in the laboratory, under polysomnography (PSG)[95]. Third, the correlation between objective and subjective sleep duration was reported to be around 0.45 by Lauderdale and colleagues[96], whilst another study found that in 34% of participants there was a discrepancy of an hour or more between sleep diaries and actigraphic sleep duration[97], and more recently, a correlation of only 0.3 was found between subjective and actigraphic sleep duration[98].

1.8.2.2 Actigraphy

Actigraphy has been used for more than twenty-five years in the assessment of sleep/wake states[99]. An actigraph is conventionally worn on the wrist of the non-dominant arm and continuously detects and records body movement (predominantly acceleration), and stores the information for long periods of time [100]. However, waist-worn actigraphs are also used for the measurement of sleep parameters, as well as for the detection of movement and activity. In addition, it is now possible to also collect data using applications on smartphones, as well as smart watches.

Actigraphy is popular for determining patterns of sleep and circadian rhythms and, unlike PSG, does not require subjects to spend time in a laboratory, can be worn for several weeks, is more affordable for data collection in large-scale studies and is less invasive than PSG, particularly for use in infant and elderly populations[101]. Although PSG is regarded as the gold standard for measuring sleep/wake behaviours, actigraphy can in fact, provide more reliable measurements, as data are collected over several days, rather than for one or two nights in the laboratory[102]. It is also likely to be more ecologically valid, as actigraphy measures habitual sleep in an individual's usual environment, as opposed to in a lab, which is an unnatural setting to sleep in and could affect sleep.

Whilst data are being recorded via actigraphs it is possible to continue with daily activities and enables individuals to remain in their natural sleep environments. Actigraphs are now used in the clinical diagnosis and evaluation of insomnia, extreme sleepiness, restless legs syndrome and circadian rhythm disorders[102].

When using actigraphy, algorithms are employed to calculate sleep and wake periods, based on individuals' activity patterns[103].

However, there are also disadvantages of using actigraphy for the measurement of sleep duration. These devices are unable to reliably differentiate sleep stages, are more expensive than self-report sleep duration measures, have at times shown poor specificity in the measurement of sleep duration, and can sometimes over- or underestimate sleep duration. For example, in a study by Paquet and colleagues[104] actigraphy showed an overall less than 50% specificity in determining sleep and also overestimated duration of sleep, in comparison with PSG. In another study of sixty-eight adult females, actigraphy underestimated sleep durations by an average of 68 minutes in those who slept for less than five hours[102]. This may be due to sleep disturbances causing increases in nocturnal movements and thus, leading to the actigraph underestimating duration of sleep.

1.9 BMI AND SLEEP DURATION

Obesity arises as a result of an energy imbalance, whereby energy intake increases relative to energy expenditure[105]. Aside from the physical and psychological health complications associated with obesity, which are mentioned earlier in section 1.2, an intriguing link between adiposity and sleep duration has been reported more recently, in both children and adults[106,107]. As the focus of this thesis is on BMI in relation to sleep duration, the literature reviewed pertains only to this association, rather than other measures of adiposity.

Both the PubMed and Web of Science databases were searched for relevant literature of the relationship between BMI and sleep duration in adults and children. Below is a detailed review of the literature, firstly in adults and then in children, followed by summary tables of key findings.

1.9.1 Literature Review Part I: current evidence in adults for the association between BMI and sleep duration

An extensive literature exists linking BMI and sleep duration in adults, particularly published in the last ten to fifteen years. Whilst cross-sectional evidence has

uncovered a consistent relationship between the two, longitudinal studies are limited and have yielded mixed findings.

1.9.1.1 Cross-sectional studies using self-reported measures of sleep

Table 1.1 summarises the key cross-sectional studies in adults that have used subjective sleep duration. There has been one systematic review, and one systematic review with meta-analysis[106,107] of the cross-sectional association between sleep duration and BMI in adults. An early review of the literature found that short sleep appeared to be independently associated with increased weight in adults in 17 of 23 cross-sectional studies[106]. Of the 23 studies included in the review, 11 observed a clear relationship between short sleep duration and higher BMI, with two studies reporting mixed findings, due to an effect in one sex and not the other. Five studies found no evidence of an association between short sleep and greater weight, with one study reporting a relationship between short sleep and decreased weight. Furthermore, six studies suggested that the association is U-shaped, such that both short and long sleep are implicated in the risk of obesity.

The only study to report that shorter sleep is associated with lower BMI was a Japanese cohort of over 100,000 participants[108]. Mean BMIs were 22.9, 22.6, 22.9 and 22.7 kg/m² in men who slept ≤4, 5, 6 and 7 hours, respectively; in women these hours of sleep corresponded to mean BMIs of 22.6, 22.9, 22.9 and 22.9 kg/m². Importantly, though, these average BMIs are indicative of a lean sample and thus, might not be directly comparable to populations such as the USA or UK.

As mentioned above, U-shaped associations between sleep duration and BMI have been suggested by a few studies. Analysis of the American Sleep Heart Health Study (SHHS), indicated that the highest mean BMIs were observed in individuals who reported sleeping for less than six hours, six to seven hours or ≥9 hours[64]. Respondents who slept for between seven and eight hours, or eight to nine hours had the lowest mean BMIs (28.4 kg/m²), in comparison with those

who slept for less than six hours and had the highest average BMIs (29.1 kg/m²). The overall sample had a mean age of 63 years and 52% of respondents were hypertensive. The highest proportion of individuals who had any CVD was observed in the shortest sleep duration group (< 6 hours), at 23%.

Findings from Canadian individuals, aged between 21 and 64 years (N=740) indicated that, compared to normal sleepers (seven to eight hours), those who reported sleeping for five to six hours, as well as those who slept for nine to ten hours, had increased odds of obesity, ORs=1.69 and 1.38, respectively[109].

Studies included in Patel et al.'s review[106] that yielded null findings are described in more detail later in this literature review. A systematic review with meta-analysis of 604,509 adults, published at approximately the same time as the review by Patel and colleagues[106], concluded that there was a cross-sectional association between short sleep and an increased risk of obesity (pooled OR=1.55)[107] across 18 studies of adults. They also observed a pooled effect size for short sleep duration of -0.35 kg/m² change in BMI per hour of sleep change. These findings were obtained through linear analyses of these data, whilst non-linear associations were not considered. Although these two reviews[106,107] largely reached the same conclusion, Patel et al.'s[106] interpretation was slightly more tentative (as discussed earlier, they stated that findings in adults were more mixed than those in children). This could be because they considered different types of cross-sectional associations in their review, such as linear and non-linear, whereas Cappuccio's[107] meta-analysis only examined linear relationships.

Since the reviews by Patel et al.[106] and Cappuccio and colleagues[107], several other cross-sectional studies on BMI and sleep duration in adults have been published. A study of 1,224 twins (mean age of 37), also showed that short sleep was associated with higher BMI, after controlling for genetic and shared environmental effects[71]. In this study, analyses were carried out in two ways. First, twins were treated as individuals and to account for the correlation structure of the data, generalised estimating equations (GEEs) were used. Then, they extended the GEE model and modelled within- and between-pair associations of sleep duration and BMI. Finally, within and between analyses were repeated after

stratifying by twin zygosity. Importantly, this study used both self-reported sleep duration and height/weight data, which the authors acknowledged as an important limitation.

Sleep duration and BMI were also inversely associated in 1,248 American middle-aged respondents[110]. In a subsample (n=441) of these respondents, actigraphic sleep duration was also assessed; the results of this part of the study are discussed below in section 1.9.1.3 ('Studies of BMI and sleep using objectively-measured sleep duration').

Data from 5,021 British civil servants, aged between 34 and 55, also found an inverse association between sleep duration and BMI. Specifically, short sleep (≤5 hours) was associated with higher BMI, as well as an increased risk of obesity (OR=1.65), compared with those who slept for seven hours[111]. Even after controlling for obstructive sleep apnoea (OSA) and physical inactivity, the cross-sectional association between obesity and sleep duration remained, in a sample of 7,641 adults[45]. Theorell-Haglow and colleagues[113] also observed that in the all-female Sleep and Health Study, short sleepers (<6 hours) had significantly higher average BMIs than long sleepers (≥9 hours).

Dashti and colleagues also observed that in 14,906 adults in the CHARGE consortium longer usual sleep duration was associated with lower BMI[114]. The most recently published study in 1,615 UK adults (mean age of 43 years) from the National Diet and Nutrion Survey Rolling Programme (NDNS-RP), also supports a negative association between sleep duration and BMI, having observed an effect of -0.46 kg/m^2 per additional hour of sleep[115]. This finding emerged from linear analyses, as their cubic spline modelling yielded no relationship between sleep duration and BMI in this sample.

However, there have also been studies that have found no relationship between sleep duration and BMI. A Japanese population-based cohort of 10,000 adults found no association between sleep duration and BMI[116]. However, respondents were largely lean, with a mean BMI of 23 kg/m^2, their average age was 55 years and, at baseline, average sleep duration was 7.6 hours.

Two studies of older adults designed to investigate predictors of sleep duration found that BMI and sleep duration were unrelated. The first study analysed data from 8,091 participants aged 55 and over across seven European countries[117]. Obesity was not associated with being in the lowest fifth percentile of night time sleep duration (defined as sleep duration in the lowest fifth percentile respective to the age group). However, being underweight (BMI <19kg/m^2) was predictive of long night time sleep (95th percentile respective to age). Importantly, though, these findings could be due to a few factors. For example, perhaps the use of weight categories (i.e. underweight, normal weight, overweight, obese), rather than modelling BMI as a continuous outcome might mean that information is lost and it thus becomes more difficult to find an association. This was also one of the first studies to investigate the relationship between BMI and sleep duration in older adults and also of importance, is that 21% of the sample were aged 75 years or over. Also, the paper did not report the prevalence of overweight and obesity in this sample, which would have been important to know, in order to compare it to other studies (i.e. they may be quite lean or quite overweight/obese), as this can have an impact on the findings.

The second study, in a community sample of 1,026 French adults over the age of 60, found that, as the obese were more likely to nap during the day, there was no evidence of an association between total sleep duration and obesity[118]. Therefore, one potential explanation is that this association between BMI and sleep duration only in the obese group may have been because they were more likely to sleep for longer during the day (one hour or more, on average). This is in line with research which suggests that obese individuals are more likely to sleep for longer during the day[119] and have shorter night time sleep durations[120]. Also, similarly to the study described above, the authors treated both BMI (exposure) and sleep duration (outcome) as categorical variables and although they were able to discern that being in the obese category was a risk factor for very short sleep (≤4 and a half hours), it is possible that categorising continuous measures could contribute to loss of information, making it harder to find an association. Findings from both of these studies, albeit null, are in line with some research

that shows this association to weaken as a function of age[121,122] (described below in 1.9.1.4).

Table 1.1 Summary table of cross-sectional findings in adults using subjective sleep duration

Type of article/study	Authors	Year	Sample size	Outcome	Main findings**
Systematic review (23 studies)	Patel & Hu	2008	1,275,797	BMI	11/23 – negative relationship between sleep duration & BMI 2/23 – mixed: effects in only one sex 4/23 – no relationship between sleep duration & BMI 1/23 – short sleep duration →decreased weight 6/23 – U-shaped relationship: short/long sleep duration → risk of obesity
Systematic review w/meta-analysis (18 studies)	Cappuccio et al.	2008	605,509	BMI/obesity	Pooled OR for association between short sleep & increased risk of obesity = 1.55 Pooled continuous effect size for association between short sleep & BMI = -0.35 kg/m²*
Observational study (in twins)	Watson et al.	2010	1,224	BMI	Shorter sleep associated with higher BMI
Observational study			1,248	BMI	Shorter sleep associated with higher BMI
Observational study	Stranges et al.	2008	5,021	BMI/obesity	Shorter sleep associated with higher BMI Shorter sleep associated with increased risk of obesity
Observational study	Theorell-Haglow et al.	2012	7,641	BMI	Shorter sleepers had significantly higher BMI than long sleepers
Observational study	Dashti et al.	2015	14,906	BMI	Longer sleep duration associated with lower BMI
Observational study	Potter et al.	2017	1,615	BMI	Shorter sleep duration associated with lower BMI
			Null findings		
Observational	Amagai et al.	2004	10,000	BMI	Respondents were mostly lean (mean BMI=23 kg/m²), mean sleep duration was 7.6 hours, both potentially indicative of a healthy sample
Observational	Ohayon et al.	2004	8,091	BMI	BMI was categorised, 21% of the sample were 75 years or older
Observational	Ohayon et al.	2005	1,026	BMI	Obese individuals slept longer during the day & less at night, both sleep duration & BMI were categorised

Note. *Did not consider non-linear association; **all effects significant/not significant at $p<0.05$.

1.9.1.2 Prospective studies using self-reported measures of sleep duration

Cross sectional studies of the association of BMI and sleep duration suggest predominantly positive associations with some evidence of non-linear and sex specific associations. However, the longitudinal evidence is equivocal, as some studies find no relationship between sleep duration and future BMI, whilst others fail to observe an association in some age groups. To date, only one meta-analysis and one review on the prospective relationship between BMI and sleep duration in adults have been published, which indicate that a negative association exists between sleep at baseline and weight gain, at follow-up[106,123]. The prospective literature of BMI and subjective sleep duration in adults is summarised in Table 1.2.

Patel et al. (2008)[106] reported two studies that indicated that this relationship weakened with age[121,122]. Briefly, Gangwisch and colleagues[122] observed that the ORs for obesity associated with short sleep (<4 hours) compared to seven hours were 3.21, 1.81 and 1.71 for those aged 32-49, 50-67 and 68-86, respectively. Hasler et al's[121] findings showed that the obesity ORs for less than six hours sleep at age 29 was 8.1 cross-sectionally, whilst it was 4.6 at age 34.

Prospective analysis over 16 years in 68,183 women from the Nurses' Health Study (NHS) found that the lowest BMI was observed in women whose sleep duration was between seven and eight hours a night. Further, women who slept ≤5 hours gained 1.14 kg more than women who consistently slept for 7 hours, over the 16-year period[124].

The findings of one study suggest that longer sleep duration increases the risk of weight gain in 740 adults[125]. Over six-year follow-up findings indicated that the risk of becoming obese was 27% for short (five to six hours), and 21% for long sleepers (nine to 10 hours), in comparison to those who slept for seven to eight hours per night; thus, providing evidence of a U-shaped, rather than a linear association. The authors stated that these findings show that emphasis should be

placed on recommending normal durations of sleep (seven to eight hours), rather than merely advocating that people sleep for longer.

There has been some prospective research in adults that has yielded null findings. The longitudinal association between sleep duration and weight gain disappeared for 1,648 Japanese males, after adjusting for time-invariant unobserved confounders[126]. The authors referred to genetic factors as potential time-invariant unobserved confounders (those which cannot be measured as part of a study), whilst other studies have also suggested social, parental and environmental factors as time-invariant confounders[127,128]. To account for this type of confounder, Nishiura and colleagues carried out two types of analysis on their data. First, they used a generalised estimating equation (GEE), which uses the population-averaged model and subsequently, they performed fixed-effects (FE) modelling. Whilst both models can account for within-person changes in repeated measurements over one wave, the GEE model assumes independence between measured exposures with error terms, yet the FE model explicitly allows for the covariance between exposures and time-invariant unobserved factors (for example, genetics) to be non-zero[129]. It was then assumed that any difference that emerged between the coefficients from the two models would mean that residual confounding cannot be discounted, and the FE model should provide a less biased result. The most important finding was that the GEE model (population-averaged) suggested a longitudinal, negative relationship between sleep duration and BMI, but the FE model yielded no association. It was therefore suggested that the longitudinal association of sleep duration with changes in BMI may frequently be overestimated by such unobserved time-invariant factors, as opposed to misclassified sleep duration and that therefore, the net effect of sleep duration on BMI may not be as large as the effect found by some studies.

Stranges and colleagues failed to find a prospective association between short sleep duration and changes in body mass index, or with obesity incidence in the Whitehall II study[107]. They stated that although they did not find a significant prospective relation, their results were in concert with those described earlier, for

example, by Hasler et al.[121], and Gangwisch et al.[122], whose research suggested that the association between sleep duration and BMI weakens with age.

Table 1.2 Summary table of prospective findings in adults using subjective sleep duration

Type of article/study	Authors	Year	Sample size	Outcome	Follow-up period	Main findings *
			Positive findings			
Observational study	Chaput et al.	2007	740	BMI	6 years	U-shaped relationship: short/long sleep duration → + BMI/+ risk of obesity
Systematic review (3 studies)	Patel & Hu	2008	78,267	BMI	9, 13 & 16 years	3/3 – short sleep duration associated with increased risk of obesity
Meta-analysis (11 studies)	Wu et al.	2014	197,906	Obesity	1 to 12 years	Pooled OR for association between short sleep duration & obesity = 1.25 (8/11 studies, as 3 affected heterogeneity)
			Null findings			
Type of article/study	Authors	Year	Sample size	Outcome	Follow-up period	Possible reasons for null association
Observational study	Nishiura et al.	2014	1,648	BMI	3 years	Authors used an FE* model as a second approach, which accounts for time-invariant covariates (e.g. genetics)
Observational study	Stranges et al.	2008	5,021	BMI	5 years	In line with some previous research, which suggests that the association weakens with age

Note. FE=fixed effects; *all effects significant/not significant at p<0.05.

1.9.1.3 Studies of BMI and sleep using objectively-measured sleep duration

It has been suggested that employing objective measures of sleep duration may help to elucidate a more accurate relationship between sleep and obesity [106] and prevent potential misclassification of sleep duration, as evidence has shown systematic discrepancies between self-reported sleep and sleep measured using actigraphy[130]. There have only been three studies in adults using objective sleep measurements, to date. These are summarised in Table 1.3 below.

1.9.1.3.1 Cross-sectional studies

A U-shaped association between sleep duration – measured with actigraphy – and BMI and obesity, was also found in a study of 983 elderly adults. Compared

to normal sleepers (7 to 8 hours), short sleepers (<5 hours) and long sleepers (>8 hours) were more likely to be obese, ORs=2.76 and 2.93, respectively[130]. However, the authors also measured sleep disturbance and found that after adjusting for it the association between short sleep duration and obesity was no longer significant, whereas there was no change to the relationship between long sleep and obesity. Importantly, there was no association between self-reported sleep duration and BMI/obesity in this study, thus the findings differed according to measurement method for sleep duration.

Objective measures were used by a Brazilian study to examine the association between BMI and sleep duration, in 1,042 adults[131], and found that those with a higher BMI had shorter sleep durations. Furthermore, a study by Mezick and colleagues[110] measured actigraphic sleep duration in 441 respondents of the MIDUS study in which they found that shorter sleep was related to a higher BMI. This finding supported the association between self-reported sleep duration and BMI yielded by an analysis of the entire sample (1,248 respondents).

As illustrated above, there is very little epidemiological cross-sectional research that has exploited objective sleep duration in adults. Whilst both of the studies described yielded results in line with the majority of studies which employ self-reported sleep duration, more research is needed to confirm these findings. Some studies, however, have found no cross-sectional relationship between BMI and sleep duration. Lauderdale and colleagues[132] were amongst the first to examine BMI and sleep duration employing objective sleep measurements, in a sample of 38 to 50 year-olds from the CARDIA Study. However, their inverse, weak correlation between sleep duration and BMI was not statistically significant. In this study, average sleep duration was only 6.1 hours, which is lower than usual for a population-based study. This may be reflective of the differences in ethnicity that they observed, for example, mean sleep duration in White women was 6.7 hours, whilst in Black men it was 5.1 hours. These disparities appeared not to be explained by social position as those with a higher income and education spent less time asleep. Also notable was that the sample size was not particularly large, with a total n of 669.

1.9.1.3.2 Prospective studies

It appears that to date, the only prospective study of BMI and objectively-measured sleep duration was carried out in the CARDIA Sleep Study[133]. Sleep duration did not predict significant prospective changes in BMI in the CARDIA Sleep Study[133]. Lauderdale and colleagues observed that cross-sectionally, shorter sleep (≤4.5 hours) was associated with the highest BMIs, whilst those who slept for more than seven and a half hours had the lowest BMIs. Thus, their cross-sectional results were in concert with several previous studies. However, their longitudinal analyses showed that over 5-year follow-up actigraphic sleep duration did not predict changes in BMI.

There are some potential explanations for these null findings. Firstly, respondents' mean age at baseline was 45 years, thus at follow-up their average age was around 50 years. As mentioned earlier, there is evidence to suggest that the magnitude of the association between sleep duration and BMI weakens with age[111,121,122]. Secondly, it is possible that five years was not sufficient to observe a significant change in BMI, as BMI tends to be fairly stable. Thirdly, this study was conducted in a sample of only 667 individuals, which may have been underpowered to detect small changes in BMI over time.

Table 1.3 Summary table of literature in adults using objective sleep duration

Type of article/study	Authors	Year	Sample size	Outcome	Main findings/ possible reasons for null findings
Positive findings – cross-sectional					
Observational	Van den Berg et al.	2008	983	BMI	U-shaped relationship: short/long sleep duration → risk of obesity (not significant after adjustment for sleep disturbance)
Observational	Moraes et al.	2013	1,042	BMI	Short sleep duration associated with higher BMI
Observational	Mezick et al.	2014	441	BMI	Short sleep duration associated with higher BMI
Null findings – cross-sectional					
Observational	Lauderdale et al.	2006	669	BMI	Mean sleep duration was low (6.1 hours); modest sample size; ethnic differences observed between White and Black individuals
Null findings – prospective					
Observational (5-year follow-up)	Lauderdale et al.	2009	667	BMI	Association weakens with age*; follow-up may not have been long enough; modest sample size

Note. *mean age at baseline was 45 years (50 years at follow-up); *all effects significant/not significant at $p<0.05$.

1.9.1.4 Sex differences in the association of BMI with (objective/subjective) sleep duration in adults

As mentioned above, there is some evidence to suggest that the association between BMI and sleep duration might be moderated by sex. For example, a study of 35,247 Japanese adults showed that both short and long sleep duration were associated with a weight increase and risk of obesity at one-year follow-up in males, but not in females[134]. Of the non-obese men at baseline, 5.8% became obese after a year and the adjusted ORs for those who slept less than five hours and five to six hours were 1.91 and 1.50, respectively.

It is possible that this study found no prospective effect in women because new overweight and obesity at one-year follow-up were, according to the authors, both quite low in women, at 12.5% and 2.4%, respectively. However, 12.5% appears, in fact, to be quite a high proportion of incident overweight at one-year follow-up. The overall percentage of obesity and overweight in women at follow-up was not reported. However, a comparison was drawn between a previous study of 24,456 Japanese women, which found that 19.4% of them were obese and

the National Government's 2006 Health and Nutrition Survey found that 21.4% of women over the age of 20 were obese. Therefore, it might be valuable to perform further prospective analyses in this sample to carry out further follow-up of the women in this sample.

Mezick and colleagues found evidence of an inverse association between actigraphy-measured sleep duration and BMI, which appeared to be stronger in females[110]. They observed that although there was an association in the overall sample, sex moderated the effect of actigraphic sleep duration on BMI. Thus, in stratified analyses actigraphy-assessed sleep duration was associated with higher BMI in women only. This was in contrast to self-reported sleep duration, for which there was no effect modification by gender on BMI.

1.9.1.5 Summary of evidence in adults

The cross-sectional systematic reviews and meta-analysis, to date, have largely found that shorter sleep is associated with a higher BMI and increased odds of being obese. Prospective research has yielded mixed findings in adults, such that some support the cross-sectional literature. It is possible that this discrepancy in findings is due to differences between studies. For example, studies categorise sleep duration differently, some studies use BMI and/or sleep duration as categorical instead of continuous and there are disparities in adjustment for covariates across studies. Also, importantly, as mentioned earlier the association between BMI and sleep duration appears to weaken with age (although few studies have been carried out in older adults).

Some cross-sectional and prospective studies observe a U-shaped relationship, such that both short and long duration of sleep are related to higher BMI and greater odds of obesity. However, the majority of studies have found a linear association, yet this is also potentially a result of most studies having not explored non-linear relationships in their data. Thus, more research is needed, in which the linear and non-linear, cross-sectional and prospective associations of BMI with sleep duration are explored in detail, in large, representative samples.

Furthermore, some studies have observed no association between sleep duration and BMI/increased risk of obesity, whilst certain studies find a cross-sectional, but not a prospective relationship. Moderation by sex has also been suggested by some research and a few studies have found an association between sleep duration and BMI in one sex, but not the other, for which some explanations have been explored earlier (in section 1.9.1.4).

Importantly, to date, studies have neither investigated the bidirectional relationship of BMI with sleep duration, nor have they performed causal modelling of this association. Thus, the direction of this association in adults remains unclear and whether BMI causes changes in sleep duration, or sleep duration might cause changes in BMI is also uncertain.

1.9.2 Literature Review Part II: current evidence in children for the association between BMI and sleep duration

There is a large body of literature on the relationship between BMI and sleep duration in children. The majority of the evidence points toward a clear link between the two, particularly in terms of long-term weight gain as a result of shorter sleep[106,107,135,136]. However, these studies have predominantly investigated whether sleep duration is associated with future higher BMI and risk of obesity and only two studies[137,138] to date, have examined the bidirectional relationship between sleep duration and BMI (discussed below). At the end of this literature review, tables are provided to summarise the evidence in children (Tables 1.4, 1.5 and 1.6). Cross-sectional and prospective studies that use subjective sleep duration are summarised in Tables 1.4 and 1.5, respectively, whilst research that uses objective sleep duration is summarised in Table 1.6.

1.9.2.1 Cross-sectional studies using subjective sleep duration

A summary table of the cross-sectional paediatric literature of BMI and subjective sleep duration is provided below (Table 1.4). Cross-sectional evidence in children predominantly supports an association between lower sleep duration and greater weight and adiposity[106,107,139,140]. The first meta-analysis (that of Cappuccio and colleagues, the adult findings of which are discussed earlier) of obesity and sleep

duration in children[107] included 30,002 children from 12 cross-sectional studies and concluded that shorter sleep was associated with significantly greater odds of obesity (pooled OR=1.89). In addition, two systematic reviews[106,135], another meta-analysis[135] and a further review[140] also concluded the same.

Some studies classified short sleep as <10 hours or ≤10 hours, whilst others defined it as <8 hours per day, ≤6 hours per night or ≤3 hours. Irrespective of this variability in defining sleep duration, results were consistent across paediatric studies, which might be because studies used age-specific cut-offs for short sleep duration, as a form of standardisation. This association appeared to be stronger in children, when compared to studies in adults, which were less uniform, suggesting that this relationship may weaken with age, when drawing specific comparisons between paediatric studies and geriatric studies. This was further supported by evidence which shows that the cross-sectional association of short sleep with weight weakened with increased age in two studies[121,122]. This was in contrast to the claim made by Cappuccio and colleagues in the earlier meta-analysis, whereby they stated that effects were of similar magnitude between children and adults[107].

However, as the meta-analyses and systematic reviews described above did not, of course, include studies that emerged after their publication, these are reviewed separately. Pileggi and colleagues[141] investigated BMI in relation to parent-reported sleep duration in 10-year old children and found that children categorised as short sleepers had significantly higher BMI standard deviation scores (SDS) (0.77 kg/m^2), as compared to normal sleepers. In a sample of obese 7- to 16-year-olds it was apparent that those who slept fewer hours (n=50) were at significantly higher risk of severe obesity[142]. However, it was unclear how many hours of sleep was classified as short sleep in this study.

A recent Chinese study of 8,760 children aged between 6 and 18 years[143] found that short sleep duration (defined as ≤7 hours) was associated with obesity among girls, but in boys this effect was only apparent in those aged between 13 and 18 years. They also observed that the odds of obesity in relation to short sleep were decreased in boys between 6 and 12 years of age. Although Cao and

colleagues'[143] overall findings are comparable to those of studies from other countries, it is important to consider these differences in the categorisation of sleep duration. Recent findings from 6,576 Chinese children[144], 303 Mexican American children[145], 17,769 Japanese children[146] and 1,810 Chilean school children[147] also support a cross-sectional relationship sleep duration and BMI/increased risk of obesity.

Findings from most cross-sectional research are suggestive of a relationship between shorter sleep and higher BMI and risk of overweight/obesity. However, for different reasons, some studies have reported null associations between sleep duration and obesity measures. Klingenberg and colleagues[148] observed no association between parent-reported sleep duration and BMI in a sample of 211 Danish 3-year olds, in analyses adjusted for multiple covariates. The lack of effect could possibly be because families in this sample tended to have high educational attainment, were wealthy and all resided in the Copenhagen region of Denmark, as well as the small sample size (n=311). This has implications for representativeness of their findings, in particular due to the potential lower risk of becoming obese and developing poor sleep habits, for example.

A recent study of 1,929 8-year old Peruvian children found that 42% percent of the sample were short sleepers (<10 hours) and had a 15% greater prevalence of obesity, compared to normal sleepers (10-11 hours)[149]. However, after adjustment for several child and family-related factors (maternal and paternal education, maternal weight, location and wealth index), they found no significant relationship between sleep duration and overweight or obesity. A potential explanation put forward for the lack of association included the definition of short vs. normal sleep duration was more conservative than other studies that yielded a significant effect.

A study of 3,086 Chinese children aged between seven and fourteen years found that, although those who were overweight or obese were less likely to sleep longer at the weekends, to compensate for insufficient sleep during the week, there was no significant relationship between weekday sleep duration and the

odds of being overweight or obese[150]. One possible explanation for this finding could be cultural factors that are not relevant to studies of Western children.

In summary, very few studies have reported null findings of the association between BMI and sleep duration in children. It is therefore important to note the potential reasons (discussed above) for these findings, i.e. they had low prevalence of obesity, were of particularly high SES and/or defined short and long sleep distinctly from other studies that did find an effect.

Table 1.4 Summary table of cross-sectional findings in children using subjective sleep duration

Type of article/study	Authors	Year	Sample size	Outcome	Main findings/possible reasons for null findings
Systematic review w/meta-analysis (12 studies)	Cappuccio et al.	2008	30,002	BMI	12/12 – shorter sleep duration associated with greater odds of obesity (pooled OR=1.89)
Systematic review w/meta-analysis (11 studies)	Chen et al.	2008	44,228	BMI	11/11 – shorter sleep duration associated with increased risk of obesity (pooled OR=1.58)
Systematic review (11 studies)	Patel & Hu	2008	26,997	BMI	11/11 – shorter sleep duration associated with increased risk of obesity
Systematic review (25 studies)	Liu et al.	2012	20,244	BMI	25/25 – shorter sleep duration associated with overweight/obesity
Observational study	Pileggi et al.	2013	542	BMI	Shorter sleep duration associated with higher BMI
Observational study	Cao et al.	2015	8,760	BMI	Shorter sleep duration associated with obesity in girls only
Observational study	Meng et al.	2012	6,576	BMI	Shorter sleep duration associated with higher BMI
Observational study	Sakamoto et al.	2017	17,769	BMI	Shorter sleep duration associated with increased risk of obesity
Observational study	Aguero et al.	2016	1,810	BMI	Shorter sleep duration associated with increased risk of obesity
			Null findings		
Observational study	Klingenberg et al.	2013	211	BMI	Families of high SES; all residents in urban area
Observational study	Carrillo-Larco et al.	2014	1,929	BMI	Authors suggested low prevalence of overweight/obesity (but in fact totalled 21% so not that low)
Observational study	Zhang et al.	2015	3,086	BMI	In Chinese culture children are under strict parental surveillance, irrespective of whether they are of normal weight/overweight/obese & all children appear to have insufficient sleep, not only overweight/obese children

*All effects significant/not significant at $p<0.05$.

1.9.2.2 Prospective studies using subjective sleep duration

Prospective studies of BMI and subjective sleep duration are summarised in Table 1.5 below. Systematic reviews and meta-analyses of prospective studies are largely in line with findings from the cross-sectional literature, such that shorter sleep is associated with changes in BMI and an increased risk of obesity[106,107,139,140,151–153]. However, discrepancies exist between studies regarding sex differences (described in 1.9.2.8 below), and there is some evidence for a U-shaped, as opposed to a linear association.

Patel and Hu[154] published the first systematic review of paediatric prospective studies and observed a strong and consistent relationship between shorter sleep and future obesity risk. Although the authors concluded that there is a longitudinal association between shorter sleep and increased risk of weight gain and obesity, they based this on the only two longitudinal studies that had been published at the time. Briefly, one of these studies followed 8,234 children from three to seven years of age and observed ORs for obesity of 1.45, 1.35 and 1.04 for those who slept either <10.5 hours, between 10.5 to 10.9 hours and 11 to 11.9 hours, compared to 12-hour sleepers[155]. The other study found that in 150 children aged between three and five years at baseline, the risk of becoming overweight at age nine and a half years, was predicted by short sleep duration. Those who became overweight slept for approximately 30 minutes less than the normal weight children[156]. Importantly, though, Patel and Hu acknowledged that neither of these studies adjusted for weight at the time when sleep duration data were collected, thus did not measure actual changes in weight.

The conclusion that shorter sleep confers an increased risk of overweight and obesity was supported by another systematic review with meta-analysis published the same year[135]. Subsequently, in 2015 two further systematic reviews with meta-analyses emerged[151,152]. Fatima and colleagues[151] found that sleep duration was inversely associated with future BMI in children, such that those who have shorter sleep durations are approximately twice as likely (OR=2.15) to become overweight/obese compared to their normal-sleeping counterparts. The authors stated that their findings concurred with those of previous meta-analyses, yet

they observed a stronger effect and speculated that this was because they weighted more heavily the three studies that used objective sleep duration, measured (rather than self- or parent-reported) BMI and had at least a three-year follow-up period. A further systematic review and meta-analysis[152] of 25 studies with a total of 56,584 children also found that over an average of 3.4 years follow-up, those who slept for approximately 10 hours, compared to those who slept 12 hours, were 75% more likely to be overweight/obese (OR=1.76). Additionally, these children had relatively greater annual BMI gain (0.13 kg/m^2) for every hour decrease in sleep duration.

The most recent meta-analyses of the association of sleep duration and change in BMI (and other measures of adiposity) support the notion that short sleep is associated with increases in future BMI[153,157]. However, there is heterogeneity in these meta-analyses which may be related to a number of factors including the definition of short and long sleep, length of follow-up time, geographic location, and ethnic group.

1.9.2.3 Bidirectional, prospective studies of BMI and sleep duration

When reviewing this literature, it was apparent that studies have largely investigated the prospective association between sleep duration and BMI over time, rather than *vice versa*, with the exception of two studies that have examined potential bidirectionality. This research is important, as it attempts to ascertain the direction of this association, which in turn, provides information on whether children's sleep duration might change as a consequence of being overweight/obese, or whether the reverse might be true[158]. Only two studies to date have investigated the bidirectional, longitudinal relationship between BMI and sleep duration in children[137,138]. Although these studies were included in some of the meta-analyses described earlier they will be described here separately.

The first to investigate the bidirectional relationship of BMI with sleep duration were Hiscock and colleagues, who observed that, in Australian children, BMI did not predict changes in sleep duration, or vice versa[137]. They performed two sets of cross-sectional and prospective analyses in 3,857 infants and 3,844 children. The

infants were between zero and one year of age at baseline, and age two to three years at follow-up; the children were aged between four and five years at baseline, and six to seven at follow-up. Prevalence of overweight was around 15% and obesity 5%, at each follow-up, but the authors observed no longitudinal association in either direction; from ages zero/one to two/three years, or four/five to six/seven years. Cross-sectionally, however, they found that obese six/seven-year-olds slept for 30 minutes less than their underweight, normal weight and overweight peers.

These negative findings contrasted with numerous epidemiological studies and this could be due to at least two reasons. Firstly, the authors used 24-hour time diaries to collect data on sleep duration whilst the majority of previous research at the time had used parent-reported sleep duration and it is this measure, rather than time diary reports, that predict obesity. Therefore, this could mean that their results were, in fact, more accurate than studies that use parent-reported sleep duration. However, the study used a bespoke measure to ascertain sleep, which has not been used in other studies and for which no validity or reliability measures were provided and may mean that the findings are uncertain.

Secondly, it is possible that the relationship between short sleep and paediatric obesity develops slightly later, which is in line with their finding from the six-to-seven-year-old children. One hypothesis could be that the relationship between shorter sleep and obesity is via eating behaviour and as such, a child would need to consume excessive amounts of food over a certain number of years (as a consequence of short sleep), which would then lead to increased weight/risk of obesity later. Also, it is possible that children who eat more when they are tired are likely to have more autonomy over what, when and how they consume food, which is a privilege that usually comes as children get older. This is supported by epidemiological research in which shorter sleep in early life (16 months) is associated with greater energy intake, but this effect emerges prior to the association with weight[159]. More specifically, this study by Fisher and colleagues[159] showed that there was no relationship between sleep duration and weight in this

sample of 1,303 British children, but the association between energy intake and sleep duration was strong.

Collings et al.[138] also investigated the bidirectional relationship between BMI and sleep duration. They analysed data from 776 South Asian and 562 White children from the Born in Bradford-1000 (BiB-1000) cohort, at ages 12, 18, 24 and 36 months of age. Their results showed that the association between BMI and parent-reported sleep duration was significant in both directions in South Asian children, but the findings were null in White children[138]. In the South Asian children BMI had a two to threefold larger effect on sleep duration, rather than the other way around (i.e. sleep duration as the exposure, which is the direction modelled in all, but two prospective epidemiological studies, to date). Finding that BMI and sleep duration are associated in both directions in South Asian children requires replication in a larger and independent sample. The findings in White children are comparable with those of the earlier study[137], even though Collings used parent-reported sleep duration, as opposed to sleep diaries.

Both of the studies described above observed no bidirectional relationship between BMI and subjective sleep duration in White children from the UK and Australia. Although each study used a distinct sleep duration measure (24-hour diaries vs. parent-reported duration) these findings have yet to be replicated in a paediatric sample using objectively-measured sleep duration.

Table 1.5 Summary table of prospective findings in children using subjective sleep duration

Type of article/study	Authors	Year	Sample size	Outcome	Follow-up period	Main findings*
Systematic review (2 studies)	Patel & Hu	2008	8,384	BMI	4 – 4.5 years	Shorter sleep associated with increased weight & risk of obesity
Systematic review w/meta-analysis (3 studies)	Chen et al.	2008	10,189	BMI	3 – 9 years	Shorter sleep associated with increased risk of becoming overweight/obese
Systematic review (22 studies)	Fatima et al.	2015	42,223	BMI	1 – 9.5 years	Shorter sleep associated with higher BMI/increased risk of overweight/obesity
Meta-analysis (11 studies)	Fatima et al.	2015	24,821	BMI	2 – 9.5 years	Shorter sleep associated with higher BMI/those who sleep for less twice as likely (pooled OR=2.15) to become overweight/obese
Meta-analysis (25 studies)	Ruan et al.	2015	56,584	BMI	0.5 – 10 years	Shorter sleep associated with greater odds of obesity (pooled OR=1.76)
Meta-analysis (12 studies)	Li et al.	2017	44,200	BMI	2 – 15 years	Shorter sleep associated with higher prospective BMI & 30% increased risk of obesity
Meta-analysis (13 studies)	Wu et al.	2017	35,540	BMI	1 – 5 years	Shorter sleep associated with greater odds of obesity (pooled OR=1.71)
Bidirectional prospective studies						
Observational study	Collings et al.	2017	776 (South Asian)	BMI & sleep duration	18, 24 and 36 months	Higher BMI associated with decreased sleep from baseline (6 months) to 12, 18 and 24 months, but not 36 months AND Longer sleep (at baseline) associated with decreased BMI at 12, 18, 24 and 36 months
Null findings						
Observational study	Hiscock et al.	2014	3,844	BMI & sleep duration	1 – 7 years	Sleep duration did not predict changes in BMI, BMI did not predict changes in sleep duration
Observational study	Collings et al.	2017	562 (White)	BMI & sleep duration	18, 24 and 36 months	Sleep duration did not predict changes in BMI, BMI did not predict changes in sleep duration

*All effects significant/not significant at $p<0.05$.

1.9.2.4 Studies using objectively-measured sleep duration

There are very few studies to date, that have used objective sleep duration to examine its relationship with BMI in children and their findings are equivocal.

Both cross-sectional and prospective studies that have used objective sleep duration measures are summarised in Table 1.6 below.

1.9.2.5 Cross-sectional studies

Chaput and colleagues[160] objectively measured sleep duration over seven days in a sample of Canadian children aged 10. After adjustment for a number of lifestyle and demographic covariates they observed that short sleepers (<10 hours) had increased odds of overweight/obesity (OR=2.08).

In 308 American children[161] between the ages of four and ten average actigraphic sleep duration was eight hours, which is markedly below the recommended amount for children. Analysis of both weekday and weekend sleep duration showed that there were no differences between normal weight, overweight and obese groups. However, the authors then examined sleep variability values within BMI SDS groups. They found that in obese children, sleep duration was more variable on weekends than on weekdays, in comparison with children in the normal and overweight BMI SDS groups. Overall, shorter sleep duration was associated with other metabolic markers, such as altered insulin, LDL and high-sensitivity CRP and thus, the authors concluded that these might be more important in this context (i.e. that shorter sleep might have a greater impact on these other metabolic markers, rather than BMI).

A Swedish study of 1,231 children[162] aged between six and ten years found objectively-measured sleep duration to be negatively associated with BMI. One important limitation of this study was the lack of sleep diary data; thus, evaluation of sleep times was more difficult. Research conducted in 303 mother-child pairs using both mother-reported and objective sleep duration suggests that BMI was associated with both of these measures[145]. However, the sleep measures were weakly correlated and the objective data appeared to provide a more reliable estimate of children's sleep durations[145]. A 2015 study using data from 6,025 children across twelve countries from the International Study of Childhood Obesity, Lifestyle and the Environment (ISCOLE) also showed an association between longer sleep duration and decreased odds of obesity (OR=0.79)[163]. Findings from Wilkie and colleagues[164] are in concert with the ISCOLE data, as

they observed a relationship between lower odds of obesity and longer sleep duration, in 374 UK children.

In a Canadian sample of 567 10-year old children no cross-sectional association was observed between actigraphic sleep duration and BMI[165]. As the effect of sleep duration on BMI was no longer significant following adjustment for several covariates (age, sex, ethnicity, family income, parental education, maturity offset, moderate to vigorous physical activity (MVPA), sleep efficiency and sleep timing) these findings were comparable to some previous research[166] in which this effect was diminished after adjustment for covariates. However, this sample had higher sleep efficiencies than others that previously objectively-measured this in children, which could in turn be explained by the fact that this cohort were relatively lean and active. However, these high sleep efficiencies might also have been influenced by the fact that data were collected using waist-worn actigraphy, which may overestimate both sleep duration and sleep efficiency[167], as compared to wrist-worn actigraphs and this might at least, in part, explain the high sleep efficiency values in this sample.

1.9.2.6 Prospective studies

Prospective studies that examine objective sleep duration in relation to BMI are still scarce, with few published to date. Using data from 304 participants of the Tucson Children's Assessment of Sleep Apnoea study, researchers found that those who slept less than seven and a half hours a night at age six were at increased odds (OR=3.3) of becoming obese 5 years later, compared to those who slept ≥9 hours per night[168]. Additionally, short sleep was associated with a mean BMI increase of 1.7 kg/m^2 at 5-year follow-up. Another study published in 2011 yielded similar results in 244 children followed from ages three to seven years[169]. From baseline to follow-up each additional hour of sleep was associated with a 0.48 kg/m^2 reduction in BMI, as well as decreased risk of being overweight.

From reviewing the extensive paediatric literature on the relationship between BMI and sleep duration, it is apparent that few studies collect objective sleep data. As mentioned earlier, one underlying reason for this is that it is still too costly to collect these data from hundreds, if not thousands, of participants.

Table 1.6 Summary table of cross-sectional & prospective findings in children using objective sleep duration

Type of article/study	Authors	Year	Sample size	Outcome	Main findings/possible reasons for null findings
Cross-sectional – positive findings					
Observational	Chaput et al.	2011	550	BMI	Shorter sleep duration associated with increased risk of obesity
Observational	Ekstedt et al.	2013	1,231	BMI	Shorter sleep duration associated with higher BMI
Observational	Martinez et al.	2014	303	BMI	Shorter sleep duration associated with higher BMI
Observational	Katzmarzyk et al.	2015	6,025	BMI	Longer sleep duration associated with decreased odds of obesity.
Observational	Wilkie et al.	2016	374	BMI	Longer sleep duration associated with decreased odds of obesity.
Cross-sectional – null findings					
Observational	Spruyt et al.	2011	308	BMI	Sleep duration was more variable in obese children on weekends vs. weekdays; sleep duration was much lower (8 hours) than the recommended amount for children; shorter sleep duration was associated with other metabolic markers, not BMI
Observational	Mcneil et al.	2015	567	BMI	Sample were lean & active compared to other studies; sleep efficiency was very high on average; waist-worn actigraphy was used, which can overestimate sleep duration/efficiency
Prospective – positive findings					
Observational	Silva et al.	2011	304	BMI	Shorter sleep duration associated with increased odds of obesity at 5-year follow-up & with increased BMI at follow-up
Observational	Carter et al.	2011	244	BMI	Longer sleep duration associated with decreased BMI at 4-year follow-up

*All effects significant/not significant at $p<0.05$.

1.9.2.7 Sex differences in the association of obesity and (subjective/objective) sleep duration in children

Some studies in children suggest that there is a marked difference in the magnitude of association between BMI and sleep duration in boys and girls. For example, one of the earlier reviews described three studies that found boys were more likely to sleep for less hours than girls[106]. Specifically, one of these studies observed distinct obesity ORs associated with <8 hours vs. >10 hours sleep, such that for boys it was 5.5 and girls 2.1[170]. A UK cross-sectional study of 1,294 children aged between 7 and 18 years revealed an association between age-adjusted BMI and sleep duration in boys, but not girls[171]. This was further supported by Chaput

and colleagues' work[172], which found an OR for obesity related to sleep duration of ≤10 hours, compared to 12 or more hours was 3.2 in girls and 5.7 in boys.

Research conducted in 6,324 children from the Australian Health and Fitness Survey observed a dose-response relationship between short sleep (<8 hours sleep vs. >10 hours sleep) and odds of being overweight/obese in boys only[173]. This is in line with research from a Canadian sample, which suggested that at ages 6 and 7, sleep duration was inversely associated with overweight/obesity in boys, but not girls[174]. They also found that sleep duration at age 2.5 years predicted overweight/obesity in boys at ages 6 and 7. Notably, the researchers also discovered that in boys, shorter sleep duration was associated with unfavourable eating behaviours and thus, at age six, they were more likely to eat at irregular times and consume too much food, too fast. This was suggestive of a mediating effect of eating behaviours between sleep duration and overweight/obesity in boys.

