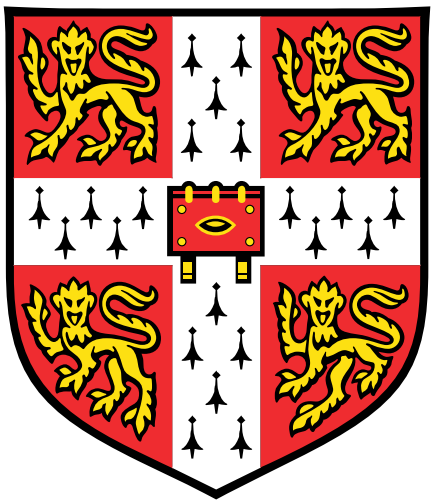
Eating behaviour and the aetiology of obesity



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This dissertation is submitted for the degree of
Doctor of Philosophy

October 2019

"I thought of that while riding my bicycle"

Albert Einstein

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared and specified in the text preceding each chapter. It is not substantially the same as any that I have submitted, or is being concurrently submitted, for a degree, diploma or other qualification at the University of Cambridge or any other University or similar institution. I further state that no substantial part of my dissertation has already been submitted, or is being concurrently submitted, for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution.

As required by the Degree Committee for the Faculties of Clinical Medicine and Veterinary Medicine, this dissertation contains fewer than 60,000 words.

Emma A. D. Clifton
October 2019

ACKNOWLEDGEMENTS

Without the help of many, and the kindness of more, this thesis would not have been possible. Foremost, my thanks go to my supervisor, Professor Ken Ong, for his patient guidance, encouragement and support throughout my PhD. Also to my second supervisor, Dr Felix Day, for fielding innumerable questions, reading and re-reading many iterations of everything and for always having time. I feel extremely fortunate to have had two such enthusiastic and knowledgeable people to guide me through my PhD and these years of my education. I learnt a huge amount, not all of which is evident on the pages of this thesis. I will remember the difference it made and make sure to pay it forward.

I am also grateful to Dr Raj Lakshman for her help on all the work related to the Baby Milk Trial; Dr Ema De Lucia Rolfe and Richard Powell for their advice regarding the use of anthropometric measures; Dr John Perry and Dr Fumiaki Imamura for their input on the GWAS of risk-taking propensity and Stephen Sharp for his guidance in developing statistical analysis plans. Also to my proof-reading team: Sally, Ian, Olivia, Hamish, Ignacio, Nicky and Connie, who saved me many hours in the preparation of this thesis.

This thesis is for my family. For my parents, Sally and Ian, who have invested so much in me and my education and continue always to encourage me to be my best. For my sister, Olivia, my brother, Hamish and my cousin, Sarah for their support and store of humour. For my grandparents, Shirley and Carl Jewell who taught me not to take things too seriously. For Christina Clifton. For my aunt and uncle, Marian and Hugh Muirhead. Finally, for Ignacio, for much emotional and culinary support during the preparation of this thesis and always.

ABSTRACT

Mounting evidence supports an association between eating behaviour (EB) and obesity. However, poor characterisation of the nature of this relationship limits its application to obesity prevention. This thesis aims to advance understanding of the association between EB and the aetiology of obesity. Across 5 studies, a combination of approaches was used to interrogate: (1) the role of EB in genetic predisposition to obesity, (2) the interaction between infant EB and maternal attitudes in the determination of infant milk intake and weight and (3) the genetic basis of EB traits and risk-taking.

First, the effect of body mass index (BMI)-related genetic variants on adult body composition was investigated using a genetic risk score (GRS) approach in the Fenland study (n=9667). The BMI-GRS primarily influenced fat mass, confirming its utility in modelling the effects of adiposity and BMI, as well as in exploring the mechanisms of genetic predisposition to obesity. Emotional eating (EE), uncontrolled eating (UE) and cognitive restraint (CR) were then modelled as potential mediators and modifiers of the BMI-GRS to BMI association amongst adults in the Fenland (n=3515) and EDEN (n=2154) studies. The association was partially mediated by EE and UE, and modified by CR. These results indicate that whilst appetitive EB traits (EE and UE) lie on the causal pathway between genetics and weight status in adulthood, restraint may protect genetically vulnerable individuals from obesity. Having demonstrated that interactions between obesity determinants can impact adult weight, I described the association of infant EB to both infant milk intake and weight in the Baby Milk Trial (n=669). I then investigated whether this could be modified by maternal factors. Positive maternal attitudes towards following healthy infant feeding guidelines attenuated the association between infant EB and both outcomes. Finally, I performed GWAS to explore the genetic basis of risk-taking and adult EB, behavioural phenotypes with a hypothesised role in the aetiology of obesity. A total of 26 genetic variants were identified in association with risk-taking (n=436,236). In aggregate, these were linked to higher BMI but heterogeneity in the impact of individual variants suggested the involvement of multiple pathways. No variants were identified for EE, UE or CR. This analysis was likely under-powered due to low sample size ($n \leq 11,843$) but indicated a genetic basis for UE that partially overlaps with that of BMI.

Abstract

Using a combination of approaches, this work demonstrates the role of EB pathways in the aetiology of obesity. The findings contribute to a deeper understanding of their likely causal role and the implications of their relationships with other behavioural traits, highlighting a range of behaviours as potential targets for obesity prevention amongst both infants and adults.

PUBLICATIONS AND PRESENTATIONS

Publications resulting from this thesis

Published work

Clifton, E.A.D., Perry, J.R.B., Imamura, F., Lotta, L.A., Brage, S., Forouhi, N.G., Griffin, S.J., Wareham, N.J., Ong, K.K., Day, F.R. (2018). Genome–wide association study for risk taking propensity indicates shared pathways with body mass index. *Communications Biology*. 1, DOI: 10.1038/s42003-018-0042-6

de Lauzon-Guillain, B.*, **Clifton, E.A.D.***, Day, F.R., Clément, K., Brage, S., Forouhi, N.G., Griffin, S.J., Akoli Koudou, Y., Pelloux, V., Wareham, N.J., Charles M-A., Heude, B.$^\wedge$, Ong, K.K.$^\wedge$. (2017). Mediation and modification of genetic susceptibility to obesity by eating behaviors. *American Journal of Clinical Nutrition*. 106, 996-1004. DOI: 10.3945/ajcn.117.157396

Clifton, E.A.D., Day, F.R., De Lucia Rolfe, E. Forouhi, N.G., Brage, S., Griffin, S.J., Wareham, N.J., Ong, K.K. (2017). Associations between body mass index-related genetic variants and adult body composition: The Fenland cohort study. *International Journal Of Obesity*. 41, 613-619. DOI: 10.1038/ijo.2017.11

In preparation or under review

Clifton, E.A.D., Ahern, A.L., Day, F.R., Sharp, S.J., Griffin, S.J., Ong, K.K., Lakshman, R. Positive maternal attitudes to healthy infant feeding guidelines attenuate the associations between infant appetitive traits and both infant milk intake and body weight. *In preparation*

Other publications

Clifton, E.A.D., Ong, K.K., Day, F.R. (2018). We uncovered the genetic basis of risk taking and found it's linked to obesity and mental illness. *The Conversation*

Lakshman, R., **Clifton, E.A.D.**, Ong, K.K. (2017). Baby-Led Weaning — Safe and effective but not preventive of obesity. *JAMA Pediatrics*. 171. 832-833. DOI: 10.1001/jamapediatrics.2017.1766

Conference presentations

Mediation and modification of genetic susceptibility to obesity by eating behaviours: The EDEN and Fenland cohort studies. (2017). The Behavior Genetics Annual Meeting. Oslo, Norway. *Oral presentation*

Eating behaviour and the genetics of obesity. (2016). Jesus College Graduate Conference. *Oral presentation*

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PRINCIPAL ABBREVIATIONS

Symbols

β	Beta; Effect estimate
r_g	Genetic correlation

Acronyms and abbreviations

BEBQ	Baby Eating Behaviour Questionnaire
BMI-GRS	Genetic risk score for BMI
BMI	Body mass index
BST	Behavioural Susceptibility Theory
CI	Confidence interval
CNS	Central nervous system
CR	Cognitive restraint
DEBQ	Dutch Eating Behaviour Questionnaire
DXA	Dual energy x-ray absorptiometry
EB	Eating behaviour
EE	Emotional eating
EF	Enjoyment of food
FR	Food responsiveness
GRS	Genetic risk score
GWAS	Genome-wide association study
HWE	Hardy-Weinberg equilibrium
MAF	Minor allele frequency

MAS	Maternal attitudes score
MR	Mendelian randomisation
PC	Principal component
QC	Quality control
RCT	Randomised controlled trial
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SDS	Standard deviation score
SE	Standard error
SiE	Slowness in eating
SNP	Single nucleotide polymorphism
SR	Satiety responsiveness
TFEQ	Three Factor Eating Questionnaire
UE	Uncontrolled eating
UKB	UK Biobank
VAT	Visceral adipose tissue
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Background

The epidemiological study of eating behaviour (EB) is a relatively new field of research. Scalable measurement tools for EB, facilitating a population-based approach, were first developed in 1970s at a time when rising levels of obesity sparked interest in the factors involved in the determination of excessive weight gain. Today mounting evidence supports an association between EB and body weight. However, the nature of the causal pathways underlying this association are poorly understood. In particular, the aetiological role of restraint over eating is debated and the relationship between EB and other determinants of obesity, including the genetic basis of body mass index (BMI), is largely unknown. Applying a combination of genetic and observational methods, the work described in this thesis aims to advance understanding of the relationship between EB and the aetiology of obesity.

This introductory chapter places the work in context. **Sections 1.2** and **1.3** provide definitions and discuss common measurement methods for both obesity and EB in large, epidemiological studies. Drawing upon both cross-sectional and longitudinal evidence, the association between EB and weight is then reviewed in **Section 1.4**. This leads into a discussion of the role of EB in the specific aetiological pathways to obesity of relevance to this thesis in **Sections 1.5** and **1.6**. Finally, **Section 1.7** describes and elaborates upon the aims of this work.

1.2 Overweight and obesity

The importance of EB to public health derives primarily from its proposed associations with weight status and, in particular, overweight and obesity. These serious medical conditions are characterised by the excessive accumulation of body fat with implications for health and well-being [1]. Unlike other major threats to global health, including tobacco use and childhood malnutrition, no country has ever achieved substantial or sustained declines in obesity prevalence, making excess body weight a major challenge for the twenty-first century [2–4]. In the following section, the measurement, classification and burden of overweight and obesity are discussed. Together, this provides a background and rationale for the study of pathways relevant to their aetiology.

1.2.1 Classification

1.2.1.1 Adults

In adulthood, overweight and obesity are typically identified using BMI, a non-invasive proxy measure of adiposity calculated by dividing a person's weight (kg) by their height squared (m^2). Amongst adults over the age of 20 years, the World Health Organisation's (WHO) classification system categorises an individual's BMI as underweight, normal weight, overweight or obese on the basis of the thresholds provided in **Table 1.1**. These thresholds are designed to reflect adiposity-related health risks associated with different levels of BMI. The relationship between BMI and adiposity is discussed in greater detail in **Chapter 3**.

Table 1.1 The WHO thresholds for the classification of BMI

	BMI range
Underweight	$<18.5 kg/m^2$
Normal weight	$18.5 kg/m^2 - 24.9 kg/m^2$
Overweight	$25.0 kg/m^2 - 29.9 kg/m^2$
Obese	$\geq 30 kg/m^2$

Adapted from WHO, 2006 [5]. World Health Organisation (WHO); Body mass index (BMI)

1.2.1.2 Children and infants

Amongst paediatric and adolescent populations under the age of 20 years, sex-specific growth charts that account for age are used to reflect degrees of adiposity. BMI-for-age reference charts are most often used to compare a child's BMI to that of the reference population mean. Both the WHO and the International Obesity Task Force (IOTF) have developed reference charts for children aged 5-19 years and 2-18 years, respectively, designed for use across populations [6, 7]. At present, the IOTF reference is the most widely used [8]. On the basis of evidence that BMI is a poor surrogate for adiposity in early life, weight-for-length growth reference or standard charts are used amongst infants [9]. In contrast to reference charts, which reflect typical growth, standard charts are designed to reflect optimal growth and are typically derived using data from healthy, breastfed infants of non-smoking mothers [10]. Although global standards exist for use across populations, including the WHO growth standard for infants aged 0-2 years, many countries use population-specific charts. In the UK, the British 1990 growth reference (UK90) is widely used [11].

Growth standard and reference charts most often classify BMI or weight-for-length on the basis of an individual's centile or standard deviation (SD) from the mean. However, given a lack of evidence for direct associations between these cut-offs and health risks, this approach has been criticised as arbitrary [7]. Obesity-related morbidity often develops in adulthood and any associations between childhood obesity and disease in later life may be mediated, or confounded, by adult weight status [7]. As a pragmatic solution, the IOTF thresholds were specifically designed to correspond to the adult BMI categories.

1.2.2 Prevalence

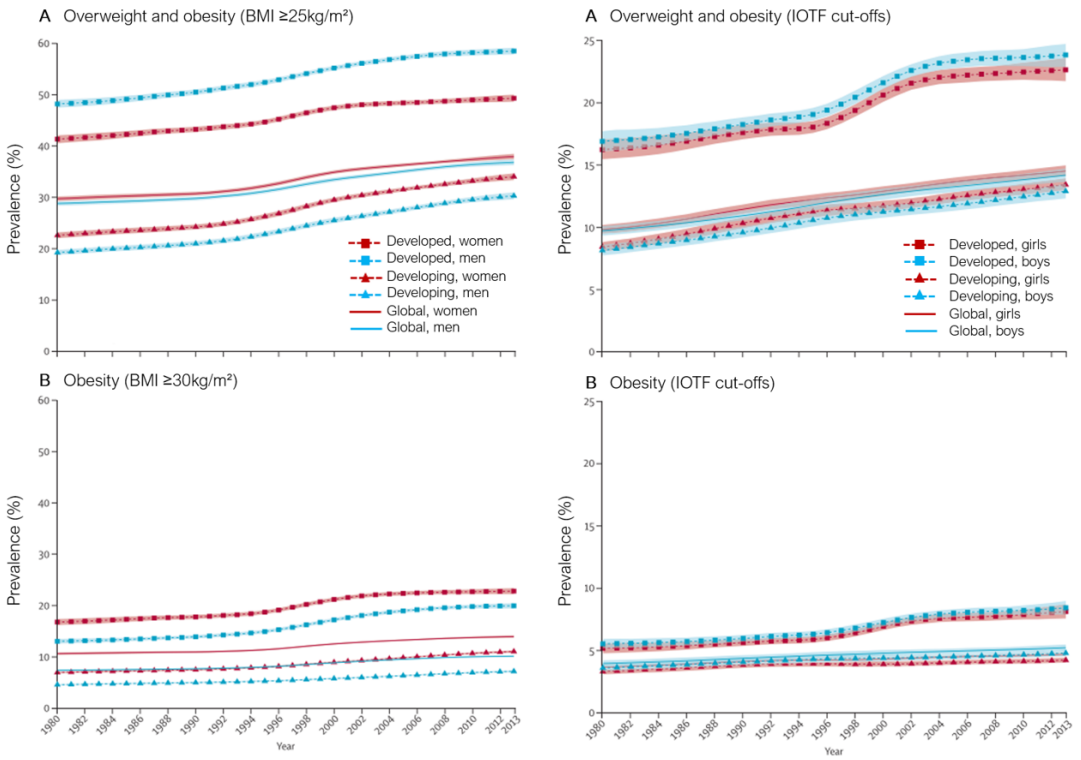
The Global Burden of Disease study (GBD) provides a comprehensive annual assessment of global health trends, incorporating data from surveys, surveillance programs, databases, reports and published studies across 195 countries and territories worldwide. In 2015, using the IOTF and WHO classification systems, the GBD study estimated the prevalence of obesity to be 5% amongst children (108 million) and 12% amongst adults (604 million) [8]. These figures reflect a doubling in the age-standardised prevalence of obesity across more than 70 of the countries included in the analysis since 1980 and continuous increases across the majority of remaining countries [8]. Alongside elevated fasting plasma glucose, high BMI is the only leading disease risk factor included in the study not to have declined in at least some GBD study regions since 2010 [12].

Figure 1.1 shows the age-standardised prevalence of overweight and obesity from 1980 to 2013, based on GBD data [4]. Globally, the prevalence has been rising since 1980 with the steepest rate of increase observed during the 1990s. Attenuation in the rate of increase post-2002 has been driven by the stabilisation of obesity prevalence in high income countries

[4]. Children and individuals in low income countries continue to experience the greatest increases in prevalence [8, 3, 4].

In the UK, the Health Survey for England provides annual BMI data from a representative sample of the population residing in England. The most recent figures show that the majority of adults over the age of 20 years are either overweight or obese (66% of men and 57% of women) and almost 1 in 5 children in England begin primary school overweight, rising to 1 in 3 by age 11 [13–15].

Even under the most optimistic assumptions, the GBD predicts that the global prevalence of obesity will continue to increase to 2040 and beyond [16]. In 2040, it is estimated that high BMI will rank second only to high blood pressure amongst the global risk factors for years of life lost across all 195 regions (**Figure 1.2**) [16].



(a) Adults aged ≥ 20 years

(b) Children aged 2-19 years

Figure 1.1 Age-standardised prevalence of overweight and obesity across 183 countries between 1980 and 2013. This figure is adapted from Ng et al, 2014 [4]. It shows the age-standardised prevalence of overweight and obesity (**A**) and obesity alone (**B**) from across 183 countries, covering 21 global regions, between 1980 and 2013. Overweight and obesity were identified using the IOTF cut-offs for children and the WHO cut-offs for adults.

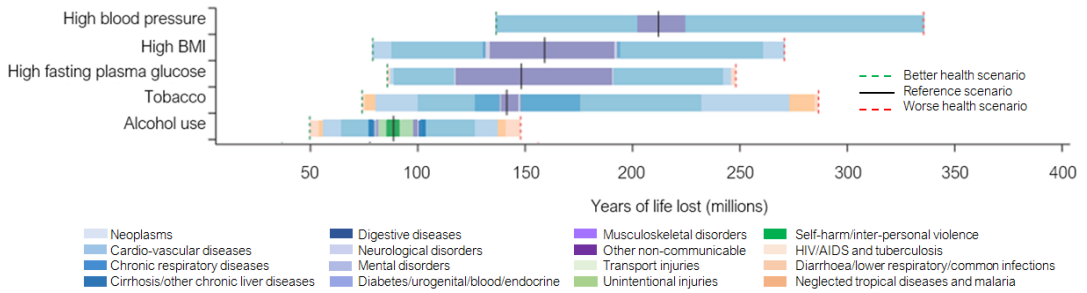


Figure 1.2 Top 5 leading risk factors contributing to projected years of life lost in 2040. Adapted from Foreman et al, 2018 [16]. The figure shows the global risk-attributable years of life lost between the 2040 reference forecast, 2040 better health scenario, and 2040 worse health scenario. The better and worse health scenarios were computed by taking the 85th and 15th percentiles of annualised rates of change observed across all locations and years in the past. The differences between the reference scenario and the better and worse health scenarios are grouped and colour-coded by cause. The black solid vertical lines represent all-cause attributable years of life lost in the 2040 reference forecast, red dashed vertical lines represent all-cause attributable years of life lost in the 2040 worse health scenario, and green dashed vertical lines all-cause attributable years of life lost in the 2040 better health scenario.

1.2.3 Consequences

Cardiovascular disease (CVD), type 2 diabetes (T2D), musculoskeletal disorders, certain cancers and asthma are well-established sequelae of high BMI, all of which can lead to premature mortality and reduced quality of life [2, 17–20]. In 2016, an estimated 4.5 million deaths and 135.4 million disability adjusted life years (DALYs) worldwide were directly attributable to obesity [2]. Beyond its implications for systemic health, high BMI can also result in adverse psychosocial outcomes, including depression, internalising disorders and poor school performance [21, 18, 22].

Given a wealth of epidemiological evidence linking both high and low BMI to adverse health outcomes, a J-shaped association between BMI and all-cause mortality has been assumed, with the lowest risks being experienced by those within the normal BMI range [23]. However, evidence suggesting improved survival amongst overweight individuals for some health conditions has challenged this assertion. For example, one study found that excess all-cause mortality amongst individuals classified as overweight or obese masked a reduced risk of mortality from non-CVD and non-cancer causes [24]. A separate study amongst 4000 American adults suggested increased mortality from T2D amongst normal weight versus obese individuals, even following adjustment for confounding [25]. Other studies have failed to identify increased all-cause mortality amongst overweight individuals [26, 27]. Together, this evidence has called into question the dangers associated with high BMI and led some to assert that the degree of concern surrounding overweight and obesity exceeds the true public health relevance of these conditions [27]. Conversely, a recent meta-analysis

of prospective observational studies showed a continuous increase in the risk of death with a BMI above 25kg/m^2 [2]. By restricting the analysis to individuals who reported no history of having smoked and those without chronic disease, this study dealt with potential sources of bias in previous studies, including smoking status and reverse causation due to pre-existing chronic disease. In combination, the weight of the observational evidence suggests that it would be premature to assert that being overweight is risk-free. More recently, this assertion has been supported by the findings of Mendelian randomisation (MR) studies.

MR is described in detail in **Section 6.3.2.9**. Briefly, where observational studies repeatedly suggest an association between an exposure and an outcome, causality is just one potential explanation. The association may also result from sources of bias, including confounding, or from reverse causation [28]. Whilst longitudinal analyses reduce the probability that reverse causality explains the results, ideally a randomised controlled trial (RCT) would be performed in order to reduce the potential impact of confounding. However, RCTs are not feasible or ethical for all exposures. MR is conceptualised as a natural RCT whereby genotype is used as a proxy for levels of an exposure. MR analyses mirror RCTs in several important ways. First, alleles are sorted independently such that the inheritance of one trait is independent of others, controlling for confounding. Second, an individual's genotype is fixed at conception, eliminating the potential for reverse causation. As the genetic basis of BMI has become better established through genome-wide association studies (GWAS), MR has been used to interrogate the association between BMI and health outcomes.

A 2019 MR analysis amongst 56,150 and 366,385 participants from the Nord-Trøndelag Health and UKB studies (including 12,915 and 10,344 deaths, respectively) identified a causal J-shaped association between BMI and all-cause mortality in the total population, and a causal linear association amongst never smokers [29]. In the total cohort, the lowest risk of all-cause mortality was observed amongst individuals with a BMI in the normal range (between 22 and 25kg/m^2). An increase of 1 genetically predicted BMI unit led to a 5% (95% confidence interval (CI): 1%, 8%) increase in mortality risk amongst overweight participants and a 9% (95% CI: 4%, 14%) higher risk of mortality amongst obese participants. The same increase in genetically predicted BMI was associated with a 34% (95% CI: 16%, 48%) reduction in risk of all-cause mortality amongst underweight participants and a 14% (95% CI: -1%, 27%) reduction amongst normal weight participants with a BMI within the lower bounds of the normal range (18.5-19.9kg/m^2) [29]. A 2018 review of MR studies found consistent support for a causal association between BMI and both T2D and hypertension [30]. Results for coronary artery disease (CAD) are less consistent but overall support a causal association, with the best powered MR studies identifying a positive association between BMI and CAD [30]. Evidence for a causal association between BMI and depression is mixed [19, 31, 21]. Overall, MR studies support a linear association between BMI and all-cause mortality amongst never smokers and have generally supported a causal role for BMI in cardio-metabolic disease.

High BMI is also associated with substantial economic costs. Globally, medical costs for obese individuals are approximately 30% higher than for those of normal weight [32]. In 2014, the annual obesity-related medical spend in the US was estimated to be $149.4 billion [33]. Additional indirect costs include productivity losses, work absenteeism, disability and premature mortality [34]. Whilst these costs are hard to quantify, estimates suggest the annual cost of obesity-related absenteeism and premature mortality in the US to be $6.38 billion and $30.15 billion, respectively [34]. On an individual level, a 2016 UK Biobank (UKB) MR study suggested that obesity is causally associated with lower income and deprivation amongst women [35].

1.2.4 Summary

In sum, overweight and obesity are serious medical conditions with manifold implications for both individuals and societies. Their global significance is compounded by increases in prevalence, particularly amongst some of the most vulnerable groups in global society (children and individuals in low income countries). Despite data detailing of the scale of the problem and accompanying large-scale characterisation of the potential causes [36], to date, no country has developed or implemented successful prevention or treatment programs for obesity at scale [37]. As such, research into the factors involved in the aetiology and maintenance of obesity is of paramount importance to global health.

1.3 Eating behaviour

Since aetiological studies of obesity began, inter-individual variations in behaviour have been an important focus for research. In particular, EB has received attention [38]. Continued focus on EB is supported by the rise of permissive food environments globally. Such environments create an enhanced opportunity for the behavioural expression of EB tendencies, thus facilitating their impact on weight and health. In the following section, a working definition of EB is provided, followed by a discussion of its questionnaire-based measurement.

1.3.1 Definition

EB is an umbrella term used to describe the habitual behavioural patterns that characterise an individual's response to food, food-related cues and food consumption. These patterns are the behavioural realisations of relatively stable underlying behavioural tendencies, referred to throughout this thesis as EB traits [39, 40]. EB traits are thought to result from the interplay between genetic and environmental influences and modulate responses to internal and external cues to commence or cease eating. Some EB traits, such as the enjoyment of food and response to feelings of satiety, emerge and can be measured during early infancy and exhibit levels of intra-individual continuity across early childhood comparable to that of stable personality traits [41]. Others, such as the exercising of conscious control over consumption, which involves the denial of internal or external cues to eat motivated by a desire to control weight or shape, rely upon the complex cognitive capacities required to engage in goal-oriented behaviour [42, 43]. These capacities arise later in development and, as such, certain EB traits are only apparent in adulthood. The following section summarises the measurement of EB by questionnaire in large population-based studies.

1.3.2 Measurement by questionnaire

Anecdotal observations suggesting that the EB of individuals who are obese is distinguishable from that of their normal weight counterparts led to the earliest attempts to formally operationalise EB in the mid-1970s. These efforts were motivated by the desire to quantify eating styles relevant to the development and maintenance of obesity. The traits most widely measured by contemporary questionnaires can broadly be traced back to three main theories of obesogenic EBs:

- ***Psychosomatic theory***. First proposed by Kaplan and Kaplan in the 1950s, this theory attributes over-eating to dysphoric emotional states. The authors contend that obese individuals misattribute unpleasant emotions to hunger or attempt to self-soothe using food [44]. The theory is reflected in measures of emotional eating (EE) [44, 45].

- ***Externality theory***. This theory suggests that obese individuals have a reduced ability to identify and respond to internal physiological signals of hunger and satiety appropriately. They thus rely upon external cues to guide the initiation and cessation of eating [46]. This theory is reflected in measures of uncontrolled eating (UE) and external eating.

- ***Restraint theory***. This theory attributes overeating to the intentional restriction of food intake motivated by a desire to influence body shape or weight [47–49]. This restriction is hypothesised to lead to overeating primarily through psychological mechanisms [50]. These include excessive eating in response to the breaking of a rigid dietary rule or a lowering of inhibitions due to, for example, the consumption of alcohol or unpleasant emotions [48]. The theory is reflected in measures of cognitive restraint (CR). Restrained eaters are thought to rely primarily on conscious control to regulate their food intake, as opposed to physiological cues. Subsequent work has shown that CR is generally driven by a desire to prevent weight gain rather than to instigate weight loss [51] and describes the subjective experience of eating less than desired [52]. This does not necessarily result in negative energy balance and reflects attempts to limit consumption regardless of the behavioural realisation of these efforts [53]. Consequently, CR and dieting are considered as distinct, if partially overlapping, concepts. In support of this conceptual distinction, restrained eaters show different reactions to food in controlled settings when compared to dieters [52]. Subsequent work, suggesting that restraint is a response to high BMI and reporting associations to healthy eating patterns such as higher fruit and vegetable intake, has called the aetiological role of restraint in obesity into question [51, 54].

These theories are not thought to be mutually exclusive and contemporary questionnaires typically assess a range of EB traits based in each of the theories. In general, EB questionnaires rely upon Likert scales, combining an individual's response across a number of items, each grading the extent to which an individual identifies with a statement, to quantify the extent to which their typical EB is characterised by a particular EB trait. In its original form, a Likert scale consisted of 5 possible responses grading the intensity of a respondent's attitude to a statement on a linear scale from *Strongly agree* to *Strongly disagree*, with the odd number of items allowing for a neutral response as the central point of the scale [55]. Subsequent Likert scales have included both greater and smaller ranges of possible responses, including the use of even numbers precluding a neutral response.

Separate questionnaires are used amongst infants, children and adults. This section will focus on the measurement of EB amongst adults and infants, as these are the groups of relevance to this thesis.

1.3.2.1 Adults

A number of questionnaires have been developed for use in adult populations. Some, such as the Restraint Scale [48, 38], Power of Food Scale [56] and Emotional Eating Scale [57], focus on one EB trait. Others use sub-scales to measure a number of separate EB traits in the same questionnaire. These include the Dutch Eating Behaviour Questionnaire (DEBQ) [58], Three Factor Eating Questionnaire (TFEQ) [47] and, more recently, the Adult Eating Behaviour Questionnaire (AEBQ) [59]. Throughout the studies that comprise this thesis, the TFEQ is used to measure EB in adults. Alongside the DEBQ, it is the most widely used adult EB questionnaire.

The Three Factor Eating Questionnaire

The original 51-item version of the TFEQ (the TFEQ-51), sometimes referred to as the Eating Inventory (EI), was published in the 1980s [47]. It was initially designed to measure restraint and problematic EBs related to restraint. The questionnaire was constructed by collating items from two existing questionnaires, the Restraint Scale (10 items) [48, 38] and Latent Obesity Questionnaire (40 items) [60], alongside 17 additional items added on the basis of the authors' clinical experience working with obese patients. The Restraint Scale measured restraint over eating with the goal of controlling weight, whilst the Latent Obesity Questionnaire identified normal weight individuals who failed to slow their eating during the course of a meal, and thus were considered to have a latent tendency to become obese which they counter-acted through restraint. Three EB traits (cognitive restraint (CR), disinihibition and hunger) were identified through factor analysis and the number of items was reduced to 51 [47].

The three EB traits can be understood as follows: CR reflects the exercising of conscious control over food intake with the intention of influencing body shape or weight (example item: I deliberately take small helpings as a means of controlling my weight) [47]. Disinhibition refers to the subjective experience of loss of control over eating (example item: Sometimes when I start eating, I just can't seem to stop) and measures reactivity to external food cues as well as eating in response to dysphoric emotions [47]. Finally, Hunger provides a more general measure of appetite and describes the experience of extreme hunger and cravings for food (example item: I often feel so hungry that I just have to eat something) [47].

The majority of the items comprising the TFEQ-51 (36 of 51) are scored by individuals selecting either *True* or *False* to indicate whether the statements apply to them. Fourteen of the remaining 15 items are measured using a 4-point Likert scale, indicating how often individuals engage in the behaviours described by the item. The final item (On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want)

and 5 means total restraint (constantly limiting food intake and never "giving in"), what number would you give yourself?) is measured on a 6-point Likert scale.

Following its publication, a number of studies designed to explore the factor structure of the TFEQ-51 were conducted. These produced inconsistent findings. Whilst some identified three distinct traits based on the 51 items [61], others did not [40]. In particular, studies variously reported that only a modest number of the 51 items loaded strongly onto any specific factor [61], that two or more related constructs might be embedded within the CR scale [62–64] and that over-eating in response to negative emotions (emotional eating) may constitute a separate factor [65, 40].

As a result of lack of coherence in the literature and the desire for a more concise questionnaire, the questionnaire was eventually revised and reduced to an 18-item version (the TFEQ-R18) in 2000 (**Appendix C.1**) [40]. The revision was based on findings amongst 4377 obese participants from the Swedish Obese Subjects study suggesting that although the CR scale was valid and should be maintained, the majority of the items assigned to the hunger and disinhibition scales reflected a single, latent construct and should be combined to form a new scale, termed *uncontrolled eating* (UE) [40]. A third cluster of items labelled *emotional eating* (EE) was also identified from items on the disinhibition scale and was assigned to its own scale. For 17 of the 18 items, the dichotomous rating system was replaced by a 4-point Likert scale from *Definitely false* (1 point) to *Definitely true* (4 points) [40]. The final item (On a scale of 1 to 8, where 1 means no restraint in eating (eating whatever you want, whenever you want it) and 8 means total restraint (constantly limiting food intake and never "giving in"), what number would you give yourself?) is measured on an 8-point scale. For this item, individuals who select 1 or 2 are coded 1, 3 and 4 are coded 2, 5 and 6 are coded 3, finally, scores of 7 and 8 are coded 4. The EB traits can be understood as follows:

- ***EE*** (3 items). Reflects the tendency to eat in response to dysphoric emotions. The 3 items comprising the scale specifically refer to loneliness, anxiety and sadness (example item: When I feel lonely, I console myself by eating).
- ***UE*** (9 items). Describes a tendency to overeat accompanied by a subjective sense of loss of control over consumption. The scale is dominated by items reflecting extreme appetite (example item: I get so hungry that my stomach often seems like a bottomless pit).
- ***CR*** (6 items). The meaning of this scale was not altered from the TFEQ-51 and the items assess the intention to restrict food intake with the objective of influencing body shape or weight (example item: I consciously hold back at meals in order not to gain weight).

In 2005, the TFEQ-R18 was further revised by adding three additional items to the EE scale in order to increase the discrimination of the scale, resulting in the TFEQ-R21 [66]. These

additional items assessed eating in response to nervousness (item: If I feel nervous, I try to calm down by eating), tension (item: When I feel tense or wound up, I often feel a need to eat) and feelings of depression (item: When I feel downhearted and depressed, I want to eat).

Despite being originally developed in an obese population, the factor structure of the TFEQ-R18 replicated the findings of an earlier study in a sample of normal weight participants [67] and has subsequently been replicated amongst both obese and normal weight populations across a range of settings [68–70].

The Dutch Eating Behaviour Questionnaire

Throughout the studies reported in this thesis, the TFEQ-R18 and TFEQ-R21 are used to measure adult EB. However, given its wide use in studies that inform the aims of this thesis, the DEBQ is mentioned here in brief. Like the TFEQ, the DEBQ was first developed in the mid-1980s but has since undergone revision. In its original form, the questionnaire was comprised of 33 self-assessed items measuring the three EB traits. These are described below. Participants are asked to select how well each of the 33 items describes their typical EB on a 5-point Likert scale from *Seldom* to *Very often*. The questionnaire was translated into English twice during the late 1980s. The second translation replaced the word *Seldom* with *Rarely* [71].

- ***EE*** (13 items). This scale resembles the EE scale of the TFEQ and measures the extent to which a person eats in response to unpleasant emotions. However, the specific emotions referred to in this questionnaire differ from the TFEQ-R18 (example item: Do you have a desire to eat when you are cross?).
- ***External eating*** (10 items). This scale resembles elements of both the UE and EE scales of the TFEQ and reflects the Externality theory of obesogenic EB. It measures behaviour around foods that are particularly appealing or accessible (example item: Can you resist eating delicious foods?).
- ***Restrained eating*** (10 items). This scale is analogous to CR scale of the TFEQ and refers to the intention to limit food intake in order to influence shape or weight (example item: Do you watch exactly what you eat?).

Most studies have confirmed the factor structure of the DEBQ, including studies from the UK [71], Spain [72], Turkey [73], Germany [74] and Malta [75]. Minor modifications have been suggested by other investigations [72].

1.3.2.2 Infants

The self-assessed nature of the adult EB questionnaires detailed above make them developmentally inappropriate for use amongst infants and children, who lack the insight and ability to understand and articulate the motivations behind their EB [76]. As concern surrounding childhood obesity grew with evidence of its rising prevalence (see **Section 1.1**), scalable methods facilitating the measurement of childhood EB based on parental report were developed. These include the Children's Eating Behaviour Questionnaire (CEBQ), the DEBQ parent-report form (DEBQ-P) and the child versions of the TFEQ-R18 and TFEQ-R21 [77, 78]. In 2011, the Baby Eating Behaviour Questionnaire (BEBQ) was developed for the measurement of EB during the period of exclusive milk-feeding [39]. It remains the only questionnaire designed to measure infant EB during this developmental period. The questionnaire items are detailed in full in **Appendix C.1**.

The BEBQ is comprised of 17 parent-assessed items measuring 4 EB traits, in addition to a single item measuring general appetite (GA). The questionnaire items are provided in full in **Appendix C.1** and were derived from items comprising the CEBQ. The authors used a combination of a review of the literature and qualitative interviews with the mothers of young children to analyse which of the 8 CEBQ-measured EB traits could be appropriately applied to infants through inclusion in the BEBQ [39]. A total of 4 EB traits were selected and the items comprising these scales were modified or excluded, based on developmental appropriateness. New items were also added where relevant. The traits are scored on a 5-point scale from *Never* to *Always* and can be understood as described in **Table 1.2**. Together Enjoyment of Food (EF) and food responsiveness (FR) are considered 'food approach' behaviours. Conversely, Slowness in Eating (SiE) and satiety responsiveness (SR) are considered to be associated with 'food avoidance'. Confirmatory factor analysis has partially replicated the factor structure of the BEBQ in an Australian sample, identifying EF, FR and SiE, but not SR [79].

Table 1.2 Description of the BEBQ eating behaviour traits

	Description	Example item
EF (4 items)	Perceived liking for milk and feeding	My baby enjoys feeding time
FR (6 items)	Drive to eat	My baby is always demanding a feed
SR (4 items)	Ease of becoming full	My baby gets full up easily
SiE (3 items)	Pace of typical feeding	My baby feeds slowly
GA (1 item)	Size of appetite	My baby has a big appetite

Based on Llewellyn et al [39]. Enjoyment of Food (EF); Food responsiveness (FR); Satiety responsiveness (SR); Slowness in Eating (SiE); General appetite (GA)

1.3.3 Strengths and limitations of questionnaire-based measures

In humans, EB can be measured either by questionnaire, through laboratory-based assessment or, in theory, in naturalistic settings. Ideal measures of EB are both valid (i.e they reflect the trait that they intend to measure) and reliable (i.e. the results from a single participant are consistent when collected on repeat occasions) (**Figure 1.3**) [80]. These concerns must also be balanced against practical considerations including cost and participant burden. Throughout this thesis, EB is measured by questionnaire. The strengths and limitations of questionnaire-based measures with respect to practicality, validity and reliability are discussed in the following section.

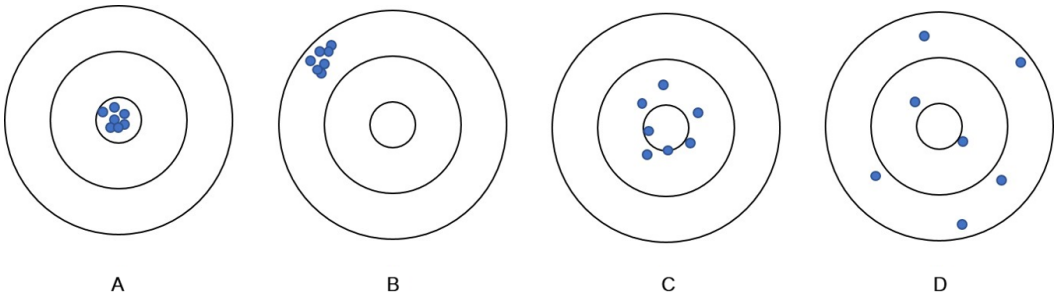


Figure 1.3 Validity and reliability. The centre of the large, white circles represent the true value. The small blue circles represent measures from a single participant taken on different occasions. (**A**) represents a valid and reliable measure. The values obtained from a single participant are consistent both with each other and the true value. (**B**) represents a measure with low validity but high reliability. The measure is repeatable over time but does not reflect the true value of the construct that it intends to measure. (**C** and **D**) represent measures with low validity and reliability. The measures do not accurately reflect the true construct that they are intended to measure, nor are they repeatable over time.

1.3.3.1 Feasibility

Questionnaire-based measures are low-cost, non-invasive, relatively rapid to complete and require no special training to administer. These qualities make them appropriate for the collection of large amounts of data and attractive to both study teams and participants. This is particularly important in light of evidence that measures perceived to invade privacy or require excessive commitment are more often refused by participants [81]. Additionally, questionnaires can be used to assess the typical patterns of EB of interest to obesity research. By contrast, laboratory-based studies typically observe a single eating episode, which may not be representative of eating in naturalistic settings [82]. Finally, questionnaires are standardised measures and results can easily be compared and combined between studies using the same questionnaire. Standardised protocols have been developed for laboratory measures of EB. However, the extent to which protocols are followed varies between studies and may complicate the comparison of results [83].

1.3.3.2 Validity and reliability

Despite their many practical advantages, there are drawbacks to questionnaire-based methods. Foremost, they are not objective and their validity and reliability requires assessment. The validity of questionnaire-based measures depends upon participant's insight into their own behaviour, or that of their child, as well as the intention to report accurately and honestly. Given widespread stigmatisation of obesity, social desirability bias, a phenomenon whereby individuals aim to project a favourable image of themselves when reporting personal details or behaviours, may influence the reporting of weight-related behaviour such as EB [84]. Obesity has been shown to predict under-reporting of total energy intake relative to objective measures [85]. However, these concerns are not unique to questionnaire-based measures and may also bias laboratory-based assessments. For example, participants in one study reported that they would eat less if they believed that their EB was being monitored. Further, manipulating their beliefs about whether they were being observed resulted in changes to their consumption [86, 82]. Another study also found that heightened awareness of observation reduced consumption of energy dense snack food, particularly amongst obese participants and those who reported high disinhibition and low restraint [87]. As such, even laboratory measures based on objective data may be biased by social desirability bias. The fact that questionnaires can be completed in relative privacy and scored by individuals blinded to the identity, and crucially the appearance, of the participant may reduce the possibility of biased reporting.

There is no gold-standard for the measurement of EB against which questionnaires can be validated. In the absence of a gold-standard, triangulation between questionnaire results, weight, food intake and laboratory-based EB assessment would ideally be performed to assess validity, alongside assessment of the internal validity of questionnaire items. In practice, associations between questionnaire-based measures of EB traits and body weight, BMI or self-reported food intake have been used to infer validity. In the case of the TFEQ-R18, these studies have widely demonstrated the questionnaire's ability to identify differences in EB in the general population that relate to both weight status and food intake [88, 89, 68, 90–92]. However, as a result of inconsistencies between self-report and laboratory-observed measures, concern still surrounds the validity of self-reported EE across questionnaires [93]. It has been proposed that self-reported EE represents a type of recall bias, whereby 'concerned eaters' retrospectively misattribute episodes of over-eating to emotional distress [93]. Given the absence of a gold-standard, it is unclear at present whether the laboratory or questionnaire-based measures of EE are at fault. In the future, naturalistic monitoring of EB through m-Health devices, such as swallowing or motion sensors [94, 95], have the potential to facilitate better validation of EB measures.

In the case of the BEBQ, good internal validity between the items of the EF, FR and SiE scales have been demonstrated [39, 79], alongside associations between the behaviours and infant

weight (**Section 1.4.2**). The validity of the BEBQ is further supported by CEBQ validation studies of the same EB traits in children [96–102].

1.3.3.3 Summary

Only a small number of studies have assessed the reliability of questionnaire-based EB measures. Those that have suggest that the BEBQ and TFEQ-R18 are both reliable [91, 103–105]. The scales comprising the questionnaires have also been shown to be internally valid [39, 79, 88]. However, there is no gold-standard measure fo r the assessment of external validity. Thus, despite consistent associations between EB and both BMI and food intake, continued scrutiny is required. Better validation in the future maybe facilitated by naturalistic monitoring using m-Health technologies.

1.4 Eating behaviour and body weight

The importance of EB to public health derives primarily from its associations with weight. In this section, the cross-sectional and prospective associations between EB and weight amongst adults and infants are discussed.

1.4.1 Adults

1.4.1.1 Emotional eating

Consistent, positive cross-sectional associations have been identified between EE, measured using the TFEQ-R18, TFEQ-R21 or DEBQ, and adult BMI. These findings are replicated across a number of studies including Swiss [106, 107], French [108], Finnish [109, 110, 68], Canadian [111], American [112, 113], German [114] and British study populations [115, 116]. However, it is notable that a number of investigations involving adolescent participants have not replicated this finding [116–118]. Indeed, one study including over 9000 Dutch adolescents with a mean age of 13 years identified a negative association between EE, measured using the DEBQ, and the probability of being overweight amongst boys [119]. A separate cross-sectional study reported that whilst EE, measured using the TFEQ-R18, was positively associated with BMI in both adults and 16-17 year old adolescents, the magnitude of this association was greatest amongst the adult participants [107]. Together, these findings suggest that either the associations between EE and BMI strengthen with age, or that insight into EE improves with age. Cross-sectional studies cannot be used to infer causality. Longitudinal investigations, whilst still subject to potential biases and confounding, are more informative in this regard. A number of longitudinal studies have indicated that EE is prospectively linked to adult weight gain [120, 121, 106, 122, 123]. However, most of these studies are relatively small-scale and have followed individuals over time periods of just 1-2 years. The largest study followed 3735 Finnish adults aged 25-74 years over 7 years [123]. Overall, the available evidence to date links EE to both BMI and weight gain in adults [122].

1.4.1.2 Uncontrolled eating

Many of the studies reporting a positive cross-sectional relationship between EE and BMI, also identify positive, cross-sectional associations between UE and adult BMI [110, 112, 114, 124, 88]. Studies using the TFEQ-51, which measures disinhibition and hunger (behaviours for which the majority of items were combined in later versions of the TFEQ under UE), have also identified positive, cross-sectional associations with BMI [125, 111, 126] and prospective associations with weight gain [127–129]. However, the results are less consistent for the external eating scale of the DEBQ. In particular, some studies amongst adolescents report

negative cross-sectional and prospective associations between external eating and BMI [118, 116, 119], whilst positive associations have been reported amongst adult populations [130, 121]. Overall, TFEQ-R18 measures of UE have shown consistent associations with weight and weight gain over time. However, measures of similar constructs, notably external eating, have produced less consistent results.

1.4.1.3 Cognitive restraint

Cross-sectional studies of the association between restraint and BMI primarily report a positive association, with higher restraint being associated with higher BMI and probability of being overweight [119, 124, 107, 110, 68, 112, 114, 92]. However, a minority of studies have found no cross-sectional association between restraint and BMI [126, 125]. Additional studies suggest that these inconsistent findings may be the result of a BMI-dependent relationship between CR and BMI. Although some investigations found consistent associations between CR and BMI amongst all BMI groups [68], others suggest that CR is positively associated with BMI amongst normal weight, but not overweight, individuals [131, 88]. Few studies have directly investigated this potential non-linear association. However, amongst 326 adults with a mean BMI of 26.6 kg/m^2 (range: 18 kg/m^2-46.5 kg/m^2), an inverted U-shaped relationship between CR and BMI was reported, with lower CR values being reported by individuals at the extremes of the study sample's BMI distribution [132].