In the ISCOLE study described earlier, longer sleep duration was associated with decreased odds of obesity in both boys and girls in this multi-country analysis[163]. However, the OR for boys was 0.83 (17% reduction in risk), whilst for girls it was 0.75 (25% reduction in risk), a result which is important, not only because this study was conducted across 12 countries, but also because they used objective sleep duration. Therefore, this study's findings differed from those of the studies discussed above, which suggested that the effect of sleep duration on obesity risk is greater for boys, rather than girls.

1.9.2.8 Summary of evidence in children

Both cross-sectionally and prospectively, evidence in children largely suggests that shorter sleep is associated with higher BMI, as well as risk of overweight/obesity. In recent years, several large-scale meta-analyses and systematic reviews have supported this finding, yet there are some differences across study results. These discrepancies in conclusions might be due to factors that have been explored above, in this literature review, in more detail. For example, similarly to adult research, there have been differences in the way that studies define short/long sleep, the analysis of BMI as a categorical (using weight

status categories) vs. a continuous measure and the inclusion of covariates is also disparate. These important factors can have an impact on study findings, all of which have been explored above in this literature review.

Some research finds that boys are, on average, more susceptible to sleep loss and potentially, increases in BMI and greater odds of overweight/obesity, which could perhaps be explained by evolutionary or physiological sleep differences. However, one of these studies, which analysed data from 6,025 children across 12 countries, found that, in fact, there was a larger effect of sleep duration on obesity risk for girls, rather than boys. Other studies find a U-shaped relationship between sleep duration and weight gain, for example, suggestive of a non-linear, rather than a linear effect. However, the majority of studies have reported a linear relationship between the two.

Unlike in adults, there have been two studies to explore the bidirectional association of sleep duration and BMI in childhood. These studies' findings were identical for children of White ethnicity, in that they yielded no prospective relationship in either direction. However, one of these studies found a relationship in both directions (BMI predicting changes in sleep duration, as well as sleep duration predicting changes in BMI) in children of South Asian ethnicity. Both of these bidirectional, epidemiological studies used only subjective sleep duration measurements; no studies have yet investigated the bidirectional association between BMI and sleep duration using objective sleep duration. Notably as well, no research has attempted to investigate whether the relationship between BMI and sleep duration might be causal, in either direction.

1.9.3 Potential explanations for sex differences in both children and adults

Eisenmann et al.[173] suggested that one explanation for sex differences in the association between BMI and sleep duration could be that females might need to experience greater sleep loss before they are affected, as they tend to be more resilient to environmental stress than males, from an evolutionary perspective. This is grounded in the theory that sex differences in early vulnerability are due

to the natural selection of optimum maternal strategies that maximise reproductive success, and that irrespective of medical care advances early life environmental stressors will always disproportionately affect males[175].

An alternative account for the difference between males and females comes from physiological and behavioural distinctions in sleep architecture between the sexes[176]. For example, there is some evidence that boys score more poorly on actigraphic sleep measures; they experience more awakenings and interruptions during sleep, whilst girls sleep for longer with fewer interruptions[177]. Further, these sex differences might influence how insufficient sleep affects phenotypes such as diet, eating behaviour and physical activity of boys differently from girls[174]. For example, perhaps boys are more sensitive to obesogenic eating behaviours when they do not have the required amount of sleep for prolonged periods. However, it might also be important to note that younger boys are likely to have their diets and eating behaviours quite closely monitored by parents/caregivers.

Explanations have also been offered for why some studies find an association between sleep duration and BMI/increased risk of obesity in females, but not males. For example, an experimental study showed that when men and women's sleep was restricted to five hours per night (compared to nine hours), both sexes were more likely to consume more calories at night, when food was available ad libitum[178]. However, men gained weight irrespective of sleep condition (five vs. nine hours), whereas women's weight only increased in the five-hour restricted sleep condition, thus the authors concluded that insufficient sleep may lead to less dietary restraint in females.

Overall, the majority of studies have observed an association between sleep duration and BMI, irrespective of sex. However, some research shows an association in males, but not females and vice versa and some potential biological explanations have been explored. More research is still required to examine this moderation by sex in very large samples and across diverse age groups. Also, the opposite direction should be investigated to ascertain whether sex might modify the effects of BMI on longitudinal changes in sleep duration.

1.9.4 Proposed pathways in the literature underlying the relationship of BMI with sleep duration

A few pathways via which sleep duration can influence the onset of obesity have been proposed. For example, there is evidence to suggest that short sleep duration is linked to changes in levels of the appetite hormones leptin (the 'satiety' hormone) and ghrelin (the 'hunger' hormone),[109,179–181] and that such alterations in appetite could mediate the relationship between sleep duration and obesity[182]. Spiegel et al[179] found that after two days of sleep restriction and two days of sleep extension, during which caloric intake and physical activity were systematically controlled, leptin (involved in the homeostatic regulation and reduction of appetite via increased satiety) levels decreased by 18%, while ghrelin (which increases hunger) increased by 28%, 24% and 23%, respectively. Importantly, though, this study had a very limited sample of 12 men and did not measure energy expenditure. This finding was, however, supported by two studies in which short sleep duration was associated with reduced leptin[109,180] (i.e. reduced satiety and higher hunger), and two reported that short sleep was associated with increased ghrelin levels[180,181] (i.e. higher hunger). In contrast, one of these studies saw no changes in the leptin levels of nine men, who had spent three nights (7 hours, 4.5 hours and total deprivation) in a sleep laboratory, separated by two weeks each time[181]. The sample analysed was particularly small and thus this study may not have been particularly well powered to detect effects.

Another pathway that could underlie the relationship between sleep duration and adiposity is related to certain obesity-related behaviours. In a study of 30,000 Japanese adults, although 80% reported getting enough sleep per night, 28% of the population reported sleeping for less than six hours nightly[183]. Findings further indicated that sleep loss was associated with an unhealthy lifestyle, such as insufficient physical activity and regular snacking between meals. Further, in the Whitehall II study Stamatakis and colleagues[184] found an association between short sleep duration and obesity-related behaviours, such as insufficient physical activity and reduced fruit and vegetable consumption. They suggested that

interventions that promote physical activity and improved nutrition should also consider sleep duration as a modifiable risk factor for obesity.

Another, more simple explanation, is that people who sleep less have more time to eat. However, the evidence in support of this explanation is not clear-cut, as individuals in the short and long sleep categories frequently have a higher BMI, compared to those who have normal sleep durations. This is supported by some evidence reviewed earlier that suggests a U-shaped relationship between BMI and sleep duration. Some research nonetheless, suggests that at least in young children, there may be partial support for this account. One such study examined the relationship between sleep and energy intake in early childhood, by analysing data from 1,303 children from the UK Gemini twin birth cohort[159]. Sleep duration data were collected from parents using a questionnaire when the children were 16 months old, whilst the diet diaries were completed when they were between 20-21 months of age. Findings revealed that shorter night-time sleep duration was associated with higher energy intake and that these infants consumed, on average, 50 kilocalories (kcal) more per day than children who had optimal durations of sleep. Although this difference appeared to be small, it equated to approximately 5% of the daily energy intake in this sample. Further analysis of the Gemini children showed that those sleeping for <10 hours, in fact, consumed an average of 120 kcal (15% of the daily intake) more at night specifically, compared to those sleeping ≥13 hours[185] .

Another conceivable explanation, which remains untested to date, is that BMI and sleep duration could have overlapping genetic factors. This is otherwise known as pleiotropy, whereby the same genes influence multiple traits[186]. The reason that this potential explanation is focused on BMI, rather than waist circumference, is that it is possible to test for pleiotropy between common genetic variants associated with BMI and sleep duration, but not WC and sleep duration. This is because there are now ninety-seven published and replicated genome-wide SNPs associated with BMI[29]. Testing for shared genetic pathways between BMI and sleep duration seems plausible, as some of the genetic variants in certain genes, such as *FTO* for example, are predominantly expressed in the

hypothalamus[30]. The hypothalamus is a key area in the brain involved in inducing sleep and promotes wakefulness.

The ventrolateral preoptic nucleus (VLPO) is a cluster of neurons in the anterior hypothalamus, which are directly connected to the brain's arousal-promoting centres and therefore, promote sleep by inhibiting these other arousal centres. Thus, it is possible that one or more SNPs in these genes may lie on the causal pathway between BMI and sleep duration and that if an individual has an elevated genetic risk of BMI, this may then confer an increased risk of shorter sleep duration. As mentioned earlier in this chapter, short sleep duration has been associated with CVD[61], T2D-mellitus[63,187], hypertension[64,188] and earlier mortality[65,66].

In summary, a few important pathways have been proposed in relation to why BMI and sleep duration are frequently associated in the epidemiological literature. However, it is clear that further research is required to investigate other previously understudied pathways and mechanisms, some of which will be explored in this thesis.

1.10 LIMITATIONS OF THE CURRENT EVIDENCE AND RATIONALE FOR THIS RESEARCH

The evidence reviewed here on the relationship between adiposity and sleep duration in children and adults has important limitations, which are discussed in this section. These limitations then lead to an outline of the rationale for the research carried out in this thesis.

The majority of research in this area has used observational epidemiological methods. These studies are unable to draw conclusions about causality, even those that employ a prospective design whereby participants may have been followed over a number of years[189]. The inability of observational studies to infer causality is due to two main reasons. Firstly, residual confounding occurs when unmeasured factors that may underlie associations cannot be accounted for in analyses and, secondly, reverse causation refers to when the 'outcome' may precede the 'exposure'. Both confounding and reverse causation can be addressed through the exploitation of genomic data, using a design called Mendelian randomisation[190] (described in more detail in Chapter 6).

Reverse causation can also be partially addressed using approaches that examine bidirectionality, which has been suggested regarding the association of adiposity measures and sleep duration[158]. However, to date, this has only been investigated in two paediatric studies[137,138], and never in adults. Furthermore, there have now been hundreds of studies that have investigated the relationship between measures of adiposity and sleep duration, yet no studies have attempted to use causal modelling to determine whether this association is causal, in either direction.

There have also been far fewer studies in older adults than in children and younger adults and none of these have used a bidirectional approach to this question. Whilst it is important to determine what effect BMI has on sleep duration, or vice versa, in children and young adults, it is both timely and important to determine the nature and size of this relationship in older adults. Although studies have suggested that the effect of sleep duration on BMI

weakens with age, the prospective effect of BMI on sleep duration has not been explored in older adults. The main reasons that this is important are twofold. The ageing population is rapidly growing, with the number of people aged over sixty expected to represent 22% of the world's population by 2050[191]. As a consequence, the second reason for more research in older people in this area, is the need to better understand the health of the elderly, as this is the time when disease and frailty are most common[192].

1.11 AIMS AND OBJECTIVES OF THIS THESIS

The overarching aim of this thesis was to examine the relationship between BMI and sleep duration using both observational and genetic epidemiological approaches. The literature on BMI and sleep duration to date, in both children and adults is mostly observational. It remains unclear whether BMI might precede changes in sleep duration, or whether sleep duration precedes changes in BMI. Also, whether this relationship is *causal* remains to be established, in either, or both directions. Thus, the research undertaken in this thesis aimed to: (i) investigate the direction of this association in both children and older adults; (ii) understand this complex relationship at both ends of the life course; (iii) and use causal analyses – Mendelian randomisation (MR) – to test the causal relationship between BMI and sleep duration. MR requires robust and replicated SNPs through GWAS (for both BMI and sleep duration), so in order to perform bidirectional MR it was also necessary to carry out a meta-GWAS of sleep duration (this project preceded the UKB 2016 GWAS published by Jones and colleagues[78]).

1.11.1 Hypotheses

The following hypotheses were tested in this thesis:

i) That in older adults, there would be a negative cross-sectional association between BMI and self-reported sleep duration (Chapter 3);

ii) That in older adults, there would be a negative prospective association between BMI and changes in self-reported sleep duration over four years, but not in the opposite direction (Chapter 3);

iii) That in children, there would be a cross-sectional negative relationship between BMI and objective sleep duration (Chapter 4);

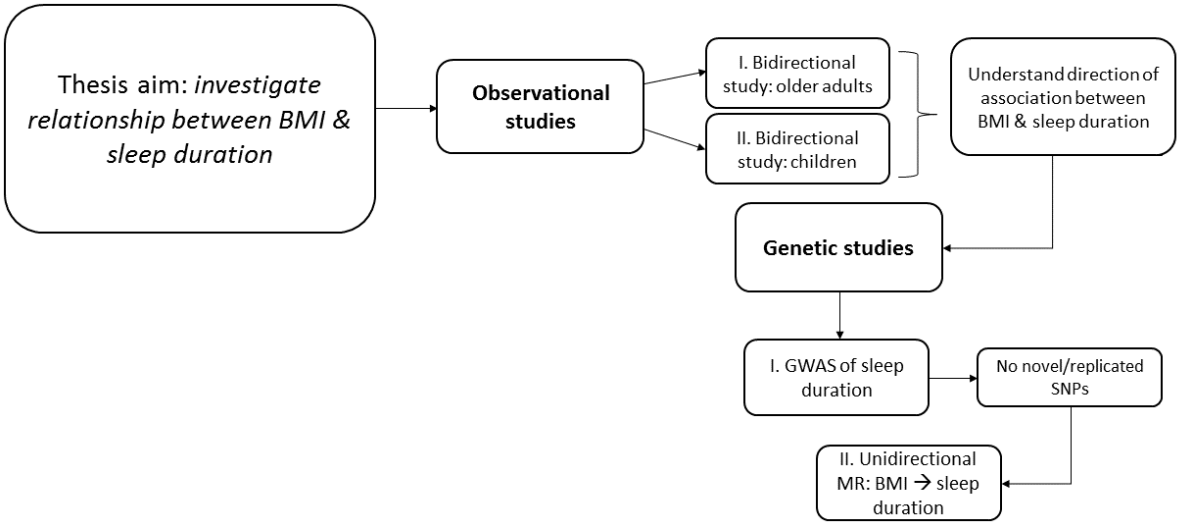
iv) That in children, there would be a prospective, negative association between objective sleep duration and change in BMI and/or a negative association between BMI and change in objective sleep duration (Chapter 4);

v) That BMI may be causally associated with self-reported sleep duration, such that a higher BMI might cause short/long sleep duration (Chapter 6).

1.11.2 Specific objectives

- Investigate the bidirectional observational association between adiposity and self-reported sleep duration, in cross-sectional and longitudinal analyses in older adults, using data from a large English community sample (Chapter 3).
- Investigate the bidirectional, observational relationship between BMI and objective sleep duration using a paediatric community sample (Chapter 4), with the aim of establishing the direction of effect in early life, given that there have only been two bidirectional studies in children, to date.
- Perform a large-scale GWAS of self-reported sleep duration in three UK population studies, as a precursor to performing a Mendelian randomisation analysis to examine whether the association between sleep duration and BMI is causal (Chapter 5).
- Perform a large-scale Mendelian randomisation study to investigate the potential causal effect of BMI on self-reported sleep duration, using the most up-to-date methodologies (Chapter 6).
- Discuss the key findings, strengths and limitations of this research; potential implications for policy and practice and prospects for future research (Chapter 7).

Figure 1.2 Work flow for the studies carried out in Chapters 3, 4, 5 and 6 in this thesis



2 DATASETS USED IN THIS THESIS

2.1 BRIEF INTRODUCTION AND OVERVIEW OF CHAPTER CONTENTS

This research thesis took a multidisciplinary approach to establishing the nature of the relationship between sleep and BMI and employed a combination of observational and genetic epidemiological methods. To maximise power for the genetic analyses, data from four epidemiological studies were used in this work: ALSPAC (mothers), ELSA and UKHLS. This chapter provides an overview of the datasets used throughout this thesis, with details of relevant phenotypic measures [height, weight, waist circumference (only ELSA analyses in Chapter 3 used WC) and sleep duration], as well as summary statistics and information about collection of DNA samples, genotyping quality control (QC) metrics and genotype imputation.

The work conducted in this thesis did not involve any subject recruitment, genotyping, QC of genetic data or genotype imputation. For the ALSPAC mothers' data, there was no involvement in preparation of the phenotypic sleep duration data either. At the end of this chapter Table 2.5 presents a summary of the phenotypic and genetic data used in this thesis.

2.2 DATASETS

2.2.1 Avon Longitudinal Study of Parents and Children (ALSPAC): mothers

ALSPAC was established with the aim of understanding how genetic and environmental factors influence health and development of parents and their children. Through an existing link with the University of Bristol it was possible to collaborate on the GWAS of sleep duration with Dr. Gibran Hemani from the Integrative Epidemiology Unit, who cleaned the sleep duration data and performed the analyses. Dr Hemani then uploaded the results to a secure server,

which were then downloaded and included in the genome-wide meta-analysis, along with two other studies.

2.2.1.1 Ethics

Ethical approval was initially granted by the Bristol and Weston Health Authority (E1808) and then the Mothers clinic was approved by the North Somerset & South Bristol Research Ethics Committee (08/H0106/96). Written informed consent was also obtained at recruitment.

2.2.1.2 Participants

Pregnant women from a specific area in the South West of England (Avon), with an expected delivery date between 1st April 1991 and 31st December 1992 were eligible, of whom 13,761 were recruited into ALSPAC. Participants have been followed up for more than 20 years, from the 8th gestational week; data have been collected using self-report, health records, biological samples and physical measurements[193].

2.2.1.3 Phenotypic data description: sleep duration

Data from ALSPAC mothers contributed to the GWAS of self-reported sleep duration (Chapter 5) in this thesis. Table 2.1 below provides summary statistics for the ALSPAC phenotypic data.

Self-reported sleep duration data was collected from ALSPAC mothers by asking them to report the number of hours and minutes they sleep for during weekdays and weekends, then a weighted average was taken:

$$\frac{(\text{weekdays} \times 5 + \text{weekends} \times 2)}{7}$$

Although this question on sleep duration has been asked at six time points, in this thesis sleep duration was analysed from the 2003 data collection. The six data collections in which sleep measures were taken are 1991 (during pregnancy), 1994, 1995, 1997, 1999 and 2003. At each time point sleep duration has been collected via questionnaire, but prior to 2003 it was measured categorically using the following groups: 0-3 hours, 4-5 hours, 6-7 hours and 8+ hours. However, in 2003,

quantitative raw sleep duration was collected via questionnaire and ALSPAC researchers state that it is preferable to use this measure, as some information can be lost when categorising this kind of variable[194]. For example, the fact that all durations greater than 8 hours are in a single category might mask U-shaped associations with other important traits[194].

Prior to performing GWAS analyses (Chapter 5) on the sleep duration phenotype, outliers were removed and thus, respondents who reported sleeping for <4 hours and >11.5 hours were excluded. Also, n=329 women who reported taking sleep medication were not included in the analyses.

Table 2.1 Summary statistics for self-reported sleep duration in ALSPAC (after cleaning, as described above)

Timepoint	N	Mean	SD
2003 weekday	7,404	7.38	1.03
2003 weekend	7,404	8.20	1.16
2003 average	7,404	7.61	0.97

Note. N= number of observations, SD= standard deviation.

2.2.1.4 Genetic data

Informed consent was obtained to take DNA from blood samples and genome-wide genotyping in 10,321 women.

2.2.1.4.1 Genotyping and QC

DNA has been collected from blood samples continuously since initial recruitment[193]. Genotyping was performed in 10,107 of the 10,321 women who had DNA available, using the Illumina 660 W-quad array, which had a total of 557,124 SNPs prior to QC. QC metrics included removing SNPs with ≥5% missingness, <1% minor allele frequency (MAF – the extent to which the less common allele occurs in the population) and those that deviated from Hardy Weinberg equilibrium (HWE – whereby genetic variation remains constant from generation to generation) at $1x10^{-6}$. Individuals with missingess of ≥5% and those with cryptic relatedness of >5% (Identity by state cut-off of 0.05) were also removed. Cryptic relatedness refers to the presence of close relationships within a sample of largely

unrelated and outbred individuals[195]. Identity by state is used to describe two identical alleles or DNA segments[195].

For the purposes of the GWAS in Chapter 5, data from 4,914 ALSPAC mothers (out of the 10,107 with GWAS data) were analysed, as this was the maximum number of individuals who had genetic data, as well as the sleep duration phenotype.

2.2.1.4.2 Imputation

Genotype imputation enables the inference of unobserved genotypes[23], that is, in the case of GWA studies, SNPs that have not been directly genotyped. Imputation is done using known haplotypes (a group of genes that are inherited together from one parent) in a particular population, which is possible, as the DNA sequence of any two individuals is 99.5% identical. However, it is the remaining variation (0.5%) that may lead to different disease risks. It is because of this that common genotype imputation reference panels, such as HapMap, 1000 Genomes and the Haplotype Reference Consortium (HRC).

Imputation is performed by using the LD structure within a sample of genotyped individuals. When choosing a reference panel it is best to select one that is a close ancestral match to the population under study, as this increases imputation accuracy[196]. Also, selecting a larger reference panel (for example, 1000 Genomes over HapMap) allows a broader range of variants to be imputed with greater accuracy[197]. Phasing (estimating haplotypes) is done to reconstruct the haplotypes, which is then compared to one of the aforementioned reference panels; finally, the LD structure is used to impute the missing genotypes.

In ALSPAC, genotype data were imputed to 1000 Genomes (Phase 1, version 3). First, SNPs were flipped to the positive strand and to hg19, GRCh37. Phasing was done using SHAPEIT, version 2[198,199] and then imputation was performed in IMPUTE2[197].

2.2.2 English Longitudinal Study of Ageing (ELSA)

Data from ELSA were used throughout this thesis, with the exception of Chapter 3. ELSA is an on-going, nationally representative panel study of health and ageing in English adults aged 50 and over.

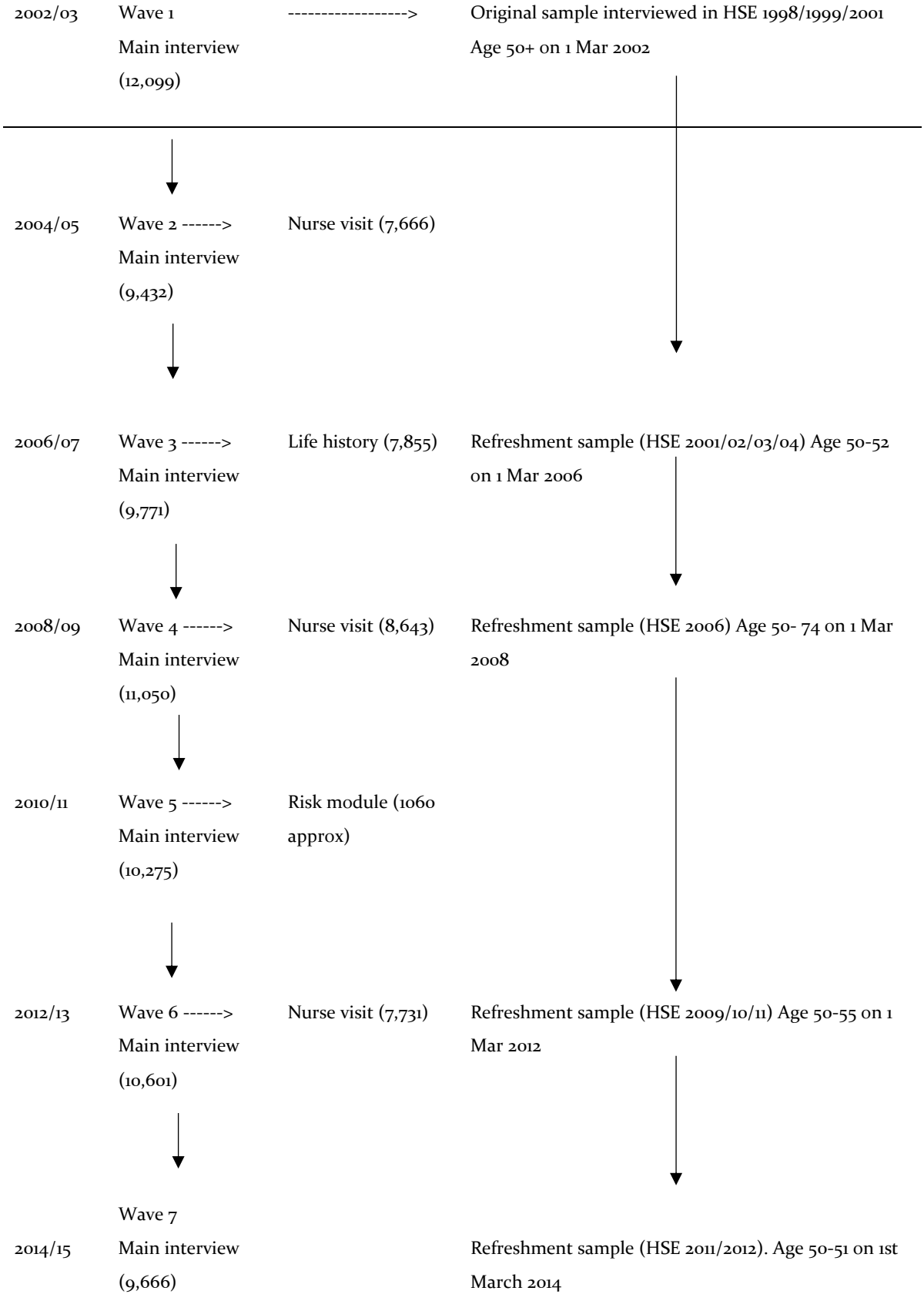
2.2.2.1 Ethics

ELSA was granted ethical approval by the London Multicentre Research Ethics Committee (MREC 01/2/91) and at each wave respondents provide informed consent.

2.2.2.2 Participants

At inception ELSA had a total of 11,391 respondents (wave 1, 2002-3), who were drawn from the Health Survey for England[200]. Data have subsequently been collected from participants at another six waves to date: 2 (2004-5), 3 (2006-7), 4 (2008-9), 5 (2010-11), 6 (2012-13) and 7 (2014-15), whilst wave 8 data collection is on-going. At every other wave (2, 4 and 6) a proportion of respondents undergo a nurse visit, in which detailed clinical and biological measurements are taken, to complement the self-reported measurements that are collected at every wave. So far, four refreshment samples have also been introduced (waves 3, 4, 6, and 7) to ensure that representation of the younger age group remains (those closer to 50) (see Figure 2.1 below).

Figure 2.1 ELSA data collection timetable



2.2.2.3 Phenotypic data description: BMI & sleep duration

Table 2.2 presents summary statistics of the ELSA phenotypic data used in this thesis.

2.2.2.3.1 BMI

At waves 2, 4 and 6, 7,666, 8,643 and 7,731 respondents received a nurse visit, respectively (there was no nurse visit at wave 1). During these visits, the nurse measured respondents' height (m) and weight (kg). Standing height was measured using a portable stadiometer standardised with head in the Frankfort plane, whilst weight was recorded to the nearest 0.1 kg, using digital scales (Tanita). BMI was subsequently derived at each wave using the formula: weight divided by height squared (kg/m^2). Mean BMIs for each wave are displayed in Table 2.2, after data were cleaned for missing values.

The work in this thesis uses data from ELSA waves 4 and 6, as these included a nurse visit and importantly, wave 4 was the first time that questions on sleep were asked. Of the 11,050 ELSA respondents at wave 4, 8,641 were interviewed and clinical measurements were obtained by a nurse; whilst at wave 6 there were 10,601 interviews and 7,731 nurse visits. There were no values that had to be removed at either wave 4 or 6 in ELSA.

2.2.2.3.2 Waist circumference (WC)

During the nurse visit, two measurements of WC were taken at the midpoint between the lower rib and the iliac crest using measuring tape. A mean of the two measurements was then used, unless they differed by more than 3 cm, in which case a third measurement was taken and then an average of the closest two measurements was used. At wave 4, two individuals whose WC values were 999.99cm were removed from the data, as these values were deemed extreme and could even be errors. WC categories were defined using the WHO definition for risk of metabolic syndrome. Categories were as follows: Not-at-risk <80 cm (females) and <94 cm (males), At increased risk>=80 cm, but <88 cm (females) and >=94 cm, but <102 cm (males), Substantially increased risk >=88 cm (females) and >=102 cm (males).

2.2.2.3.3 Sleep duration

Participants were asked to self-report their sleep duration at waves 4 and 6. They were asked: "How many hours of sleep do you have on an average weeknight?" Mean sleep duration for waves 4 and 6 can be seen in Table 2.2, after the variables were cleaned for missing data. Individuals who slept for < 2 hours or >12 hours were excluded (1 hour=7 individuals, 1.5 hours =1 individual, 13 hours=2 individuals, 13.5 hours=1 individual, 14 hours=2 individuals). This differed somewhat from the phenotype inclusion criteria for ALSPAC. The decision was made to remove the n=13 individuals who slept for <2 hours and >12 hours in ELSA because in adults >50 years of age these values are more likely to reflect errors and/or illness. In ALSPAC it was also more important to keep as many participants in the sample as possible, as they contributed only to the GWAS in this thesis (Chapter 5) for which ample power is required.

Table 2.2 Summary statistics of BMI and sleep duration in ELSA across waves for all respondents (after data cleaning, as described above)

Wave	N	Mean (SD)
	BMI (kg/m²)	
2	7,225	27.93 (4.89)
4	8,262	28.27 (5.30)
6	7,693	28.21 (5.10)
	WC (cm)	
2	7,416	95.67 (13.18)
4	8,409	96.97 (13.65)
6	7,866	96.38 (13.94)
	Sleep duration (hours)	
4	10,566	6.85 (1.34)
6	9,964	6.84 (1.33)

Note. N=number of observations, SD=standard deviation.

2.2.2.4 Genetic data

At waves 2 and 4 written consent was obtained to extract DNA from blood samples (obtained during the nurse visit); from 5,662 at wave 2 and a further 1,945 individuals at wave 4. Therefore, genotype data was available for a total of 7,607 individuals.

2.2.2.4.1 Genotyping and QC

Genotyping was carried out at UCL Genomics and the QC procedure was performed by Drs. Delilah Zabaneh and Ghazaleh Fatemifar. Genotyping was performed in two batches (5,662 individuals from wave 2 and 1,945 from wave 4) using the Illumina HumanOmni 2.5M platform. QC was done on each batch separately, prior to merging them. Both SNP and individual missingness were set to 5% and therefore, individuals who had more than 5% missing genotypes were excluded, as well as SNPs that had more than 5% missing genotype rates. MAF and HWE were not included in the QC and thus these checks were performed as part of the GWAS (Chapter 5). Individuals who reported non-White/European ethnicity were removed (n=133). It is important to remove these individuals of different genetic ancestry (known as population stratification or substructure), as it can lead to spurious associations due to differences in ancestry, rather than true associations. PLINK 1.9 was used to estimate relatedness with one individual of a related pair kept in the data, thus 40 were excluded during this QC step. A sex check was also carried out using PLINK and discrepancies were corrected, which means that participants' sex was either confirmed or refuted (versus self-reported sex), using DNA. After excluding related individuals and ethnic outliers the total sample size with genetic data was 7,412.

2.2.2.4.1.1 Imputation

Prior to imputing the genotypes, family groups with genetic relatedness discordant with stated relationships were removed, as well as SNPs with a MAF of ≤1%. Genotypes were imputed to the European component of 1000 Genomes, Phase 1. Phasing was carried out using MaCH1 software[201] and imputation was performed in Minimac[202]. Imputation was carried out in chunks of 1000 markers, with an overlap of 250 markers between chunks. After imputation chunks were

merged with the 'best' (highest R^2) kept in the overlapped segments. All imputed genotypes had an R^2 of ≥0.3.

2.2.3 Trondheim Early Secure Study (TESS)

Data from waves 2, 3 and 4 from the TESS study were used to investigate the bidirectional epidemiological relationship between BMI and objective sleep duration in children (Chapter 4). TESS comprises a prospective community sample of children born in Trondheim, Norway, in 2003 and 2004 and its primary aim was the assessment of mental health in children. A letter of invitation was sent, which included the Strengths and Difficulties Questionnaire (SDQ), version 4-16[203] for participants' parents to complete. This was administered because the SDQ is a commonly-used screening tool to detect conduct and emotional problems, which was one of the primary emphases of TESS.

2.2.3.1 Ethics

Ethical approval for TESS was granted by the Regional Committee for Medical and Health Research Ethics, Mid-Norway. Written informed consent has been obtained at each data collection, from children's parents/primary caregivers.

2.2.3.2 Participants

Data have been collected from children at ages 4 (wave 1), 6 (wave 2), 8 (wave 3) and 10 (wave 4), to date. From the 2003 and 2004 birth cohorts (N=3,456) in Trondheim, 1,250 children were recruited into TESS at wave 1, by means of a letter of invitation, which included the SDQ, as mentioned above. Thus, all children in these birth cohorts were invited to participate in TESS and of the 3,456 children, 3,358 attended the well-child clinic at age four for a routine health check, from whom the 1,250 who were recruited into TESS were drawn. Due to the purposeful oversampling of children with behavioural and emotional problems, sample weights are always applied in all TESS analyses. At waves 2, 3 and 4, 795, 699 and 702 children took part in the study, respectively.

2.2.3.3 Phenotypic data description: BMI & sleep duration

Summary statistics for BMI and sleep duration are presented in Table 2.3.

2.2.3.3.1 BMI

Height (m) and weight (kg) were measured at each wave by a nurse, using a digital stadiometer (Heightronic digiral stadiometer: QuickMedical, Model 235A) and electronic weighing scales (Tanita BC420MA). Then BMI was calculated using the standard formula: weight (kg) / height (m^2). BMI standard deviation scores (SDS) were derived at each age, using the British growth curve reference for those aged between birth and 23 years[87]. Mean BMIs are displayed in Table 2.3. BMI SDS at waves 2 and 3 were created by TESS researchers, yet BMI SDS values at wave 4 were calculated as part of the work in this thesis, as this had not previously been done. To do this, the LMS Growth[87] Microsoft Excel add-in was used, in which the raw BMI data (wave 4=age 10) were inputted and BMI SDS values were calculated according to the British 1990 growth reference[87]. A BMI SDS value of 0 means that the child's BMI is in line with the reference data average, whilst a value >0 means they have a higher BMI than the reference data average and values <0 mean that their BMI is lower than the reference data average. BMI SDS data are also used to derive weight status in children. International Obesity Task Force (IOTF) weight status categories were also derived using LMS Growth in Excel, which use age- and sex-specific BMI centile values associated with BMIs of 25 (overweight in adults) and 30 (obese in adults) at age 18y[204]. The BMI data had already been cleaned (by TESS Research Assistants) and therefore, a dataset was provided that was ready for analysis.

2.2.3.3.2 Sleep duration

Sleep duration was objectively measured using the ActiGraph™ GT3X accelerometer (Manufacturing Technology Incorporated, Fort Walton Beach, FL, USA). Participants (children) wore the actigraphs on their hip for 7 consecutive days, including whilst asleep, and were only required to remove them whilst showering or bathing. Only data from participants with ≥ 3 days of recordings were included, as Acebo and colleagues recommend that at least 3 nights are necessary to ascertain reliable individual differences. Sleep duration was converted from raw data by employing Sadeh's algorithm[205], once time in bed and out of bed had been manually set by examining each night, using ActiLife

software. A technician who was experienced with accelerometers set this manually, aided by the sleep detection algorithm provided by ActiLife. Bedtime was assumed by a sharp decrease in activity, which is characteristic of bedtime, in so far as it is often preceded by a marked increase about 20 minutes prior to this. Bedtime was recorded when there were approximately five consecutive epochs with less than 100 counts per epoch. Sadeh's algorithm automatically differentiates prolonged sitting from sleep. In addition to actigraphy, a questionnaire was supplied for parents to complete, asking whether participants had been ill and more/less active than usual during the seven days of data collection. However, parents were not asked to log children's bedtimes and wake times, but this will be included in TESS from age 14 onwards. TESS sleep duration data had already been cleaned and prepared for analysis (by the actigraphy technician) and a clean dataset was provided for the analyses in this thesis. Mean sleep durations are displayed below in Table 2.3.

Table 2.3 Summary statistics for BMI and sleep duration in TESS across waves

Wave	N	Mean (SD)
BMI-SDS		
2	552	-0.11 (0.88)
3	509	0.09 (0.90)
4	686	0.12 (1.01)
Sleep duration (hours)		
2	674	9.62 (0.72)
3	547	9.09 (0.57)
4	633	9.19 (0.70)

Note. BMI-SDS= BMI standard deviation scores, N=number of observations, SD=standard deviation, wave 2=age 6, wave 3=age 8, wave 4=age 10.

2.2.4 The UK Biobank Study

Summary-level data from the UK Biobank (UKB) study were used for the Mendelian randomisation analyses in Chapter 6. UKB comprises 500,000 individuals between the ages of 40 and 69 years, who have undergone detailed phenotyping and genotyping.

2.2.4.1 Ethics

UKB participants provided full informed consent. More details on the complex Ethics and Governance Framework in UKB can be found in Sudlow et al. (2015)[206].

2.2.4.2 Participants

The 500,000 participants were assessed between 2006 and 2010 across 22 UK assessment centres, with the aim of covering a diversity of socioeconomic and ethnic backgrounds. Data have been collected via self-completion touch-screen questionnaires, a computer assisted interview, as well as functional and physical measures and blood, saliva and urine samples.

2.2.4.3 Phenotypic data description: sleep duration

In UKB sleep duration was ascertained by asking participants for the average number of hours that they slept in a 24-hour period. For GWAS analyses (from which summary statistics were used in Chapter 6 for the purposes of MR), Jones and colleagues[78] derived their sleep duration measure by excluding those who slept for >18 hours, then adjusted for age, sex and study centre, obtained the model residuals and then applied an inverse-normal transformation.

2.2.5 The UK Household Longitudinal Study (UKHLS)

Data from UKHLS[207] were used in the GWAS (Chapter 5), Mendelian randomisation and polygenic risk score analyses (Chapter 6). UKHLS is an ongoing, nationally representative panel study of over 40,000 UK households, which collects social and economic data, as well as behavioural and health data.

2.2.5.1 Ethics

UKHLS received ethical approval from the University of Essex Ethics Committee and nurse visits (waves 2 and 3) have been approved by the National Research Ethics Service.

2.2.5.2 Participants

Data are collected annually from UKHLS respondents; it comprises a General Population Sample (GPS), a stratified clustered random sample of representative

UK households (joined in 2009-10) and participants from the well-established British Household Panel Survey (BHPS)[207]. UKHLS has collected data at six waves, to date, with 50,994 (wave 1), 54,597 (wave 2), 49,739 (wave 3), 47,157 (wave 4), 44,903 (wave 5) and 45,290 (wave 6) observations.

In addition to the main interview at waves 2 (GPS sample component) and 3 (BHPS sample component), 26,961 and 8,914 participants, respectively, were eligible (had been interviewed in English, were aged over 16 years, were not pregnant at the time and lived in England, Wales or Scotland) to take part in the nurse health assessment. Thus, these participants had objective measurements of anthropometry taken by a nurse and blood samples were also collected for extraction of DNA. Of those eligible at wave 2, 10,175 participated and at wave 3, 5,053 participated, of whom 10,175 and 3,342 individuals provided blood samples.

2.2.5.3 Phenotypic data description: BMI & sleep duration

Table 2.4 presents summary statistics for the UKHLS phenotype data used in this thesis.

Table 2.4 Summary statistics for BMI and sleep duration in UKHLS for participants with phenotype data

Wave	N	Mean (SD)
	BMI (kg/m^2)	
2/3	9,660	28.07 (5.60)
	Sleep duration (hours)	
1	5,754	6.85 (1.44)
4	8,855	6.61 (1.36)

Note. N=number of observations for individuals who were visited by a nurse, SD=standard deviation, wave 2/3= indicates that BMIs were combined into one variable, from the waves 2 and 3 nurse visits, as respondents had their height/weight measured at either one of these waves.

2.2.5.3.1 BMI

Height (m) was measured during the nurse visits at waves 2 and 3, using a portable stadiometer with the respondent's head in the Frankfort plane. One

measurement, to the nearest millimetre was recorded. Weight (kg) was measured using a digital floor Tanita scale (BF 522) during the nurse visits. Participants whose weight was greater than 130kg were asked to estimate their weight, as the scales are not accurate above this level. However, there was only n=1 respondent with a weight value >130 kg and they were excluded, as participants estimating their own weight is unlikely to be reliable. Subsequently, BMI was calculated using the standard formula (weight in kg/height^2 in cm).

2.2.5.3.2 Sleep duration

Self-reported sleep duration was measured at waves 1 and 4. At each wave participants were asked: "How many hours of actual sleep did you usually get at night during the last month? This may be different than the actual number of hours you spent in bed". Individuals who reported sleeping for <2 hours or >12 hours were excluded (1 hour = 27 individuals, 13 hours = 1 individual, 16 hours = 1 individual, 17 hours = 2 individuals, 18 hours = 1 individual). The reason for this was that these sleep duration values were considered extreme, as it is possible that these values were errors or could reflect illness in these respondents. Thus, sleep duration was cleaned in the same way as in ELSA.

2.2.5.3.3 Phenotypic data included in this thesis

For the purposes of this thesis, data from UKHLS respondents were used *only* if they had phenotypic data and genotypic data. This is because UKHLS was included in both of the genetic studies (Chapters 5 and 6). Thus, Table 2.4 displays summary statistics for BMI and sleep duration for individuals who had phenotype and genotype data.

As BMI was collected from two different nurse visits (waves 2 and 3), to maximise the sample size for the genetic analyses, both waves were used. Thus, as different respondents received a nurse visit at wave 2 from those in wave 3, the sample size was maximized for BMI by combining these participants.

This collapsed variable that combined all respondents who had a BMI measurement was provided, alongside the genetic data. Similarly, to maximise the sample size for sleep duration from waves 1 and 4, an overall sleep duration

variable was created that took into account respondents who had sleep duration at either wave 1 or 4. Thus, if a participant completed the sleep duration question at wave 1, but they were no longer in the study at wave 4, their wave 1 measurement was used, and if a participant entered the study at wave 4 and hence, lacked sleep duration at wave 1, then their wave 4 response was analysed. Wave 1 was prioritised, as it had a larger sample size than wave 4; specifically, at wave 1, 5,486 respondents had both genetic data and sleep duration, whilst at wave 4, 5,001 respondents had genetic and sleep duration data. Therefore, if a participant had sleep duration data at wave 1, that was used for analysis, whereas for those who did not have sleep duration measurements at wave 1, but did have them at wave 4, those data were used.

During the wave 2 and 3 nurse visits participants provided informed consent to have a blood sample taken and to have DNA extracted from it for use in scientific research. Of the 13,517 individuals who consented to giving a blood sample, 10,484 White/European individuals were genotyped.

2.2.5.3.4 Genotyping & QC

The genotyping and QC of the data were led by Prof. Eleftheria Zeggini's group at the Wellcome Trust Sanger Institute. Genotyping was done using the Illumina Infinium HumanCoreExome BeadChip Kit, which includes a panel of more >240,000 common and rare exonic variants, as well as >250,000 genome-wide SNPs. Individual-level QC included the following filters: call rate of <98%, gender discrepancies, duplicate individuals as per an identity by descent (IBD: refers to a matching DNA segment shared by at least two people, which is inherited from a common ancestor with no recombination, that is when offspring are produced with trait combinations that are distinct from those of either parent) cut-off >0.9, ethnic outliers. A total of 9,965 individuals survived this QC. During the SNP-level QC, variants with a HWE p-value $<1x10^{-4}$, call rate <98%, poor genotype clustering values (<0.4), alongside Y-chromosome and mitochondrial variants. This left a total of 525,314 SNPs that passed QC.

As UKHLS is a household study, further relatedness exclusions were applied prior to GWAS analyses (Chapter 5). There are different methods for dealing with relatedness; the chosen method here was that recommended by Yang et al., 2010[19], in which an identity-by-descent cut-off is chosen to remove closely related individuals prior to performing the main analyses [208]. In this method, the genetic relationship matrix (GRM) was estimated between pairs of individuals from a set of SNPs. Then an identity by descent (IBD) cut-off of 0.025 was used, which excludes individuals who are related up to third or fourth cousins[209]. Thus, of the original sample (n=9,994) who were genotyped and passed QC, IBD excluded n=1,001 individuals (one per related pair).

2.2.5.3.4.1 Imputation

Prior to imputation family groups with genetic relatedness discordant with stated relationships were removed, as well as SNPs with a MAF of ≤1%. Genotypes were imputed to the European component of 1000 Genomes, Phase 1. Phasing was carried out using MACH1 software and imputation was carried out in Minimac. Imputation was performed in chunks of 1000 markers, with an overlap of 250 markers between chunks. Post imputation chunks were merged with the 'best' (highest R^2) kept in the overlapped segments. All imputed genotypes had an R^2 of ≥0.3.

Table 2.5 Summary of datasets

Study	Phenotype – n (mean, SD)				n Genotyped	Imputation	n for analyses* (chapter)
	BMI**	Sleep duration	WC	Wave			
ALSPAC	N/A	7,404 (7.61, 0.97)	N/A	2003	10,321	1k Genomes	4,914 (5)
ELSA	8,262 (28.27, 5.30) 7,693 (28.21, 5.10)	10,566 (6.85, 1.34) 9,964 (6.84, 1.33)	7,866 (96.38 ,13.94)	4 6	7,607	1k Genomes	5,015 (3) 6,028 (5,6) 5,296 (6)##
TESS	552 (-0.11, 0.88) 509 (0.09, 0.90) 686 (0.12, 1.01)	674 (9.62, 0.72) 547 (9.09, 0.57) 633 (9.19, 0.70)	N/A	2 3 4	N/A	N/A	794*** (4)
UKHLS	9,660 (28.07, 5.60)	5,574 (6.85, 1.44) 8,855 (6.61, 1.36)	N/A	2/3# 1 4	10,484	1k Genomes	8,608 (5,6) 6,811 (6)##

Note. ALSPAC= Avon Longitudinal Study of Parents and Children, ELSA= English Longitudinal Study of Ageing, TESS= Trondheim Early Secure Study, UKHLS= UK Household Longitudinal Study, *number of individuals with complete data for relevant analysis, **BMI-SDS, ***these analyses were performed using a maximum likelihood approach and therefore, all available data were used, #BMI measurements were collected at a nurse visit during either wave 2 or 3 (for different participants), see section on UKHLS above for more details, ##number of individuals included in observational analysis in Mendelian randomisation study in Chapter 6.

2.3 CHAPTER SUMMARY

- An overview of the datasets used in this thesis was provided.
- A description of each sample was given including ethics and participant information.
- Information was provided about how the main phenotypic data analysed in this thesis were collected: BMI and sleep duration, including summary statistics.