In keeping with the initial conceptualisation of CR as an obesogenic behaviour, CR has been found to predict weight gain in some studies [127, 110, 133, 134]. However, approximately half of studies find no prospective association between CR and weight gain based on both objective measures of BMI [131] and retrospective report [128, 121]. A growing number of studies suggest that increases in BMI prospectively predict increases in CR, indicating that CR may represent a reaction to weight gain, as opposed to a causal factor [131, 135–137]. For example, a study amongst 3735 Finnish adults aged 25-74 years showed that whilst baseline CR did not predict BMI change over 7 years, BMI change predicted increases in CR over the same time period [138]. The same study indicated that genetic risk of obesity, modelled using a genetic risk score for BMI (BMI-GRS), was associated with higher CR, suggesting that CR might provide a proxy for susceptibility to weight gain. In this study individuals with high BMI-GRS scores gained more weight between the age of 20 years and the baseline assessment than those with low BMI-GRS scores. However, the effect was less pronounced amongst those with higher CR. A recent review of the effect of dietary restraint on BMI change amongst non-obese individuals (BMI 18.5-29.9 kg/m^2) concluded that restraint is a weak and inconsistent positive predictor of weight gain and concurred that CR may represent a proxy measure of susceptibility to weight gain [51]. Overall, the available evidence suggests that CR is inconsistently associated with weight gain and could represent a response to weight status.

1.4.2 Infants

To date, the majority of studies of infant EB traits are cross-sectional and many are small scale. In general, these investigations report negative associations between weight and the 'food avoidance' scales of the BEBQ (SR and SiE) and positive associations between weight and the 'food approach' scales (FR and EF) [139, 79]. Amongst 4634 infants from the UK Gemini twin cohort and 167 infants from the Australian New Beginnings: Healthy Mothers and Babies study, respectively, negative associations were reported between both SR and SiE with weight [139, 79]. The UK study also demonstrated small but statistically significant positive associations between the food approach scales and weight. Whilst the Australian study replicated the positive association between EF and weight, no association between FR and weight was found [79]. A separate study of 85 mother-infant dyads from the UK and Israel suggested weak associations between the BEBQ EB traits and infant weight z-score amongst infants aged 2-6 months [140].

The BEBQ was designed to quantify EB traits measured using the CEBQ in childhood amongst infants (see **Section 1.3.2**). Given that the CEBQ predates the BEBQ, a greater number of studies have investigated the consequences of FR, SR, SiE and EF in children. Amongst children, EF and FR have been consistently positively linked to BMI across a range of cross-sectional studies [96–101]. The same studies have shown negative associations between both SR and SiE and childhood BMI. For example, higher BMI standard deviation scores (BMI-SDS) were positively associated with lower SR and higher FR in both 3-5 year olds and 8-11 year olds in one study [102].

In light of the reported cross-sectional associations, longitudinal studies are needed to help clarify the direction of causality between infant EB and body weight. These investigations generally suggest that infant EB prospectively influences weight gain [141, 142]. For example, one study showed that appetite measured at 3 months was associated with the degree of weight gain from 3-9 months, as well as demonstrating a positive association with weight at both 9 and 15 months [141]. A separate study amongst 210 infants from the GUSTO cohort found that SR at 3 months was negatively associated with BMI at 6 months and with less rapid weight gain from 3-6 months [143]. Conversely, FR at 3 months was positively associated with BMI at 6 months and with more rapid weight gain from 3-6 months [143]. However, this longitudinal evidence does not conclusively demonstrate causality. It remains possible that infant EB and weight gain share a common aetiology, that weight in very early life influences the development of EB or that the relationship is bi-directional. For example, amongst 4350 mother-infant dyads from the Generation R Study, more rapid fetal growth from late pregnancy to birth was associated with reduced SR at 4 years [144]. Similarly, higher birth weight was associated with a more appetitive EB profile, characterised by higher FR and EF, and lower SR scores at 4 years [144]. These findings suggest that intrauterine growth may potentially impact the development of EB.

Some evidence also suggests an effect of infant weight on EB traits. Accelerated weight gain from 0-5 years was associated with reduced SR and higher daily calorie consumption at 5 years in one study and children who grew rapidly from 6-36 months showed lower SR and higher FR scores at 3-6 years in another study [145, 146]. Lack of baseline EB data complicate the drawing of definitive conclusions from these studies. One study has explored the relationship between EB and weight in both directions, concluding that whilst weight predicts appetite from 3-15 months, appetite predicts weight to a greater extent [141]. Amongst children, the association between EB and BMI also appears to be bi-directional. Higher scores on the appetitive EB trait scales (FR and EF) have been reported both as a cause and consequence of weight gain. In one study, between the ages of 4-8 years, high FR predicted a steeper increase in BMI-SDS, whilst BMI-SDS also predicted increases in FR and decreases in SR [147]. In another study, being at risk of becoming overweight at age 5 (defined as a BMI >85th percentile) predicted the emergence of dietary restraint and disinhibited eating at age 9 [148].

1.4.3 Summary

In sum, consistent evidence supports a role for both UE and EE in weight gain in adult populations across diverse settings. Food approach behaviours (EF and FR) also promote weight gain in children and infants, whilst EB associated with food avoidance (SR and SiE) are typically associated with lower BMI in these age groups. Studies suggest that whilst associations between body weight and EB are likely to be bi-directional in infancy and childhood, the impact of EB on body weight exceeds that of body weight on EB. The literature provides less clarity regarding the role of CR, a trait measured exclusively in adulthood. CR generally demonstrates positive cross-sectional associations with BMI at least amongst normal weight individuals. However, associations may be BMI-dependent and prospective data suggests that the association may be the result of reverse causality, with high levels of CR representing a response to high weight status.

1.5 Eating behaviour and the aetiology of obesity

Together **Sections 1.2**, **1.3** and **1.4** demonstrate the importance of the relationship between EB and weight to public health, as well as highlighting areas where further research is needed to clarify associations. Here the role of EB in selected aetiological pathways to obesity is elaborated. **Section 1.5.1** discusses the role of EB in genetic predisposition to obesity, **Section 1.5.2** reviews the interplay of infant EB and parent control over infant feeding in the development of obesity and **Section 1.5.3** discusses the potential contribution of GWAS studies of EB traits to the understanding of obesity.

1.5.1 Eating behaviour and the genetic aetiology of obesity

Obesity is a heritable phenotype. A meta-analysis of BMI GWAS studies, published in 2015, identified 97 genetic variants reaching genome-wide significance, together explaining ~ 2.7% of inter-individual variation in BMI [149]. The mechanisms through which these variants act to influence body weight are largely unknown. However, it has been proposed that EB traits provide one plausible mechanism. The predominant theory regarding the relationship between EB and the genetics of BMI is depicted in **Figure 1.4**. The theory proposes that the 'appetitive' EB traits (i.e. EB traits associated with increases in food consumption as a result of responsiveness (or lack of responsiveness) to cues of hunger and satiety) lie on the causal pathway between genetics and BMI, partially mediating genetic predisposition to obesity [102].

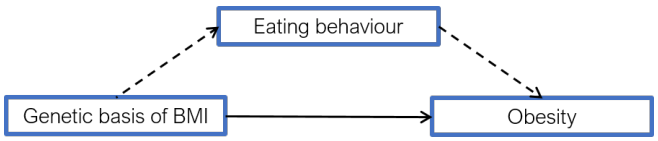


Figure 1.4 Eating behaviour as a mediator of genetic susceptibility to obesity.

Evidence in support of this theory arises from several sources. First, in aggregate, the 97 variants implicated by BMI-associated loci demonstrate enriched expression in the central nervous system (CNS) [149]. This suggests a role for cognitive pathways, and hence for behaviour, in the determination of BMI (**Figure 1.5**) [149]. Further, the enrichment is particularly pronounced in the hypothalamus, pituitary gland, hippocampus and limbic system, areas of the brain with established roles in the central regulation of eating [149].

Second, twin studies have demonstrated shared genetic influences for appetitive traits and weight during infancy [139]. Amongst 4634 twins from the Gemini cohort study, the genetic correlation between the BEBQ measured EB traits and weight at 3 months was between 0.22 and 0.37, suggesting that the genetic basis of EB and weight is partially shared, at least

during infancy [139]. Moreover, indirect evidence that EB mediates genetic predisposition to obesity derives from the observation that hyperphagia in monogenic obesity syndromes drives weight gain [150]. This is described more fully in **Section 1.5.3.1**.

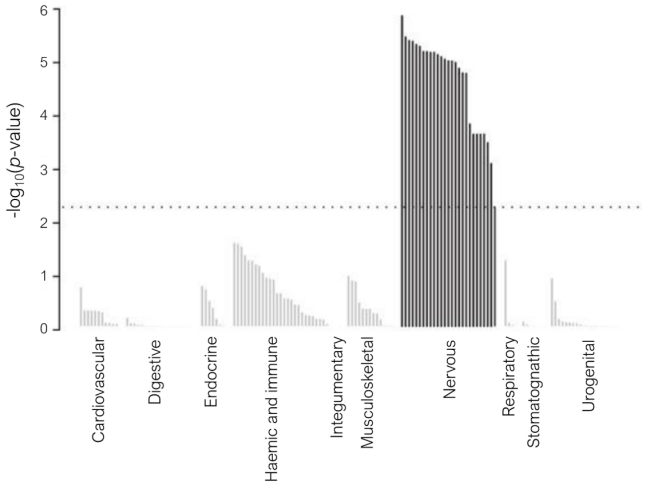


Figure 1.5 Tissues enriched for genes implicated by BMI-associated loci. Adapted from Locke et al [149]. The graph shows that genes within BMI-associated loci show enriched expression in the CNS. Tissues are sorted by physiological system along the x-axis and plotted against the -$\log_{10}$(p-value) on the y-axis. The horizontal dotted line represents statistically significant enrichment. Significantly enriched tissues are displayed in black.

The findings of gene discovery studies for BMI have facilitated candidate gene analyses for EB and energy intake, based on BMI-associated variants. Together, the findings of these studies support a role for EB in genetic predisposition to obesity. *FTO* has the greatest magnitude of effect of any known common variant on BMI [149]. As a consequence, it has attracted much research attention. Amongst children, the BMI increasing *FTO* allele has been linked to increased FR, decreased SR and increased palatable food consumption after a meal [151–154]. In adults, *FTO* has been linked to higher total energy intake [155], binge eating [156] and CR [112]. *MC4R* has been linked to both UE [157] and EE [158], *NMB* to disinhibition and hunger [159], *HTR2A* to food reinforcement [160] and *MTCH2* to EE [112].

Studies of the aggregated effects of BMI-associated variants on EB have also shown positive associations with EE and UE in adulthood [161, 112], negative associations with SR in childhood and positive associations with appetite in infancy [162, 163]. Prior to the study reported in **Chapter 4** (published in 2017), direct testing of mediation of genetic predisposition to obesity by EB had been performed in three studies: two in children and one in adults [163, 164, 161]. Amongst 2258 children with a mean age of 10 years, SR mediated the association between 28 BMI-associated loci and weight [163]. A second study in children did not replicate these findings, reporting that the association between 32 BMI-associated loci and weight gain was not mediated by EB traits amongst 652 children aged 6-8 years [164]. Both studies were conducted in European cohorts and derived EB measures using the

CEBQ. It is possible that, as a result of lower sample size, the latter study was not powered to detect a true mediating effect. Alternatively, mediation may not be present in younger age groups, the mediation effect may be of weight status established in early life and not of weight gain or the findings of the earlier study may be spurious. A 2019 study reported that the association between appetite, measured by a single item, partially mediated the association between 16 BMI-associated loci and BMI amongst 1142 French children aged 2-5 years [162]. This suggests that the discrepancy between the results of the earlier studies is not due to differences in the age of the children.

The single pre-existing study amongst adults tested for mediation of the association between 90 BMI-associated loci and BMI by UE and EE in two Finnish cohorts, comprised of 4632 and 1231 individuals, respectively [161]. The BMI-GRS to BMI association was mediated by EE in both cohorts and by UE in one cohort. As a result of the small number of studies conducted, the limited number of BMI-associated loci included and the lack of investigation of the role of CR in genetic predisposition to obesity, more research is required to provide clarity.

1.5.1.1 Summary

Whilst several lines of evidence suggest that EB lies on the causal pathway between the genetics of BMI and realised weight, no studies prior to that reported in **Chapter 4** had investigated mediation of genetic predisposition to obesity by EB traits based on the full range of 97 BMI-associated variants. Further, possible relationships between EB and genetic predisposition to obesity, beyond mediation, had not been interrogated. This is of particular importance to the understanding of the role of CR in obesity. Whilst it is possible that cross-sectional associations between CR and BMI are explained by a causal influence of CR on BMI, the evidence is mixed. If CR represents a weight-limiting strategy for those predisposed to obesity, it may modify, rather than mediate, genetic predisposition. This is explored in **Chapter 4**.

1.5.2 Infant eating behaviour, parental feeding styles and obesity

1.5.2.1 Rapid infant weight gain and obesity

The first 1000 days, from conception to the age of 2 years, have been identified as a critical period for determining vulnerability to overweight and obesity in later life [37, 165, 166]. The best established risk factor operating during this period is rapid infant weight gain. A systematic review of the risk factors for childhood obesity in early life published in 2016, identified 46 studies including high infant weight and rapid infant growth as an exposure, 45 of which reported significant positive associations [165]. The review concluded that rapid

infant weight gain is the only postnatal factor, besides infant birthweight, with consistent evidence supporting a causal association with childhood obesity. More recently, a 2018 systematic review replicated this finding in adults, reporting an association between rapid infant weight gain and adult obesity [166].

In early life, there is a close association between energy intake and weight. By contrast, studies have not been able to provide clear evidence of a substantive link between energy expenditure and infant weight outcomes [167, 168]. As a result, rapid weight gain during infancy is thought to be primarily determined by factors influencing infant energy intake. Mounting evidence indicates that these include both infant and parental influences [37, 165]. In **Chapter 5** the independent and interacting roles of maternal attitudes to following healthy infant feeding recommendations and infant EB in the determination of infant milk intake and weight is interrogated. Here, the role of infant EB and parental feeding behaviour in the determination of infant weight, is elaborated.

1.5.2.2 Parental feeding styles and responsive feeding

As detailed in **Section 1.4**, the role of restraint over eating in obesity is contentious. Whilst some researchers conceptualise restraint as a problematic EB trait that disposes to a loss of control over eating and, hence, obesity [38], others argue that it represents a response to weight gain [51]. In infancy, the role of parental control over infant feeding, whereby a parent restricts the amount of food an infant consumes in order to prevent excessive weight gain, is similarly contentious. Parents and other caregivers make decisions regarding how, how often and how much to feed their infants. Four parenting styles, related to the implementation of a range of care-giving activities, including feeding, have been identified and elaborated in the literature. These comprise authoritative, authoritarian, neglectful and permissive styles [169, 170].

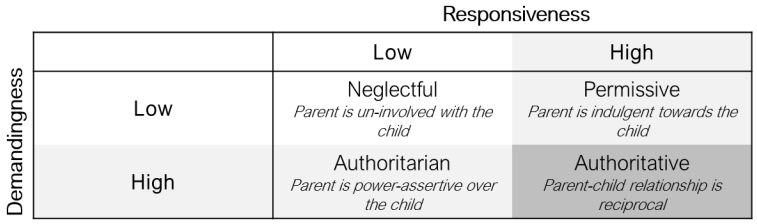


Figure 1.6 Parenting styles matrix. Based on Sokol et al [169]. The matrix shows 4 parenting styles: Authoritative, Authoritarian, Permissive and Neglectful, illustrating how they are defined with relation to responsivity and demandingness.

Each style is characterised by different degrees of responsiveness which can be understood as the degree of nurturing and warmth parents display towards the child, and demandingness, the extent to which a parent establishes and enforces boundaries for the child. The relationship of these dimensions to parenting styles is depicted in **Figure 1.6**. As shown

in **Figure 1.6**, neglectful parents are neither demanding nor responsive to their child. This parenting style is characterised by a pervasive lack of involvement in care-giving [171]. By contrast, authoritative parents are both demanding and responsive. Parents employing this style convey clear standards, monitor their child's behaviour and enforce boundaries. However, their interactions with their children are also characterised by high levels of warmth. They are able to respond to their child's needs in a way that is neither overly intrusive nor restrictive [171]. In common with the authoritative style, authoritarian parenting is characterised by high levels of control and boundary enforcement. However, parents employing this style exhibit low levels of warmth and responsiveness [171]. Finally, parents employing a permissive parenting style are warm and responsive towards their children but do not define, enforce or monitor boundaries for the child's behaviour. This style avoids parent-child conflict [171].

A systematic review of longitudinal evidence published in 2017 concluded that an authoritative parenting style (high levels of both warmth and control) may be associated with reduced risk of obesity and overweight in children [169]. However, the role of parental control in infant feeding is debated. It has previously been assumed that infants have a natural ability to self-regulate their food intake and do not require high levels of external control to achieve optimal growth. As such, high levels of parental control have been hypothesised to impair the natural development self-regulation and lead to excessive weight gain. Thus, research to date has primarily focused on the promotion of responsive parenting and the prevention of excessive parental control [172].

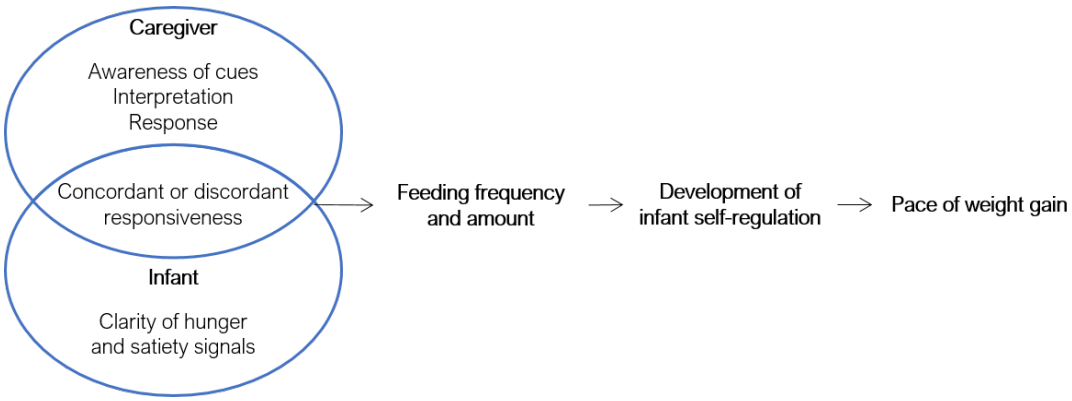


Figure 1.7 Responsive feeding. Based on DiSantis et al [172]. The figure illustrates the parent-child interaction and its hypothesised consequences for infant weight gain.

Responsive parenting as applied to infant feeding is known as responsive feeding and consists of parental awareness and appropriate interpretation of infant cues of hunger and satiety coupled with consistent, developmentally appropriate responses [172]. In order for a successful, responsive interaction to occur, the infant must also be active by providing clear, accurate signals of their needs. If an optimal interaction is achieved, it is hypothesised to facilitate the development of nascent infant self-regulatory capacity, which

in turn diminishes the risk of rapid infant weight gain (**Figure 1.7**) [172]. Non-responsive feeding practices are those which rely primarily upon maternal control to determine how and when an infant is fed. Non-responsive styles include restriction, pressurisation, active encouragement to eat, praise for eating or using food as a comfort or reward [173].

1.5.2.3 Responsive feeding and infant weight

Cross-sectional studies have broadly supported a role for non-responsive feeding practices in adverse infant weight outcomes. A 2011 review of the evidence regarding responsive feeding and childhood overweight in high income countries identified consistent associations between pressurised and restrictive feeding, both non-responsive styles predominantly identified through parent-report questionnaires, and both lower and higher infant weight, respectively [174]. This replicated the results of a 2004 review which reported a positive association between restrictive feeding practices and infant weight based upon 22 studies [175]. Few studies use objective measures of parental feeding behaviour and thus may be subject to reporting bias. However, a review of observational studies published in 2015 replicated these findings, reporting a positive association between restrictive feeding practices and body weight amongst children aged 2-6 years [176]. Further, a 2018 study using video recordings of laboratory-based parent-child feeding interactions also suggested that parental discouragement (and encouragement) to eat is associated with higher BMI z-score [177]. The majority of the studies included in all three reviews (25/31, 19/22 and 12/13) were cross-sectional and it is thus unclear whether these styles were the cause or effect of childhood weight outcomes.

Indeed, a number of longitudinal investigations have indicated that maternal feeding practices represent a response to a child's characteristics, such as weight status, as opposed to a cause [178]. Amongst 1920 8 month old infants enrolled in the Gemini cohort, maternal pressure to eat was associated with lower birthweight and lower infant appetite at 3 months, whilst maternal restriction was associated with high infant appetite at 3 months, suggesting that parents were adjusting their feeding style in response to their child's EB and birthweight [179]. Further studies are needed to provide greater clarity as longitudinal evidence has also suggested that restriction precipitates the tendency to over-eat, particularly amongst children with low inhibitory control [171].

In addition to evidence that non-responsive styles might constitute reactions to infant characteristics as opposed to aetiological factors involved in weight gain, the assumption that infants naturally self-regulate has been called into question. An RCT of an intervention designed to promote baby-led weaning, whereby children self-feed, suggests that children over-consume if given control over their intake [180]. The BLISS intervention, designed to promote baby-led approaches to complementary feeding was delivered through 5 or more group sessions and three face-to-face contacts from 5 to 9 months. Whilst no differences in

mean BMI *z*-score were observed between the intervention and control arms of the trial at 12 or 24 months, children in the intervention arm demonstrated higher probability of being overweight at 24 months, as well as lower SR and food fussiness and higher EF [181, 180]. These findings indicate that some degree of control over infant feeding might be beneficial and calls into question the wisdom of an entirely responsive approach, as infants may over-consume relative to their needs.

Several RCTs of interventions designed to promote responsive parenting have been conducted, providing varying degrees of support for the role of responsive feeding in infant weight gain. These include both the NOURISH and INSIGHT trials. The NOURISH trial intervention achieved changes in maternal behaviours with mothers in the intervention arm reporting more responsive feeding behaviours than those in the control arm [173]. However, these changes did not result in differences to infant's BMI *z*-score or probability of being classified as overweight at 2 or 5 years old [173, 182]. By contrast, the INSIGHT trial of a separate responsive parenting intervention reduced the pace of infant weight gain from 6 months to 1 year and the probability of being classified as overweight at 1 year [183]. However, by age 3 years, whilst the children in the responsive parenting group had a lower mean BMI *z*-score, they did not differ from children in the control arm with respect to mean BMI percentile or probability of being overweight or obese [184].

1.5.2.4 Summary

Overall, the relationship between infant EB, parental feeding practices and infant weight gain remains unclear. In particular, whether parental control over infant feeding is detrimental to the development of self-regulation and leads to over-eating, or represents a reaction to infant characteristics, remains uncertain. Further research is required, particularly in light of the importance of this developmental period to later life obesity risk. **Chapter 5** investigates the role of a measure of parental control (parental attitudes towards following healthy infant feeding guidelines) in the determination of infant milk intake and body weight.

1.5.3 The genetics of eating behaviour and obesity

As described in **Section 1.4**, the relationship between EB and weight may result from bi-directionality or reverse causality, particularly in the case of CR. The results of GWAS studies can help to elucidate the underlying biology of traits, including the biological pathways involved. They can also be used to identify traits that share a genetic basis and to interrogate causal relationships through MR (this method is elaborated in **Chapter 6**). Thus, GWAS could be applied both to understand the genetic basis of EB and to explore the role of EB in the aetiology of obesity.

1.5.3.1 The heritability of eating behaviour

Twin studies and evidence from monogenic obesity syndromes together indicate a genetic basis for EB. A number of investigations have used family and twin designs to estimate the heritability of EB. The majority have found evidence that CR, UE, EE, and related EB phenotypes, are heritable. Heritability estimates for EE from twin studies fall between 9% and 60% [66, 115]. The heritability estimates for UE, and its related traits (external eating, disinhibition and hunger), are comparable and fall between 0% and 69% [185, 186, 115]. Notably with regards to this thesis, TFEQ-R18 measures of UE have been estimated to be 45-69% hertiable [115], whilst measures of hunger assessed using the TFEQ-51 have evidenced lower heritability [186–188]. Overall, findings from functional MRI studies further support the heritability of EB, suggesting that brain responses to food cues are under genetic influence [189]. In the case of CR, heritability estimates from twin studies range from 0% in one study using the TFEQ-51 [186] to around 50% in studies using the TFEQ-R18 or TFEQ-21 [66, 115, 188]. A study using the DEBQ to measure restraint fell in the middle of this range, providing a heritability estimate of 31% [190]. Taken together, twin studies suggest that EB traits are heritable in adulthood.

Studies amongst infants and children have shown mixed results. Amongst 2402 British twin pairs aged under 3 months, the BEBQ has been used to demonstrate the heritability of SiE (84%), SR (72%), FR (59%) and EF (53%) [104]. Further, amongst children, a twin study involving almost 5500 British twin pairs determined that FR and SR were 75% and 63% heritable, respectively [102]. Traits related to questionnaire measures of EB, including eating rate and eating in the absence of hunger, have also exhibited evidence of heritability in twin and family studies [191, 192]. However, two recent twin studies suggest that there is a low genetic influence over EE in childhood. Amongst 2054 5 year old twins from the Gemini study, emotional over- and under-eating were both estimated to be 7% (95% CI: 6%-9%) heritable [193]. This finding was replicated amongst a sample of almost 400 twins from another British cohort, selected from lean or obese families, which detected no evidence for the heritability of emotional over- or under-eating [194]. Overall, existing studies suggest that the EB traits measured in infancy are heritable. However, emotional under-eating and over-eating in childhood are likely to be more substantively influenced by environmental factors.

Certain monogenic obesity syndromes, characterised by extreme, early-onset obesity resulting from a mutation in a single gene, also suggest a genetic basis for EB. For example, extreme obesity in association with congenital leptin deficiency is secondary to pathological hyperphagia [150]. Leptin therapy typically results in weight loss amongst these individuals, achieving its effects through the normalisation of appetite. The therapy has no demonstrable effects on either basal metabolic rate or energy expenditure [150]. Similarly, mutations in the *MC4R* gene which result in obesity are also associated with hyperphagia [150]. Associations between *MC4R* mutations and reduced SR in children have been reported [195].

Conversely, most studies of associations with adult TFEQ-measured EB traits have reported no associations with *MC4R* mutations [195].

Together, these studies suggest a genetic basis for EB traits and thus indicate that genetic approaches may be informatively applied to the study of EB.

1.5.3.2 The genetic basis of eating behaviour

Despite evidence for a genetic basis of EB, prior to the study reported in **Chapter 7**, no GWASs of EB traits measured in the general population have been reported. In part, this is due to the large sample sizes required for well-powered GWAS coupled with the relatively small sample sizes with overlapping genome-wide genotyping and EB trait information available in existing large-scale studies [196]. In the Fenland study, for example, despite a total sample size of over 12,000, just 3515 participants completed the TFEQ-R18 and EB trait data was not collected as part of UKB. By contrast, pathologies of eating have been the subject of several published GWAS.

A 2016 GWAS of food addiction, measured using the Yale Food Addiction Scale, amongst 9000 participants identified no genome-wide significant results [197]. The existence of food addiction is debated and its relationship to EB traits measured in the general population remains largely unknown, limiting the interpretation of these findings with regards to the EB traits of interest to this thesis [198, 199]. The largest GWAS of anorexia nervosa (AN) was reported amongst 3500 cases and 11,000 controls of European ancestry in 2017 [200]. This study identified a single genome-wide significant locus on chromosome 12 (rs4622308) which had previously been associated with both type 1 diabetes and rheumatoid arthritis [200]. AN demonstrated significant negative genetic correlations with BMI, insulin, glucose, and lipid phenotypes and a positive genetic correlation with high density lipoprotein (HDL) cholesterol levels and psychiatric traits [200]. Whilst these results are informative, suggesting the potential for a shared genetic basis for eating-related behaviour and metabolic traits, including BMI, eating disorders represent clinically significant aberrations in EB. They are serious mental illnesses characterised by persistent, pathological alterations in EB and associated with a core psychopathology of overvaluation of shape or weight [201, 202]. The extent to which eating disorders are continuous with non-pathological EB traits is the subject of ongoing debate and, as such, GWAS results for AN cannot be extrapolated.

1.5.3.3 Summary

Despite evidence suggesting a genetic basis for adult EB, no GWAS studies have been conducted. This results, in part, from low sample sizes with intersecting genome-wide genotyping and EB data in individual studies and represents a significant gap in the existing literature.

1.6 Risk-taking propensity and the aetiology of obesity

As reported in **Section 1.5.1**, genes implicated by BMI-related genetic loci demonstrate enriched expression in the CNS, implicating behavioural pathways in the aetiology of obesity [149]. Alongside EB traits, other behaviours also represent potential pathways. The tendency to take risks has been shown to demonstrate positive cross-sectional associations with overweight and obesity [203, 204]. Whether these associations are causal and whether they are independent of EB is not yet known. In the following section, the definition and measurement of risk-taking propensity is discussed and its association with BMI is reviewed.

1.6.1 Definition and measurement

Risk-taking propensity describes an underlying tendency to engage in reward-seeking actions despite the possibility of negative consequences [205]. The willingness to take risks in light of a known balance of potential positive and negative consequences is considered the core characteristic of risk-taking propensity and a feature that distinguishes this phenotype from other, related traits [206]. For example, risk-taking is closely linked to impulsivity, a multi-faceted construct defined by high levels of urgency (the tendency to experience frequent, strong impulses under conditions of negative mood), lack of perseverance when tasks are boring or difficult, lack of premeditation and sensation-seeking (the pursuit and enjoyment of new experiences) [207, 208]. Impulsivity and risk-taking propensity are distinguished by the fact that risk-taking propensity does not require risk-engagement to be unplanned or motivated by the seeking of novelty. However, the distinction is not clear-cut. Some researchers contend that risk-taking behaviour is rooted primarily in sensation-seeking and thus encompasses some aspects of impulsivity [209]. In light of its associations with realised risk-taking behaviours, here impulsivity is considered a subset of risk-taking propensity [210, 211].

There is no gold-standard measurement of risk-taking propensity. However, several behavioural and self-report measures are widely used. Amongst behavioural measures, the most commonly employed are the Iowa Gambling Task (IGT) and the Balloon Analogue Risk Task (BART). In the IGT, participants are given access to several decks of cards alongside a starting amount of money. They are asked to pick 100 cards from the decks. Each of the cards represents a monetary loss or gain. The decks each have an equal probability of yielding losses or gains [212]. However, as participants discover during the course of the task, some decks are high-risk, high-reward decks (meaning that the profits and losses are both high. However, the value of the losses in these decks exceeds the value of the gains) whilst others are low-risk, low-reward decks (meaning that they yield lower gains but also lower losses. However, the value of the gains exceeds the value of the losses). In the long-run, the high-risk decks will result in a net loss, whilst the low-risk decks will result in net gains.

A participant's tendency to risk high losses in the pursuit of high gains, rather than to rely on the low-risk decks, is considered a measure of their propensity to take risks.

In the BART, participants are instructed to blow up virtual balloons by clicking a button on a computer [213]. Each pump inflates the virtual balloon and the participant receives money for the number of pumps he or she delivers. However, the balloon will eventually explode at an unspecified point, if over-inflated. If this happens the participant will receive no money. Over repeated trials, participant risk-taking propensity is inferred from the willingness to risk balloon explosion [213].

Studies assessing the validity of the IGT and BART measures of risk-taking propensity have generally found both measures to be associated with risk-taking behaviour (such as substance and alcohol abuse) but not with each other [214]. It has been proposed that, whilst BART measures intentional risk-taking, the time it takes to learn which decks are risky in the IGT means that IGT may reflect unintentional risk-taking, particularly in the early stages of the task [214].

Self-report measures of risk-taking propensity also exist. These vary in complexity from single question measures to multi-item questionnaires. For example, Dohmen and colleagues measure risk-taking propensity in response to a single question: "How do you see yourself? Are you generally a person who is fully prepared to take risks or do you try to avoid taking risks? Please tick a box on the scale, where the value 0 means not at all willing to take risks and the value 10 means very willing to take risks" [215]. This measure has been shown to predict smoking and other risk behaviours, but is not widely used [216]. UKB measures risk-taking on the basis of a single, un-validated question with a binary (yes/no) response: "Would you describe yourself as someone who takes risks?". By contrast, the Domain Specific Risk Taking scale (DOSPERT) [217] and Sensation seeking scale [218] measure risk-taking propensity using multiple items. The items are combined to develop a risk-taking propensity score, with domain specific scores indicating risk-taking propensity specific to particular domains of behaviour (financial, ethical, health/safety, social, and recreational) in the DOSPERT questionnaire.

1.6.2 Risk-taking propensity and obesity

1.6.2.1 The association between risk-taking propensity and health

Overall, risk-taking propensity is considered an important risk-factor for behaviours that impact health, including smoking, alcohol use, drug use, sexual behaviours and driving safety. For example, higher levels of risk-taking propensity measured through BART and self-report questionnaires have been linked to higher rates of smoking, unprotected sex, driving dangerously and alcohol use [216, 219, 213, 220]. A 2017 systematic review of 17 studies identified impaired IGT performance (indicating higher risk-taking propensity)

amongst individuals with gambling disorder and alcohol use disorder [221]. Whilst these findings have not always been replicated [216], there is a general consensus that risk-taking propensity is associated with health-related behaviour.

1.6.2.2 The association between risk-taking propensity and body weight

More recently, several studies have reported cross-sectional associations between the propensity to take risks and obesity [203, 204]. For example, amongst 121 participants, overweight and obese men took more risks in the IGT and obese women exhibited higher impulsivity, relative to those of normal weight [203]. In another study, compared to their normal weight peers, adolescents with a BMI above the 99^{th} percentile for their age and sex reported greater odds of a range of risk-taking behaviours, including smoking and having used drugs or alcohol before their last sexual encounter [222]. Other findings suggest that obese individuals are more likely to neglect long-term outcomes in decision-making, making them more prone to impulsive actions [207]. Obesity has also been associated with risky decision-making, inferred from lower scores on the IGT [223, 224], and lower scores on measures of executive functioning [225].

A 2018 meta-analysis of 72 studies including a total of 4900 overweight and obese participants reported that overweight was associated with reductions in inhibition, whilst obesity was associated with broad impairments to executive functioning (the ability to organise and inhibit sets of actions in order to achieve a goal) [226, 227]. A systematic review of the association between personality and obesity, published in 2015, identified neuroticisim, impulsivity, conscientiousness and self-control as personality traits of relevance to obesity [204]. Specifically, neuroticism (a measure of negative emotionality, particularly in response to adverse experiences [228]) and impulsivity (the tendency to act without forethought and to exhibit a lack of behavioural inhibition [229]) were identified as risk factors for the emergence of obesity [204]. Conversely, conscientiousness (a measure of goal-directed behaviour [230]) and self-control were found to protect against weight gain [204]. In some conceptual models, including the Five Factor Personality model, impulsivity is considered a component of neuroticism [228] and studies suggest that impulsivity may partially underlie the link between neuroticism and obesity [204, 230]. For example, analyses in the Baltimore Longitudinal Study of Ageing, a cohort with follow-up data spanning more than 50 years, showed that positive longitudinal associations between neuroticism and weight gain were primarily explained by inter-individual differences in impulsivity [231].

Few studies have explored the underlying personality traits associated with EB. However, two investigations have reported positive cross-sectional associations between both external eating and EE with neuroticism amongst students and obese adults, respectively [230, 232]. The larger of these studies reported that, amongst obese participants, the association was driven by impulsiveness [230]. In the same study, restraint was linked to

higher conscientiousness, lower neuroticism and more extraversion and openness [230]. A single study amongst 55 adult women has also reported that high levels of impulsivity interact with food cravings to increase reactions to palatable food cues [233]. Other studies have also linked impulsivity to eating-related traits and behaviours associated with higher energy intake, including snacking [234, 235]. In particular, attentional impulsivity (the inability to stay focused) has been positively associated with measures of the salience of external food cues, such as the pleasantness of high-calorie foods, perceptions of hunger, disinhibition and external eating [236, 237]. It has been hypothesised that high attentional impulsivity might increase susceptibility to palatable food cues, inducing over-eating and leading to weight gain over time [238]. However, the observation of ADHD-like symptoms (characterised by high impulsivity) in the majority (~ 80%) of homozygous carriers of *MC4R* mutations, who suffer early-onset severe obesity, suggests the possibility of reverse causality or shared pathways [239].

1.6.2.3 The genetics of risk-taking propensity and the aetiology of obesity

Whilst studies suggest an association between risk-taking propensity and obesity, the direction of causality, the presence of confounding and the potential mechanisms of this association, including EB, require further investigation. In these regards, GWAS and downstream analyses can be used to inform understanding. Heritability estimates for risk-taking range between 0-55%, indicating that it may be possible to study risk-taking from a genetic perspective [240–242].

Prior to the work reported in **Chapter 6**, several gene discovery studies of risk-taking had been conducted. The first was a candidate gene study in 23andMe, which explored associations between *CADM2* and a range of personality traits, including risk-taking propensity assessed by the question: *Overall, do you feel comfortable or uncomfortable taking risks?* [243]. Amongst 140,500 participants in this study, *CADM2* was linked to risk-taking propensity [243]. A GWAS of risk-taking propensity has also been conducted among 116,225 UKB participants based on the question: *Would you describe yourself as someone who takes risks?* [242]. The study identified two genome-wide significant loci, one within *CADM2* and the other within the human leukocyte antigen (HLA) region on chromosome 6. A novel positive genetic correlation between risk-taking and obesity was reported in this study, suggesting a shared genetic basis for these traits [242].

The risk-taking propensity phenotype is now available in a greater number of UKB participants, facilitating a larger GWAS study of this trait which may be better powered to identify a larger number of genetic variants. This would enable a better understanding of the underlying biology of risk-taking propensity, as well as having the potential to inform a deeper understanding of the association between risk-taking and obesity.

1.6.3 Summary

Cross-sectional associations between measures of risk-taking propensity and obesity suggest that risk-taking behaviour might play a causal role in the development of obesity. However, obesity has previously been shown to have implications for cognition, raising the possibility of reverse causality [244]. Further, poor sleep quality has been linked to both obesity and risk-taking [245, 246]. Thus there is a possibility that confounding may explain the observed correlations. Better characterisation of the genetic basis of risk-taking propensity has the potential to facilitate understanding of the relationship between risk-taking and obesity, including exploration of the role of EB in this pathway.

1.7 Thesis Aims

The over-arching aim of this PhD is to contribute to the understanding of the association between EB and the aetiology of obesity. Within this remit, three sub-aims are interrogated through 5 studies. These are depicted in **Figure 1.8**. The diagram is not intended to represent all possible relationships between the included variables, but depicts those of central relevance to this thesis. The aims are elaborated in **Figure 1.9**.

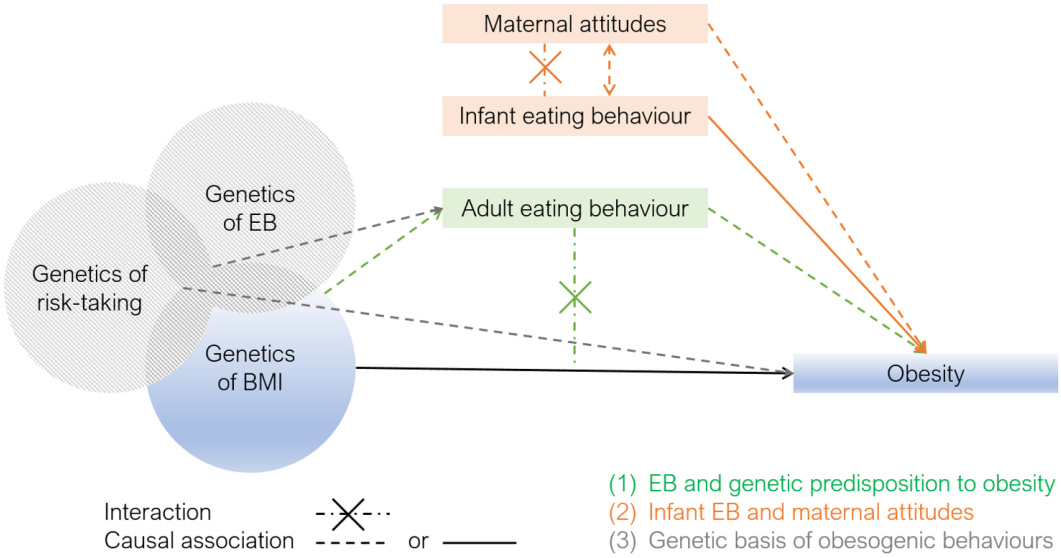


Figure 1.8 Aims of the thesis. Associations relevant to this thesis are indicated by arrows or lines in the figure. The dashed lines or arrows indicate novel contributions to the literature, whilst the solid lines represent established associations replicated by this work. The arrows represent hypothesised or reported causal associations, whilst the lines represent interactions. Under Aim 3, GWAS are performed to elucidate the genetics of both EB and risk-taking propensity, thus these circles are dashed. The genetic variants involved in the determination of BMI will be taken from a previous GWAS meta-analysis [149].

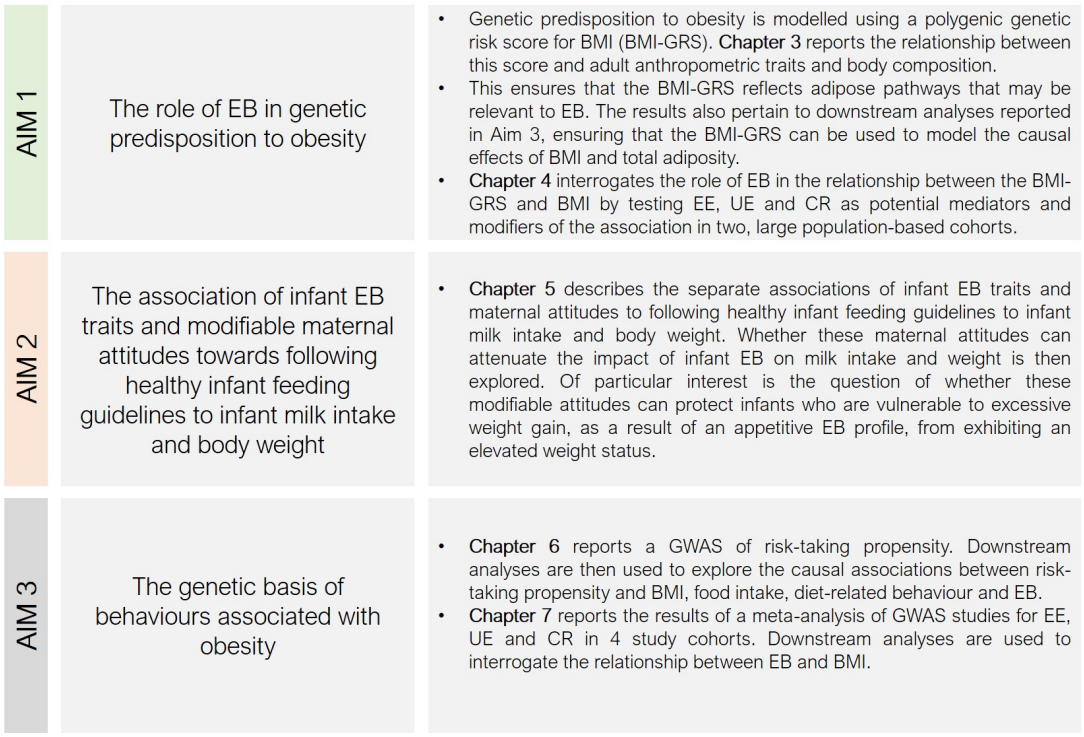


Figure 1.9 Elaboration of the aims of the thesis.

CHAPTER 2

DATA SOURCES

The work reported in this thesis is based primarily upon data from two population-based cohort studies and one randomised controlled trial (RCT). General information regarding these studies, including recruitment, data collection and laboratory methods, are provided in this chapter. Details of sub-populations, phenotypes and other study populations pertinent to particular studies are discussed within the relevant chapters.

2.1 The Fenland study

2.1.1 Background

The Fenland study is a population-based cohort study designed to investigate the independent and interacting effects of environmental, lifestyle and genetic influences on the development of obesity, type 2 diabetes and related metabolic disorders. Relatively young individuals were recruited to the cohort to facilitate investigation of the early processes and pathways involved in metabolic illness, unaffected by therapy or co-existing disease.

The Fenland study has two distinct phases. Phase I, during which baseline data was collected from participants, took place between 2005 and 2015. Phase II was launched in 2014 and involved repeating the measurements collected during Phase I, alongside the collection of new measures. All participants who had consented to being re-contacted after their Phase I assessment were invited to participate in Phase II. At least 4 years must have elapsed between visits. As a result of this stipulation, recruitment to Phase II is ongoing.

2.1.2 Participants

Adults born between 1950 and 1975 and registered at participating general practices in Cambridge, Ely, Wisbech and the surrounding Cambridgeshire region were eligible for inclusion in the study [247]. Individuals fulfilling these criteria were identified through National Health Service (NHS) primary care practice lists and invited to participate in the study through their general practitioner (GP) [247]. Exclusion criteria were: clinically diagnosed diabetes, inability to walk unaided, terminal illness (life expectancy <1 year at the time of recruitment), clinically diagnosed psychotic disorder, pregnancy or lactation.

A total of 46,020 individuals were approached, resulting in the enrolment of 12,435 participants (response rate: 27%).

2.1.3 Data collection

Participants attended one of three Medical Research Council (MRC) Epidemiology Unit testing centres where anthropometric and body composition measures were taken, blood samples were collected and questionnaires were completed. The following discussion describes the collection of data relevant across the studies reported in this thesis. Phenotypes relevant to specific studies are described within the relevant chapters.

2.1.3.1 Anthropometric and body composition measures

Anthropometric and body composition measures were collected by trained staff following standard, established protocols [247]. Volunteers were barefoot and wore light clothing during the assessment. Weight was measured to the nearest 100g using a calibrated scale (Tanita model BC-418 MA; Tanita^{TM}, Tokyo, Japan) and height was measured to the nearest 0.1cm using a calibrated wall-mounted stadiometer (Seca 240; Seca^{TM}, Birmingham, UK). Waist (WC) and hip circumference (HC) were measured to the nearest 0.1cm using a non-stretchable fibre-glass insertion tape (D-loop tape; Chasmors Ltd, London, UK). Waist and hip measurements were each taken twice. If measures differed by >3cm, a third measurement was taken. The mean of the two or three measurements was recorded and used in the analyses reported in this thesis. Waist-to-hip ratio (WHR) was calculated by dividing WC by HC.

Full body dual-energy x-ray absorptiometry (DXA) scans (GE Lunar Prodigy Advanced, GE Medical Systems, Hartfield, UK) were used to derive fat, lean and bone mass measurements across body regions. Scans were performed for all consenting participants weighing ≤ 140kg. Beyond this threshold, the scanner was considered insufficiently precise to warrant performing the scan. Fat, lean and bone mass measures in the total body, trunk, android, gynoid and leg regions were generated and appendicular lean mass (lean mass in the legs + lean mass in the arms) was calculated. The DXA software (enCORE software version 14.10.022 to 16, GE Medical Systems) also generated estimates of visceral adipose tissue within the android region (VAT) for individuals whose girth allowed them to fit within the scanning area and who had >1g of VAT. Using these VAT measurements alongside total android fat mass, values for subcutaneous adipose tissue within the android region (SAT) were generated (SAT = android fat mass−VAT) and VAT/SAT ratio was calculated.

2.1.3.2 Eating behaviour

Eating behaviour (EB) traits were assessed using the 18-item version of the Three Factor Eating Questionnaire (TFEQ-R18), completed during the baseline assessment. The questionnaire is provided in full in **Appendix C.1** [40] and described in detail in **Chapter 1**.

2.1.3.3 Genetic data

DNA extraction from ethylenediamine tetraacetic acid (EDTA) whole blood was performed using a standard technique at Whatman BioSciences (Cambridge, UK). DNA was genotyped at the MRC Epidemiology Unit (Cambridge, UK) on one of three platforms: the Affymetrix UK Biobank Axiom array (n=9,368), the Affymetrix Genome-Wide Human SNP 5.0 array (n=1,402) or the Illumina Infinium CoreExome-24 array (n=1,664). Standard sample-level quality control (QC) procedures were applied (call rate: ≥ 95%, minor allele frequency

(MAF): >0.1%, p-value for deviation from Hardy-Weinberg equilibrium (P_{HWE})> 5×10^{-6}). Missing genotypes and those not directly measured were imputed via IMPUTE version2 [248] based on the 1000 Genomes (1000G) Project European haplotype reference [249]. The exact 1000G version used was dependent upon the array and date of imputation. All required single nucleotide polymorphisms (SNPs) within the sub-populations comprising this thesis could be imputed in this manner with sufficient accuracy (imputation information value >0.4).

2.1.4 Funding

The Fenland study is funded by the MRC (MCU106179471, MCUU12015/1, MCPC13046).

2.1.5 Ethical approval

Written informed consent was attained from all participants and the study was approved by the Cambridge Local Research Ethics Committee.

2.2 The UK Biobank study

2.2.1 Background

UK Biobank (UKB) is a large, population-based prospective cohort study, established in the early 2000s to facilitate investigation of the determinants of human health, morbidity and mortality in middle to old age [250]. Data on an extensive list of exposures, coupled with prolonged follow-up of cause-specific outcomes is held and remains fully open access for research deemed to be in the public interest [251].

2.2.2 Participants

Recruitment took place between 2006 and 2010. Individuals who lived within ~ 25 miles of an assessment centre were identified through centralised NHS primary care registrations and invited to participate. Those aged 40-69 years without pre-existing health conditions were targeted in order that the study population was both old enough that a reasonable number of incident disease outcomes could be anticipated during the early years of follow-up and young enough that the initial assessment took place before illness had impacted upon exposures [252]. Approximately 9.2 million invitations were mailed, resulting in the enrolment of 503,325 participants (a response rate of ~ 5%) [253]. Participants were aged 37-73 years (99.5% between 40 and 69 years) and had no known pre-existing health conditions.

2.2.3 Data collection

Participants attended one of the 22 UKB assessment centres for a baseline assessment. A detailed catalogue of the data collected and protocols for each variable are available on the UKB data showcase at: http://biobank.ctsu.ox.ac.uk/crystal/. Briefly, participants completed a touchscreen questionnaire designed to assess socio-demographic characteristics, early life exposures, medical history, lifestyle factors, cognitive function, hearing and psychosocial characteristics. Aspects of this questionnaire were clarified and elaborated upon in individual interviews with trained staff. In addition, a range of basic physical measurements were taken. Blood, urine and saliva samples were also collected [253]. The collection of data relevant across the studies reported in this thesis is described below. Specific phenotypes of relevance to particular studies are described within the relevant chapters.

2.2.3.1 Anthropmetric and body composition measures

Body size and composition measurements were collected by trained staff using standard procedures. Standing height was measured barefoot using a calibrated wall-mounted stadiometer (Seca^{TM} 240cm height measure) [254]. Weight and bioimpedance data were collected using a Tanita^{TM} BC-418MA body composition analyser (Tanita, Tokyo, Japan) [254]. Participants were weighed barefoot and without heavy outer clothing. BMI was calculated by bioimpedance and derived from anthropometric measures using the equation: weight(kg)/height(m^2).

2.2.3.2 Genetic data

The majority of participants (n=487,409) were genotyped using the Affymetrix Applied Biosystems UK Axiom array (Santa Clara, CA, USA). This array was designed to maximise marker overlap with the existing Affymetrix Applied Biosystems UL BiLEVE Axiom Array, which was used to genotype the first 49,950 participants [255]. The arrays are very similar, with >95% shared content [256]. SNPs were excluded prior to imputation if they were multi-allelic, had missing data or a MAF of <1%. Phasing was performed using a modified version of the SHAPEIT2 algorithm. Imputation was performed using IMPUTE v2 and a merged reference panel comprised of the 1000 Genomes Project Phase 3 and UK10K haplotype reference panels.

2.2.4 Funding

UKB was established by the Wellcome Trust, MRC, Department of Health, Scottish Government and Northwest Regional Development Agency. Funding was also contributed by the Welsh Assembly Government and the British Heart Foundation.

2.2.5 Ethical approval

Ethical approval was obtained from the North West Multi-centre Research Ethics Committee (reference number 06/MRE08/65), the National Information Governance Board for Health and Social Care in England and Wales and the Community Health Index Advisory Group in Scotland.

2.3 The Baby Milk Trial

2.3.1 Background

The Baby Milk Trial is an RCT that aims to evaluate the efficacy, cost-effectiveness and acceptability of a multi-component intervention designed to reduce formula-milk intake and prevent excessive weight gain amongst formula-fed infants [257]. In England, almost 75% of mothers initiate breastfeeding at birth but only 44% are still breastfeeding at 6-8 weeks [258]. Thus, the majority of infants receive formula milk within 2 months of birth. Given the high prevalence of formula-feeding, the promotion of healthy growth amongst formula-fed infants is a public health priority, alongside support for breastfeeding,.

2.3.2 Participants

Healthy, full-term infants who were fully or partially formula-fed within 14 weeks of birth were eligible for inclusion. Exclusion criteria comprised: low birth weight (<2500g), pre-term birth (<37 weeks gestation), the use of special formulas (soya-based, lactose-free, hydrolysed or anti-reflux), major malformations and the presence of hormonal or metabolic disease that might interfere with growth or nutrition [257]. Participants were recruited through: GP practices (n=279; 42%), a mail-out using the centralised NHS integrated database SystmOne (n=183; 27%), research staff on a postnatal hospital ward (n=157; 23%), referral from health visitors and community midwives (n=12; 2%) or self-referral (n=38; 6%). A total of 2133 mother-infant dyads were assessed for eligibility, resulting in the randomisation of 669 parent-child sets [259].

2.3.3 Data collection

2.3.3.1 Anthropometric measures

Anthropometric measurements were collected by trained research staff using standard operating procedures. The measurement team was blind to the trial group allocation of the infants and both the measurement team and parents were advised not to discuss group allocation. Infant weight was measured to the nearest 0.01kg using Seca^{TM} Infant Electronic Scales whilst infants were undressed. Infant supine length was measured to the nearest 0.5cm on a Kiddimeter^{TM} or Starters mat^{TM} whilst the infant wore only a nappy. Parental weight and body fat was measured using a Tanita^{TM} scale and height was measured using a Seca^{TM} wall-mounted stadiometer [257].

2.3.3.2 Infant eating behaviour

Infant EB traits were assessed using the retrospective version of the Baby Eating Behaviour Questionnaire (BEBQ), a validated, 17-item, parent-report questionnaire, completed at the 6 month follow-up assessment [39]. The questionnaire is provided in full in **Appendix C.1** and described in detail in **Chapter 1**.

2.3.4 Funding

The Baby Milk Trial is funded by the National Prevention Research Initiative (http://www.npri.org.uk. Grant no. MR/J000361/1). The work was undertaken under the auspices of the Centre for Diet and Activity Research (CEDAR), a UKCRC Public Health Research Centre of Excellence which is funded by the British Heart Foundation, Cancer Research UK, Economic and Social Research Council, MRC, the National Institute for Health Research, and the Wellcome Trust. The Funding Partners relevant to this award are: Alzheimer's Research Trust, Alzheimer's Society, Biotechnology and Biological Sciences Research Council, British Heart Foundation, Cancer Research UK, Chief Scientist Office (Scottish Government Health Directorate), Department of Health, Diabetes UK, Economic and Social Research Council, Health and Social Care Research and Development Division of the Public Health Agency (HSC RD Division), MRC, The Stroke Association, Wellcome Trust, Welsh Assembly Government and World Cancer Research Fund [257].

2.3.5 Ethical approval

Ethical approval was obtained from the Cambridgeshire 4 Research Ethics Committee (Ref:10/ H0305/9), and informed written consent was attained from all participants. The trial was registered at Current Controlled Trials ISRCTN20814693 on 3rd October 2010.

CHAPTER 3

GENETIC SUSCEPTIBILITY TO OBESITY AND ADULT BODY COMPOSITION

Publications

Clifton E. A. D., Day F. R., De Lucia Rolfe E., Forouhi N. G., Brage S., Griffin S. J., Wareham N. J. and Ong K. K. (2017). Associations between body mass index-related genetic variants and adult body composition: the Fenland cohort study. *International Journal of Obesity*. 41(4), 613-619. DOI: 10.1038/ijo.2017.11. [260].

Contributions

I planned this project and devised the analysis plan in collaboration with my supervisors. I generated the BMI-GRS, conducted the statistical analyses and jointly interpreted the results. I wrote this chapter and the resulting manuscript.

3.1 Summary

BMI is a widely used surrogate measure of adiposity but does not distinguish fat from lean or bone mass. The genetic determinants of BMI are thought to predominantly influence adiposity. However, this has not been confirmed. The utility of genetic risk scores for BMI (BMI-GRSs), comprised of known BMI-related genetic variants, in exploring the relationships of measured BMI and adiposity to health-related outcomes, including eating behaviour (EB), depends upon the extent to which these scores reflect adiposity. In this study, the association between BMI-related genetic variants and body composition was characterised amongst 9667 adults aged 29-64 years from the Fenland study. The results showed that a weighted BMI-GRS, comprised of 96 BMI-related genetic variants, was positively associated with fat, lean and bone mass across all body regions. Associations of the greatest magnitude were identified with fat mass, intermediate associations with lean mass and associations of the lowest magnitude with bone mass. All of the 28 SNPs that showed nominally significant associations with BMI in this participant group (p<0.05) were positively associated with fat mass and 13 demonstrated adipose-specific effects. Together, these findings indicate that the genetic determinants of BMI are associated with adult body composition in ways that mirror measured BMI. Together they influence adiposity to a greater extent than either lean or bone mass and are not associated with body fat distribution. As such, the BMI-GRS can be used to model the effects of measured BMI and total body adiposity on health and other outcomes in adulthood, as well as to investigate the mechanisms of adipose pathways, including EB traits in **Chapter 4**.

3.2 Background

As described in **Chapter 1**, overweight and obesity describe states of excess adiposity that confer risks to health [1]. They are most often identified using BMI which is calculated by dividing an individual's weight (kg) by their squared height (m^2). The utility of BMI stems from its ability to approximate total body adiposity on the basis of scalable, non-invasive anthropometric measurements alone. Whilst BMI is designed to reflect adiposity and demonstrates strong, positive, linear associations with total body fat in largely sedentary populations across diverse settings [261–263], it is also positively influenced by fat-free mass [264]. Fat-free mass is comprised of both lean and bone mass. In the general population, these components of body mass demonstrate less inter-individual variability than fat mass, and thus contribute less to variability in BMI [265]. However, both traits can lead to the misclassification of an individual's adiposity-related health risk when using BMI under certain circumstances [266–271]. For example, non-significant associations between BMI and adposity have been reported amongst elite middle-distance runners [272]. Further, Pacific Islanders and those of Asian ethnicity demonstrate higher and lower fat-free mass relative to Europeans at the same levels of BMI, respectively [263, 273].

A further limitation to the ability of BMI to fully convey adiposity-related health risk is insensitivity to body fat distribution [274, 275]. Robust evidence from large-scale studies supports an independent association between fat stored in the android region (**Figure 3.1**), measured using waist circumference (WC), and type 2 diabetes (T2D), cardiovascular disease (CVD) and all-cause mortality [266, 268]. Further cross-sectional and longitudinal evidence together indicate that the type of abdominal fat (visceral versus subcutaneous) also impacts health [276]. Over 5 years of follow-up amongst 3100 Framingham Heart Study participants with a mean age of 50 years, visceral adipose tissue in the android region (VAT) was positively associated with incident CVD, after adjustment for both known clinical risk-factors and total adiposity (hazard ratio = 1.44 (95% CI: 1.08,1.92)) [277]. Further, a meta-analysis of 41 studies comparing pre-diabetic and diabetic individuals to non-diabetics indicated greater differences in visceral than subcutaneous adipose tissue (SAT) [278].

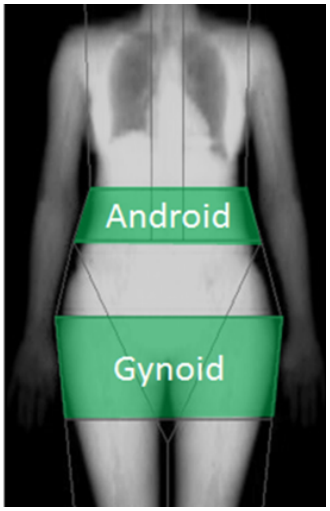


Figure 3.1 Android and gynoid fat. Android fat is located around the body trunk, whilst gynoid fat is located around the hips and thighs.

Genetic factors contribute to the determination of BMI and estimates from twin studies indicate that heritability falls between 31% and 90% [279]. In 2015, a large GWAS meta-analysis

including over 300,000 individuals identified 97 independent, genome-wide significant BMI-related genetic variants, together accounting for ~ 2.7% of inter-individual variability in BMI [149]. It is thought that, in aggregate, the association of these variants with body composition mirrors that of measured BMI. Thus, that they reflect adiposity to a greater extent than lean or bone mass, and are not associated with body fat distribution. In order to assess the utility of BMI-related genetic variants in modelling the effects of measured BMI and adiposity on health, it is important to determine whether, in combination, their relationships to body composition mirror those of measured BMI. This also has implications for studies designed to interrogate the mechanisms of genetic susceptibility to obesity using genetic risk scores for BMI (BMI-GRSs), including that reported in **Chapter 4**. In this study, under the assumption that the BMI-GRS primarily reflects adipose pathways, EB traits are modelled as mediators and modifiers of the BMI-GRS to BMI association.

The majority of previous studies exploring the relationship between BMI-related genetic variants and body composition have focused on single genes. For example, one study amongst 4500 female British twins (mean age: 53 years) investigated the associations between 5 single nucleotide polymorphisms (SNPs) in the *FTO* haplotype block and hip circumference (HC), WC, waist-to-hip ratio (WHR), lean and fat mass [280]. The study reported positive associations between all 5 of the SNPs and both WC and HC, 4 of the SNPs and both BMI and lean mass and 3 of the SNPs and fat mass. None of the SNPs were associated with WHR. In a separate study amongst 1890 European and African-American adolescents (mean age 16 years), 2 variants near *MC4R* were shown to be associated with weight, WC, body fat percentage (BF%), VAT and SAT measures [281]. Another study also examined the relationships between 8 BMI-associated SNPs and both BF% and height, reporting directionally consistent associations between all 8 SNPs and BF% and weaker, but generally positive, associations with height amongst 14,000 European participants [282]. A smaller investigation reported positive associations between 31 of 32 BMI-associated SNPs with adiposity amongst 8000 European adults from the Atherosclerosis Risk in Communities (ARIC) study [283]. Whilst knowledge of the associations between single variants and body composition is informative in elucidating the mechanisms of particular variants, it does not fully inform understanding of the overall utility of a BMI-GRS comprised of multiple variants.

A limited number of studies have employed a GRS approach, summarising the combined effect of multiple BMI-related genetic variants in a single score. A study published in 2014 reported significant, positive associations between an unweighted 29 SNP BMI-GRS and both WC and HC, but not WHR, amongst 787 Algerian adults [284]. This finding indicates that genetic susceptibility to obesity, like measured BMI, may be associated with total adiposity but not body fat distribution. The results of a separate study, also published in 2014, supported these findings amongst 1500 French adults using an unweighted BMI-GRS comprised of 31 SNPs, in addition to demonstrating a positive association between the score and body fat percentage (BF%) [285]. A 2015 investigation partially replicated the

results, reporting a positive association between a 90 SNP BMI-GRS and WC amongst 4632 and 1200 Finnish adults from the DILGOM and FinnTwin12 studies [161]. This study did not examine any other anthropometric or body composition traits.

Overall, few previous studies have investigated the combined effect of BMI-related genetic variants on body composition. Those that have are based on both a limited number of known BMI-related genetic variants and a limited range of body composition measures. No previous investigations have explored the association of the 97 known BMI-related genetic variants with fat, lean and bone mass across body regions, or characterised the associations between individual SNPs and each of these outcomes.

The present investigation reports the association between BMI-related genetic variants, summarised in a weighted BMI-GRS, and fat, lean and bone mass, alongside anthropometric measures, across body regions in a large, adult sample. A secondary analysis was performed to characterise the associations between individual SNPs and total body fat, lean and bone mass.

3.3 Participants and methods

3.3.1 Participants

The study population of the present analysis comprised 9667 individuals (53% women) aged 29-64 years with complete body composition and genome-wide genotype information enrolled in the Fenland study. For a full description of the Fenland study, see **Section 2.1**.

3.3.2 Methods

3.3.2.1 Construction of a weighted genetic risk score for BMI

Genotyping was performed as described in **Section 2.1**. A weighted BMI-GRS was then calculated for each participant. The 97 BMI-related SNPs reported in the 2015 Locke et al. BMI GWAS meta-analysis were considered for inclusion in the score [149]. One SNP, rs2033529 (nearest gene: *TDRG1*), was tri-allelic and could not be incorporated. The remaining 96 were included and the BMI-GRS was constructed according to a previously reported method [283].

Each participant was assigned a value of 0, 1 or 2 for each of the 96 SNPs, indicating the number of BMI-increasing alleles. This value was multiplied by the European-only sex-combined effect estimate for the BMI-increasing allele reported by Locke et al. [149]. Finally, the products across all 96 SNPs were summed for each participant to give a single, aggregated score reflecting genetic susceptibility to obesity.

The European sex-combined estimates were selected to weight the score as they most closely reflected the demography of the Fenland study population (of the participants included in this study 99% identified as white and 92% as white British (**Appendix A.1**)). In the absence of sex-specific effects for the majority of the 96 included loci (only two show evidence of heterogeneity in the discovery sample), the sex-combined effect estimates were used [149]. These estimates have greater precision than the sex-specific estimates due to the larger sample size from which they are calculated.

The effect estimates from Locke et al. were generated using inverse normally transformed residual measurements and cannot be translated to BMI units. To aid the interpretation of the results, the BMI-GRS for each participant was multiplied by the standard deviation (SD) increase in BMI per unit increase in the BMI-GRS, adjusted for age, in the Fenland study population. This scaling of the score was performed separately in each sex, as the main analyses were sex-stratified. After this adjustment, 1 BMI-GRS unit corresponds to 1 SD of BMI-GRS predicted BMI in this sample.

Overall, the formula for the BMI-GRS was as follows:

$$GRS_j = (\sum_{i=1}^{96} s_{ij} w_i) \times \beta_{GRS}$$

The GRS_j refers to the BMI-GRS for individual j; 96 reflects the number of SNPs included in the score; s_{ij} is the number of BMI-increasing alleles at SNP i for individual j; w_i is the effect estimate of SNP i on inverse normally transformed BMI, as reported by Locke et al.; β_{GRS} is the regression coefficient of the weighted BMI-GRS on BMI z-score, adjusted for age, in this Fenland study population (β_{GRS} = 0.94 in men; 0.83 in women). This last parameter was included to align all effect estimates to a +1 SD change in BMI in this population.

3.3.2.2 The assessment of anthropometric and body composition measures

Anthropometric and body composition measures were collected as described in **Section 2.1**. Anthropometric measures were collected using standard protocols and body composition measures were collected through DXA scanning. The following variables were included in this analysis: BMI (kg/m^2), weight (kg), WC (cm), HC (cm), WHR (WC/HC), height (cm) and BF%. Fat, lean and bone mass measurements in the total body, trunk, android and gynoid regions were included alongside bone and fat mass in the legs and appendicular lean mass. Android and gynoid measures were taken from the regions highlighted in **Figure 3.1**. Trunk measurements are generated by summing measurements from the torso and pelvic region and appendicular lean mass comprises the sum of total lean mass in the arms and legs. Finally, VAT (kg) and SAT (kg) were included alongside VAT/SAT ratio.

3.3.2.3 The association between the BMI-GRS and body composition

The BMI-GRS was tested for cross-sectional associations with the anthropometric and body composition variables in sex-stratified, age-adjusted linear regression models. Besides age, no further covariates were added to the models. As genotype is fixed at conception and remains constant throughout life, the association between the BMI-GRS and the outcomes should not be vulnerable to confounding. This is discussed in greater detail in **Section 6.3.2.9**. The distributions of the residuals from these regressions were checked to ensure that the anthropometric and body composition variables did not require transformation and a Bonferroni corrected p-value of $p < 1.04 \times 10^{-3}$, corrected for 48 tests (24 in each sex), was used to account to multiple testing in the assessment of significance.

The analysis was sex-stratified. Whilst just 2 of the 96 BMI-related SNPs demonstrated heterogeneity between the sexes for BMI in the discovery sample, genes implicated by some of the included SNPs have previously shown sex-specific effects on body composition measures, including WHR, VAT, SAT and BF% [149, 286–288]. For example, *IRS1* is more

strongly associated with BF% in men than women [289, 290]. Further, measured BMI has sex-specific associations with some of the included body composition measures. At similar levels of BMI, women typically have more lower extremity fat whilst men have higher VAT mass [291]. As a result, it was plausible that the BMI-related genetic variants included in this analysis could have heterogeneous effects on body composition.

To facilitate comparison of the effect estimates derived from variables measured in different units, the anthropometric and body composition variables were all standardised to *z*-scores (mean=0; SD=1).

In order to aid the assessment of the validity of the findings, the pattern of missing data was interrogated for body composition variables missing information for ≥ 1% of participants. Individuals with missing values were compared to the rest of the cohort for sex, age, ethnicity and BMI using logistic regression, Chi-squared tests or Fisher's exact tests, as appropriate.

3.3.2.4 The association between measured BMI and body composition

The association between measured BMI and the anthropometric and body composition variables was analysed for the purposes of comparing the associations to that of the BMI-GRS with the same outcomes. The main analysis (described above) was repeated, replacing the BMI-GRS with measured BMI in the models. Measured BMI was standardised (mean=0; SD=1).

3.3.2.5 Sensitivity analyses

Two sensitivity analyses were performed. First, to ensure that heterogeneity in the effects of variants comprising the BMI-GRS between the sexes did not influence the results, the main analysis was repeated using sex-specific BMI-GRS scores. These were weighted using the European-only sex-specific effect estimates from Locke et al [149]. Second, the main analysis was repeated amongst participants with self-reported white ethnicity.

3.3.2.6 The association between individual genetic variants and body composition

The relationship between the BMI-related SNPs and body composition was investigated on an individual SNP basis to identify individual SNPs or SNP groups associated with particular components of body composition. The sample size of this analysis was only a fraction of that of the GWAS meta-analysis from which the SNPs were identified and was not powered to detect associations between all 96 SNPs and BMI individually. Only SNPs that demonstrated nominally significant associations with BMI in this cohort were included in this analysis.

SNPs were considered in linear regression models characterising their age and sex-adjusted associations with total body fat, lean and bone mass. Body composition measures were standardised to z-scores and, in the absence of significant heterogeneity in the associations between the BMI-GRS and body composition or an interaction between sex and the BMI-GRS, both sexes were combined to maximise power. The results were used to construct a heat map colour-coding the z-statistic for the association of each SNP with total body fat, lean and bone mass. To avoid spurious precision, z-statistic values between -0.5 and 0.5 were displayed as neutral.

All analyses were performed in Stata version 14 (StataCorp LCC, College Station, TX) and figures were produced using R version 3.2.2.

3.4 Results

3.4.1 Characteristics of the study participants

Table 3.1 Descriptive characteristics of the Fenland study participants (n=9667)

	Men				Women				
	Total (%)	Mean (SD)	Min.	Max.	Total (%)	Mean (SD)	Min.	Max.	p-val.[a]
Sex	4522 (46.8%)	–	–	–	5145 (53.2%)	–	–	–	–
Age	–	48.5 (7.5)	29.4	63.7	–	48.6 (7.4)	30.0	64.0	0.43
White British	3999/4334 (92.3%)	–	–	–	4527/4922 (92.0%)	–	–	–	0.60
BMI (kg/m^2)	4522	27.2 (3.9)	15.3	49.1	5145	26.5 (5.2)	14.5	52.5	<0.01
BMI status[b]									
Underweight	15 (0.3%)	–	–	–	42 (0.8%)	–	–	–	<0.01
Normal weight	1326 (29.3%)	–	–	–	2325 (45.2%)	–	–	–	<0.01
Overweight	2208 (48.8%)	–	–	–	1694 (32.9%)	–	–	–	<0.01
Obese	973 (21.5%)	–	–	–	1084 (21.1%)	–	–	–	0.59
Weight (kg)	4522	86.1 (13.4)	42.9	139.0	5145	71.3 (14.4)	38.2	138.7	<0.01
HC (cm)	4520	103.1 (6.8)	76.2	144.7	5135	103.5 (10.3)	75.5	168.0	0.02
WC (cm)	4521	97.0 (11.2)	65.6	184.2	5142	85.5 (12.5)	59.0	141.0	<0.01
WHR	4519	0.9 (0.1)	0.7	1.9	5133	0.8 (0.1)	0.6	1.1	<0.01
Height (cm)	4522	177.7 (6.8)	129.5	200.5	5145	164.1 (6.3)	140.4	189.8	<0.01
Body fat %	4522	29.0 (6.0)	9.1	47.2	5145	37.5 (7.1)	7.7	58.6	<0.01
Total body (kg)									
Fat mass	4522	25.4 (8.3)	5.8	60.6	5145	27.5 (10.2)	2.9	79.3	<0.01
Lean mass	4522	57.5 (6.8)	31.3	84.8	5145	41.5 (5.3)	27.4	69.0	<0.01
Bone mass	4522	3.15 (0.39)	1.72	4.92	5145	2.39 (0.31)	1.42	3.66	<0.01
Trunk (kg)									
Fat mass	4522	14.6 (5.6)	1.9	41.0	5145	13.5 (6.2)	1.0	46.8	<0.01
Lean mass	4522	27.1 (3.2)	13.9	40.1	5145	20.4 (2.6)	12.9	36.0	<0.01
Bone mass	4522	1.0 (0.2)	0.4	1.6	5145	0.7 (0.1)	0.4	1.3	<0.01
Android (kg)									
Fat mass	4522	2.6 (1.2)	0.2	7.7	5145	2.2 (1.2)	0.1	8.9	<0.01
Lean mass	4522	4.3 (0.6)	2.1	6.7	5145	3.2 (0.4)	2.0	6.1	<0.01
Bone mass	4522	0.1 (0.0)	0.0	0.1	5145	0.1 (0.0)	0.0	0.1	<0.01
Gynoid (kg)									
Fat mass	4522	3.8 (1.2)	0.8	9.6	5145	5.0 (1.7)	0.3	13.5	<0.01
Lean mass	4522	9.2 (1.2)	3.6	14.2	5145	6.6 (0.9)	3.8	10.7	<0.01
Bone mass	4522	0.3 (0.1)	0.1	0.5	5145	0.2 (0.0)	0.1	0.4	<0.01
Leg (kg)									
Fat mass	4522	7.5 (2.3)	1.1	23.5	5145	10.3 (3.6)	1.0	30.3	<0.01
Bone mass	4522	1.2 (0.2)	0.1	2.0	5145	0.9 (0.1)	0.5	1.4	<0.01
Appendicular lean (kg)	4522	27.1 (3.7)	9.0	43.2	5145	18.2 (2.9)	10.4	32.2	<0.01
VAT (kg)	4517	1.4 (0.8)	2×10^{-3}	5.6	4929	0.6 (0.5)	1×10^{-3}	4.2	<0.01
SAT (kg)	4517	1.2 (0.5)	0.1	4.6	4929	1.6 (0.7)	0.1	6.7	<0.01
VAT/SAT ratio	4517	1.2 (0.8)	2×10^{-3}	10.1	4929	0.4 (0.3)	8×10^{-4}	3.8	<0.01

Standard deviation (SD); body mass index (BMI); hip circumference (HC); waist circumference (WC); waist-to-hip ratio (WHR); visceral adipose tissue (VAT); subcutaneous adipose tissue (SAT)

[a] p-value refers to the difference in mean values between men and women calculated using a 2 sample Student's t-test, Mann-Whitney-U test or Chi-squared test

[b] WHO BMI categories: Underweight <18.5kg/m^2; Normal weight ≥18.5 and <25.0kg/m^2; Overweight ≥25kg/m^2 and <30kg/m^2; Obese ≥30kg/m^2

– Not applicable

The characteristics of the 9667 Fenland participants of the present analysis are summarised in **Table 3.1**. Approximately half were women (n=5142; 53%) and, reflecting UK population norms, the majority of participants were either overweight or obese (3181 men (70%) and 2778 women (54%)) [14]. All anthropometric and body composition measures exhibited statistically significant sexual dimorphism (p<0.05) and the majority of the participants self-reported their ethnicity as white (9137; 99%). Detailed information regarding self-reported ethnicity is provided in **Appendix A.1**.

VAT, SAT and VAT/SAT ratio were the only variables with ≥ 1% missing data (n=221 missing; 2% of the cohort). The majority of missing data resulted from VAT measures of <1g (n=207; 94%) as the DXA software was not able to estimate values for these individuals. The remaining participants with missing values were too large in relation to the scanner for accurate estimates to be made. Compared to the rest of the cohort, individuals with missing data exhibited higher mean age (48.6 versus 44.7 years; p<0.01), were more likely to be female (98% versus 52%; p<0.01) and had a lower median BMI (21.5kg/m^2 versus 26.3kg/m^2; p<0.01).

3.4.2 The BMI-GRS and body composition

The BMI-GRS was positively associated with all the included anthropometric and body composition variables (**Figure 3.2**; **Table 3.2**). Of the 48 associations tested (24 in each sex), 43 were statistically significant at the Bonferroni-corrected p-value threshold for 48 tests ($p < 1.04 \times 10^{-3}$). Only VAT/SAT ratio (men: β=0.06 SDs (95% CI: -0.12, 0.24); p=0.54; women: 0.28 SDs (95% CI: 0.09, 0.47); $p = 4.29 \times 10^{-3}$), android bone mass in men (β=0.29 SDs (95% CI: 0.10, 0.48); $p = 2.74 \times 10^{-3}$) and height (men: β=-0.02 SDs (95% CI: -0.21, 0.17); p=0.85; women: 0.10 SDs (95% CI: -0.10, 0.30); p=0.31), did not reach statistical significance.

The BMI-GRS demonstrated associations of the greatest magnitude with the adiposity variables, intermediate associations with the lean mass variables and associations of the lowest magnitude with the bone mass variables and height (**Figure 3.2**). This pattern was replicated across all body regions, as well as in the total body measures. Alongside the effect estimates for the age-adjusted regressions of the BMI-GRS on the body composition variables, **Figure 3.2** displays the variance in each of the body composition variables explained by measured BMI (R^2). The R^2 values mirror the association of the BMI-GRS with body composition.

The effect estimates for the BMI-GRS on the fat mass variables were comparable across body regions. For men, the highest effect estimate was for SAT mass and the lowest was for VAT mass. For women, the highest estimate was for total body fat and the lowest was for VAT mass. In both sexes, effect estimates were greater for SAT than for VAT mass. For each SD increase in BMI-GRS predicted BMI, we observed a 0.98 SD (95% CI: 0.79, 1.16) increase in SAT mass for men and a 0.92 SD (95% CI: 0.72, 1.12) increase for women. The

corresponding VAT estimates were 0.62 SDs (95% CI: 0.44, 0.80) and 0.70 SDs (95% CI: 0.51, 0.90) for men and women, respectively.

The BMI-GRS demonstrated significant positive associations with WHR: β=0.61 SDs (95% CI: 0.43, 0.79), $p = 5.33 \times 10^{-11}$ for men and 0.41 SDs (95% CI: 0.21, 0.60), $p = 4.24 \times 10^{-5}$ for women. Directionally consistent but not statistically significant associations were observed for VAT/SAT ratio in both sexes: β=0.06 SDs (9% CI: -0.12, 0.24), p=0.54 for men and 0.28 SDs (95% CI: 0.09, 0.47), $p = 4.29 \times 10^{-3}$ for women.

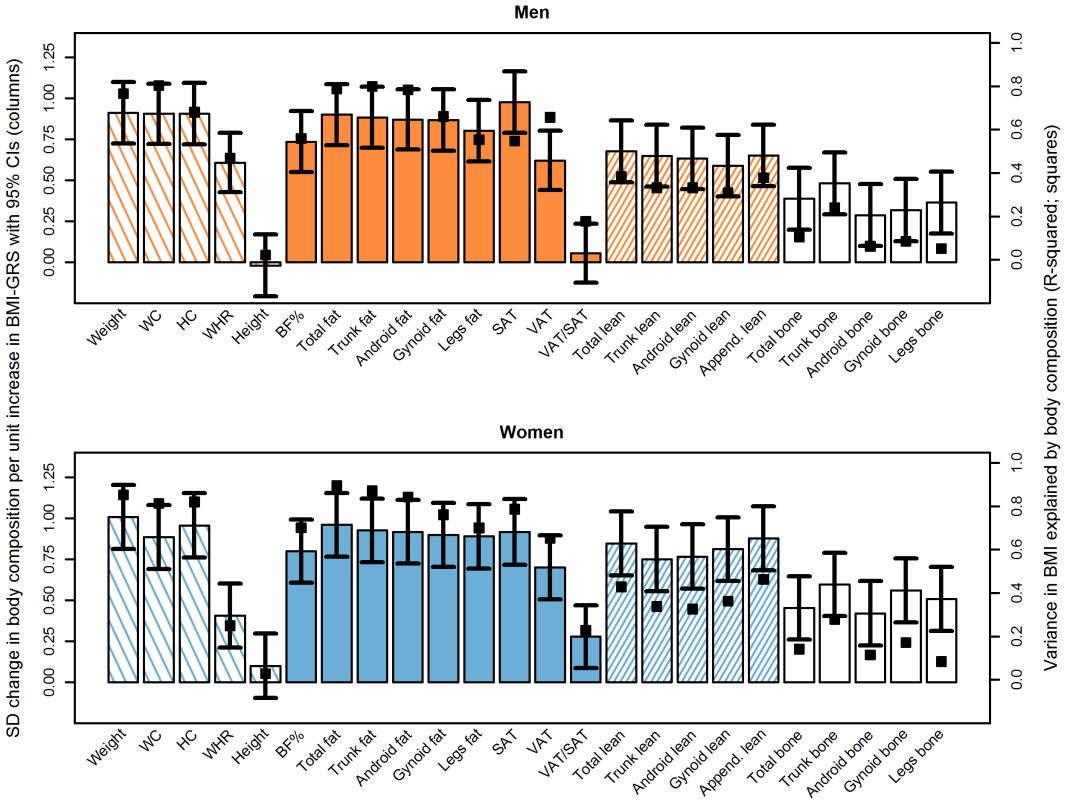


Figure 3.2 Associations of the BMI-GRS and measured BMI to body composition. The **columns** display the effect estimates from age-adjusted linear regressions of the BMI-GRS on the specified anthropometric or body composition variable z-score. The units are: SD change in the body composition variable per unit increase in BMI-GRS predicted BMI. The bars represent the 95% CIs for these estimates. The **squares** display the variance in BMI explained by the body composition variable from age-adjusted linear regression of body composition on BMI z-scores on a scale of 0-1 (the R^2 values). Anthropometric, adposity, lean and bone measures are grouped and distinguished by shading.

Table 3.2 Associations between the BMI-GRS and body composition

	Men		Women	
	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value
Weight	0.91 (0.72, 1.10)	2.2×10^{-21}*	1.01 (0.81, 1.20)	9.9×10^{-24}*
WC	0.91 (0.72, 1.09)	9.6×10^{-22}*	0.89 (0.69, 1.08)	6.2×10^{-19}*
HC	0.91 (0.72, 1.09)	4.1×10^{-21}*	0.96 (0.76, 1.20)	1.6×10^{-21}*
WHR	0.61 (0.43, 0.79)	5.3×10^{-11}*	0.41 (0.21, 0.60)	4.2×10^{-5}*
Height	-0.02 (-0.21, 0.17)	0.85	0.10 (-0.10, 0.30)	0.31
BF%	0.74 (0.55, 0.92)	9.1×10^{-15}*	0.80 (0.61, 0.99)	4.1×10^{-16}*
Total fat	0.90 (0.71, 1.09)	3.8×10^{-21}*	0.96 (0.77, 1.16)	6.1×10^{-22}*
Trunk fat	0.88 (0.70, 1.07)	1.3×10^{-20}*	0.93 (0.73, 1.12)	1.0×10^{-20}*
Android fat	0.87 (0.69, 1.06)	3.2×10^{-20}*	0.92 (0.72, 1.11)	2.5×10^{-20}*
Gynoid fat	0.87 (0.68, 1.06)	1.8×10^{-19}*	0.90 (0.70, 1.09)	3.7×10^{-19}*
Legs fat	0.80 (0.62, 0.99)	7.2×10^{-17}*	0.89 (0.69, 1.09)	8.6×10^{-19}*
SAT	0.98 (0.79, 1.16)	2.2×10^{-24}*	0.92 (0.72, 1.12)	3.7×10^{-19}*
VAT	0.62 (0.44, 0.80)	1.9×10^{-11}*	0.70 (0.51, 0.90)	2.2×10^{-12}*
VAT/SAT	0.06 (-0.12, 0.24)	0.54	0.28 (0.09, 0.47)	4.3×10^{-3}
Total lean	0.68 (0.49, 0.86)	1.9×10^{-12}*	0.85 (0.65, 1.04)	2.7×10^{-17}*
Trunk lean	0.65 (0.46, 0.84)	1.6×10^{-11}*	0.75 (0.56, 0.95)	7.6×10^{-14}*
Android lean	0.63 (0.45, 0.82)	4.3×10^{-11}*	0.77 (0.57, 0.96)	2.5×10^{-14}*
Gynoid lean	0.59 (0.40, 0.78)	6.9×10^{-10}*	0.81 (0.62, 1.01)	3.0×10^{-16}*
Append. lean	0.65 (0.46, 0.84)	9.6×10^{-12}*	0.88 (0.68, 1.07)	1.1×10^{-18}*
Total bone	0.39 (0.20, 0.58)	5.7×10^{-5}*	0.45 (0.26, 0.65)	4.0×10^{-6}*
Trunk bone	0.48 (0.29, 0.67)	6.0×10^{-7}*	0.60 (0.40, 0.79)	1.4×10^{-9}*
Android bone	0.29 (0.10, 0.48)	2.7×10^{-3}	0.42 (0.22, 0.62)	2.8×10^{-5}*
Gynoid bone	0.32 (0.13, 0.51)	9.7×10^{-4}*	0.56 (0.36, 0.76)	2.2×10^{-8}*
Legs bone	0.36 (0.18, 0.55)	1.6×10^{-4}*	0.51 (0.31, 0.70)	3.4×10^{-7}*

Confidence interval (CI); waist circumference (WC); hip circumference (HC); waist-to-hip ratio (WHR); body fat percentage (BF%); visceral adipose tissue (VAT); subcutaneous adipose tissue (SAT); Appendicular (Append.)

[a] Effect estimates (Beta) are the age-adjusted SD change in the body composition variable per SD increase in genetically predicted BMI from the age-adjusted linear regression of the scaled BMI-GRS on body composition *z*-score

* $p<1.04\times10^{-3}$

3.4.3 Measured BMI and body composition

Measured BMI was positively associated with all the anthropometric and body composition variables tested, except for height, which demonstrated a small, negative association with BMI in both sexes (**Figure 3.3**; **Table 3.3**). All of the associations were statistically significant after Bonferroni correction for multiple testing ($p < 1.04 \times 10^{-3}$).

Mirroring the association between the BMI-GRS and body composition, measured BMI showed associations of the greatest magnitude with the adiposity variables, intermediate associations with the lean mass variables and associations of the lowest magnitude with the bone mass variables and height. This pattern was replicated across all body regions, as well as in the total body measures.

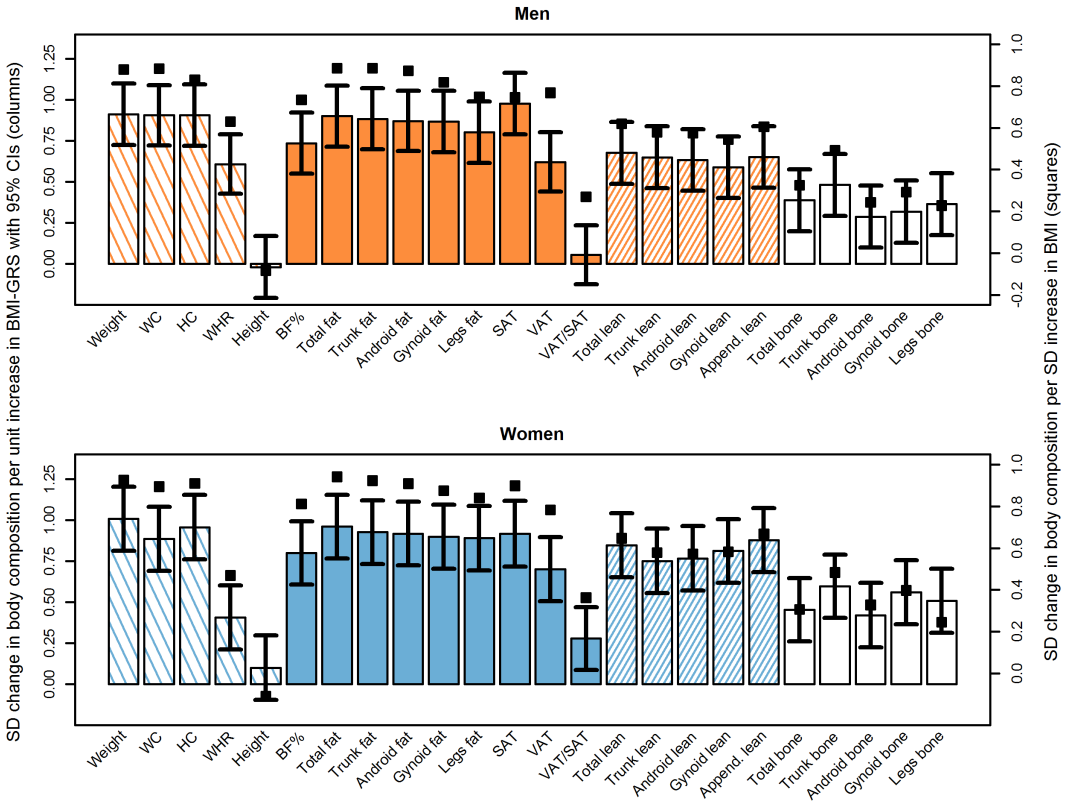


Figure 3.3 Body composition predicted by the BMI-GRS (columns) and body composition predicted by BMI (squares). The columns display the effect estimates from age-adjusted linear regressions of the BMI-GRS on the specified anthropometric or body composition variable z-scores, with 95% CIs. The effect estimates represent the SD change in each body composition variable per unit increase in BMI-GRS predicted BMI. The squares display the effect estimates from the age-adjusted linear regressions of BMI z-scores on the specified body composition variable z-scores. The effect estimates are SD change in body composition per SD increase in BMI. Anthropometric, adposity, lean and bone measures are grouped and distinguished by shading.

Table 3.3 Associations between measured BMI and body composition

	Men		Women	
	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value
Weight	0.88 (0.87, 0.89)	$<1.0\times10^{-25}$*	0.93 (0.92, 0.94)	$<1.0\times10^{-25}$*
WC	0.88 (0.87, 0.90)	$<1.0\times10^{-25}$*	0.89 (0.88, 0.91)	$<1.0\times10^{-25}$*
HC	0.83 (0.81, 0.85)	$<1.0\times10^{-25}$*	0.91 (0.90, 0.92)	$<1.0\times10^{-25}$*
WHR	0.63 (0.61, 0.65)	$<1.0\times10^{-25}$*	0.47 (0.45, 0.49)	$<1.0\times10^{-25}$*
Height	-0.08 (-0.11, -0.05)	2.7×10^{-8}*	-0.11 (-0.14, -0.08)	6.1×10^{-15}*
BF%	0.73 (0.71, 0.75)	$<1.0\times10^{-25}$*	0.81 (0.80, 0.83)	$<1.0\times10^{-25}$*
Total fat	0.89 (0.87, 0.90)	$<1.0\times10^{-25}$*	0.94 (0.93, 0.95)	$<1.0\times10^{-25}$*
Trunk fat	0.89 (0.87, 0.90)	$<1.0\times10^{-25}$*	0.92 (0.91, 0.93)	$<1.0\times10^{-25}$*
Android fat	0.87 (0.86, 0.89)	$<1.0\times10^{-25}$*	0.91 (0.90, 0.92)	$<1.0\times10^{-25}$*
Gynoid fat	0.82 (0.80, 0.83)	$<1.0\times10^{-25}$*	0.87 (0.86, 0.89)	$<1.0\times10^{-25}$*
Legs fat	0.75 (0.73, 0.77)	$<1.0\times10^{-25}$*	0.84 (0.82, 0.86)	$<1.0\times10^{-25}$*
SAT	0.75 (0.73, 0.77)	$<1.0\times10^{-25}$*	0.90 (0.89, 0.91)	$<1.0\times10^{-25}$*
VAT	0.77 (0.75, 0.79)	$<1.0\times10^{-25}$*	0.78 (0.77, 0.8)	$<1.0\times10^{-25}$*
VAT/SAT	0.27 (0.24, 0.30)	$<1.0\times10^{-25}$*	0.36 (0.34, 0.39)	$<1.0\times10^{-25}$*
Total lean	0.62 (0.6, 0.64)	$<1.0\times10^{-25}$*	0.65 (0.63, 0.67)	$<1.0\times10^{-25}$*
Trunk lean	0.58 (0.56, 0.60)	$<1.0\times10^{-25}$*	0.58 (0.56, 0.60)	$<1.0\times10^{-25}$*
Android lean	0.58 (0.55, 0.60)	$<1.0\times10^{-25}$*	0.57 (0.55, 0.59)	$<1.0\times10^{-25}$*
Gynoid lean	0.54 (0.52, 0.57)	$<1.0\times10^{-25}$*	0.58 (0.56, 0.61)	$<1.0\times10^{-25}$*
Append. lean	0.61 (0.58, 0.63)	$<1.0\times10^{-25}$*	0.67 (0.65, 0.69)	$<1.0\times10^{-25}$*
Total bone	0.32 (0.30, 0.35)	$<1.0\times10^{-25}$*	0.31 (0.28, 0.33)	$<1.0\times10^{-25}$*
Trunk bone	0.49 (0.47, 0.52)	$<1.0\times10^{-25}$*	0.48 (0.46, 0.51)	$<1.0\times10^{-25}$*
Android bone	0.24 (0.21, 0.27)	$<1.0\times10^{-25}$*	0.33 (0.30, 0.35)	$<1.0\times10^{-25}$*
Gynoid bone	0.29 (0.26, 0.32)	$<1.0\times10^{-25}$*	0.4 (0.37, 0.42)	$<1.0\times10^{-25}$*
Legs bone	0.23 (0.20, 0.26)	$<1.0\times10^{-25}$*	0.25 (0.22, 0.27)	$<1.0\times10^{-25}$*

Confidence interval (CI); waist circumference (WC); hip circumference (HC); waist-to-hip ratio (WHR); body fat percentage (BF%); visceral adipose tissue (VAT); subcutaneous adipose tissue (SAT); Appendicular (Append.)