- Where necessary (all datasets, with the exception of TESS), an overview of the genetic data was provided, including genotyping, QC and imputation methods.

3 INVESTIGATING THE BIDIRECTIONAL ASSOCIATION OF ADIPOSITY AND SLEEP DURATION IN OLDER ADULTS

Results from the work in this chapter are published in: **Garfield, V.**, Llewellyn, C. H., Steptoe, A. & Kumari, M. Investigating the Bidirectional Associations of Adiposity with Sleep Duration in Older Adults: The English Longitudinal Study of Ageing (ELSA). *Sci. Rep.* **7,** (2017).

3.1 BRIEF INTRODUCTION AND OVERVIEW OF CHAPTER CONTENTS

As mentioned in Chapter 1 when reviewing the literature on BMI and sleep duration, it appeared that the magnitude of this relationship might differ by age group such that associations diminish in older age groups. As stated earlier in this thesis, it has been hypothesized that greater BMI may precede shorter sleep duration or that shorter sleep duration may precede weight gain. Thus, it is important to determine the direction of this association in older adults because if the direction of this relationship is established it will enable health professionals to understand better where to target interventions in older age groups, when disease and frailty are most likely to occur.

Cross-sectional evidence in older adults primarily suggests that short sleep is associated with greater adiposity[106,107]. However, the prospective relationship is less clear in this age group. For example, analyses of the Whitehall II study found no evidence of an association between shorter sleep duration and changes in BMI or obesity incidence over 4 years, in a sample with a mean age of fifty-six years[111], yet prospective analysis of 3,576 Spanish older adults suggests that a sleep duration of less than or equal to 5 hours, as well as a sleep duration of 8 or 9 hours is associated with obesity and with weight gain over a period of two years, but only in females[210].

This study is also very timely since the UK has an increasing ageing population. Over twenty-three million people are currently aged 50 and over, which constitutes more than a third of the UK population[211]. Projections suggest that by 2040, 24.2% of the population will be aged 65 or over[212]. Adults between the ages of fifty-five and sixty-four are the most likely to be obese, and whilst obesity prevalence decreases, the percentage of overweight adults increases with age[213], whilst sleep duration decreases as a function of age[67]. Furthermore, a recent report suggests that sleep deprivation costs the UK economy £40 billion annually[59]. These authors also reiterate the importance of sufficient sleep in relation to decreased risk of hypertension[64], T2D[63,187], CVD[61] and mortality[66].

It appears that no studies have to date, tested the prospective, bidirectional relationship between adiposity and sleep duration in a single study, in older adults. Also, importantly, BMI may not be the most optimal adiposity indicator in older adults, due to its reduced ability to predict body fat in this population[88,90]. This is because muscle mass decreases as a function of age, which is known as sarcopenia[88,90]. Few studies have investigated the association of alternative adiposity measures with sleep duration, such as waist circumference (WC), with evidence emerging in favour of a cross-sectional[110,214], but not a prospective relationship[215]. No studies have previously examined the association between WC and potential change in sleep duration over time, nor have they incorporated bidirectional analyses. Also, evidence suggests that there is a relationship between lower socioeconomic position (SEP) and sleep duration[64,216–219], depression and sleep duration[60,220], SEP and obesity[221,222], and BMI and depression[223] and thus, these factors should be explored as important covariates in this relationship.

This chapter begins with specific aims for the study presented here, followed by the methods used to examine this bidirectional relationship of adiposity and sleep duration in older adults. There is then a description of results from both cross-sectional and longitudinal analyses. This is followed by a discussion of the findings, in which potential mechanisms for the association of adiposity and

sleep duration are put forward. The discussion ends with directions for future research and the final section of this chapter provides a summary of key points.

3.2 AIMS OF THIS CHAPTER

The aim of this chapter was to investigate the following two hypotheses: i) in cross-sectional analyses baseline adiposity (BMI and WC) is negatively associated with baseline sleep duration and ii) prospectively, that the relationship between adiposity (BMI and WC) and self-reported sleep duration, would be bidirectional, such that greater adiposity at baseline is associated with shorter sleep duration at follow-up, and that shorter sleep duration at baseline is associated with great adiposity at follow-up.

3.3 METHODS

For the work carried out in this chapter, I designed the study, performed all data cleaning and all of the statistical analyses.

3.3.1 Sample: ELSA

In this study data were analysed from 5,015 respondents from waves 4 and 6 of ELSA; inclusion of respondents was based on whether they had complete data for measures of adiposity, sleep duration and all covariates at both waves of data collection. Thus, after each variable was cleaned (details in Chapter 2), all variables were merged into one file, individuals who did not have two BMI and WC measures, two sleep duration measures and all covariates were excluded from the analyses (n=1,090).

3.3.2 Measures

Details of how each measure was cleaned and prepared are in Chapter 2.

BMI

BMI was derived from height and weight using the standard formula: weight divided by height squared (kg/m^2).

WC

An average of two WC measurements taken during the nurse visits at waves 4 and 6 was used (see Chapter 2). These categories were defined using the WHO definition for risk of metabolic syndrome[224].

Sleep duration and change in sleep duration

Respondents were asked 'How many hours of sleep do you have on an average week night?' (Further details on cleaning and preparing the measure are in Chapter 2). Change in sleep duration from baseline to follow-up was calculated by subtracting sleep duration at wave 6 from sleep duration at wave 4.

Covariates

Demographic, socio-economic and health behaviour measures collected at wave 4 were used as covariates in the analyses. The covariates included in these analyses were chosen on the basis that they might be associated with the outcome of interest (sleep duration or BMI/WC).

Demographics

Age was recorded as a continuous number (in years) until 90 years, with ages above 90 collapsed to the value of 91. Socio-economic position was determined by quintiles of non-pension wealth, which is regarded as the most salient measure of standard of living in older age groups[225].

Health behaviours

Frequency of alcohol consumption within the last 12 months [categorised to less than daily; daily (5-7 times per week)] was measured with the question: 'Thinking now about all kinds of drinks, how often have you had an alcoholic drink of any kind in the last 12 months?' The category of 'less than daily' also included respondents who were non-drinkers.

Participants were first asked whether they had ever been a smoker and those who responded 'yes' were asked if they 'smoke cigarettes at all nowadays' during the wave 4 data collection. Thus, responses were combined from these two questions

and a third variable was derived, whereby respondents were categorised into: never smoked, ex-smoker and current smoker.

Physical activity level (Sedentary; Low; Moderate; High) was derived by summarising responses to the level of work activity and the 'type and amount of physical activity involved in daily life' questions (how often respondents participated in mild/moderate/vigorous sports or activities). These were subsequently categorised into: Sedentary (mild exercise, one to three times per week), Low (mild, but no vigorous activity at least once a week), Moderate (moderate activity more than once a week, or vigorous activity between one to three times per month) and High (heavy manual work or vigorous activity more than once per week).

Health problems

Long-standing illness was assessed with the following question: 'do you have any long-standing illness, disability or infirmity? By long-standing I mean anything that has troubled over a period of time, or that is likely to affect over a period of time', to which they could answer 'No' or 'Yes'). If respondents answered 'yes' then a further question was asked about whether the illness/illnesses limit activities in any way.

Depressive symptoms (measured with the Centre for Epidemiologic Studies – Depression Scale – CESD – 8 item scale) were also assessed by questionnaire. CES-D responses were summed (with the exception of the item: "whether respondent felt their sleep was restless in the past week") to obtain a total score, which was then dichotomized using a cut-off of >=3, which has recently been used in other ELSA publications[226]. Due to removal of the sleep item, it seemed plausible to check whether retaining at cut-off of >=3 was appropriate or whether this should be lowered to >=2. However, the cut-off of >=3 was retained, as lowering the cut-off to >=2 meant that this was unlikely to be accurately capturing those who had depressive symptoms because the percentage of those without depressive symptoms changed from 67% to 2%. This was due to the large

number of respondents who scored 2 and would thus, be classed as 'depressed' using this lower cut-off.

Season

As sleep duration can differ depending on the season, a dichotomous season variable was created using the date that respondents completed their interview, which resulted in a categorisation of 0= "BST" (British Summer Time) and 1= "GMT" (Greenwich Mean Time). However, in linear regression models adjusted for age and sex, no association was observed between season at baseline and sleep duration at wave 4 [B (unstandardised coefficient) = 0.022, (95% CI= -0.047; 0.091), *P*=0.533] or wave 6 [B= 0.009, (95% CI= -0.079; 0.061), *P*=0.800], and were therefore not included in subsequent analyses.

3.3.3 Statistical analyses

Analyses were performed in STATA, version 13.

3.3.3.1 Power calculations

Observed (post-hoc) power was calculated using G*Power, by taking the R^2 values from the simple linear regression models of exposures on outcomes in this study. This was done in the exact same way for the cross-sectional and bidirectional prospective regression models. The parameters used to calculate power were: effect size F^2, % error probability, total sample size and number of tested predictors. F^2 for the effect size was calculated as: R^2 / 1 - R^2, by taking the R^2 from simple (unadjusted) cross-sectional and prospective bidirectional models.

The alpha level was set at 0.05, the total n was 5,015 and the number of tested predictors was 1 for cross-sectional and prospective analyses. Power calculations indicated that there was sufficient power to detect the observed effect sizes (Table 3.2).

Table 3.1 Power calculations for cross-sectional and bidirectional prospective analyses in ELSA

Model	Effect size F^2*	% error probability	N	No. tested predictors	Power
Cross-sectional	0.0025	0.05	5015	1	94.2%
Prospective BMI on sleep duration	0.0041	0.05	5015	1	99.4%
Prospective Sleep duration on BMI	0.0021	0.05	5015	1	88.6%

Note. *For cross-sectional models this (F^2) was calculated as: R^2/ 1 – R^2 = 0.0025 / 1 – 0.0025 = 0.0025; for prospective models of BMI on sleep duration this was calculated as: R^2/ 1 – R^2 = 0.0041 / 1 – 0.0041 = 0.0041; for prospective models of sleep duration on BMI this was calculated as: R^2/ 1 – R^2 = 0.0021 / 1 – 0.0021 = 0.0021. All of these R^2 values were taken from results of simple linear regression models (exposure → outcome only) in ELSA.

3.3.3.2 Univariate associations

For examination of baseline sample characteristics sleep categories were created to examine potential differences in adiposity and covariates, as well as to examine linearity of adiposity, according to how much sleep participants reported. More specifically, these categories were defined as 1= ≤5 up to 5.4 hours, 2= 5.5 hours up to and including 7 hours, 3= 7.5 hours up to and including 9 hours and 4= >9 hours.

Then, one-way ANOVAs were used to compare means for age, BMI and WC, whilst Chi-squared tests were used to examine differences in categorical demographic variables (smoking status, alcohol consumption, long-standing illness, wealth, sex, ethnicity and depressive symptoms) across the 4 sleep duration groups. Depressive symptoms are also described by sex in the Results, due to known differences in prevalence of depression in men and women[227]. Also, two-sample t-tests were performed to examine whether there were significant

differences between baseline and follow-up mean BMI and WC to establish the amount of change over time.

3.3.3.3 Regression models

Regression analyses were performed to examine the linear cross-sectional and bidirectional prospective relationships of BMI with sleep duration. BMI, WC and sleep duration were treated as continuous variables and thus, linear models were tested, both cross-sectionally and prospectively. In all linear regression models the coefficients presented in Tables 3.2, 3.3 and 3.4 represent unstandardised coefficients (B). Multicollinearity was tested in all regression models using the variance inflation factor (VIF) to examine the extent to which predictors were correlated. A VIF of 1 indicates no correlation, whilst values >10 are generally cause for concern[32].

3.3.3.3.1 Cross-sectional models

To examine whether there was an inverse cross-sectional relationship between adiposity and sleep duration, four regression models were performed, with BMI or WC as the exposure and sleep duration as the outcome.

Model 1 was minimally-adjusted (age, sex, wealth, ethnicity). Model 2 was adjusted for the covariates in model 1 + health behaviours (model 1 + alcohol consumption, smoking status, physical activity levels). Model 3 was adjusted for the covariates in model 1 + health problems (model 1 + depressive symptoms, long-standing illness). Model 4 was the final model and was fully-adjusted for all covariates [model 1 (demographics) + model 2 (health behaviours) + model 3 (health problems)]. These models were adjusted hierarchically to examine whether there was confounding in the association of adiposity and sleep duration, by important behavioural and health factors.

3.3.3.3.2 Prospective models

Prospectively, the association between baseline adiposity (BMI and WC) and changes in sleep duration, as well as baseline sleep duration and changes in adiposity were investigated. To examine change in sleep duration, linear regressions were performed with adiposity measures (BMI or WC) as the

exposure, sleep duration as the outcome, and models were adjusted for covariates as well as baseline sleep duration. Conversely, in analyses to examine changes in adiposity, BMI or WC at follow-up was analysed as the outcome with sleep duration at baseline adjusted for BMI or WC at baseline as the exposure. Aside from this difference, Models 1 to 4 were identical to the cross-sectional models described above.

3.3.3.3.3 Non-linear models

Quadratic regression modelling was used to investigate potential non-linear associations in cross-sectional and prospective, bidirectional relationships between adiposity measures (BMI and WC) and sleep duration. Models were run to examine the possible quadratic cross-sectional relationship between BMI and WC as exposures with sleep duration (outcome) at wave 4 (baseline) of ELSA. These models varied in adjustments, which were as follows: Model 1= age, sex, wealth and ethnicity, Model 2= Model 1 + health behaviours (alcohol consumption, physical activity and smoking status), Model 3= Model 1 + health problems (depressive symptoms, long-standing illness), Model 4= Model 1 + Model 2 + Model 3. Covariates were grouped into these categories (demographics, health behaviours and health problems), as the aim was to examine whether clusters of factors might affect the association between the exposure and outcome.

As the aim of this study was to test the bidirectional association of adiposity with sleep duration, two sets of quadratic analyses were performed on the prospective data. To investigate whether there was a U-shaped prospective relationship between BMI/WC at baseline and follow-up sleep duration, quadratic (BMI^2 and WC^2) terms were created and included in the regression models. Analyses were then performed to investigate the potential U-shaped association of sleep duration-squared at baseline with BMI and WC at follow-up. In models where BMI^2 or WC^2 predicted changes in sleep duration covariates were identical to the cross-sectional models described above, but included additional adjustments for baseline sleep duration (Model 1= demographics + baseline sleep duration; Model 2= model 1 + health behaviours + baseline sleep duration; Model 3= model 1 +

health problems + sleep duration; Model 4= model 1 + model 2 + model 3). In regression models that predicted changes in adiposity (BMI or WC), a sleep duration² term was created to test for a U-shaped association between sleep duration and adiposity. Four models were run for BMI and WC separately, with adjustments for covariates as above, but also included adjustments for baseline BMI or WC to investigate change.

3.3.3.3.4 Difference in coefficients before and after adjustments for covariates

To test for confounding of the relationship between adiposity and sleep duration by demographics, health behaviours and health problems, the percentage reduction in the regression coefficient following adjustment was calculated by comparing the coefficient for each exposure from models with and without adjustment for covariates. For example, if the unstandardised coefficient changes from B=0.60 to B=0.40 from one model to the next, this would mean that there is a 33% reduction in the coefficient. This is illustrated as follows:

B_1 =0.60 (B from Model 1)

B_2=0.40 (B from Model 2)

To calculate the percentage change:

$[(B_1 - B_2) / B_1] = [(0.60 - 0.40) / 0.60] = 0.33 \rightarrow 33\%$

3.3.3.3.5 Moderation by age and sex

Cross-sectional interactions with age and sex

To investigate potential moderation by age and sex, interaction terms between baseline adiposity (BMI and WC) and sleep duration with age and sex were created. Analyses were not stratified if there was no significant interaction between these variables, as this is not common practice in epidemiological analyses[228], nor is it statistically correct. The pursuit of stratified analysis, in the absence of a significant interaction can yield significant subgroup effects, but these are likely to be due to chance[228].

Prospective interactions with age and sex

An interaction term was also created to examine the potential effect modification of sleep duration by age and sex on adiposity measures. Then, linear regression modelling was used to examine whether there were any differential effects of BMI by age or sex on sleep duration and changes in sleep duration. Linear regressions were also performed to examine whether there were any differential effects of sleep duration by age and sex on adiposity.

3.4 RESULTS

3.4.1 Sample characteristics

Compared with all participants at wave 4 of ELSA, those included in this study were wealthier, slightly older and less likely to report having a long-standing illness (all $p<0.05$).

Table 3.1 shows baseline (wave 4) characteristics of participants, according to their sleep duration category. It can be seen that the majority of respondents reported sleeping between 6 to 7 hours (53.06%) or 8 to 9 hours (33.12%), whilst only a small proportion slept for ≤5 hours (12.6%) and even fewer reported sleeping for >9 hours (1.22%). Although not presented in a table, a chi-squared test showed that there were significant differences ($p<0.001$) between the number of females vs. males, who reported depressive symptoms: 1,032 (37.16%) females out of 2,777 vs. 578 (25.83%) males out of 2,238. This is in relation to an earlier point about differences between males and females in prevalence estimates of depression[227].

One-way ANOVAs showed that there were significant differences across sleep duration categories for both age and BMI, such that respondents who slept >9 hours were on average, older than the rest of the sample and that those who slept for ≤5 hours had on average, the highest BMIs. There were, however, no significant differences in baseline waist circumference across the four sleep duration categories. Pearson's correlations between BMI and WC (at baseline) for males and females were $r=0.87$ ($p<0.001$) and $r=0.86$ ($p<0.001$), respectively.

Chi-squared analyses showed that sex, smoking status, alcohol consumption, limiting illness, wealth, depressive symptoms and physical activity levels, were all significantly associated with sleep duration. Short sleepers (≤5 hours) were significantly more likely to be females, ex-smokers, less wealthy, consume less alcohol, report a long-standing illness and engage in 'moderate' physical activity (Table 3.1). There was no evidence of multicollinearity in any of our cross-sectional or prospective regression models, as all VIF values were around 1 when tested.

Mean BMI values were 28.20 kg/m² and 28.17 kg/m², at baseline and follow-up respectively, whilst average duration of sleep was 6.86 hours at baseline and 6.87 hours at follow-up indicating no change over time for either BMI or sleep duration, across the sample as a whole. Respondents who slept for five hours or less had the highest mean BMI both at baseline (28.72 kg/m²) and follow-up (28.71 kg/m²), whilst those who slept between eight and nine hours had the lowest mean BMI at baseline (27.99 kg/m²), which remained identical at follow-up (27.99 kg/m²). Overall, mean WC at baseline was 96.49 cm and 96.09 cm at follow-up.

Figures 3.1 and 3.2 depict baseline and follow-up sleep duration by weight status. In Figure 3.1 it can be seen that respondents in the normal weight group had the longest mean sleep duration at baseline (6.93 hours), whilst at follow-up the longest mean sleep duration was observed in the underweight group (7.14 hours). The shortest duration of sleep at baseline was observed in the underweight group (6.77 hours) and at follow-up, those with the shortest average sleep duration were respondents in the obese category (6.76 hours). In Figure 3.2 it can be seen that at baseline, those with the longest average sleep duration were respondents in the not-at-risk group (6.95 hours), which was identical at follow-up. Similarly, at both baseline and follow-up, respondents with the shortest mean sleep durations were those at substantially increased risk (6.82 and 6.80 hours).

Table 3.2 Sample characteristics at baseline by sleep duration category (N=5,015)

	≤5 hrs	6-7 hrs	8-9 hrs	>9 hrs	Total	P
	n=632	n=2,661	n=1,661	n=61	N=5,015	
Age (years)**	65.25 (8.56)	64.29 (8.43)	65.15 (8.03)	68.51 (8.86)		<0.001
Sex**						<0.001
Male	219 (34.65)	1,243 (46.71)	755 (45.45)	21 (35.43)	2,238 (44.63)	
Female	413 (65.35)	1,418 (53.29)	906 (54.55)	40 (65.57)	2,777 (55.37)	
Ethnicity						>0.05
White	616 (97.47)	2,615 (98.27)	1,635 (98.43)	60 (98.36)	4,926 (98.23)	
Non-white	16 (2.53)	46 (1.73)	26 (1.57)	1 (1.64)	89 (1.77)	
			BMI (kg/m²)*			
	28.75 (5.54)	28.10 (4.91)	27.99 (4.78)	28.09 (5.76)	5,015	<0.05
			WC (cm)			
Males	103.72 (12.91)	102.22 (11.28)	101.36 (11.13)	102.55 (8.65)	102.08 (11.39)	<0.05
Females	93.47 (13.31)	91.63 (12.69)	91.76 (12.72)	94.32 (16.29)	91.99 (12.86)	<0.05
Smoking status*						
Never smoked	244 (38.61)	1,160 (43.59)	739 (44.49)	26 (42.62)	2,169 (43.25)	
Ex-smoker	290 (45.89)	1,198 (45.02)	751 (45.21)	23 (37.70)	2,262 (45.10)	<0.05
Current smoker	98 (15.51)	303 (11.39)	171 (10.30)	12 (19.67)	584 (11.65)	
Alcohol consumption*						
Less than daily	517 (81.80)	2,042 (76.74)	1,257 (75.68)	47 (77.05)	3,863 (77.03)	
Daily (5-7 days/week)	115 (18.20)	619 (23.26)	404 (24.32)	14 (22.95)	1,152 (22.97)	<0.05
Wealth quintile**						
Lowest	154 (24.37)	313 (11.76)	166 (9.99)	13 (21.31)	646 (12.88)	<0.001
Others	478 (75.63)	2,348 (88.24)	1 518 (90.01)	48 (78.69)	4,369 (87.12)	
Long-standing illness**						
No	246 (38.92)	1,323 (49.72)	842 (50.69)	20 (32.79)	2,431 (48.47)	
Yes	386 (61.08)	1,338 (50.28)	819 (49.31)	41 (67.21)	2,584 (51.53)	<0.001
CES-D**						
No	293 (46.36)	1,859 (69.86)	1,221 (73.51)	32 (52.46)	3,405 (67.90)	<0.001
Yes (score >=3)	339 (53.64)	802 (30.14)	440 (26.49)	29 (47.54)	1,610 (32.10)	
PA levels*						
Sedentary	36 (5.70)	64 (2.41)	36 (2.17)	4 (6.56)	140 (2.79)	
Low	185 (29.27)	497 (18.68)	285 (17.16)	22 (36.07)	989 (19.72)	<0.05
Moderate	291 (46.04)	1,440 (54.11)	956 (57.56)	22 (36/07)	2,709 (54.02)	
High	120 (18.99)	660 (24.80)	384 (23.12)	13 (21.31)	1,177 (23.47)	

Note. BMI = Body Mass Index; WC= waist circumference, Means (SDs) or n (%), PA = physical activity, CES-D = Centre for Epidemiologic Studies of Depression Scale.

3.4.2 Cross-sectional associations: BMI and sleep duration at baseline and WC and sleep duration at baseline

3.4.2.1 BMI and sleep duration at baseline

Model 1 (with adjustment for age, sex, wealth, ethnicity) revealed a small, inverse linear relationship between BMI and sleep duration, which was attenuated and no longer significant in Model 2 (Model 1 with additional adjustment for health behaviours; Model 1 to Model 2 = 11% decrease in the coefficient), and further

weakened in Model 3 (Model 1 with adjustment for health problems; Model 1 to Model 3 = 34% decrease in the coefficient). In the final model adjusted for all covariates this effect was again, attenuated (Model 1 to Model 4 = 36% decrease in the coefficient) (Table 3.2). There was no interaction between sex (p=0.822) or age (p=0.366) and BMI at baseline on sleep duration, in any of the four models. Quadratic cross-sectional regression models were also performed to test for a U-shaped relationship between BMI and sleep duration, but this was not significant (p=0.987).

3.4.2.2 WC and sleep duration at baseline

The pattern of results for waist circumference and sleep duration was almost identical to that of BMI and sleep duration (Table 3.2). In a model adjusted only for demographics there was a significant, negative association between WC and sleep duration (Model 1), which was attenuated with inclusion of health behaviours in Model 2 (Model 1 to Model 2 = 22% decrease in the coefficient). With adjustments for health problems, the coefficient was again, reduced (Model 1 to Model 3 = 39% decrease in the coefficient) and a final model including all covariates resulted in further attenuation (Model 1 to Model 4 = 44% decrease in the coefficient). We observed no evidence of a U-shaped association between baseline WC and sleep duration (p=0.103), nor did we find a significant interaction of age (p=0.084), or sex (p=0.300) with baseline WC on sleep duration.

Table 3.3 Cross-sectional associations between adiposity and sleep duration at Wave 4 of ELSA (N=5,015)

BMI (baseline)			
Sleep duration (baseline)	B (minutes)	95% CI	P
Basic model (1)	-0.44	-0.014 – -0.000	0.033
Adjusted for health behaviours (2)	-0.39	-0.013 – -0.000	0.065
Adjusted for health problems (3)	-0.29	-0.012 – -0.002	0.167
Fully-adjusted model (4)	-0.28	-0.012 – 0.002	0.190
Waist circumference (baseline)			
Sleep duration (baseline)	B (minutes)	95% CI	P
Basic model (1)	-0.18	-0.006 – -0.000	0.034
Adjusted for health behaviours (2)	-0.14	-0.005 – 0.000	0.087
Adjusted for health problems (3)	-0.11	-0.005 – -0.000	0.198
Fully-adjusted model (4)	-0.10	-0.004 – 0.001	0.270

Note. This table presents the cross-sectional association between baseline BMI/WC as exposures and baseline sleep duration as the outcome, with different levels of adjustment for covariates. (1) Adjusted for age, sex, wealth, ethnicity; (2) Basic model + physical activity, smoking status, alcohol consumption; (3) Basic model + long-standing illness, depressive symptoms; (4) Basic model + health behaviours + health; B (Unstandardized coefficient) = difference in sleep duration (minutes) per difference in WC (cm), 95% CI= 95% confidence interval, P= regression p-value.

3.4.3 Prospective associations I: BMI and changes in sleep duration; WC and changes in sleep duration

3.4.3.1 BMI and changes in sleep duration

The first set of prospective analyses performed had baseline BMI as the exposure and follow-up sleep duration as the outcome, the results of which are shown in Table 3.3. Model 1 revealed a negative association between baseline BMI and follow-up sleep duration, such that a higher BMI was associated with increasingly shorter sleep at wave 6. In Model 4, the longitudinal association between BMI and sleep duration was only slightly attenuated (Model 1 to Model 4, 12.5% non-significant decrease in the coefficient, $p>0.05$).

3.4.3.2 WC and changes in sleep duration

On average, the change in sleep duration from baseline to follow-up was -0.42 minutes per unit increase in BMI. A very similar pattern of associations was observed between WC and changes in sleep duration, such that for every centimetre increase in WC at baseline, sleep duration at follow up decreased, on average, by 0.18 minutes. Although very small, this effect remained significant after adjustment for all covariates in Model 4 and the coefficient was identical throughout the models (Table 3.3). The mean change in sleep duration from baseline (6.867 hours) to follow-up (6.872 hours) was 0.005 hours (0.3 minutes), standard deviation = 1.13 hours (67.5 minutes). This indicated that, on average, durations of sleep did not change over the 4-year follow-up period.

There were no interactions of baseline age or sex with BMI and WC on follow-up sleep duration in any of the 4 models ($p>0.05$). There was no evidence of a quadratic association between baseline BMI or WC and follow-up sleep duration ($p>0.05$).

3.4.4 Prospective associations II: Sleep duration and changes in BMI; sleep duration and WC

3.4.4.1 Sleep duration and changes in BMI

The longitudinal analyses presented in Table 3.4 revealed no significant associations between sleep duration and future BMI. Across all 4 models, there was no evidence of a linear association between sleep duration at baseline and BMI at 4-year follow-up. Nor was a U-shaped association observed ($p>0.05$). Although age at baseline was strongly associated with BMI at follow-up [B=-0.020 kg/m^2, (95% CI= -0.027; -0.013) $P<0.001$] after adjustment for BMI at baseline and all other covariates, there were no interactions between baseline age or sex with sleep duration on BMI at follow-up in any of the 4 models (all $p>0.05$).

3.4.4.2 Sleep duration and changes in WC

Similarly, there was no significant association between sleep duration at baseline and changes in waist circumference (Table 3.4) in any of the 4 regression models (all $p>0.05$). There were also no significant interactions between age and baseline

sleep duration, or sex and baseline sleep duration on follow-up WC, nor was there evidence of a quadratic association (all p>0.05). Thus, results are presented for males and females together, with the exception of WC in the sample characteristics table (Table 3.1), which is presented separately for males and females, across sleep duration categories.

Table 3.4 Prospective associations between adiposity at Wave 4 (baseline) and change in sleep duration at Wave 6 (follow-up) of ELSA (N=5,015)

	BMI (baseline)		
Sleep duration (follow-up)	B (minutes)	95% CI	P
Basic model (1)	-0.48	-0.014 – -0.003	0.004
Adjusted for health behaviours (2)	-0.48	-0.014 – -0.003	0.004
Adjusted for health problems (3)	-0.48	-0.013 – -0.002	0.012
Fully-adjusted model (4)	-0.42	-0.013 – -0.002	0.013
Waist circumference (baseline)			
Sleep duration (follow-up)	B (minutes)	95% CI	P
Basic model (1)	-0.18	-0.006 – -0.000	0.005
Adjusted for health behaviours (2)	-0.18	-0.006 – -0.000	0.007
Adjusted for health problems (3)	-0.18	-0.005 – -0.000	0.015
Fully-adjusted model (4)	-0.18	-0.005 – -0.000	0.016

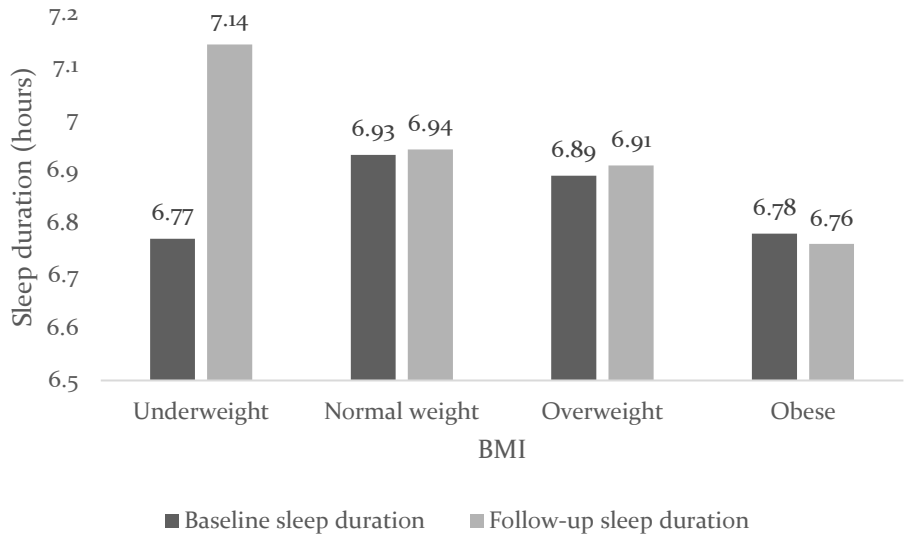
Note. This table presents the prospective association between baseline BMI/WC as exposures and follow-up sleep duration as the outcome, with different levels of adjustment for covariates. (1) Adjusted for age, sex, wealth, ethnicity and baseline sleep duration; (2) Basic model + physical activity, smoking status, alcohol consumption, baseline sleep duration; (3) Basic model + long-standing illness, depressive symptoms, baseline sleep duration; (4) Basic model + physical activity, smoking status, alcohol consumption, long-standing illness, depressive symptoms, baseline sleep duration; B (Unstandardized coefficient) = change in sleep duration (minutes) per unit change in BMI (kg/m^2) or WC (cm), 95% CI =95% confidence interval, P= regression p-value.

Table 3.5 Prospective associations between sleep duration at Wave 4 (baseline) and change in adiposity at Wave 6 (follow-up) of ELSA (N=5,015)

Sleep duration (baseline)			
BMI (follow-up)	B (kg/m²)	95% CI	P
Basic model (1)	0.005	-0.039 – 0.053	0.755
Adjusted for health behaviours (2)	0.008	-0.038 –0 .054	0.725
Adjusted for health problems (3)	0.009	-0.044 – 0.055	0.712
Fully-adjusted model (4)	0.009	-0.037 – 0.055	0.696
Sleep duration (baseline)			
Waist circumference (follow-up)	B (cm)	95% CI	P
Basic model (1)	-0.06	-0.195 – 0.082	0.426
Adjusted for health behaviours (2)	-0.05	-0.189 – 0.089	0.480
Adjusted for health problems (3)	-0.06	-0.197 – 0.082	0.416
Fully-adjusted model (4)	-0.05	-0.194 – 0.085	0.447

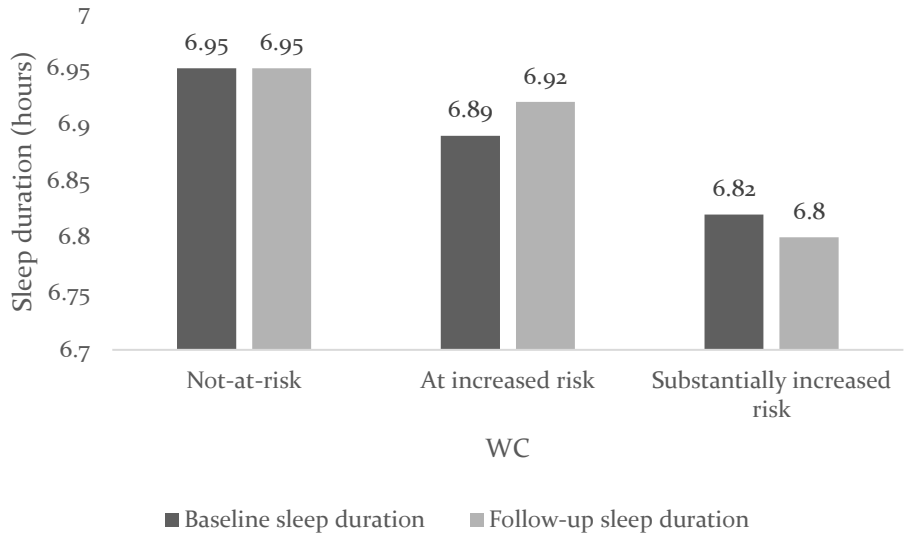
Note. This table presents the prospective association between baseline sleep duration (exposure) and follow-up BMI/WC (outcome), with increasing levels of adjustment for covariates. (1) Adjusted for age, sex, wealth and baseline BMI or WC; (2) adjusted for age, sex, physical activity, smoking status, alcohol consumption, baseline BMI or WC; (3) adjusted for age, sex, long-standing illness, depressive symptoms, baseline BMI or WC; (4) adjusted for age, sex, wealth, physical activity, smoking status, alcohol consumption, long-standing illness, depressive symptoms, baseline BMI or WC; B (Unstandardized coefficient) = change in BMI (kg/m2) or WC (cm) per change in sleep duration (minutes), 95% CI= 95% confidence interval, P= regression p-value.

Figure 3.1 Baseline and follow-up sleep duration, by BMI category at baseline



Note. Underweight <18.5 kg/m² (n=31), Normal weight≥18.5 kg/m² & ≤24.9 kg/m² (n=1,317), Overweight ≥25 kg/m² & <30 kg/m² (n=2,154), Obese≥30 kg/m² (n=1,513).

Figure 3.2 Baseline and follow-up sleep duration, by WC category at baseline



Note. Not-at-risk <80 cm (females) and <94 cm (males) [n=1,031], At increased risk>=80 cm, but <88 cm (females) and >=94 cm, but <102 cm (males) [n=1,267], Substantially increased risk >=88 cm (females) and >=102 cm (males) [n=2,717].

3.5 DISCUSSION

3.5.1 Key findings

In this large, nationally representative study of older adults, findings suggest that cross-sectionally, while both BMI and WC are inversely associated with sleep duration, these relationships are largely accounted for by variations in health status and health behaviours. Prospectively, greater BMI and WC at baseline were associated with sleep duration over a 4-year period, independently of adjustment for a variety of covariates. In contrast, sleep duration at baseline was not associated with changes in BMI or WC over the follow-up period. The richness of the available dataset enabled analyses that accounted for a number of factors, including wealth, illness and depressive symptoms, and health behaviours.

3.5.2 Cross-sectional findings

Cross-sectional results indicate that adiposity measures (BMI and WC) are not associated with self-reported sleep duration, after adjustment for a wide range of covariates. This finding is consistent with two earlier large-scale studies, which did not find an association between adiposity and sleep duration[117,229]. However, this result does not accord with evidence in favour of this cross-sectional relationship in older adults [210,230]. One of these studies, which found a significant association of BMI and WC with sleep duration in older adults made no adjustment for physical long-standing illness or socioeconomic position in their analysis[230], which could in part explain the discrepancy between the current findings and theirs. The authors also used a measure of self-reported sleep duration by which respondents were only asked to report how many hours they had slept on the two nights prior to the interview[230] rather than the more general sleep duration question in ELSA which asked about the number of hours sleep on an average weeknight. The other study, mentioned above[210] also differed regarding inclusion of covariates in comparison to the present findings. Covariates that these authors adjusted for that were not included in the current study were the following: coffee consumption, educational level, social network (not online, but ascertained from data on social links), perceived health status,

waking up during the night and whether participants were taking anxiolytic medication. The authors observed that their cross-sectional relationship of sleep duration and adiposity remained robust to this wide range of adjustments. However, the present study included adjustment for physical activity, wealth and ethnicity, which were not adjusted for in the study by Lopez-Garcia and colleagues[210]. A further disparity was the mean age, as well as age range in this sample (72 years) as compared to the present study (65 years). Also, the current study included respondents aged 50 or older, whereas Lopez-Garcia et al.'s study included only those who were 60 or older.

Furthermore, as mentioned above, health behaviours also impacted the association between BMI and sleep duration at baseline. Specifically, the cross-sectional models that examined whether BMI/WC were associated with sleep duration adjusting only for demographics (wealth, ethnicity, age and sex) revealed a significant effect (Appendix 8.1, Table 8.1). However, subsequent models that adjusted for health behaviours (PA, smoking and alcohol consumption) in addition to demographics, showed a clear attenuation of the association between sleep duration and adiposity such that it was no longer significant (Appendix 8.1, Table 8.2). On closer inspection, it was apparent that PA attenuated the relationship between adiposity (BMI and WC) and sleep duration, as associations were observed between PA and sleep duration (p=0.005 for BMI and p=0.006 for WC), whilst this was not the case for alcohol consumption or smoking status (Appendix 8.1, Table 8.2). It is possible that this represents mediation by PA, such that respondents with higher BMIs, or shorter sleep durations may engage in lower levels of PA, which could lead to shorter sleep duration. Evidence suggests that in older adults, PA may protect against developing insomnia, for example[231].

Associations in these data concur with several reports that associations exist between disadvantaged socioeconomic position and sleep duration[64,216–219] (Appendix 8.1, Table 8.1), and depression and sleep duration[60,220] (Appendix 8.1, Table 8.3). There is also previous evidence for an association between socioeconomic position and obesity[221,222], as well as BMI and depression[223].

When tested there was no statistically significant evidence of a cross-sectional U-shaped relationship between BMI and sleep duration. However, the finding of longest sleep duration in those with BMI between 18.5 and 24.9 kg/m² agrees with previous research[71,106,107,112,133]. This may reflect reverse causation, as long-standing illness is prevalent in older age groups and may lead to weight loss. These findings support this notion because adjustment for health problems attenuated observed associations.

Interactions between BMI/WC and sleep duration, and both age and sex were tested, but were not significant. The rationale for investigating these potential effect modifiers stemmed from some previous literature, which suggests that this association changes with age and that there may be sex differences. A handful of previous studies found that the relationship between BMI and sleep duration diminishes with age[111,121,122].

An early study in this area showed that there was an interaction between age and sleep duration, and their association with adiposity, such that after age 34 years there was no association between sleep duration and adiposity[121]. Gangwisch and colleagues[122] also found that the ORs for obesity in short sleepers (<4 hours) in comparison with those who slept seven hours substantially decreased with age: the OR for ages 32-49 was 3.21, whilst at ages 68-86 it was 1.71. These findings emerged from stratified regression analyses, after the authors found a significant age-by-sleep duration interaction and its association with obesity. However, this was not apparent in ELSA as no significant age-by-adiposity, or age-by-sleep duration was found, thus suggesting that across all ages (range of 50 to 90 years and over) the magnitude of association appeared to be the same.

In ELSA, there was also no evidence of an interaction between sex and adiposity and the effect on sleep duration, nor was there an interaction between sex and sleep duration and the effect on adiposity. A Japanese study suggested that at one-year follow-up sleep duration was associated with increased weight and increased risk of obesity in men, but not in women[134]. Another study, by Mezick et al.[110] found that sex moderated the effect of objectively measured sleep duration on BMI, such that there was only an association in females. However,

this was not the case for self-reported sleep duration, as there appeared to be no effect modification by sex. Thus, the lack of any significant sex interactions in ELSA is in agreement with this earlier study, as self-reported sleep duration was also used.

3.5.3 Prospective findings I: BMI and WC, and changes in sleep duration

This study was the first to perform bidirectional analyses of adiposity and sleep duration in a large sample of older adults. Longitudinal analyses of adiposity at baseline with change in sleep duration between baseline and follow-up revealed a negative association, such that higher BMI and WC were associated with decreased length of sleep. However, it is important to note that these effect sizes were very small and are unlikely to be clinically meaningful. Also, on average, no change between baseline and follow-up sleep duration was observed, which might explain why the magnitude of effect was small.

Finding that adiposity and sleep duration were associated in a prospective analysis accords with previous research, which found evidence of an association between average changes in weight gain and average change rates in sleep duration[121,232]. The present study found that both BMI and WC were associated with future sleep duration in a sample whose average age was 65 years, but it is important to note that this effect size was very small (0.42 minutes). This result is in line with a study in younger adults, which suggests that the association between adiposity and changes in sleep duration was stronger than the opposite relationship, which examined sleep duration and changes in adiposity[121].

In linear models, despite a small effect, both BMI and waist circumference remained associated with change in sleep independently of a wide range of covariates (measured at baseline), including health and health behaviours. However, these effects were small and residual confounding cannot be discounted, as it was not possible to examine all other factors that might explain the observed association between adiposity and sleep duration.

For example, an important factor that the analyses were not able to account for was napping. A recent study found that both longer sleep duration and prolonged

midday napping of greater than or equal to ninety minutes, are potential risk factors for incidence of metabolic syndrome[233], compared to their counterparts who napped for less than, or equal to thirty minutes and reported night time sleep durations of between 6 to 7 hours. A recent study in the UK Biobank found that, interestingly, the relationship between a genetic risk score of BMI and BMI and WC, is moderated by sleep duration and daytime napping [234]. These effects were observed independently of diet, socio-demographic factors and comorbidities[234].

As some previous research has shown that the association between adiposity and sleep duration may be U-shaped, prospective quadratic modelling was performed for both BMI and WC in relation to sleep duration. Quadratic models showed that there was no significant U-shaped association between adiposity and changes in sleep duration in ELSA and therefore suggest that this relationship is linear in nature.

3.5.4 Prospective associations II: sleep duration and changes in BMI and WC

In ELSA, there was no evidence of an association between sleep duration and change in BMI from baseline to follow-up, nor between baseline sleep duration and change in WC at follow-up. That sleep duration was not associated with change in BMI or WC specifically in older adults, accords with some[235,236], but not all previous reports[210]. One potential explanation for this may relate to the stability of adiposity in this age group, as neither average BMI nor WC changed greatly in 4 years. This was confirmed by a paired samples t-test, which showed that there was no significant difference between BMI at baseline and follow-up in the analytic sample ($p=0.33$). Thus, perhaps further follow-up of the participants could reveal associations with BMI that were not yet apparent. WC showed greater change over the follow-up period (baseline vs. follow-up, $p<0.001$), such that, on average, WC decreased from 96.49 cm (SD=13.21) at baseline to 96.09 cm (SD=13.54) at follow-up, yet evidence suggests that the WCs of older adults tend to increase, rather than decrease, over time[237]. However, there was still no effect

of sleep duration on WC and both adiposity measurements yielded consistent findings.

Secondly, it is suggested that the magnitude of the association between sleep duration and changes in adiposity measures declines with age[121,122]. This may explain why the results presented here, where mean age is 65 years, and in other studies such as the Whitehall II[111], are null. Thus, these data suggest that obesity may be a target to ameliorate co-morbidities that occur due to poor sleep, but that sleep duration is not a target to prevent obesity in older age groups. This non-significant prospective association between sleep duration and two adiposity measures is an important one, particularly as the majority of previous research in this area has yielded positive findings. It is possible that differences in inclusion of covariates may have contributed to this discrepancy in results, and/or that these effects have disappeared by the time data are collected from individuals in later life (for example, ELSA and WHII). In comparing the present results with those of the WHII paper[111] the authors also adjusted their analyses for a list of covariates that are similar to those adjusted for in ELSA. There were small differences in adjustments, for example in ELSA ethnicity and long-standing illness were included, whilst in WHII adjustment was made for medications, such as cardiovascular drugs and hypnotics. Also, notably, in ELSA and WHII the sleep duration question asked is identical, whilst other studies included in the most recent meta-analysis of prospective studies in adults[238] shows that in the other 10 studies sleep duration was asked slightly differently, which may affect the results. For example, three of these studies[239–241] asked participants to report their average daily sleep duration, two asked about both weekday and weekend sleep duration and then took a weighted average[134,242], two studies asked for average sleep duration in a 24 hour period[124,243] and the remaining studies asked participants to report average night time duration of sleep[244–246]. Also, of these studies, only two included ethnicity as a covariate[243,246] and only two, besides the WHII paper[111], included adjustments for medications[124,244]. Notable is that the study which had the fewest covariates (age, sex, baseline BMI, length of follow-up and SES) and

the smallest sample size (n=151), had the largest effect size (OR=2.97) for sleep duration and risk of obesity at follow-up[239].

Also, a quadratic sleep duration term was included in separate models, adjusted for covariates, the results of which were not significant. Thus, there was no U-shaped relationship between sleep duration and changes in BMI or WC.

3.5.5 Potential unexplored mechanisms for the association between adiposity and sleep duration in older adults

In this section, potential mechanisms that might underlie the relationship between adiposity and sleep duration are explored. However, the magnitude of effect between baseline BMI/WC and changes in sleep duration was miniscule, which is important to note.