[a]Effect estimates (Beta) are the age-adjusted SD change in body composition per SD increase in measured BMI from the age-adjusted regression of the BMI *z*-score on body composition *z*-score

* $p<1.04\times10^{-3}$

3.4.4 Sensitivity analyses

The associations between the BMI-GRS and body composition followed similar patterns in both sexes and no statistically significant differences between the effect estimates were identified. Sensitivity analyses using sex-specific BMI-GRSs, weighted using the sex-specific estimates from Locke et al. [149] were conducted (**Appendix A.2** & **A.3**). Further sensitivity analyses were conducted amongst participants who reported white ethnicity (**Appendix A.4** & **A.5**). Neither analysis altered the conclusions.

3.4.5 Individual genetic variants and body composition

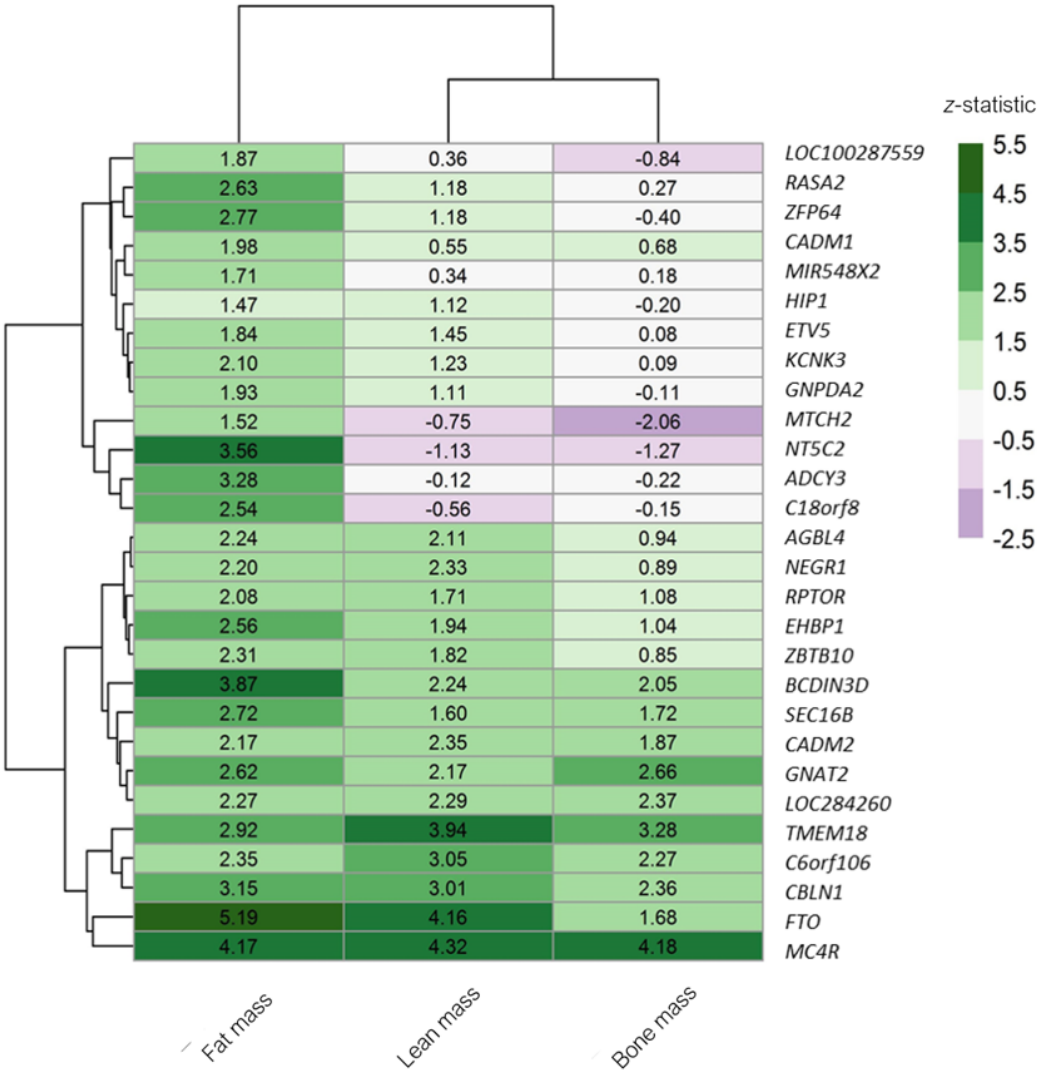


Figure 3.4 Heat map of the associations between the 28 SNPs that exhibited nominally significant associations with BMI in this cohort, clustered by their associations with fat, lean and bone mass. The values and colour-coding indicate the *z*-statistic (Beta/SE) from the age and sex-adjusted linear regressions of each SNP on the standardised body composition variables (mean=0; SD=1).

Of the 96 SNPs included in this analysis, 28 demonstrated nominally significant associations with measured BMI in this cohort (p<0.05). All 28 exhibited positive associations with fat mass, of which 22 were nominally significant. Positive associations with lean mass were observed for 24 of the 28 SNPs (86%), of which 11 were nominally significant, and 20 of the 28 SNPs (71%) demonstrated positive associations with bone mass, of which 8 were nominally significant. All 28 SNPs demonstrated the greatest magnitude of association with fat mass (**Appendix A.6**).

A heat map was constructed to display the associations of the 28 SNPs with total body fat, lean and bone mass (**Figure 3.4**). The primary clustering of body composition variables on the x-axis separated fat from lean and bone mass. The primary clustering of the SNPs on the y-axis separated 15 SNPs associated with a global increase in all three body composition measures, from 13 SNPs with apparent adipose-specific effects. *MTCH2* is notable amongst these, as it demonstrated a nominally significant, negative association with bone mass: β=-0.78 SDs of bone mass (95% CI: -1.52, -0.04); p=0.04 (**Appendix A.6**).

3.5 Discussion

3.5.1 Summary and context of the main findings

This analysis characterised the associations between BMI-related genetic variants and adult anthropometric and body composition traits. Amongst 9667 predominantly white British adults from the Fenland study, the BMI-GRS demonstrated positive, age-adjusted associations with total and regional fat, lean and bone mass. Associations of the greatest magnitude were observed for adiposity variables, intermediate associations were observed for lean tissue variables, and associations of the lowest magnitude were observed for bone variables and height. This pattern was replicated across all body regions and mirrors the relationship between measured BMI and body composition both in this participant group and other adult populations [292, 293]. This finding confirms the utility of the BMI-GRS in modelling the effects of measured BMI on outcomes of interest, as well as supporting its use in interrogating the mechanisms of adipose pathways, including EB in **Chapter 7**.

Corroborating and extending the results of other studies, we found a positive association between the BMI-GRS and BF% [283, 282, 285, 294]. The most recent study of a BMI-GRS and BF% in a European population reported that each BMI-increasing allele was associated with a 0.14% (95% CI: 0.05, 0.24) increase in BF% (p=0.004) [285]. The study included 31 SNPs combined to form an unweighted score and 1578 adult participants. Our results confirm this association using a weighted BMI-GRS comprised of a greater number of variants and a sample size over 6 times as large. Further, consistent with previous studies, we found significant, positive associations between the BMI-GRS and WC, HC and weight [284, 285]. We did not find an association with height. In the present study, the BMI-GRS was positively associated with WHR. The findings from two previous studies of a BMI-GRS on WHR were directionally consistent but not statistically significant in either BMI-adjusted or BMI-unadjusted analyses [284, 285]. These previous investigations included 1578 and 740 participants, respectively. Thus this study is better powered to detect associations.

The association of the BMI-GRS with VAT/SAT ratio did not reach statistical significance. This novel finding suggests that the genetic regulation of BMI may be independent of the mechanisms that regulate the relative distribution of visceral and subcutaneous fat in the abdominal region. This supports investigations using different methods that show only modest overlap between the genetic regulation of BMI and VAT/SAT ratio. Only 7 of 32 BMI-related loci were associated with VAT/SAT ratio among European adults in one study [287] and 1 of 12 BF%-associated SNPs was associated with VAT/SAT ratio in a separate study [290]. Discrepancy between the findings for WHR and VAT/SAT ratio speculatively indicate that WHR and VAT/SAT may measure different aspects of central adiposity. While WHR provides a measure of central relative to peripheral fat, VAT/SAT ratio measures the relative distribution of internal and subcutaneous fat within the abdominal region. An alternative

explanation for the findings is that differential measurement error is not consistent between WHR and VAT/SAT.

In this cohort, 28 SNPs exhibited nominally significant associations with BMI on an individual basis. All 28 were positively associated with fat mass and showed a greater magnitude of association with fat than with lean or bone mass. The SNPs clustered into two main groups by their associations with body composition. Approximately half (n=15) were associated with a global increase in fat, lean and bone mass, whilst the remaining 13 exhibited more adipose-specific effects. This supports evidence from a recent GWAS of lean mass, suggesting some but not total overlap between the genetics of fat and lean mass [295]. In particular, *FTO* was 1 of 5 genome-wide significant SNPs associated with lean mass, and was also strongly associated with lean mass in the present study. This corroborates the observation of concurrent reductions in both lean and fat mass observed amongst *FTO*-deficient mice [296]. The SNP near *MC4R* showed significant positive effects on fat, lean and bone mass in this study. This is in keeping with the observation that *MC4R*-deficient individuals exhibit elevations in both fat and fat-free mass [150].

3.5.2 Strengths and limitations

This study was the first to examine the relationship between a BMI-GRS and fat, lean and bone mass across body regions. We present novel findings representing an extension of previous investigations through increased sample size, SNP number and body composition outcome measures. However there are limitations. The results pertain only to adults aged 29-64 years. Whilst many genetic variants are associated with BMI throughout life [297], associations between specific BMI-related variants and some body composition phenotypes are age-dependent [298, 299]. For example, a meta-analysis of data from 4 birth cohort studies did not find an association between a BMI-GRS (comprised of 16 adult BMI SNPs) and BF% in infancy and early childhood [298]. Other studies have reported inconsistent associations between specific SNPs and BMI at different stages of adulthood [299, 300]. Larger studies may be powered to explore relationships in different phases of adulthood and the present results should not be extrapolated to children or the elderly. However, the gene discovery sample included adults of all ages and consistency in the results across all body regions indicate robust findings that may be generally applied to early and middle-aged adults.

The findings cannot be extrapolated to individuals >140kg, non-Europeans or those with metabolic disease. The sample size of this investigation limited its power to robustly detect associations between the individual SNPs and body composition. Finally, the cross-sectional nature of the study did not facilitate exploration of causal relationships between the components of body composition.

3.5.3 Conclusions

In combination, 96 BMI-related genetic variants are positively associated with adult adiposity, with intermediate effects on lean mass and weaker effects on bone mass. This pattern mirrors the relationship between measured BMI and body composition in this age group. The findings support the use of the BMI-GRS in the causal modelling of the impact of adult BMI and total body adiposity on health and other outcomes. Further, the results suggest that the BMI-GRS can be used to interrogate the mechanisms of BMI and adipose pathways. This is highly relevant to the study of EB in **Chapter 4** and applies also to the Mendelian randomisation (MR) investigations reported in **Chapters 6** and **7**. The results indicate that BMI-related SNPs may be associated either with a global increase in mass or adipose-specific effects. As a result, MR analyses using BMI-related genetic variants will need to carefully consider possible heterogeneous effects. Future studies are needed both to replicate this finding and to further explore the associations and mechanisms of individual SNPs.

CHAPTER 4

MEDIATION AND MODIFICATION OF GENETIC SUSCEPTIBILITY TO OBESITY BY EATING BEHAVIOUR TRAITS

Publications

de Lauzon-Guillain, B.*, **Clifton, E.A.D.***, Day, F.R., Clément, K., Brage, S., Forouhi, N.G., Griffin, S.J., Akoli Koudou, Y., Pelloux, V., Wareham, N.J., Charles, M.A., Heude, B.$^\wedge$ and Ong, K.K.$^\wedge$. (2017). Mediation and modification of genetic susceptibility to obesity by eating behaviors. *Am J Clin Nutr.* 106,966-1004. DOI: 10.3945/ajcn. 117.157396. [301].

* joint first authorship
$^\wedge$ joint last authorship

Contributions

I planned this project and devised the analysis plan in collaboration with my supervisors. I generated the EB trait scores and BMI-GRS, conducted the statistical analyses in the Fenland study and jointly interpreted the results. I wrote this chapter as well as the introduction, methods, Fenland study results and discussion sections of the resulting manuscript. The EDEN study results were first drafted by the EDEN study collaborators and were then re-written by my supervisors and I.

4.1 Summary

A number of genetic variants demonstrate robust, genome-wide significant associations with BMI, primarily reflecting an impact on adiposity (**Chapter 3**). However, the mechanisms through which these variants act to influence BMI, and the factors with which they interact, are not well understood. **Chapter 3** illustrates that genetic susceptibility to obesity, summarised through a weighted genetic risk score for BMI (BMI-GRS) comprised of 96 BMI-related genetic variants, primarily reflects fat mass and can thus be used to interrogate the mechanisms of adipose pathways. In this study, eating behaviour (EB) traits were modelled as potential mediators and modifiers of genetic susceptibility to obesity. Amongst 3515 and 2154 participants from the Fenland and EDEN studies, the Sobel test was used to assess the mediating effect of the three TFEQ-R18 measured EB traits (emotional eating (EE), uncontrolled eating (UE) and cognitive restraint (CR)) on the BMI-GRS to BMI association. In addition, interaction terms were used to assess modification of the association by each of the EB traits in turn. In both cohorts, the BMI-GRS to BMI association was partially mediated by both EE and UE in separate sex-combined analyses. CR did not mediate the association, except amongst EDEN women. CR modified the association between the BMI-GRS and BMI amongst men in both cohorts and Fenland women. In these participant groups, the association between the BMI-GRS and BMI was strongest in the lowest tertile of CR, and weakest in the highest tertile. Together, the findings indicate that genetic susceptibility to obesity is partially mediated by the appetitive EB traits (EE and UE), whilst high levels of cognitive control over eating (CR) may attenuate the impact of BMI-related genetic variants on realised BMI in adulthood. Interventions designed to support CR, or to reduce appetitive EB traits, might help to protect genetically vulnerable individuals from obesity.

4.2 Background

A 2015 GWAS meta-analysis of BMI, including over 300,000 participants, identified 97 genetic variants demonstrating individual, genome-wide significant associations with BMI in adulthood [149]. However, the mechanisms through which these variants influence body weight are not well understood [149]. This represents an important gap in the literature, limiting the application of GWAS findings to interventions designed to prevent or reverse excessive weight gain. **Chapter 3** demonstrated that known BMI-related genetic variants are primarily associated with fat mass. As such, these variants can used to interrogate the mechanisms of the genetic pathways involved in adiposity, including EB.

Here, we investigated the role of EB traits (EE, UE and CR) in the mediation and modification of genetic susceptibility to obesity in adulthood. These traits can be measured using the TFEQ-R18 (**Appendix C.1**) and are described in greater detail in **Chapter 1**. Briefly, EE is designed to measure overeating in response to dysphoric emotional states. It is assessed by combining participant responses across three questionnaire items measuring the tendency to eat in the context of loneliness, anxiousness and sadness (example item: when I feel anxious, I find myself eating) [40]. UE is measured through 9 items and quantifies the tendency to overeat, generally in response to external food cues and ignoring internal signals of satiety, accompanied by a subjective loss of control over consumption (example item: Sometimes when I start eating, I just can't seem to stop) [40]. Finally, CR is measured through 6 items and conveys an individual's intention to restrict their food intake with the objective of influencing their shape or weight (example item: I deliberately take small helpings as a means of controlling my weight) [40]. Together, EE and UE are considered appetitive EB traits, whereas recent research suggests that CR may represent a conscious response to weight status [302, 138, 51]. As outlined in **Chapter 1**, twin studies amongst adult populations suggest that the TFEQ-R18 measured EB traits have a genetic basis [124, 66]. Although specific genetic variants have yet to be identified (**Chapter 7**), these findings indicate that genetic approaches can be used to study the relationships between EB and other traits, including BMI.

The Behavioural Susceptibility Theory (BST) of obesity posits that genetic susceptibility to obesity is mediated by appetitive EB traits, such as EE and UE [102]. In support of this theory, twin studies have demonstrated shared genetic influences on appetitive traits and weight during infancy [139] and associations between specific BMI-related genetic variants and aspects of EB have been reported. These are outlined in **Chapter 1**. Whilst these same statistical associations would be anticipated if changes in BMI resulted in alterations to appetitive EB traits, longitudinal studies in adults suggest that EE and UE are the cause, rather than the consequence, of weight change. Further, in aggregate, genes implicated by BMI-related genetic variants show enriched expression in regions of the brain with an established role in the central regulation of eating [149]. Indeed, a functional Magnetic

Resonance Imaging (fMRI) investigation amongst 44 adolescent women reported that responsivity of the brain's reward circuitry to food cues was positively associated with future weight gain [303]. It is still possible that a bi-directional association exists between EB and obesity, whereby increases in BMI enhance or perpetuate appetitive EB traits. Knowledge of the biology of EB traits through studies including that reported in **Chapter 7**, will help to inform understanding in the future. However, at present, the weight of evidence suggests that EE and UE can be modelled on the causal pathway to BMI.

The possibility that EB traits mediate genetic susceptibility to obesity has been directly examined in three studies pre-dating the work described in this chapter. Two were conducted amongst children and one amongst adults [163, 164, 161]. The earliest study, published in 2014, reported partial mediation of a 28 SNP BMI-GRS to BMI association by satiety responsiveness (SR) amongst 2258 children with a mean age of 10 years [163]. SR is measured by the parent-completed children's eating behaviour questionnaire (CEBQ) and describes a child's receptivity to feelings of fullness (example item: my child cannot eat a meal if s/he has had a snack just before) [77]. It is negatively correlated with weight [163]. The study did not interrogate other EB traits as mediators of the association. A later study, published in 2016, did not detect mediation of the association between a 32 SNP BMI-GRS and weight gain by child EB traits amongst 652 children aged 6-8 years [164]. Both studies were conducted amongst European participants and derived EB measures using the CEBQ. There are several possible explanations for the discrepancy in results. It is possible that, as a result of lower sample size, the 2016 study was not powered to detect a true mediating effect, that mediation is not present in a younger age group, that the mediating effect is of weight status established in early life and not of weight gain or that the findings of the earlier study were spurious. A third study amongst children, published in 2019, following the work reported in this chapter, showed that appetite, measured by a single item, partially mediated the association between a 16 SNP BMI-GRS and BMI amongst 1142 French children aged 2-5 years [162]. This indicates that the age of participants in the earlier studies does not explain the discrepancy in results.

The single pre-existing study amongst adults tested for mediation of a 90 SNP BMI-GRS to BMI association by EE and UE in two Finnish cohorts, comprised of 4632 and 1231 individuals, respectively [161]. The BMI-GRS to BMI association was mediated by EE in both cohorts and by UE in the larger of the two cohorts. A separate study, in adults, described the association of a 32 SNP BMI-GRS with UE and EE, reporting positive associations between the score and both traits, but did not explicitly test for mediation [112].

No prior studies had examined mediation of the BMI-GRS to BMI association by CR, or modification of this association by any EB traits. Further, no studies had used all 96 bi-allelic BMI-associated variants in their analysis. Here EE, UE and CR were tested as potential mediators and modifiers of genetic susceptibility to obesity, summarised as a 96 SNP BMI-GRS, in two large, well-characterised population-based adult cohort studies.

4.3 Participants and methods

4.3.1 Participants

4.3.1.1 The Fenland study

The Fenland study population of this analysis comprised 3515 individuals (53% women) aged 35-64 years with intersecting EB, genotype and BMI data. For a full description of the Fenland study, see **Section 2.1**.

4.3.1.2 The EDEN study

The EDEN study (Etude des Déterminants pré et postnatals de la santé et du développement de l'ENfant (Study of pre- and early postnatal determinants of child health and development)) is a French prospective cohort study established in 2003 to assess the pre and postnatal determinants of childhood growth, development and health [304]. Recruitment took place between 2003 and 2006. All pregnant women who attended prenatal clinics at two French university hospitals, located in Nancy and Poitiers, France, prior to 24 weeks amenorrhoea were invited to participate. Of the 3758 women approached, 2002 were recruited to the cohort (a response rate of 53%). Exclusion criteria comprised: multiple pregnancy, maternal diabetes diagnosed prior to pregnancy, illiteracy in French or plans to move outside the region in the next 3 years. On several occasions during pregnancy and follow-up, mothers were asked to complete questionnaires regarding their health and lifestyle, including their EB. A clinical examination was organised between 24 and 28 weeks amenorrhoea where a blood sample was taken for genotyping. Fathers were invited to participate at any time during the mother's pregnancy. The study population for this analysis comprised 2154 individuals (56% women) aged 18-56 years with complete EB, genotype and BMI data.

The EDEN study was approved by the Ethics Committee of the University Hospital of Kremlin-Bicêtre on 12th December 2002. Data files were declared to the National Committee for Processed Data and Freedom. Written informed consent was obtained from both parents.

4.3.2 Methods

4.3.2.1 Construction of a weighted genetic risk score for BMI

In Fenland, genome-wide genotyping was performed using the Affymetric UK Biobank Axiom array, as described in **Section 2.1**. In EDEN, genotyping for 27 BMI-related genetic

variants was performed at the MRC Epidemiology Unit, Cambridge (iPLEX platform, Sequenom). These 27 variants were taken from Speliotes et al. (2010) [283].

In both cohorts, a BMI-GRS was constructed using the method described in **Section 3.3.2.1**. Briefly, the number of BMI-increasing alleles for each participant at each locus (0,1 or 2) was multiplied by the effect estimate for the BMI-increasing allele at that locus. The products at each locus were then summed to create a score for each participant. In Fenland, the 96 biallelic BMI-related SNPs identified in the 2015 Locke et al. GWAS meta-analysis, and used to create the BMI-GRS in **Chapter 3**, were included in the score. Given the predominance of white British participants in the Fenland sample, the Fenland BMI-GRS was weighted by the European-only, sex-combined effect estimates from Locke et al. [149]. In EDEN, the BMI-GRS comprised 27 BMI-related genetic variants identified by Speliotes et al. and weighted by their European-only, sex-combined effect estimates in that study [283].

The 27 loci that comprised the EDEN BMI-GRS were all present in the Fenland BMI-GRS. Further, they were amongst the most strongly associated signals in the Fenland score [149]. Thus they explain a larger proportion of the variance in BMI than the additional 69 SNPs that comprise the Fenland BMI-GRS. The 96 SNP Fenland BMI-GRS explained 4% of the variance in BMI amongst Fenland men and 1% amongst Fenland women. The corresponding figures for the 27 SNP EDEN BMI-GRS amongst EDEN participants were 3% and 1%. The units of the effect estimates used to weight the Fenland and EDEN BMI-GRSs were different. To facilitate comparison between the studies, the BMI-GRSs were standardised by z-score transformation in both cohorts.

4.3.2.2 The assessment of eating behaviour

The EB traits were measured using the TFEQ-R18 in Fenland and the TFEQ-R21 in EDEN [40, 66]. The questionnaires are described in greater detail in **Chapter 1**. Both questionniares measure EE, UE and CR. The TFEQ-R21 was developed from the TFEQ-R18 by adding 3 additional items to the EE subscale of the questionnaire with the intention of improving the discrimination of the scale. All other items are identical between the two questionnaires. The TFEQ-R18 was initially developed in an obese population but has been validated for use in normal weight populations and can accurately distinguish different patterns of EB in the general population [68, 89]. The factor structure of the TFEQ-R21 has also been replicated in a population-based study of Swedish male twins [66].

Fenland participants completed the TFEQ-R18 at their baseline assessment. In the EDEN cohort, parents completed the TFEQ-R21 at their child's 2 year follow-up assessment. In both studies, subscale scores were generated for each participant and transformed to a 0-100 scale using the following equation [89]:

$$\frac{raw\ score - lowest\ possible\ raw\ score}{possible\ raw\ score\ range} \times 100$$

The *raw score* refers to the mean of the items that comprise the EB trait scale multiplied by the total number of items on the trait's subscale. This step accounts for missing data. The *lowest possible raw score* refers to the lowest possible raw score a participant could receive for the subscale. For example, as each item on the subscales is scored from 1 to 4, if the subscale is comprised of 3 items, the lowest possible raw score would be 3 (indicating a mean score of 1 on the subscale items, multiplied by the 3 items on the subscale). The *possible raw score range* is the highest possible raw score on the subscale, minus the lowest possible score on the subscale.

The scaled scores were then standardised to a mean of 0 and SD of 1. Cronbach's alpha was used to test the inter-correlations between the individual questionnaire items of each EB trait separately in both cohorts. On the basis of convention, a threshold of 0.6 was chosen for inclusion of items [305]. The observed between-item correlations within each EB trait fell between 0.75 and 0.87 in Fenland, and between 0.76 and 0.93 in EDEN, suggesting a high level of reliability of all the EB trait scales.

4.3.2.3 The assessment of body mass index

In Fenland, anthropometric measurements were taken during participant's baseline visit as described in **Section 2.1**. Weight was measured to the nearest 0.1kg using electronic scales (TANITA model BC-418 MA; Tanita^{TM}, Tokyo, Japan) and height was measured to the nearest 0.1cm using a wall-mounted stadiometer (SECA 240; Seca^{TM}, Birmingham, UK). Participants were measured and weighed barefoot and were asked to remove heavy items of clothing.

In EDEN, maternal weight was measured to the nearest 0.1kg using electronic scales (Terraillon SL-351, Hanson Ltd, Hemel Hempstead, UK) at the 1 and 3 year follow-up visits. At 2 year follow up, mothers reported their current weight but were not weighed. Paternal weight was measured at baseline, self-reported or reported by the mother. Parental heights were measured to the nearest 0.2cm using a wall-mounted stadiometer (SECA 206, Seca^{TM}, Hamburg, Germany). In instances where paternal heights were not measured objectively, they were self-reported or reported by the mother. Participants were weighed and measured barefoot and were asked to remove heavy items of clothing. For the mothers, an attempt was made to ensure that the weight used was collected as close to the time of the EB data collection as possible. In order of preference, self-reported weight at 2 year follow-up (55%), the mean of weight measured at 1 year follow-up and weight measured at 3 year follow-up (15%) or weight measured at 1 year follow-up (30%) was used. At the baseline assessment, mothers also self-reported their pre-pregnant BMI. This was used in sensitivity

analyses. Amongst fathers for whom anthropometric measurements during pregnancy were unavailable, we used self-reported height and weight at baseline (11%), or father's height and weight reported by the mother at baseline (6%).

4.3.2.4 The analysis of mediation

Mediation occurs when the relationship between an exposure (in this case, the BMI-GRS) and an outcome (in this case, BMI) is explained by the presence of a third variable (the mediator) which lies on the causal pathway between them (**Figure 4.1**). The mediator may be wholly or partly responsible for the association between the exposure and the outcome.

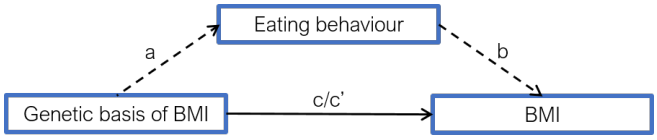


Figure 4.1 Depiction of the mediation analysis. *Path a* shows the association between the genetic basis for BMI (the BMI-GRS) and EB; *Path b* shows the association between EB and BMI; *Path c* shows the association between the BMI-GRS and BMI; *Path c'* represents the association between the BMI-GRS and BMI, adjusted for EB.

To test the theory that EB is a mediator of genetic predisposition to obesity, the association between the BMI-GRS and BMI was analysed in linear regression models with the BMI-GRS modelled as the exposure and BMI as the outcome. The models were adjusted for age, sex and, in the EDEN cohort, recruitment centre. This constituted the *base model*, represented by *Path c* in **Figure 4.1**.

If an EB trait was associated with both the exposure (BMI-GRS) and the outcome (BMI), in separate linear regression models adjusted for age, sex and, in EDEN, recruitment centre, we tested for mediation. The *base model* was adjusted for the EB trait. The presence of mediation was established using the Sobel test [306, 307] and quantified using the mediation ratio [308]. The mediation ratio was calculated using the equation: $((\beta - \beta^{'})/\beta)$, where β is the effect estimate for the BMI-GRS from the regression of the BMI-GRS on BMI (adjusted for age, sex and, in EDEN, recruitment centre) (*Path c* in **Figure 4.1**) and $\beta^{'}$ is the effect estimate for the BMI-GRS from the same regression after the model is additionally adjusted for EB (*Path c'* in **Figure 4.1**).

4.3.2.5 The analysis of effect modification

Effect modification occurs when the association between an exposure and an outcome differs depending on the level of a third variable (the modifier) [309]. Standard approaches to the assessment of mediation, including the Sobel test, assume no interaction between

the exposure and the mediator and do not provide a method to test for mediation and modification simultaneously [310]. Thus separate models were used to test for modification. Conditions can exist whereby mediation and modification occur along the same pathway (e.g. mediated modification and moderated mediation). However, a single variable cannot simultaneously be considered as both a mediator and a modifier of the same association [309]. As the association between genetic susceptibility to obesity and EB is uncertain, we separately modelled the EB traits as both mediators and modifiers.

To test whether the association between the BMI-GRS and BMI was modified by EB, an interaction term (BMI-GRS × EB trait score) was added to the *base model* for each EB trait. Effect modification was identified if the effect estimate for the interaction term was statistically significant (p<0.05). In order to better characterise the interaction, if effect modification was identified, the cohort was split into tertiles based on scores for the modifying EB trait. The BMI-GRS to BMI association was then tested separately within each tertile of the EB trait score.

4.3.2.6 Sensitivity analyses

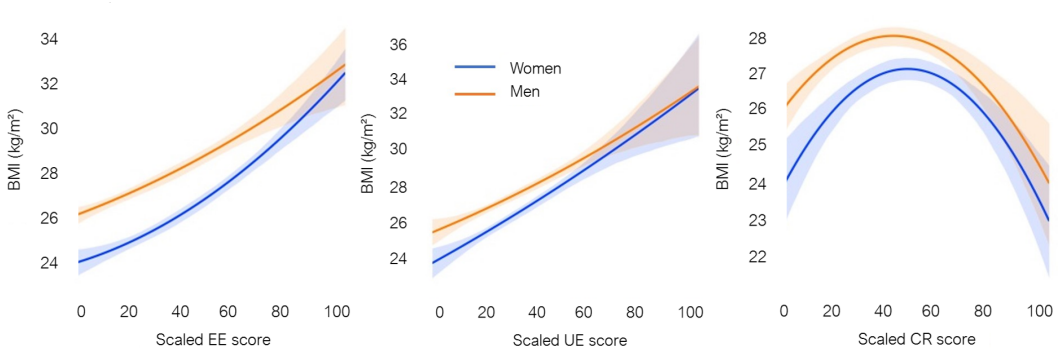
In the EDEN cohort, the main analyses were repeated using maternal pre-pregnant BMI, reported at baseline. This analysis was performed in order to ascertain whether the results were influenced by recent pregnancy, which may be associated with both EB traits and BMI.

4.3.2.7 Preliminary analyses

Sex stratification. As described in **Chapter 3**, most known genetic determinants of BMI do not exhibit heterogeneous effects between the sexes [149]. In the Locke et al. discovery sample, just 2 of the 97 identified variants demonstrated evidence of heterogeneity [149]. However, their associations to EB traits may be sex-specific. In order to determine whether the analyses should be stratified by sex, we tested for modification of the age-adjusted BMI-GRS to EB trait association by sex in linear regression models in both cohorts. This analysis indicated that sex modified the relationship between the BMI-GRS and CR in Fenland (p-value for the interaction term (p-*interaction*)=0.02) but not UE (Fenland: p-*interaction*=0.34; EDEN: p-*interaction*=0.89), EE (Fenland: p-*interaction*=0.26; EDEN: p-*interaction*=0.57) or CR in EDEN (p-*interaction*=0.12). Although sex did not modify the association between the BMI-GRS and CR in EDEN, as a result of the presence of modification in the larger Fenland study, the analyses of mediation and modification by CR were sex-stratified in both cohorts.

Linear regression. To determine whether the relationship between EB traits and BMI could be appropriately modelled using linear regression, we plotted the associations between the EB and BMI (**Figure 4.2**). The associations of EE and UE to BMI were approximately linear, suggesting that linear regression is appropriate. However, the relationship between CR and

BMI appeared non-linear (**Figure 4.2c**). To account for this, both CR and its quadratic term (CR × CR) were added to regression models when testing for mediation by CR. **Figure 4.2** shows the association between EB and BMI in Fenland. The associations in EDEN were consistent (**Appendix B.1**).



(a) Emotional eating **(b)** Uncontrolled eating **(c)** Cognitive restraint

Figure 4.2 The association between the EB traits and BMI in the Fenland study. The graphs plot the EB trait scores (0-100) on the x-axis against BMI (kg/m^2) on the y-axis. The association amongst women is shown in blue and the association amongst men is shown in orange. The shaded areas indicate 95% CIs.

Associations between the EB traits. If EB traits were identified as mediators of the BMI-GRS to BMI association, we planned to determine whether their mediating effect was independent or occurred through a shared mechanism. In order to ascertain whether this additional analysis was necessary, we explored the correlations between the EB traits. In both cohorts, all three EB traits were positively correlated with each other in the sex-combined sample. The appetitive traits, EE and UE, were particularly highly correlated (**Table 4.1**). To ascertain whether any mediating effects of the appetitive EB traits were independent, or occurred through a shared mechanism, we planned additional analyses that simultaneously accounted for both EE and UE in the same model. First, mediation was assessed in models where both EB traits were controlled for simultaneously, representing the specific effects of each EB, whilst controlling for the other. Second, we modelled the mediating effect of the residuals from a model predicting EE from UE (and vice-versa) on the BMI-GRS to BMI relationship. This was designed to examine the mediating effect of the component of each EB that occurs independently of the other EB.

Analyses were performed using Stata version 14 (StataCorp LCC, College Station, TX) in Fenland and SAS version 9.3 (SAS, Cary, NC) in EDEN. Figures were produced using R version 3.3.2.

Table 4.1 Correlations between emotional eating, uncontrolled eating and cognitive restraint

	Total cohort[a]			Men			Women		
	EE	UE	CR	EE	UE	CR	EE	UE	CR
Fenland									
Emotional eating	1.00	–	–	1.00	–	–	1.00	–	–
Uncontrolled eating	0.64*	1.00	–	0.65*	1.00	–	0.65*	1.00	–
Cognitive restraint	0.16*	0.03*	1.00	0.16*	0.05	1.00	0.04	< -0.01	1.00
EDEN									
Emotional eating	1.00	–	–	1.00	–	–	1.00	–	–
Uncontrolled eating	0.60*	1.00	–	0.60*	1.00	–	0.67*	1.00	–
Cognitive restraint	0.37*	0.21*	1.00	0.34*	0.20*	1.00	0.31*	0.23*	1.00

Correlations were assessed using the Pearson's correlation coefficient

Emotional eating (EE); Uncontrolled eating (UE); Cognitive restraint (CR)

* $p < 0.05$

[a] Total cohort refers to the combined cohort including both men and women

– Not applicable

4.4 Results

4.4.1 Characteristics of the study participants

The study population of the present analysis comprised 3515 Fenland participants (1869 women; 53%) and 2154 EDEN participants (1200 women; 56%) (**Table 4.2**). The mean age in Fenland (51 years amongst both sexes) was higher than in EDEN (men: 32 years; women: 30 years) and the prevalence of obesity (men: 24%; women: 22%) was approximately double that in EDEN (men: 9%; women: 11%). The Fenland participants also scored higher on all three EB traits. In both cohorts, women reported higher scores for all EB traits than men. In the combined cohort of both sexes, which was larger and thus had a greater power to detect correlations than the sex-stratified cohort, all the EB traits were positively correlated with each other. However, the appetitive traits (EE and UE) were more strong correlated with each other than with CR (**Table 4.1**).

Table 4.2 Descriptive characteristics of the Fenland (n=3515) and EDEN (n=2154) study participants

	Fenland		EDEN	
	Men (n=1646)	Women (n=1869)	Men (n=954)	Women (n=1200)
Age (years)	50.7 (7.3)	50.9 (7.2)	32.2 (5.6)	29.9 (4.7)
BMI (kg/m^2)	27.6 (4.2)	26.6 (5.3)	25.2 (3.6)	24.1 (4.8)
BMI status				
Underweight	0.4% (7)	1.2% (22)	0.7% (7)	6.2% (74)
Normal weight	27.2% (449)	43.9% (821)	51.6% (492)	60.6% (727)
Overweight	48.2% (793)	33.3% (622)	38.7% (369)	21.8% (262)
Obese	24.1% (397)	21.6% (404)	9.0% (86)	11.4% (137)
Emotional eating	27.1 (24.8)	42.2 (28.0)	16.2 (21.1)	34.5 (27.4)
Uncontrolled eating	29.1 (17.5)	31.0 (17.7)	23.3 (18.5)	23.4 (17.7)
Cognitive restraint	35.5 (19.0)	45.8 (19.1)	20.7 (18.0)	32.7 (21.2)

Values are mean (SD) or % (n). WHO BMI categories: Underweight <18.5kg/m^2; Normal weight 18.5–24.9kg/m^2; Overweight ≥ 25kg/m^2; Obese ≥ 30kg/m^2. EB traits scores are scaled from 0-100

4.4.2 The analysis of mediation

4.4.2.1 The association between eating behaviour traits and BMI

EE and UE demonstrated positive linear associations with BMI in both cohorts (**Table 4.3**; **Figure 4.2**). When both EE and UE were considered simultaneously in the same model,

both maintained significant but reduced associations with BMI in Fenland (EE: β =1.35 kg/m^2 (95% CI: 1.15, 1.55); p<1x10^{-10}. UE: β =0.65kg/m^2 (95% CI: 0.45, 0.85); p<1x10^{-10}) (**Appendix A.7**). In EDEN, only EE remained associated with BMI (EE: β =1.08 kg/m^2 (95% CI: 0.85, -1.31); p<1x10^{-10}. UE: β =0.22kg/m^2 (95% CI: -0.02, 0.46); p=0.05).

The BMI-GRS to CR association was modified by sex (see **Section 4.3.2.7**). As a result, CR was analysed in men and women separately. In both cohorts, and amongst both men and women, there was a quadratic association between CR and BMI (**Table 4.4**, all p<0.001 for the quadratic term). At lower levels of CR, CR was positively associated with BMI, but at higher levels of CR, the association between CR and BMI became negative in Fenland and plateaued in EDEN (**Figure 4.2c**; **Appendix B.1**). When the cohorts were separately stratified into two groups based on BMI, comprising normal weight participants (BMI <25kg/m^2) and overweight/obese participants (BMI ≥25kg/m^2), a positive linear association between CR and BMI was found amongst all groups with BMI <25kg/m^2 (Fenland: men: β =0.24kg/m^2 (95% CI: 0.10, 0.37); $p = 7.0 \times 10^{-4}$; women: β =0.25kg/m^2 (95% CI: 0.13, 0.36); $p = 2.0 \times 10^{-5}$. EDEN: men: β =0.57kg/m^2 (95% CI: 0.43, 0.72); $p < 1.0 \times 10^{-10}$; women: β =0.69kg/m^2 (95% CI: 0.55, 0.83); $p < 1.0 \times 10^{-10}$). However, amongst overweight and obese participants, a negative association between CR and BMI was found amongst Fenland men ($\beta = -0.22$kg/m^2 (95% CI: -0.43, -0.00); p=0.05) and women ($\beta = -0.64$kg/m^2 (95% CI: -0.97, -0.33); $p = 8.3 \times 10^{-5}$). No association was found between CR and BMI amongst overweight and obese participants in EDEN (men: β =0.01kg/m^2 (95% CI: -0.13, 0.16); p=0.85. Women: $\beta = -0.08$kg/m^2 (95% CI: -0.28, 0.11); p=0.40).

4.4.2.2 The association between the BMI-GRS and eating behaviour traits

In both cohorts and in both sexes combined, the BMI-GRS was positively associated with both EE and UE (EE: Fenland: p=0.02; EDEN: p=0.01. UE: Fenland: $p = 5.0 \times 10^{-4}$; EDEN: p=0.04). Amongst men in both cohorts, the BMI-GRS was not associated with either the linear or quadratic CR term p>0.05) (**Table 4.4**). Amongst women in both cohorts, the BMI-GRS was positively associated with the linear CR term (p<0.05) but not to the quadratic CR term (p>0.05). These results are shown in **Table 4.4**.

Individual SNP to EB associations are generally under-powered due to limited sample sizes in both cohorts. However, in Fenland, 9 of the 96 BMI-associated SNPs included in the BMI-GRS showed nominally significant associations (p<0.05) with EE (6 positive), 8 with UE (5 positive) and 5 with CR (1 positive) (**Appendix A.9**). In EDEN, 4 of the 27 SNPs included in the BMI-GRS showed nominally significant associations with EE (3 positive), 3 with UE (1 positive) and 2 with CR (2 positive) (**Appendix A.10**).

4.4.2.3 Mediation by the eating behaviour traits

Emotional eating and Uncontrolled eating. In both cohorts, EE and UE partially mediated the association between the BMI-GRS and BMI (**Table 4.3**). For EE, the mediation ratio (Sobel test *p*-value) was 10% (p=0.02) in Fenland and 11% (p=0.01) in EDEN. For UE, the corresponding values were 12% (p=0.0006) in Fenland and 6% (p=0.04) in EDEN. Controlling for UE, EE did not independently mediate the BMI-GRS to BMI association in either cohort. Controlling for EE, UE no longer mediated the association in EDEN. However, it remained a partial mediator in Fenland (mediation ratio: 3% (Sobel test p-value = 0.02)) (**Appendix A.7**). When the residuals from the regression of EE on UE (and UE on EE) were tested as a mediator of the association between the BMI-GRS and BMI, only the residuals from the regression on EE on UE, representing the independent effects of UE, in Fenland appeared to mediate the association (mediation ratio: 4% (Sobel test p-value = 0.01)) (**Appendix A.8**).

Cognitive restraint. The quadratic CR term did not meet the pre-defined conditions to be analysed as a mediator in Fenland or amongst EDEN men (see **Section 6.3.2**) as it was not associated with both the BMI-GRS and BMI (**Table 4.4**). Despite the non-linearity of the association between CR and BMI, amongst EDEN women only, the linear CR term was associated with both the BMI-GRS and BMI. In this group only, the linear CR term appeared to mediate the association between the BMI-GRS and BMI (mediation ratio=19%; Sobel test p-value=0.0009).

Table 4.3 Mediation of the BMI-GRS to BMI association by EE and UE

	BMI-GRS to EB		EB to BMI		BMI-GRS to BMI		BMI-GRS to BMI (adj. for EB)			
	Effect size[a] (95% CI)	*p*-val.	Effect size[b] (95% CI)	*p*-val.	Effect size[c] (95% CI)	*p*-val.	Effect size[d] (95% CI)	*p*-val.	Sobel test *p*-val.	Mediation ratio (%)
Emotional eating										
Fenland (n=3515)	0.04 (0.01, 0.07)	0.02	1.78 (1.63, 1.94)	$< 1 \times 10^{-10}$	0.70 (0.54, 0.85)	$< 1 \times 10^{-10}$	0.63 (0.48, 0.78)	$< 1 \times 10^{-10}$	0.02	10.0%
EDEN (n=2154)	0.06 (0.01, 0.10)	0.01	1.22 (1.04, 1.39)	$< 1 \times 10^{-10}$	0.62 (0.44, 0.80)	$< 1 \times 10^{-10}$	0.55 (0.38, 0.72)	4×10^{-10}	0.01	10.8%
Uncontrolled eating										
Fenland (n=3515)	0.06 (0.03, 0.09)	5×10^{-4}	1.50 (1.35, 1.65)	$< 1 \times 10^{-10}$	0.70 (0.54, 0.85)	$< 1 \times 10^{-10}$	0.61 (0.46, 0.76)	$< 1 \times 10^{-10}$	6×10^{-4}	12.0%
EDEN (n=2154)	0.04 (0.00, 0.09)	0.04	0.92 (0.74, 1.10)	$< 1 \times 10^{-10}$	0.62 (0.44, 0.80)	$< 1 \times 10^{-10}$	0.58 (0.40, 0.75)	1×10^{-10}	0.04	6.4%

Eating behaviour (EB); genetic risk score for BMI (BMI-GRS); body mass index (BMI)
Effect sizes are from linear regression models adjusted for age, sex and (in EDEN) recruitment centre
The BMI-GRS and EB trait scores were standardised to *z*-scores. BMI was in kg/m^2
[a] SD change in EB per SD increase in the BMI-GRS; [b] change in BMI (kg/m^2) per SD increase in EB; [c] change in BMI (kg/m^2) per SD increase in the BMI-GRS; [d] change in BMI per SD change in the BMI-GRS, adjusted for EB

Table 4.4 Mediation of the BMI-GRS to BMI association by CR

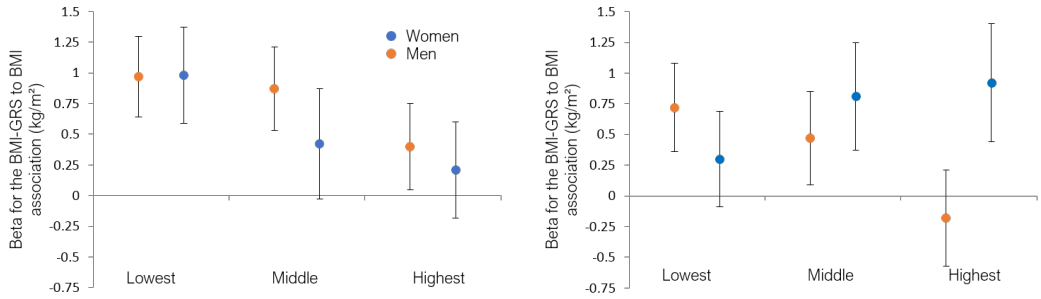
	BMI-GRS to EB		EB to BMI		BMI-GRS to BMI		BMI-GRS to BMI (adj. for EB)			
	Effect size[b] (95% CI)	*p*-val.	Effect size[c] (95% CI)	*p*-val.	Effect size[d] (95% CI)	*p*-val.	Effect size[e] (95% CI)	*p*-val.	Sobel test *p*-val.	Mediation ratio (%)
Cognitive restraint (linear term)										
Men										
Fenland (n=1646)	0.00 (-0.05, 0.05)	0.94	0.14 (-0.06, 0.35)	0.17	0.79 (0.60, 0.99)	$< 1 \times 10^{-10}$	–	–	–	–
EDEN (n=954)	0.04 (-0.02, 0.10)	0.21	0.98 (0.76, 1.20)	$< 1 \times 10^{-10}$	0.37 (0.15, 0.60)	1×10^{-3}	–	–	–	–
Women										
Fenland (n=1869)	0.07 (0.03, 0.12)	9×10^{-4}	0.10 (-0.15, 0.35)	0.42	0.61 (0.38, 0.85)	5×10^{-7}	–	–	–	–
EDEN (n=1200)	0.10 (0.04, 0.15)	6×10^{-4}	1.62 (1.36, 1.88)	$< 1 \times 10^{-10}$	0.80 (0.53, 1.07)	6×10^{-9}	0.65 (0.39, 0.90)	$< 1 \times 10^{-10}$	9×10^{-4}	19.0%
Cognitive restraint (quadratic term)[a]										
Men										
Fenland (n=1646)	0.00 (-0.01, 0.01)	0.96	-1.80 (-2.53, -1.07)	2×10^{-6}	0.79 (0.60, 0.99)	$< 1 \times 10^{-10}$	–	–	–	–
EDEN (n=954)	-0.02 (-0.09, 0.04)	0.51	-0.34 (-0.55, -0.14)	9×10^{-4}	0.37 (0.15, 0.60)	1×10^{-3}	–	–	–	–
Women										
Fenland (n=1869)	-0.01 (-0.02, 0.01)	0.31	-2.16 (-3.02, -1.30)	8×10^{-7}	0.61 (0.38, 0.85)	5×10^{-7}	–	–	–	–
EDEN (n=1200)	-0.01 (-0.08, 0.05)	0.71	-0.49 (-0.71, -0.27)	1×10^{-5}	0.80 (0.53, 1.07)	6×10^{-9}	–	–	–	–

Eating behaviour (EB); genetic risk score for BMI (BMI-GRS); body mass index (BMI). Effect sizes and 95% CIs are from linear regression models adjusted for age, sex and (in EDEN) recruitment centre. The BMI-GRS and TFEQ scores were all standardised to *z*-scores (mean=0; SD=1). [a] Effect estimates refer to the quadratic term. Models were additionally adjusted for the linear term; [b] SD change in EB per SD increase in the BMI-GRS; [c] change in BMI (kg/m^2) per SD increase in EB; [d] change in BMI (kg/m^2) per SD increase in the BMI-GRS; [e] change in BMI (kg/m^2) per SD increase in the BMI-GRS adjusted for EB. – Not applicable. The EB was not associated with both the BMI-GRS and BMI and, as such, could not be considered as a mediator

4.4.3 The analysis of effect modification

A nominally significant interaction between CR and the BMI-GRS on BMI was observed amongst men in both cohorts (Fenland: *p-interaction*=0.04; EDEN: *p-interaction*=0.0001) and Fenland women (*p=interaction*=0.0004) but not EDEN women (*p=interaction*=0.15). EE and UE did not interact with the BMI-GRS in either cohort (all $p>0.05$).

Amongst all groups demonstrating evidence of a BMI-GRS×CR interaction, grouping the participants into tertiles by CR score showed that the association between the BMI-GRS and BMI was strongest in the lowest tertile of CR and weakest in the highest tertile (**Figure 4.3**; **Table 4.5**).



(a) Fenland **(b)** EDEN

Figure 4.3 Association between the BMI-GRS and BMI within tertiles of CR. The graphs plot the effect estimates and 95% CIs from the linear regression of the BMI-GRS on BMI (y-axis) by tertiles of CR (x-axis). The units are change in BMI (kg/m^2) per SD increase in the BMI-GRS. The regressions were sex-stratified and age-adjusted. In EDEN, recruitment centre was also included in the models.

Table 4.5 The BMI-GRS to BMI association within tertiles of cognitive restraint

		Men		**Women**	
		Effect size[a] (95% CI)	*p*-value	Effect size[a] (95% CI)	*p*-value
Fenland	Lowest CR tertile	0.97 (0.64, 1.30)	<0.0001	0.98 (0.59, 1.37)	<0.0001
	Middle CR tertile	0.87 (0.53, 1.21)	<0.0001	0.42 (-0.03, 0.87)	0.07
	Highest CR tertile	0.40 (0.05, 0.75)	0.03	0.21 (-0.17, 0.60)	0.28
EDEN	Lowest CR tertile	0.72 (0.36, 1.07)	<0.0001	0.30 (-0.08, 0.69)	0.10
	Middle CR tertile	0.47 (0.09, 0.85)	0.02	0.81 (0.36, 1.25)	0.0004
	Highest CR tertile	-0.18 (-0.57, 0.21)	0.40	0.92 (0.44, 1.40)	0.0002

Cognitive restraint (CR)

[a]Effect sizes are from the linear regression of the BMI-GRS on BMI, adjusted for participant age. The units are change in BMI (kg/m^2) per SD increase in the BMI-GRS

4.4.4 Sensitivity analyses

In EDEN, using the self-reported pre-pregnant BMI of women in place of the self-reported 2 year post-partum BMI, did not substantially alter the results of the mediation analysis. EE, UE and the linear CR term each remained partial mediators of the BMI-GRS to BMI association explaining 11%, 7% and 22% of the association, respectively (Sobel test *p*-values: 0.01, 0.03 and <0.01) (**Appendix A.11**).

4.5 Discussion

4.5.1 Summary and context of the main findings

This analysis showed that genetic susceptibility to obesity is both mediated and modified by different aspects of EB. These findings were based on 3515 predominantly white British adults aged 35-64 years from the Fenland study and 2154 French adults aged 18-56 years from the EDEN study. In these groups, appetitive EB traits (EE and UE) partially mediated the association between the BMI-GRS and BMI whilst, with the exception of EDEN women, the association was modified by CR. The results highlight the importance of behavioural pathways in the aetiology of obesity. Relationships between other aspects of behaviour and body weight are explored in **Chapters 5** and **6**.

4.5.1.1 Appetitive traits.

In both the Fenland and EDEN cohorts, EE and UE demonstrated positive linear associations with both BMI and the BMI-GRS, allowing them to be tested as mediators. Although the present investigation was cross-sectional, precluding the drawing definitive causal inferences, the positive association between BMI and these traits is consistent with reports that appetitive EB traits predict weight gain in adulthood [106, 120, 123, 127]. In keeping with the weight of evidence that appetitive EB traits impact BMI, we modelled EB as the exposure and BMI as the outcome in all models. Future prospective research and genetic studies (such as **Chapter 7**) are needed to further confirm this direction of association.

The finding that EE and UE partially mediated genetic susceptibility to obesity corroborates a growing body of evidence in support of BST [102]. This theory is outlined in **Chapter 1** and proposes that appetitive EB traits lie on the causal pathway between BMI-related genetic variants and obesity. Previous evidence in support of BST includes enriched expression of genes associated with obesity-related genetic variants in brain regions involved in appetite regulation [149] and reported relationships between single BMI-related genetic variants and appetitive aspects EB [158, 151, 159, 160]. Explicit testing for mediation of genetic susceptibility to obesity by appetitive traits had only previously been reported by three studies, one in adults and two in children. Our findings extended these reports, testing CR as a mediator and exploring modification of this pathway by EE, UE and CR for the first time. Following the publication of our study, two further investigations have been reported.

The single previous adult study tested for mediation of the association between a 90 SNP BMI-GRS and BMI by UE and EE in two Finnish cohorts: DILGOM (adults aged 25-74 years) and FinnTwin12 (adults aged 21-26 years) [161]. In keeping with our findings, EE partially mediated the association in both cohorts. However, UE was a partial mediator in DILGOM only. The lack of mediation by UE in FinnTwin12 may reflect differences between

the FinnTwin12 participants and other cohorts. Notably, the FinnTwin12 participants had a mean age of 22 years, 8 years younger than the mean age amongst EDEN women (mean age: 30 years), who constitute the next youngest group. Further research is therefore needed to determine whether age influences the mediating effect of UE. However, results in children suggest this is an unlikely explanation. The finding may also be explained by low levels of obesity in FinnTwin12 (6% of men and 5% of women compared to 20% of men and 23% of women in DILGOM). CR was not tested as a mediator.

In 2018, following the publication of the present study, the mediation of genetic susceptibility to obesity by EB traits was tested in the Quebec Family Study [311]. Amongst 750 adults with a mean age of 44 years, the association between an unweighted 97 SNP BMI-GRS and BMI was partially mediated by disinhibition and susceptibility to hunger, measured using the TFEQ-51. Following failures to replicate the factor structure of the TFEQ-51, the questionnaire was revised and reduced to create the TFEQ-R18 [40]. During this revision, items on the disinhibition and hunger scales were primarily assigned to UE and reflect appetitive EB traits [40]. Thus these findings can broadly be considered to replicate ours. This study is the only other investigation to test CR as a mediator. It replicated our findings suggesting that CR does not mediate genetic susceptibility to obesity. The study did not explore effect modification.

Amongst children, mediation of a 28 SNP BMI-GRS to BMI association by SR was demonstrated in 8-11 year olds (n=2258) [163]. SR measures sensitivity to feelings of hunger and fullness and is only measured in adults using the Adult Eating Behaviour Questionnaire (developed in 2016) [59]. No formal comparison studies have been reported comparing SR to either UE or EE. However, the items that comprise the SR scale of the CEBQ are comparable to some items that comprise the UE scale of the TFEQ-R18. A second study amongst 6−8 year olds did not detect mediation of the association between a 32 SNP BMI-GRS and weight gain by EB (n=662) [164]. It is possible that this study was not powered to detect mediation, or that mediation is not evident for weight gain. A third study published in 2019 showed that appetite, measured by a single item, partially mediated the association between a 16 SNP BMI-GRS and BMI amongst 1142 children aged 2-5 years from the EDEN study [162]. Together, these findings suggest that the mediating effect of appetitive EB traits demonstrated in adulthood may also be evident amongst young children. Future research is needed to highlight specific aspects of childhood EB that mediate genetic susceptibility to obesity and how these relate to EB traits in adulthood. Studies using weight gain, as opposed to weight or BMI, as the outcome are also needed amongst both adults and children.

4.5.1.2 Cognitive restraint.

Our results suggest that the relationships between CR, the BMI-GRS and BMI are distinct from those of EE and UE. First, the association between CR and BMI was quadratic. Amongst

underweight and normal weight participants (BMI <25kg/m^2), there was a positive linear association, whilst amongst overweight and obese participants (BMI ≥ 25kg/m^2), the association was negative in Fenland women and non-significant in all other groups. Previously, studies have assessed the association between CR and BMI in linear models. Whilst some have identified a positive association [119, 124, 107, 110, 68, 112, 114, 92], others have reported no association [126, 125]. Two previous studies have suggested that CR is positively associated with BMI amongst normal weight but not overweight individuals [131, 88]. Thus these findings contribute to research suggesting that the association between CR and BMI is BMI-dependent.

We do not conclude that CR is a positive mediator of the genetic susceptibility to higher BMI, as indicated by EDEN women. Supported by evidence that changes in BMI predict changes in CR, we speculate that CR represents a response to increasing BMI amongst normal weight individuals [136–138]. This is supported by evidence from other studies suggesting that CR is more often motivated by a desire to prevent weight gain than to instigate weight loss [312, 51]. Further, in a previous study, higher scores for restraint were identified in non-obese adults who reported a history of obesity compared to non-obese adults with no history of obesity [313]. This speculatively suggests that CR might be an effective means of controlling the weight amongst normal weight individuals with a propensity to, or history of, weight gain. Conversely, the abandonment of restraint might contribute to overweight and obesity, explaining the quadratic association in our study [131, 88]. Longitudinal data with repeated assessments of EB and BMI, as well as genetic evidence, are needed to better understand this relationship.

In support of a limiting effect of CR on BMI, we show for the first time that CR modifies the association between the BMI-GRS and BMI. In all groups showing an interaction between the BMI-GRS and CR on BMI (men in both cohorts and Fenland women), the effect of the BMI-GRS on BMI was strongest amongst those with the lowest levels of CR and was weakest amongst those with the highest levels of CR. This novel finding suggests that CR may protect genetically susceptible individuals from excessive weight gain. Following the publication of this investigation, our finding of an interaction between the BMI-GRS and CR has been replicated using weight gain as the outcome. In a cohort of ~ 5000 Finnish adults aged 25-74 years enrolled in the DILGOM study, the association between the BMI-GRS and annual weight gain from age 20 years to baseline was modified by CR [138]. Dietary restraint might be beneficial to weight control. Whilst interventions to alter CR have not yet been developed, other behaviours that encourage control over consumption are modifiable. The role of maternal attitudes that are amenable to change in modifying determinants of infant weight gain is explored in **Chapter 5**.

4.5.2 Strengths and limitations

This study was conducted amongst two large, well phenotyped, population-based cohorts. Both used the same validated measure of EB, facilitating direct comparison of results. Given the public health significance of obesity and mixed messages regarding the role of restraint over eating, with some evidence suggesting that restraint leads to weight gain, the findings make an important contribution to the literature.

The main limitation was the cross-sectional design of the analyses. We chose to model EB traits as the cause, rather than the outcome, of BMI. This decision was based on evidence from longitudinal research suggesting a prospective association between appetitive traits and weight gain in adulthood and the expression of genes associated with BMI-related genetic variants in regions of the brain involved in appetite regulation. However, further longitudinal research is needed, especially in the case of CR. Genetic evidence could also be used in the future to help clarify causality (as in **Chapters 6** and **7**). Further, whilst the results in the two cohorts are broadly consistent, no modifying effect of CR was identified amongst EDEN women. EBs and BMI were assessed in EDEN women at 2 years post-partum, a time when CR and weight are plausibly still altered by pregnancy. However, the results were similar when using self-reported pre-pregnant BMI and it remains uncertain why CR might have a different relationship with BMI in this group, particularly in light of replication of the CR modification results in a subsequent study published in 2018 [138].

4.5.3 Conclusions

Our results indicate that genetic susceptibility to obesity in adulthood is partially mediated by appetitive EB traits and modified by CR. These findings support the view that appetitive traits lie on the causal pathway between genetics and weight status and suggest that they may provide a target for obesity prevention. Further, the findings demonstrate a novel relationship between the genetic determinants of obesity and CR, indicating that CR may protect genetically vulnerable individuals from obesity. This challenges the assertion that high levels of CR increase obesity risk and highlights CR as an additional target for obesity prevention.

CHAPTER 5

MATERNAL ATTITUDES TO FOLLOWING HEALTHY INFANT FEEDING GUIDELINES AND THE ASSOCIATIONS BETWEEN INFANT EATING BEHAVIOUR, MILK INTAKE AND BODY WEIGHT

Publications

This study is being prepared for submission:

Clifton, E.A.D., Amy, A.L., Day, F.R., Sharp, S.J., Griffin, S.J., Ong, K.K. and Lakshman, R. (2019). Positive maternal attitudes to healthy infant feeding guidelines attenuate the associations between infant appetitive traits and both infant milk intake and body weight.

Contributions

I planned this project and devised the analysis plan in collaboration with my supervisors. I cleaned and prepared the Baby Milk Trial dataset such that it could be used in this and other projects, including the main trial paper. I generated the EB trait and maternal attitudes scores, conducted the statistical analyses and jointly interpreted the results with my supervisors. I wrote this chapter and the resulting manuscript, which is being prepared for submission.

5.1 Summary

Infancy is increasingly recognised as a critical period for the development of lifetime obesity risk. The findings reported in **Chapter 4** suggest that whilst appetitive eating behaviour (EB) traits lie on the causal pathway between genetics and body weight in adulthood, the determinants of obesity can also be modified by exerting control over consumption. However, exercising restraint requires cognitive capabilities that do not arise during infancy. Whether there are modifiable maternal factors that interact with infant appetitive EBs, and could attenuate their associations with milk intake and body weight in early life, is not yet known. Amongst 669 infants enrolled in the Baby Milk Trial, we demonstrated age and sex-adjusted cross-sectional associations between two infant EB traits (infant food responsiveness (FR) and satiety responsiveness (SR)), measured using the Baby Eating Behaviour Questionnaire (BEBQ), and both infant milk intake and body weight. We then analysed whether maternal attitudes to following healthy infant feeding guidelines were associated with these outcomes and, secondarily, whether they modified the effect of the infant EB traits. Positive maternal attitudes to following healthy infant feeding guidelines were associated with lower infant milk intake and body weight. Further, maternal attitudes attenuated the positive association between infant FR and infant milk intake (p=0.049) and the negative association between infant SR and infant body weight (p=0.01). Overall, in this formula-fed cohort, positive maternal attitudes to following healthy infant feeding guidelines attenuated the association between infant EB traits and both milk intake and body weight. These findings indicate that promoting positive maternal attitudes to feeding guidelines may help infants to achieve a healthy weight through ensuring appropriate milk consumption, regardless of their EB tendencies.

5.2 Background

The mediation and modification of genetic susceptibility to obesity by adult EB traits is characterised and described in **Chapter 4**. The findings support other evidence suggesting that appetitive EB traits lie on the causal pathway to obesity, indicating that EE and UE partially mediate the effect of the genetic determinants of BMI. Further, CR was shown to modify genetic susceptibility to obesity. Whilst interventions designed to support CR may help genetically vulnerable adults to avoid or reverse obesity in the future, cognitive strategies are not applicable to early life due to their dependence on self-awareness and self-control, abilities that arise later in development [314]. Rapid weight gain during the first 1000 days, from conception to the age of 2 years, is considered an important determinant of lifetime obesity risk [165, 37, 166]. Whether known determinants of infant weight gain for which there are no existing interventions, interact with modifiable factors to influence milk intake and body weight is not yet known. Here, we investigated the associations between infant EB traits, maternal attitudes to following healthy infant feeding guidelines, infant milk intake and infant body weight.

Infant EB and its relationship to weight is described in detail in **Section 1.4.2**. Briefly, EB in infancy is typically assessed using the BEBQ, a parent-report questionnaire that measures 4 EB traits: food responsiveness (FR; 6 items), satiety responsiveness (SR; 3 items), enjoyment of food (EF; 4 items) and slowness in eating (SiE; 4 items) (**Appendix C.1**). Higher scores on the FR and EF scales of the questionnaire are considered to be associated with a greater tendency towards 'food approach'. FR conveys an infant's desire to eat (example item: my baby is always demanding a feed) and EF measures perceived liking for milk and feeding (example item: my baby enjoys feeding time). Higher scores on SiE and SR are together considered to be associated with greater 'food avoidance' and describe the pace of typical eating (example item: my baby feeds slowly) and ease of becoming full (example item: baby gets full up easily), respectively [39].

Infant EB traits demonstrate longitudinal stability between the ages of 3 and 15 months [104]. In separate studies amongst young children, the same traits have also been reported to track between 2-5 years, 3-4 years and 4-11 years [315, 316, 41]. Infant EB profiles characterised by high drive to eat (high FR) and muted response to feelings of fullness during feeding (low SR) are prospectively associated with more rapid weight gain during the first 15 months of life [142]. Further, infant appetite measured at 3 months predicts weight gain over the first 2 years of life [143]. Although some evidence suggests that the relationship between infant appetite and infant weight may be bi-directional, the prospective association of infant EB to weight gain is stronger than the association of weight gain to EB [141].

In light of their links to weight gain, in theory, infant EB traits provide a potential infant-specific target for obesity prevention. However, their determinants are elusive. Twin studies suggest that they are heritable and share a genetic basis with weight but no gene discovery

analyses have been performed and specific genetic variants have yet to be identified [104]. Amongst adults, this is addressed in **Chapter 7**. The role of other potential determinants of infant EB traits is supported by mixed evidence. For example, in separate studies, previous breastfeeding has been positively associated with SR measured at 6-12 months and 18-24 months, and with SiE [317, 145, 181]. However, these studies may be vulnerable to reverse-causality, whereby breastfeeding is stopped amongst infants with low SR and SiE. Furthermore, no impact on FR or EF was identified and a separate study reported decreased SR and increased FR amongst previously breastfed infants, suggesting a more appetitive profile [39].

Parental feeding styles and practices have also been analysed as potential determinants of infant EBs. Amongst 323 mother-child dyads, maternal restriction, emotional feeding and encouragement to eat were associated with increases in EF, EE and general appetite in children, whilst maternal monitoring was associated with reductions in 'food approach' EBs (EF and FR), amongst 2-3 year olds [318]. The same study showed that parental practices are also responses to child EB. Overall, whilst maternal concerns and behaviours have been correlated with infant EB, the totality of the evidence leaves the directions of effect unclear [175]. A 2004 systematic review based on 22 studies concluded that parental restriction was associated with higher childhood weight [175]. However, 19 of the 22 studies were cross-sectional. Amongst over 400 Australian mother-infant dyads, the mothers of infants with higher SR and lower EF reported greater concern about their infant becoming underweight whilst the mothers of infants with higher FR reported more concern about their infant becoming overweight [319]. This suggests that parental concern and feeding responses may be reactions to, rather than causes of, infant eating characteristics. In sum, as a result of poor understanding of their determinants, there are no known interventions that reliably influence the development of infant EB traits.

Alongside infant EB traits, parental feeding styles have separately been associated with infant weight trajectories in prospective studies [141, 320]. Unlike infant EBs, parental feeding behaviours are modifiable. Interventions designed to influence these behaviours have successfully promoted healthy infant weight gain in randomised controlled trials (RCTs) [183, 321, 259]. For example, an intervention designed to promote responsive parenting reduced infant weight gain up to the age of 6 months, probability of overweight at 1 year and BMI z-score at 3 years, relative to a home-safety intervention [183, 184]. Further, an RCT of an intervention designed to promote responsive feeding, reduce milk intake and promote weight monitoring slowed infant weight gain and reduced milk intake amongst infants aged up to 6 months, relative to general advice [321, 259]. These trials tested interventions that balanced responsivity to a child's cues of hunger and satiety with a degree of parental control over consumption.

In light of evidence that infants gain weight at a similar pace whether or not they are given control of their consumption through practices such as baby-led weaning, parental attitudes

and behaviours may be important to the avoidance of overfeeding [180, 322]. This may be particularly pertinent amongst infants with more appetitive EB profiles, who have a tendency to over-consume. The results of the Baby Milk Trial indicate that parental attitudes to following infant feeding guidelines are modifiable [321]. However, little is known about their relationship to infant feeding and body weight.

Here were explored the associations between maternal attitudes to following healthy infant feeding guidelines, infant EB traits, infant milk intake and infant body weight amongst formula-fed infants enrolled in the Baby Milk Trial. The study was designed to determine whether the impact of infant EB traits can be modified by maternal attitudes.

5.3 Participants and methods

5.3.1 Participants

Participants in this analysis comprised 669 mother-infant dyads enrolled in the Baby Milk Trial (mean infant age: 2 months). A full description of the Baby Milk Trial is provided in **Section 2.3.1**.

5.3.2 Methods

5.3.2.1 The assessment of infant eating behaviour

Infant EB was assessed using the retrospective version of the BEBQ, completed at the 6 month follow-up assessment (**Appendix C.1**). The questionnaire is described in greater detail in **Chapter 1**. Briefly, the BEBQ is a parent-report measure used to derive scores for 4 infant traits through 18 items: FR (6 items), SiE (4 items), SR (3 items) and EF (4 items) [39]. A single item measures general appetite. Each item is scored on a 5-point Likert scale, where higher scores indicate higher levels of the EB to which the item corresponds. The mean score for items comprising each of the 4 EB traits was calculated, resulting in a score between 1 to 5 for each of the 4 EB traits. In order to limit the number of tests performed, it was decided *a priori* that only FR and SR would be included in the analyses. These are the most widely studied of the infant EBs.

Cronbach's alpha was used to test the inter-correlations between the individual questionnaire items comprising each scale. For both FR and SR, these were 0.80, suggesting a high level of internal consistency between the items comprising each EB trait.

5.3.2.2 The assessment of maternal attitudes to infant feeding guidelines

Maternal attitudes to following healthy infant feeding guidelines were assessed at baseline, following recruitment to the Baby Milk Trial, but prior to intervention exposure. An 11-item self-report questionnaire designed to measure theory-based constructs surrounding parental attitudes to infant feeding was used (**Appendix C.2**) [323]. Each item was scored on a 5 point scale from *Strongly disagree* (scored as 1) to *Strongly agree* (scored as 5), with higher scores reflecting greater endorsement of the item. The questionnaire was used to generate scores on three sub-scales: self-efficacy (SE; 4 items), outcome expectancy (OE; 5 items) and intention (2 items).

SE and OE are important constructs in Social Cognitive Theory (SCT), which emphasises their role as mediators of behavioural change. In support of this theory, higher SE and

OE scores have been associated with greater success in changing behaviour [324]. The intentions measurement was derived from the Theory of Planned Behaviour [325]. This theory hypothesises that, alongside perceptions of control, intentions to perform behaviour account for considerable variance in realised actions. A meta-analysis of 94 studies together comprising 8461 participants, illustrated that intentions, identified by planning, enhanced the likelihood of goal achievement beyond motivational components alone [326]. In the context of infant feeding, it has been hypothesised that SE, OE and intentions are determinants of parental feeding behaviours [257].

SE describes an individual's belief that they are capable of organising and executing the actions required to manage a particular situation [327]. In the context of infant feeding, it describes parental confidence in their ability to monitor their child's feeding and growth and to overcome barriers to these activities such that their child gains weight appropriately (example item: I am confident that I can follow the new feeding recommendations, even if my baby cries between feeds). OE describes an individual's understanding of the probable outcome of following a particular course of action [327]. For example, the degree to which a parent expects their child to gain weight healthily if they follow feeding guidelines (example item: If I follow the new feeding recommendations, my baby's growth will be optimal). Intention describes how strongly a parent plans to follow feeding guidelines (example item: I intend to follow the new feeding recommendation).

Beyond the Baby Milk Trial, these constructs have not previously been applied to infant EB. This questionnaire is un-validated and has yet to be used in other studies. As such, the independence of the three theory-based attitudes that it measures had not previously been assessed. In order to explore the factor structure of the questionnaire and to determine whether three distinct attitudes could be identified in this sample, a factor analysis of the 11 items was performed. The analysis suggested the existence of one underlying factor with an eigenvalue >1.0 (eigenvalue= 4.12) (**Table 5.1**). Ten of the 11 items demonstrated strong, positive loadings onto this single factor (**Appendix A.12**). One item on the SE scale, (It would be difficult for me to follow the feeding recommendations if my partner and family do not support me), demonstrated a weak, negative loading onto this factor. The identified factor explained just 1% of the variance in the item (**Appendix A.12**).

Based on the findings of the factor analysis it was concluded that the questionnaire did not measure three distinct maternal attitude constructs, as hypothesised. Instead, the majority of the items (10/11) likely reflect a single construct. As a result, we generated a combined maternal attitudes score (MAS) by taking the mean of the 10 items that loaded positively and strongly onto the single identified factor. We excluded the 11^{th} item. Higher scores reflect more positive attitudes to following healthy infant feeding guidelines.

Cronbach's alpha for the 10 items comprising the MAS was 0.9, indicating high internal consistency.

Table 5.1 Factor analysis of the maternal attitudes questionnaire

Factor	Eigenvalue
Factor 1	4.12
Factor 2	0.99
Factor 3	0.41
Factor 4	0.14
Factor 5	0.07
Factor 6	-0.01
Factor 7	-0.12
Factor 8	-0.12
Factor 9	-0.15
Factor 10	-0.18
Factor 11	-0.20

5.3.2.3 The assessment of infant milk intake and body weight

Infant milk intake. Infant milk intake was measured using parent-completed questionnaires delivered at baseline, prior to intervention exposure, when infants were approximately 2 months old (mean age=2.3 months (SD=0.9)). Values for typical total daily milk consumption were calculated for each infant by summing the volume of: formula-milk, expressed breastmilk and milk from direct breastfeeds consumed over a typical 24 hour period. Formula-milk and expressed breastmilk intake were both assessed by multiplying the number of parent-reported feeds in 24 hours by the average parent-reported quantity consumed per feed. To calculate the quantity of milk consumed from direct breastfeeds, the following equation was applied, based on the estimation that infants aged under 7 months drink approximately 13.5ml/minute during breastfeeding [328]:

$$\text{(Number of feeds in 24 hours)} \times \text{(Average feed duration (mins)} \times 13.5\text{(ml/min))}$$

We excluded three mother-infant dyads from the milk intake analysis. The calculated values of total milk consumption for these infants were <300ml/day. These values were substantially lower than the lowest retained value (340.8ml/day) and were considered to be implausibly low.

Infant weight. Infant weight was measured at baseline by trained research assistants at a research clinic using standard operating procedures (see **Section 2.3.1**). Infants were weighed naked using a Seca Infant Electronic Scale to the nearest 0.01kg. Weight standard deviation scores (SDSs) were calculated using the WHO 2006 Growth Standard and adjusted for infant age and sex.

5.3.2.4 The association of infant EB and maternal attitudes to infant milk intake and body weight

Age and sex-adjusted linear regression models were used to test the associations between infant EBs and milk intake. Infant sex was tested as a modifier of this association with the intention of running sex stratified models if there was evidence of modification. The same model, replacing infant EB with the MAS, was used to investigate the association between the maternal attitudes and milk intake. The analyses were repeated using infant weight SDS as the outcome.

At the time of the baseline assessment, participants had not been exposed to the intervention. Thus the entire cohort was analysed together, without stratification or adjustment for intervention group. The residuals from the regressions were checked for normality to ensure that linear models were appropriate.

5.3.2.5 The analysis of effect modification

Effect modification is described in **Section 4.3.2.5**. Briefly, effect modification is said to occur when the relationship between an exposure and an outcome differs by levels of a third variable (the modifier). In this instance, the MAS was analysed as an effect modifier of the association between infant EB and both infant milk intake and body weight. If a main effect of both the infant EB and MAS on infant milk intake or body weight SDS was found, we tested the interaction between the EB and MAS in the following linear regression model:

$$\text{Milk intake or weight} \sim (\text{Infant EB} \times \text{MAS}) + \text{Infant EB} + \text{MAS} + \text{Infant sex} + \text{Infant age}$$

In order to interrogate the presence of differential effects between the groups, if an interaction was detected, the cohort was divided into tertiles according to MAS. The association between the infant EB and milk intake (or body weight SDS) was then tested separately within each tertile.

5.3.2.6 Sensitivity analysis

Sensitivity analyses were performed, repeating the main analysis with additional adjustment for maternal BMI, maternal age, maternal education level and maternal self-reported ethnicity.

5.4 Results

5.4.1 Characteristics of the study participants

A total of 669 mother-infant dyads were included in the analysis (**Table 5.2**). There was an approximately even split between female and male infants (n=308 female; 46%). The mean age of the mothers at the time of the baseline assessment was 31.6 years (SD=5.8) and the majority reported their ethnicity as white (n=617 (94% of 653 who reported their ethnicity)). FR, SR and MAS were each provided on a scale of 1-5. The mean milk intake of infants was 900.0ml/day (SD=214.4) and mean weight SDS, based on the WHO 2006 Growth Standard, was -0.11SDs (SD=0.9). All infants were receiving formula milk at baseline. A small minority were also receiving some breastmilk (n=87; 13%).

Table 5.2 Descriptive characteristics of the Baby Milk Trial participants (n=669)

		Total (n=669)
Infant characteristics		
Age (months)		2.3 (0.9)
Female		308 (46%)
Weight (kg)		5.5 (0.9)
Weight SDS[a]		-0.1 (0.9)
Infant EB[b]	Food responsiveness	2.1 (0.7)
	Satiety responsiveness	2.4 (0.7)
Milk intake (ml/day)		900.0 (214.4)
Receiving any breastmilk		87 (13%)
Maternal characteristics		
Attitudes score (MAS)[b]		3.4 (0.6)
Age (yrs)		31.6 (5.8)
White ethnicity		617 (94%)
Education group	Degree or higher	243 (38%)
	A-level/below degree	142 (22%)
	GCSE/vocational	246 (38%)
	Below GCSE	11 (2%)
BMI (kg/m^2)		27.9 (5.5)
Paternal characteristics		
BMI (kg/m^2)		28.0 (5.2)

Eating behaviour (EB); Body mass index (BMI); standard deviation score (SDS); Values are n (%) or mean (SD)
All information was collected at baseline, excluding infant EB, which was collected at 6 month follow-up
[a] Weight SDS was based on the WHO 2006 Growth Standard and adjusted for infant sex and age
[b] Infant EB traits and the MAS are on a 1-5 scale

5.4.2 Infant EB traits and the maternal attitudes score

The infant EB scores were negatively correlated with each other (Pearson's correlation coefficient=-0.28; p<0.0001). There was no association between either of the infant EB traits and the MAS (all $p \geq 0.05$) (**Table 5.3**).

Table 5.3 Associations between infant EB traits and the MAS

	Beta-MAS (95% CI)	p-value
Food responsiveness	-0.04 (-0.11, 0.02)	0.19
Satiety responsiveness	0.02 (-0.05, 0.02)	0.02

Maternal attitudes score (MAS)
Effect estimates and p-values are from the linear regression model: MAS ~ infant EB (FR or SR) + infant sex + infant age
Units of the effect estimate are change in MAS per 1 point increase in FR or SR

5.4.3 Infant EB traits and the maternal attitudes score to infant milk intake and body weight

Table 5.4 Associations between infant EB traits and the MAS with infant milk intake and body weight SDS

	Milk intake		Body weight SDS	
	Beta (95% CI) (ml/day)[a]	p-value	Beta (95% CI) (SDs)[b]	p-value
Infant EB				
Food responsiveness	42.6 (18.7, 66.5)	<0.001	0.26 (0.16, 0.35)	<0.001
Satiety responsiveness	-40.1 (-63.6, -16.6)	0.001	-0.18 (-0.27, -0.08)	<0.001
Maternal attitudes score	-68.4 (-96.6, -40.2)	<0.001	-0.13 (-0.25, -0.02)	0.03

Eating behaviour (EB); standard deviations (SDs); standard deviation score (SDS)
Effect estimates and p-values are from the linear regression model: [infant milk intake or weight SDS] ~ [infant EB or MAS] + infant sex + infant age
[a]Units of the effect estimate are change in milk intake (ml/day) per 1 point increase in FR, SR or the MAS
[b]Units of the effect estimate are change in infant weight SDS (SDs) per 1 point increase in FR, SR or the MAS

Both infant EB and the MAS were associated with infant milk intake and infant weight SDS in separate linear regression models, adjusted for infant age at baseline and infant sex (**Table 5.4**). Infant FR was positively associated with these outcomes, whilst infant SR and

the MAS were both negatively associated (all p<0.05). There was no evidence that infant sex modified the associations (**Appendix A.14** and **A.13**).

5.4.4 The analysis of effect modification

We tested the associations between the infant EB traits to infant milk intake and weight SDS for effect modification by the MAS. The results are reported separately for milk intake and body weight.

5.4.4.1 Infant milk intake

There was evidence of an interaction between infant FR and the MAS on infant milk intake (p = 0.049) (**Table 5.5**). When the cohort was split into tertiles according to the MAS, the association between FR and milk intake was only significant amongst infants whose mothers were members of the lowest MAS tertile (Beta = 66.8ml/day per unit increase in FR (95% CI: 17.8, 115.8); p = 0.01). In the middle and highest MAS tertiles, there was no significant association between infant FR and infant milk intake (Middle tertile: Beta = 24.1ml/day (95% CI: -12.5, 60.7); p = 0.20; Highest tertile: Beta = 36.0ml/day (95% CI: -4.0, 76.1); p = 0.08) (**Figure 5.1**; **Appendix A.15**). There was no evidence of an interaction between infant SR and the MAS on infant milk intake (p = 0.28) (**Table 5.5**).

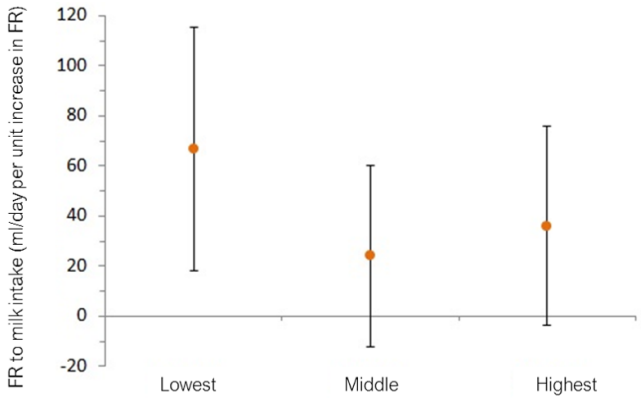


Figure 5.1 The association between infant food responsiveness and infant milk intake by tertiles of the MAS. The association between infant FR and infant milk intake within tertiles of the MAS is plotted on the y-axis. The tertiles of the MAS are shown on the x-axis. The graph shows attenuation of the FR to milk intake association with higher MAS. Effect estimates are ml/day per 1 point increase in FR and are displayed with 95% CIs.

Table 5.5 The interaction between infant EB traits and the MAS in the determination of infant milk intake

	β-infant EB(^{a}) (95% CI)	p-value	β-MAS(^{b}) (95% CI)	p-value	β-interaction(^{c}) (95% CI)	p-value
Food responsiveness						
MAS	174.7 (35.3, 314.2)	0.01	11.9 (-78.3, 102.0)	0.80	-40.1 (-80.1, -0.13)	0.049
Satiety responsiveness						
MAS	-113.9 (-251.5, 23.6)	0.10	-124.9 (-220.3, -29.4)	0.01	21.3 (-17.4, 60.1)	0.28

Eating behaviour (EB); maternal attitudes score (MAS)

Effect estimates and p-values are from the regression: Infant milk intake ~ (Infant EB × MAS) + infant EB + MAS + infant sex + infant age. The units are change in infant milk intake (ml/day) per 1 point increase in ^{a}infant EB, ^{b}the MAS or ^{c}the combined effect of infant EB and MAS

5.4.4.2 Infant body weight SDS

There was evidence of an interaction between infant SR and the MAS on infant weight SDS (p = 0.01) (**Table 5.6**). When the cohort was split into tertiles based on the MAS, the association between infant SR and weight SDS was strongest amongst infants whose mother's MAS scores were in the lowest tertile (Beta = -0.28 SDs/unit increase in infant SR (95% CI: -0.47, -0.10); p=0.003) and weakest in the highest tertile (Beta = -0.02 SDs (95% CI: -0.19, 0.14); p=0.77) (**Figure 5.2**; **Appendix A.16**;). There was no interaction between infant FR and the MAS on infant body weight SDS (p = 0.13) (**Table 5.6**).

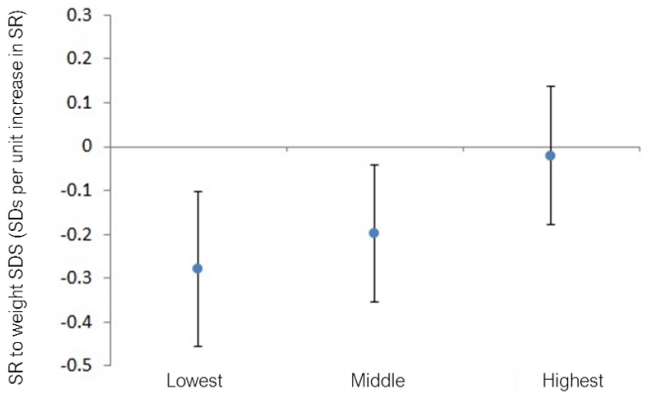


Figure 5.2 The association between infant satiety responsiveness and infant weight SDS by tertiles of the MAS. The association between infant SR and infant weight SDS within tertiles of the MAS is plotted on the y-axis. The tertiles of the MAS are shown on the x-axis. The graph shows attenuation of the SR to weight SDS association by higher MAS. Effect estimates are SDs of infant weight per 1 point increase in SR and are displayed with 95% CIs.

Table 5.6 The interaction between infant EB traits and the MAS in the determination of infant weight SDS

	β-infant EB[a] (95% CI)	p-value	β-MAS[b] (95% CI)	p-value	β-interaction[c] (95% CI)	p-value
Food responsiveness						
MAS	0.69 (0.11, 1.27)	0.02	0.17 (-0.21, 0.54)	0.38	-0.13 (-0.29, 0.04)	0.13
Satiety responsiveness						
MAS	-0.94 (-0.15, -0.37)	<0.01	-0.64 (-1.04, -0.23)	<0.01	0.22 (0.06, 0.38)	0.01

Eating behaviour (EB); maternal attitudes score (MAS)

Effect estimates and p-values are from the regression: Infant weight SDS ~ (Infant EB × MAS) + infant EB + MAS + infant sex + infant age. The units are change in infant weight SDS (SDs) per 1 point increase in [a]infant EB, [b]the MAS or [c]the combined effect of infant EB and MAS

5.4.5 Sensitivity analyses

The associations between the infant EBs and MAS with infant milk intake and infant body weight SDS were not substantively altered after adjustment for maternal BMI, maternal age, maternal education and maternal ethnicity. All of the associations remained directionally consistent and statistically significant following the addition of these variables to the models (**Appendix A.17**). The adjustments did not substantively alter the results of the interaction analyses. The MAS×SR interaction term remained statistically significant in the infant SR to infant weight SDS regression (p=0.01), although the FR×MAS interaction no longer reached statistical significance in the infant FR to infant milk regression (p=0.07) (**Appendix A.18**).

5.5 Discussion

5.5.1 Summary and context of the main findings

Amongst 669 mother-infant dyads enrolled in the Baby Milk Trial, infant EB traits (FR and SR) and maternal attitudes to following healthy infant feeding guidelines were separately associated with both infant milk intake and body weight during the period of exclusive milk-feeding. Whilst infant SR and maternal attitudes were negatively associated with both outcomes, FR was positively associated. Beyond their separate associations, there was evidence of an interaction between infant EB traits and maternal attitudes. Specifically, more positive maternal attitudes towards following healthy infant feeding guidelines attenuated the positive association between infant FR and infant milk intake, such that the magnitude of the positive association between FR and milk intake was greatest in the lowest tertile of the maternal attitudes score and was non-significant in both the middle and highest tertiles. Positive maternal attitudes also attenuated the negative association between infant SR and infant body weight, such that the magnitude of the negative association between SR and infant body weight was highest in the lowest tertile of the maternal attitudes score and lowest in the highest tertile.

Together, the findings highlight maternal attitudes to following healthy infant feeding guidelines as a potential novel determinant of infant weight and milk intake with the ability to modify the impact of infant EB traits on these outcomes. As such, these attitudes represent a possible target for interventions designed to promote healthy infant weight.

Recent data from the UK in 2017 indicates that 75% of children aged 0-12 months consume more than the average estimated energy requirement and weigh above the median WHO growth standard [329]. Given the known association between infant weight gain and obesity, this raises concern about lifetime obesity risk [141, 165]. Mounting evidence suggests that over-feeding can be driven either by infants or their caregivers [180]. For example, formula-fed infants typically exhibit more rapid weight gain trajectories than their breastfed counterparts [330, 331]. This is thought to be driven, in part, by parent-led over-feeding, including the encouragement to empty bottles [332]. In addition, trial evidence suggests that allowing infants to control their intake through encouraging self-feeding during weaning does not aid the development of self-regulation but rather leads to infant-driven over-feeding [180]. Insufficient weight gain during infancy can also result from either infant or parent-driven factors and weight faltering can adversely impact immunity, linear growth, final height and cognitive development [333–336].

In light of evidence that consumption and growth depend on both parent and infant driven factors, infant feeding outcomes are increasingly considered the joint responsibility of infants and their caregivers. Specifically, infants are responsible for detecting and accurately signalling feelings of hunger and satiety whilst caregivers are responsible for interpreting

and responding appropriately to these signals (**Chapter 1**) [173, 182]. Our results support this assertion. Consistent with previous research, we report positive associations between infant's drive to eat (FR) and both milk intake and weight SDS, whilst infant's sensitivity to feelings of fullness (SR) demonstrated negative associations with both outcomes [141–143]. Further, we demonstrated a negative association between maternal attitudes to following healthy infant feeding guidelines and these outcomes for the first time. This supports experimental research suggesting that parental behaviours can limit infant food intake and weight gain [183, 337]. Alongside their separate effects, the results demonstrated an interplay between parents and infants in determining food intakes and body weight for the first time. The finding that both obesity-increasing (FR) and decreasing (SR) infant EB traits are modified by maternal attitudes is consistent with evidence that interventions to support healthy feeding interactions prevent both infant weight faltering and infant overweight [333].

Corroborating the findings reported in **Chapter 4**, the results of this study demonstrate that the determinants of obesity interact. Both investigations illustrate that influences within conscious control, that promote the monitoring and regulation of energy intake (CR and maternal attitudes to feeding guidelines, respectively), can attenuate the impact of variables that are either unmodifiable (genetic susceptibility to obesity) or cannot be modified by existing interventions (infant FR and SR) on body weight.

5.5.2 Strengths and limitations

This study draws on the detailed assessment of infant milk intakes, weight and EB from the Baby Milk Trial. Infant weight was measured by trained researchers, using established protocols, and infant EB traits were measured using a widely used and well-validated questionnaire. The study also applies a recently developed instrument to assess maternal attitudes to following infant feeding guidelines for the first time and illustrates the importance of these attitudes to infant weight and milk intake.

There were several limitations. The study population was limited to formula-fed infants. Whilst formula feeding is common practice both in the UK and globally and facilitated more accurate quantification of milk intake in this analysis [338], the results may not be generalisable to infants who are breastfed. Further, the associations and interactions reported here are cross-sectional. We have assumed that infant EBs and maternal attitudes influence infant milk intake and weight SDS with minimal reverse causality. Robust evidence supports a causal impact of infant EBs on weight and consumption [141]. However, it is possible that infant weight and milk intake influenced the maternal attitudes. The lack of an association between maternal attitudes and infant EB in this sample supports the notion that maternal attitudes may be independent of infant factors at this time point. However, longitudinal analyses are required to provide clarity in this regard.

There are also limitations to the measurement of infant EB and maternal attitudes. Infant EB was measured at the 6 month follow-up assessment but referred to EB in the first 3 months, during the period of exclusive milk feeding. The mothers filled in a retrospective version of the BEBQ, and the importance of referring exclusively to the first 3 months of life was stressed. The questionnaire is validated and used widely amongst infants, even beyond 6 months of age, and indeed was developed amongst 8 month old infants [39]. However, it is possible that subsequent infant EB and experiences at 6 months may have impacted maternal responses. The maternal attitudes questionnaire has not been validated against realised feeding behaviour. The associations of the maternal attitudes score to infant milk intake and weight in the anticipated directions is reassuring in this regard. However, these associations require replication and the aspects of feeding behaviour impacted by the attitudes should be explored. Further, the questionnaire items were found to represent a single underlying construct. Future research is needed to measure and understand the separate implications of SE, OE and intention with regards to infant feeding.