3.5.5.1 Sleep apnoea as a possible explanation

In Chapter 1, shared genetic aetiology was discussed as a potential explanation for the association between BMI and sleep duration. Additionally, it is possible that obstructive sleep apnoea (OSA) is another plausible explanation for the prospective associations of adiposity measures and sleep duration in older adults OSA is a condition that causes the airways to collapse or become blocked whilst sleeping and is markedly prevalent in obese adults[247]. Older people with higher BMIs and/or WCs may have a higher percentage of visceral fat than their leaner counterparts, which has been found to be a significant risk factor for OSA[247,248]. Therefore, they may develop OSA, which could subsequently affect their sleep duration.

Evidence suggests that when objectively measuring sleep duration, very short sleep (mean duration of 3 hours) is associated with greater OSA severity[249], which could also be applicable to self-reported sleep duration. Another recent study found self-reported short sleep duration and OSA to be independently associated with visceral obesity, in adults aged between forty and sixty-nine years[250]. Meta-analytic evidence also finds that OSA is predictive of CVD and all-cause mortality[251]. However, in ELSA waves 4 (baseline) and 6 (follow-up) there are no

questions asked about sleep apnoea, thus it was not possible to perform analyses to investigate this further.

3.5.6 Study strengths

This study has several strengths. As both adiposity (BMI and WC) and sleep duration data were collected at two time points in ELSA, it was possible to investigate changes in both adiposity and sleep duration by incorporating these baseline measures as covariates in all prospective, bidirectional modelling.

It was also important to analyse two measures of adiposity, particularly WC, which is deemed better for measuring adiposity in older adults, as BMI can be less accurate in this population, mainly due to sarcopenia[88,90]. Thus, the fact that WC and sleep duration were related in an identical way to BMI and sleep duration also strengthens the study conclusions. However, no relationship was observed between baseline sleep duration and changes in BMI or WC, thus this association is likely to be weak in comparison in older adults. This is in line with literature reviewed in Chapter 1, which suggests that the prospective relationship between sleep duration and changes in adiposity in fact weakens with age.

A further strength is that BMI and WC were measured by a nurse, rather than self-reported, which reflects an improvement on some earlier studies in this area[71,252]. Large-scale epidemiological studies suggest that BMI calculated from self-reported height and weight may overestimate values at the lower end of the scale (<22 kg/m^2) and underestimate values at the upper end of the scale (particularly for BMI values >28 kg/m^2)[253]. Adults over the age of 55 are also more likely to underestimate their BMI, in comparison to those aged between 42 and 55[253].

A further strength is that ELSA is broadly representative of the English population aged 50 years and older[254] , which is important, as it allows generalisability of the findings. The richness of the available dataset enabled analyses that accounted for a number of factors, including wealth, illness and depressive symptoms, and health behaviours.

3.5.7 Study limitations

Sleep duration was self-reported, which may be prone to error and bias[130]. In older adults, it may not be optimal to rely on self-reports of sleep duration, as sleep disruptions due to OSA and insomnia are common and can lead to erroneous estimates of time spent asleep[154]. For example, research within the same ageing sample showed an association between objective sleep duration and obesity, but no relationship was found when self-report measurements were analysed in relation to obesity[130]. It is important to note that for a within-person analysis it may be less important that self-reported sleep duration may be error-prone, as the associated measurement error terms remain consistent. However, this does not rule out that for example, there could be a differential effect of adiposity (BMI, WC) on how respondents report their sleep durations.

Data were only available from waves 4 and 6 of ELSA; hence there were only four years between baseline and follow-up, which may have contributed to the trivial change observed in sleep duration and adiposity. This could perhaps be related to findings that usual sleep parameters do not significantly change in adults after the age of sixty[255]. It was also not possible to examine all potential mediators of the prospective association between adiposity measures and sleep duration. For example, respondents might sleep poorly due to their own or partner's snoring or other symptoms of sleep apnoea, as mentioned above. Additionally, information on daytime napping was not available, which is particularly pertinent in older adults. Despite ELSA's representativeness of the English ageing general population, these findings may not be entirely applicable to non-white ethnic groups and to adults below the age of 50. It is also possible that the restriction of sleep duration to between 2 and 12 hours may have impacted the results, although there were only 12 respondents whose durations of sleep were outside of this range.

3.5.8 Future directions

The present study was the first to examine the association of adiposity and sleep duration using bidirectional analyses. However, there are some important directions to consider for future research.

These findings should be replicated with objective sleep duration, perhaps by means of actigraphy, as it is rapidly becoming less expensive. For example, since this work was completed, the UKB have released wrist actigraphy data collected from approximately 100,000 individuals, which will enable large-scale analyses of objective sleep duration and BMI. This is important, as it has been reported that the agreement between objective and subjective sleep duration is at best, modest[96].

Evidence suggests that length of average sleep is unlikely to change drastically in healthy adults over the age of sixty. However, to confirm the results from this study it may be important to carry out a similar study using a longer follow-up time. This could potentially be done using the ELSA sample, as wave 8 data is newly available and includes sleep duration, as well as crucial questions about shift work and napping, for example. Thus, it would be possible to investigate whether factors such as these do in fact, mediate the association between adiposity and changes in sleep duration. However, as the amount of change in both BMI and sleep duration over the 4-year follow-up period was very small, it would have been difficult to use other methods to examine this bidirectional relationship.

In relation to an earlier point, in future it would also be of interest to further explore the interrelationship between BMI, socioeconomic position, depression and sleep duration.

3.6 CHAPTER SUMMARY

- Analyses were performed to investigate the cross-sectional and bidirectional prospective relationship of BMI and WC with self-reported sleep duration in older adults.
- Using data from waves 4 and 6 of ELSA linear and non-linear regression analyses were performed to establish the direction of this association.
- After adjustment for a wide range of covariates, there was no evidence of a significant cross-sectional association between adiposity and sleep duration.

- Prospective models indicated that there was a significant relationship between BMI at baseline and very small changes in sleep duration, but the opposite relationship was not significant.
- Although some potential explanations for this association were proposed, such as sleep apnoea and share genetic aetiology, it is unlikely that we would need to target adiposity in order to promote longer sleep duration in older adults, as these effects are very small.

4 INVESTIGATING THE BIDIRECTIONAL ASSOCIATION OF BMI AND SLEEP DURATION IN EARLY LIFE

4.1 BRIEF INTRODUCTION AND OVERVIEW OF CHAPTER CONTENTS

Chapter 1 introduced and provided a review of the literature to date, on the relationship between adiposity (typically measured by BMI, waist circumference or another inexpensive method of data collection) and sleep duration (usually measured by means of self-report, with the exception of a small number of studies which have used objective sleep duration). Whilst reviewing this literature, several systematic reviews and meta-analyses of cross-sectional and prospective studies predominantly suggest that shorter sleep is related to changes in BMI and increased risk of obesity[106,107,139,140,151–153]. However, all of these studies examined whether sleep duration might predict changes in BMI or increased risk of obesity, whilst there appear to have been only two studies (described below), to date, to examine both processes in a single sample[137,138].

Hiscock and colleagues[137] were the first to investigate the bidirectional association of BMI with sleep duration in children. They found that BMI did not predict changes in sleep duration, nor did they observe that sleep duration was associated with changes in BMI, in a large sample of Australian children.

More recently, another study provided support for Hiscock's findings[138]. These researchers found that BMI did not predict changes in sleep duration, nor did sleep duration predict changes in BMI, in White children from the Born in Bradford Study. However, they observed associations in both directions in South Asian children.

It is important to investigate the direction of this association further, using a distinct approach from previous studies. Both the Hiscock et al. and Collings et al. studies analysed subjective measures of sleep duration and thus, no research

has yet investigated the bidirectional relationship of BMI with objective sleep duration in a paediatric sample. The sample used in this chapter has prospective data available in children, as well as carefully-measured actigraphically assessed sleep duration and measures of height and weight. Thus, it provided a unique opportunity to investigate the bidirectional, prospective association of BMI and objective sleep duration at three time points. Establishing the direction of effect between BMI and objective sleep duration in children is important, as this could be different from the relationship between BMI and subjective duration of sleep because objective sleep is likely to be measured with more precision.

As mentioned in Chapter 1, the remainder of this thesis focuses on understanding the association between BMI and sleep duration in adults and therefore, this is the only study in a paediatric sample. Funding was provided by the ESRC for an Overseas Institutional Visit (OIV) and due to an existing collaboration with Dr Clare Llewellyn, I visited the Department of Psychology at the Norwegian University of Science and Technology (NTNU) for three months (March 2017 – June 2017) to work with Dr Silje Steinsbekk and Prof. Lars Wichstrom. Data were available from the comprehensively-phenotyped study that this research group houses: the Trondheim Early Secure Study (TESS), and enabled the investigation of the prospective relationship of BMI with objective sleep duration, for the first time, in a paediatric sample.

4.2 AIMS OF THIS CHAPTER

The study presented in this chapter had three aims:

- Examine the cross-sectional associations between BMI and objectively measured sleep duration at ages 6 and 8y.
- Examine the association between BMI at age 6y and changes in sleep duration between 6 and 8y, as well as the association between BMI at age 8y with changes in sleep duration between 8 and 10y.
- Examine the association between sleep duration at age 6y with changes in BMI between 6 and 8y, and the association between sleep duration at age 8y and changes in BMI between 8 and 10y.

4.3 METHODS

For the work carried out in this chapter I designed the study, derived BMI-SDS for the age 10 data and performed all statistical analyses.

4.3.1 Sample

The Trondheim Early Secure Study (TESS) comprises a community sample of Norwegian children born in 2003 and 2004 (see Chapter 2 for more detailed information about TESS). Data have been collected at ages 4, 6, 8 and 10 years, to date. From these two birth cohorts, 1250 (97.2%) children were recruited into TESS at wave 1 (age 4) by means of a letter of invitation, which included the Strengths and Difficulties Questionnaire (SDQ), version 4-16[203]. The SDQ is a screening tool for behavioural and emotional problems, which was one of the primary focuses of TESS. Therefore, due to the oversampling of children with such problems, sample weights are needed in all analyses of the TESS data. Ethical approval for TESS was granted by the Regional Committee for Medical and Health Research Ethics, Mid-Norway and written informed consent was obtained from parents. Here data were analysed from waves 2 (baseline), 3 (baseline + first follow-up) and 4 (follow-up) and there was a total of 794 children included in the study. Thus, two separate cross-sectional and prospective studies were carried out, as children develop and *may* change (in terms of weight and sleep duration) between the ages of 6y and 10y. For this reason a 2-year follow-up period was used, rather than a single, 4-year follow-up.

4.3.2 Measures

4.3.2.1 Body Mass Index (BMI)

Height (metres) and weight (kg) at ages 6y, 8y and 10y were collected by a health nurse, using digital scales (Heightronic digital stadiometer: QuickMedical, Model 235A and Tanita BC420MA). BMI was then calculated with the standard formula: weight (kg) divided by height (m^2). BMI standard deviation scores (SDS) were derived in TESS using the British growth curve reference for children aged from birth to twenty-three years[87]. The British reference data were used because they are deemed to be more reliable than the Norwegian reference data and are

therefore used in TESS published studies (for example, Steinsbekk et al[256].). Further details on BMI, BMI-SDS and how weight status categories were derived in TESS are provided in Chapter 2.

4.3.2.2 Sleep duration

Sleep duration was objectively measured using the ActiGraph™ GT3X accelerometer (Manufacturing Technology Incorporated, Fort Walton Beach, FL, USA). Participants wore the actigraphs on their hip for 7 consecutive days, including whilst asleep, and were only required to remove them whilst showering or bathing. More details on this measure are provided in Chapter 2.

4.3.2.3 Covariates

Covariates included in the analyses were demographics: age (continuous), sex (0=male, 1=female), a measure of socioeconomic status (SES), ethnicity and season. Parental occupation was used as a measure of SES, coded as 0=skilled professionals and leaders, 1=unskilled. This was recoded from the International Classification of Occupations, which is measured on a 6-point scale (1=Manual workers, 6=Leaders)[257]. If participants' parents were living together the parent with the highest occupation was selected. Ethnicity was originally coded from 1 to 12 (1=Norway, 2=Nordic countries, 3=Western Europe, USA, Canada, New Zealand, Australia, Israel, 4=Eastern Europe, 5=Balkans (Former Yugoslavia, Romania, Bulgaria), 6=Turkey, 7=North Africa, 8=South Africa [country], 9= Rest of Africa, 10= Central and South America, 11=Asia/Rest of Oceania, 12= Sami (indigenous Norwegian population). However, as there were only n=11 across all categories that did not fall into either 1 or 2 the ethnicity variable was recoded as 1=Nordic and 2=Non-Nordic.

A season variable was created from the month when the sleep duration data were collected at both waves 2 and 3 (ages 6y and 8y). The rationale for including season as a potential covariate was that there is some evidence to suggest that children's sleep duration varies between seasons, particularly in Northern Europe and Scandinavia. A recent study in 730 Danish children, aged 8-11y found that

objective sleep duration was approximately 2% longer during winter, compared to spring[258].

The variable was originally coded as a number between 1 and 12 to indicate the month of assessment, which was subsequently recoded into an ordinal measure with 4 categories, one per season. In creating these categories a similar approach to that of Kolle and colleagues[259] was used, who defined the Norwegian seasons as: Autumn (September, October, November); Winter (December, January, February); Spring (March to mid-June); and summer was not included, as they did not collect data in July or August. However, in TESS data were collected during July and August and were thus, also categorised as Summer. The season variables for ages 6y and 8y were coded as: 1=spring, 2=summer, 3=autumn, 4=winter. Season was included as a covariate, as linear regressions showed that there was a significant cross-sectional association between both season and sleep duration at age 6y (B= 0.10, 95% CI= 0.011; 0.190, P=0.027), and season and sleep duration at age 8y (B=0.21, 95% CI=0.122; 0.292, P<0.001). Both of these regression models were adjusted for age, sex, ethnicity and SES.

4.3.3 Statistical analyses

4.3.3.1 Power calculations

Using G*Power post-hoc power was calculated for cross-sectional and bidirectional prospective analyses, by using the following parameters: effect size F^2, % error probability, total sample size, number of tested predictors and total number of predictors. F^2 for the effect size was calculated as: R^2 / 1 - R^2, by taking the R^2 from an unadjusted (no covariates included) cross-sectional and prospective models from the results of the ELSA study (Chapter 3). The alpha level was set at 0.05, the total n was 794 and the number of tested predictors was 1 for cross-sectional and prospective analyses. Power calculations indicated that there was limited power to observe similar effects to those in the ELSA study in Chapter 3 (Table 4.1), which was unsurprising, given the very small effects found in ELSA.

Table 4.1 Power calculations for cross-sectional and bidirectional prospective analyses in TESS

Model	Effect size F^{2*}	% error probability	N	No. tested predictors	Power
Cross-sectional	0.0025	0.05	794	1	29%
Prospective BMI on sleep duration	0.0046	0.05	794	1	48%
Prospective Sleep duration on BMI	0.0046	0.05	794	1	48%

Note. *For cross-sectional models this (F^2) was calculated as: R^2/ 1 – R^2 = 0.0025 / 1 – 0.0025 = 0.0025; for prospective models of BMI on sleep duration this was calculated as: R^2/ 1 – R^2 = 0.0046 / 1 – 0.0046 = 0.0046; for prospective models of sleep duration on BMI this was calculated as: R^2/ 1 – R^2 = 0.0021 / 1 – 0.0021 = 0.0021 . All of these R^2 values were taken from results of the simple linear regression (unadjusted) models in the ELSA study in Chapter 3.

4.3.3.2 Full Information Maximum Likelihood (FIML) estimation

Statistical analyses were all performed in STATA, version 14. Due to the limited sample size for a complete case analysis (CCA) of n=452, analyses were performed using full information maximum likelihood (FIML), yielding an analysis sample of n=794. Maximum likelihood estimation is a powerful method for handling missing data and is less biased than ad hoc techniques such as pairwise deletion, mean imputation and list wise deletion[260]. FIML is sometimes also preferred over multiple imputation (MI) for various reasons, one particularly important reason being that MI produces different results for the same data every time because it involves random draws[260]. FIML does not suffer from this limitation. Furthermore, analyses were all weighted using sample weighting in STATA to ensure representativeness of the general population, due to the oversampling of children with emotional and behavioural problems in TESS. Associations between BMI and sleep duration were tested separately for cross-sectional associations at both ages 6 years and 8 years and subsequently, separate models were run to try to establish the direction of effect.

4.3.3.3 Comparison of sample characteristics across sleep duration categories

To compare baseline characteristics, the sample at age 6y was divided into two categories of sleep duration. These sleep categories were defined in line with the latest recommendations from the American Academy of Sleep Medicine, which state that, for every 24 hours, sleep duration should be between 9 and 12 hours for children aged 6 to 12 years old[261]. Thus, <9 hours was defined as "short sleep" and ≥9 hours as "typical sleep".

One-way ANOVAs were then used to compare participants on age and BMI (and BMI-SDS), whilst Chi-squared tests were used to compare sex, ethnicity, SES and weight status across sleep duration categories (Table 4.2).

4.3.3.4 Change in BMI and sleep duration between ages 6 and 8, and 8 and 10

To examine whether BMI and sleep duration changed substantially between ages 6 and 8 years, and 8 and 10 years, Pearson's correlations were calculated and subsequently, paired samples t-tests were also performed. In STATA, first, weighted means for both BMI and sleep duration were obtained using the sample weight that is applied to all TESS analyses and then linear combinations of these variables (for example, BMI at age 8 – BMI at age 6) were estimated to compute the respective t-and p-values.

4.3.3.5 Linear models

Linear regression models were performed to examine the cross-sectional relationship between BMI SDS with sleep duration, separately, at both ages 6 and 8 (waves 2 and 3, respectively). As mentioned above, these models were all adjusted for age, sex, ethnicity, parental SES and season.

Then linear regressions were performed to examine the prospective bidirectional associations between BMI and BMI SDS, and sleep duration. To investigate the relationship between BMI and changes in sleep duration, first sleep duration was regressed on BMI and BMI SDS using age 6y (wave 2) 6 as the baseline and age 8y (wave 3) as follow-up, followed by age 10y (wave 4) sleep duration regressed on

BMI and BMI SDS at age 8y (wave 3). These models were adjusted for age, sex, SES, ethnicity, season and baseline sleep duration.

To examine the opposite relationship age 8y BMI and BMI SDS were regressed on age 6y sleep duration, followed by age 10 BMI and BMI SDS regressed on age 8y sleep duration. These regression models were adjusted for age, sex, SES, ethnicity, season and baseline BMI or BMI SDS.

4.3.3.6 Non-linear models

A quadratic term was included for exposures in both cross-sectional and bidirectional prospective analyses. Thus, in cross-sectional regressions a BMI-squared (BMI^2) was included, as well as in prospective associations to examine the change in sleep duration from baseline BMI. In prospective analyses to examine changes in BMI from baseline sleep duration, a sleep duration-squared (sleep $duration^2$) term was included. Subsequently, one cross-sectional linear regression model was performed, adjusted for age, sex and parental SES. Additionally, the interactions between sex and baseline BMI and sleep duration (ages 6y and 8y) were tested, as some previous research has found differences between boys and girls.

4.4 RESULTS

4.4.1 Sample characteristics

Table 4.2 presents time-invariant characteristics in TESS. There were no significant differences between these groups in terms of sex, SES, ethnicity (all $p>0.05$). Tables 4.3, 4.4 and 4.5 present descriptive statistics for age, season, BMI, BMI-SDS and weight status at ages 6, 8 and 10 years. At 6y there was a small but significant difference in age between shorter and longer sleepers in TESS at age 6y, such that children who slept for longer were younger. However, there were no differences between the two sleep duration groups across any other time-varying characteristics at age 6y. At age 8y (Table 4.4) there was a nominally significant difference in mean age between the two sleep categories. There was a significant difference in the percentage of children who slept for <9 hours vs. ≥9 hours

across the four seasons (spring, summer, autumn, winter) at age 8y. There was no difference in BMI, BMI-SDS or weight status categories between the two sleep groups at age 8 (all p>0.05). At age 10y (Table 4.5) the two sleep duration groups differed significantly in terms of BMI and BMI-SDS, such that those who slept <9 hours had a higher mean BMI/BMI-SDS than those who slept for ≥9 hours. Similarly to age 8y, there was a significant difference in the number of children who slept <9 hours in comparison. ≥9 hours across the four seasons at age 10y.

Table 4.2 Sample characteristics in TESS by sleep duration category, at age 6y

	<9 hours (n=108)	≥9 hours (n=686)	Total (Max. N=794)	*P*
Sex – n (%)				
Boys	62 (57.41)	329 (48.03)	391 (49.31)	0.070
Girls	46 (42.59)	356 (51.97)	402 (50.69)	
SES – n (%)				
Skilled	81 (77.14)	502 (74.93)	583 (75.23)	0.625
Unskilled	24 (22.86)	168 (25.07)	192 (24.77)	
Ethnicity				
White	104 (98.11)	637 (98.62)	741 (98.54)	0.695
Non-white	2 (1.89)	9 (1.39)	11 (1.46)	
Mean age (SD)	6.05 (0.21)	6.01 (0.16)	752	0.044
Season – n (%)				
Spring	31 (32.39)	190 (31.61)	221 (31.71)	
Summer	32 (33.33)	135 (22.46)	167 (23.96)	0.058
Autumn	18 (18.75)	124 (20.63)	142 (20.37)	
Winter	15 (15.63)	152 (25.29)	167 (23.96)	
BMI – mean (SD)	15.49 (1.55)	15.59 (1.47)	15.54 (1.51)	0.523
BMI-SDS – mean (SD)	-0.30 (0.90)	-0.11 (0.85)	-0.14 (0.86)	0.506
Weight status – n (%)				
Underweight	13 (16.05)	45 (9.34)	58 (10.30)	
Normal weight	63 (77.78)	399 (82.78)	462 (82.06)	0.173
Overweight/obese	5 (6.17)	38 (7.88)	43 (7.64)	

Note. Overweight + obese weight status categories were collapsed as there were only n=3 overweight and n=2 obese participants.

Table 4.3 Descriptive statistics for exposure/outcome measures at ages 8y and 10y in TESS

	Sleep duration (age 8)			
	<9 hours (n=205)	≥9 hours (n=481)	Total (Max. N=686)	*P*
BMI – mean (SD)	16.72 (1.99)	16.55 (1.96)	628	0.297
BMI-SDS – mean (SD)	0.15 (0.94)	0.07 (0.88)	509	0.298
	Sleep duration (age 10)			
BMI – mean (SD)	17.76 (2.59)	17.40 (2.36)	686	0.028
BMI-SDS – mean (SD)	0.22 (1.05)	0.06 (0.99)	686	0.038

4.4.2 Cross-sectional linear and non-linear associations of BMI and sleep duration at ages 6 and 8

Cross-sectional linear regressions yielded no significant associations between BMI and sleep duration, at either ages 6y and 8y (Table 4.4). These models were adjusted for age, sex, SES ethnicity and season, and were performed for both BMI and BMI SDS. There was no evidence of a U-shaped (quadratic) relationship between BMI and sleep duration at either age (p>0.05).

Table 4.4 Cross-sectional associations of BMI and BMI SDS with sleep duration at ages 6 and 8

Sleep duration	B (minutes)	95% CI	P
	Age 6		
Model 1 - BMI (age 6)	0.01	-0.067; 0.095	0.724
Model 2 - BMI-SDS (age 6)	0.02	-0.067; 0.012	0.590
	Age 8		
Model 3 - BMI (age 8)	-0.03	-0.124; 0.060	0.500
Model 4 - BMI-SDS (age 8)	0.03	-0.070; 0.129	0.560

Note. Models 1 and 2= adjusted for age, sex, SES, ethnicity and season (age 6); Models 3 and 4= adjusted for age, sex, SES, ethnicity and season (age 8); exposure in Models 1 and 3 = BMI, exposure in Models 2 and 4= BMI SDS; B (Unstandardized coefficient) = difference in sleep duration (minutes) per difference in BMI, 95% CI =95% confidence interval, P = regression p-value.

4.4.3 Bidirectional prospective linear and non-linear associations of BMI and sleep duration

In prospective analyses using age 6 as a baseline and age 8 as follow-up, there was no significant relationship between BMI (and BMI SDS) and changes in actigraphic sleep duration, in unadjusted models that included only the exposure, outcome and baseline outcome ($p>0.05$). In further models adjusted for age, sex, SES, season, ethnicity and baseline sleep duration (Table 4.5). Furthermore, there was no significant association between sleep duration assessed at age 6y and changes in BMI, in a model adjusted for the same covariates as mentioned above, but with baseline BMI rather than sleep duration. The pattern of results for BMI-SDS in relation to sleep duration was identical to BMI and thus, there was no association between BMI-SDS at age 6y and change in sleep duration at age 8, nor was there any relationship between sleep duration at age 6y and change in BMI-SDS at age 8y.

There was also no significant U-shaped association between BMI at age 6y and sleep duration at age 8y, in either direction ($p>0.05$). Although Pearson's correlations showed that BMI was largely stable between the ages of 6y and 8y, $r=0.85$, $p<0.001$, a paired samples t-test indicated that there was also change over time, with a difference of 1.15 kg/m² (15.56 kg/m² at age 6y vs. 16.58 kg/m² at age 8y), which was, in fact, significant ($p<0.001$).

Duration of sleep was much less stable between these ages, $r=0.27$, $p<0.001$. Mean sleep duration at age 6y was 9.62 hours, whilst at age 8y it decreased to 9.09 and a paired samples t-test confirmed that this difference of approximately 28 minutes, was significant ($p<0.001$).

Results from linear regressions which used age 8y as a baseline and age 10y as follow-up, were identical to the previous bidirectional analyses (age 6y to 8y). Thus, BMI at age 8y did not predict changes in sleep duration at age 10y, nor did sleep duration at age 8y predict changes in BMI at age 10y, irrespective of level of adjustment for covariates (Table 4.6). Results also showed no significant quadratic associations between BMI and sleep duration in either direction

(p>0.05). Again, Pearson's correlations indicated that participants' BMIs were fairly stable between ages 8y and 10y (r=0.86, p<0.001), but a paired samples t-test suggested that there was nevertheless change over time and the difference in mean BMIs (16.60 kg/m² at age 8 vs. 17.49 kg/m² at age 10) was significant (p<0.001). The correlation between age 8y and age 10y sleep duration was much smaller, indicating less stability over this developmental period (r=0.32, p<0.001), although a paired samples t-test revealed that this difference (average decrease of 3.56 minutes between age 8 and age 10 sleep duration) was not significant (p=0.088).

Table 4.5 Bidirectional prospective models of BMI and sleep duration from age 6 to 8

	BMI (age 6) → Sleep duration (age 8)		
	B (minutes)	95% CI	P
Model 1	0.02	-0.07; 0.11	0.694
Model 2	0.03	-0.057; 0.129	0.451
	BMI-SDS (age 6) → sleep duration (age 8)		
Model 3	0.02	-0.08; 0.13	0.685
Model 4	0.02	-0.086; 0.121	0.737
	Sleep duration (age 6) → BMI (age 8)		
	B (kg/m²)	95% CI	P
Model 5	-0.02	-0.06; 0.03	0.467
Model 6	-0.01	-0.058; 0.030	0.527
	Sleep duration (age 6) → BMI-SDS (age 8)		
Model 7	-0.03	-0.08; 0.01	0.148
Model 8	-0.01	-0.092; 0.065	0.736

Note. Model 1= baseline sleep duration (age 6); Model 2= adjusted for age, sex, SES, ethnicity, season and baseline sleep duration (age 6); Model 3= baseline sleep duration (age 6); Model 4= adjusted for age, sex, SES, ethnicity, season and baseline sleep duration (age 6); Model 5= adjusted for baseline BMI (age 6); Model 6= adjusted for age, sex, SES, ethnicity, season and baseline BMI (age 6); Model 7= adjusted for baseline BMI (age 6), Model 8= adjusted for age, sex, SES, ethnicity, season and baseline BMI (age 6; B (unstandardised coefficient in minutes) = change in sleep duration per unit change in BMI (Models 1 and 2), outcome in Models 1 to 4= sleep duration at age 8y, outcome in Models 5 and 6 = BMI at age 8y, outcome in Models 7 and 8 = BMI-SDS at age 8y.

Table 4.6 Bidirectional prospective models of BMI and sleep duration from age 8 to 10

BMI (age 8) → sleep duration (age 10)			
	B (minutes)	95% CI	P
Model 1	-0.04	-0.12; 0.04	0.318
Model 2	-0.04	-0.120; 0.040	0.332
BMI-SDS (age 8) → sleep duration (age 10)			
Model 3	-0.00	-0.09; 0.08	0.997
Model 4	0.07	-0.016; 0.160	0.107
Sleep duration (age 8) → BMI (age 10)			
	B (kg/m²)	95% CI	P
Model 5	0.00	-0.07; 0.07	0.998
Model 6	0.00	-0.068; 0.074	0.934
Sleep duration (age 8) → BMI-SDS (age 10)			
Model 7	0.02	-0.04; 0.09	0.449
Model 8	-0.03	-0.133; 0.071	0.547

Model 1= baseline sleep duration (age 8); Model 2= adjusted for age, sex, SES, ethnicity, season and baseline sleep duration (age 8); Model 3= baseline sleep duration (age 8); Model 4= adjusted for age, sex, SES, ethnicity, season and baseline sleep duration (age 8); Model 5= adjusted for baseline BMI (age 8); Model 6= adjusted for age, sex, SES, ethnicity, season and baseline BMI (age 8); Model 7= adjusted for baseline BMI (age 8), Model 8= adjusted for age, sex, SES, ethnicity, season and baseline BMI (age 8); B (unstandardised coefficient in minutes) = change in sleep duration per unit change in BMI (Models 1 and 2), outcome in Models 1 to 4= sleep duration at age 10y, outcome in Models 5 and 6 = BMI at age 10y, outcome in Models 7 and 8 = BMI-SDS at age 10y.

4.5 DISCUSSION

4.5.1 Summary of findings

In this study, analyses were performed to examine the cross-sectional, as well as the bidirectional, prospective association between BMI and actigraphic sleep duration in a Norwegian sample. The purpose of this study was to understand the nature of the direction of this relationship in children, which has only been investigated by two previous studies[137,138], but never with objectively measured

sleep duration. This study in TESS yielded no significant cross-sectional associations between BMI and actigraphic sleep duration at ages 6y or 8y. There was also no evidence of a prospective relationship in either direction between BMI and actigraphic sleep duration, using age 6y as a baseline and age 8y as follow-up, or using age 8y as a baseline and age 10y as follow-up. There was also no evidence of U-shaped relationships between BMI and actigraphic sleep duration in TESS.

Correlations between BMI at ages 6y and 8y, and ages 8y and 10y in TESS were very strong, indicating that there was very little change in children's BMIs over 4 years. This was not the observation for actigraphic sleep durations, which changed to a greater extent, as expected, between ages 6y and 8y, and 8y and 10y.

4.5.2 Evaluation of findings in relation to previous research

4.5.2.1 Cross-sectional findings

As mentioned earlier, large-scale meta-analyses on BMI and sleep duration in children have predominantly concluded that shorter sleep is associated with higher BMI and increased risk of obesity[106,107,135,151,153,157]. The findings in this study accord with one study from another Scandinavian population, in which Klingenberg et al.[148] observed no cross-sectional association between parent-reported sleep duration and BMI in 211 Danish children. One reason for this null finding could be that the families recruited are mostly of high SES and live in the capital city region, thus these children are less likely to become obese and have poor sleeping habits. The TESS sample possesses important similarities to the Danish sample used in the earlier study, as for example, 86.4% of the children slept for ≥9 hours, which is in line with the recommendations for the amount of sleep in this age group[261]. There is some evidence to support this notion of social differences in children's sleep patterns. Findings from 11,500 ALSPAC children showed that those who were more likely to go to bed later and wake up later were those in low-income homes, yet there was little difference in total sleep duration with children from higher income homes[262]. However, it was also

observed that children of older mothers (>35 years) had shorter sleep durations, and that children in larger families were more likely to have later bedtimes[262].

BMIs did not differ across the sleep duration categories in the TESS sample and the prevalence of overweight/obesity was only 7.6% at age 6y and 6.77% at age 8y. It is therefore a possibility that no association was observed between BMI and sleep duration in TESS, as participants are largely lean and also had healthy sleep durations, on average, with approximately one in eight children slept for less than 9 hours at age 6y. In order to observe an effect between BMI and sleep duration it may be necessary to have greater variation in weight and sleep duration. In particular, the proportion of overweight/obese children in TESS is very different from that of the UK paediatric population. Statistics from the 2016-17 National Child Measurement Programme show that at ages 4/5y one in three children are overweight/obese and at ages 10/11y one in three are overweight/obese[263].

Thus, it is possible that the TESS sample is somewhat too homogenous and healthy to have observed a cross-sectional association between BMI and sleep duration. This work adds to a small body of null research on the cross-sectional relationship between BMI and sleep duration in childhood.

4.5.2.2 Bidirectional prospective findings

This study is not the first to find a non-significant prospective association between BMI and sleep duration in children. For example, the only two previous studies to investigate the bidirectional relationship between BMI and sleep duration in children yielded non-significant findings[137,138]. Hiscock and colleagues[137] observed that BMI did not predict changes in sleep duration, or vice versa, in a large sample of Australian children. This was supported by a more recent study on the bidirectional association of BMI and sleep duration, which found no significant effects in either direction, in a sample of 562 White children, who were followed up at ages 12, 18, 24 and 36 months[138]. Importantly, both of these studies used parent-reported sleep duration, in the form of sleep duration diaries. Thus, this is a fundamental difference between the analyses in TESS and

earlier studies, yet similar findings were observed across studies. This is important because if an association truly exists in either direction in childhood, it would have likely emerged using objective measurement of sleep duration, due to its improved precision over subjective measures (self or parent-reported).

Another study, which analysed both parent-reported, as well as accelerometer-estimated sleep duration in 311 young Danish children, found that neither measure of sleep was associated with BMI[148]. These authors found that parent-reported sleep duration at 9 months of age was not related to BMI at 18 months or 3 years of age, nor was accelerometer-measured sleep duration was associated with BMI cross-sectionally, at age 3. A recent Chinese study also found no significant relationship between risk of being overweight or obese and weekday sleep duration[150] in 3,086 children, which could be due to ubiquitous insufficient weekday sleep amongst Chinese children.

Overall, given that the present findings alongside results from the two earlier bidirectional studies, conducted in samples across three different countries (UK, Australia and Norway) suggest a null association (in White children), irrespective of sleep duration measurement (objective vs. subjective), it is possible that earlier non-bidirectional studies have overestimated the size of this effect.

4.5.3 Could sleep dimensions, other than duration be more important in relation to childhood obesity?

Jarrin and colleagues[166] found that other sleep dimensions, such as quality, pattern and disturbances may in fact be more important in relation to obesity. They reached this conclusion, as in their study the association between sleep duration with BMI was attenuated following adjustments for covariates, whereas this was not the case for the other sleep dimensions. Recently, researchers also observed that, in a sample of 236 children aged between 6 to 10 years, it was bedtime and sleep timing that were significantly associated with weight, rather than sleep duration[264]. Specifically, children classed as 'late sleepers' were significantly heavier than their 'normal sleeper' counterparts. In a Chinese sample of more than 5,000 children aged between 9 to 12 years findings suggest that

although sleep duration is related to BMI, later bedtime is also associated with a higher BMI[265], which provides some support for the previously described study by Thivel and colleagues[264]. Furthermore, evidence from 11,945 children from the Millenium Cohort Study (MCS) found that at age five, those who had irregular bedtimes were also the most likely to be obese[266].

The underlying mechanisms for how phenotypes such as bedtime and sleep timing may impact BMI are not fully understood. However, evidence suggests that children who have later bedtimes, compared to those with earlier bedtimes, are more likely to have higher BMIs, be inactive and have longer screen times[166]. Thus, it is possible that delaying sleep in children may mean that they spend more time exposed to an obesogenic environment and may for example, not have breakfast and be overall, more sedentary.

It is important, however, to acknowledge that studies have mainly focused on the relationship between sleep duration and BMI and thus, little is known about the association between other sleep parameters with BMI, which could also be important. However, this could be partly due the fact that self-reported sleep duration, in particular, is very easy to measure. The majority of large-scale studies collect this type of measure, as it can usually be captured with a single question asking how long participants usually sleep for, or by asking them the time they usually go to sleep and wake up and then deriving a proxy for duration of sleep. This was also the case in the TESS study, in which data on sleep quality, pattern, timing, etc were not available at waves 2, 3 and 4. If these data are collected in future waves it would be important to test the association between BMI and these other sleep parameters, using a bidirectional approach, similarly to the present analyses.

4.5.4 Study strengths

The study reported here has important strengths. It was the first to investigate the bidirectional relationship between BMI and objectively measured sleep duration in children, using actigraphic data; similar previous studies have only employed subjective sleep duration[137,138]. Actigraphic sleep duration can provide

more accurate estimates of sleep duration in comparison to subjective measures[97]. Van den Berg and colleagues[97] examined the agreement between actigraphic and self-reported sleep duration in adults from the Rotterdam Study. They observed that in 34% of participants, actigraphic and subjective sleep duration differed by more than an hour and thus recommended that multiple indicators of sleep duration be used in research. However, this is not always possible, mainly due to costs and time constraints when collecting data from large samples.

As indicated by the power calculations in the Methods section, there was sufficient power to detect associations between BMI and sleep duration of at least the size observed in ELSA (Chapter 3). Further, as TESS participants have been followed throughout childhood, it was possible to perform both cross-sectional and longitudinal analyses, using a robust approach, FIML, which maximised all data points available in the study.

4.5.5 Study limitations

The TESS sample is a particularly homogenous one, as indicated in the sample characteristics of the Results section. The proportion of overweight/obese children in TESS was only 7.6% at age 6 and 6.77% at age 8, with 92% of participants in the normal weight category at age 6 and 93.23% at age 8. It is likely that these low overweight/obesity prevalence estimates contributed to the null finding of a relationship between BMI and objective sleep duration in this study. Participants' BMIs only changed by 1.15 kg/m^2 from ages 6y to 8y and 0.89 kg/m^2 from ages 8y to 10y, and t-tests showed that these differences were significant. Mean durations of sleep did change, by approximately 28 minutes, over the 2-year follow-up period between ages 6 and 8, which was shown with t-test analyses.

Furthermore, there was no measure of parent-reported sleep duration available at these ages in TESS, which would have enabled a direct comparison with the actigraphic sleep duration measures. Also, there is some evidence to suggest that waist actigraphy may overestimate sleep duration in children, compared with

wrist actigraphy[167]. The present findings may not be applicable to children of non-Nordic backgrounds, which is supported by a previous study of the bidirectional association of BMI and sleep duration in South Asian children, as the authors found a prospective relationship in both directions. It is also important to mention that power to detect similar effects to those observed in ELSA (Chapter 3) was limited.

4.5.6 Future directions

There are some important considerations for future research in this area. In order to confirm, or refute emerging evidence that other sleep parameters are related to adiposity and an increased risk of obesity[166,264], future research could involve similar bidirectional analyses to those performed here, but with a focus on other such sleep measures, as well as longitudinal data, larger sample sizes and other ethnic groups.

Further research could also be performed using the TESS cohort, as data collection is on-going and the team plan to follow participants throughout adolescence and early adulthood. It might be of interest to repeat these bidirectional analyses at slightly older ages, as meta-analytic evidence also suggests a link between sleep duration and BMI in adolescents[267]. This is important, as sleep patterns, weight and body composition can change as participants enter puberty and adolescence. As part of this, analyses could also be performed to examine the relationship of other adiposity indicators, available in TESS, in relation to sleep parameters. Also, given the potential discrepancies between wrist and waist actigraphy in measuring sleep duration, future research should use wrist actigraphy, where possible.

It is also important that future studies use a more efficient analytical approach than the present study. For example, a parallel process latent growth curve model may be more appropriate for studies in which repeated measurements are available because it would allow the modelling of outcomes (in this case, BMI and objective sleep duration), as a function of both time and exposures/covariates. This kind of approach is also particularly effective to

investigate change and potential inter-individual variation in any observed change.

4.6 CHAPTER SUMMARY

- In this chapter, data were analysed from a Norwegian paediatric cohort to investigate the bidirectional relationship between BMI and actigraphic sleep duration at ages 6, 8 and 10.
- Both linear and non-linear analyses and found that there was no cross-sectional association between BMI and sleep duration at age 6 or 8.
- Prospective, bidirectional analyses also yielded non-significant results in both directions, at ages 6 to 8, and 8 to 10.
- Future studies ought to investigate the bidirectional association of other sleep dimensions (pattern, quality, bedtime, timing) with adiposity measures in children.

5 GENOME-WIDE ASSOCIATION STUDY (GWAS) OF SELF-REPORTED SLEEP DURATION

Results from the GWAS analyses performed in ELSA and UKHLS contributed to the following publication: **Garfield, V.,** Fatemifar, G., Dale, C., Smart, M., Bao, Y., Llewellyn, C., Steptoe, A., Zabaneh, D. & Kumari, M. Assessing Potential Shared Genetic Aetiology between Body Mass Index (BMI) and Sleep Duration in 142,209 individuals. *Under review in Scientific Reports.*

5.1 BRIEF INTRODUCTION AND DESCRIPTION OF CHAPTER CONTENTS

As outlined in Chapter 1 (section 1.7: Genetic determinants of sleep duration*)* there have only been a handful of GWAS of sleep duration, with only one replicated SNP to date[78]. As mentioned in Chapter 1, undertaking a large GWAS of self-reported sleep duration is important, given the current molecular genetic literature on this phenotype. Also, to potentially investigate the causal relationship between self-reported sleep duration and BMI, more robustly associated SNPs (with sleep duration) are needed, as this is a core assumption underlying Mendelian randomisation (more details in Chapter 6) – a genetic epidemiological method, which enables the use of genetic variants as instrumental variables (IVs) to examine causation between an exposure and outcome of interest. This chapter presents findings of a meta-genome-wide association (GWA) study, which was performed across three large-scale UK studies, in collaboration with a researcher from the University of Bristol.

5.2 AIMS AND OBECTIVES

The main aim of this chapter was to perform a large-scale meta-GWAS of self-reported sleep duration.

The specific objectives were fourfold:

- Identify novel common genetic variants (SNPs) associated with self-reported sleep duration by performing a meta-analysis of three large-scale population-based studies.
- Replicate previously reported loci associated with sleep duration.
- Investigate whether any genome-wide suggestive SNPs were significant expression quantitative trait loci (eQTL). An eQTL is a genomic locus that explains a portion of the variance of a gene expression phenotype.
- Estimate the SNP heritability of sleep duration.

5.3 METHODS

For the work carried out in this chapter, I performed all of the data cleaning and analyses, with the exception of the GWAS in ALSPAC, which was performed by Dr Gibran Hemani (University of Bristol). I did not, however, carry out the initial genotype QC or the imputation for any of the studies included in this meta-GWAS.

5.3.1 Samples

Across the ALSPAC, ELSA and UKHLS samples, inclusion of participants in the GWAS analyses was dependent on them having genotypic data, as well as self-reported sleep duration (phenotypic) and all covariate data. Within ALSPAC, only genotyped and phenotyped *mothers* were included. See Table 5.1 in the Results section for sample characteristics.

5.3.2 Phenotype

For detailed descriptions of the sleep duration phenotype QC and preparation, see Chapter 2. Data from ALSPAC mothers were analysed for this GWAS, with an analytical sample of n=4,914; an analytical sample of 6,028 was used in the ELSA and data from n=8,608 individuals from UKHLS were analysed in the GWAS.

5.3.3 Covariates

Association tests and genome-wide complex trait analyses (GCTA) were adjusted for age, sex and the first 10 principal components (PCs) in ELSA and UKHLS.

Age and sex were included as covariates to ensure that associations between SNPs and sleep duration would not be due to variance explained by age and sex. These covariates were included because, although genotypes are unconfounded by factors such as age and sex, they were included in the genetic association analyses to account for any additional variance, which will in turn, ensure that the GWAS signals are less confounded.

The first 10 PCs were used as covariates to adjust for any residual population structure during the association tests between SNPs and sleep duration, by minimising the chances of detecting spurious associations that might be due to genetic ancestry[268]. Principal component analysis (PCA) is the most commonly implemented approach for identifying related individuals in genetic association studies[269]. A multivariate statistical approach, PCA produces uncorrelated variables (principal components) from a data matrix that contains observations from several supposedly correlated variables. PCs are calculated so that PC1 explains as much as possible variance in the data in a single component, which is followed by PC2, etc. In its application to the detection of genetic ancestry, observations are individuals and genetic markers are the potentially correlated variables[269]. These PCs are usually created for each sample, using genome-wide genetic data from common reference panels, such as the HapMap or 1000 Genomes genotype data, for which ancestry is already known. These common panels enable the detection of continental-level ancestry, as it contains genotype data from Europe, Asia and Africa. Due to the fact that these ancestral groups are very divergent, the first two PCs will sufficiently cluster individuals in these populations. This model can also be applied to individuals in GWAS to predict their PC scores and therefore, enable them to be clustered ancestrally, alongside samples from the reference panel, such as HapMap or 1000 Genomes.

5.3.4 Statistical Analysis

Across all three studies the minor allele frequency (MAF) filter was set at 1% and a threshold of $P < 5x10^{-8}$ was used to identify SNPs reaching genome-wide significance. This MAF threshold is used in GWAS to identify common genetic variants associated with a trait of interest and >1% is recommended, as GWAS has

low power to detect rare variants[270]. The significance threshold of $P<5x10^{-8}$ has become the most widely used in GWAS and applies a Bonferroni correction that accounts for multiple testing in GWAS, under the assumption that there are a million independent SNPs in the human genome[268]. However, SNPs that were associated with sleep duration at $P<5x10^{-6}$ as 'suggestive' of significance in this study.

Genomic inflation factor (Lambda genomic control= λ_{GC}) and quantile-quantile (Q-Q) plots were used to examine whether there is inflation due to population stratification.

The Q-Q plot ranks p-values for each genetic variant from smallest to largest and plots these against expected values from a chi-squared distribution. λ_{GC} was obtained for each study separately (see Appendix 8.1 for individual study Q-Q plots) and for the meta-analysis, using LD score regression (described in more detail below in section 5.3.4.4), implemented in LD Hub. Also, within each study, phenotypic variance (in sleep duration) explained by single top SNPs was calculated using the following formula, as previously reported by[271]:

$$R^2 \cong \frac{2\hat{\beta}^2 MAF(1-MAF)}{2\hat{\beta}^2 MAF(1-MAF) + (se(\hat{\beta}))^2 2NMAF(1-MAF)}$$

Whereby, R^2 for a given SNP based on the effect estimate for its association with self-reported sleep duration (beta or $\hat{\beta}$), respective standard error ($se(\hat{\beta})$), minor allele frequency (MAF), and sample size (N).