A final note pertains to the recruitment of mother-infant pairs to the study. As mothers were responsible for completing the questionnaires, the study was only able to examine maternal attitudes. In all instances the mothers were the primary caregivers and this is generally representative of infants in the UK at age 2 months, when paternity leave has typically ended. We anticipate that paternal attitudes, and the attitudes of other caregivers, would also influence infant feeding and body weight where responsibilities are shared or mothers are not the primary caregivers.

5.5.3 Conclusions

For the first time, this study demonstrated an interaction between maternal and infant factors in the determination of infant milk intake and body weight. The findings showed that modifiable maternal attitudes to following healthy infant feeding guidelines are associated with lower milk intake and weight. Further, in the same way that CR modified the impact of genetic susceptibility to obesity on BMI in **Chapter 4**, maternal attitudes modified the impact of infant EB traits on both milk intake and weight. Interventions designed to promote positive maternal attitudes to following healthy infant feeding guidelines may support healthy infant weight and intake amongst formula-fed infants during a critical period of development for the determination of lifetime obesity risk. The findings support the results of **Chapter 4**, demonstrating interactions between determinants of obesity and highlighting promising intervention targets that may influence the impact of known but, as yet, unmodifiable determinants of obesity.

CHAPTER 6

THE GENETIC DETERMINANTS OF RISK-TAKING PROPENSITY AND SHARED PATHWAYS WITH BODY MASS INDEX

Publications

Clifton, E.A.D., Perry, J.R.B., Imamura, F., Lotta, L.A., Brage, S., Forouhi, N.G., Griffin, S.J., Wareham, N.J., Ong, K.K., and Day, F.R. (2018). Genome–wide association study for risk-taking propensity indicates shared pathways with body mass index. *Communications Biology*. https://doi.org/10.1038/s42003-018-0042-6. [339].

Clifton, E.A.D., Ong, K.K., Day, F.R. (2018). We uncovered the genetic basis of risk-taking and found it's linked to obesity and mental illness. *The Conversation*

Contributions

I planned this project and devised the analysis plan in collaboration with my supervisors. I generated the risk-GRS, jointly conducted the statistical analyses and interpreted the results with my supervisors. I wrote this chapter and the resulting manuscript.

6.1 Summary

Previous studies have linked risk-taking to both obesity and eating behaviour (EB). However, the direction of causality and mechanisms of this association are not yet understood. **Chapters 4** and **5** indicate that separate behavioural phenotypes are linked to the aetiology of obesity in different ways and that a better understanding of these pathways can highlight potential targets for obesity prevention. Here, a genetic approach was used to investigate the likely causal association between risk-taking, EB and BMI. A GWAS of risk-taking propensity was conducted amongst 436,236 white European participants enrolled in the UK Biobank (UKB) study. Genome-wide associations were identified at 26 loci ($p < 5 \times 10^{-8}$), 24 of which were novel. These loci implicated genes exhibiting enriched expression in the GABA and GABA receptor pathways. Modelling the effect of risk-taking on BMI using Mendelian Randomisation (MR) indicated a positive effect (0.25 approximate SDs of BMI (SE: 0.06); $p < 7 \times 10^{-5}$), whilst a reverse MR indicated no effect of BMI on risk-taking. Within the MR of risk-taking to BMI, the impact of individual risk-associated SNPs was highly heterogeneous. This suggests a complex relationship between the traits, arising from multiple shared pathways as opposed to a single causal mechanism. Positive genetic correlations were identified between risk-taking and WHR, childhood obesity, ever smoking, attention-deficit hyperactivity disorder (ADHD), bipolar disorder (BPD) and schizophrenia, alongside a negative genetic correlation with age at first birth amongst women. A genetic risk score for risk-taking (risk-GRS) showed positive associations with EE amongst men in the Fenland study cohort. Together, these findings confirm the utility of GWAS in exploring the relationship between behaviour and obesity and suggest that the behavioural pathways involved in risk-taking propensity may play a role in obesity, smoking and psychiatric disorders.

6.2 Background

Risk-taking propensity describes a tendency to engage in reward-seeking actions despite the possibility of negative consequences [205]. Whilst risk-taking typically peaks during adolescence, inter-individual differences demonstrate longitudinal stability and risk-taking propensity is considered a stable trait, representing an established risk factor for a range of health-related behaviours including smoking, alcohol use and binge-eating [240, 340–342, 206, 343]. Together **Chapters 4** and **5** indicate that behavioural pathways are involved in the aetiology of obesity. Increasingly, research suggests a specific association between risk-taking and BMI [203, 204].

Cross-sectional associations between risk-taking and obesity have been reported across a range of experimental and observational studies. Amongst 121 participants, overweight and obese men took more risks in a laboratory-based gambling task and obese women exhibited higher impulsivity, relative to those of normal weight [203]. In addition, compared to their normal weight peers, adolescents with a BMI above the 99th percentile for their age and sex exhibit greater odds of a range of risk-taking behaviours, including smoking and having used drugs or alcohol before their last sexual encounter [222]. Other findings suggest that obese individuals are more likely to neglect long-term outcomes in decision-making, making them more prone to impulsive actions [207].

Whilst studies suggest an association between risk-taking propensity and obesity, the direction of causality and potential mechanisms of this association, including EB, require further investigation. Aspects of impulsivity, a trait closely linked to risk-taking, have been consistently associated with measures of dietary and eating-related behaviour linked to over-eating, including snacking [234, 235]. In particular, attentional impulsivity (the inability to stay focused) has been positively associated with measures of the salience of external food cues, such as the pleasantness of high-calorie foods, perceptions of hunger, disinhibition and external eating [236, 237]. It has been hypothesised that high attentional impulsivity might increase susceptibility to palatable food cues, inducing over-eating and leading to weight gain over time [238]. However, the observation of ADHD-like symptoms in the majority (~ 80%) of homozygous carriers of *MC4R* mutations, who suffer early-onset severe obesity, suggests the possibility of reverse causality or shared pathways [239]. One approach to exploring the causal relationships between risk-taking, EB and BMI is MR using genetic variants associated with risk-taking as instrumental variables.

Heritability estimates from twin studies of risk-taking, using both experimental and self-report measures, range between 0-55%, indicating that it may be possible to study risk-taking from a genetic perspective [240, 241]. Further, gene discovery studies of risk-related behaviours have been reported. Among 125,667 adults enrolled in UKB, 38 loci were identified for age at first sexual intercourse [344]. One SNP identified in this analysis is intronic to *CADM2* (rs57401290) and has subsequently been associated with risk-taking assessed

by the question: *Overall, do you feel comfortable or uncomfortable taking risks?* in an independent sample of 140,487 participants from 23andMe using a phenome-scan for associations between *CADM2* and a range of personality traits (rs1865251; correlation (r^2) with rs57401290 = 0.78) [243]. A GWAS of risk-taking propensity has been conducted among 116,225 UKB participants based on the question: *Would you describe yourself as someone who takes risks?*. The study identified two genome-wide significant loci, one within *CADM2* and the other within the human leukocyte antigen (HLA) region on chromosome 6. Genetic correlations between risk-taking and schizophrenia, BPD, ADHD, post-traumatic stress disorder, smoking and obesity were reported [242].

To identify genetic variants robustly associated with risk-taking propensity, we performed the largest GWAS to-date amongst 436,236 white Europeans from UKB. The findings were linked to other genome-wide results for obesity and other outcomes, as well as for gene expression. A risk-GRS was used to examine associations with EB, food-related behaviour and dietary intake.

6.3 Participants and methods

6.3.1 Participants

6.3.1.1 UK Biobank

Genome-wide genotype and risk-taking data from 436,236 white European participants aged 40-69 years in UKB was included in this analysis. Further details of UKB, including the recruitment and genotyping methods are provided in greater detail in **Chapter 2**.

6.3.1.2 The Fenland study

The Fenland study population of the present analysis comprised up to 11,441 individuals (52% women) aged 30-64 years with complete genome-wide genotype, dietary intake, food-related behaviour and EB data. For a full description of the Fenland cohort, including recruitment see **Chapter 2**.

6.3.2 Methods

6.3.2.1 The assessment of risk-taking propensity in UKB

As part of their baseline assessment, UKB participants completed a touchscreen questionnaire including the question: *Would you describe yourself as someone who takes risks?*. Possible responses were: Yes, No, Don't know or Prefer not to answer. A total of 482,173 participants responded either Yes (n=129,877; 27%) or No (n=352,296; 73%). Those who answered Don't know or Prefer not to answer (n=19,538) were excluded from this analysis.

The same question was posed again to a sub-set of the participants during follow-up assessments. The baseline assessments took place between 2006−2010, the first and second repeat assessments were taken from 2012−2013 and 2014 onwards, respectively. As the sample size substantially decreased between follow-ups, the baseline responses of all participants were used in the primary GWAS analysis.

6.3.2.2 The assessment of eating behaviour in the Fenland study

Emotional eating (EE), uncontrolled eating (UE) and cognitive restraint (CR) were measured at baseline using the TFEQ-R18 (**Appendix C.1**) [40]. These EB traits and the scoring of the TFEQ, are described in more detail in **Chapter 1**. As in **Chapters 4** and **7** the EB trait scores were scaled to give a score between 1 and 100 [89].

A total of 3515 participants (53% women) aged 35–64 years with intersecting EB and genotype data were included in the analysis. The EB analyses were sex-stratified based on evidence that EE, UE and CR all significantly higher amongst women. In this sample, the *p*-value for the difference between men and women were $p<0.0001$ for UE and EE and $p<0.01$ for CR. Further, the findings of **Chapter 4** suggest that sex may modify the association between BMI-associated loci and CR.

6.3.2.3 The assessment of food-related behaviour in the Fenland study

Food-related behaviour was measured as part of the baseline general questionnaire administered to Fenland participants. To assess snacking while watching television, participants answered: *Apart from meals, how often do you snack on foods while watching television?*. Possible answers were: Never or rarely, Occasionally, Usually, Always. To assess frequency of eating home-cooked meals, participants answered the question: *When you eat your main meal at home, how often do you usually eat home-cooked meals?*. Possible answers were: Never or less than once a month, 1–2 times/week, 3–5 times/week, 5+ times/week. Finally, to assess the frequency with which participants typically eat breakfast, participants answered: *How often do you usually eat breakfast?*. Possible answers were: Never or less than once a month, 1–2 times/week, 3–5 times/week, 5+ times/week.

As the food-related behaviour groups were not continuous and their distributions were markedly non-normal with the majority of participants selecting the most healthy response available, we coded the variables into binary variables for analysis in logistic regression models. In general, 0 represented the more healthy response and included all participants who selected the most healthy option, and 1 indicated the less healthy response and included participants who selected the remaining options (**Table 6.1**). For frequency of eating breakfast, the coding was reversed, such that 0 represented those who rarely skip breakfast and 1 represented those who regularly skip breakfast.

A total of up to 11,441 participants (53%) women, aged 30-64 years had intersecting food-related behaviour and genetic data and were included in this analysis.

Table 6.1 Coding of food-related behaviours in the Fenland study

	Coded 0	Coded 1
Home-cooked food	≥ 5 times/week	< 5 times/week
Snacking in front of the TV	Never or rarely	Occasionally, usually or always
Frequency of skipping breakfast	< 2 times/week	≥ 2 times/week

6.3.2.4 The assessment of diet in the Fenland study

Habitual daily calorie, fat, protein, carbohydrate, fruit, vegetable and fibre intakes were measured using the food frequency questionnaire (FFQ) completed by Fenland participants at baseline. The FFQ is a validated 130-item semi-quantitative questionnaire that records habitual, self-reported intake over the previous year. Food intake frequency was converted to daily energy (kcal/day) and nutrient intakes (g/day) using FETA 2.53 software [345]. A total of 8981 participants (53% women) aged 30–64 years had intersecting genotype and dietary data and were included in the analysis.

6.3.2.5 Genotyping, imputation and quality control

The 2017 imputed genetic data, based on the Haplotype Reference Consortium (HRC) panel release from UKB, comprising 7,736,308 million SNPs, was used in the GWAS analysis of risk-taking propensity. Genotyping, imputation, phasing and quality control (QC) are described in greater detail in **Chapter 2**. Briefly, 487,409 of the UKB participants were genotyped using the Affymetrix Applied Biosystems UK Axiom array (Santa Clara, CA, USA), designed to optimise imputation performance in GWAS studies. A small number (n=49,950) were genotyped using the Affymetrix Applied Biosystems UL BiLEVE Axiom Array [255]. These arrays share 95% of their marker content [256]. SNPs were excluded prior to imputation if they were multi-allelic, had missing data or had a minor allele frequency (MAF) of < 1%. Phasing was performed using a modified version of the SHAPEIT2 algorithm. Imputation was performed using IMPUTE 2 and a merged reference panel comprised of the 1000 Genomes Project Phase 3 and UK10K haplotype reference panels. In addition to UKB QC procedures, we defined a white European ancestry set based on *k*-means clustering using the first 5 genetic principle components (PCs). Individuals who genetically appeared to be white European but did not specify their ethnicity were included in the analysis. However, all those who specifically self-identified as non-white European were excluded, regardless of genetic information.

6.3.2.6 Genome-wide association study

Main analysis. GWAS are used to identify individual genetic variants associated with a trait of interest. Participants are genotyped at points of the genome that commonly show inter-individual variation (i.e. where the less common allele occurs at a frequency of >1% of the population). The association between all of these genotyped points and the trait of interest are then evaluated. Due to the large number of tests being performed, a *p*-value of 5×10^{-8} is conventionally used as the threshold for significance in GWAS.

In this analysis, the top 10 PCs were significantly, but minimally, associated with the odds of risk-taking, indicating the presence of population substructure. This is shown in **Ap-**

pendix A.19. GWAS testing for associations between SNPs and self-reported risk-taking was performed using a linear mixed model (LMM) implemented in BOLT-LMM [346]. This approach minimises the effect of population structure by adjusting for the top 10 PCs identified and any additional substructure, as well as permitting the inclusion of related individuals in the analysis, thus maximising statistical power.

Loci were established through distance-based clumping, using a distance of 1Mb. Sex, age and genotyping array were included as covariates. SNPs were filtered based on an imputation information quality (info) of > 0.5 and MAF > 1%. Individuals were excluded based on ancestry, withdrawal from the UKB study, sex mismatch or failure of genetic QC. A total of 436,236 individuals of white European ancestry and 7,736,308 variants were included.

Heritability analyses were performed using restricted maximum likelihood implemented in BOLT-LMM, which computes heritability on the observed scale [346]. Genetic variance was calculated for all genotyped autosomal SNPs for which QC was performed, adjusting for chip status, age, sex and the top 10 genetically determined PCs (n=612,622). Only unrelated individuals of white European ancestry were included in the heritability analysis (n=339,414).

The assessment of repeated measures of risk-taking in UKB. We assessed the stability of the measure of risk-taking used in UKB by comparing participant's responses at baseline to those taken during the first and second follow-up assessments. We report the percentage of participant's who recorded consistent responses, alongside a *p*-value calculated using repeat measures ANOVA.

Quasi-replication. In the absence of an appropriate data set in which to directly replicate our risk-taking results, we conducted a quasi-replication using a closely related phenotype, *Ever smoking*. Given lack of access to an independent data set in which to conduct suitably powered analyses, this was conducted in the same European ancestry UKB sample and was used to look up our genome-wide significant SNPs for risk-taking. The UKB sample of the *Ever smoking* analysis comprised 450,406 individuals (207,229 ever smokers (46%) and 243,177 never smokers). *Ever smoking* was considered an appropriate phenotype for quasi-replication as a result of its known associations to measures of risk-taking. Across a number of studies, smoking status has been shown to predict other risk-taking behaviours, including seat-belt wearing, speeding and risky sexual activity [347, 348].

6.3.2.7 Pathway and tissue enrichment analysis

MAGENTA was used to implement a gene set enrichment analysis-based approach testing the genome-wide discovery data for associations with biological pathways defined in GoTerm, PANTHER, KEGG, Biocarta, Reactome and Ingenuity. MAGENTA maps each

gene in the genome to a single index SNP with the lowest p-value within a window ranging from 110kb upstream to 40kb downstream of the gene. This p-value, representing a gene score, is then corrected in a regression model for confounding factors such as gene size, SNP density and linkage disequilibrium (LD)-related properties. Each mapped gene in the genome is then ranked by its adjusted gene score. The observed number of gene scores in a given pathway with a ranked score above the 75^{th} percentile threshold is calculated. This observed statistic is then compared to one calculated from randomly permuted pathways of an identical size. The comparison generates an empirical Gene Set Enrichment Analysis (GSEA) p-value for the pathway. An individual pathway was defined as being significantly enriched when it reached a false discovery rate (FDR) < 0.05 in either analysis.

Tissue enrichment analysis was performed using the genotype-tissue expression (GTEx) database [349]. This approach uses stratified LD score regression, a method for partitioning heritability from GWAS summary statistics, to test whether trait heritability is enriched in regions surrounding genes with the highest specific expression in a given tissue [350]. Significance thresholds were established using a Bonferroni correction for the number of tests performed.

6.3.2.8 The analysis of genetic correlations

Linkage disequilibrium (LD) score regression was used to identify genetic correlations (r_g) between risk-taking and 12 traits of interest [351]. These traits were defined *a prioi* and comprised a range of adiposity-associated phenotypes, risk-related behaviours and psychiatric disorders. Genetic information regarding these traits was accessed through publicly available databases. The traits comprised: BMI, WHR, childhood obesity, birth weight, type 2 diabetes, age at first birth, ever smoking, years of schooling, anorexia nervosa, ADHD, BPD and schizophrenia.

6.3.2.9 Mendelian randomisation analysis of risk-taking and BMI

When observational studies consistently report an association between two variables, such that the finding is unlikely to be spurious, causality is just one potential explanation. The association may also result either from confounding or reverse causation. Confounding describes a situation whereby the association between an exposure and an outcome is explained by a third, extraneous variable. This variable, the confounder, is associated with both the exposure and the outcome but does not lie on the causal pathway between them [28]. Reverse causality describes a situation whereby the variable assumed to be the exposure is, in reality, the outcome [28].

(a) Confounding **(b)** Reverse causality

Figure 6.1 Confounding and reverse causality. The diagram illustrates the concepts of confounding and reverse casuality. **(a)** depicts confounding whereby the association between an exposure and an outcome is explained, fully or in part, by the presence of a third variable known as the confounder. The confounder is associated with both the exposure and the outcome but does not lie on the causal pathway. **(b)** depicts reverse causality whereby the hypothesised outcome variable exerts an influence on the assumed exposure.

Randomised controlled trials (RCTs) can be used to help overcome these sources of bias. Individuals are randomly assigned to either an intervention or control arm, a process which diminishes the potential for unmeasured confounding to bias the results. Delivery of the exposure subsequent to randomisation helps to eliminate reverse causality. However, RCTs are not feasible or ethical for all exposures. MR is conceptualised as a natural RCT whereby genotype is used as a proxy for levels of an exposure. As illustrated in **Figure 6.2**, MR analyses mirror RCTs in several important ways. First, alleles are sorted independently such that the inheritance of one trait is independent of others, controlling for confounding. Second, genotype is fixed at conception, eliminating the potential for reverse causality.

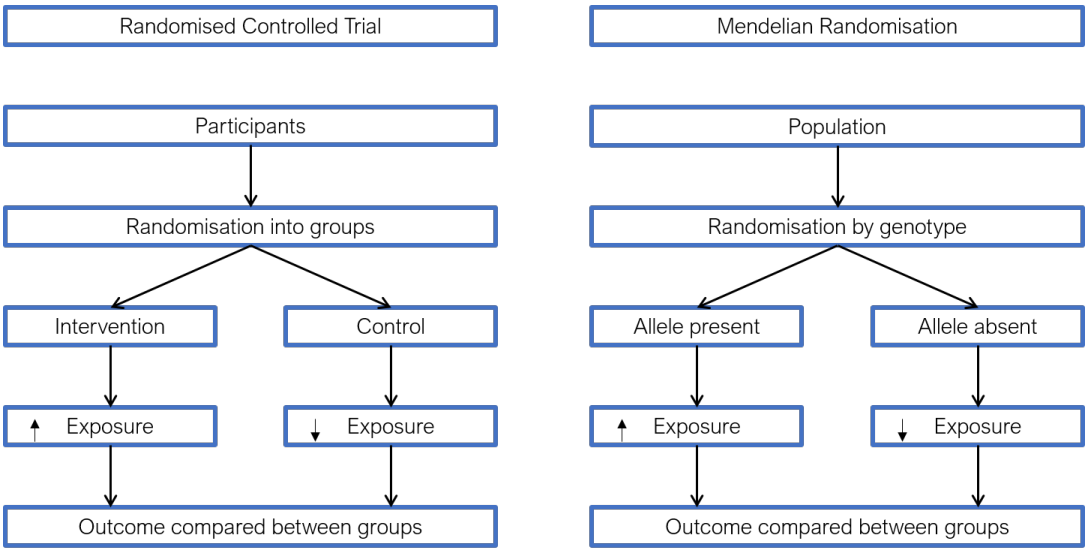


Figure 6.2 Comparison of Mendelian randomisation and Randomised controlled trials. Adapted from Burgess et al, 2012 [352]. The diagram compares MR analyses to the design of RCTs.

Conventional inverse variance weighted MR. In this study, we conducted a bi-directional MR analysis of risk-taking to BMI using all genome-wide significant variants for risk-taking from the present GWAS. An unpublished GWAS meta-analysis of BMI using UKB plus GIANT

data and comprising a total of 772,825 individuals provided effect estimates for BMI. For the risk-taking to BMI analyses, SNPs were aligned to the risk-increasing allele. For the BMI to risk-taking MR, SNPs were aligned to the BMI-increasing allele. We first used conventional inverse variance weighted (IVW) MR. This analysis performs a linear regression of the genetic associations with the exposure on the genetic associations with the outcome of interest, weighting by the inverse-variance of the genetic associations with the outcome. This ensures that effect estimates with higher precision carry more weight in the overall regression, regardless of effect size.

In order to be valid, the genetic instruments used to estimate MR exposures must meet the following instrumental variable assumptions [353]. These are depicted in **Figure 6.3**.

- (1) They must be robustly associated with the exposure.
- (2) They must not be associated with confounders of the association between the exposure and the outcome.
- (3) They must only be associated with the outcome through the exposure.

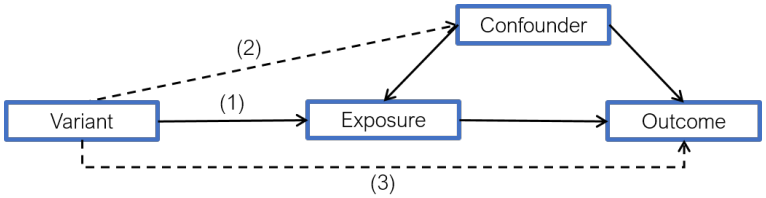


Figure 6.3 Assumptions of Mendelian randomisation. Adapted from Bowden et al, 2016 [354]. The assumptions of MR are indicated by the numbers and correspond to the list above. (1) depicts the robust associations assumed between the genetic variant and the exposure; (2) represents an association between the variant and a confounder, which invalidates the variant and (3) represents associations between the variant and the outcome, which invalidates the variant.

Conventional IVW MR assumes that all genetic variants included in the analysis are valid instruments on the basis of these assumptions, thus that they predict the exposure with precision and do not exert an influence on the outcome through pathways that are not under investigation in the MR analysis. Only assumption (1) can be tested directly. Horizontal pleiotropy can affect MR when variants used to model the exposure influence the outcome variable through biological pathways that are independent of the exposure. If the combination of these pleiotropic effects is directional (i.e. it has a mean that differs from 0), the IVW MR estimate will be biased. Thus, two pleiotropy-robust MR analyses, MR Egger and weighted median MR, were performed to detect violations of, and conduct analyses robust to, assumptions (2) and (3).

MR Egger. The MR Egger method is similar to that of conventional IVW MR but provides a quantitative estimate of directional pleiotropy and an effect estimate which accounts for its

presence [355]. In contrast to conventional IVW MR, in MR Egger analyses, the regression is not constrained to pass through the origin. In the absence of directional pleiotropy, when the gene-exposure association is 0, the gene-outcome association should also be 0 and the y-intercept should pass through the origin. Departure of the y-intercept from 0 indicates directional pleiotropy and quantifies its presence [355]. The MR Egger effect estimate is provided by the slope of the regression and is robust even in the event that all variants used to model the exposure impact the outcome through pleiotropic pathways [355]. However, MR Egger relies on the assumption that the variant-exposure association is independent of the direct effects of variants on the outcome. This is known as the InSIDE (Instrument Strength Independent of Direct Effect) assumption. The drawback of this method is low statistical power, susceptibility to weak instruments (which tend to bias results toward the null) and the inability to test the InSIDE assumption [356].

Weighted median MR. Weighted median MR complements MR Egger. In this method, the MR estimates (the ratio of the gene-outcome to gene-exposure ratios) are ordered by magnitude and weighted by the inverse of the variance of the ratio estimate [354]. To account for unbalanced heterogeneity, the contribution of genetic variants with heterogeneous ratio estimates is then down-weighted and the median estimate is taken [354]. Unlike MR Egger which allows all variants to have pleiotropic effects, the weighted median method requires a minimum of 50% of variants to be valid. However, it is more robust to violation of the InSIDE assumption and allows for variants that are invalid as a result of violations to any of the instrumental variable assumptions depicted in **Figure 6.3**. This method also provides greater precision than MR Egger if all genetic variants have similar magnitudes of association with the exposure [354, 356].

Leave-one-out analyses. Finally, to identify specific SNPs associated with risk-taking or BMI that might drive overall effects evident in the MR analysis, a *leave-one-out* analysis was planned. The MR of risk-taking to BMI was repeated with each of the genome-wide significant SNPs for risk-taking removed, in turn.

It is important to note that MR is also limited by factors beyond pleiotropy that cannot be controlled for but should be considered. Canalisation and compensation might mitigate the effects of genetic changes on outcomes. Further, complexity in the biology of exposures may make causal inferences about the dimensions of a trait that are important to an outcome difficult to infer without biological knowledge [356].

All MR analyses were conducted in R version 3.3.1.

6.3.2.10 The analysis of the genetic risk score for risk-taking

To characterise the effect of risk-taking propensity on EB traits and food-related behaviour, a weighted risk-GRS was constructed amongst Fenland participants (n=11,249) using the

summary statistics from the present GWAS for weighting. The 26 SNPs showing genome-wide significant associations with risk-taking in this analysis were included in the score. The score was constructed in the same way as the BMI-GRS in **Chapters 3** and **7**. Briefly, at each SNP, the number of risk-increasing alleles (0, 1 or 2) was multiplied by the effect estimate for the risk-increasing allele from this GWAS. The products across all 26 risk-associated SNPs were then summed for each participant.

The association between the risk-GRS and the EB traits was examined in Fenland using sex-stratified, age-adjusted linear regression models. The association between the risk-GRS and both the dietary and food-related behaviour variables was analysed in Fenland using age and sex-adjusted linear or logistic regression models, as appropriate. Outcome variables were log-transformed if they were not normally distributed, in order to improve the normality of the residuals. The following 12 traits were analysed using the risk-GRS: EE, UE, CR, total calorie intake per day (kcal/day), fat intake (g/day), fibre intake (g/day), protein intake (g/day), carbohydrate intake (g/day), fruit and vegetable intake (g/day), snacking while watching TV, frequency of skipping breakfast (times/week) and number of home cooked meals (times/week). The analysis was conducted in Stata version 14 (StataCorp LCC, College Station, TX).

6.4 Results

6.4.1 Characteristics of the study participants

The GWAS sample comprised 436,236 UKB participants of white European ancestry (235,954 women; 54%). Of these, 113,882 (26%) responded Yes and 322,354 (74%) responded No to the question: *Would you describe yourself as someone who takes risks?*. The mean age of participants at enrolment was 56.8 years (SD=8.0).

Table 6.2 Descriptive characteristics of UKB participants by answer to the question: *Would you describe yourself as someone who takes risks?* (n=436,236)

	Yes (n=113,882)	No (n=322,354)	p-value[a]
Female	44,982 (39.5%)	190,972 (59.2%)	$< 1 \times 10^{-200}$
Age (years)	55.8 (8.2)	57.1 (7.9)	$< 1 \times 10^{-200}$
BMI (kg/m^2)	27.7 (4.7)	27.3 (4.8)	3×10^{-81}
Age at first birth (years)[b]	25.2 (4.9)	25.4 (4.5)	1×10^{-20}
Ever smoked	60,670 (53.3%)	139,579 (43.3%)	$< 1 \times 10^{-200}$
Alcohol frequency	Median: *3 or 4 times a week*	Median: *Once or twice a week*	$< 1 \times 10^{-200}$
Drug addiction	372 (0.33%)	343 (0.11%)	4×10^{-32}
Any eating disorder	252 (0.08%)	83 (0.07%)	0.22
Schizophrenia	348 (0.12%)	134 (0.11%)	0.17
Depression	19,222 (6.18%)	7,041 (5.96%)	2×10^{-14}
Age completed education	16.7 (2.4)	16.6 (2.1)	7×10^{-6}

Body mass index (BMI)

Values are mean (SD) or n (%), except for alcohol frequency where the responses were on a 6-point scale ranging from *Never* to *Daily or almost daily*. Possible responses were: Never, Special occasions only; 1-3 times/month; 1-2 times/week; 3-4 times/week; Daily or almost daily.

[a]Age and sex-adjusted models were used to calculate the p-value from the regression of risk-taking to the variable (linear for continuous phenotypes; logistic for binary phenotypes and ordered categorical for alcohol frequency)

[b]Data for women only, the p-value is from a model with only age adjustment

All data regarding health and health-related behaviour was collected by self-report

Compared to non-risk-takers, those who self-identified as risk-takers were more likely to be male, younger and to have a higher BMI (**Table 6.2**). They were also more likely to report specific risk-taking behaviours, such as ever having smoked or experienced substance addiction. Amongst women who reported having had children, risk-takers gave birth to their first child at a younger age. Whilst these differences were significant, in many cases, their magnitude was small. We did not find any association between risk-taking and clinical eating disorders or schizophrenia, both of which were reported by very small numbers of individuals. However, there was a positive association between risk-taking and depression. Surprisingly, risk-takers reported a slightly older age at leaving education. However, the SD for this outcome was larger amongst the risk-takers, indicating greater variability (Levene's test $p < 1 \times 10^{-8}$).

The assessment of repeated measures of risk-taking. Risk-taking propensity was recorded on repeat occasions amongst a sub-set of participants. Repeat measures ANOVA showed that risk-taking propensity at each time point was associated with risk-taking propensity at later time points ($p = 6.02 \times 10^{-6}$). Overall, the consistency of responses was moderate. Of all UKB participants with repeated risk-taking measures, including those of non-European ancestry, 16,385 out of 19,006 (86%) recorded the same response between baseline and their first follow-up, 10,102 of 12,084 (84%) recorded the same response between baseline and their second follow-up and 3300 of 3816 (86%) recorded the same response between their first and second follow-ups.

6.4.2 Genomic loci

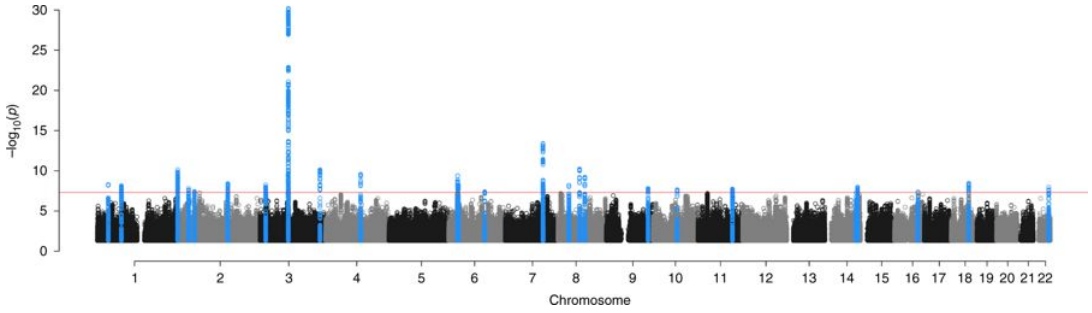


Figure 6.4 Manhattan plot of the GWAS of risk-taking propensity. The plot illustrates the results of the GWAS of 436,236 participants of white European ancestry in UKB. Negative log-transformed p-values for each SNP (y-axis) are plotted by chromosomal position (x-axis). The red-dashed line indicates the threshold for statistical significance ($p = 5 \times 10^{-8}$). The blue dots indicate variants within a 1Mb region of a genome-wide significant signal.

In this analysis, 26 loci were associated with risk-taking propensity ($p < 5 \times 10^{-8}$) (**Figure 6.4**; **Table 6.3**). We observed a low intercept value for the LD score regression GWAS (1.02, SE: 0.01), indicating that the vast majority of test statistic inflation (lambda genomic control (GC)=1.37) is due to polygenicity rather than population structure. The effect estimates (odds of self-reported risk-taking propensity) ranged from 1.022 to 1.049 per allele. The strongest signal, rs6762267, lies intronic in *CADM2* on chromosome 3. This SNP is in high LD with both SNPs previously reported in association with risk-taking, which were also intronic to *CADM2* (rs57401290: $r^2 = 0.78$; rs13084531: $r^2 = 0.49$) [344, 242]. Other correlated *CADM2* variants have also previously been reported in association with BMI (rs13078960: $r^2 = 0.21$) [149], educational attainment (rs62263923: $r^2 = 0.27$; rs55686445: $r^2 = 0.27$) [357, 358] and alcohol consumption (rs9841829: $r^2 = 0.49$) [359]. The second strongest signal identified (rs727644) lies intronic in *FOXP2*, which has previously been associated with age at first birth in women (rs10953766: $r^2 = 0.14$) [360].

Other notable association signals include rs58560561 within *SDCCAG8*, which has been reported in association with educational attainment (rs2992632: $r^2 = 0.76$) [357]; rs6923811

near *POM121L2* and rs3117340 near *OR14J1*, which have both been reported in association with autistic spectrum disorder (rs141342723: $r^2 = 0.13$; rs115329265: $r^2 = 0.24$, respectively) [361]; and rs4801000 near *TCF4* (rs9636107: $r^2 = 0.46$) and rs283914 within *TBC1D5* (rs4330281: $r^2 = 0.58$), which have been reported in association with schizophrenia [362]. In addition, *NEGR1* has previously been reported in association with BMI [149], although our signal appears independent of that reported signal (rs3101336; r^2 with our signal (rs4233093)=0.02).

Table 6.3 Twenty-six genome-wide significant loci for risk-taking propensity

rsID	Chr.	Pos.	Gene	SNP location	Alleles	Allele freq.	OR	95% CI	*p*-val.	Disorders and phenotypes
rs6762267	3	85513115	*CADM2*^{N,E}	Intronic	C/A	0.38	1.049	1.041–1.058	6.60×10^{-31}	–
rs727644	7	114109349	*FOXP2*^{N,E}	Intronic	G/A	0.60	1.031	1.023–1.040	4.00×10^{-14}	Speech & language disorder
rs62519827	8	65481947	*CYP7B1*^{E,M}	Intergenic	T/C	0.89	1.042	1.029–1.055	6.00×10^{-11}	Spastic paraplegia
rs9841382	3	181408124	*SOX2-OT*^{N}	Intronic	C/T	0.14	1.038	1.026–1.049	7.10×10^{-11}	CNS abnormalities; developmental delay
rs58560561	1	243537729	*SDCCAG8*^{N,E}	Intronic	G/T	0.65	1.028	1.019–1.036	7.20×10^{-11}	Educational attainment; Bardet–Biedl syndrome
rs992493	4	106180264	*TET2*^{N}	Intronic	T/C	0.19	1.033	1.023–1.043	2.50×10^{-10}	–
rs6923811	6	27289776	*POM121L2*^{N}	Intergenic	T/C	0.68	1.027	1.019–1.036	3.90×10^{-10}	Autistic spectrum disorder
rs7817124	8	81404008	*ZBTB10*^{N}	Intronic	C/G	0.24	1.030	1.020–1.039	6.10×10^{-10}	—
rs4801000	18	53456943	*TCF4*^{N}	Intergenic	G/A	0.34	1.025	1.017–1.034	3.40×10^{-9}	Schizophrenia
rs4653015	1	33776431	*ZNF362*^{E}	Intergenic	T/C	0.26	1.027	1.018–1.037	3.80×10^{-9}	—
rs12476923	2	145830053	*DKFZp686O1327*^{N}	Intronic	A/C	0.34	1.025	1.017–1.034	4.70×10^{-9}	—
rs283914	3	17330649	*TBC1D5*^{N,E}	Intronic	T/C	0.53	1.024	1.016–1.032	5.30×10^{-9}	Schizophrenia
rs4233093	1	73446245	*NEGR1*^{N}	Intergenic	A/G	0.52	1.024	1.016–1.032	5.30×10^{-9}	Neuronal growth
rs7829912	8	33479228	*DUSP26*^{N}	Intergenic	T/C	0.56	1.024	1.016–1.032	5.90×10^{-9}	—
rs3117340	6	29210596	*OR14J1*^{N}	Intergenic	G/T	0.62	1.024	1.016–1.033	7.00×10^{-9}	Autistic spectrum disorder; sensory experience
rs1381287	14	98597552	*RP11-61O1.1*^{N,E}	Intergenic	T/C	0.46	1.023	1.015–1.032	9.90×10^{-9}	—
rs28520003	22	46411969	*LINC00899*^{E}	Intergenic	G/A	0.69	1.025	1.016–1.034	1.10×10^{-8}	—
rs12115650	9	126367705	*DENND1A*^{N}	Intronic	G/A	0.72	1.026	1.017–1.035	1.50×10^{-8}	—
rs11226319	11	104221573	*PDGFD*^{N}	Intergenic	A/G	0.16	1.032	1.021–1.043	1.50×10^{-8}	Neocortical development
rs1358391	7	115111838	*SNORA25*^{N}	Intergenic	G/T	0.51	1.023	1.015–1.031	1.50×10^{-8}	—
rs12617392	2	27336827	*CGREF1*^{N,E}	Intronic	C/A	0.56	1.023	1.015–1.031	1.80×10^{-8}	—
rs542883	2	45143382	*SIX3*^{N,E}	Intergenic	C/G	0.56	1.023	1.015–1.031	2.20×10^{-8}	Holoprosencephaly
rs10823791	10	73338334	*CDH23*^{N}	Intronic	T/A	0.40	1.023	1.015–1.031	3.60×10^{-8}	Usher syndrome; deafness
rs34905321	6	109131107	*ARMC2*^{N}	Intergenic	T/C	0.57	1.022	1.014–1.031	3.90×10^{-8}	—
rs891124	16	71440756	*CALB2*^{N}	Intergenic	T/C	0.71	1.024	1.016–1.033	4.10×10^{-8}	—
rs35914833	14	94182383	*PRIMA1*^{N}	Intergenic	T/C	0.68	1.024	1.015–1.033	5.00×10^{-8}	—

Chromosome (Chr.); Position (Pos); Odds ratio (OR); Confidence interval (CI); Central nervous system (CNS)
^{N}Nearest gene; ^{E}eQTL; ^{M}Missense
[a] Effect allele/other allele
[b] Effect allele frequency

Several of the genes that co-locate with risk-taking signals are reported to be mutated in rare disorders of central nervous system (CNS) functioning and neuro-developmental delay. For example, *CDH23* is mutated in Usher syndrome, characterised by profound deafness [363], *CYP7B1* is mutated in a rare form of spastic paraplegia [364], *SIX3* is mutated in holoprosencephaly resulting in major mental retardation [365] and mutations in *FOXP2* are associated with speech and language disorder 1 [366]. Moreover, mutations in *SOX2-OT* are associated with CNS abnormalities and neuro-developmental delay [367] and mutations in *SDCCAG8* are associated with Bardet–Biedl Syndrome, features of which include obesity and neuro-developmental delay [368]. Other signals co-localise near genes that regulate CNS or sensory neural function. These include, *NEGR1* which is involved in neuronal growth [369], *OR14J1*, which is involved in sensory experience [370] and *PDGFD*, which is involved in human neocortical development [371]. One lead SNP (rs62519827) is in high LD ($r^2 = 0.98$)

with a missense variant (rs62519835) in *BHLHE22*, which encodes a transcription factor involved in neuronal differentiation and is also an eQTL for *CYP7B1*.

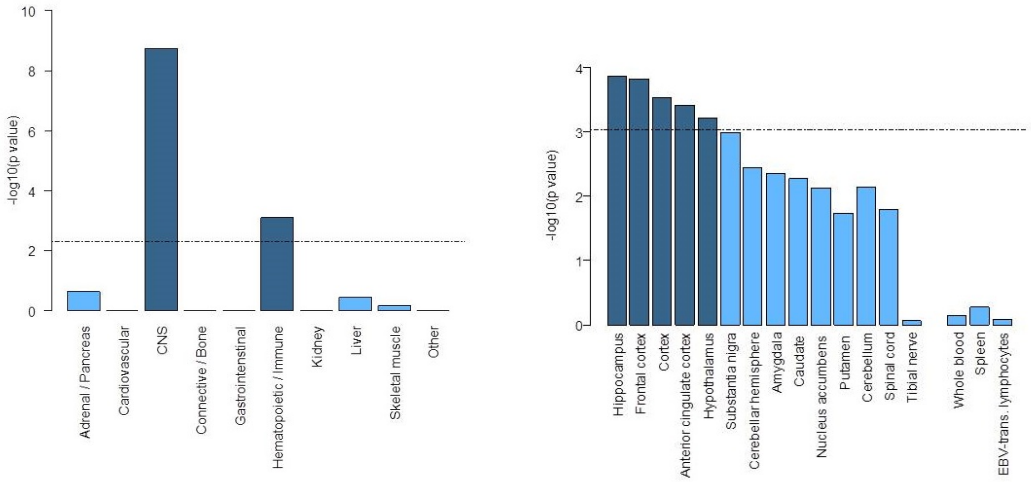
6.4.2.1 Quasi-replication

Whilst a true replication of our results was not possible due to lack of available, independent data, we conducted a GWAS of *Ever smoking* in UKB in order to look up genome-wide significant SNPs for risk-taking. The sample comprised 207,229 ever smokers (46%) and 243,177 never smokers. The results are presented in **Appendix A.20**. Eleven of the 26 risk-taking SNPs showed Bonferroni significant associations with ever smoking (corrected for 26 tests: $p < 0.0019$) and 13 reached nominal significance ($p < 0.05$). All nominally significant SNPs demonstrated directionally consistent associations between risk-taking and smoking. In total, 21 of the 26 SNPs were directionally consistent.

6.4.2.2 Chip heritability

The chip heritability estimate for risk-taking propensity in UKB was 8.4% (95% CI: 8.0%, 8.8%).

6.4.3 Pathways and tissues associated with risk-taking



(a) Tissues **(b)** Brain regions

Figure 6.5 Tissues showing enriched expression of genes implicated by risk-associated loci. The dotted lines indicate statistical significance (Bonferroni-corrected *p*-value of partitioned heritability calculated by stratified LD score regression). **Figure 6.5a:** GTEx analysis indicates that genes implicated by risk-associated loci show enriched for expression in the CNS and hematopoietic/immune system. **Figure 6.5b:** GTEx analysis indicates genes within risk-associated loci show enriched expression in specific brain regions.

Tissue enrichment analysis using the GTEx database indicated that genes co-located with risk-taking variants were enriched for expression in the CNS ($p = 1.80 \times 10^{-9}$) and immune system ($p = 8.20 \times 10^{-4}$) (**Figure 6.5a**). Of specific CNS tissues, the hippocampus, frontal cortex, cortex, anterior cingulate cortex and hypothalamus showed enrichment of expression after correction for multiple testing (**Figure 6.5b**).

To identify mechanisms that influence risk-taking propensity, we performed a systematic test of all annotated biological pathways for enrichment of genes located near risk-associated variants using MAGENTA. Two overlapping pathways were associated with risk-taking: the gamma aminobutyric acid (GABA) pathway (false discovery rate (FDR) based on 75% cutoff=0.006) and GABA receptor pathway (FDR based on 75% cutoff=0.04). Overlap between these pathways is depicted in **Figure 6.6**.

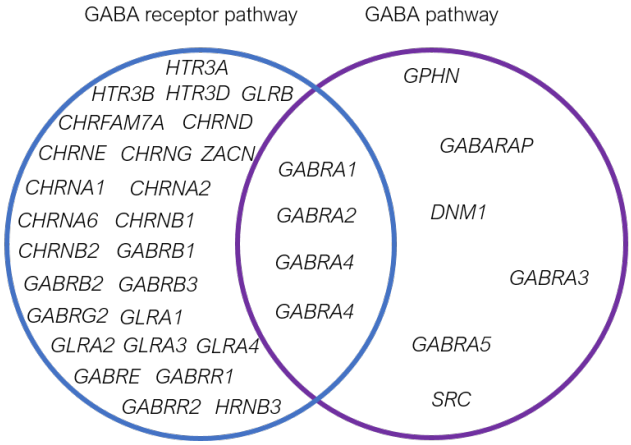


Figure 6.6 Overlap between genes in the GABA and GABA receptor pathways

6.4.4 Genetic correlations

The genetic correlations between risk-taking propensity and 12 adiposity-related, risk-behaviour and psychological traits were calculated using LD score regression. After Bonferroni correction for multiple testing, risk-taking propensity showed positive genetic correlations with: WHR, childhood obesity, ever smoking, ADHD, BPD and schizophrenia; and negative genetic correlations with age at first birth in women (all $p< 0.004$). A nominally significant, positive genetic correlation was also observed between risk-taking and BMI (p=0.03) (**Table 6.4**; **Figure 6.7**).

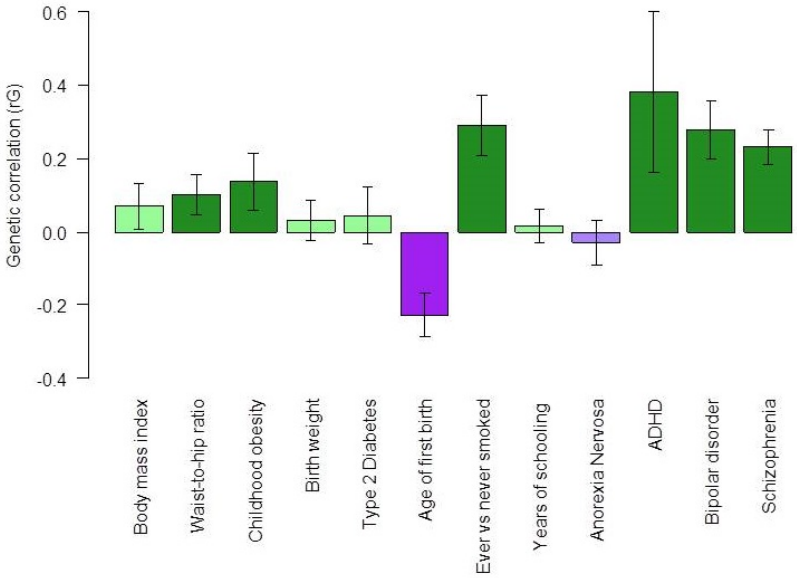


Figure 6.7 Genetic correlations between risk-taking propensity and selected traits. Whole-genome LD score regression tested genome-wide SNP associations for risk-taking against similar data for 12 BMI-related traits. Error bars show the 95% CIs for these estimates. Green indicates a positive association and purple indicates a negative association. Dark colours indicate a significant association, after adjustment for multiple testing. After correction for multiple testing, WHR, childhood obesity, age at first birth, ever smoking, ADHD, bipolar disorder and schizophrenia remained significant.