5.3.4.1 ALSPAC association analysis

The GWAS analysis in ALSPAC was performed by a collaborator, Dr Gibran Hemani (University of Bristol). Linear association tests were performed using the frequentist method in SNPTEST between genotyped and imputed SNPs, and untransformed sleep duration. Dr Hemani then sent the ALSPAC summary estimates. A Manhattan plot of ALSPAC results is presented in Appendix 8.1.

5.3.4.2 ELSA and UKHLS association analyses

The analytical approach adopted in ELSA and UKHLS was identical: linear regressions

(as sleep duration was measured in hours, as a continuous trait), which assumed additive effects between (genotyped and imputed) SNPs and untransformed sleep duration were implemented in the snpStats R package[272]. An additive model was chose for this GWAS study because it is powered to detect additive and dominant effects, but might be underpowered for the detection of recessive effects[21]. Individual Manhattan plots from ELSA and UKHLS GWASs are presented in Appendix 8.1.

5.3.4.3 Meta-analysis of ALSPAC, ELSA and UKHLS

An inverse-variance fixed effects meta-analytical approach[273] was adopted using METAL[274], as the phenotype was very similar across the three studies (N=19,550) and individuals were all of European ancestry. Underlying this model is the assumption that studies included in the meta-analysis have a common genetic effect, thus any differences in study findings are due to sampling variation. When performing the analysis, this approach weights the effect sizes according to the inverse of their standard errors. In total, 9,498,728 SNPs were included in the meta-analysis.

5.3.4.4 Genome-wide Complex Trait Analysis (GCTA) and LD Score (LDSC) regression

The Genome-wide Complex Trait Analysis (GCTA) package[19] was used to perform restricted maximum likelihood (REML) analysis to estimate the amount of phenotypic variance (SNP heritability) in sleep duration explained by all the genotyped SNPs in each study separately. Prior to running the REML analyses it was necessary to estimate the genetic relationship matrix (GRM) between pairs of individuals. Power was calculated using the online GCTA GREML Power calculator[275] for different heritability estimates (Table 5.1).

LDSC regression is an alternative method, which uses GWAS summary statistics to estimate the SNP heritability of complex traits[28]. This method involves regressing summary statistics from GWAS (from millions of SNPs) and measures to what extent each SNP is able to tag other variants locally (or, its 'LD score'). The slope of the LDSC regression model can then be rescaled to provide a heritability estimate of a trait, accounted for by all SNPs used in the estimation of the LD scores [28]. LD Hub is a web interface, which allows the uploading of summary statistics from association analyses

(from each study separately, as well as results from the meta-analysis) in order to automate the LD score regression analytical process[20]. SNP heritability of sleep duration was estimated using this method in addition to GCTA for two reasons: 1) to compare the results and 2) to calculate the heritability of sleep duration across all three studies using the meta-analysis results.

Table 5.1 Power calculations for different heritability estimates of self-reported sleep duration using GCTA GREML

Study	Sample size	Heritability estimate (h^2)	Power
ALSPAC	4,914	20%	87%
		15%	64%
		10%	34%
		7%*	19%
ELSA	6,028	20%	97%
		15%	82%
		10%*	48%
		7%	27%
UKHLS	8,608	20%	99%
		15%	98%
		10%	78%
		7%*	48%

Note. *indicates actual h2 obtained from GCTA GREML analyses.

5.4 RESULTS

5.4.1 Main results

5.4.1.1 Sample characteristics

As per Table 5.2 below, the mean sleep duration across the samples included in the current meta-GWAS (ALSPAC, ELSA and UKHLS) were largely comparable to those included in the earlier CHARGE meta-GWAS.

Table 5.2 Characteristics of current GWAS samples vs. samples included in the CHARGE sleep duration GWAS

Study	N	Mean age (SD)	Sex, %Female	Mean sleep duration, hours (SD)
ALSPAC	4,914	39.4	100%	7.61 (0.95)
ELSA	6,028	67.09 (9.90)	54.66%	6.61 (2.41)
UKHLS	8,608	53.67 (16.15)	56.42%	6.61 (1.30)
		Samples in the CHARGE GWAS		
ARIC	3578	62.6 (5.6)	53.20%	7.4 (1.1)
CHS	1515	77.9 (4.6)	62.10%	7.3 (1.3)
FHS	7531	51.3 (13.2)	54.10%	7.9 (1.3)
HABC	1661	73.8 (2.8)	47.00%	7.0 (1.2)
HBCS	1175	69.0 (2.7)	60.70%	8.2 (1.1)
HPFS	3542	56.0 (8.7)	0.00%	7.2 (0.9)
InCHIANTI	1205	68.3 (15.5)	55.40%	6.8 (1.5)
MrOS	2354	76.7 (5.7)	0.00%	7.0 (1.2)
NHS	6638	54.4 (6.7)	100.00%	7.0 (0.9)
QFS	865	41.1 (15.4)	56.30%	7.7 (1.1)
QIMR	2286	34.5 (14.3)	74.20%	7.7 (1.0)
RS I	2834	76.1 (6.3)	59.50%	6.8 (1.3)
RS II	1425	68.9 (7.6)	57.60%	6.9 (1.3)
SHIP	2859	49.4 (16.5)	57.90%	7.5 (1.3)
SOF	3303	77.0 (5.1)	100.00%	7.0 (1.2)
TwinsUK	1531	53.1 (12.6)	86.10%	6.8 (0.8)
WiSC	850	55.7 (7.5)	45.60%	7.1 (0.9)
YFS	2028	37.7 (5.0)	54.90%	7.4 (0.8)

Note. Sample characteristics were not available in the UKB GWAS paper[78] and are therefore not presented.

5.4.1.2 Main results

In the analyses, there appeared to be little evidence of population stratification, as shown in the Q-Q plot in Figure 5.1 and the calculated Lambdas: λ_{GC} ALSPAC= 1.00, λ_{GC} ELSA= 1.00, λ_{GC}UKHLS= 1.00, λ_{GC} Meta-analysis= 1.02. In the case of these studies, the Q-Q plot showed that the majority of the variants are distributed along the null line, with 15 SNPs towards the top end at $p<10^{-7}$.

No novel or previously reported genome-wide significant SNPs associated with self-reported sleep duration emerged in the meta-analysis, thus associations are presented where $p<5*10^{-6}$, resulting in 34 'suggestive' SNPs in Table 5.3. Of these loci presented in Table 5.3, 25 are located on chromosome 19, 4 on chromosome 14, 2 on chromosome 12,

and one each on chromosomes 3 and 5, respectively, which can also be seen in the Manhattan plot in Figure 5.2. Taken together, these variants explained a modest proportion of the phenotypic variance in self-reported sleep duration: ALSPAC= 3%, ELSA = 3%, UKHLS= 4%. Therefore, in ALSPAC and ELSA the percentage of variance explained by these SNPs was less than half the size of the SNP h^2 estimate of 7%, whilst in UKHLS the variance explained by these 34 SNPs was slightly larger (4%), which was expected due to the larger sample size.

The top SNP as presented in Table 5.3 was rs72781084 on chromosome 5 ($p=8.49*10^{-7}$), an intron within the Polymerase (RNA) III (DNA Directed) Polypeptide G (*POLR3G*) gene, which was associated with a 4-minute decrease in sleep duration. In these data, this variant had minor allele frequencies of between 0.23 in ALSPAC and ELSA, and 0.24 in UKHLS, and explained 0.12%, 0.19% and 0.09% of the phenotypic variation in sleep duration in each study, respectively. No variants within this gene have previously been associated with any sleep phenotypes in a GWAS.

Twenty-four of the SNPs on chromosome 19 are intronic variants whilst rs855632 is an exonic synonymous variant, within the Signal-induced proliferation-associated 1 like 3 (*SIPA1L3*) gene. All of these SNPs have MAFs of between 0.42 and 0.45 across the three studies. Using the SNP Annotation and Proxy Search tool (SNAP: http://www.broadinstitute.org/mpg/snap/ldsearchpw.php) and applying a threshold of $R^2=0.8$, pairwise LD was obtained for these SNPs, the results of which are in Appendix 8.1 and show that 19 of these variants are in high LD ($R^2= 0.93 - 1.00$). The effect allele for each of the 25 SNPs on chromosome 19 was associated with a 3-minute increase (17 SNPs) or decrease (8 SNPs) in sleep duration, effect sizes which are comparable to SNPs significantly associated with this phenotype in recently published in large-scale studies[74,78].

rs118167883 and rs117831282 on chromosome 12 are both intergenic variants, which are not in LD. The former is located between the PDZ Domain Containing Ring Finger 4 (*PDZRN4*) and the Glucoside Xylosyltransferase 1 (*GXYLT1*) genes, whilst the latter is between the Ethanolamine Kinase 1 (*ETNK1*) and Sex Determining Region Y – Box 5 (*SOX5*) genes. In these data, rs118167883 was associated with a 20-minute increase in

sleep duration, whilst rs117831282 was associated with a 14-minute decrease in sleep duration; however, the MAFs of these variants are low at 0.01 and 0.02, respectively. These small MAFs indicate that, whilst such variants are considered to be 'common' and appropriate for inclusion in genome-wide analyses, rs118167883 was only present in 195 individuals and rs117831282 in 391 individuals in this study and thus, their effect sizes should be interpreted with caution. The potential implication of this is that these particular analyses may not have been the most suitable for SNPs with low MAFs and perhaps a more appropriate test would be one for analysis of rare variants. These SNPs explained 0.15% and 0.17% of the phenotypic variance in sleep duration within ALSPAC, 0.12% and 0.11% in ELSA, and both explained only 0.08% in UKHLS.

On chromosome 3, it appeared that rs116728846, an intergenic variant located between the Vent Homeobox Pseudogene 7 (*VENTXP7*) and the SGOL1 Antisense RNA 1 (*SGOL1-AS1*) was associated with a 15-minute increase in sleep duration. Similarly to the variants on chromosome 12 the minor allele frequencies for this SNP were low: 0.01 in ALSPAC and UKHLS, and 0.02 in ELSA, and it only explained between 0.01% and 0.12% of the phenotypic variance in sleep duration in these samples. Thus, this effect size of 15 minutes should be interpreted cautiously, as this SNP was only present in ~281 individuals out of the 19,500 in the meta-GWAS (49 individuals in ALSPAC, 60 individuals in ELSA and 172 individuals in UKHLS).

Of the four SNPs on chromosome 14, rs138098759, rs2749493 and rs1958962 were associated with a 6-minute decrease in sleep duration per allele, whilst rs1953188 was associated with a 6-minute increase in sleep duration. Only rs1958962 and rs1953188 are in high LD, R^2=1.00. These SNPs all have MAFs of 0.08 in these samples, indicating that they are present in 1,564 individuals from the overall meta-analytic sample, and explained between 0.08% and 0.16% of the variation in sleep duration. All of these SNPs are intergenic variants located between the F-Box Protein 33 (*FBXO33*) and the (uncharacterised) *LOC644919* RNA gene.

The meta-analysis also found an intergenic variant on chromosome 16, rs55950229 to be associated with a 4-minute decrease in sleep duration. This SNP's minor allele frequency is 0.20 in ALSPAC and ELSA, and 0.21 in UKHLS;

and it explained between 0.05% and 0.23% of the phenotypic variance in sleep duration. rs55950229 lies between the MicroRNA 4719 (*MIR4719*) and MON1 Secretory Trafficking Family Member B (*MON1B*) genes.

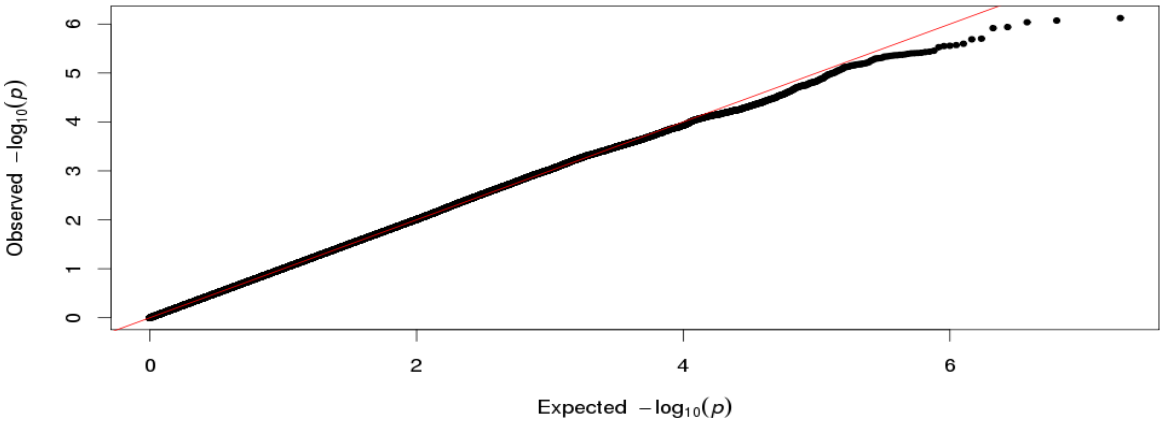
The Co-expression database (COXPRESdb)[276] was used to examine which genes are co-expressed with the *SIPA1L3* and *POLR3G* genes, which are presented in two network diagrams in Appendix 8.1. There is an enrichment of genes in adhesion processes and RNA modification.

Table 5.3 34 'top' SNPs associated with sleep duration in meta-analysis of ALSPAC, ELSA and UKHLS (N=19,550) at suggestive p-value threshold of <5x10^{-6}

							Meta-analysis		
SNP	Functional class	Gene/locus	Chr	Position	A1*	A2	Effect	SE	P-value
rs118167883	intergenic	*PDZRN4,GXYLT1*	12	42149726	T	C	0.329	0.070	2.78E-06
rs117831282	intergenic	*ETNK1,SOX5*	12	23497814	T	C	-0.233	0.051	4.47E-06
rs116728846	intergenic	*SGOL1-AS1,VENTXP7*	3	21051974	C	G	0.249	0.055	6.41E-06
rs138098759	intergenic	*FBXO33,LOC644919*	14	40171948	A	G	-0.104	0.023	4.87E-06
rs2749493	intergenic	*FBXO33,LOC644919*	14	40176497	A	G	-0.103	0.023	4.23E-06
rs1953188	intergenic	*FBXO33,LOC644919*	14	40187953	A	G	0.104	0.023	6.60E-06
rs1958962	intergenic	*FBXO33,LOC644919*	14	40179804	T	C	-0.104	0.023	3.94E-06
rs55950229	intergenic	*MIR4719,MON1B*	16	76930106	T	C	-0.072	0.015	3.51E-06
rs72781084	Intronic	*POLR3G*	5	89789454	A	G	-0.072	0.015	8.49E-07
rs8112798	Intronic	*SIPA1L3*	19	38661228	A	G	-0.055	0.012	4.95E-06
rs8109799	Intronic	*SIPA1L3*	19	38661480	A	G	-0.054	0.012	5.09E-06
rs9941474	Intronic	*SIPA1L3*	19	38658607	A	G	-0.054	0.012	3.73E-06
rs332849	Intronic	*SIPA1L3*	19	38629126	T	C	-0.054	0.012	9.57E-06
rs6508765	Intronic	*SIPA1L3*	19	38644528	T	C	-0.054	0.012	6.05E-06
rs855632	exonic (synonymous)	*SIPA1L3*	19	38652993	T	C	-0.054	0.012	3.97E-06
rs332850	Intronic	*SIPA1L3*	19	38629630	A	T	-0.053	0.012	8.63E-06
rs332848	Intronic	*SIPA1L3*	19	38630160	A	G	-0.053	0.012	8.46E-06
rs8101826	intronic	*SIPA1L3*	19	38631193	T	C	0.053	0.012	4.56E-06
rs332851	intronic	*SIPA1L3*	19	38634474	T	C	0.053	0.012	7.48E-06
rs8100144	intronic	*SIPA1L3*	19	38645276	T	C	0.054	0.012	6.87E-06
rs2099340	intronic	*SIPA1L3*	19	38647545	A	G	0.054	0.012	6.69E-06
rs2384778	intronic	*SIPA1L3*	19	38637945	T	C	0.054	0.012	6.93E-06
rs8109695	intronic	*SIPA1L3*	19	38637746	A	G	0.054	0.012	4.05E-06
rs2569412	intronic	*SIPA1L3*	19	38639903	T	C	0.054	0.012	7.66E-06
rs332864	intronic	*SIPA1L3*	19	38642874	T	C	0.054	0.012	6.39E-06
rs332855	intronic	*SIPA1L3*	19	38638176	A	T	0.054	0.012	7.62E-06
rs4802251	intronic	*SIPA1L3*	19	38643951	A	G	0.055	0.012	6.27E-06
rs332856	intronic	*SIPA1L3*	19	38639940	A	C	0.055	0.012	6.69E-06
rs332858	intronic	*SIPA1L3*	19	38641264	T	C	0.055	0.012	6.61E-06
rs2005055	intronic	*SIPA1L3*	19	38642044	T	C	0.055	0.012	6.58E-06
rs10404957	intronic	*SIPA1L3*	19	38634020	T	C	0.055	0.012	3.87E-06
rs8111180	intronic	*SIPA1L3*	19	38627138	A	G	0.055	0.012	4.62E-06
rs332843	intronic	*SIPA1L3*	19	38603259	C	G	0.056	0.012	8.26E-06
rs332844	intronic	*SIPA1L3*	19	38601728	A	G	0.056	0.012	9.41E-06

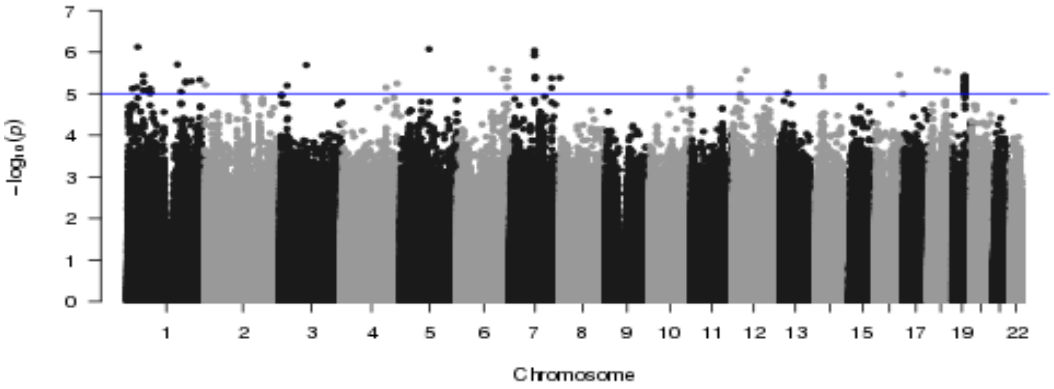
Note. *A1= effect allele, A2= alternative allele, SE= standard error, p-value= association p-value.

Figure 5.1 Q-Q plot of p-values for meta-analysis of ALSPAC, ELSA and UKHLS



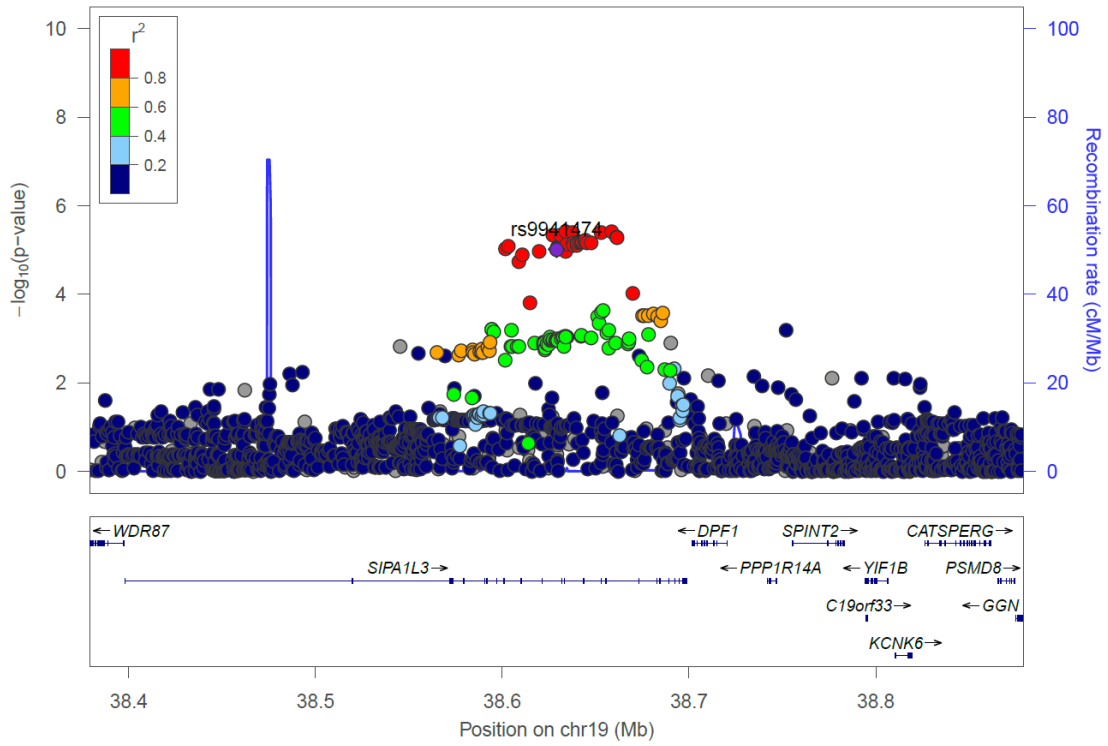
Note. Q-Q plot depicts the observed $-\log_{10}$ p-values on the Y-axis and the expected $-\log_{10}$ p-values on the X-axis.

Figure 5.2 Manhattan plot for meta-analysis of sleep duration in ALSPAC, ELSA and UKHLS



Note. Manhattan plot depicts $-\log_{10}$ p-values on the Y-axis, with the blue line set at $p<5x10^{-5}$ and along the X-axis are chromosomes (1-22).

Figure 5.3 Locus zoom plot of GWAS results



5.4.2 Previously associated loci in CHARGE and UK Biobank: how are they associated with sleep duration in the present GWAS?

As mentioned in the *Introduction* of this chapter, the most recently published GWAS in 127,573 UK Biobank participants[78] was the first to replicate a signal (rs62158211) for self-reported sleep duration. rs62158211 is in high LD with rs1823125 (R^2=0.95) and rs1807282 (R^2=1.00), which were previously reported in the CHARGE consortium GWAS[74]. An intron within the *PAX8* gene, rs62158211 was present in the ELSA and UKHLS samples and in the present meta-analysis a similar effect size was observed, albeit with a very large standard error (UKBiobank effect =-2.34 minutes, SE=0.30 minutes vs. present meta-analysis effect = -1.80 minutes, SE=1.14). Proxies for rs62158211 (rs1823125 and rs1807282) were identified but were not present in the ALSPAC dataset.

Further, the two novel loci reported by Jones and colleagues[78], rs17190618 and rs1380703 are both introns in the Vaccinia Related Kinase 2 (*VRK2*) gene and were associated with a 2-minute decrease and a 1.5-minute increase in self-reported sleep duration, respectively. In the current meta-analysis these SNPs were associated with a 2-minute

decrease and a 1.4-minute increase in sleep duration, but not significantly (p=0.061 and p=0.087, respectively).

Previously, Gottlieb et al., 2014[74] performed a genome-wide association analyses in ~47,000 individuals in the CHARGE consortium and found seven novel loci associated with self-reported sleep duration, four of which are on chromosome 2 and 3 on chromosome 6. In CHARGE, the four SNPs on chromosome 2, rs1191685, rs1823125, rs1807282 and rs1964463 were all associated with a 3-minute increase in self-reported sleep duration. The present meta-analysis found that rs1191685 was associated with a 2-minute increase in sleep duration, whilst rs1823125, rs1807282 and rs1964463 were associated with increases in sleep duration of 1.44, 2 and 1 minutes, respectively. However, these combined effects were only for ELSA and UKHLS, as none of these SNPs or any proxies in LD of R^2>0.80 were present in ALSPAC, with the exception of rs1964463, for which no proxies were available. Minor allele frequencies (MAFs) for these variants were comparable between CHARGE and the present samples. As the effect alleles for these three variants in CHARGE were the opposite allele to those in these data the signs of the coefficients were changed here for the purposes of reporting and comparing the results.

In addition, the three loci of interest on chromosome 6 (rs4587207, rs4248149, rs2394403) were looked up in this study and they were not present. The Single Nucleotide Polymorphisms Annotator (SNiPA: http://snipa.helmholtz-muenchen.de/snipa/index.php) was used to search for proxies for these variants. The best proxy (with the highest r^2= 1), which was in high LD with all three of these SNPs was rs147772769 (Appendix 8.1). This variant was present in ALSPAC and ELSA, but not in UKHLS. However, the effect of rs147772769 in these two studies was in the same direction as in Gottlieb's[74] study: in ALSPAC this effect was -0.02, in ELSA it was -0.001 and in the previous GWAS it was -0.05.

5.4.3 Do the 'top' (suggestive) 34 SNPs from the present meta-analysis replicate in the latest UKB GWAS?

The summary statistics from the latest UKB GWAS by Jones and colleagues[78] were freely available alongside the publication and were therefore downloaded to

look up the suggestive loci from the current meta-GWAS. However, none of these SNPs were significantly associated with self-reported sleep duration in UKB at either genome-wide or suggestive significance. The lowest p-value was 0.042 for rs7278108 4, which had a directionally consistent effect in UKB in comparison to the present study, -0.004 vs. -0.072, respectively.

5.4.4 Expression quantitative trait loci (eQTLs) associated with 'top' SNPs

The Genotype-Tissue Expression portal (http://www.GTEXportal.org) was used to search for significant expression quantitative trait loci (eQTLs) in tissue for each of the top SNPs. rs7278108 4, on chromosome 5, which was the most strongly associated SNP with sleep duration, was significantly associated with expression levels in the following tissues: colon, thyroid and adrenal gland.

Fourteen of the SNPs on chromosome 19 were significantly associated with expression levels in the pancreas, whilst 2 variants were associated with expression levels in the tibia nerve. No significant eQTLs for any of the other top SNPs were found.

5.4.5 SNP heritability in ALSPAC, ELSA and UKHLS

Restricted maximum likelihood (REML) analyses in GCTA showed that the SNP heritability of self-reported sleep duration in these samples was low and not statistically significant. This was further confirmed by estimating SNP heritability using LD score regression (see Table 5.4) in each sample. However, the heritability estimate from LD score across all three samples albeit low at 7%, was significant. The recent UK Biobank GWAS found the SNP heritability of sleep duration to be 7% in a sample that was approximately 10 times greater than the GWAS reported here. This is therefore, consistent with what was found in the present study.

Table 5.4 Heritability of self-reported sleep duration in ALSPAC, ELSA and UKHLS samples

Method	ALSPAC			ELSA			UKHLS			All 3 studies		
	h^2	SE	P-value	h^2	SE	P-value	h^2	SE	P-value	h^2	SE	P-value
GCTA*	0.07	0.0564	0.17	0.103	0.067	0.058	0.075	0.108	0.238	N/A	N/A	N/A
LD score	0.188	0.107	0.57	0.055	0.076	0.080	0.041	0.055	0.064	0.074	0.021	0.0005

Note. There are no GCTA estimates for h^2 in all 3 studies combined, as it requires IPD.

5.5 DISCUSSION

5.5.1 Summary of findings

In a genome-wide meta-analysis of 19,550 individuals of European ancestry, no novel genetic variants associated with self-reported sleep duration were found, nor were any SNPs published in recent large-scale GWA studies of sleep duration[74] replicated at genome-wide level. These analyses were relevant at the time they were performed, as the UKB sleep duration GWAS[78] had not been published (date of publication: August, 2016) and one of the main aims of this project was to carry out bidirectional Mendelian randomisation analyses in Chapter 6.

An intron (rs72781084) in the *POLR3G* gene was the most strongly associated SNP with a 4-minute decrease in sleep duration ($p=8.49x10^{-7}$). This variant was also found to be significantly associated with expression quantitative trait loci (eQTLs) in the colon, the thyroid and the adrenal gland. A further 33 SNPs were suggestively ($p<5x10^{-6}$) associated with sleep duration, albeit not at the genome-wide significance level ($p<5x10^{-8}$). Of these variants, twenty-five are introns in the *SIPA1L3* gene on chromosome 19; four are on chromosome 14, two on chromosome 12, and one each on chromsomes 3, 5 and 16. Aside from the introns in the *SIPA1L3* and *POLR3G* genes, the other SNPs found are all intergenic. Significant associations with expression quantitative trait loci (eQTLs) in the pancreas were found for twenty-two of the introns in the *SIPA1L3* gene. Most of the variants on chromosome 19 are in high linkage disequilibrium and the lead SNP was identified as rs332858. Of the additional one-hundred and

twenty genetic variants that were suggestively associated with sleep duration ($P<5x10^{-6}$), there were another four intronic SNPs in the *SIPA1L3* gene.

As mentioned above, rs727810 84 is an intronic variant which was suggestively associated with a decrease in sleep duration in the present GWAS, yet it has not previously been associated with sleep duration. The *POLR3G* gene has not previously been linked to any sleep phenotypes. The *SIPA1L3* gene has not been related to any aspects of sleep in the GWAS literature. Located on chromosome 12, rs118167883 is an intergenic variant between the *PDZRN4* and *GXYLT1* genes, whilst rs117831282 is found between the *ETNK1* and *SOX5* genes, yet neither of these SNPs has been associated with any sleep phenotypes. rs55950229 lies between the *MIR4719* (a microRNA gene) and *MON1B* (protein coding) genes on chromosome 16. To date, this variant has not been associated with any sleep phenotypes in GWAS.

It is important to note that it can be problematic to interpret GWAS results that are below the significance threshold of $p<5x10^{-8}$. This may mean that SNPs detected to be associated with sleep duration at this less stringent threshold are more likely to be spurious signals and these usually require further validation. In the present meta-GWAS this is a plausible explanation for the loci that were suggestively associated with sleep duration, as upon closer inspection of these signals, it emerged that none of them had been related to any sleep mechanisms or pathways in the literature.

5.5.2 Significant eQTLs in the SIPA1L3 and POLR3G genes

Several genetic variants in the *SIPA1L3* were significant eQTLs in the pancreas. Evidence from a recent review of over one-hundred thousand individuals, suggests that duration and quality of sleep are both significant predictors of type 2 diabetes[187], a condition in which individuals build up insulin resistance, causing the pancreas to work harder to produce higher than normal levels of insulin.

5.5.3 Heritability of self-reported sleep duration

Across the samples included in this study, self-reported sleep duration was only 7-10% heritable, but these were not significant when using GCTA, which is likely due to the limited power. Using the LD Score method heritability across all three studies was still low at 7%, but was significant. However, twin studies have previously reported that genetic factors explain between thirty to fifty per cent of the variance in sleep duration[69–71]. One reason for this could be the fact that the CTM assumes a lack of, or minimal gene*environment interactions and that therefore, all genetic risk is additive, which can in turn, produce inflated heritability estimates[277]. It is also possible that the CTM estimates include rare, as well as common variants.

In a sample of approximately 120,000 individuals from the UK Biobank[78] SNP heritability of self-reported sleep duration was 7%, which accords with what was found in the present study. However, this estimate reached statistical significance in the UK Biobank, which is not surprising as their study had a much larger sample size and thus, more power.

There are some key points to be noted in relation to the heritability of self-reported sleep duration. Firstly, the present estimates accord with the very limited number of genome-wide significant loci that have been found to date in GWAS. If genetic factors only explain a very small proportion of the variation in a phenotype then it is plausible that we fail to detect a large number of common variants associated with this phenotype. Secondly, it is likely that the genetic component of self-reported sleep duration is highly complex with potentially many variants of very small effect sizes (polygenic trait) and that they may still not have been discovered using GWAS. Thirdly, it is also possible that rare genetic variants account for a proportion of the heritability of self-reported sleep duration and/or that there may be epigenetic factors that play a role in sleep duration, but they have not yet been described. Therefore, even though the present GWAS did not have substantial power to detect SNP heritability using GCTA, the results of GCTA REML analyses appear to suggest that the genetic component of this phenotype might not be

accounted for by common variants of small effect size. Fourthly, finding that self-reported sleep duration is not highly heritable does not rule out the fact that objectively measured sleep duration, using methods such as actigraphy or polysomnography, might yield higher estimates of SNP heritability, in comparison to self-reported sleep duration. To date, research into the molecular genetics of objective sleep measures remains limited. However, a candidate polymorphism study found a significant association between a specific polymorphism (rs324981) and objective sleep duration, as measured by actigraphy, in a sample of four-hundred and thirty-six individuals over the age of sixty[278]. In the present meta-analysis this variant was associated with a -0.5-minute decrease in sleep duration, although not at the GWAS significance level. Fifthly, the study reported here, alongside other large-scale GWA studies of self-reported sleep duration to date, have focused on examining heritability in individuals of Northern European ancestry. Therefore, it cannot be assumed that in populations of different ancestry heritability estimates of sleep measures are equal. For example, Americans of African descent reportedly have shorter sleep durations compared to European Americans[279], and a recent review suggests that being of African American, or sub-Saharan African descent may predispose to shorter sleep duration[280].

5.5.4 Issues with self-reported sleep duration as a phenotype

Self-reported sleep measures are widely used in observational and genetic epidemiological research as they are inexpensive and easy to administer. Within the context of GWAS, obtaining both objective sleep duration as well as genotype data from large samples of participants remains on the whole, financially unfeasible, and only one, small-scale GWAS of objective sleep measures has been published, to date. Across the majority of studies, sleep duration is assessed by asking respondents how many hours and/or minutes they sleep on an average night, or they are asked to report the times that they go to bed and wake up, from which sleep duration is estimated. The only GWAS of actigraphic sleep duration to date found one novel SNP in the Zinc Finger MYM-Type Containing 4 (*ZMYM4*) gene[281]. However, this study's sample size was only 956 individuals,

aged between 40 to 79 years and thus, requires stringent replication in future studies.

5.5.5 Study strengths

This study possesses important strengths. The sleep duration phenotype definition was almost identical across the three samples included in this meta-GWAS, whilst for example, Gottlieb et al., 2014 [282] meta-analysed data from eighteen studies that did not ask about sleep duration in the same manner. The fact that it was possible to meta-analyse data from three studies is also a strength; each study on its own had extremely limited power, as single GWA studies only have the ability to detect large effects [283]. Also, genotypes were imputed to the same reference panel (1000 Genomes), and all of the same covariates were available across all three studies.

5.5.6 Study limitations

However, there are also methodological limitations concerning this GWAS. As discussed earlier in more depth, the phenotype was self-reported across ALSPAC, ELSA and UKHLS. Crucially, this study also had limited statistical power to both detect novel, and replicate existing loci associated with self-reported sleep duration, particularly as previous larger GWAS of 47,000[282] and 127,573 individuals[78] have only detected a total of nine independent variants for sleep duration. Thus, due to the lack of genome-wide significant hits further analyses were limited, such as pathway analyses, gene enrichment analyses, genetic correlations with other brain phenotypes of interest, amongst others. Furthermore, given the markedly low and non-significant SNP heritability, a polygenic risk score was not created, as this is commonly done when the variance can be at least partially explained by genetic factors. Also, the only study in which individuals were removed due to reporting the use of sleep medication was ALSPAC, but exclusions based on taking sleep medication were not made in ELSA or UKHLS. The ELSA participants also had a different age and sex distribution from the other two studies, which makes the findings somewhat less representative of the general population. Another limitation is the use of different cut-offs for sleep

duration ALSPAC, as compared to ELSA and UKHLS, which introduced noise to the phenotype.

5.5.7 Future directions

Future studies should aim to perform genome-wide analyses using objective sleep duration in large samples. Furthermore, it is important to perform larger genetic association studies to potentially identify common variants; novel and/or previously-identified, associated with other self-reported sleep parameters. For example, there have yet to emerge SNPs related to sleep quality, as measured by validated self-report scales. This is particularly significant, as evidence suggests that for example (and of interest to the wider research carried out in this thesis), it is sleep quality, rather than sleep duration, that is linked to higher BMI and increased risk of obesity[166,284].

Finally, a newer method for estimating SNP heritability has recently emerged[285]. This technique is able to describe the variation of heritability, dependent on genotype certainty, MAF and LD. The authors re-estimated the SNP heritability for 19 traits and observed that their method yielded estimates that were on average, 43% higher than GCTA. The application of this novel approach may be important to confirm heritability estimates of self-reported sleep duration, as the present study, as well as other published studies have shown that SNP heritability is currently estimated to be approximately 7%.

5.6 CHAPTER SUMMARY

- A meta-GWAS of self-reported sleep duration was performed across three population-based studies.
- No novel genome-wide significant loci associated with sleep duration emerged.
- This GWAS did not replicate any previously reported sleep duration loci.
- SNP-based heritability of self-reported sleep duration was low in these samples.

- Due to all of the above, the decision was made that it would not be feasible to perform a Mendelian randomization study of sleep duration on BMI, as one of the core assumptions was not met (lack of robustly replicated SNPs associated with the exposure of interest, in this case sleep).

6 IS THE ASSOCIATION BETWEEN BMI AND SELF-REPORTED SLEEP DURATION CAUSAL? A MENDELIAN RANDOMISATION STUDY

Results from analyses performed in this chapter contributed to the following publication: **Garfield, V.,** Fatemifar, G., Dale, C., Smart, M., Bao, Y., Llewellyn, C., Steptoe, A., Zabaneh, D. & Kumari, M. Assessing Potential Shared Genetic Aetiology between Body Mass Index (BMI) and Sleep Duration in 142,209 individuals. *Resubmitted to Genetic Epidemiology*.

6.1 BRIEF INTRODUCTION AND OVERVIEW OF CHAPTER CONTENTS

In Chapter 3, observational regression analyses were performed to investigate the nature of the direction of the relationship between BMI and self-reported sleep duration in a community sample of older English adults. These findings suggested that the direction was from BMI and WC to very small decreases in sleep duration over a 4-year follow-up period: BMI [B = -0.42 minutes, (95% CI = -0.013; -0.002), p = 0.013] and WC [B = -0.18 minutes, (95% CI = -0.005; -0.000), p = 0.016], independently of several demographic, health behaviour and problematic health covariates. Though, one crucial limitation of these analyses, even when they are performed using prospective data, is their limited ability to allow the inference of causation, due to confounding and possible reverse causation (discussed in more detail in Chapter 1 and below). Also, despite the fact that the effects observed between adiposity and changes in sleep duration in ELSA (Chapter 3) were small, these analyses were performed only in an ageing sample and thus, it is important to investigate causality using data from a sample of a wider age range.

Mendelian Randomisation has been proposed as a method to address causality using genetic markers as instruments. When these analyses were planned, there had been no previous attempt to investigate the causal relationship between BMI

and sleep duration. Then, the first MR of BMI and self-reported sleep duration was published[78], but the analyses presented in this chapter differ somewhat from the previous study.

Briefly, in this chapter the causal relationship between BMI and self-reported sleep duration was investigated, using genetic data. This chapter begins by asking why we would want to use genetics to investigate causation, followed by an overview of Mendelian randomisation and polygenic risk scoring (PRS) and the aims of this chapter. Then, there is a detailed account of the methodologies and statistical analyses used, followed by results, which are divided into *observational* and *genetic*. Subsequently the main findings are discussed, in light of previous evidence and cover relevant strengths and limitations. The chapter ends with a summary of what has been presented.

6.2 MENDELIAN RANDOMISATION: A GENETIC TOOL FOR ASSESSING CAUSALITY

Mendelian randomisation (MR) was proposed to investigate causation between two traits, which have been consistently associated in the observational epidemiological literature[286]. This method has been increasingly used in the last decade with 1,060 publications indexed under 'Mendelian randomization' and 151 under 'Mendelian randomisation' in PubMed Central (as of October 2017). MR has been called 'nature's randomised trial'[287], as it provides an alternative to the RCT and enables researchers to exploit large-scale studies in which participants have undergone detailed genotyping.

6.2.1 A brief overview of Mendelian randomisation (MR)

MR – the random assortment of genes, passed on from parents to offspring during conception and gamete formation[286] – exploits the unique properties of genetic variants. What makes genetic variants unique is that they are unlikely to be associated with common confounders and we have them from birth throughout the life course[286]. Due to these properties, MR uses common genetic variants (SNPs) from published GWAS as proxies for an exposure, to investigate cause and effect between said exposure and an outcome of interest[288]. Through

Mendel's second law – the law of independent assortment – genotypes transferred from parent to child are independent of one another, making MR analogous to the randomized controlled trial (RCT), as one allele (out of a possible two) is randomly allocated during meiosis and passed on during gamete formation[288]. This has been called 'nature's randomised trial'[287], as genotypes are unlikely to be confounded in the same way that phenotypes are. For example, BMI and sleep duration may be associated because they are both associated with physical activity (PA). Therefore, it is possible to use genetic variants associated with BMI, rather than BMI itself, to examine its association with sleep duration, whilst removing PA (the confounder) from the equation. With the aid of MR in the last decade, genetic epidemiology has contributed to furthering our understanding of modifiable risk factors of disease, as evidenced by >1000 published MR studies, and can be seen in the directed acyclic graph (DAG) in Figure 6.1.

Figure 6.1 Directed acyclic graph (DAG) of MR with BMI and sleep duration, showing how all parts of the model are interlinked

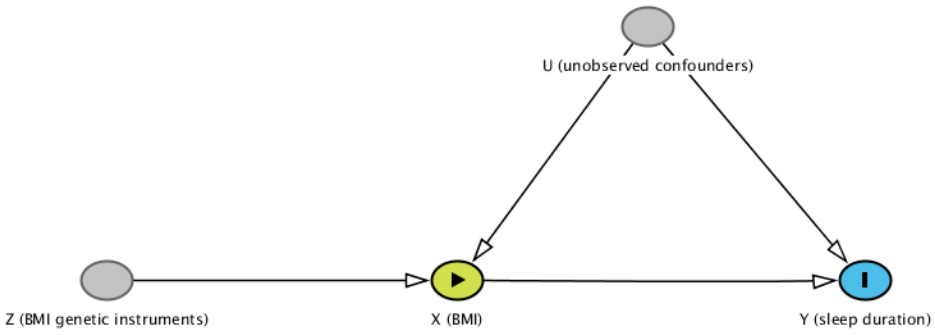


Figure 6.1 illustrates how Mendelian randomisation enables analyses to investigate causal association between BMI and sleep. In both cases, the genetic instruments – SNPs – (Z) are specific to the exposure (X) and not associated with confounders (U). This implies that any association observed between the genetic score (Z) and the outcome (Y) is due to the exposure (X).

6.2.1.1 Principles of MR

Two core underlying principles are fundamental to the MR technique:

I. *Eliminating reverse causation* and II. *randomisation*[286,289]. These two principles enable genetic epidemiology to go a step further than traditional observational epidemiology.

I. Eliminating reverse causation

Longitudinal studies provide only a partial solution to the problem of reverse causation – which implies that observational studies cannot confirm that the outcome in fact precedes the exposure, rather than the other way around and thus, the direction of association is unclear[290,291]. However Mendelian randomisation is able to overcome this hurdle by using genes as instrumental variables. The observed association between a genetic variant and a phenotype is a result of the specific effect of the said genetic variant. In line with the 'Central Dogma' of molecular biology[292] – genomic DNA is transcribed to messenger ribonucleic acid (mRNA) and then translated to a protein – the reverse association (phenotype causes the genotype) is not possible.

II. Randomisation

A confounder is a third factor that is associated with both the exposure and outcome of interest. Therefore the confounder (for example, depression may confound the BMI-sleep duration relationship) exerts the actual effect on the outcome, rather than the measured exposure[291]. Through Mendel's second law (see above) it is possible to exploit the random assignment of genes in a bid to reduce and potentially eliminate confounding. In this context, randomisation serves to overcome the problem of confounding, in a similar way to the randomised control trial (RCT) design, as genotypes are randomly distributed in the population and theoretically, should only be associated with a specific trait (although, in practice, the majority of complex traits in fact, follow a model of polygenic inheritance). In practice, when examining genetic markers, association analyses are performed to confirm that they are not associated with confounders.

6.2.1.2 Assumptions of MR

Three core assumptions underlie the Mendelian randomisation approach to ensure a reliable causal association. First, there should be a sufficiently robust

association between the specific genetic variant and the exposure of interest. To investigate causality between BMI and sleep duration there should be robustly-associated SNPs with BMI, which come from the most recent meta-GWAS of BMI[293]. Second, the chosen genetic variant should be unrelated to typical confounding factors. Thus, in the case of BMI and sleep duration, this assumption implies that the BMI SNPs are not associated with common confounders of this association, such as PA. Third, there must be independence between the genetic variant and the outcome, i.e. there must be no horizontal pleiotropy[288]. This means that the BMI genetic variants should not be directly associated with sleep duration, but only indirectly (through the exposure: BMI).

Assumption I implies that the relationship between the genetic variant and the exposure is reliable and can be quantified, but SNPs identified using GWAS usually only explain a small proportion of the variance. This is evidenced by the most recently published BMI meta-GWAS, in which 97 SNPs explain 2.7% of the variance in BMI[293]. However, published GWAS provide a basis for the selection of genetic variants. This assumption is tested by ensuring that the SNPs for the exposure of interest (in this case, BMI) are associated with the BMI phenotype in the data under study. As described further down (Methods section) in the present study a 2-sample summary-level MR was performed and thus, the effects of the BMI SNPs on BMI were taken from the latest published GWAS'[293] summary statistics. As described below (6.2.1.3, under 'Winners' curse'), this was to ensure that a true 2-sample approach was used.

For assumption II to be met there should be evidence, which suggests that the genetic variant is not affected by the usual confounders that are known to influence the exposure-disease association. This is tested by selecting common confounders of the relationship under study (for example, in the case of BMI → sleep duration it may be that physical activity is a confounder) and examining whether the SNPs for the exposure (BMI) are associated with such confounders. If they are then this constitutes a violation of this assumption. Importantly, though, in the present study it was not possible to test this assumption, due to

the use of summary-level data, which were used to ensure that the sample size was large enough.

Assumption III is met if the genotype does not directly affect the outcome (disease) of interest, nor should there be any mediating effect other than via the exposure. This assumption refers to what is known as 'horizontal pleiotropy' and can be tested and corrected for using different MR approaches, which are discussed below in section 6.2.1.3 and then in more detail in the Methods section.

Related to the first assumption is an issue central to this doctoral research: a lack of published GWAS studies that have identified and replicated genetic variants associated with sleep duration. As mentioned above, investigation of the reverse relationship (from BMI to sleep duration) is possible, as published GWAS have now replicated and identified SNPs robustly associated with BMI[86]. However, the sleep duration GWAS literature is limited, making it difficult to perform a Mendelian randomisation to examine the causal association between sleep duration/disturbance and BMI. This was the rationale for the meta-GWAS performed in Chapter 5, but no previously-associated loci for self-reported sleep duration were replicated, nor did any novel associations emerge. Thus, genetic variants robustly associated with sleep duration have not been discovered, to date, and therefore, the decision was made not to perform MR analyses to investigate potential causality in the direction of sleep duration to BMI.

Figure 6.2 Diagram illustrating violations of the assumptions described above

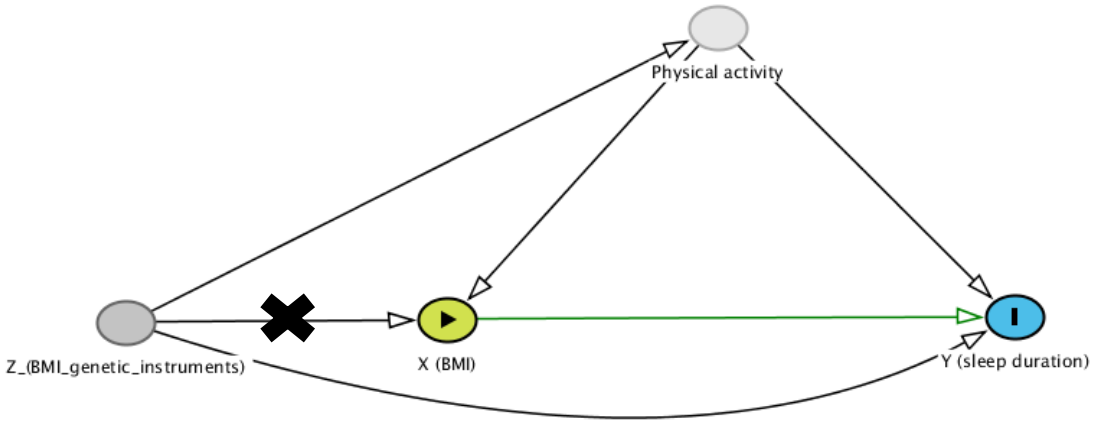


Figure 6.2 illustrates a violation of the core assumptions of MR. Firstly, the 'x' between the BMI SNPs and X (BMI) indicates that these SNPs are not in fact associated with BMI (the exposure) and thus, a violation of this assumption. Secondly, the arrow from the BMI SNPs to sleep duration (the outcome) and thus, indicates that these variants are directly associated with sleep duration, rather than via BMI. Thirdly, the arrow between the BMI genetic instruments and physical activity depict another assumption violation, as these SNPs should not be related to confounders of the BMI-sleep duration association. The arrow from BMI to sleep duration is green because it indicates that it is the causal pathway that is under study.

6.2.1.3 Potential problems when performing MR analyses

i. *Pleiotropy* – is when a single gene influences more than one trait, thus if a genetic variant has a direct effect on the outcome it can invalidate the MR, as a core assumption is violated. A valid instrumental variable (IV) is one that meets the core MR assumptions: it is not associated with confounders of the exposure-outcome association; it is associated with the exposure of interest; it is independent of the outcome under study, conditional on the exposure and unobserved confounders[294].

MR Egger[294] – is a modification of Mendelian randomisation and was adapted from meta-analysis. Its main aim is to detect horizontal pleiotropy between the genetic instruments and the outcome, whilst retaining its ability to provide an estimate of the underlying causal effect. Part of MR-Egger's flexibility relates to the fact that it is able to estimate the causal relationship between two traits of interest, even in a scenario whereby 100% of the IVs under study are invalid (do not meet all three of the required MR assumptions). MR-Egger can also provide informative pleiotropy statistics, in the form of Cochran's Q and Higgins' I^2. If the value of I^2 is >75(%) then this indicates a cause for concern, as it implies that there is a high level of heterogeneity in a study (pleiotropy, in this case). Similarly, if the Cochran's Q p-value is significant then this is also problematic and provides confirmation of heterogeneity.

Weighted median[295] – this is a further methodological development that can help with the issue of with pleiotropy in MR studies. Bowden and colleagues[295] proposed that researchers now apply this as an additional MR sensitivity analysis, alongside MR-Egger. This technique is as efficient as the inverse variance weighted (IVW) method, yet it is flexible enough to allow the inclusion of up to 50% of IVs with invalid weights and provide a causal estimate in the presence of balanced pleiotropy. IVW meta-analysis is used in MR to obtain an overall estimate of the causal estimates (SNP-outcome / SNP-exposure) from each individual SNP and is also known as 'conventional MR'[296]. However, the Weighted median approach is different from MR-Egger, as the former allows only up to 50% IVs with invalid weights, whilst the latter allows all of the IVs themselves to be invalid and can still estimate the causal effect of X on Y. More details, including the mathematics behind the different methods (IVW, MR-Egger and Weighted median) are explained below in the Methods section.

ii. Linkage disequilibrium (LD) – defined as the non-random association between alleles at distinct loci across the population[297]. If the genetic variant is in LD with another variant associated with the outcome of interest the IV regression may produce a confounded estimate of the causal relationship between the exposure and outcome. This is because, for example, if a chosen genetic variant (SNP_1) is in

LD with another genetic variant (SNP_2) then it is possible that SNP_2 has a direct or indirect effect on the outcome[298]. Another possibility is that SNP_2 exerts an influence on a confounder of the exposure-outcome association[298].

iii. Population stratification – within a given population individuals' ancestry is usually genetically heterogenous[299]. The relevance to MR is that population stratification might lead to confounded results, which is especially possible in a case where the genetic variant (IV)-modifiable risk factor association was collected in a sample population that is different from the genetic variant-outcome association[300]. This is because population subgroups might have different rates of disease and different allele frequencies and thus this can produce spurious (confounded) associations between a genotype and disease/trait in the entire population[300].

iv. Winner's curse – this refers to a situation whereby several of the genetic variants' true effect sizes (for the exposure) are similar, the one with the strongest association in the dataset under analysis may be overestimated[301]. This is likely to occur if the SNPs for the exposure were discovered in the data under study[301]. Two-sample MR is an approach in which the associations between genetic variants (IVs) and the exposure and outcome of interest come from two separate non-overlapping data sources. As such, two-sample MR reduces potential 'winner's curse' that can underestimate a true causal effect in one-sample MR and it also diminishes weak instrument bias[302]. This method is described in more detail below in the Methods section.

v. Dynastic effects – where the offspring's outcome phenotype is also influenced by the parental exposure caused by the parent's genotype and may thus, affect the magnitude of the causal estimate[303]. The effect may be inflated, as the child might have greater exposure, or it may be smaller if the parental genotype creates a hostile environment *in utero*, from which the foetus then develops to protect against[303].

vi. Canalisation – a compensatory mechanism for disruptive environmental or genetic factors[304]. This may occur for certain risk factors, whereby an individual

develops such compensatory mechanisms as a response to higher or lower levels of a specific risk factor[304] (for example, increased or reduced BMI).

6.2.2 The Polygenic Risk Score (PRS)

The use of polygenic risk scores (PRS) is now a widely-used approach to aggregate data from GWAS, as a lot of complex traits appear to be polygenic. They are used to predict an individual's genetic predisposition to a particular trait, as well as to uncover potential genetic overlap between two traits[305]. PRS analyses can now be performed using either individual-participant data (IPD) or summary-level data, due to recent advances in bioinformatics techniques.

Until recent years, a PRS tended to include only SNPs that had been identified as GWAS significant ($P<5x10^{-8}$) in a large-scale study and would then use their effect sizes as external weights when creating a PRS. However, effects have been observed between a PRS with an inclusion threshold as high as $P<0.5$ and thus this approach is now also widely used[306]. In PRS analyses, the aim is to examine whether two traits of interest possess underlying shared genetic factors. It differs from a genetic correlation (r_g) analysis, as this includes all genotyped SNPs on a particular genotyping array or all SNPs from GWAS results. A PRS analysis, however, aims to investigate whether traits X and Y share underlying common genetic variants, using a high-resolution best-fit approach[307].

6.3 AIMS OF THIS CHAPTER

The aims of the research carried out in this chapter were fourfold:

- Perform cross-sectional, observational analyses to examine the association between BMI and self-reported sleep duration in two UK population studies.
- Perform 2-sample Mendelian randomisation analyses in a large sample of UK adults, exploiting both individual participant data (IPD) and summary-level data from published GWAS.

- Examine potential horizontal pleiotropy between BMI and sleep duration, using multiple methods, such as MR-Egger regression and a weighted median.
- If substantial horizontal pleiotropy is detected using the methods mentioned in iii) then perform additional analyses to investigate this relationship, by means of polygenic risk scoring.

6.4 METHODS

For the work carried out in this chapter, I designed the study, performed all of the phenotypic data cleaning and all observational and genetic analyses in ELSA and UKHLS. However, as the UKB data used were only summary-level data, I was not involved in any phenotype or genotype cleaning or any other individual-level data handling of this sample.

6.4.1 Samples

This study included summary-level data from 127,573 UK Biobank participants, as well as IPD from 2 population studies. For observational purposes, data were analysed from 5,296 individuals from the English Longitudinal Study of Ageing (ELSA) and 6,811 participants from the UK Household Longitudinal Study (UKHLS) (see Table 6.1). For genetic analyses, data were used from individuals included in the ELSA and UKHLS sleep duration GWAS analyses from Chapter 5, which were 6,028 and 8,608, respectively. The reason for this was that for observational analyses the sample sizes were slightly reduced, data were required from participants who had data on BMI, sleep duration, covariates and genotype data (see Table 6.1). These inclusion criteria were so that in ELSA and UKHLS, both observational and genetic analyses were performed on the same individuals.

Table 6.1 Details of samples included in this study, with respective n for different analyses

Study	Type of data	GWAS N*	Observational analysis n**
ELSA	IPD	6,028	5,296
UKHLS	IPD	8,608	6,811
UKB	Summary***	127,573	N/A

Note. *Individuals GWAS of sleep duration, due to available genotype, sleep duration phenotype and covariate data; **summary statistics from sleep duration GWAS by Jones et al[78] downloaded from: http://www.t2diabetesgenes.org/data/; ***individuals included in the observational analysis of BMI and sleep duration, due to availability of BMI and sleep duration phenotypic data, as well as genetic data.

6.4.2 Exposure and outcome

The main exposure was researcher-measured BMI (kg/m^2) (in ELSA, UKHLS and UKB) and the single outcome was self-reported sleep duration. ELSA respondents were asked: 'How many hours of sleep do you have on an average week night?'. UKHLS participants were asked: 'How many hours of actual sleep did you usually get at night during the last month? This may be different than the actual number of hours you spent in bed.' More details on the sleep duration measures are in Chapter 2. Briefly, in for the UKB sleep duration (from which GWAS summary statistics were used here) was ascertained by asking participants for the average number of hours that they slept in a 24-hour period. More details of the phenotype derivation for Jones et al.'s sleep duration GWAS are in Chapter 2.

6.4.3 Genotyping

Full genotyping details for ELSA and UKHLS are in Chapter 2. Studies were genotyped using a genome-wide, Metabochip or Exome array (see Chapter 2).

6.4.4 Statistical analyses

Analyses were performed using a combination of R, version 3.3.2, PRSice, version 1.25[307] and PLINK, version 1.9 (www.cog-genomics.org/plink/1.9)[308].

6.4.4.1 Observational analyses

Firstly, in studies for which IPD (ELSA and UKHLS) were available, observational analyses were performed using linear regressions, with adjustments for age and sex. This was because the inclusion of multiple covariates can prove difficult in this type of observational analysis, as studies often measure demographic and lifestyle factors in different ways, and in an MR study it is more important to combine datasets to increase power. These results were subsequently combined in a fixed-effects meta-analysis to obtain an overall observational estimate across studies. Heterogeneity between studies was assessed by means of Cochran's Q and I^2.

6.4.4.2 Genetic analyses

Selection of BMI SNPs and genetic instrument creation: 97 SNPs were selected from the published GIANT consortium GWAS (Appendix 8.3) which included up to 339,224 participants from 125 independent studies[29]. Where the target SNP was not available in IPD data, proxy SNPs in linkage disequilibrium (LD) with the target SNP were analysed, using a threshold of $R^2>0.8$ (Appendix 8.3).

Proxies were found using two online tools: SNP Annotation and Proxy Search (SNAP)[309], and Single Nucleotide Polymorphisms Annotator (SNiPA)[310]. SNPs that did not contribute to the genetic instrument are in Table 6.2 below, along with the reason why.

Table 6.2 BMI SNPs not included in MR study

SNP	Reason not included
rs11057405	No proxy available in UKHLS
rs10733682	UKHLS does not have this SNP or a proxy for it
rs11727676	ELSA does not have this SNP or a proxy for it
rs12016871	UKHLS does not have this SNP or a proxy for it
rs13107325	ELSA does not have this SNP or a proxy for it
rs13191362	UKHLS does not have this SNP or a proxy for it
rs17001654	UK-Biobank does not have this SNP or a proxy for it

rs2075650	UK-Biobank does not have this SNP or a proxy for it
rs2080454	UKHLS does not have this SNP or a proxy for it

6.4.4.2.1 2-Sample MR analyses

6.4.4.2.1.1 Associations between BMI SNPs and BMI (SNP-exposure association)

Results were extracted for the association between 97 BMI SNPs and BMI (for both men and women) from the GIANT GWAS paper[29]. Linear regressions were also performed between each BMI SNP and BMI in the whole sample (n=142,209) and subsequently combined in a fixed-effects meta-analysis to obtain one estimate, the results of which are in Appendix 8.3. The percentage of variance explained in BMI by the BMI genetic instruments was obtained by performing a multivariable linear regression between the BMI SNPs and BMI and taking the R^2 value. I^2 and a p-value for Cochran's Q test were obtained to quantify the amount of heterogeneity of these associations between the studies (Appendix 8.3).

6.4.4.2.1.2 Associations between BMI SNPs and sleep duration (SNP – outcome association)

Using an additive model, linear regressions were performed between each individual SNP, and sleep duration in ELSA and UKHLS. To examine this association in UK Biobank summary statistics were downloaded from the latest sleep duration GWAS[78] and the results extracted for up to 97 BMI SNPs.

Subsequently, the results from the 3 studies were combined in a fixed-effects meta-analysis to obtain one estimate for each of the SNPs, for which I^2 and Cochran's Q were also obtained to quantify heterogeneity between studies (Appendix 8.3). In IPD the proportion of variance in sleep duration explained by the BMI genetic instruments was obtained by entering all of the SNPs into a multivariable linear regression with sleep duration as the outcome and multiplying the R^2 by 100.

6.4.4.2.1.3 Instrumental variable (IV) analyses

Three types of analysis were implemented to estimate the potential causal association of BMI on sleep duration in this study: Inverse-variance weighted (IVW) method, MR-Egger method and the weighted median, as detailed below. These analyses were performed in STATA version 14 using the *mrrobust* package (https://github.com/remlapmot/mrrobust). The final genetic instrument comprised 88 BMI SNPs, thus 9 variants were excluded for various reasons, which are detailed in Table 6.2 above.

I. Inverse-variance weighted (IVW) analysis

IVW is analogous to 2-stage least squares (2SLS). MR using IPD data, which was until recently, the most commonly employed MR. 2SLS estimates the causal effect ($\hat{\beta}_G$) of the exposure on the outcome with the following equation:

$$Y = \alpha + \hat{\beta}_G \hat{X} + \varepsilon$$

where α intercept term and ε the associated error term from the second stage regression. $\hat{X}$ represents the predicted value of the exposure (BMI) calculated in the first stage, as a result of X on a weighted gene score Z[311].

However, the IVW method was used here to calculate a combined estimate of the causal relationship between BMI and sleep duration for each variant: SNP – sleep duration / SNP – BMI. IVW performs a weighted linear regression of the genetic associations with the outcome (BMI SNPs → sleep duration) on the associations between the genetic variants and the exposure (BMI SNPs → BMI) [$\sigma_{Y_j}^{-2}$], under a fixed-effects meta-analysis. The intercept is constrained to equal zero in this model and it is assumed that all genetic variants are valid IVs and that they are not in LD (uncorrelated). Therefore, the causal estimate under an IVW model is calculated as:

$$\hat{\beta}_{IVW} = \frac{\sum_j \hat{\beta}_{Y_j} \hat{\beta}_{X_j}^2 \sigma_{Y_j}^{-2}}{\sum_j \hat{\beta}_{X_j}^2 \sigma_{Y_j}^{-2}}$$

where $\hat{\beta}_{x_j}$ is the coefficient from the regression of the exposure (BMI) on genetic variant *j*, $\hat{\beta}_{y_j}$ represents the coefficient from the regression of the outcome (sleep duration) on genetic variant *j*[312], alongside the respective standard error term, σ_{Y_j}.

II. MR-Egger analysis

MR-Egger was performed as a sensitivity analysis to account for potential horizontal pleiotropy, as it is still able to provide a valid causal estimate[13]. As per Burgess & Bowden[311], the IVW causal estimate described above, can also be calculated using an identical weighted linear regression ($\hat{\beta}_{Y_j}$ on $\hat{\beta}_{X_j}$), but without an intercept term and instead uses the $\sigma_{Y_j}^{-2}$ term as weights. In this scenario, in which the intercept is not forced to be zero, an MR-Egger regression is performed, in which a causal estimate ($\hat{\beta}_E$) is obtained using the following equation:

$$\hat{\beta}_{Y_j} = \hat{\alpha}_E + \hat{\beta}_E \hat{\beta}_{X_j}$$

where, as per the IVW equation described above, $\hat{\beta}_{Y_j}$ represents the coefficient from the regression of sleep duration on variant *j* and $\hat{\beta}_{X_j}$ is the coefficient from regressing BMI on variant *j*, with a respective standard error term σ_{Y_j}. However, the new terms introduced in the MR-Egger equation are interpreted as: $\hat{\alpha}_E$ is the intercept term, which denotes average horizontal pleiotropy across all SNPs and its respective P-value indicates whether this form of pleiotropy exists in the causal relationship of exposure (BMI) and outcome (sleep duration). Under the null hypothesis $\hat{\alpha}_E = 0$, but if this is not the case then it means that there is some degree of horizontal pleiotropy. If the accompanying P-value for the MR-Egger intercept is small (≤0.05) the implication is that horizontal pleiotropy is present, and that caution should be taken when drawing causal conclusions of the association under study. MR-Egger also makes an additional assumption, which is that associations between SNPs and the exposure (BMI SNPs → BMI), indicative of the strength of the instruments (BMI SNPs) are independent of the direct

associations between the SNPs and the outcome (BMI SNPs → sleep duration). This is referred to as the Instrument Strength Independent of Direct Effect (InSIDE) assumption[295], under which it is assumed that stronger SNPs have more reliable estimates of the causal relationship, compared to weaker SNPs. As mentioned above, the average pleiotropic effect of SNPs is then accounted for via the Egger intercept term and any dose-response relationship in the SNP associations is evidential of a causal effect.

III. Weighted median analysis

The Weighted median estimator was also implemented in this study. The weighted median approach first estimates the causal effect $\hat{\beta}$ of each SNP *j* via the ratio method ($\hat{\beta}_j = \hat{\beta}_{Y_j}/\hat{\beta}_{X_j}$). Weights are obtained by using the inverse variance of these ratio estimates, $\acute{w}_j = \hat{\beta}_{X_j}\sigma_{Y_j}^{-2}$, where then $s_j = \sum_{k-1}^{j} w_k$ represents the sum s_j $(= 1)$ of the standardised weights w_j up to and including the weight of the *j*th ordered ratio estimate. If k represents the largest integer, whereby s_j up to and including the kth ($s_k = \sum_{j \leq k} w_j$) estimate is <0.5, a causal effect $\hat{\beta}_{WM}$ of the association between BMI and sleep duration will be interpolated between the kth and $(k + 1)$ ratio estimates, as per the following equation:

$$\hat{\beta}_{WM} = \hat{\beta}_k + (\hat{\beta}_{k+1} - \hat{\beta}_k) \times \frac{0.5 - s_k}{s_{k+1} - s_k}$$

The main strength of the Weighted median is the ability to provide a valid causal estimate when up to 50% of the SNP weights under analysis are invalid[295] and it is more robust than MR-Egger[295]. This is because MR-Egger has been shown to produce considerably less precise estimates (with very large standard errors) and has substantially reduced power to detect a causal effect[295].

Importantly, the *mrrobust* package also outputs pleiotropy statistics for each method (IVW, MR-Egger and Weighted median), which are interpreted alongside the main results from each approach. These statistics

include an I^2 and a Cochran's Q test p-value for heterogeneity. The higher the I^2, the greater the degree of heterogeneity, whilst the Cochran's Q test p-value should be not significant (>0.05) if there is no heterogeneity.

6.4.4.2.2 Further follow-up genetic analyses: polygenic risk scoring and genetic correlation

Genetic correlation (r_g) of BMI and sleep duration

To estimate the r_g between BMI and sleep duration LD Score regression[28] was used, implemented in LD Hub[20], using summary statistics from GWA analyses of sleep duration in ELSA, UKHLS and UKB, to examine the underlying genetic correlation (r_g) of BMI with sleep duration in the whole sample. A genetic correlation (r_g) is the extent to which SNPs that contribute to variation in one trait (in this case, BMI) also contribute to variation in a second trait (here, self-reported sleep duration). An r_g is interpreted similarly to a Pearson's correlation, as it ranges from 1 to -1, with values that are closer to 1 indicating that there is potentially a high proportion of overlap between two traits, whilst values closer to zero mean that the traits are unlikely to have a large amount of shared genetic aetiology.

Briefly, LDSC regression involves regressing summary statistics from GWAS (from millions of SNPs) and measures to what extent each SNP is able to tag other variants locally (or, its 'LD score'). The slope of the LDSC regression model can then be rescaled to provide a heritability estimate of a trait, accounted for by all SNPs used in the estimation of the LD scores[28]. LD Hub[20] is a web tool where researchers can upload their GWAS summary statistics and obtain LDSC heritability estimates, as well genetic correlations between their trait and dozens of other traits of interest.

Polygenic risk scores (PRSs) of BMI and associations with sleep duration

A PRS can be used to infer whether two traits possess shared underlying genetic factors and therefore, in the present study, whether the same genetic variants might influence BMI and self-reported sleep duration. To ensure that the most high-resolution PRS is produced, it is important to use a range of different p-

value cut-offs, rather than merely GWAS significance ($p<5x10^{-8}$). The aim of these PRS analyses was to find the best-fit model, or the highest possible proportion of variance in sleep duration, explained by BMI SNPs. This was achieved by not only selecting SNPs at the very stringent GWAS significance ($p<5x10^{-8}$), but by using a range of p-value thresholds (Table 6.4).

Sleep duration GWA analyses in ELSA and UKHLS were adjusted for the first 10 principal components to account for population stratification, as well as age and sex (Chapter 5). The UKB sleep duration GWAS was adjusted for age, sex and study centre and these authors used linear mixed modelling in BOLT-LMM[21], which is able to take into account potential relatedness between individuals, as part of the analysis. Then summary statistics were downloaded from the most recent large-scale consortium meta-GWAS of BMI[15] from up to 339,224 individuals and the β coefficients were used as external weights in the PRSs.

PRSice[307] software is implemented in R and also exploits specific PLINK (version 1.9) functions, as well as the summary-statistic function from the Genetics ToolboX[313] (gtx) R package. A total of 9 PRSs were created at p-value thresholds of 1.0, 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, 0.01 and 0.001 (Table 6.2). At each threshold SNPs were clumped by LD using a cut-off of r^2=0.1 and a window of 250kb, to ensure that only independent SNPs were included, as recommended by the creators of PRSice[307].

6.4.4.3 Power calculation

Using the online MR power calculation tool: http://cnsgenomics.com/shiny/mRnd/[314] power to detect causal estimates was calculated, given a sample size of 142,209, the proportion of variance in the exposure (BMI) explained by the genetic instruments (R^2), βyx for the true underlying causal association (unstandardised coefficient (B) =0.84, taken from Jones et al.'s[78] IVW causal estimate of BMI on sleep duration and multiplied by 60 to convert it to minutes), B_{OLS} for the observational association of BMI and sleep duration, variance of the exposure (BMI SD^2 = 25.81) and variance of the outcome (sleep duration SD^2= 1.64). The estimates for the observational

relationship, as well as SDs to calculate variance of the exposure and outcome, were taken from pooled ELSA and UKHLS results (Table 6.1). The power calculation indicated that with a sample size of 142,209 yielded 100% power to detect a potential causal association of β=0.84[78], between BMI and sleep duration, assuming a type-1 error rate of 0.05.

6.5 RESULTS

6.5.1 Sample characteristics

Table 6.3 shows sample characteristics for individuals from ELSA and UKHLS included in the observational analyses. Mean sleep duration and BMI was similar across both studies, with the highest mean BMI observed in ELSA.

Table 6.3 Participant characteristics for IPD studies (N=12,107)

Study	Mean sleep duration* (SD)	Mean BMI** (SD)	Mean age (SD)
ELSA	6.86 (1.27)	28.14 (5.11)	66.7 (9.16)
UKHLS	6.63 (1.29)	28.01 (5.05)	52.76 (15.98)
Both studies	6.74 (1.28)	28.07 (5.08)	59.73 (12.57)

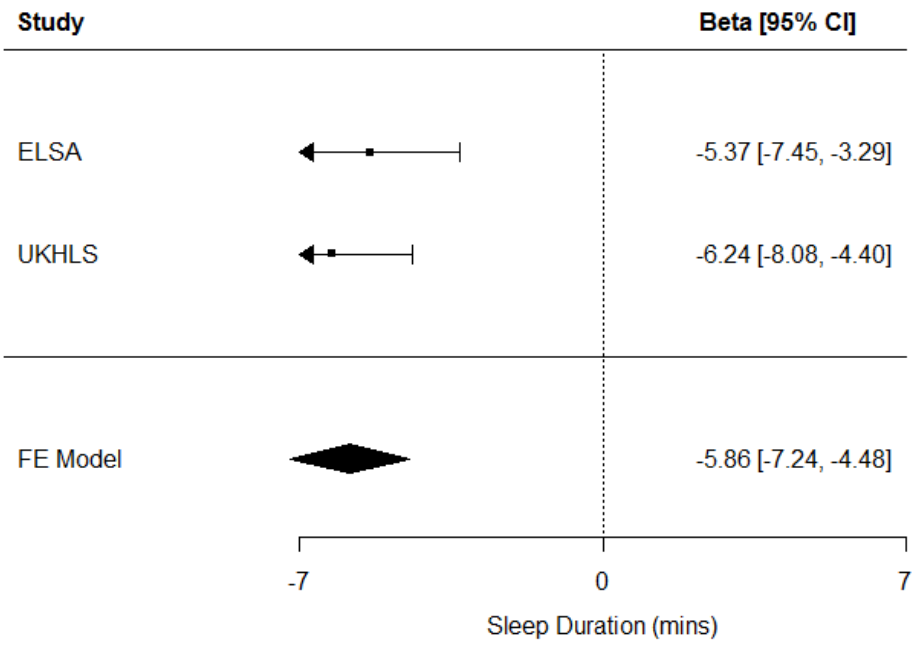
Note. *Hours, **kg/m²

6.5.2 Cross-sectional, observational results

The observational estimates presented in Figure 6.3 are adjusted for age and sex and depict a negative, cross-sectional association between BMI and self-reported sleep duration, such that for every SD increase in BMI (kg/m²) there is a mean sleep duration difference of 5 and 6 minutes in ELSA and UKHLS, respectively. The overall estimate showed that for every SD increase in BMI (5.08 kg/m²) there was a mean difference in sleep duration of 5.86 minutes. Cochran's Q and I^2 revealed that there were no issues with heterogeneity between the 2 studies (Cochrans's Q p-value=0.70, I^2=0). At first glance the ELSA cross-sectional finding appears to be completely inconsistent with the cross-sectional BMI-sleep duration result found in Chapter 3. However, the observational cross-sectional model tested here (Chapter 6) differed from that of the minimally-adjusted model in Chapter 3 and importantly, although the sample sizes were similar, this

was not a 100% overlapping sample. In a sensitivity analysis, an identical regression model to the present one (with standardised BMI as the exposure and sleep duration as the outcome and adjusted for age and sex only) was performed in the ELSA sample from Chapter 3 (n=5,015). The results of this model were β= -3.72 minutes, 95%CI= -0.10; 0.03, p=0.001, an inconsistency (of approximately 1 minute difference in effect sizes) which seemed plausible, given that the samples did not consist of exactly the same individuals.

Figure 6.3 Observational association between (standardised) BMI and sleep duration in IPD studies (N=12,107)



6.5.3 Genetic results

6.5.3.1 Results of 2-sample MR

The percentage of variance in BMI explained by the BMI SNPs was 1.15% (93 SNPs out of 97) in ELSA and 1.8% (86 SNPs out of 97) in UKHLS. Figure 6.4 presents the causal association between BMI (kg/m²) and sleep duration (minutes), as a result of IVW, MR-Egger and weighted median analyses.

6.5.3.1.1 MR assumptions

Assumption I – BMI SNPs should be robustly associated with BMI

It was ensured that this assumption was met by using summary statistics from the latest GIANT BMI GWAS to extract associations between the genome-wide BMI SNPs and BMI. This approach was taken because a 2-sample MR was implemented in this study.

Assumption II – BMI SNPs should not be associated with common confounders of the BMI → sleep duration relationship

As mentioned earlier in this chapter, it was not possible to test this assumption here, as this was a 2-sample summary-level MR study. This was because the majority of the data were contributed by summary statistics from the latest UKB sleep duration GWAS.

Assumption III – BMI SNPs should not be directly associated with sleep duration, but should only be associated with sleep duration via BMI

This assumption was tested by implementing three MR methods in total, namely IVW, MR-Egger and a weighted median. The IVW is the most 'conventional MR' approach, whilst MR-Egger and the weighted median are commonly-used sensitivity analyses that are able to (in different ways) correct for horizontal pleiotropy. This is described in more detail below.

6.5.3.1.2 Main MR results

Inverse-variance weighted (IVW) approach

The IVW yielded a result that was consistent with a causal effect of BMI on sleep duration, such that for every additional kg/m^2 sleep duration decreased by 3.23 minutes (Figure 6.4). However, the I^2 heterogeneity statistic for the IVW = 100% and the Cochran's Q test p-value was <0.001, which indicated the presence of substantial balanced pleiotropy.

Weighted median approach

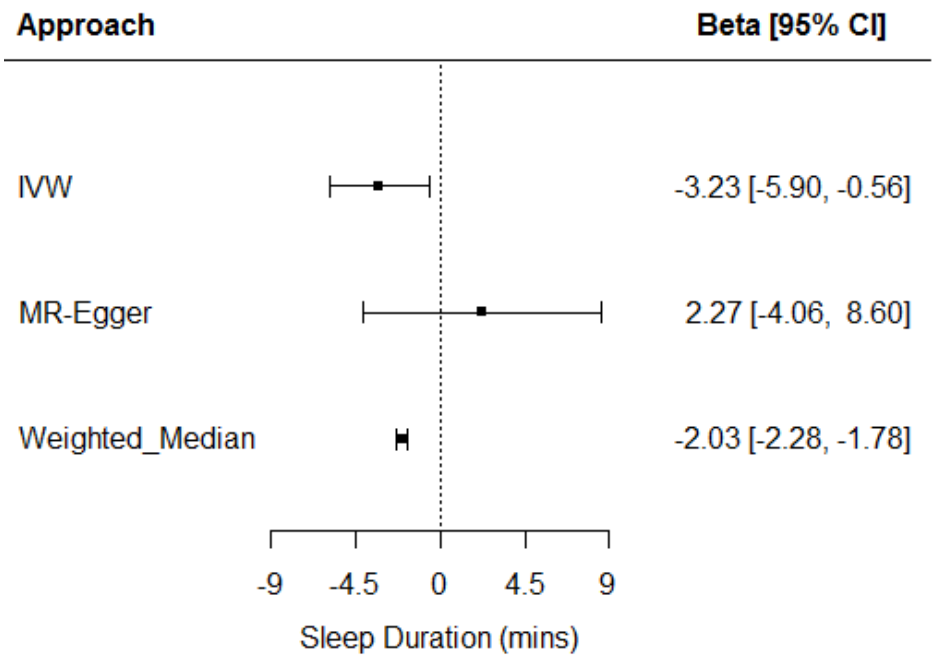
The weighted median estimate was smaller (by approximately 1 minute) than the IVW, at a decrease in sleep duration of 2.03 minutes per additional kg/m^2 of BMI

and was also consistent with a significant causal effect of BMI on sleep duration (Figure 6.4).

MR-Egger approach

MR-Egger suggested there was substantial directional pleiotropy (I^2>70% and Cochran's Q test p-values <0.001), thus violating one of the core underlying instrumental variable assumptions. The MR-Egger intercept p-value also confirmed this (p=0.06, which is close to the threshold of 0.05) and the causal estimate yielded by MR-Egger was positive, whereas the weighted median and IVW estimates were negative, which is consistent with the observational results (Figure 6.4).

Figure 6.4 Causal association between BMI and sleep duration using IVW, MR-Egger and Weighted median analyses in IPD + summary-level data



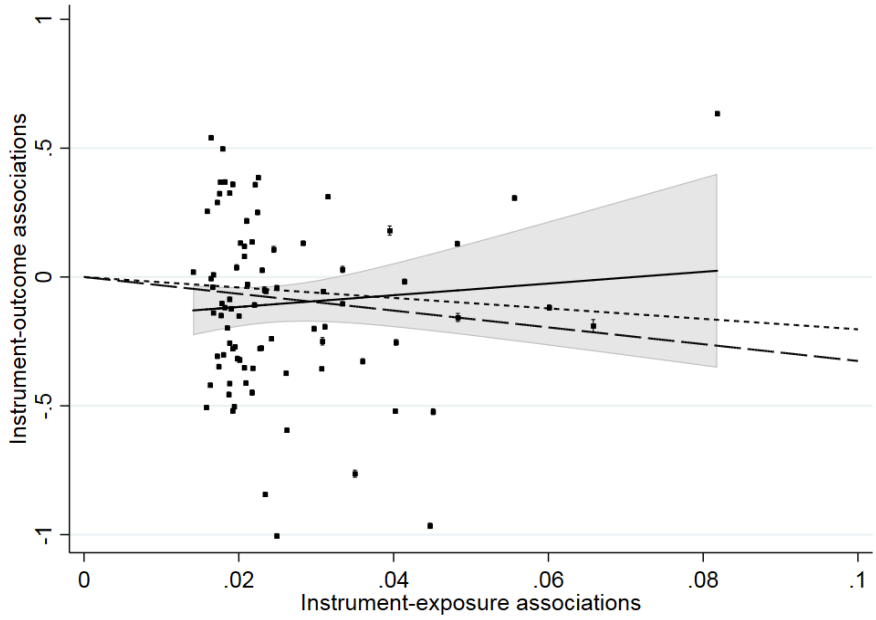
Note. Datasets include ELSA (n=6,028), UKHLS (n=8,608) (IPD) and UKB (summary-level data) (127,573).

Additional graphical sensitivity checks

To further examine the results of the three MR approaches, the plots below were created. Figure 6.4 presents a scatter plot of the BMI instruments – exposure (BMI) association and the BMI instruments – outcome (sleep duration)

association. As such, the MR-Egger, IVW and weighted median slopes are interpreted as the unit change in the outcome (hours of sleep duration) for every unit increase in the exposure (BMI) due to the BMI genetic variants. In this plot each BMI genetic variant is a data point and it shows that multiple variants violate MR assumption III and are therefore, subject to horizontal pleiotropy, which is also confirmed because the MR-Egger (solid line) intercept only *just* passes through zero. Also, in line with the description of results earlier, the MR-Egger slope is positive, whilst the IVW and weighted median slopes are negative.

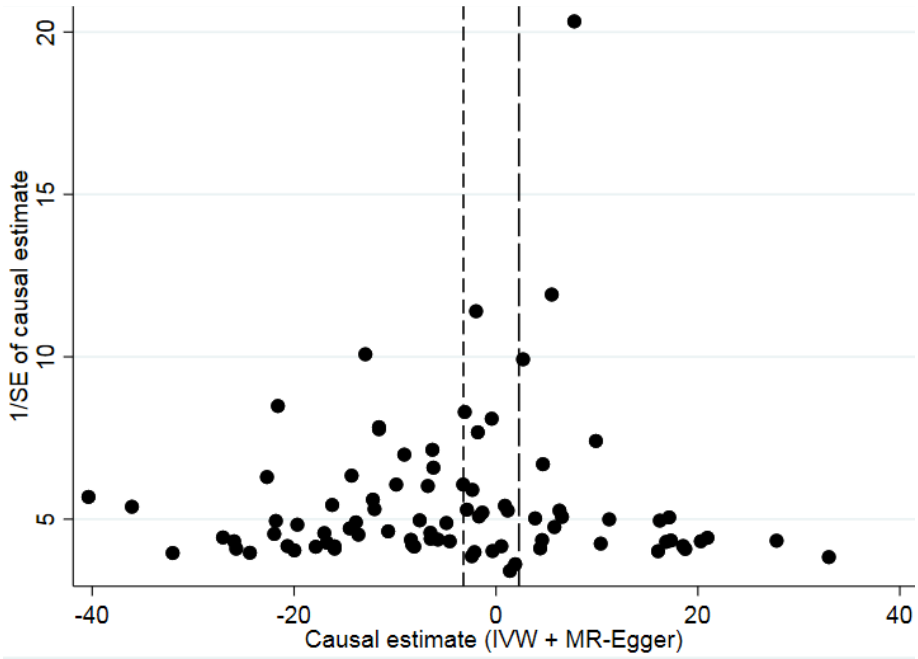
Figure 6.5 Associations between BMI SNPs and BMI (X) and sleep duration (Y)



Note. The main line represents the MR-Egger result and the other lines are the IVW (long dash) and the weighted median (short dash).

Figure 6.5 presents a funnel plot of the IVW and MR-Egger causal estimates and similarly to the scatter plot above, each data point is a BMI genetic variant. The x-axis represents the estimate of the gene-outcome association divided by the estimate of the gene-exposure association (Wald ratio). The funnel plot is asymmetric, which is confirmatory of the fact that multiple BMI genetic variants have remarkably strong effects on sleep duration given that their precision is low. Also, as per the results description and the scatter plot above, the MR-Egger and IVW estimates are on opposing sides of zero and are thus, inconsistent with one another.

Figure 6.6 Funnel plot of MR-Egger and IVW causal estimates against the precision of each of these estimates



Note. Line with longer dashes = MR-Egger, line with shorter dashes = IVW.

6.5.3.2 Results of polygenic risk score (PRS) and genetic correlation (rG) analyses

6.5.3.2.1 Genetic correlation (rG) of BMI and sleep duration

To estimate the rG between BMI and sleep duration LDSC[17] was used, implemented in LD Hub[22], by using summary statistics from GWA analyses of sleep duration in ELSA, UKHLS and UKB. The rG between BMI and sleep duration was was -0.067, p=0.09.

6.5.3.2.2 Polygenic risk scores (PRSs) of BMI and associations with sleep duration

Of the 9 PRSs, a PRS of BMI at a p-value inclusion threshold of 0.01 explained the highest proportion of the variance in sleep duration (0.02%). This PRS was negatively associated with sleep duration (β=-1.75, p= 6.13×10^{-7}) (Table 6.4).

*Table 6.4 PRS analyses of BMI & sleep duration in 142,209 individuals after clumping SNPs by LD**

P-val. threshold	No. SNPs in model	Coeff. (SE)	P-val.	(Pseudo) R^2	N
1.0	54,505	-0.62 (0.18)	0.0003	$6.06x10^{-5}$	142,209
0.5	36,688	-0.64 (0.18)	0.0003	$6.94x10^{-5}$	142,209
0.4	31,254	-0.59 (0.19)	0.0008	$7.49x10^{-5}$	142,209
0.3	25,196	-0.67 (0.20)	0.0003	$8.16x10^{-5}$	142,209
0.2	18,195	-0.62 (0.21)	0.002	$8.22x10^{-5}$	142,209
0.1	10,477	-0.80 (0.24)	0.0005	$8.44x10^{-5}$	142,209
0.05	6,006	-1.33 (0.29)	$2.36x10^{-6}$	0.0001	142,209
0.01**	2,024	-1.75 (0.67)	$6.13x10^{-7}$	0.0002	142,209
0.001	536	-2.07 (0.43)	0.004	$4.83x10^{-5}$	142,209

Note. *Clumping parameters are r^2=0.1 and 250kb, Coeff. = unstandardized coefficient in minutes of sleep duration; **best threshold with 0.02% of the variance in sleep duration explained by this PRS.

6.6 DISCUSSION

6.6.1 Summary of findings

A large-scale two-sample MR study was conducted to examine the potential causal relationship between general adiposity (BMI) and self-reported sleep duration. In doing so, three distinct MR methods were applied to obtain a complete picture of this complex, potentially causal association. Observationally, the cross-sectional pooled estimate of ELSA and UKHLS showed a mean difference in sleep duration of 6 minutes for every standard deviation increase in BMI (5.08 kg/m^2).

However, the MR findings suggest that there is still a degree of uncertainty regarding the causal association between BMI and sleep duration and that future research should perform even more in-depth analyses. The IVW approach indicated that there was a large amount of heterogeneity. MR-Egger results suggested no causal association between BMI and sleep duration and indicated that there was likely to be a substantial proportion of directional pleiotropy. The Weighted median results were consistent with a causal effect of BMI on sleep duration and thus supported the IVW result. Importantly, the sensitivity analyses performed in the present study were able to test and correct for horizontal, but not vertical pleiotropy. Unmeasured horizontal pleiotropy violates a core MR assumption, in that the genetic variant(s) are associated with the outcome through more than one independent causal pathway (in the present case, via a pathway other than BMI). However, vertical pleiotropy would not confer a violation of MR assumptions and is present when the genetic variant(s) affect the outcome via a mediator (i.e. a factor that lies on the causal pathway).

Subsequently, follow-up genetic analyses were performed to investigate whether BMI and sleep duration might have shared underlying genetic factors. The PRS that fitted the data best was at a p-value threshold of 0.01, which was negatively associated with sleep duration and explained only 0.02% of its variance. The genetic correlation between BMI and sleep duration was -0.067 and not significant (p=0.09), a result, which is in line with the small variance explained (0.02%) in the PRS analyses.

6.6.2 Observational findings

Only the two studies which had IPD available were included in the observational analysis. Cross-sectional analyses in ELSA and UKHLS showed that there was a negative relationship between BMI and sleep duration, after adjusting for age and sex. A subsequent fixed-effects pooled analysis yielded an overall negative cross-sectional effect of BMI on sleep duration with a total sample size of 12,107 individuals. This finding is also consistent with large epidemiological meta-analyses[106,107] of BMI and sleep duration.

6.6.3 MR findings

Although there appeared to be a causal association, as suggested by the IVW and MR-Egger results there was a large proportion of horizontal pleiotropy. This was apparent from the heterogeneity statistics (I^2 and Cochran's Q test p-value) outputted by the IVW and MR-Egger methods. This metric was adapted from the meta-analysis literature[315] to assess pleiotropy in the context of MR. MR-Egger requires that the effects of each instrument (SNP) on the exposure are independent of supposed pleiotropic effects on the outcome, which is known as the 'InSIDE assumption'[295], as mentioned earlier. Although this assumption may seem somewhat unintuitive, its plausibility has been supported by evidence from a study in which associations of SNPs with different phenotypes were largely uncorrelated[316].

Results from the IVW analysis showed that there was a significant negative causal effect of BMI on sleep duration in this sample of 142,209 individuals. However, the I^2 produced alongside this result indicated that there was a high proportion of pleiotropy. Fundamentally, the IVW approach assumes that each SNP is a valid IV. Thus, if the three main assumptions hold (mentioned earlier) then the IVW can estimate the true causal effect of the exposure on the outcome. However, in this study it was not possible to test whether the BMI SNPs were associated with confounders, as these data were not available for the UKB study because summary data were used to maximise statistical power. A related and important point here is a potential violation of the InSIDE assumption (not possible to test in a 2-sample MR), whereby the pleiotropic effects of the SNPs on the outcome act via one particular confounder[317] (for example, physical activity, in the case of BMI and sleep duration). Therefore, if this was tested in future and the BMI SNPs were associated with sleep duration, via physical activity this would mean that the InSIDE assumption is violated.

To overcome some of the issues with IVW, both MR-Egger[294] and the Weighted median[295] were implemented as sensitivity analyses. If the IVW causal estimate of BMI on sleep duration is in fact true, results from the MR-Egger and Weighted median approaches should be almost indistinguishable from it. MR-Egger

calculates the average pleiotropic effect across SNPs; a small p-value (near or ≤0.05) as well as an intercept that is different from zero indicate the presence of directional pleiotropy[294]. Results from MR-Egger analyses suggested no causal association between BMI and sleep duration (p=0.481) and the coefficient was positive, as opposed to the IVW and weighted median estimates, which were negative. The MR-Egger also revealed that there was likely to be directional pleiotropy underlying this relationship, as the intercept's p-value was 0.06. It is important to note, though, that the 95% CI around the Egger estimate was particularly wide (-4.06; 8.60). This was not unexpected, as it is reliant on genetic variants having different effect strengths on the exposure[317]. Also, the MR-Egger has reduced power, compared to the other approaches, is more susceptible to a violation of assumption II *(BMI SNPs should not be associated with common confounders of the BMI → sleep duration relationship)* and may be more of a problem for weaker instrumental variables[317]. Therefore, it seems plausible that the Egger estimate in the present study may be biased, as it is inconsistent with the observational result, as well as the IVW and weighted median MR results.

A Weighted median estimator[295] was also used to ensure that the conclusions drawn from the results were robust and to assess the presence of potential balanced pleiotropy. The Weighted median is distinct from MR-Egger, as it is able to provide reliable causal estimates when up to fifty per cent of the IV weights are invalid. The Weighted median yielded a significant, negative causal estimate of the relationship between BMI and sleep duration. However, taken together, the three approaches showed that there is still uncertainty surrounding the causal relationship between BMI and self-reported sleep duration. Therefore, it may be unlikely that the BMI genetic variants exert their influence on self-reported sleep duration via BMI. This was partially supported by an additional analysis in which associations between the BMI SNPs and sleep duration were tested in the IPD studies (ELSA and UKHLS, n=12,107), whilst adjusting for BMI and then IVW, MR-Egger and Weighted median MR analyses were performed. Results showed that none of the MR estimates were significant, which was not surprising, as this analysis was likely to be underpowered with such a small

sample size. Out of the 88 BMI SNPs, four were significantly associated with sleep duration in a model adjusted for measured BMI (Appendix 8.3). It is also important to note that findings from the only previous MR of BMI and self-reported sleep duration differed from those of the present study. Specifically, the earlier study found, using IVW that there was no causal relationship between BMI and sleep duration, whereas the present study found the opposite. However, the MR-Egger analyses from both studies yielded an inverted effect in comparison to the IVW estimate.