Table 6.4 Genetic correlations between risk-taking propensity and selected traits

	Genetic correlation (r_g)	SE	p-value
BMI	0.0705	0.0323	0.03*
WHR	0.1019	0.0277	0.0002**
Childhood obesity	0.137	0.04	0.0006**
Birth weight	0.0319	0.0274	0.24
Type 2 Diabetes	0.0439	0.0401	0.27
Age of first birth	-0.2287	0.0302	3.6×10^{-14}**
Ever smoked	0.2901	0.0414	2.5×10^{-12}**
Years of schooling	0.0176	0.0232	0.45
Anorexia Nervosa	-0.0302	0.0323	0.35
ADHD	0.3807	0.1115	0.0006**
Bipolar disorder	0.2788	0.0403	4.4×10^{-12}**
Schizophrenia	0.2317	0.0245	3.2×10^{-21}**

Body mass index (BMI); Waist-to-hip ratio (WHR); Attention deficit hyperactivity disorder (ADHD); Standard error (SE)

*Nominally significant (p<0.05)

**Bonferroni significant (p<0.004), corrected for 12 tests

6.4.5 Genome-wide significant signals for BMI

Of the 26 risk-associated loci, 4 demonstrated genome-wide significant associations with BMI (**Table 6.5**), including two novel signals: rs891124, which is an eQTL for *CALB2* and rs35914833 at *PRIMA1*. These loci were derived from a combination of UKB and the GIANT consortium data and have not been reported in any previous BMI GWAS studies. Signals at *CADM2* and *ZBTB10* have previously been associated with BMI [149].

The risk-increasing variants at *CADM2*, *CALB2* and *PRIMA1* were associated with higher BMI. However, the risk-increasing variant at *ZBTB10* was associated with lower BMI. Signals at *CALB2*, *ZBTB10* and *PRIMA1* showed nominally significant associations ($p< 0.05$) with TV snacking, skipping breakfast and daily energy intake, respectively (**Table 6.5**). None of the 4 loci were associated with EE, UE or CR (all $p> 0.05$) (**Table 6.6**).

Table 6.5 Associations between the 4 risk-taking loci that were genome-wide significant signals for BMI and food-related behaviour in the Fenland study

		BMI		TV snacking		Home-cooked meals		Skipping breakfast		Energy (kcal/day)	
Variant	Gene	Beta (SE)	*p*-value	Beta (SE)	*p*-value	Beta (SE)	*p*-value	Beta (SE)	*p*-value	Beta (SE)	*p*-value
rs891124	*CALB2*	0.01 (0.002)	3.5×10^{-10}	0.12 (0.05)	0.02*	0.04 (0.03)	0.21	0.01 (0.03)	0.86	3.86 (11.0)	0.73
rs35914833	*PRIMA1*	0.02 (0.002)	5.3×10^{-14}	−0.05 (0.05)	0.34	−0.03 (0.03)	0.33	−0.04 (0.03)	0.20	30.3 (11.0)	0.01*
rs6762267	*CADM2*	0.02 (0.002)	1.7×10^{-15}	0.09 (0.05)	0.07	0.02 (0.03)	0.45	0.03 (0.03)	0.36	12.3 (10.2)	0.23
rs7817124	*ZBTB10*	−0.01 (0.002)	1.8×10^{-9}	0.09 (0.06)	0.10	−0.03 (0.03)	0.36	0.08 (0.03)	0.02*	12.4 (11.5)	0.28

Body mass index (BMI); Standard error (SE)

SNPs were aligned to the risk-increasing allele. Effect estimates (Beta and SE) were derived from linear or logistic regressions of the variant to the named trait, adjusted for age and sex. Beta from logistic regressions are odds ratios (TV snacking, home-cooked meals and skipping breakfast). Beta for linear regressions are SD change in BMI per risk-increasing allele or change in energy intake (kcal/day) per risk increasing allele. BMI was a continuous outcome standardised within the BMI meta-analysis. TV snacking was coded: 0: never/rarely; 1: occasionally/ usually/ always. Home-cooked food was coded: 0: 5+ home-cooked meals/week; 1: 0−5 home-cooked meals/week. Skipping breakfast was coded: 0: <2 times/week; 1: ≥2 times/week

*Nominally significant ($p< 0.05$)

Table 6.6 Associations between the 4 risk-taking loci that were genome-wide significant signals for BMI and EB traits in the Fenland study

		BMI		Emotional eating		Uncontrolled eating		Cognitive restraint	
Variant	Gene	Beta (SE)	*p*-value	Beta (SE)	*p*-value	Beta (SE)	*p*-value	Beta (SE)	*p*-value
rs891124	*CALB2*	0.01 (0.002)	3.5×10^{-10}	1.12 (0.71)	0.11	0.48 (0.47)	0.30	0.03 (0.51)	0.96
rs35914833	*PRIMA1*	0.02 (0.002)	5.3×10^{-14}	0.06 (0.71)	0.93	-0.01 (0.47)	0.98	-0.11 (0.51)	0.83
rs6762267	*CADM2*	0.02 (0.002)	1.7×10^{-15}	0.95 (0.65)	0.15	0.69 (0.43)	0.11	0.33 (0.47)	0.48
rs7817124	*ZBTB10*	-0.01 (0.002)	1.8×10^{-9}	0.31 (0.73)	0.67	0.17 (0.48)	0.72	0.37 (0.52)	0.48

Body mass index (BMI); Standard error (SE)

SNPs were aligned to the risk-increasing allele. Effect estimates (Beta and SE) were derived from linear regressions of the variant to the named trait, adjusted for age and sex, and represent SD change in BMI per risk-increasing allele or change in EB score per risk-increasing allele. BMI was a continuous outcome standardised within the BMI meta-analysis. The EBs were scaled from 0-100

*Nominally significant ($p< 0.05$)

6.4.6 Bi-directional MR analyses of risk-taking propensity and BMI

Using results from the present GWAS and an unpublished meta-analysis of BMI involving 772,825 individuals from GIANT and UKB, we conducted a bi-directional MR analysis of risk-taking and BMI. In the IVW model, genetically predicted risk-taking propensity predicted higher BMI (0.25 approximate SDs of BMI (SE=0.06); $p = 6.7 \times 10^{-5}$), while genetically predicted BMI did not predict risk-taking propensity (p=0.23) (**Table 6.7**). Neither the MR Egger nor the weighted median MR results were significant.

Table 6.7 Bi-directional MR analyses of risk-taking and BMI

Analysis	Beta (SE)	p-value
Risk-taking to BMI		
Conventional MR (IVW)	0.251 (0.063)	6.7×10^{-5}
MR Egger	0.885 (0.985)	0.37
Weighted Median MR	0.091 (0.121)	0.45
BMI to risk-taking		
Conventional MR (IVW)	0.004 (0.004)	0.23
MR Egger	0.002 (0.017)	0.88
Weighted median MR	−0.008 (0.007)	0.26
Between SNP heterogeneity[a]	N/A	9.9×10^{-8}

Body mass index (BMI); Mendelian Randomisation (MR); Inverse-weighted variance (IVW); Standard error (SE); Not applicable (N/A)
MR Egger intercept was not significant
[a] The p-value refers to the Cochran's Q statistic from the conventional IVW risk-taking to BMI MR analysis

A high level of between SNP heterogeneity was detected using Cochran's Q statistic applied to the IVW risk-taking to BMI MR analysis ($p = 9.9 \times 10^{-8}$), with individual risk-increasing alleles showing strong associations with either higher or lower BMI (**Figure 6.8**). We performed a *leave-one-out* analysis, whereby we repeated the MR analysis of risk-taking to BMI 26 times with each of the genome-wide significant SNPs for risk-taking removed in turn. The results suggested that all 4 of the individually genome-wide significant SNPs for BMI had a substantial effect on the heterogeneity of the data (**Appendix B.2**). We performed a further MR analysis of risk-taking to BMI excluding the 4 risk-taking SNPs that were also genome-wide significant for BMI, and found no association between risk-taking propensity and BMI (β from IVW MR=0.01 (SE=0.07); p=0.91) and no evidence of heterogeneity (p=0.24). Similarly, a random effects IVW MR model, combining the estimates calculated when treating each risk-associated SNP as an individual instrument, also provided no evidence for an overall causal relationship between risk-taking and BMI (**Appendix B.3**).

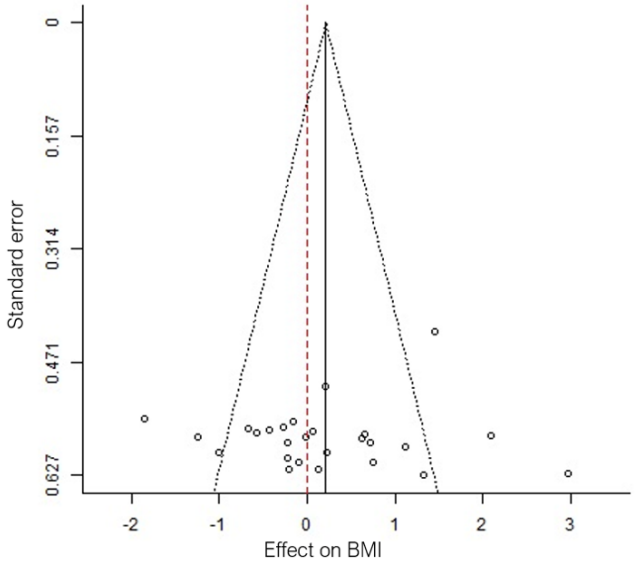


Figure 6.8 Funnel plot showing the heterogeneity in the association between the 26 genome-wide significant SNPs for risk-taking and BMI. Each data point represents one of the 26 SNPs. The SNP-specific MR estimate for the association of risk-taking with BMI (approximate SDs of BMI per risk-increasing allele) (x-axis) is plotted against the SE of this association (y-axis). The summary estimate for all 26 SNPs combined is marked by the solid black line. The dotted lines, originating from the summary estimate and marking a triangle, represent the expected 95% CIs of the combined effect estimate, assuming the variants have an effect on BMI. The vertical red-dotted line indicates the null.

6.4.7 Risk-taking propensity, eating behaviour and dietary patterns

An analysis of the relationship between genetically predicted risk-taking (risk-GRS) and both EB and dietary patterns was performed amongst the Fenland study participants. Genetically predicted risk-taking propensity showed positive associations with EE in men, after adjustment for multiple testing, and nominally significant positive associations with total daily kcal, fat and protein intake in the combined cohort of men and women (**Table 6.8**).

The ranges of the EB trait scores were as follows: CR: 0–100; UE: 0–96.3; EE: 0–100. The food-related behaviour variables were initially ordered categorical variables. However, their distributions were markedly non-normal. To account for this, they were dichotomised and logistic regression was performed. In all cases, the category containing the majority of participants was split from the rest of the sample. This was designed to increase the sample size of the comparison group and to maximise power. The analysis revealed a nominally significant positive association between the risk-GRS and odds of skipping breakfast more than twice a week (OR=1.05 (95% CI: 1.02, 1.07)). No associations were observed between the risk-GRS and UE, CR, total daily fibre, fruit and vegetable or carbohydrate intake. Genetically predicted risk-taking propensity did not predict the odds of eating home-cooked meals or snacking in front of the television (**Table 6.8**).

Table 6.8 Association between the risk-GRS and diet, food-related behaviours and EB traits in the Fenland study

	Total (n)	Beta (95% CI)	r^2	p-value
All participants: nutrient intake				
Energy (kcal/day)	8981	803.5 (140.1, 1466.8)	0.042	0.02*
Total fat (g/day)[a]	8981	0.52 (0.12, 0.92)	0.042	0.01*
Fruit and vegetables (g/day)[a]	8844	0.46 (-0.07, 0.99)	0.044	0.09
Protein (g/day)[a]	8981	0.36 (0.06, 0.66)	0.010	0.02*
Fibre (g/day)[a]	8981	0.28 (-0.10, 0.66)	0.005	0.15
Carbohydrates (g/day)[a]	8981	0.25 (-0.10, 0.60)	0.028	0.16
All participants: food-related behaviours[b]				
TV snacking	4414	1.03 (0.99, 1.06)	–	0.46
Home cooked food	11,439	0.99 (0.97, 1.01)	–	0.59
Skipping breakfast	11,441	1.05 (1.02, 1.07)	–	0.03*
Men only: eating behaviours				
Emotional eating (0-100)	1646	94.6 (35.7, 153.6)	0.007	0.002**
Cognitive restraint (0-100)	1646	-2.62 (-48.0, 42.7)	0.005	0.91
Uncontrolled eating (0-100)	1646	32.0 (-9.3, 73.3)	0.019	0.13
Women only: eating behaviours				
Emotional eating (0-100)	1869	-21.2 (-82.7, 40.6)	0.002	0.50
Cognitive restraint (0-100)	1869	-21.2 (-63.3, 20.8)	0.005	0.32
Uncontrolled eating (0-100)	1869	14.8 (-24.0, 53.6)	0.013	0.45

All models were linear or logistic regressions of the risk-GRS to the variable, adjusted for age and sex. Sex-stratified models were only adjusted for age

TV snacking was coded: 0: never/rarely; 1: occasionally/ usually/ always. Home-cooked food was coded: 0: 5+ home-cooked meals/week; 1: 0−5 home-cooked meals/week. Skipping breakfast was coded: 0: <2 times/week; 1: ≥2 times/week

*Nominally significant (p<0.05)

**Bonferroni significant after adjustment for 15 tests (p<0.003)

[a]Log-transformed

[b]Logistic regression. Effect estimates are odds ratios

6.5 Discussion

6.5.1 Summary and context of the main findings

Amongst 436,236 adult UKB participants, this analysis identified 26 genetic loci associated with self-reported risk-taking propensity, 24 of which are novel. The results support the utility of gene discovery in investigating the biological pathways of health-related behaviours, as well as the mechanisms of their association with health outcomes. Of particular relevance to the aims of this thesis, we were able to use genetic instruments to interrogate the association between risk-taking and obesity reported in observational studies using both MR and GRS approaches [341, 203, 372].

As anticipated for a behavioural trait, the findings suggest that the genetics of risk-taking act primarily through the CNS. In addition to the cortex, 4 specific brain regions exhibiting enriched expression for genes associated with risk-taking propensity were identified. These comprised the pre-frontal cortex, hippocampus, anterior cingulate cortex and hypothalamus, all regions that have previously been implicated in risk-related traits through functional magnetic resonance imaging (fMRI) studies. Decreases in pre-frontal cortex activation during experimental risk-taking tasks have been linked to declines in self-reported risk-taking behaviour in adolescents [373], the hippocampus has an established role in behavioural inhibition (the tendency to withdraw from unfamiliar situations, people or environments) [374], the anterior cingulate cortex has been implicated in assessing the value of exercising control whilst performing a task [375], and the hypothalamus is involved in the processing of innate and learned fear, including fear of pain, predators and aggression [376]. Additionally, enriched expression of risk-associated genes in the immune system supports growing evidence suggesting a role for the immune system in human behaviour [377]. Research has primarily concerned clinically relevant mood and behavioural aberrations, including depression [378]. However, an association between immune function and personality has been proposed [379].

Genetic correlations between risk-taking and schizophrenia, BPD and ADHD confirm the findings of a smaller, overlapping GWAS of risk-taking among 116,255 UKB participants [242]. Given the genetic and symptomatic overlap between major mental disorders, as well as diagnostic migration and co-segregation within families, traits with trans-diagnostic relevance are important to understanding shared vulnerabilities and mechanisms.

Of particular relevance to this thesis and to the application of GWAS to the EB traits in **Chapter 7**, we were able to interrogate the relationship between risk-taking and obesity in downstream analyses. We observed novel genetic correlations between risk-taking and both childhood obesity and WHR, suggesting a shared genetic basis for these traits. We also observed a nominally significant, positive genetic correlation with adult BMI. This finding is in partial agreement with the results of a smaller GWAS of risk-taking propensity

in an overlapping UKB participant group which reported a Bonferroni significant genetic correlation between BMI and risk-taking propensity [242]. The IVW MR analysis linked some risk-taking pathways to BMI in adulthood. The high levels of heterogeneity in this analysis indicate that genetic correlation, which assumes a linear association between effect sizes for both traits across the genome, may not adequately summarise the complex relationship between risk-taking and BMI.

Of the risk-taking to BMI MR analyses, only the IVW MR generated a significant result. Whilst this analysis assumes the absence of horizontal pleiotropy, it has the highest statistical power of the MR analyses performed [356]. The finding of 4 SNPs with strong, genome-wide associations with both risk-taking and BMI, but variable directional consistency, supports the finding of between SNP heterogeneity, and suggests the existence of diverse, pleiotropic pathways linking these two traits, as opposed to a single causal mechanism.

In order to elucidate pathways that may be involved in the association between risk-taking and BMI, we conducted a risk-GRS analysis interrogating the association of risk-taking propensity to EB, dietary patterns and food-related behaviour. The results suggested that risk-taking propensity may be associated with EE in men, as well as higher daily calorie, fat and protein intake and greater odds of regularly skipping breakfast in both sexes. These findings require replication but speculatively indicate that obesogenic EBs and dietary practices could provide a mechanism through which some facets of risk-taking propensity are related to BMI. **Chapter 7** uses genetic instruments to further address these questions with reference to risk-taking and EB.

6.5.2 Strengths and limitations

This is the largest gene discovery effort for risk-taking propensity to-date, increasing the sample size of the largest previous study by approximately 4-fold. Given the importance of risk-taking phenotype to a range of important health-related behaviours and outcomes, including obesity, this study makes an important contribution to the literature. The large sample size facilitated the identification of a larger number of genetic variants with smaller effect sizes than previous efforts. These variants could then be used in downstream analyses to interrogate the relationship between risk-taking and BMI for the first time. Further strengths include the use of a single study from which all participants were drawn (UKB). This ensured that there were no differences in study design, conduct, measurement or processing techniques between studies.

The main limitation was the measurement of risk-taking propensity, which was self-reported and based on the answer to a single, un-validated question. However, responses were moderately stable in the sub-set of participants with repeated measures. Moreover, self-identification as a risk-taker was associated with classically risk-taking behaviours, including alcohol consumption, smoking, drug addiction and age at first birth, in the anticipated

ways. There is no gold-standard measurement for risk-taking propensity. Studies most often rely upon self-report but behavioural measures, derived from laboratory tasks, are also widely used. Where studies rely on self-report, questionnaires typically comprise multiple items [380]. Research amongst those involved in extreme sports cautions against assuming psychological or behavioural homogeneity in risk-taking populations [381]. While some risk-takers in these studies take impulsive risks, others take planned risks in response to feelings of confidence and self-efficacy, justified by experience and the development of expertise [381, 382]. Some researchers argue that impulsivity and risk-taking propensity are distinct and governed by related, but separate, neurobiological mechanisms [383, 384, 208]. The use of a single question and lack of clarifying questions to determine why respondents self-identify as risk-takers is an important limitation of this study that precludes a discussion of the facets of risk-taking propensity that are being captured, or how these are related to health and other outcomes. This is one potential explanation for the heterogeneity observed in the MR results, with some risk-increasing alleles being associated with reductions in BMI. It is possible that, whilst some aspects of risk-taking propensity are related to obesity, others are either protective or not involved. Future research is needed to provide clarity. A further limitation was the inability to replicate the results in an independent dataset, due to lack of available data on risk-taking propensity in any independent cohorts. Whilst we performed a quasi-replication in a related phenotype (*Ever smoking*), future studies are needed to directly replicate the findings.

6.5.3 Conclusions

This study advances understanding of the genetic basis for risk-taking, identifying 26 risk-associated loci and highlighting a common genetic basis for risk-taking and a range of health-related phenotypes. Building on observational research, the findings also indicate that the association between risk-taking and BMI is likely the result of shared biological pathways, as opposed to a single, causal mechanism. However, some aspects of risk-taking propensity may dispose to obesity through dietary decisions and behaviour. This finding requires replication and it is likely that many pathways, some associated with lower BMI, are involved. Overall the findings confirm the utility of gene discovery in illuminating the relationship between behaviour and obesity.

CHAPTER 7

THE GENETIC DETERMINANTS OF EATING BEHAVIOUR

Publications

There are, as yet, no publications associated with this work.

Contributions

I planned this project and devised the analysis plan in collaboration with my supervisors. I conducted the GWAS analysis in the Fenland cohort, identified the other participating cohorts and liaised with their analysts to perform individual cohort GWAS analyses. I performed collation, quality control and meta-analysis of the study-level results for all contributing cohorts. I performed all the downstream analyses, jointly interpreted the results with my supervisors and wrote this chapter. At present, there is no resulting manuscript.

7.1 Summary

Building upon the findings of **Chapter 6** which illustrated the utility of genome-wide gene discovery studies to the investigation of the relationship between behavioural traits and obesity, this chapter describes the first GWAS of emotional eating (EE), uncontrolled eating (UE) and cognitive restraint (CR). Separate GWAS of white, European adults were conducted in 4 population-based study cohorts with intersecting genome-wide genotyping and eating behaviour (EB) information. The included studies comprised: the Fenland study, FinnTwin12, the Nurse's Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). The results were then meta-analysed, resulting in a final sample size of up to 11,843 participants for each of the three EB traits. No genome-wide significant associations were identified. However, the chip heritability estimate for UE was 11% (95% CI: 3%, 19%) and positive genetic correlations between UE and both BMI and waist-to-hip ratio (WHR) indicate a shared genetic basis for these traits. The chip heritability estimates for EE and CR were non-significant (2% (95% CI: -8%, 12%) and 1% (95% CI: -7%, 9%), respectively). Mendelian randomisation (MR) analyses demonstrated a positive effect of BMI on all three EB traits. Whilst the impact of individual variants was highly heterogeneous, sensitivity analyses further suggested that BMI is causally implicated in EB. MR analyses also showed a positive effect of risk-taking propensity on UE, but not EE or CR. Overall, the findings suggest that UE is a heritable trait that shares a genetic basis with BMI and may be positively influenced by risk-taking propensity. Larger studies may be better powered to identify specific variants and further illuminate the biology of EE, UE and CR.

7.2 Background

Chapter 6 supports the utility of genome-wide association studies (GWAS) in illuminating the underlying biology of behavioural traits and clarifying their associations with health. Several lines of evidence indicate a genetic basis for EB, suggesting that a GWAS could be used to study EB traits. These are summarised in **Chapter 1**. Briefly, amongst adults, heritability estimates from twin studies range between 9%-60% for EE [66, 115], 45%-69% for UE [115] and cluster around 50% for CR [115, 66, 188]. Moreover, known BMI-associated genetic variants show enriched expression in the CNS, broadly suggesting a role for behavioural pathways. This enrichment is particularly pronounced in brain regions with an established role in the central regulation of eating [149]. Moreover, studies using a BMI-GRS approach, including **Chapter 4**, have indicated that appetitive EB traits mediate genetic predisposition to obesity, further indicating a role for genetics in EB [161, 301, 311].

No GWAS studies of EE, UE or CR have previously been reported. Besides isolating specific genetic variants and illuminating biological pathways, GWAS has the potential to inform several outstanding questions in EB research. First, whether all EB traits have a genetic basis is debatable. In particular, some twin studies have not found restraint to be heritable. For example, a 2003 study amongst 580 female twins did not identify evidence of heritabiliy for CR, measured using the TFEQ-51 [186]. Twin studies amongst toddlers and young children also suggest that emotional under and overeating are not substantively influenced by genetics in early life [385, 193, 194]. Whether this is also true in adulthood requires further analysis. Second, the extent to which EB traits reflect distinct biological pathways is also a matter of debate. EE and UE demonstrate strong, positive correlations and it has been asserted that they may not represent separate constructs [185]. Finally, studies including **Chapter 4**, typically model EB as a cause of weight gain. The extent to which reverse causality explains associations between EB and BMI is not known. In particular, mounting evidence supports the view that CR represents a marker of previous weight gain, as opposed to an aetiological factor in the development of obesity [138, 51]. Bi-directional MR studies, facilitated by gene discovery, could help to disentangle cause and effect.

The development of validated questionnaires has facilitated the measurement of EB in large-scale studies, making GWAS of EB traits a possibility. The most widely used and studied questionnaire in adult populations is the TFEQ-R18 (described in detail in **Chapter 1** and provided in **Appendix C.1**). The questionnaire has been validated in both obese and healthy weight populations across a range of settings [68] and measures three EB traits: EE (3 items), UE (9 items) and CR (6 items) [40].

In order to specify the genetic basis of EB traits, we performed the first GWAS of EE, UE and CR amongst over 11,500 white European participants from 4 population-based cohorts. Downstream analyses were designed to investigate the relationship between the EBs, as well as their associations to BMI and risk-taking.

7.3 Participants and methods

7.3.1 Participants

To maximise the sample size of the analysis, data from 4 separate study cohorts was meta-analysed. A literature search was used to identify studies with intersecting genome-wide genotyping data and TFEQ-R18 or TFEQ-R21-measured EB traits. A total of three cohorts, alongside the in-house Fenland study cohort, responded to the request. All included studies measured EB using the TFEQ-R18, genotyped samples on a genome-wide array and imputed samples to the HRC reference panel. All included participants were of self-reported white, European ethnicity.

7.3.1.1 The Fenland study

The Fenland study population of the present analysis comprised 3515 individuals, 1869 women (53.2%) and 1646 men, aged 35-64 years with complete genome-wide genotype and EB information. A detailed description of the Fenland study is provided in **Section 2.1**.

7.3.1.2 FinnTwin12

FinnTwin12 is an ongoing cohort study of Finnish twins born between 1983 and 1987 comprising ~ 2700 families. The first phase took place when the twins were aged 11-12 years [386]. The TFEQ-R18 was administered during the fourth phase of the study, conducted between 2006 and 2008 when twins were 21–26 years old. The majority of the twins were successfully recontacted for this study phase (n=1347 individual twins, 50% of the overall sample and 73% of the target sample). A total of 1295 participants provided blood and/or saliva samples for genotyping [387]. The study population of the present analysis comprised 1238 individuals, 670 women (54.2%) and 568 men, aged 21-26 years with complete genome-wide genotype and EB information.

7.3.1.3 The Health Professionals Follow-up Study (HPFS)

The HPFS was established in 1986. Over 50,000 male health professionals aged 40-75 years and residing in the US completed a medical and lifestyle questionnaire. Follow-up questionnaires were mailed every two years and blood samples for genotyping were collected from ~ 18,000 men between 1993 and 1996 [112]. In 2010, the TFEQ-R18 was included as a supplementary questionnaire mailed to participants for whom genome-wide genotype data was available [112]. Intersecting EB and genotype information for the present analysis was available for 2696 men.

7.3.1.4 The Nurse's Health Study (NHS)

The NHS was established in 1976. Over 100,000 female nurses residing in 11 large US states completed a mailed medical and lifestyle questionnaire [388]. Follow-up questionnaires were sent every two years. In 2010, the TFEQ-R18 was included as a supplementary questionnaire mailed to participants for whom genome-wide genotype data was available [112]. Intersecting EB and genotype information for the present analysis was available for 4869 women.

7.3.2 Methods

7.3.2.1 The assessment of eating behaviour

All included cohorts measured EB using the TFEQ-R18 (**Appendix C.1**). This questionnaire measures three EB traits: EE (3 items), UE (9 items) and CR (6 items). The traits and their scoring is described in greater detail in **Chapter 1**. As in other chapters, in all cohorts, the EB scores for each participant were scaled to 1-100 [89].

7.3.2.2 Genome-wide association analyses

Genotyping, imputation and quality control (QC) procedures were applied independently in each study guided by an analysis plan sent to study analysts (**Appendix C.3**). Centralised QC was also performed prior to meta-analysis. The analysis plan described phenotype measurement, GWAS instructions, standard QC procedures, imputation requirements and the statistics and file format required. SNPs were filtered prior to imputation on the basis of call rate (>95%), minor allele frequency (MAF; >1%) and Hardy Weinberg Equilibrium (HWE) p-value $>1 \times 10^{-6}$. Following imputation, mono-morphic SNPs and variants with an imputation quality score of <0.3 were excluded. Individual samples were filtered on the basis of missingness (>5% of genotypes), relatedness, population stratification, gonosomal abnormalities, sex-mismatch, duplication of samples or outlying ethnicity. All studies were then imputed to the most up-to-date HRC imputation panel.

In light of evidence that women typically score higher on all EB traits than men and that the association between the BMI-GRS and CR is modified by sex (**Chapter 4**) [301], the GWAS were initially sex-stratified. This resulted in a total of 6 GWAS in each cohort. The association between SNPs and each of the three EB traits was analysed using an additive model including age and study-specific covariates, as appropriate (e.g. study site). We conducted the analysis in the Fenland cohort and included the first three principal components (PCs) as covariates in the model. We did not include any other study-specific covariates. The results of the individual GWAS analyses were hosted on an Secure File Transfer Protocol (SFTP) site.

7.3.2.3 Quality control

After the analysts responsible for conducting the GWAS in each of the 4 cohorts had loaded files to the SFTP site, file and meta-level QC were performed prior to meta-analysis [389].

File-level QC

File-level QC steps were performed using the EasyQC protocol developed by Winkler and colleagues [389]. Files were checked to ensure correct naming of variables and alignment of alleles to the same strand, as specified in the analysis plan. SNPs with missing or invalid data, including mislabeled alleles or non-sensical values, were then removed alongside any remaining mono-morphic SNPs and SNPs on the sex chromosomes. A minimum minor allele count of 7 and participant number of 30 individuals were applied to each SNP. Files were also checked to ensure that the QC procedures specified in the analysis plan had been correctly followed. SNPs with a call rate of <95%, imputation quality of <0.3, HWE p-value of $<1 \times 10^{-6}$ or MAF <1% were removed. The data in each file was then reduced such that 4 significant digits were given for effect estimates, SEs, p-values and effect allele frequencies (EAF).

Meta-level QC

Following file-level QC, between-study (meta-level) comparisons of statistics were made to identify study-specific problems. The following plots were generated in each study file:

SE-N plots. The inverse median of the SE of effect estimates across all SNPs was plotted against the square root of the sample size. The inverse proportionality between the median SE and the square root of the sample size derives from the fact that the sampling variance of a linear regression–derived effect estimate of a specific SNP depends on the variance of the phenotype, the variance of the SNP genotype and the sample size [389]. Studies are expected to fall on a diagonal, with larger studies towards the top right and smaller studies towards the bottom left. Significant deviations from this pattern indicate a problem. This step did not indicate problems with any study files.

P-Z plots. These plots compare the p-value for each SNP against the p-values calculated from the z-statistics (Beta/SE). Results are expected to correspond exactly. Deviations indicate a problem with the reported p-values, effect estimates or SEs. This step did not indicate problems with any study files.

EAF plots. The EAFs were plotted against those reported in the 1000 Genomes phase 3 reference panel. Results are expected to line up on a diagonal and deviations from this

pattern indicate strand issues, miscoding of alleles or ancestry errors. This step did not indicate problems with any study files.

Quantile-Quantile (QQ) plots. QQ plots display the expected -log10 of the *p*-values based on a theoretical chi-squared distribution (*x*-axis) against the observed -log10 of the *p*-values (*y*-axis). Results are expected to line up on a diagonal, indicating no significant difference from the null expectation, with a possible small deviation to the top right of chart, indicating a small number of possible, true associations. An early deviation from the null (as shown in **Figure 7.1**), indicates that a number of low or moderate *p*-values are more significant than expected. Confounding is likely and population stratification, in particular, may be suspected.

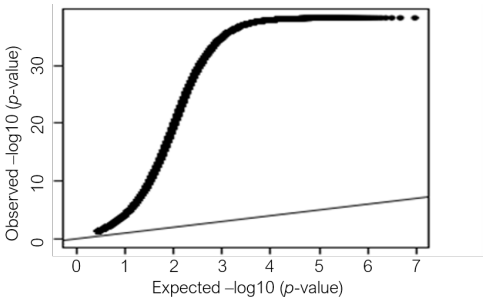


Figure 7.1 QQ-plot highlighting problems with HPFS data genotyped on the Illumina platform.

This step indicated problems with the HPFS files genotyped on the Illumina platform for all three EB traits. **Figure 7.1** exemplifies these issues, depicting the QQ plot of the HPFS Illumina file for EE. As a result of issues highlighted by the QQ plots, the HPFS files genotyped on the Illumina platform for all three EB traits were excluded prior to meta-analysis. The remaining HPFS files were genotyped on the Affymetrix platform. This led to the loss of 475 participants from the CR analysis and 474 participants from both the EE and UE analyses.

7.3.2.4 GWAS meta-analysis

The meta-analyses for all three EB traits were conducted using the inverse standard error (SE) weighted approach in the METAL package [390]. This approach weights the effect estimates for each SNP by the corresponding SE. In effect, this adjusts the effect estimate for the sample size. The package also implements Cochran's *Q* test for heterogeneity to identify heterogeneity between effect estimates in different files. Following meta-analysis, results were retained if they represented SNPs (as opposed to insertions or deletions), their MAF was >1% and the SNP was present in all study files. This left ~ 8million SNPs in each meta-analysed file. Distance-based clumping was used to identify independent signals. SNPs were considered representative of the same signal if they fell within 1000 kb of the lead SNP, taken to be the SNP with the lowest *p*-value.

In keeping with the analysis plan, sex-specific meta-analyses were performed first. The combined results files for men and women were then meta-analysed. This step identified no evidence of heterogeneity between the sexes. To increase power, downstream analyses were performed using the sex-combined results.

7.3.2.5 The analysis of genetic correlations

To quantify the proportion of loci shared between the EB traits and both BMI and WHR, genetic correlations (r_g) were assessed using LD score regression. These were performed in LDHub using the publicly available genetic information for BMI and WHR accessible through the database [351]. Genetic correlations between the EB traits were performed using LDSC software version 1.0.0, following the procedure described by Bulik-Sullivan et al. [351]. This method was also used to generate chip heritability estimates for the EB traits.

7.3.2.6 Mendelian randomisation analysis of BMI to the eating behaviour traits

Section 6.3.2.9 describes MR in detail. Conventional IVW MR was used to investigate the relationship between BMI and EB. MR Egger and weighted median MR were performed as sensitivity analyses. The SNP effect estimates for BMI were regressed on the SNP effect estimates for each of the EBs in turn. SNPs were aligned to the BMI-increasing allele. The BMI-associated SNPs included in this analysis were taken from the 2015 Locke et al. GWAS meta-analysis for BMI [149]. All 96 bi-allelic SNPs showing genome-wide significant associations with BMI were included and weighted by the European-only, sex-combined effect estimates [149]. The analysis was performed in R version 3.2.2.

7.3.2.7 Individual BMI-associated SNPs and eating behaviour traits

The association between the 96 bi-allelic BMI-associated SNPs and the EB traits was investigated on an individual, SNP by SNP basis. Effect estimates and SEs were taken from the sex-combined meta-analysed GWAS summary statistics for EB in this analysis. SNPs were aligned to the BMI-increasing allele and a z-statistic was generated for each SNP (Beta/SE). As in **Chapter 3**, the results were used to construct a heat map colour-coding the z-statistic for the association of each SNP with EB. To avoid spurious precision, z-statistics between 0.5 and 0.5 were displayed as neutral. This analysis was performed in Stata version 14 (StataCorp LCC, College Station, TX) and the heatmap was constructed in R version 3.2.2.

7.3.2.8 Mendelian randomisation analysis of risk-taking to the eating behaviour traits

Conventional IVW MR, MR Egger and weighted median MR were used to interrogate the relationship between risk-taking propensity and EB, as described in **Section 7.3.2.6**. The SNP effect estimates for risk-taking propensity were regressed on the SNP effect estimates for each of the EBs in turn. All 26 SNPs that demonstrated genome-wide significant associations with risk-taking propensity in **Chapter 6** were included in this analysis [339]. SNPs were aligned to the risk-increasing allele and weighted by the sex-combined effect estimates [339]. The analysis was performed in R version 3.2.2.

7.4 Results

7.4.1 Characteristics of the study participants

Four study cohorts were included in the GWAS meta-analysis. The total sample comprised 11,843 participants (7404 women (62.5%)).

Table 7.1 Studies included in the GWAS meta-analysis

Study	Total (N)	Age (years)	BMI (kg/m^2)	EE (0-100)	UE (0-100)	CR (0-100)
Fenland						
Men	1646	50.7 (7.3)	27.6 (4.2)	27.1 (24.8)	29.1 (17.5)	35.5 (19.0)
Women	1869	50.9 (7.2)	26.6 (5.3)	42.1 (28.0)	31.0 (17.7)	45.8 (19.1)
FinnTwin12						
Men	568	22.4 (0.7)	24.1 (3.6)	16.1 (18.7)	34.4 (17.5)	26.2 (17.8)
Women	670	22.4 (0.7)	22.8 (3.9)	36.4 (25.9)	33.9 (17.0)	40.5 (21.3)
HPFS						
Men	2221	74.3 (7.3)	—	17.6 (22.0)	20.0 (15.9)	46.5 (21.6)
NHS						
Women	4869	66.9 (6.6)	—	32.0 (27.4)	23.4 (17.0)	48.4 (20.2)

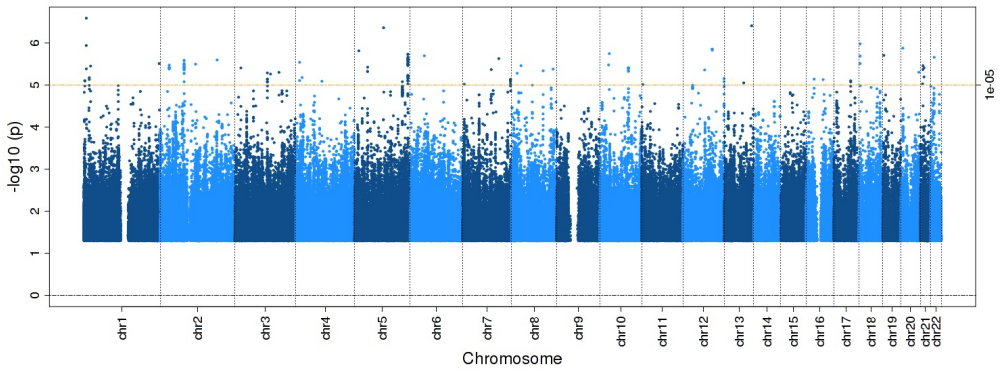
Numbers are N or Mean (SD)
Missing data or not applicable (—); Body mass index (BMI); Emotional eating (EE); Uncontrolled eating (UE); Cognitive restraint (CR); Health Professionals Follow-up Study (HPFS); Nurse's Health Study (NHS)
Age refers to the age of participants when their EB data was collected

Table 7.1 highlights variation in the age of participants in the different cohorts when their EB data was collected. FinnTwin12 had the youngest participants (mean age: 22.4 years (SD: 0.7 years)) and the HPFS study had the oldest participants (mean age: 74.3 years (SD: 7.3 years)). In Fenland, the mean BMI of both men and women was overweight (27.6kg/m^2 and 26.6kg/m^2, respectively). The mean BMI of participants in the FinnTwin12 study was within the WHO normal weight range (22.4kg/m^2 for both men and women). No BMI data was provided for participants in the HPFS or NHS cohorts.

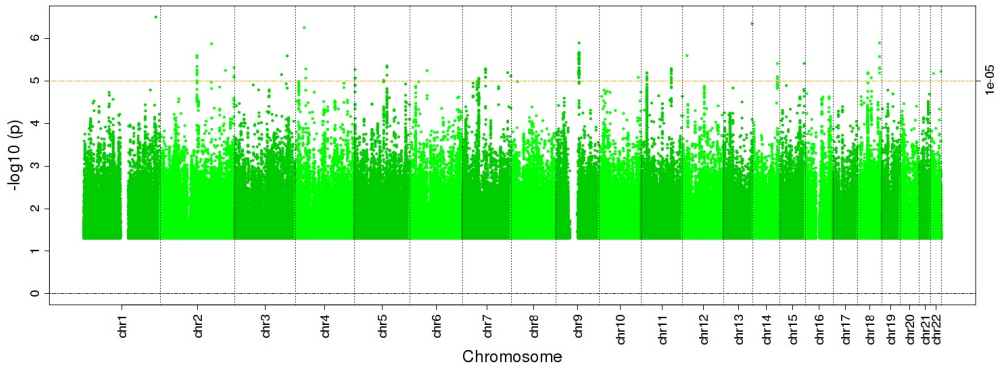
7.4.2 Genomic loci

The sex-specific GWAS meta-analyses for all three EB traits identified no associations that reached the threshold for genome-wide significance ($p<5\times10^{-8}$) (**Appendix B.4**, **B.5** and **B.6**). Meta-analysis of men and women together showed no heterogeneity in results. As would be expected by chance in the absence of any heterogeneity, the mean heterogeneity p-value was p=0.50 and 5% of the p-values were <0.05 for all three EB traits. As such, men and

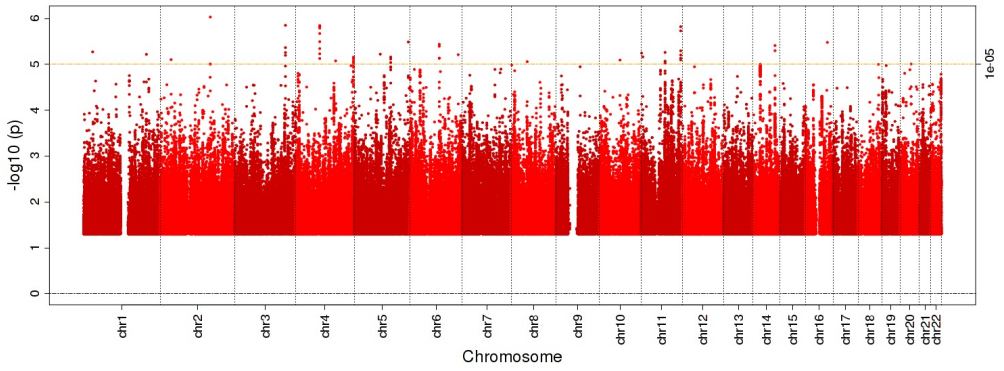
women were analysed together. No loci were associated with EE, UE or CR at the threshold for genome-wide significance (p<5 × 10^{-8}) in the sex-combined analysis (**Figure 7.2**). To increase power, all subsequent analyses were based on the sex-combined cohort.



(a) Emotional eating. (n=11,809)



(b) Uncontrolled eating. (n=11,827)



(c) Cognitive restraint. (n=11,843)

Figure 7.2 Manhattan plots showing the results of the sex-combined GWAS meta-analyses for EE, UE and CR. The plots show the results of the GWAS meta-analysis amongst participants from the Fenland, FinnTwin12, HPFS and NHS studies. Each dot represents a SNP. SNP chromosomal positions (x-axis) are plotted against the negative log-transformed p-values for the association of each SNP with EB (y-axis). The horizontal yellow line on each chart indicates a p-value of 1 × 10^{-5}). No genome-wide significant loci were identified.

7.4.3 Chip heritability

UE demonstrated the highest heritability estimate of the EB traits (11% (95% CI: 3%, 19%)). The estimated heritability of EE and CR was 2% (95% CI: −8%, 12%) and 1% (95% CI: (−7%, 9%), respectively (**Table 7.2**). The low heritability estimates, particularly for EE and CR, adversely affect the reliability of the downstream MR analyses reported in **Section 7.4.5**.

Table 7.2 Chip heritability estimates for the eating behaviour traits

	Heritability	SE
Emotional eating	0.02	0.05
Uncontrolled eating	0.11	0.04
Cognitive restraint	0.01	0.04

Standard error (SE)

7.4.4 Genetic correlations

7.4.4.1 Genetic correlations between EE, UE and CR

The genetic correlation (r_g) between EE and UE was positive, but not significant due to a very large SE (r_g (SE) = 1.08 (1.29); p-value=0.40). We were unable to estimate genetic correlations for CR as a result of the low heritability estimate for this trait.

7.4.4.2 Genetic correlations of eating behaviour with BMI and WHR

Table 7.3 Genetic correlations of eating behaviour with BMI and WHR

	r_g (SE)	p-value
Emotional eating		
BMI	1.22 (2.01)	0.54
WHR	—	—
Uncontrolled eating		
BMI	0.43 (0.10)	6.5×10^{-6}
WHR	0.29 (0.09)	0.002
Cognitive restraint		
BMI	—	—
WHR	0.81 (1.85)	0.66

Genetic correlation (r_g); Standard error (SE); Body mass index (BMI); Waist-to-hip ratio (WHR); — Missing

UE showed positive genetic correlations with both BMI (p=6.4 × 10^{-6}) and WHR (p=0.002). These were statistically significant after correction for multiple testing (Bonferroni corrected p-value for 6 tests <0.02) (**Table 7.3**). The low heritability estimates for EE and CR impacted the analysis and LDHub was not able to estimate the genetic correlation between EE and WHR, or CR and BMI. EE and CR did not show evidence of genetic correlation with either BMI or WHR.

7.4.5 Mendelian randomisation analyses of BMI to EB

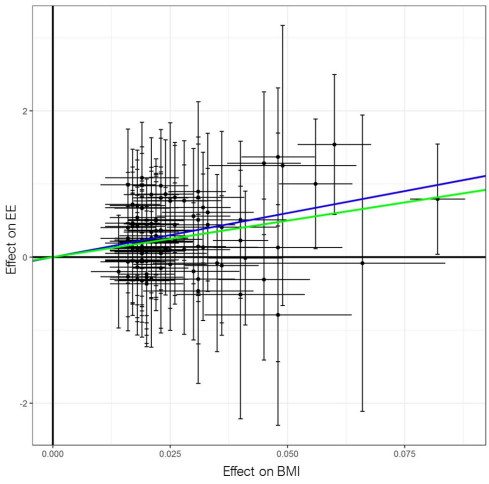
Using results from the present GWAS meta-analysis alongside those from the 2015 Locke et al. meta-analysis of BMI GWAS studies [149], we conducted a uni-directional MR of BMI to EB. Given that no genome-wide significant loci for EB were identified, bi-directional MR was not possible. The 96 bi-allelic SNPs identified in the BMI meta-analysis were included alongside their European-only sex-combined effect estimates and SEs. Effect estimates and SEs from the current study were also taken from the sex-combined analysis. SNPs were aligned to the BMI-increasing allele. The results are shown in **Table 7.4** and **Figure 7.3**.