PRS and rG analyses were also performed to examine whether BMI and sleep duration were likely to have a shared common genetic aetiology. After considering a few of the most well characterised BMI genes, it appeared that shared biological pathways could be a likely explanation for this relationship. For example, the fat-mass and obesity associated (*FTO*) gene is of interest here, as it is expressed in the hypothalamus[29] and neurons in the ventrolateral preoptic nucleus (VLPO) are instrumental in promoting sleep, by shutting off other arousal centres in the brain[318]. Specifically, rs1558902, an intron in the *FTO* gene was included in our best-fit PRS and its association with sleep duration in the meta-GWAS of all three studies was -0.68 minutes. Thus, although this effect was not genome-wide significant it is consistent with the expected direction of effect, such that higher genetic risk of obesity is associated with less sleep.

Results from PRS analyses showed that there was a negative association between a BMI PRS comprised of ~2,000 SNPs, and sleep duration, but it accounted for only 0.02% of its variance. LDSC findings were consistent with this, such that the non-significant rG of BMI and sleep duration was -0.067, a result which was very similar to the rG (-0.05) reported by Jones and colleagues[78]. These findings suggest that BMI and self-reported sleep duration might not possess shared genetic aetiology, but perhaps these results should be interpreted with some degree of caution. For example, although all three samples included in this study asked participants a very similar question on sleep duration, the UKB phenotype was derived distinctly from that of ELSA and UKHLS.

As summary statistics were included from the UKB sleep duration GWAS it was not possible to modify the phenotype for inclusion in the analyses. Briefly, Jones and colleagues[78] excluded individuals who reported sleep durations greater than 18 hours; they then adjusted for age, sex and study centre, obtained the model residuals and subsequently applied inverse-normalisation to ensure a normal distribution. Although it could be argued that the 2 to 12-hour range allowed for in ELSA and UKHLS is somewhat liberal, this was the result of restricting the original sleep duration phenotype to +/- 4 SDs. Thus, one possible explanation for the very small amount of variance explained in sleep duration could be that it is indicative of large variation in this phenotype, between the IPD samples (ELSA and UKHLS) and UK Biobank.

6.6.4 Study strengths

This study possesses important strengths. This was the largest Mendelian randomisation study of BMI and sleep duration in adults, to date. A 2-sample approach was employed, which decreases the chance of obtaining biased results. This is because in a 2-sample MR setting, no data from individuals who contributed to the latest BMI GWAS[86] were analysed, none of these studies (ELSA, UKB and UKHLS) were part of this meta-GWAS.

6.6.5 Study limitations

One of the limitations of this study was the considerable overlap between the sample analysed and that of Jones and colleagues[78]. However, the present study also included data from two other general population samples and also performed comprehensive PRS analyses to assess shared genetic aetiology underlying BMI and sleep duration. Sleep duration was self-reported, which might suffer from measurement error and bias and has been shown to correlate only modestly with actigraphic sleep duration[97]. These analyses were conducted with data from White/European individuals and may therefore not be applicable to other ethnic groups. Also, as it was not possible to access to the UKB IPD observational analyses were not performed in this study. Jones et al did not report findings from observational analyses of BMI and sleep duration, which

meant it was not possible to make comparisons with the ELSA and UKHLS results.

6.6.6 Future directions

In future, it would be important to perform similar analyses in children and adolescents, as genetic effects can differ over the life course. Research suggests that some complex traits – including BMI – may be more heritable in younger people. For example, Evans and colleagues[319] found that SNP heritability of BMI was higher in ALSPAC children, compared to an adult sample. Although an individual's DNA remains unaltered throughout their life, differential levels of gene expression are linked to several disease states and cellular responses[320] and it would be of interest to investigate this more in future.

It is also important to investigate potential shared genetic aetiology between BMI and objectively measured sleep duration, as evidence suggests that there is at best, moderate agreement between subjective and objective measures[98,130]. Objectively measuring sleep duration in large samples is now possible, unlike a few years ago. For example, the UKB have recently released data on actigraphic sleep duration collected in 100,000 individuals.

Shared genetic aetiology between BMI and other sleep phenotypes, such as pattern, bedtime, timing, quality and disturbance should also be investigated. Although it is likely that an individual's sleep duration correlates with some, if not all other sleep measures, research suggests that each of these phenotypes should be treated independently[56]. There are some challenges to be considered when measuring sleep dimensions other than duration, particularly when combining multiple studies to increase statistical power. Studies are less likely to have administered the same measure of sleep quality, for example, compared to duration.

Future research may also benefit from the inclusion of samples which have derived their sleep duration phenotype more similarly. As mentioned earlier, the present study used data from three samples, one of which used particularly

unconventional exclusion criteria for hours of sleep (>18 hours)[78], even though they subsequently applied inverse-normalisation to assure a normally distributed measure.

Further MR studies that investigate the causal association between BMI and self-reported sleep duration could also consider performing further sensitivity analyses. For example, if substantial heterogeneity is identified amongst BMI SNPs then it would be plausible to remove some of the more heterogeneous SNPs (i.e. have an I^2 value of >75%).

Finally, the UKB have recently released genetic data from 500,000 (the previous release was n=150,000) individuals and thus, these analyses could be repeated in this much larger sample in future.

6.7 CHAPTER SUMMARY

- Observational data in IPD revealed a negative relationship between BMI and sleep duration in 12,107 individuals.
- A comprehensive set of 2-sample MR analyses in 142,209 individuals suggests that there is still a degree of uncertainty in terms of the causal association between BMI and self-reported sleep duration.
- MR findings showed substantial pleiotropy between BMI and sleep duration.
- A polygenic risk score of BMI was significantly related to sleep duration, but only explained a small proportion of its variance.
- Future research should investigate shared genetic aetiology between BMI and other sleep phenotypes, use objective sleep duration and employ samples in which sleep duration is derived uniformly.

7 GENERAL DISCUSSION

7.1 SYNTHESIS OF EVIDENCE GENERATED

The obesity epidemic has coincided with a chronic reduction in sleep duration, largely, but not exclusively, in Western societies[59]. The work undertaken in this thesis used a combination of observational and genetic epidemiological methods, in an attempt to better understand the complex relationship between BMI and sleep duration. The majority of this work focused on adults, with one study that analysed data from a paediatric sample. Below are the specific objectives that were outlined in Chapter 1, followed by the key findings that addressed them:

I. Establish the direction of effect between BMI/WC and self-reported sleep duration in older adults, by performing bidirectional, epidemiological analyses, with adjustment for a wide range of important covariates (Chapter 3). *This research showed that in older adults, higher BMI leads to small decreases (<0.5 minutes) in self-reported sleep duration over time but sleep duration was not associated with prospective changes in BMI.*

II. Ascertain the direction of effect between BMI and objectively-measured sleep duration in childhood, by analysing data from a paediatric sample and using bidirectional modelling, with adjustment for important covariates (Chapter 4). *This work was the first to examine the bidirectional association between BMI and objectively measured sleep duration in childhood and found no effect in either direction, from ages six to eight and eight to ten years.*

III. Perform genome-wide analyses to find novel, as well as replicate previous, common genetic variants associated with self-reported sleep duration, with the aim of using them in bidirectional MR analyses (Chapter 5). *No novel genetic variants were identified in this meta-GWAS, nor were previous sleep duration variants replicated. This was*

likely due to limited statistical power, as the sample size was very modest compared to other meta-GWAS of self-reported sleep duration.

IV. Use Mendelian randomisation to investigate whether there is a causal relationship between BMI and self-reported sleep duration in a large sample of adults (Chapter 6). *MR analyses suggested that the association between BMI remains uncertain with respect to whether it is causal or not. Also, these phenotypes likely possess only a small amount of genetic overlap (genetic correlation was -0.067 and not significant and the variance in sleep duration explained by BMI SNPs was 0.02%).*

7.2 JUDGING THE EVIDENCE

To draw definitive conclusions about the work that was carried out in this thesis, it is necessary to judge the findings in relation to the following limitations pertaining to measurement, design and generalisability: measurement of sleep duration; methods for assessing causality; confounding; and generalisability of the findings. General limitations that arose around these themes will be discussed; specific limitations pertaining to each study have been discussed in each chapter.

7.2.1 Measurement of sleep duration

In Chapters 3, 5 and 6 sleep duration was measured using self-report. Taken together, the analyses in this thesis that investigated the association between BMI and self-reported sleep duration (or self-reported sleep duration and BMI), whether observational or genetic, seemed to suggest that this association is likely to be very small, or absent.

Importantly, in Chapters 3 and 6, observational cross-sectional models of BMI and sleep duration that were adjusted only for age and sex showed revealed a much larger effect size, as compared to models adjusted for other demographics, as well as health behaviours and health problems. This finding confirmed that the cross-sectional relationship between BMI and sleep duration became attenuated following the inclusion of these important covariates in the model.

The null (multiply-adjusted) cross-sectional findings are in support of at least three epidemiological studies of BMI and self-reported sleep duration in adults, which found no association between the two[116–118]. However, several studies in adults reveal a cross-sectional association between BMI and self-reported sleep duration[71,106,107,110,113–115,235]. One reason for this discrepancy in results across cross-sectional studies in adults could be the age of participants. For example, in the early meta-analysis of adults by Cappuccio and colleagues[107] the majority of the studies included were conducted in younger or middle-aged adults. Later studies were also largely carried out in younger adults, with mean ages of 37y[71], 54y[110], 43y[114,115] and 55y[235], whereas the mean age of the ELSA participants used in this work was 65y. Prospectively, there have been no previous bidirectional studies of BMI and self-reported sleep duration in adults and earlier research largely focused on the prospective association between sleep duration and changes in BMI. However, a systematic review[106], a Canadian population-based study[109] and the most recent large-scale meta-analysis[123] showed an association between short sleep and greater BMI/increased risk of obesity. Importantly, in the Canadian study participants were on average 41y of age, in the meta-analysis most studies were in younger or middle-aged adults and the studies in the review had samples with mean ages of ~40y. However, at least two previous longitudinal studies in adults found no relationship between sleep duration and BMI[235,321], in line with evidence from this thesis. One of these studies[321] used a fixed effects model to account for unobserved time-invariant covariates (for example, genetics) and it was the results of this analysis that, contrary to their generalised estimating equation analysis, completely attenuated the prospective association between sleep duration and BMI, even though the respondents were aged between 19y and 39y. The other study found that sleep duration did not predict changes in BMI, which is important because at follow-up participants's mean age was 56y at baseline, whilst at follow-up they were in their early to mid-sixties.

The majority of paediatric cross-sectional studies also suggest an association between BMI and self-reported sleep duration[106,107,135,140,141,143,144,146,147]. Nevertheless, three cross-sectional studies in children produced null findings[148–

[150], which are in agreement with the results in TESS. Three systematic reviews and four meta-analyses in children suggest that subjective (usually parented-reported) sleep duration does predict changes in BMI and an increased risk of obesity[106,135,151–153,157], but the paediatric work in this thesis used objectively-measured sleep duration and thus, there is more detailed discussion below about the bidirectional findings in TESS and comparison to previous similar studies.

Another important reason for discrepancies in findings could be the differences in adjustments for confounders across studies, which is discussed in more detail below (section 7.2.3).

As mentioned earlier in this thesis, evidence suggests only modest agreement between subjective and objective sleep duration, when assessed in the same individuals[96–98]. Specifically, when asked, people are likely to overestimate their hours of sleep per night, as compared to actigraphy[96–98]. Despite these issues, as mentioned in Chapter 1 of this thesis, there are several advantages of using self-reported sleep measures. The main advantages of self-report sleep duration measures are: they are inexpensive and easy to administer, particularly in large studies, as one or two questions can usually be asked as part of a larger questionnaire; they are simple to code and to analyse, as a typical question asks about the number of hours an individual sleeps for, thus researchers can choose to use this or convert to minutes if they prefer. There have also not been any GWAS to date that have used large samples to find common genetic variants associated with objective sleep duration, but it is likely that these will become available in the near future (this is discussed in more detail in the section on Future directions below). It is also important to note, however, that when analysing within-person observational data, the poor agreement between subjective and objective sleep measures are likely to be less of a problem. This is because the error terms associated with the measurement remain consistent and thus, do not invalidate the findings.

In Chapter 4, objective sleep duration was used to investigate the bidirectional association with BMI in children. The current findings are in agreement with the only two prior studies that examined the bidirectional relationship of BMI with

(subjective) sleep duration and it therefore, appears that the association between BMI and sleep duration, and vice versa, may have previously been overestimated. The first of these by Hiscock and colleagues[137] found no relationship in either direction between BMI and sleep duration, in an Australian sample of approximately 3,800 children. The second study showed no association in either direction between BMI and sleep duration in 526 children from the ethnically White component of the BiB study[138].

It was also not possible to check the correlation between parent-reported sleep duration and actigraphic sleep duration, as the former was not collected; nor were parents asked to log the children's bedtimes and wake times, which may have improved and aided the estimation of sleep duration. More generally, actigraphy also has its own limitations when measuring sleep duration. For example, Spruyt and colleagues[322] examined the concordance between wrist actigraphy and polysomnography (PSG) in a sample of 149 healthy children, aged between four years and nine years of age. Their findings suggested that the actigraph significantly underestimated sleep duration by approximately 30 minutes, in comparison to PSG, further supported by only a modest correlation of 0.47 between these two measures.

7.2.2 Approach taken to assess causality

The RCT is widely accepted as the gold standard for causality, whereby participants in the treatment and control arms of the trial are not to differ on any parameter, other than the fact that they have been randomly assigned to either group. As detailed in the introduction in Chapter 6, MR can be thought of as 'Nature's randomised trial'[287], as alleles are randomly allocated at conception. Thus, this provides support for the application of MR in its ability to assess causality.

The ELSA bidirectional study (Chapter 3), which found that BMI led to small changes in self-reported sleep duration in older adults over four-year follow-up formed the basis of the rationale for the MR study in Chapter 6. Findings from all of the three MR approaches performed in 142,209 adults suggested that we are

still uncertain about whether the relationship between BMI and self-reported sleep duration is causal. This was judged to be the case, after taking into account the whole picture from all of the MR methods (inverse-variance weighted, MR-Egger and weighted median) that were implemented. MR results showed that there was a high proportion of horizontal pleiotropy. Then, follow-up analyses using genetic correlation and comprehensive polygenic risk scoring analyses revealed that the BMI SNPs (with inclusion of only independent SNPs) explained a very small amount of the variance in sleep duration.

Also, it must be noted that, as mentioned earlier (Chapter 6), a core assumption of MR is that the genetic variants for the exposure (in this case, BMI) should not be associated with confounders of the relationship under study. It was not possible to test this in the MR analyses used in this thesis because a two-sample MR design was implemented and the majority of the data came from summary statistics from the UK Biobank GWAS study[323]. It was not possible to access the UKB IPD for this study.

Triangulation of findings has been highlighted as crucial in aetiological epidemiology[324]. Triangulation means that different methods are applied in order to strengthen causal conclusions, particularly if various approaches point towards the same conclusion. In attempting to triangulate findings, Lawlor and colleagues[324] suggest implementing some of the following methods to the same research question: multivariable regression modelling, cross-cohort comparisons, MR, instrumental variable (IV) analysis of intermediate (exposure) in an RCT, negative control studies, RCTs and within-sibship comparisons. Of these approaches, MR and multivariable regression modelling were used in this thesis, yet there is scope to apply some of the other methods to BMI and sleep duration.

One suggestion could be performing a cross-cohort comparison. For example, the ELSA bidirectional results from Chapter 3 with results of the same analyses from another ageing cohort, but potentially from another ethnicity or culture. The cross-cohort comparison could be performed in for example, the Irish Longitudinal Study on Ageing (TILDA)[325], as they have collected self-reported sleep duration and weight/height in a similar way to ELSA and also collect data in

Irish people who are over the age of 50. Specifically, TILDA administered a question about sleep duration that is very similar to the one in ELSA, as it asks about how many hours participants sleep on a weeknight. Participants in TILDA also undergo measurements of their height and weight by a research nurse, which is also how these measures are collected in ELSA.

Another relevant approach could be a within-sibship comparison to compare outcome data (for example, sleep duration) from sibling pairs that are discordant for the exposure (for example, BMI), whilst controlling for observed and unobserved shared (familial) confounders. One such study in the G1219 Longitudinal Twin Study cohort used a discordant twin design that investigated the relationship between BMI and sleep quality[326]. This study found that siblings who had a higher BMI reported poorer sleep quality, but the effect of sibling difference in BMI on sleep quality was attenuated (not significant) when adjusting for depression, anxiety and general health. Also, participants who had more symptoms of any of these three conditions were more likely to report poorer sleep quality. This study is an interesting example of how the discordant twin design removes confounding by all factors that are completely shared by twin pairs, yet it is unable to shed any light on causation if the data used are cross-sectional.

7.2.3 Confounding

As mentioned in Chapter 1, confounding remains an issue central to observational epidemiology. In Chapter 3, when investigating the bidirectional relationship of BMI and sleep duration, models were adjusted for a wide range of demographics, health problems and health behaviours, after which a small effect of BMI on sleep duration (over 4-year follow-up) remained. As with the majority of observational research, these analyses did not control for all possible confounding and therefore, there may be other unobserved factors that might affect the association between BMI and self-reported sleep duration in older adults. However, the MR analyses performed in Chapter 6 do not suffer from the same issues with confounding as observational methods, as MR uses genetic variants as instrumental variables and these are unlikely to be associated with

potential confounders of the relationship between BMI and sleep duration. Therefore, perhaps greater weight should be given to the MR findings. However, as mentioned above, it was not possible to examine associations between the BMI SNPs and confounders, given that summary-data was used in combination with IPD to maximise the sample size. This is because MR studies require large sample sizes to ensure precision of estimates[327], as often genetic variants used in MR (as IVs for the exposure) do not have large effects, which results in a weak instrument and the need for very large samples[327].

Another important point is that in multiply adjusted models, no cross-sectional relationship was observed between BMI and sleep duration in ELSA (Chapter 3), which is in contrast to the large-scale systematic review[106], and meta-analysis[107] published in 2008. One of the main reasons for this discrepancy in findings may be related to confounding. For example, of the studies included in this review, and meta-analysis, at least three made no adjustments for *any* covariates, but still found a cross-sectional association between BMI and sleep duration in adults[64,328,329], whilst some other studies included only sex as a covariate[116,328,330,331], with two others that adjusted for age and sex[180,332], and one study adjusted for sex and sleep disorders[120]. Few studies included a more comprehensive list of covariates[117,118,132,333,334] and in fact, these studies' findings were generally less straightforward than those with an inferior set of covariates. Specifically, one of these studies found a relationship between BMI and sleep duration in one sex, but not the other; another of them observed an association between long sleep duration and BMI, as opposed to short sleep duration and BMI. Notable is also that in their pooled analyses of cross-sectional studies in adults, Cappuccio and colleagues[107] included exclusively unadjusted estimates, due to such inconsistencies in covariates across studies. It was the result of this pooled analysis which led them to conclude that cross-sectional studies of BMI and sleep duration in adults show a robust association between the two. Therefore, it is possible that in several earlier published cross-sectional studies of BMI and sleep duration this relationship is confounded by important factors that have not been accounted for.

7.2.4 Generalisability

It is important to consider the generalisability of the findings of this thesis, particularly in terms of the samples used. It has been suggested that findings from the ALSPAC mothers are generally applicable to the majority of UK women, as well as women in other high-income countries. However, the majority of families are White and there are slightly lower levels of deprivation as compared to the general population[193]. ELSA is broadly representative of the English population, when comparing socio-demographic characteristics with national census data[200]. UKHLS is an annual survey, which is representative of the UK population[335]. The UKB study comprises a very large sample of 500,000 UK adults, but it is important to acknowledge the potential selection bias that it suffers from. This is because, although the sample is large and was recruited both fast and efficiently, the response rate was only 5.5%, which is particularly low[336]. One of the main impacts that this has on health research is that due to selective probabilities that operate in drawing particular individuals to participate in this kind of study, diseases may appear to be associated when they are not[336].

In summary, from judging the evidence presented here, it should be concluded that the true relationship between BMI and sleep duration is small in magnitude in both children and adults, irrespective of whether subjective or objective sleep duration is used; it is unlikely to be causal in nature, and these phenotypes do not possess much underlying common genetic aetiology.

7.3 RECOMMENDATIONS FOR POLICY AND PRACTICE

7.3.1 Recommendations for adults

This work found no association between sleep duration and changes in BMI in adults over the age of 50. This finding is in line with at least two previous studies in older adults that found no relationship between sleep duration and changes in weight[235,236].Thus, the suggestion that older people should sleep for longer to prevent weight gain, needs revisiting.

As results from this thesis suggest that the negative, prospective association between BMI and self-reported sleep duration was very small in older adults, on average, this age group may not benefit from interventions that target their weight in order to improve sleep duration. Since one of the potential underlying explanations for the association between BMI and sleep duration could be related to obstructive sleep apnoea (OSA) (discussed in Chapter 3), it is possible that individuals with OSA who are overweight/obese may benefit from interventions that target their weight with the aim of also improving their sleep duration. However, measures of OSA were not collected in waves 4 or 6 of ELSA and thus, not included in these analyses.

7.3.2 Recommendations for children

Meta-analyses of prospective studies to date largely suggest that public health strategies should recommend the following, for obesity prevention: *sufficient* sleep[135,152,157]; regular[153] or earlier bedtimes and later wake-up times[135]; and/or behavioural interventions aimed at increasing duration of sleep[151]. Paediatric findings from this thesis are in support of the only two previous bidirectional studies of BMI and sleep duration in children[137,138] and suggest that designing interventions to increase sleep duration to prevent obesity may be premature. Therefore, it would perhaps be more practical for public health efforts to focus on ensuring that health professionals are aware of up-to-date guidelines and recommendations on children getting *sufficient* sleep, as this comes with many other health benefits. It is important, however, to ensure that health professionals do not simply recommend longer sleep duration, as sleeping for too many hours has also been associated with poorer health outcomes in adulthood[61,62,63]. Findings from this work do not suggest that weight loss interventions would help prevent short sleep in children and as such, it is important to inform these children's parents/caregivers that improvements in sleep are unlikely as a result of this kind of intervention.

7.4 FUTURE DIRECTIONS

7.4.1 Future directions for observational epidemiology

7.4.1.1 Further prospective analyses

Bidirectional analyses in both Chapters 3 and 4 exploited all of the prospective data that were available for BMI and sleep duration in ELSA and TESS, respectively. Despite the fact that the TESS analyses yielded null findings in both directions, future research should also investigate this association with further prospective data as it becomes available in TESS. This is important because previous bidirectional studies have also failed to find these effects in children (of White ethnicity)[137,138]. Similarly, the analyses performed in Chapter 3 in ELSA, could be repeated using data from wave 8, which is now available and would provide an eight-year follow-up period from when sleep duration was first asked. This may help shed more light on the relationship between BMI and sleep duration in older age, as there was not a lot of change in either of these phenotypes over four years of follow-up, it is possible that greater change might be observed over a longer time period.

7.4.1.2 Replication of findings

In relation to the null findings in TESS (Chapter 4), these analyses should be performed in an independent cohort, as the two previous studies to perform bidirectional analyses of BMI and sleep duration used self-reported sleep duration and are thus, might not be completely comparable with the TESS findings, although the same conclusion was reached in all three studies. Also, future work ought to investigate the bidirectional association of BMI and objective sleep duration in children from other ethnic backgrounds, as well as in a sample that is perhaps less lean and healthy and in which the socioeconomic background of participants is more diverse (most participants were from affluent families). As Norway is a social democracy and thus, provides a lot of support for families in general, it may be that to observe an effect between BMI and sleep duration, we need to examine this relationship in samples from countries with a greater degree of inequity, such as the USA or the UK, for example.

Although one of the two previous bidirectional studies on BMI and sleep duration in children used data from a UK cohort including both Whites and South Asians and found only an association in the latter participants[138], the BiB study is not representative of the entire UK population, as the city of Bradford has particularly high levels of poverty[337]. The other only paediatric bidirectional study of BMI and sleep duration to date, had null findings in both directions and analysed data from White Australian children[137].

The findings in ELSA (Chapter 4) should also be replicated, perhaps using data from another ageing study, such as TILDA. These analyses should also be performed using data in older adults from other ethnic groups, as 98% of ELSA are White individuals. For example, similar bidirectional analyses could be performed in for example, the Longitudinal Ageing Study in India[338], as this study was designed to be comparable to HRS. Importantly, some evidence suggests that there are ethnic differences in adult sleep duration, with non-White ethnic groups at increased risk of being short/long sleepers, in comparison to White individuals[339].

7.4.1.3 The association of BMI with sleep parameters, other than sleep duration

Future research should investigate other sleep parameters, such as quality, chronotype, bedtime, disturbance and latency and their potential effects on BMI and/or whether BMI might differentially affect any of these measures. For the work undertaken in this thesis it would have proved difficult to perform genetic analyses (Chapters 5 and 6) of other sleep measures apart from duration using the datasets for which access was granted. This is because studies do not measure these phenotypes uniformly, as there are at least a handful of validated questionnaires for the assessment of distinct sleep parameters. Thus, harmonisation of data across studies becomes difficult, which is necessary to have adequate power to perform both observational and genetic epidemiological analyses. However, studies such as the UKB provide a way to overcome some of these issues, as both genetic and phenotypic data are available on 500,000 individuals from the general population. The UKB has also collected self-reported

sleep measures of chronotype, daytime napping, narcolepsy (daytime dozing), sleeplessness/insomnia, snoring and how difficult participants find it to get up in the mornings. Thus, it would be possible to perform MR analyses of BMI on these other sleep phenotypes, as to date, no papers have examined the potential causal associations using the entire UKB sample of 500,000 individuals.

7.4.1.4 Self-reported vs. objective sleep duration

As mentioned earlier, the use of self-reported sleep duration may not be optimal for several reasons. It is prone to measurement error, as individuals may not provide accurate reports of their average sleep duration, and agreement with objective measurements is only moderate[96–98]. Although the gold-standard method is PSG, this remains expensive and needs to be administered in a lab setting, which means that it is still not an option for large-scale epidemiological studies. Lab studies may also lack ecological validity[340]. However, waist and wrist actigraphy provide an alternative that is in between self-reported sleep duration and PSG, as they are not as costly as PSG, but are an improvement on subjective sleep reports. Some evidence suggest that actigraphy is in fact, more reliable than PSG, as data are collected over a period of several days and not just one or two nights in a laboratory[102]. However, both methods still only involve the collection of sleep data over limited periods of time, which might not be typical for that individual, but this is why usually a questionnaire is administered alongside actigraphy. Another idea is whether in the near future it might be possible to gain access to objectively-measured habitual sleep patterns in the population, using data from for example, wrist-worn devices like the Apple Watch, or similar. This kind of device routinely measures people's sleep patterns when worn during the night and is able to record and track trends in sleep.

Thus, future work investigating BMI in relation to sleep duration should use objective measurements, where possible. Studies such as the the UK Biobank are beginning to collect actigraphic sleep duration from large numbers of individuals.

7.4.1.5 The association between sleep duration and other measures of adiposity

Future work in this area should also ascertain whether sleep duration is also associated with other measures of adiposity. In Chapter 3, it was confirmed that in ELSA there was a prospective relationship between BMI *and* WC with sleep duration, which strengthened the findings, particularly as the magnitude of the effect was consistent for BMI and WC. However, the TESS analyses (Chapter 4) were performed using BMI only (TESS did not collect WC); thus, future work could involve analysing other measures of adiposity in relation to sleep duration in children, such as for example, body-fat percentage. This is important, as BMI is unable to distinguish muscle mass from fat mass. In terms of the relationship between BMI and sleep duration, one possibility is that there may be confounding by specific body composition measures, but not due to fatness. For example, having greater muscle mass increases BMI, but not because of fat. Longitudinal evidence in 244 children from New Zealand showed that objectively-measured sleep duration at ages 3y to 5y (an average of the two was taken) was negatively associated with fat mass index at age 7 in multiply-adjusted models (sex, maternal education, maternal BMI, income, ethnicity, birth weight, smoking during pregnancy, physical activity, TV viewing, fruit-vegetable intake and non-core foods intake)[341]. The authors also found a negative prospective relationship between sleep duration and BMI, but their findings suggested that these differences in body weight (between children with varying hours of sleep) were accounted for by increased fat mass deposition.

7.4.2 Future directions for genetic epidemiology

7.4.2.1 Advances in Mendelian randomisation methodology

MR is constantly being developed by statisticians who have now been working with it for a number of years. Although the causal analyses conducted in this thesis employed three MR methods and findings are in line with those from the largest previous study, which found no causal effect of BMI on self-reported sleep duration, there is scope for future MR work.

Importantly, there have been recent advances in MR methods, which could be applied in the future. One recent area of development is a novel approach to dealing with horiztontal pleiotropy in MR[342]. Van Kipperslius and Rietveld[342] call this method Pleiotropy robust Mendelian randomisation (PRMR), as it is able to estimate the degree of pleiotropy and also correct for it, whilst producing unbiased estimates of a causal effect between an exposure and outcome of interest. Thus, in addition to IVW, MR-Egger and the weighted median methods, future MR studies that estimate the causal effect of BMI on sleep parameters could also use the PRMR approach, as an additional sensitivity analysis with the aim of ensuring that all approaches lead to the same conclusion.

Furthermore, future MR studies in this area could take into account potential mediators, which is another area of recent progress[343,344]. Of particular importance here is the distinction between the total effect and the direct effect, as the latter suggests that the potential causal pathway between the exposure and outcome may operate partly through a mediator (indirect effect)[344]. The validity of this approach has recently been shown using the example of age at menarche and risk of breast cancer, whereby BMI mediates this association, although a direct effect was also observed, independently of BMI[344].

This is potentially an important advance, as, of late, it has been suggested that MR may not be a valid causal analysis tool when the exposure that is instrumented is not closely related to a physiological phenotype[345]. This was recently argued in response to a large-scale MR study which found a causal relationship between educational attainment and CHD[345]. The argument put forward was that there must be mediators on the causal pathway from educational attainment causing CHD, thus the method discussed above provides a way of testing this. Therefore, it is recommended that future studies that use MR estimate the causal relationship between BMI and a sleep-related outcome potentially investigate relevant mediators that may lie on the causal pathway. For example, a recent study highlighted the importance of specific energy balance-related behaviours in this context[346]. Using data from 5,900 adults from five European countries (including the UK), their findings showed that work-related

sedentary behaviour significantly mediated the relationship between self-reported sleep duration and BMI. Although they found that dietary habits and physical activity were not significant mediators, an important limitation was that the questions asked about dietary habits were relatively simple and did not capture for example, caloric intake. Their data were also cross-sectional, which precluded any conclusions about temporality and of course, causation. Therefore, it would be of interest to test this using MR to examine whether BMI genetic variants are causally associated with sleep duration, via their effect on work-related sedentary behaviours. The main hypothesis would be that a higher BMI is associated with increased work-related sedentary behaviour, which in turn leads to shorter sleep duration.

In addition, future research in this area should consider a multivariable MR approach to further investigate the causal relationship between adiposity and sleep, and sleep and adiposity. This is important, due to the limitations of both BMI and sleep duration as phenotypes (as discussed earlier). Multivariable MR uses several genetic variants associated with multiple relevant risk factors of interest to simultaneously estimate the causal relationship between each risk factor and the outcome[347]. A future multivariable MR study in the context of this thesis' topic may include multiple measures of adiposity, such as waist circumference, waist-hip-ratio and body fat percentage and simultaneously estimate their individual causal effects on a sleep phenotype of interest (other than duration). Similarly, a multivariable MR study in the other direction may investigate the causal effect of for example, sleep quality, timing and bedtime on a measure of adiposity.

7.4.2.2 New developments in molecular methods to estimate heritability

Recently, a new method for the estimation of SNP heritability was proposed. Speed and colleagues[285] analysed imputed genetic data for 19 physical, neurological and psychiatric phenotypes. They derived a model that describes with more certainty how heritability may vary depending on MAF, LD and genotype uncertainty. Their model led to estimates of SNP heritability that were on average, 43% greater than those produced by GCTA. In future, it is important

that SNP heritability is estimated using this new method, as a standalone, or in addition to GCTA and LDSC.

7.4.2.3 Using MR to investigate causality of BMI and sleep parameters other than duration

Meta-analyses and systematic reviews that conclusively find a prospective observational association between BMI and other sleep parameters (timing, bedtime, disturbance, quality, latency) other than duration, and/or vice versa (how sleep parameters might influence BMI) are still needed. This is important, as MR studies are usually carried out once an observational relationship between a modifiable exposure and an outcome of interest, has been established. An interesting future direction could subsequently be to investigate whether there is a causal relationship between BMI and such sleep parameters. MR analyses in the opposite direction will prove difficult until replicated genetic variants for such sleep parameters have been found.

7.4.2.4 Application of MR to investigate the causal effect of BMI on sleep duration in younger populations

Although the findings from Chapter 4 suggested that BMI does not predict changes in sleep duration in children, these findings, using objective sleep measurements, should be replicated in a non-Norwegian paediatric sample, to draw definitive conclusions. It is also important that the causal effect of BMI on sleep duration is investigated in children, adolescents and young adults, as the MR analyses in this thesis were only performed in middle-aged and older individuals. An important rationale for this is that the effects of the BMI SNPs on BMI are stronger in younger age groups[348,349]. This would be possible, given that there are now several paediatric cohorts with measures of sleep duration, BMI and GWAS data.

7.5 OVERALL CONCLUSIONS

This thesis performed a comprehensive investigation of the relationship between BMI and sleep duration. As the first study to investigate this bidirectional relationship in adults, findings revealed no prospective association of sleep

duration and BMI, in accordance with two earlier epidemiological studies[235,236], yet BMI predicted very small changes in self-reported sleep duration over four years. There is no prospective relationship between BMI and objective sleep duration, in either direction, in middle childhood, in line with previous null bidirectional paediatric findings[137,138]. Common genetic variants implicated in sleep duration are likely to have very small effects and thus, can prove difficult to detect using GWAS. This work also suggested, via Mendelian randomisation analyses, that there is still a degree of uncertainty regarding whether the association between BMI and self-reported sleep duration is causal or not. Also, analyse showed that these phenotypes possess only a small amount of shared genetic aetiology. Overall, this thesis made novel contributions in dissecting this complex relationship and investigated it from a previously-unexplored perspective, by using observational epidemiology with complementary genetic epidemiological methods. Taken together, the results of this thesis suggest that the association of BMI with sleep duration is small in magnitude, if present at all. Therefore, these, and other earlier findings strongly suggest that the public health focus on improving sleep duration as an obesity prevention initiative might need reviewing.

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8 APPENDICES

8.1 ADDITIONAL TABLES FOR CHAPTER 3

Tables 8.1 to 8.4 present the results of all cross-sectional associations between body mass index (BMI), waist circumference (WC) and sleep duration in ELSA, with varying levels of adjustment for covariates. As previously discussed in Chapter 3 of this thesis, these results show that health behaviours and health problems played a role in the attenuation of these associations between adiposity and sleep duration in ELSA.

Table 8.1 Cross-sectional models of BMI/WC and sleep duration in ELSA, adjusted for demographics

Sleep duration	B (minutes)	95% CI	P
BMI	-0.44	-0.014 – -0.000	0.033
Age	0.23	-0.015 - -0.014	0.066
Sex	-5.21	-9.314 - -1.108	0.013
Wealth	7.63	6.118 – 9.139	<0.001
Ethnicity	-15.10	-30.535 – 0.336	0.055

Sleep duration	B (minutes)	95% CI	P
WC	-0.18	-0.006 – -0.000	0.034
Age	0.24	-0.350 - -0.014	0.050
Sex	-7.11	-11.567 - -2.659	0.002
Wealth	7.63	6.117 - 9.136	<0.001
Ethnicity	-15.22	-30.660 - 0.211	0.053

Note. These models were adjusted for age, sex, wealth and ethnicity; B (Unstandardized coefficient) = difference in sleep duration (minutes) per difference in WC (cm), 95% CI= 95% confidence interval, P= regression p-value.

Table 8.2 Cross-sectional models of BMI/WC and sleep duration in ELSA, adjusted for demographics and health behaviours

Sleep duration	B (minutes)	95% CI	P
BMI	-0.39	-0.013 – -0.000	0.065
Age	0.28	-0.797 – 0.040	0.026
Sex	-4.92	-9.098 - -0.743	0.021
Wealth	6.91	5.317 – 8.509	<0.0001
Ethnicity	-15.29	-30.747 - 0.166	0.053
Physical activity	4.19	1.257 - 7.126	0.005
Alcohol consumption	0.41	-4.619 – 5.437	0.873
Smoking status	-2.62	-5.760 - 0.518	0.102

Sleep duration	B (minutes)	95% CI	P
WC	-0.14	-0.005 – 0.000	0.087
Age	0.30	0.047 - 0.547	0.020
Sex	-6.45	-11.015 - -1.888	0.006
Wealth	6.94	5.344 – 8.531	<0.001
Ethnicity	-15.36	-30.816 – 0.100	0.051
Physical activity	4.13	1.183 - 7.075	0.006
Alcohol consumption	0.53	-4.497 - 5.553	0.837
Smoking status	-2.50	-5.631 - 0.633	0.118

Note. These models were adjusted for age, sex, wealth, ethnicity, physical activity, alcohol consumption and smoking status; B (Unstandardized coefficient) = difference in sleep duration (minutes) per difference in WC (cm), 95% CI= 95% confidence interval, P= regression p-value.

Table 8.3 Cross-sectional models of BMI/WC and sleep duration in ELSA, adjusted for demographics and health problems

Sleep duration	B (minutes)	95% CI	P
BMI	-0.29	-0.012 – -0.002	0.167
Age	0.31	0.067 – 0.557	0.013
Sex	-3.19	-7.291 - 0.904	0.127
Wealth	6.38	4.855 - 7.907	<0.001
Ethnicity	-10.18	-25.535 - 5.178	0.194
Longstanding limiting illness	-4.15	-8.337 - 0 .036	0.052
Depressive symptoms	-19.37	-23.86 - -14.88	<0.001

Sleep duration	B (minutes)	95% CI	P
WC	-0.11	-0.005 – -0.000	0.198
Age	0.32	0.077 – 0.567	0.010
Sex	-4.35	-8.817 - 0.105	0.056
Wealth	6.39	4.870 - 7.919	<0.001
Ethnicity	-10.26	-25.620 - 5.089	0.190
Longstanding limiting illness	-4.09	-8.300 - 0 .106	0.056
Depressive symptoms	-19.35	-23.844 - -14.864	<0.001

Note. These models were adjusted for age, sex, wealth, ethnicity, longstanding limiting illness and depressive symptoms; B (Unstandardized coefficient) = difference in sleep duration (minutes) per difference in WC (cm), 95% CI= 95% confidence interval, P= regression p-value.

Table 8.4 Cross-sectional models of BMI/WC and sleep duration in ELSA, fully adjusted for demographics, health behaviours and health problems

Sleep duration	B (minutes)	95% CI	P
BMI	-0.28	-0.012 – 0.002	0.190
Age	0.32	0.090 – 0.589	0.008
Sex	-3.12	-7.294 - 1.048	0.142
Wealth	6.03	4.428 - 7.626	<0.001
Ethnicity	-10.49	-25.88 - 4.893	0.181
Physical activity	2.45	-0.506 - 5.401	0.104
Alcohol consumption	-0.04	-5.032 - 4.955	0.988
Smoking status	-1.62	-4.749 - 1.506	0.310
Longstanding limiting illness	-3.59	-7.814 – 0.634	0.096
Depressive symptoms	-18.84	-23.357 - -14.317	<0.001

Sleep duration	B (minutes)	95% CI	P
WC	-0.10	-0.004 – 0.001	0.270
Age	0.35	0.099 - 0.597	0.006
Sex	-4.11	-8.672 - 0.454	0.078
Wealth	6.05	4.455 - 7.648	<0.001
Ethnicity	-10.53	-25.921 - 4.856	0.180
Physical activity	2.42	-0.537 - 5.388	0.109
Alcohol consumption	0.05	-4.944 - 5.038	0.985
Smoking status	-1.53	-4.651 - 1.588	0.336
Longstanding limiting illness	-3.58	-7.813 – 0.657	0.098
Depressive symptoms	-18.84	-23.358 - -14.318	<0.001

Note. These models were adjusted for age, sex, wealth, ethnicity, physical activity, alcohol consumption, smoking status, longstanding limiting illness and depressive symptoms; B (Unstandardized coefficient) = difference in sleep duration (minutes) per difference in WC (cm), 95% CI= 95% confidence interval, P= regression p-value.

8.2 ADDITIONAL FIGURES AND TABLES FOR CHAPTER 5

Figures 8.1, 8.3 and 8.5 present Manhattan plots of individual GWAS analyses in the ALSPAC, ELSA and UKHLS studies. It can be seen that no genome-wide significant SNPs were found to be associated with self-reported sleep duration in any of the three studies. Figures 8.2, 8.4 and 8.6 depict Q-Q plots of GWAS p-values in each study and in line with the λ_{GC} for each study (reported in Chapter 5) there appear to be no issues with underlying population stratification in any of these studies. Figures 8.7 and 8.8 present network plots for genes that are co-expressed with the SIPA1L3 and POLR3G genes, as the 'top' 34 SNPs that emerged as suggestive in the meta-GWAS were either in or nearby one of these genes. Table 8.5 presents proxy SNPs that are in high LD with the 'top' 34 SNPs in the meta-GWAS, at an R^2 of 0.8 or above.

Figure 8.1 Manhattan plot of sleep duration GWAS results in ALSPAC

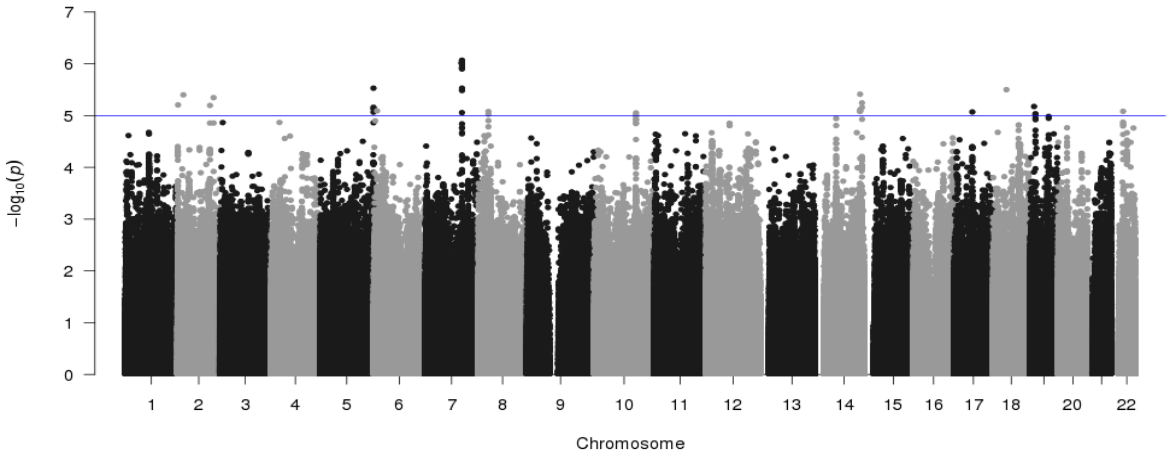

Figure 8.2 Q-Q plot of GWAS p-values in ALSPAC

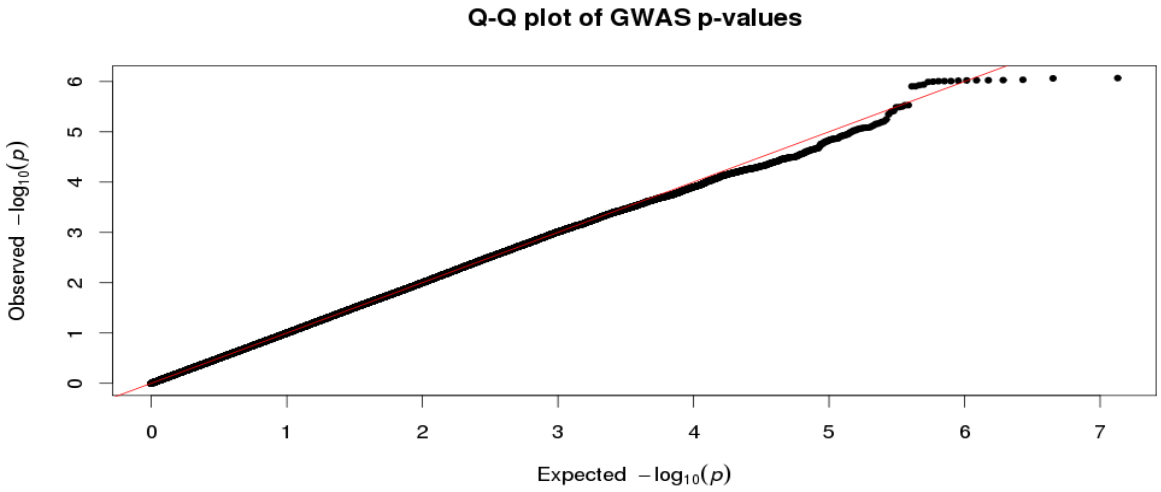

Figure 8.3 Manhattan plot of sleep duration GWAS results in ELSA

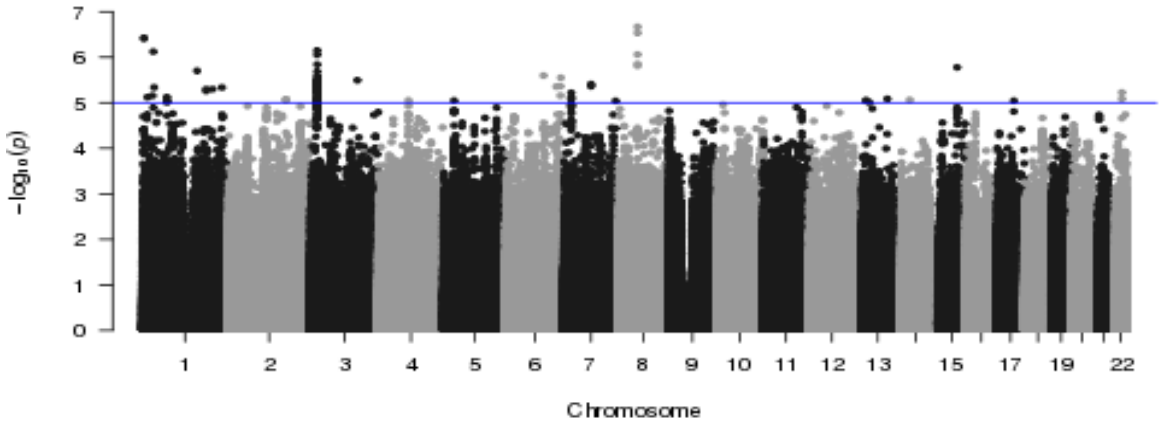

Figure 8.4 Q-Q plot of GWAS p-values in ELSA

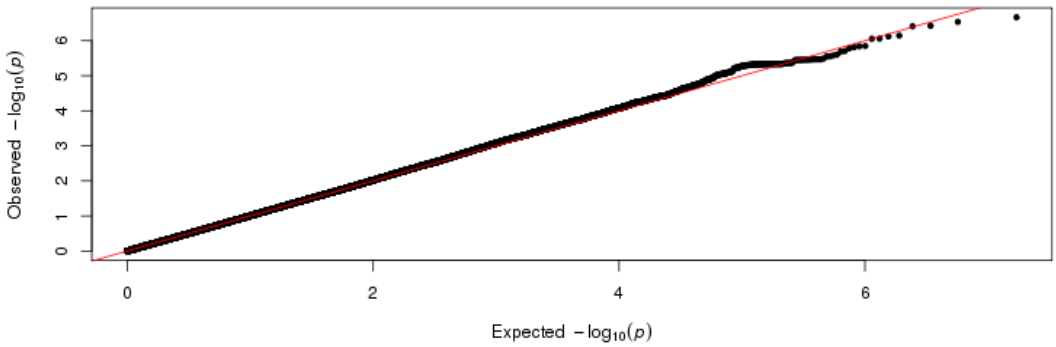

Figure 8.5 Manhattan plot of sleep duration GWAS results in UKHLS

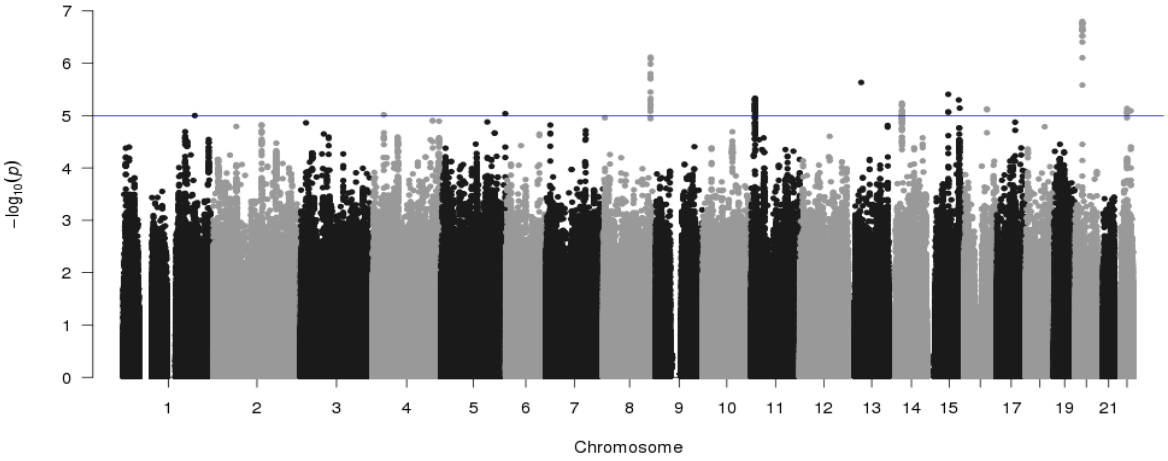

Figure 8.6 Q-Q plot of GWAS p-values in UKHLS

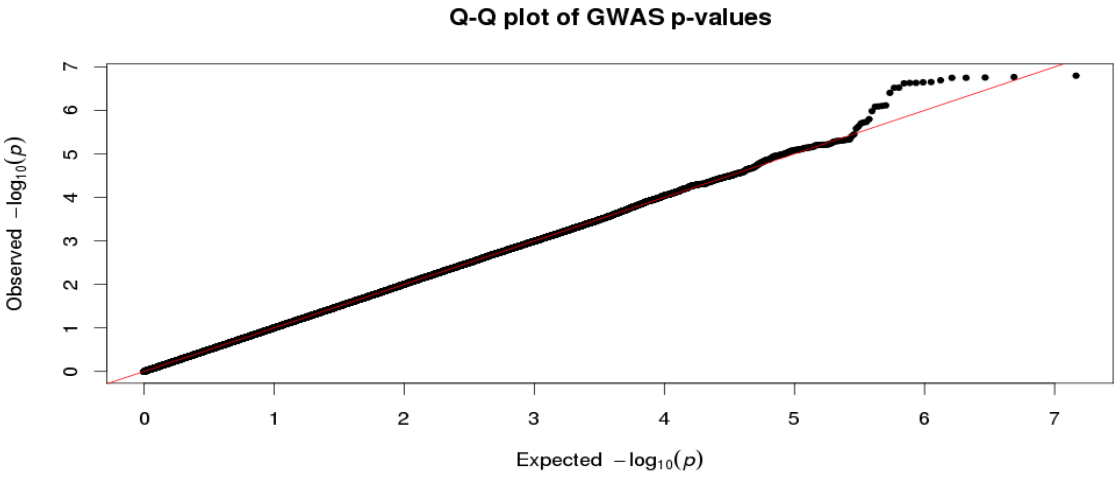

Figure 8.7 Network for genes co-expressed with SIPA1L3 gene

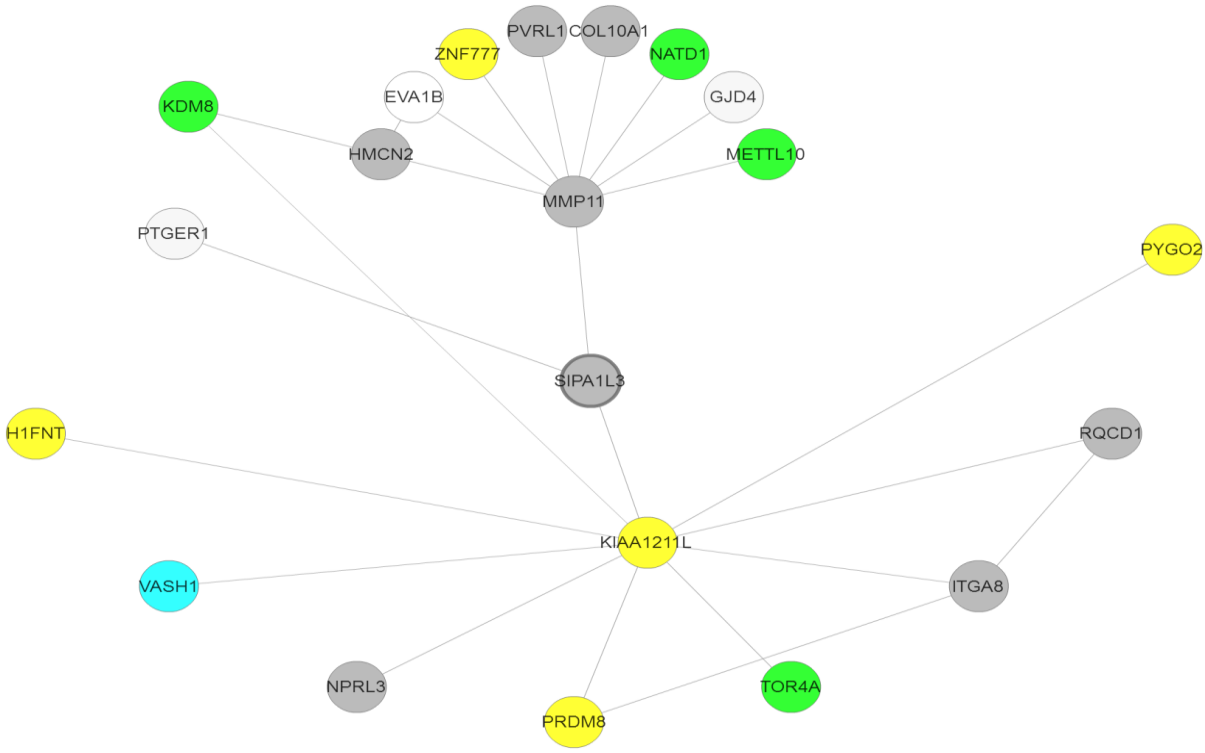

Figure 8.8 Network for genes co-expressed with POLR3G

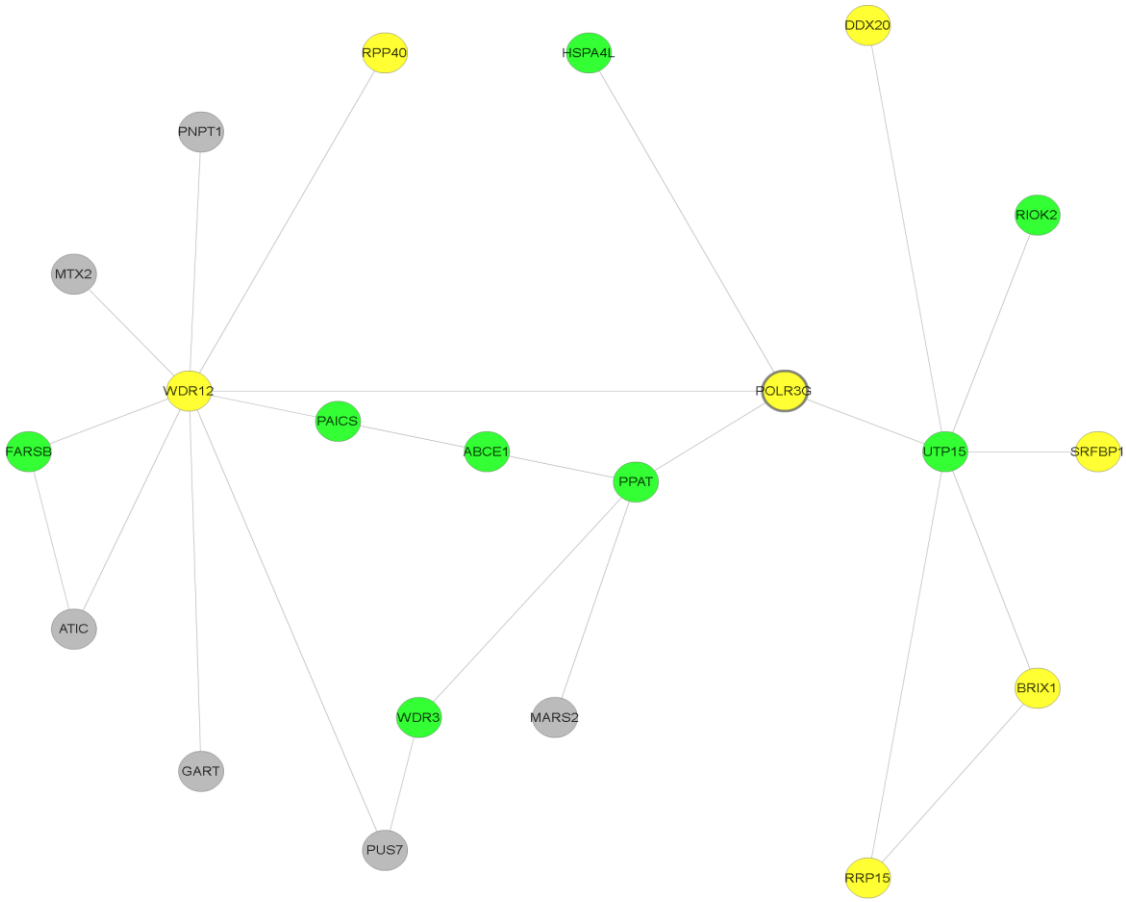

Table 8.5 Pairwise LD for 'top' SNPs in meta-analysis of ALSPAC, ELSA and UKHLS

SNP	Proxy	Distance (base pairs)	R-squared	Chromosome
rs8109799	rs332849	4048	0.967	19
rs8109799	rs4802251	5429	0.967	19
rs8109799	rs8111180	9743	0.967	19
rs8109799	rs332864	13090	0.901	19
rs9941474	rs8111180	1034	1	19
rs9941474	rs4802251	5348	1	19
rs9941474	rs332849	14825	1	19
rs9941474	rs332864	23867	0.934	19
rs332849	rs4802251	9477	1	19
rs332849	rs8111180	13791	1	19
rs332849	rs332864	9042	0.934	19
rs6508765	rs4802251	3702	1	19
rs6508765	rs332849	5775	1	19
rs6508765	rs8111180	8016	1	19
rs6508765	rs332864	14817	0.934	19
rs855632	rs8111180	26901	0.935	19
rs855632	rs4802251	31215	0.935	19
rs855632	rs332849	40692	0.935	19
rs855632	rs332864	49734	0.87	19
rs332848	rs332849	1077	1	19
rs332848	rs4802251	8400	1	19
rs332848	rs8111180	12714	1	19
rs332848	rs332864	10119	0.934	19
rs8101826	rs4802251	3471	1	19
rs8101826	rs332849	6006	1	19
rs8101826	rs8111180	7785	1	19
rs8101826	rs332864	15048	0.934	19
rs8100144	rs4802251	454	0.934	19
rs8100144	rs8111180	3860	0.934	19
rs8100144	rs332849	9931	0.934	19
rs8100144	rs332864	18973	0.868	19
rs2099340	rs8111180	1033	0.967	19
rs2099340	rs4802251	3281	0.967	19
rs2099340	rs332849	12758	0.967	19
rs2099340	rs332864	21800	0.901	19
rs8109695	rs332849	4011	0.935	19

rs8109695	rs4802251	5466	0.935	19
rs8109695	rs8111180	9780	0.935	19
rs8109695	rs332864	13053	0.87	19
rs2569412	rs332849	3594	0.967	19
rs2569412	rs332864	5448	0.967	19
rs2569412	rs4802251	13071	0.967	19
rs2569412	rs8111180	17385	0.967	19
rs332864	rs332849	9042	0.934	19
rs332864	rs4802251	18519	0.934	19
rs332864	rs8111180	22833	0.934	19
rs332855	rs332864	8487	0.967	19
rs332855	rs332849	17529	0.967	19
rs332855	rs4802251	27006	0.967	19
rs332855	rs8111180	31320	0.967	19
rs4802251	rs8111180	4314	1	19
rs4802251	rs332849	9477	1	19
rs4802251	rs332864	18519	0.934	19
rs332856	rs332864	8235	0.967	19
rs332856	rs332849	17277	0.967	19
rs332856	rs4802251	26754	0.967	19
rs332856	rs8111180	31068	0.967	19
rs332858	rs332864	5614	0.967	19
rs332858	rs332849	14656	0.967	19
rs332858	rs4802251	24133	0.967	19
rs332858	rs8111180	28447	0.967	19
rs8111180	rs4802251	4314	1	19
rs8111180	rs332849	13791	1	19
rs8111180	rs332864	22833	0.934	19
rs332843	rs332849	2687	1	19
rs332843	rs4802251	6790	1	19
rs332843	rs8111180	11104	1	19
rs332843	rs332864	11729	0.934	19
rs332844	rs332849	1907	1	19
rs332844	rs4802251	7570	1	19
rs332844	rs8111180	11884	1	19
rs332844	rs332864	10949	0.934	19

8.3 ADDITIONAL FIGURES AND TABLES FOR CHAPTER 6

Table 8.6 below lists all of the BMI SNPs used in the Mendelian randomisation analyses in Chapter 6, with details of proxies used where the target SNP was not available in ELSA, UKB or UKHLS. Proxies were selected at an R^2 threshold of at least 0.8 to ensure that they were in high LD with the target SNP. Table 8.7 presents associations between BMI SNPs (from Locke et al. GWAS) and BMI in GIANT and for comparison, Table 8.8 shows the associations between BMI SNPs and BMI in ELSA and UKHLS (for which individual-level data were available), Table 8.9 presents the pooled associations between these BMI SNPs and BMI in the IPD samples used in the MR analyses (ELSA + UKHLS). UKB was not included, as only summary data were used. Figure 8.9 depicts a bar chart that shows the variance explained (R^2) at different p-value thresholds between the chosen limits of 0.5 and 0.001 in the polygenic risk score (PRS) analyses.

Table 8.6 GWAS SNPS associated with BMI from Locke et al., 2015 and proxies used in ELSA, UK Biobank and UKHLS Mendelian randomisation analyses

SNP ID	Chromosome	Position	Gene	Proxy (r^2 >0.8)		
				ELSA	UKB	UKHLS
rs657452	1	49,362,434	*AGBL4*			
rs11583200	1	50,332,407	*ELAVL4*			
rs2820292	1	200,050,910	*NAV1*			
rs11126666	2	26,782,315	*KCNK3*			
rs11688816	2	62,906,552	*EHBP1*			rs2539984
rs1528435	2	181,259,207	*UBE2E3*			
rs7599312	2	213,121,476	*ERBB4*			
rs6804842	3	25,081,441	*RARB*			
rs2365389	3	61,211,502	*FHIT*			
rs3849570	3	81,874,802	*GBE1*	rs7620240		
rs16851483	3	142,758,126	*RASA2*			
rs17001654	4	77,348,592	*SCARB2*			
rs11727676	4	145,878,514	*HHIP*			
rs2033529	6	40,456,631	*TDRG1*		rs1579557	
rs9400239	6	109,084,356	*FOXO3*			
rs13191362	6	162,953,340	*PARK2*			
rs1167827	7	75,001,105	*HIP1*			
rs2245368	7	76,446,079	*DTX2P1*			rs7804663
rs2033732	8	85,242,264	*RALYL*			
rs4740619	9	15,624,326	*C9orf93*			
rs6477694	9	110,972,163	*EPB41L4B*			
rs1928295	9	119,418,304	*TLR4*			
rs10733682	9	128,500,735	*LMX1B*			
rs7899106	10	87,400,884	*GRID1*			
rs17094222	10	102,385,430	*HIF1AN*			
rs11191560	10	104,859,028	*NT5C2*			rs5011520
rs7903146	10	114,748,339	*TCF7L2*			
rs2176598	11	43,820,854	*HSD17B12*			
rs12286929	11	114,527,614	*CADM1*			
rs11057405	12	121,347,850	*CLIP1*			
rs10132280	14	24,998,019	*STXBP6*			
rs12885454	14	28,806,589	*PRKD1*			
rs3736485	15	49,535,902	*DMXL2*			
rs758747	16	3,567,359	*NLRC3*			
rs2650492	16	28,240,912	*SBK1*			
rs9925964	16	31,037,396	*KAT8*		rs2288004	
rs1000940	17	5,223,976	*RABEP1*			
rs1808579	18	19,358,886	*C18orf8*			
rs7243357	18	55,034,299	*GRP*			
rs17724992	19	18,315,825	*PGPEP1*			

rs977747	1	47,457,264	*TAL1*		
rs1460676	2	164,275,935	*FIGN*		
rs17203016	2	207,963,763	*CREB1*		
rs13201877	6	137,717,234	*IFNGR1*		
rs1441264	13	78,478,920	*MIR548A2*		
rs7164727	15	70,881,044	*LOC100287559*		
rs2080454	16	47,620,091	*CBLN1*		
rs9914578	17	1,951,886	*SMG6*		
rs2836754	21	39,213,610	*ETS2*		
rs492400	2	219,057,996	*USP37*		
rs16907751	8	81,538,012	*ZBTB10*		
rs9374842	6	120,227,364	*LOC285762*		
rs9641123	7	93,035,668	*CALCR*		
rs9540493	13	65,103,705	*MIR548X2*		
rs4787491	16	29,922,838	*INO80E*		
rs6465468	7	95,007,450	*ASB4*		
rs7239883	18	38,401,669	*LOC284260*		
rs3101336	1	72,523,773	*NEGR1*		
rs12566985	1	74,774,781	*FPGT*		
rs12401738	1	78,219,349	*FUBP1*		
rs11165643	1	96,696,685	*PTBP2*		
rs17024393	1	109,956,211	*GNAT2*		
rs543874	1	176,156,103	*SEC16B*		rs506589
rs13021737	2	622,348	*TMEM18*		
rs10182181	2	25,003,800	*ADCY3*		
rs1016287	2	59,159,129	*FLJ30838*		
rs2121279	2	142,759,755	*LRP1B*		
rs13078960	3	85,890,280	*CADM2*		
rs1516725	3	187,306,698	*ETV5*	rs10513801	
rs10938397	4	44,877,284	*GNPDA2*	rs13130484	
rs13107325	4	103,407,732	*SLC39A8*		
rs2112347	5	75,050,998	*POC5*		
rs205262	6	34,671,142	*C6orf106*		
rs2207139	6	50,953,449	*TFAP2B*		
rs17405819	8	76,969,139	*HNF4G*		
rs10968576	9	28,404,339	*LINGO2*		
rs4256980	11	8,630,515	*TRIM66*		
rs11030104	11	27,641,093	*BDNF*		
rs3817334	11	47,607,569	*MTCH2*		
rs7138803	12	48,533,735	*BCDIN3D*		
rs12016871	13	26,915,782	*MTIF3*		
rs12429545	13	53,000,207	*OLFM4*		
rs11847697	14	29,584,863	*PRKD1*		
rs7141420	14	78,969,207	*NRXN3*		

rs16951275	15	65,864,222	*MAP2K5*
rs12446632	16	19,842,890	*GPRC5B*
rs3888190	16	28,796,987	*ATP2A1*
rs1558902	16	52,361,075	*FTO*
rs12940622	17	76,230,166	*RPTOR*
rs6567160	18	55,980,115	*MC4R*
rs29941	19	39,001,372	*KCTD15*
rs2075650	19	50,087,459	*TOMM40*
rs2287019	19	50,894,012	*QPCTL*
rs3810291	19	52,260,843	*ZC3H4*
rs7715256	5	153,518,086	*GALNT10*
rs2176040	2	226,801,046	*LOC646736*
rs6091540	20	50,521,269	*ZFP64*

Table 8.7 Associations between BMI SNPs and BMI in GIANT

SNP	A1	A2	A1 Frequency	N	Beta	SE	L_95CI	U_95CI	P-value
rs3101336	C	T	0.65	316872	0.033	0.003	0.027	0.039	2.66E-26
rs7243357	T	G	0.87	322107	0.022	0.004	0.014	0.030	3.86E-08
rs11030104	A	G	0.8	322103	0.041	0.004	0.033	0.049	5.56E-28
rs12446632	G	A	0.87	316758	0.04	0.005	0.030	0.050	1.48E-18
rs1516725	C	T	0.91	320644	0.045	0.005	0.035	0.055	1.89E-22
rs16951275	T	C	0.78	322098	0.031	0.004	0.023	0.039	1.91E-17
rs12885454	C	A	0.63	320823	0.021	0.003	0.015	0.027	1.94E-10
rs13021737	G	A	0.88	318287	0.06	0.004	0.052	0.068	<1.0E-40
rs9400239	C	T	0.7	321988	0.019	0.003	0.013	0.025	1.61E-08
rs2287019	C	T	0.85	300921	0.036	0.004	0.028	0.044	4.59E-18
rs16907751	C	T	0.96	307752	0.035	0.007	0.021	0.049	1.25E-07
rs2820292	C	A	0.51	321707	0.02	0.003	0.014	0.026	1.83E-10
rs1928295	T	C	0.58	321979	0.019	0.003	0.013	0.025	7.91E-10
rs12940622	G	A	0.54	322032	0.018	0.003	0.012	0.024	2.49E-09
rs17724992	A	G	0.69	319588	0.019	0.004	0.011	0.027	3.41E-08
rs2033732	C	T	0.76	321406	0.019	0.004	0.011	0.027	4.89E-08
rs9374842	T	C	0.74	322008	0.019	0.004	0.011	0.027	9.67E-08
rs17405819	T	C	0.63	322085	0.022	0.003	0.016	0.028	2.07E-11
rs16851483	T	G	0.09	233929	0.048	0.008	0.032	0.064	3.55E-10
rs6804842	G	A	0.58	321463	0.019	0.003	0.013	0.025	2.48E-09
rs2365389	C	T	0.66	316768	0.02	0.003	0.014	0.026	1.63E-10
rs10132280	C	A	0.67	321797	0.023	0.003	0.017	0.029	1.14E-11
rs1808579	C	T	0.53	322032	0.017	0.003	0.011	0.023	4.17E-08
rs7141420	T	C	0.62	321970	0.024	0.003	0.018	0.030	1.23E-14
rs7599312	G	A	0.71	322024	0.022	0.003	0.016	0.028	1.17E-10
rs2112347	T	G	0.63	322019	0.026	0.003	0.020	0.032	6.19E-17
rs29941	G	A	0.67	321970	0.018	0.003	0.012	0.024	2.41E-08
rs1167827	G	A	0.54	306238	0.02	0.003	0.014	0.026	6.33E-10
rs6091540	C	T	0.73	321975	0.019	0.004	0.011	0.027	8.02E-08
rs12429545	A	G	0.1	312934	0.033	0.005	0.023	0.043	1.09E-12
rs11688816	G	A	0.46	322051	0.017	0.003	0.011	0.023	1.89E-08
rs4740619	T	C	0.53	321887	0.018	0.003	0.012	0.024	4.56E-09
rs7903146	C	T	0.75	322130	0.023	0.003	0.017	0.029	1.11E-11
rs7164727	T	C	0.78	321312	0.018	0.003	0.012	0.024	6.83E-08
rs4787491	G	A	0.61	267491	0.016	0.003	0.010	0.022	2.24E-06
rs9925964	A	G	0.61	318385	0.019	0.003	0.013	0.025	8.11E-10
rs13201877	G	A	0.08	322095	0.023	0.005	0.013	0.033	2.35E-07
rs7239883	G	A	0.32	321909	0.016	0.003	0.010	0.022	1.63E-07
rs6465468	T	G	0.33	307937	0.017	0.004	0.009	0.025	2.30E-06
rs6477694	C	T	0.36	322048	0.017	0.003	0.011	0.023	2.67E-08

rs3810291	A	G	0.63	296261	0.028	0.004	0.020	0.036	4.81E-15
rs11126666	A	G	0.31	321979	0.021	0.003	0.015	0.027	1.33E-09
rs11165643	T	C	0.58	320730	0.022	0.003	0.016	0.028	2.07E-12
rs4256980	G	C	0.73	320028	0.021	0.003	0.015	0.027	2.90E-11
rs2033529	G	A	0.26	321917	0.019	0.003	0.013	0.025	1.39E-08
rs1528435	T	C	0.58	321924	0.018	0.003	0.012	0.024	1.20E-08
rs3849570	A	C	0.37	284339	0.019	0.003	0.013	0.025	2.60E-08
rs13078960	G	T	0.18	322135	0.03	0.004	0.022	0.038	1.74E-14
rs492400	C	T	0.33	321090	0.016	0.003	0.010	0.022	4.17E-07
rs1000940	G	A	0.23	321836	0.019	0.003	0.013	0.025	1.28E-08
rs2836754	C	T	0.65	320231	0.016	0.003	0.010	0.022	4.16E-07
rs977747	T	G	0.47	322086	0.017	0.003	0.011	0.023	8.65E-08
rs17024393	C	T	0.04	297874	0.066	0.009	0.048	0.084	7.03E-14
rs1441264	A	G	0.55	310286	0.018	0.003	0.012	0.024	6.04E-08
rs9914578	G	C	0.17	321126	0.02	0.004	0.012	0.028	8.99E-08
rs17094222	C	T	0.21	321770	0.025	0.004	0.017	0.033	5.94E-11
rs2650492	A	G	0.31	319464	0.021	0.004	0.013	0.029	1.91E-09
rs3736485	A	G	0.43	321398	0.018	0.003	0.012	0.024	7.41E-09
rs12401738	A	G	0.43	322070	0.021	0.003	0.015	0.027	1.14E-10
rs10968576	G	A	0.29	322061	0.025	0.003	0.019	0.031	6.61E-14
rs12286929	G	A	0.43	321903	0.022	0.003	0.016	0.028	1.31E-12
rs7715256	G	T	0.45	322084	0.016	0.003	0.010	0.022	1.70E-07
rs17203016	G	A	0.2	316466	0.021	0.004	0.013	0.029	8.14E-08
rs1016287	T	C	0.33	321969	0.023	0.003	0.017	0.029	2.25E-11
rs11583200	C	T	0.38	322095	0.018	0.003	0.012	0.024	1.48E-08
rs2176040	A	G	0.39	321972	0.014	0.003	0.008	0.020	6.06E-06
rs11191560	C	T	0.06	321893	0.031	0.005	0.021	0.041	8.45E-09
rs3817334	T	C	0.45	321959	0.026	0.003	0.020	0.032	5.14E-17
rs758747	T	C	0.27	308688	0.023	0.004	0.015	0.031	7.47E-10
rs657452	A	G	0.42	313651	0.023	0.003	0.017	0.029	5.48E-13
rs9540493	A	G	0.45	318961	0.017	0.003	0.011	0.023	1.42E-07
rs1460676	C	T	0.22	322089	0.02	0.004	0.012	0.028	8.98E-07
rs3888190	A	C	0.36	321930	0.031	0.003	0.025	0.037	3.14E-23
rs7899106	G	A	0.05	321770	0.04	0.007	0.026	0.054	2.96E-08
rs2121279	T	C	0.12	322065	0.025	0.004	0.017	0.033	2.31E-08
rs205262	G	A	0.27	315542	0.022	0.004	0.014	0.030	1.75E-10
rs543874	G	A	0.27	322008	0.048	0.004	0.040	0.056	2.62E-35
rs6567160	C	T	0.28	321958	0.056	0.004	0.048	0.064	<1.0E-40
rs10182181	G	A	0.5	321759	0.031	0.003	0.025	0.037	8.78E-24
rs12566985	G	A	0.43	319282	0.024	0.003	0.018	0.030	3.28E-15
rs7138803	A	G	0.44	322092	0.032	0.003	0.026	0.038	8.15E-24
rs2207139	G	A	0.1	322019	0.045	0.004	0.037	0.053	4.13E-29

rs2176598	T	C	0.2	316848	0.02	0.004	0.012	0.028	2.97E-08
rs10938397	G	A	0.43	320955	0.04	0.003	0.034	0.046	3.20E-38
rs1558902	A	T	0.45	320073	0.082	0.003	0.076	0.088	<1.0E-40

Note. N= number of observations, A1=effect allele, A2=other allele, SE=standard error, L_95CI= lower 95% confidence limit, U_95_CI=upper 95% confidence limit.

Table 8.8 Associations between BMI SNPs and BMI in meta-analysis of ELSA & UKHLS studies (n=12,107)

SNP	Effect	SE	L_95_CI	U_95_CI	P-value
rs3101336	-0.315	0.063	-0.439	-0.191	<0.001
rs7243357	-0.288	0.082	-0.448	-0.128	<0.001
rs11030104	-0.258	0.076	-0.407	-0.110	0.001
rs12446632	-0.224	0.087	-0.395	-0.054	0.010
rs1516725	-0.221	0.114	-0.444	0.003	0.053
rs16951275	-0.191	0.072	-0.333	-0.049	0.008
rs12885454	-0.189	0.064	-0.315	-0.062	0.003
rs13021737	-0.188	0.082	-0.349	-0.027	0.022
rs9400239	-0.177	0.068	-0.309	-0.044	0.009
rs2287019	-0.175	0.079	-0.330	-0.019	0.027
rs16907751	-0.156	0.105	-0.361	0.049	0.135
rs2820292	-0.155	0.062	-0.277	-0.033	0.013
rs1928295	-0.148	0.062	-0.269	-0.028	0.016
rs12940622	-0.143	0.062	-0.264	-0.023	0.020
rs17724992	-0.141	0.070	-0.277	-0.004	0.044
rs2033732	-0.139	0.070	-0.277	-0.002	0.047
rs9374842	-0.136	0.073	-0.280	0.007	0.062
rs17405819	-0.135	0.067	-0.266	-0.003	0.045
rs16851483	-0.134	0.107	-0.344	0.076	0.212
rs6804842	-0.121	0.062	-0.243	0.000	0.050
rs2365389	-0.121	0.062	-0.242	0.000	0.051
rs10132280	-0.109	0.071	-0.247	0.029	0.123
rs1808579	-0.104	0.063	-0.228	0.019	0.097
rs7141420	-0.103	0.061	-0.224	0.017	0.094
rs7599312	-0.090	0.069	-0.225	0.045	0.192
rs2112347	-0.089	0.063	-0.213	0.035	0.161
rs29941	-0.084	0.065	-0.212	0.044	0.198
rs1167827	-0.072	0.062	-0.193	0.049	0.244
rs6091540	-0.066	0.067	-0.198	0.066	0.329
rs12429545	-0.060	0.091	-0.238	0.118	0.507
rs11688816	-0.052	0.062	-0.173	0.069	0.404
rs4740619	-0.046	0.061	-0.166	0.074	0.451
rs7903146	-0.046	0.068	-0.180	0.088	0.503
rs7164727	-0.046	0.066	-0.176	0.084	0.492
rs4787491	-0.041	0.062	-0.162	0.080	0.507
rs9925964	-0.038	0.064	-0.165	0.088	0.554
rs13201877	-0.030	0.093	-0.212	0.153	0.748
rs7239883	-0.027	0.063	-0.151	0.098	0.675
rs6465468	-0.017	0.070	-0.155	0.121	0.809

rs6477694	-0.016	0.065	-0.143	0.110	0.800
rs3810291	-0.010	0.070	-0.148	0.128	0.886
rs11126666	-0.009	0.070	-0.147	0.129	0.895
rs11165643	-0.003	0.062	-0.125	0.119	0.964
rs4256980	-0.001	0.066	-0.130	0.128	0.987
rs2033529	0.005	0.067	-0.127	0.137	0.938
rs1528435	0.013	0.063	-0.110	0.137	0.832
rs3849570	0.014	0.082	-0.146	0.175	0.860
rs13078960	0.018	0.075	-0.130	0.166	0.811
rs492400	0.025	0.062	-0.096	0.146	0.683
rs1000940	0.031	0.067	-0.101	0.163	0.646
rs2836754	0.031	0.065	-0.095	0.158	0.626
rs977747	0.032	0.062	-0.090	0.153	0.608
rs17024393	0.035	0.193	-0.344	0.415	0.855
rs1441264	0.039	0.066	-0.092	0.169	0.560
rs9914578	0.039	0.076	-0.110	0.189	0.605
rs17094222	0.047	0.080	-0.110	0.203	0.560
rs2650492	0.048	0.071	-0.092	0.188	0.502
rs3736485	0.059	0.062	-0.063	0.180	0.346
rs12401738	0.062	0.064	-0.063	0.187	0.333
rs10968576	0.064	0.065	-0.064	0.192	0.328
rs12286929	0.064	0.061	-0.056	0.184	0.294
rs7715256	0.076	0.062	-0.047	0.198	0.226
rs17203016	0.076	0.076	-0.072	0.225	0.311
rs10162287	0.087	0.067	-0.044	0.218	0.194
rs11583200	0.091	0.062	-0.031	0.214	0.142
rs2176040	0.094	0.064	-0.031	0.219	0.142
rs11191560	0.094	0.117	-0.135	0.323	0.420
rs3817334	0.096	0.063	-0.027	0.220	0.126
rs758747	0.100	0.076	-0.050	0.249	0.192
rs657452	0.102	0.062	-0.020	0.224	0.102
rs9540493	0.124	0.062	0.003	0.246	0.045
rs1460676	0.125	0.084	-0.039	0.290	0.136
rs3888190	0.132	0.069	-0.003	0.267	0.056
rs7899106	0.136	0.136	-0.131	0.403	0.318
rs2121279	0.137	0.093	-0.046	0.319	0.142
rs205262	0.155	0.072	0.015	0.295	0.030
rs543874	0.164	0.077	0.014	0.314	0.033
rs6567160	0.170	0.072	0.029	0.311	0.018
rs10182181	0.181	0.062	0.060	0.302	0.003
rs12566985	0.184	0.062	0.063	0.306	0.003
rs7138803	0.194	0.064	0.070	0.319	0.002

rs2207139	0.195	0.083	0.033	0.358	0.018
rs2176598	0.200	0.071	0.060	0.339	0.005
rs10938397	0.256	0.078	0.103	0.409	0.001
rs1558902	0.325	0.062	0.203	0.447	<0.001

Table 8.9 Pooled associations (meta-analysis) between BMI SNPs (88) and sleep duration in ELSA, UKB and UKHLS

SNP	Effect	SE	L_95_CI	U_95_CI	I-sq. (P-het)
rs7243357	0.63	0.01	-0.46	-0.44	0% (0.375)
rs2365389	0.54	0.00	-0.16	-0.14	0% (0.383)
rs1016287	0.50	0.00	-0.28	-0.27	0% (0.389)
rs11847697	0.39	0.01	-0.16	-0.12	0% (0.396)
rs10132280	0.37	0.00	0.02	0.04	0% (0.409)
rs2820292	0.37	0.00	-0.28	-0.26	0% (0.424)
rs1516725	0.36	0.01	-0.53	-0.51	0% (0.424)
rs2836754	0.36	0.00	0.53	0.55	0% (0.429)
rs11583200	0.33	0.00	-0.16	-0.14	0% (0.442)
rs12566985	0.32	0.00	-0.25	-0.23	0% (0.453)
rs17203016	0.31	0.00	0.21	0.23	0% (0.456)
rs2287019	0.31	0.01	-0.34	-0.32	0% (0.475)
rs7141420	0.29	0.00	-0.06	-0.05	0% (0.502)
rs977747	0.26	0.00	0.00	0.02	0% (0.509)
rs10968576	0.25	0.00	-1.01	-1	0% (0.528)
rs3736485	0.22	0.00	0.36	0.38	0% (0.541)
rs12429545	0.19	0.01	0.02	0.04	0% (0.547)
rs205262	0.18	0.00	0.35	0.37	0% (0.560)
rs1928295	0.14	0.00	-0.42	-0.4	0% (0.563)
rs16951275	0.13	0.00	-0.2	-0.18	0% (0.574)
rs7715256	0.13	0.00	-0.43	-0.41	0% (0.596)
rs758747	0.13	0.00	0.38	0.39	0% (0.603)
rs4256980	0.12	0.00	-0.42	-0.4	0% (0.605)
rs9540493	0.11	0.00	-0.32	-0.3	0% (0.614)
rs7138803	0.08	0.00	0.3	0.32	0% (0.619)
rs17094222	0.04	0.00	-0.05	-0.03	0% (0.627)
rs11030104	0.03	0.01	-0.03	-0.01	0% (0.657)
rs2033732	0.03	0.00	0.35	0.37	0% (0.670)
rs2650492	0.02	0.00	0.11	0.13	0% (0.676)
rs6477694	0.01	0.00	-0.36	-0.34	0% (0.690)
rs2207139	-0.01	0.01	-0.98	-0.96	0% (0.697)
rs9914578	-0.02	0.00	-0.33	-0.31	0% (0.705)
rs12940622	-0.03	0.00	-0.13	-0.11	0% (0.728)
rs12286929	-0.04	0.00	0.13	0.14	0% (0.767)
rs6465468	-0.04	0.00	-0.05	-0.03	0% (0.782)
rs1167827	-0.05	0.00	0.12	0.14	0% (0.783)
rs17024393	-0.06	0.01	-0.21	-0.17	0% (0.785)
rs9374842	-0.06	0.00	-0.47	-0.45	0% (0.791)
rs29941	-0.09	0.00	0.36	0.38	0% (0.797)

rs16851483	-0.10	0.01	-0.17	-0.14	0% (0.799)
rs2176598	-0.10	0.00	-0.33	-0.31	0% (0.809)
rs1441264	-0.11	0.00	0.32	0.33	0% (0.821)
rs9641123	-0.12	0.00	0.19	0.2	0% (0.827)
rs2033529	-0.12	0.00	-0.13	-0.11	0% (0.834)
rs3849570	-0.12	0.00	-0.26	-0.25	0% (0.886)
rs17405819	-0.12	0.00	0.24	0.26	0% (0.894)
rs3888190	-0.14	0.00	-0.06	-0.05	0% (0.903)
rs1460676	-0.14	0.01	0.03	0.05	0% (0.905)
rs3101336	-0.15	0.00	-0.11	-0.1	0% (0.906)
rs6804842	-0.15	0.00	-0.21	-0.19	0% (0.911)
rs7239883	-0.16	0.00	-0.01	0	0% (0.914)
rs2121279	-0.19	0.01	0.1	0.12	0% (0.916)
rs7903146	-0.19	0.00	-0.85	-0.84	0% (0.924)
rs2176040	-0.20	0.00	0.01	0.03	0% (0.933)
rs11191560	-0.20	0.01	-0.26	-0.23	0% (0.943)
rs1808579	-0.24	0.00	-0.15	-0.13	0% (0.944)
rs2112347	-0.25	0.00	-0.38	-0.37	0% (0.984)
rs13078960	-0.25	0.00	-0.21	-0.19	0% (0.994)
rs3810291	-0.26	0.00	0.12	0.14	0% (0.995)
rs13201877	-0.27	0.01	-0.06	-0.04	0% (0.998)
rs7164727	-0.28	0.00	-0.31	-0.29	14% (0.312)
rs10938397	-0.28	0.00	-0.53	-0.51	16% (0.306)
rs1558902	-0.28	0.00	0.63	0.64	19% (0.290)
rs3817334	-0.30	0.00	-0.6	-0.59	19% (0.291)
rs13021737	-0.31	0.01	-0.13	-0.11	21% (0.283)
rs1000940	-0.32	0.00	-0.53	-0.51	25% (0.266)
rs543874	-0.32	0.00	0.12	0.14	33% (0.226)
rs657452	-0.33	0.00	-0.28	-0.27	36% (0.209)
rs12885454	-0.35	0.00	0.07	0.09	39% (0.192)
rs12401738	-0.35	0.00	-0.04	-0.02	39% (0.192)
rs10182181	-0.35	0.00	-0.36	-0.35	41% (0.182)
rs6091540	-0.36	0.00	0.32	0.33	43% (0.174)
rs4787491	-0.37	0.00	0.25	0.26	44% (0.168)
rs11126666	-0.41	0.00	-0.36	-0.34	44% (0.169)
rs7899106	-0.41	0.01	0.16	0.2	46% (0.157)
rs2245368	-0.42	0.01	-0.13	-0.11	46% (0.159)
rs12446632	-0.45	0.01	-0.26	-0.24	47% (0.149)
rs9400239	-0.46	0.00	-0.09	-0.08	53% (0.118)
rs1528435	-0.50	0.00	-0.11	-0.09	57% (0.097)
rs17724992	-0.51	0.00	-0.51	-0.49	67% (0.049)
rs492400	-0.52	0.00	-0.51	-0.5	67% (0.050)
rs4740619	-0.52	0.00	0.49	0.51	70% (0.342)

rs9925964	-0.52	0.00	-0.29	-0.27	71% (0.031)
rs6567160	-0.59	0.00	0.3	0.32	73% (0.023)
rs7599312	-0.76	0.00	-0.12	-0.1	74% (0.022)
rs11165643	-0.84	0.00	-0.36	-0.35	76% (0.015)
rs11688816	-0.97	0.00	0.28	0.3	77% (0.014)
rs16907751	-1.01	0.01	-0.78	-0.75	81% (0.006)

Note. SE=standard error, L_95_CI=lower 95% confidence limit, U_95_CI=upper 95% confidence limit, I-sq=% of heterogeneity, as per the I^2 statistic, P-het= p-value for heterogeneity.

Figure 8.9 Bar chart showing variance explained at different p-value thresholds between 0.5 and 0.001 (corresponding to Table 6.2 in Chapter 6)

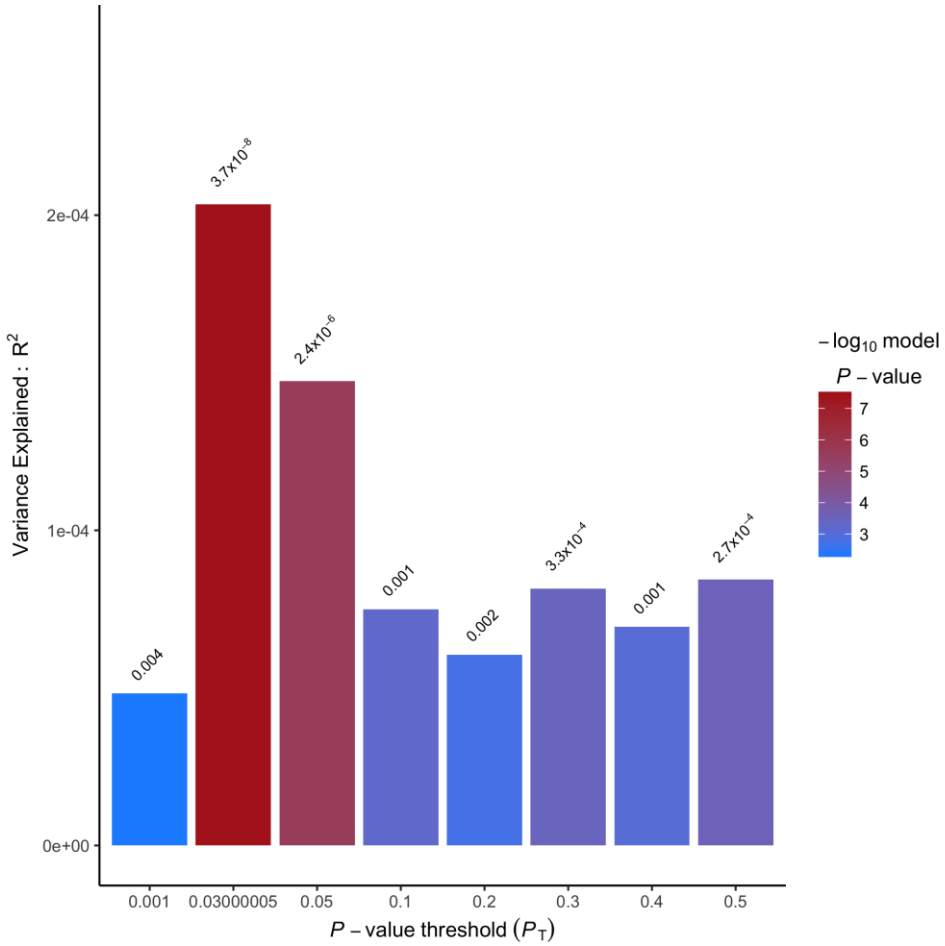

Figure 8.10 Forest plot for SNPs associated with sleep duration, after adjustment for BMI, in IPD (ELSA + UKHLS)