Table 7.4 Mendelian randomisation analyses of BMI to eating behaviour

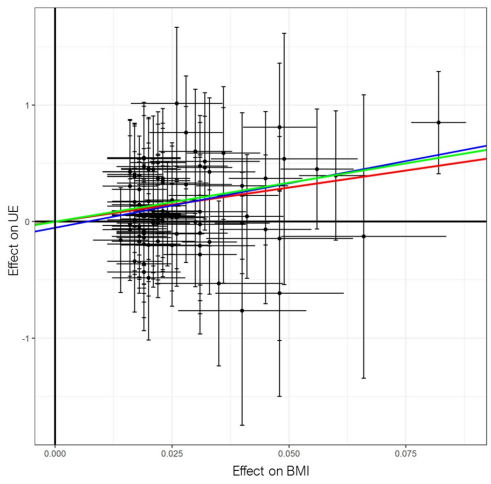
Analysis	Beta (SE)	p-value
BMI to Emotional eating		
Conventional MR (IVW)	12.03 (1.64)	<0.00001**
MR Egger	12.07 (4.03)	0.003**
Weighted Median MR	9.99 (2.86)	0.0005**
BMI to Uncontrolled eating		
Conventional MR (IVW)	5.85 (1.09)	<0.00001**
MR Egger	7.62 (2.69)	0.005**
Weighted median MR	6.66 (1.57)	0.00002**
BMI to Cognitive restraint		
Conventional MR (IVW)	6.50 (1.14)	<0.00001**
MR Egger	8.01 (3.04)	0.009*
Weighted median MR	7.16 (1.85)	0.001**

Body mass index (BMI); Mendelian Randomisation (MR); Inverse-weighted variance (IVW); Standard error (SE).
Beta units are genetically predicted change in TFEQ-R18 EB score per genetically predicted 1 unit increase in BMI (BMI units from Locke et al are inverse normally transferred residual BMI measurements)
* Nominally significant (p<0.05)
** Bonferroni significant after adjustment for 9 tests (p<0.009)

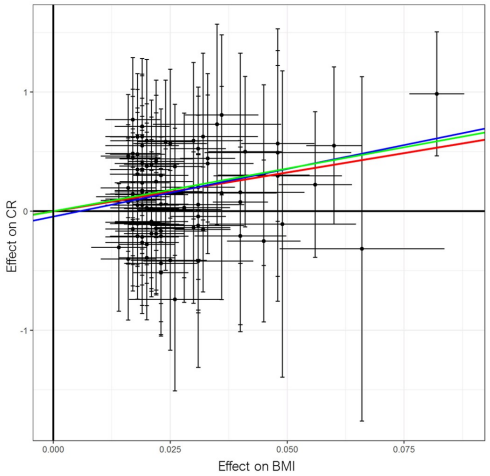
For each of the EB traits, the three MR analyses conducted showed nominally significant positive associations between BMI and EB (p<0.05). Eight of the 9 analyses were also significant at a Bonferroni p-value corrected for 9 tests (p<0.006). The one exception to this was the MR Egger for BMI to CR, which was not significant at this level (p=0.009). The MR Egger is the least powered of the MR analyses.



(a) Emotional eating



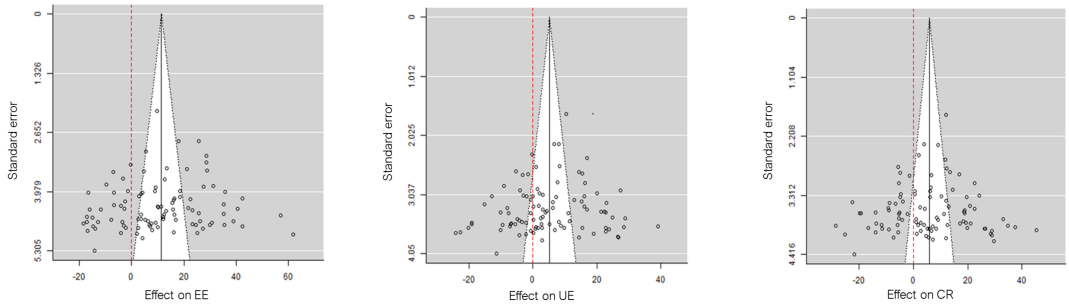
(b) Uncontrolled eating



(c) Cognitive restraint

Figure 7.3 Dosage plots showing the results of the Mendelian randomisation analyses of BMI to EB. Each dot represents one of the 96 BMI-associated SNPs, 95% CIs are represented by black lines. The effect of each SNP on BMI on the x-axis is plotted against its effect on EB on the y-axis. The coloured lines represent the MR results. Red represents the IVW MR, blue represents the MR Egger and green represents the weighted median MR. All three MRs are present on each plot. However, where results overlap, some lines are not visible.

The between-SNP heterogeneity estimate derived from the IVW MR analyses using Cochran's Q statistic was significant for all the EB traits (EE: p=5.6 × 10^{-245}; UE: p=9.3 × 10^{-221} ; CR: p=7.0 × 10^{-250}). Thus, despite the aggregated positive association, some BMI-increasing alleles are negatively associated with particular EB traits. This may reflect horizontal pleiotropy or lack of precision in the effect estimates for EB due to the low sample size and power of the EB GWAS meta-analysis. Given this uncertainty, at these levels of heterogeneity, only the weighted median MR analysis can be considered robust.



(a) Emotional eating **(b)** Uncontrolled eating **(c)** Cognitive restraint

Figure 7.4 Funnel plots showing heterogeneity in the association between the 96 bi-allelic BMI-associated SNPs and the EB traits. Each data point represents one of the 96 BMI-associated SNPs. The SNP-specific MR estimate for the association of BMI with EB (x-axis) is plotted against the SE of this association (y-axis). The summary estimate for all 96 SNPs combined is marked by the solid black line. The grey-dotted lines, originating from the summary estimate and marking a triangle, represent the expected 95% CIs of the combined effect estimate. The vertical red-dotted line indicates the null.

7.4.6 BMI-associated SNPs and eating behaviour traits

To depict the effect of BMI-associated SNPs on EB in more detail, a heat map was constructed. The map displays the associations between the 96 bi-allelic BMI-associated SNPs with EE, UE and CR (**Figure 7.5**). SNPS were aligned to the BMI-increasing allele.

The primary clustering of EB traits on the x-axis separated CR from the appetitive traits (EE and UE). The primary clustering of SNPs on the y-axis separated a group of 14 SNPs associated with an increase in all three EB traits from the remaining 82 SNPs. A second group of 18 SNPs was identified that are positively associated with UE and, to a lesser extent, EE, but are either negatively or neutrally associated with CR. The remaining 64 BMI-associated SNPs show weak associations with EB. These findings support the results of the MR analyses, highlighting heterogeneity in the influence of BMI-associated SNPs on EB.

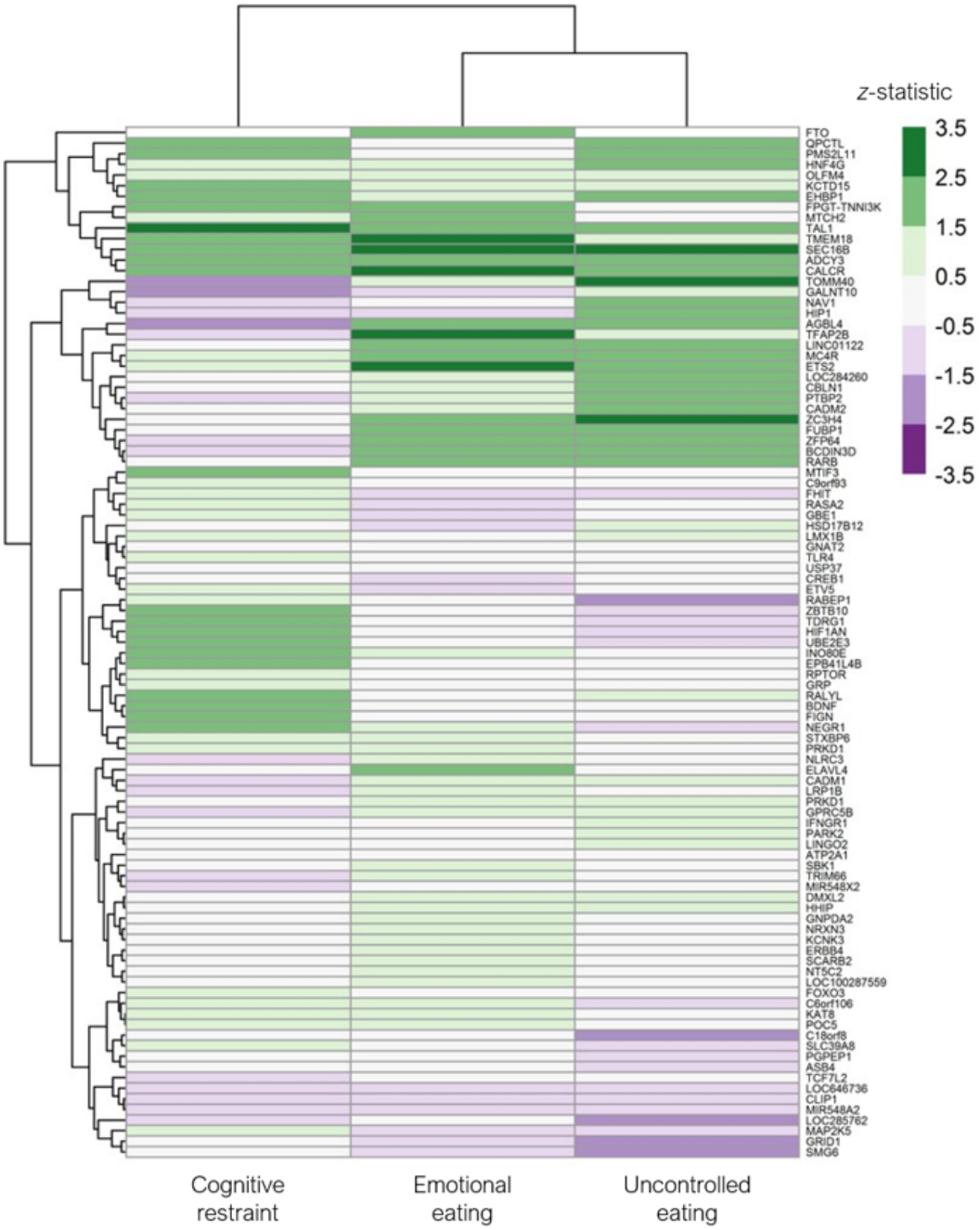


Figure 7.5 Heat map of the 96 bi-allelic BMI-associated SNPs clustered by their associations with the EB traits. The values and colour-coding indicate the *z*-statistic (Beta/SE) from the age and sex-adjusted linear regression of each SNP on the standardised EB traits (mean=0; SD=1).

7.4.7 Mendelian randomisation analyses of risk-taking to EB

Using results of the present GWAS meta-analysis alongside those from the GWAS of risk-taking reported in **Chapter 6**, a uni-directional MR of risk-taking to the EB traits was conducted. The 26 SNPs which showed genome-wide associations with risk-taking in **Chapter 6** were included. The results are shown in **Table 7.5** and **Appendix B.7**.

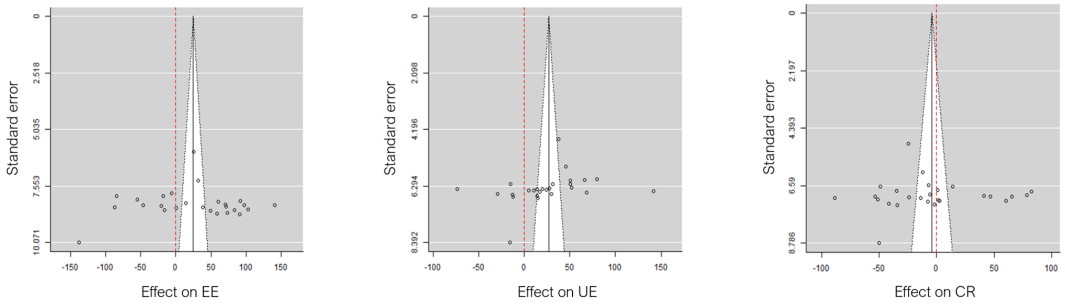
Table 7.5 Mendelian randomisation analyses of risk-taking to eating behaviour

Analysis	Beta (SE)	*p*-value
Risk-taking to Emotional eating		
Conventional MR (IVW)	25.0 (13.1)	0.06
MR Egger	9.49 (60.7)	0.88
Weighted Median MR	29.1 (19.2)	0.13
Risk-taking to Uncontrolled eating		
Conventional MR (IVW)	29.0 (7.61)	0.0001**
MR Egger	110.4 (35.0)	0.002**
Weighted Median MR	30.6 (10.8)	0.005**
Risk-taking to Cognitive restraint		
Conventional MR (IVW)	−5.07 (9.04)	0.58
MR Egger	−30.6 (41.7)	0.46
Weighted Median MR	−10.2 (13.3)	0.44

Mendelian Randomisation (MR); Inverse-weighted variance (IVW); Standard error (SE)

** Bonferroni significant after adjustment for 9 tests (p<0.009)

The IVW, MR Egger and weighted median MRs showed statistically significant, positive associations between risk-taking and UE at the Bonferroni corrected p-value threshold for 9 tests (p<0.006). The MR analyses of risk-taking to EE and CR did not yield significant results. The p-values for the IVW MR were p=0.06 and p=0.58, respectively. A significant degree of between SNP heterogeneity was detected in all of the IVW analyses (EE: $p < 1 \times 10^{-200}$; UE: $p = 2.9 \times 10^{-200}$; CR: $p = 2.5 \times 10^{-193}$). This was unsurprising given that 4 of the SNPs included in this analysis are also genome-wide significant for BMI (**Chapter 6**) and BMI SNPs demonstrate heterogeneous associations with EB (**Section 1.4**). Individual risk-associated SNPs were associated with higher or lower levels of EB. **Figure 7.6** displays the large SNP effect estimates for EB, likely the result of low precision, as well as between-SNP heterogeneity.



(a) Emotional eating **(b)** Uncontrolled eating **(c)** Cognitive restraint

Figure 7.6 Funnel plot showing heterogeneity in the association between the 26 risk-associated SNPs and the eating behaviour traits. Each data point represents one of the 26 risk-associated SNPs. The SNP-specific MR estimate for the association of risk-taking with EB (x-axis) is plotted against the SE of this association (y-axis). The summary estimate for all 26 SNPs combined is marked by the solid black line. The grey-dotted lines, originating from the summary estimate and marking a triangle, represent the expected 95% CIs of the combined effect estimate. The vertical red-dotted line indicates the null.

7.5 Discussion

7.5.1 Summary and context of the main findings

Here we report the results of the first genome-wide gene discovery study of EE, UE and CR. The genetic basis of these traits was assessed in GWAS meta-analyses amongst over 11,500 white European adults from 4 study cohorts.

No genetic variants showing genome-wide significant associations with any of the EB traits were identified. However, UE was estimated to be 11% heritable (95% CI: 3%, 19%) and demonstrated a shared genetic basis with both BMI and WHR. This estimate is comparable to the heritability estimate of 8% (95% CI: 7%-9%) for risk-taking estimated from the UKB sample in **Chapter 6** and is generally in line with chip heritability estimates for other behavioural and personality traits estimated on the observed scale [391, 392]. As an increasing number of studies collect intersecting EB and genome-wide genotype information, it is likely that larger investigations will be powered to detect specific associations for UE in the future. The heritability estimates for both EE and CR were negligible at 2% (95% CI: −8%, 12%) and 1% (95% CI: −7%, 9%), respectively. In light of the fact that the majority of twin studies in adulthood suggest a genetic basis for these traits, this finding was unanticipated [66, 124]. Given the wide 95% CIs, it is likely that the analysis lacked the power to detect a genetic basis for these traits. However, it is also plausible that EE and CR are primarily determined by environmental factors in adulthood. Twin studies suggesting that emotional over and under-eating in early childhood are learnt behaviours provide some tentative support for this suggestion with respect to EE [193, 385, 194].

EE and UE show consistent, positive phenotypic correlations across a range of studies, leading some researchers to assert that they reflect a single underlying construct [185]. **Chapter 4** reported that EE does not mediate the association between the BMI-GRS and BMI independently of UE, suggesting that the elements of EE relevant to the genetics of BMI might be captured by UE. However, EE and UE showed no evidence of genetic correlation in the present analysis. The analysis was under-powered due to the low heritability estimate for EE, and should not be considered conclusive evidence of no genetic overlap. However, it does not directly support the assertion that the traits reflect a single construct from a genetic perspective [185].

EB traits have typically been considered to be a cause, rather than a consequence, of obesity and have been modelled as such throughout the studies that comprise this thesis. In the MR analyses, BMI-increasing alleles were, in aggregate, positively associated with all of the EB traits, suggesting that BMI might play a causal role in EE, UE and CR. The main MR analyses were supported by the results of MR Egger and weighted median MR, adding weight to this finding. In the case of CR, this interpretation supports mounting evidence that high BMI leads people to consciously limit their food intake and thus that CR is a response to weight

status, rather than risk-factor for weight gain [51]. However, if, as other studies, including **Chapter 4**, have suggested, some BMI-associated loci are linked to BMI through a primary association with appetitive EB traits, the same MR results would be expected. In the absence of robust genetic instruments to model EB traits, all that can be robustly concluded from these results are that EB and BMI lie on the same causal pathway. Overall, in the absence of reliable genetic proxies for EB, the conclusions that can be drawn from the MR analysis are limited.

Not all BMI-associated loci were associated with EB in the anticipated ways. Whilst BMI-increasing alleles at some loci show the anticipated positive associations with the EB traits, others show neutral or negative associations with one, or all, of the EB traits. Notwithstanding power limitations, this suggests that whilst some of the pathways involved in BMI may be relevant to EB, others are likely to be unrelated and may instead reflect different aspects of health-related physiology or behaviour. Taken together with the MR analyses reported in **Chapter 6**, which indicated a high level of heterogeneity in the influence of individual risk-associated SNPs with BMI, these results suggest that behavioural traits have complex associations with BMI. Better powered studies are needed to provide more precise SNP effect estimates for EB, diminishing the possibility that the MR findings are the result of error.

The findings reported in **Chapter 6** suggested that risk-taking may influence food intake, with risk-prone individuals consuming more calories per day [339]. To explore the possibility of a link between risk-taking and EB traits, we performed MRs of risk-taking to the EB traits. These analyses demonstrated a significant positive association between risk-associated SNPs and UE. Although individual SNPs showed evidence of heterogeneity, the overall association persisted in sensitivity analyses, suggesting a true, causal association. No association between risk-taking and EE or CR was identified. This contrasts to the results reported in **Chapter 6** which suggest a causal role for risk-taking in EE using a GRS approach, but detected no association with UE. The present analysis included a greater sample size which would be expected to increase the power of the analysis and may explain differences in the UE results between the studies. It may be that the risk to EE association reported in **Chapter 6** is spurious. However, the sparse genetic profile of the EB traits was used to reflect EB in the MR analysis and this is likely to be weak relative to the phenotypic measure used in **Chapter 6**, particularly for EE for which no genetic basis was identified in the present study. As such, it remains plausible that risk-taking is causally involved in appetitive EB. Further research is needed to provide clarification.

7.5.2 Strengths and limitations

This study represents the only genome-wide discovery effort for EE, UE and CR to date. An acceptable GWAS sample size was achieved by combining data from 4 separate cohorts. A

substantial strength of the study was that all the cohorts used the same, widely used and validated questionnaire (the TFEQ-R18) to measure three well studied EB traits. Further, the analysts for each study followed a pre-defined protocol, ensuring the harmonisation of procedures. This controlled for issues including population stratification by restricting the sample to those of white European ancestry. The results of the individual GWAS studies underwent rigorous central QC prior to meta-analysis. Although the study did not detect any associations between genetic loci and EB, the results were used in downstream analyses providing insights into the relationship between EB and obesity, WHR and risk-taking.

A significant limitation of the present analysis was its sample size. Whilst the study included over 11,500 participants, by the standards of contemporary GWAS, and particularly GWAS meta-analyses, it was not a large study and was under-powered to detect specific variant associations. In part, the sample size was limited by the range of methods used to assess EB and the lack of clarity regarding how they relate to each other. These include different questionnaires (such as the DEBQ and TFEQ-51), as well as laboratory-based measures. The study was restricted to cohorts measuring EB using the TFEQ-R18 or TFEQ-R21 and required that both EB and genome-wide genetic information were present in the same cohort. A final note pertains to the age of the participants which varied from a mean of 22.4 years in the FinnTwin12 cohort to a mean of 74.3 years in the HPFS cohort. Given evidence that the heritability of behavioural traits can change with age [393], this may have limited the power of the study to detect associations relevant at different points of adulthood.

7.5.3 Conclusions

This study was designed to identify the genetic basis of EE, UE and CR and use these results to elucidate the biological pathways involved in EB, as well as to interrogate the relationship between EB and health. No specific genetic variants were identified in association with any of the EB traits and the analysis was likely under-powered due to low sample size. However, the results indicate that UE is heritable and shares a genetic basis with both BMI and WHR. Future studies, including a greater number of participants, will be better powered to identify specific associations for UE and may also detect a genetic basis for EE and CR.

CHAPTER 8

DISCUSSION, CONCLUSIONS AND IMPLICATIONS

8.1 Summary of the aims, rationale and methods

The central aim of this thesis is to advance understanding of the relationship between eating behaviour (EB) and the aetiology of obesity. Within the remit of this over-arching goal, three inter-related research aims were identified. These were to explore: (1) the role of EB in genetic predisposition to obesity, (2) the interaction between infant EB and modifiable maternal attitudes to following healthy infant feeding guidelines on infant body weight and milk intake and (3) the genetic basis of behaviours relevant to obesity. The relationship between these aims is depicted in **Figure 8.1** and elaborated below. The dashed lines and shading represent novel associations investigated and reported in this thesis. The solid lines represent previously established associations replicated within specific chapters.

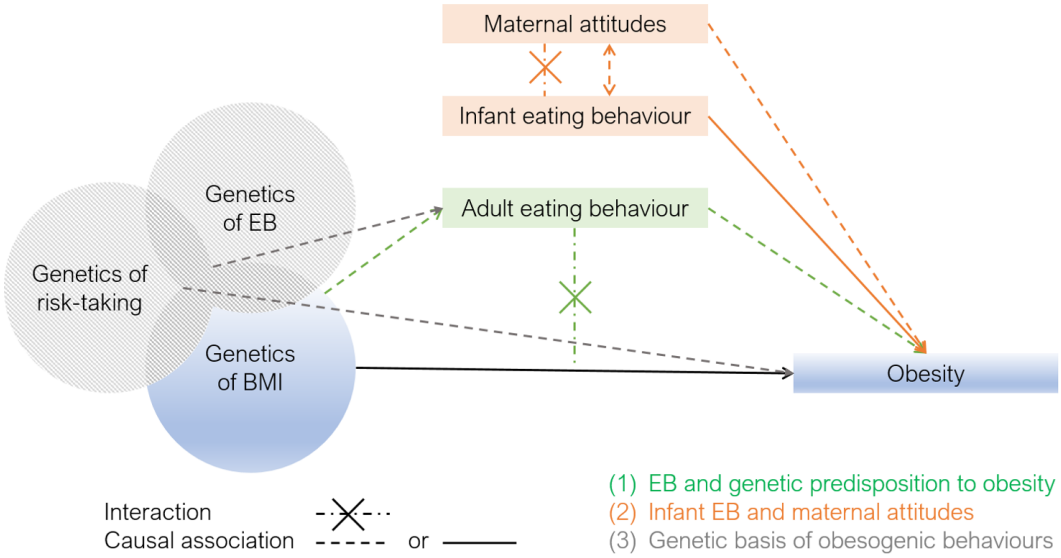


Figure 8.1 Diagrammatic representation of the aims of the thesis. Aim 1 is represented in green, Aim 2 in orange and Aim 3 in grey. The diagram is not intended to represent all relationships between the included variables, but depicts those of central relevance to this thesis. All associations (indicated by arrows or lines) were analysed within the thesis. The dashed lines and arrows indicate novel contributions to the literature, whilst the solid lines represent established associations replicated in this work. Arrows represent hypothesised causal associations, whilst lines represent interactions. Under Aim 3, GWAS were performed to elucidate the genetic basis of EB traits and risk-taking propensity. Genetic variants involved in the determination of BMI were taken from a previous GWAS meta-analysis [149] and used across the individual studies comprising this thesis.

8.1.1 Aim 1: The role of EB in genetic predisposition to obesity

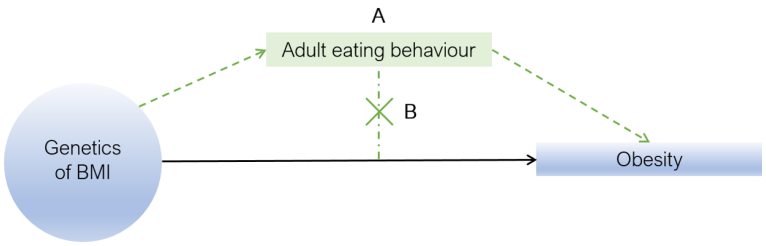


Figure 8.2 The role of EB in genetic predisposition to obesity. Under this aim, adult EB traits measured by the TFEQ-R18 (emotional eating (EE), uncontrolled eating (UE) and cognitive restraint (CR)) were modelled as potential mediators (**A**) and modifiers (**B**) of genetic susceptibility to obesity. Genetic variants involved in the determination of BMI were taken from a previous GWAS meta-analysis [149].

Pathway and tissue expression analyses of BMI GWAS results, candidate gene studies and evidence from monogenic obesity syndromes together suggest that EB traits lie on the causal pathway between genetics and obesity [149, 150]. Prior to the work reported in **Chapter 4**, two previous studies in adult populations had directly tested this assertion by modelling appetitive adult EB traits (emotional eating (EE) and uncontrolled eating (UE)) as potential mediators of the association between a genetic risk score for BMI (BMI-GRS) and measured BMI. Both found that these traits partially mediate the association [112, 161]. However, there were several limitations to these investigations. Both studies relied on the untested assumption that the BMI-GRS reflects adiposity pathways, included a limited number of the 97 known BMI-related genetic variants in the BMI-GRS (32 and 90, respectively) and only modelled EB traits as mediators of genetic predisposition to obesity. Further, the first study modelled the EB traits as mediators in the same model and thus was unable to differentiate between the separate effects of the traits [112]. The second study reported conflicting results for UE, finding that it mediated the BMI-GRS to BMI association in one study cohort, but not the other [161].

Chapters 3 and **4** were designed to address these gaps in the literature and to inform a more complete understanding of the relationship between EB and genetic predisposition to obesity. BMI-GRSs, summarising the combined effect of BMI-related genetic variants on BMI, reflect all genetic pathways involved in the determination of obesity that current GWAS have the power to detect. **Chapter 3** interrogated the assumption that genetic predisposition to obesity, expressed by a BMI-GRS comprised of the 96 biallelic BMI-related variants reported by Locke et al. [149], can be used to understand adiposity pathways, such as EB. The relationship between the BMI-GRS and both anthropometric traits and body composition was analysed in sex-stratified, age-adjusted linear regression models in the Fenland cohort (n=9667).

Chapter 4 then modelled the appetitive EB traits (EE and UE) alongside cognitive restraint (CR), as potential mediators and modifiers of genetic predisposition to obesity separately in the Fenland (n=3515) and EDEN (n=2154) studies. Using a 96 SNP BMI-GRS, the Sobel test and mediation ratio were used to identify and quantify mediation, whilst statistical interaction analyses were used to detect effect modification. This study included a greater number of BMI-related genetic variants than previous investigations and represented the first time that CR was modelled as a mediator of genetic predisposition to obesity and that modification of BMI-GRS to BMI association by EB traits had been considered.

8.1.2 Aim 2: The interaction between infant EB traits and modifiable maternal attitudes on infant milk intake and body weight

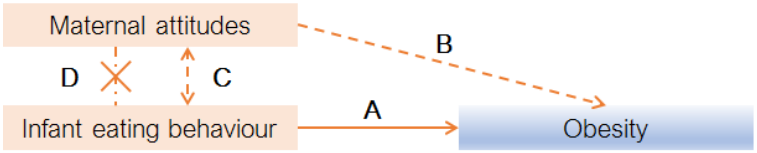


Figure 8.3 Infant EB traits and maternal attitudes to following healthy infant feeding guidelines. Under this aim, the association between two infant EB traits measured by the BEBQ (food responsiveness (FR) and satiety responsiveness (SR)) and both infant milk intake and body weight was described (**A**), alongside the separate association between maternal attitudes to following healthy infant feeding guidelines and these outcomes (**B**). The association between maternal attitudes and infant EB was then modelled (**C**) and, finally, the interaction between infant EB and maternal attitudes on infant milk intake and body was interrogated (**D**).

The first 1000 days from conception to 2 years are considered a critical period for development of obesity risk and consistent evidence links infant weight trajectories during this period to lifetime obesity [37, 165]. As such, infancy represents an important developmental period during which obesity prevention has the theoretical potential to be particularly effective. However, the determinants of infant weight gain and status, and the relationships between them, are incompletely understood. Under Aim 2, the role of infant EB traits and maternal attitudes to following healthy infant feeding guidelines in determining infant milk intake and body weight was investigated.

Infant EB traits are known, heritable influences on weight in early life [104, 141, 139, 143]. Associations between parental factors, such as feeding styles, and infant weight gain have also been demonstrated primarily through intervention studies that target parental feeding behaviours [183]. Whilst infant and parental factors have separately been implicated in weight outcomes, prior to the work reported in **Chapter 5**, the impact of maternal attitudes to infant feeding and the interactions between infant and maternal factors had not been explored. This is particularly important because whilst infant EB traits cannot yet be modified by interventions, parental behaviours and attitudes are modifiable.

Chapter 4 introduced the notion that behaviours extraneous to the causal pathways between determinants of BMI and realised weight status can protect vulnerable individuals from obesity. In particular, restraint over eating (measured by CR) was shown to interact with genetic susceptibility to obesity, attenuating its effect on realised BMI. This evidence indicates that restriction over eating may intervene in pathways to obesity. As such, it was hypothesised that it may be possible to protect infants with an appetitive EB profile from obesity by targeting parental factors that promote control over infant consumption in accordance with healthy guidelines. In **Chapter 5**, the association between infant EB traits and both infant milk intake and weight was described amongst the Baby Milk Trial participants (n=669). Further, a score reflecting maternal attitudes to following healthy infant feeding guidelines was generated based on a recently designed self-report questionnaire [323]. The associations of the maternal attitudes score to these infant outcomes was also described. The interaction between infant EB traits and the maternal attitudes score was then assessed using an interaction term added to the separate age and sex-adjusted linear regression of these traits on infant milk intake and body weight.

8.1.3 Aim 3: The genetic basis of behaviours associated with obesity

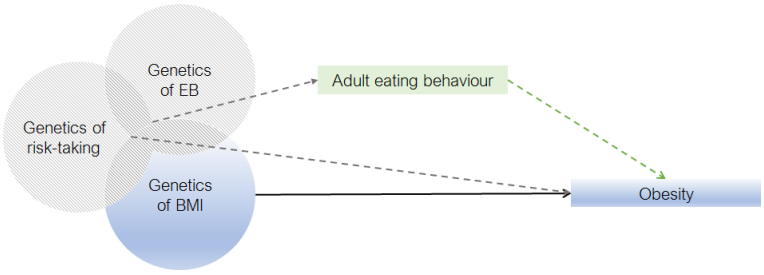


Figure 8.4 The genetic basis of behaviours associated with obesity. Under this aim, the genetic basis of risk-taking propensity and EB traits measured by the TFEQ-R18 (EE, UE and CR) was investigated through GWAS. In downstream analyses, the genetic correlation between these phenotypes, as well as between the phenotypes and BMI, was performed and Mendelian randomisation (MR) was used, where possible, to interrogate causal associations.

A range of behaviours have been implicated in the aetiology of obesity. However, the biological mechanisms underlying these associations, as well as the directions of association, have yet to be conclusively determined. In the case of EB, the role of CR is particularly debatable. Whilst CR was initially conceptualised as a problematic EB, recent studies including **Chapter 4** suggest that it may represent a response, as opposed to a cause, of susceptibility to obesity [301]. In order to advance understanding of the mechanisms and causality in associations between behaviour and obesity, GWAS of risk-taking propensity (**Chapter 6**) and EB traits (EE, UE and CR) in adulthood (**Chapter 7**) were performed. Risk-taking propensity was selected as a phenotype of interest on the basis of an existing body of literature linking risk-taking to health and health-related behaviours, notably including

both obesity and EB. This literature is summarised in **Chapter 6**. Alongside the central aim of exploring the mechanisms of the relationship between risk-taking and obesity, this study was also designed to confirm the utility GWAS to the study of behaviour and obesity.

Prior to the work reported in this thesis, no GWAS studies of EE, UE or CR had been conducted. In part, this reflects the limited availability of intersecting EB and genome-wide genotype information. Lack of knowledge regarding the genetic basis of these traits limits understanding of the biological pathways involved in EB, as well as the biological distinction or overlap between EB traits. The lack of genetic characterisation also precludes Mendelian randomisation (MR) analyses based on genetic variants which could be used to elucidate the associations between EB, weight and other health outcomes.

A GWAS meta-analysis of the EB traits measured by the TFEQ-R18 (EE, UE and CR) was facilitated by combining data from 4 cohorts of white European ancestry (n>11,500 for each EB trait). The study was conceived to explore the biological pathways involved in EB and their relationship to obesity. Work published during the progress of this thesis added additional interest to the findings. First, twin studies indicating that emotional under and overeating amongst toddlers and young children are not substantively heritable added intrigue to the heritability estimates for EE in adulthood [385, 193, 194]. Further, **Chapter 4** suggested that EE and UE might influence BMI through overlapping pathways, contributing to ongoing debate regarding the distinction between these traits. Some researchers now argue that they reflect a single underlying construct [185]. Understanding the genetic determinants of EE and UE would provide insights into their underlying biology, contributing to the debate on their independence.

8.2 Summary of the main findings

8.2.1 Aim 1: The role of EB in genetic predisposition to obesity

8.2.1.1 Genetic susceptibility to obesity reflects adiposity to a greater extent than lean or bone mass

In **Chapter 3**, amongst 9667 participants from the Fenland study, the BMI-GRS comprised of 96 biallelic BMI-associated genetic variants [149], was associated with adult body composition and anthropometric measures, such as height and waist-to-hip ratio (WHR), in ways that mirror the associations between measured BMI and these traits. In particular, the BMI-GRS reflected variation in adiposity to a greater extent than either lean or bone mass, and was not associated with height or body fat distribution. The results were consistent amongst both men and women. A total of 26 SNPs included in the BMI-GRS demonstrated nominally significant associations with BMI in this study sample. Approximately half of these showed adipose-specific effects, whilst the remainder were associated with a global increase in fat, lean and bone mass. The study showed that a BMI-GRS, based on all known, biallelic BMI-associated genetic variants (n=96), can be used to reflect genetic predisposition to total adiposity in white, European populations of both sexes. This supports the utility of the score as a tool to examine the causal effect of adiposity and measured BMI on outcomes of interest in future studies. It also supports the use of the BMI-GRS as tool to investigate the mechanisms of adiposity pathways, such as EB.

8.2.1.2 Appetitive EB traits mediate genetic predisposition to adult obesity

Chapter 4 built directly upon these findings. The results indicated that the appetitive EB traits, EE and UE, partially mediate the association between the BMI-GRS and BMI amongst adult participants from the Fenland and EDEN study cohorts (n=3515 and 2154, respectively). By contrast, in 3 of 4 population groups studied (Fenland men and women and EDEN men), CR was not found to be a mediator of the association, suggesting that the mediation effect is particular to appetitive EB traits. It is unclear why EDEN women demonstrated different results from the other three population groups studied. Subsequent work in other cohorts has also suggested that CR does not mediate genetic predisposition to obesity [311, 138]. Thus we do not conclude that CR was a true mediator, even amongst EDEN women. Adjusting for both EE and UE in the same model provided evidence for overlap in the pathways through which these traits mediate genetic predisposition to obesity. Specifically, following adjustment for UE, EE was no longer a mediator in either cohort. Given that the appetitive EB traits together mediated just a portion (~10%) of the relationship between the BMI-GRS and BMI, the results support the involvement of other pathways. These may reflect unmeasured EB traits, other behaviours or non-behavioural pathways.

8.2.1.3 Cognitive restraint modifies genetic predisposition to adult obesity

Amongst three of the 4 population groups studied (Fenland men and women, as well as EDEN men), CR modified the association between the BMI-GRS and BMI. At high levels of CR, the positive association between the BMI-GRS and BMI was attenuated. Coupled with the mediation results, these findings indicate that EB traits have diverse associations with genetic predisposition to obesity and that CR is distinct from EE and UE. Whilst the appetitive traits likely lie on the causal pathway, CR may interact with genetic predisposition to influence realised weight.

8.2.2 Aim 2: The interaction between infant EB traits and modifiable maternal attitudes on infant milk intake and body weight

8.2.2.1 Infant EB traits and maternal attitudes to following healthy infant feeding guidelines are separately associated with infant milk intake and body weight

In **Chapter 5**, infant food responsiveness (FR) was positively associated with infant milk intake and body weight amongst 2 month old infants enrolled in the Baby Milk Trial (n=669). Further, infant satiety responsiveness (SR) was negatively associated with both outcomes. Maternal factors were also shown to be important. Specifically, maternal attitudes to following infant healthy feeding guidelines, summarised as a single score reflecting the strength of intentions to follow healthy feeding guidelines, feelings of self-efficacy in following guidelines and the expectation of positive results, were negatively associated with infant milk intake and body weight.

8.2.2.2 The association of infant EB traits to infant milk intake and body weight is modified by maternal attitudes during a critical period of development

This built upon **Chapter 4**, drawing upon the conclusion that obesity determinants can interact to influence BMI. Thus, the impact of unmodifiable determinants, or those for which interventions have yet to be developed, can be altered by interventions targeting modifiable traits. Maternal attitudes to following healthy infant feeding guidelines interacted with infant EB traits, modifying their association with milk intake and weight. Positive maternal attitudes reduced the magnitude of the positive association between infant FR and milk intake, as well as the magnitude of the negative association between infant SR and body weight. The maternal attitude score was not associated with infant EB. These results provide evidence that modifiable maternal factors, that do not influence infant EB traits directly, can modify the effect of infant EB traits on health-related outcomes in early life and have the potential to prevent high weight, as well as weight faltering. The findings require replication and longitudinal analyses are needed to explore relationships over time.

8.2.3 Aim 3: The genetic basis of behaviours associated with obesity

8.2.3.1 GWAS provide insights into the relationship between behaviour and obesity

Risk-taking propensity. A total of 26 genetic variants associated with risk-taking propensity were identified, 24 of which were novel. Together, these variants show enriched expression in both the CNS and immune system. Enrichment in the CNS implicates behavioural pathways. However, future studies are required to clarify the role of the immune system in risk-taking. In contrast to a previous study in a smaller, overlapping sample [242], the genetic correlation between risk-taking and adult obesity did not reach statistical significance at the Bonferroni corrected level in our study, suggesting that these traits may not share a substantive genetic basis. However, this may vary by age as there was a genetic correlation between risk-taking and childhood obesity. Bi-directional MR provided evidence that BMI does not influence risk-taking. Thus, correlations between risk-taking and BMI observed in epidemiological studies are likely driven by risk-taking. However, the association is complex and results from shared biological pathways, some of which operate in opposing directions, as opposed to a single, causal mechanism. It seems likely that different aspects of risk-taking propensity are associated with BMI in different ways. Future studies are needed to refine measurements of risk-taking such that the behaviours relevant to health, and their biological pathways, can be identified.

Eating behaviour traits. Having established that a GWAS approach could be used to advance understanding of the relationships between behaviour and obesity in **Chapter 6**, **Chapter 7** reported the results of the first GWAS of EE, UE and CR. No specific genetic variants were identified. The study was likely under-powered and highlights the need for large GWAS sample sizes to detect associations for behavioural traits. However, the results suggested that UE is a heritable trait. Downstream analyses further indicated that UE shares a genetic basis with both BMI and WHR, and that it is positively influenced by risk-taking propensity.

8.2.4 Summary

Overall, the findings indicate that different aspects of EB have different relationships to genetic susceptibility to obesity in adulthood. Whilst appetitive EB traits lie on the causal pathway between genes and BMI, restraint may modify these innate pathways. In infancy, the impact of EB traits on milk intake and weight can also be modified, as illustrated by the interaction between maternal attitudes and infant EBs on these outcomes. Well-powered gene discovery studies of obesity-related behaviours have the potential to provide insights into the mechanisms underlying the associations between behaviour and obesity. However, large sample sizes are required to identify specific genetic variants. Risk-taking propensity and obesity likely share biological pathways, and UE shares a genetic basis with BMI.

8.3 Implications and future research

The results reported in **Section 8.2** have implications for obesity prevention, the conceptualisation of EB traits and future research. These are discussed in turn in the following section.

8.3.1 Implications for obesity prevention

Foremost, the results of **Chapters 4** and **5** together highlight the likely causal associations between appetitive EB traits and obesity in both infancy (FR and SR) and adulthood (EE and UE). They thus suggest that these EB traits provide a target for obesity prevention. Another central conclusion of the work is that the impact of obesity determinants that are either unmodifiable or that we do not yet know how to change, can be altered throughout the life-course, without direct or invasive intervention designed to change the pathways themselves. The results of **Chapter 4** show, for the first time, that CR modifies genetic predisposition to obesity. This indicates that CR represents a potential target for obesity prevention in adulthood that may be of particular benefit to individuals who are genetically susceptible to obesity. The findings corroborate a mounting body of evidence suggesting that CR does not lead to weight gain, as initially hypothesised, but may instead be beneficial to obesity prevention [51]. However, future research is needed to clarify the prospective relationship between CR and weight. In particular, whether CR can be used to prevent weight gain, to promote weight loss or both. Further, given the quadratic association between CR and BMI observed in **Chapter 4**, it is important to determine if CR has different prospective associations with BMI across the BMI spectrum. Modifiable environmental determinants of CR, as well as the factors, such as dichotomous thinking, that differentiate CR from dieting and make it a better tool for controlling weight, also require identification [394, 395].

The results of **Chapter 5** indicate that maternal attitudes to following healthy infant feeding guidelines could also represent an intervention target with the potential to reduce the impact of infant EB traits on milk intake and weight. The maternal attitudes explored in this thesis were strengthened amongst mothers in the intervention arm of the Baby Milk Trial, indicating that they are amenable to change and providing direction as to how this may be achieved [259]. However, the findings require replication in other cohorts, as well as amongst breastfed infants and infants of different ages. Wider use of the maternal attitudes questionnaire would facilitate this.

8.3.2 Theoretical implications

The work described in this thesis contributes to several ongoing discussions in the EB literature. These are elaborated here.

8.3.2.1 Behaviour and genetic predisposition to obesity

Beyond confirming that appetitive EB traits mediate genetic predisposition to obesity in adulthood, the results highlight other, novel relationships between EB and the genetics of obesity. In particular, the results suggest that CR interacts with the BMI-GRS. Whilst this requires replication in other cohorts, it indicates that to fully understand the relationship between EB and the genetics of obesity, there is a need to explore associations beyond mediation. The mediation results from our analysis are consistent across a number of studies [112, 161, 311]. However, no investigations have previously or subsequently explored effect modification. A 2018 study of the relationship between EB and the BMI-GRS to BMI relationship, published after the investigation reported in **Chapter 4**, again only assessed mediation [311]. The EB questionnaires developed through the 1970s and 1980s were designed to better understand the EB of obese individuals and leave a legacy of focus on obesogenic EB traits which may restrict research if the potential limiting effect of certain EB traits on weight is not actively considered.

8.3.2.2 Behaviour and complex associations with obesity

Chapters 6 and **7** both highlight that the biology of behavioural phenotypes is highly complex. Single number summaries of complex behaviours are likely to mask multiple biological pathways, which may have different, and even opposing, associations with specific health outcomes. Previous research has also shown that sub-types of impulsivity are associated with UE in different ways [396] and divergent associations between different aspects of CR (e.g. rigid versus flexible restraint) and BMI have been demonstrated in a separate study [311]. This study found that flexible restraint was negatively associated with BMI, rigid control demonstrated a positive association and other aspects of restraint showed no evidence of association [311]. Together, these findings suggest that associations between crudely measured behavioural traits and health-related phenotypes, such as BMI, might mask the importance of specific pathways which can only be isolated through greater scrutiny. Efforts should be made to identify the particular aspects of behaviour that are driving associations such that interventions can be optimised. Moreover, behavioural measures should be subject to continued scrutiny. For example, analysing the factor structure of the maternal attitudes questionnaire in **Chapter 5** indicated that the items measured a single construct, rather than three distinct attitudes. In instances where there is clear evidence that questionnaire measures do not reflect biological reality, and are instead conflating several biologically distinct phenotypes or unnecessarily separating measures of the same underlying trait, questionnaires should be revised and refined.

8.3.2.3 The understanding of eating behaviour traits

The work reported in this thesis makes three central contributions to literature on the understanding of EB traits. First it contributes to the ongoing debate regarding the distinction between EE and UE. Second, it illuminates the relationship between CR and obesity. Finally, it has implications for the presentation of EB as immutable traits largely controlled by genetic influences. These are elaborated below.

The distinction between EE and UE

Citing the high correlation between EE and UE, some researchers have argued that these traits, alongside other measures designed to quantify behaviours surrounding overeating, reflect a single latent construct [185]. Following this argument, the present separation of these traits is an example of the *jangle fallacy* whereby different names are used for the same underlying construct, creating artificial divisions in research that complicate the interpretation of findings [185].

Here, the finding that EE and UE are highly correlated was replicated. Further, the results indicate that they are likely to share some biological pathways with respect the mediation of genetic predisposition to obesity (**Chapter 4**). However, evidence that these traits might reflect distinct aetiologies, with UE having a more substantial genetic basis than EE, was also provided in **Chapter 7**. There are explanations for this finding that are consistent with the argument that they should be combined. The GWAS in **Chapter 7** may simply have been underpowered to accurately detect and estimate heritability for EE. Alternatively, UE and EE may measure different extremities of the same underlying trait or EE may cover a more restricted spectrum of the underlying trait which UE summarises more fully, making the analysis of UE better powered in the limited sample size available [397]. These ideas require further research. However, at present, conflating EE and UE may limit research attempting to uncover the aetiology and implications of EB. As noted, future research is needed to better understand the components of behavioural measures that are important to specific health outcomes. Whether or not EE is a component of UE, having an isolated measure reflecting the tendency to overeat in response to dysphoric emotions may be helpful in this regard.

Cognitive restraint and obesity

All three EB traits measured by the TFEQ, were initially conceptualised as obesogenic behaviours, high levels of which would be expected to result in weight gain. Longitudinal studies have broadly continued to support this view with regards to UE and EE [106, 127, 128]. However debate still surrounds the role of CR in weight gain. Some studies report that obesity predicts increases in CR [135], whilst others report a prospective association

between CR and weight gain [134] and others still report that CR is associated with weight loss [398].

In this thesis, a quadratic association between CR and BMI is reported, suggesting that the relationship between CR and BMI is BMI-dependent. Low levels of CR were reported amongst individuals at the extreme ends of the BMI spectrum (**Chapter 4**). Whilst it is possible that CR is causally linked to increases in BMI amongst normal weight individuals, a positive linear association, such as that demonstrated by EE and UE, would be anticipated if CR was simply, causally linked to weight gain across the BMI spectrum. The positive association between CR and BMI amongst normal weight participants in **Chapter 4** is interpreted as indicating that CR is a response to weight gain that may prevent individuals from becoming overweight or obese. This corroborates the findings of a 2013 review of prospective studies of the association between CR and weight gain amongst normal weight adults [51]. In 19 of the 20 studies included in the review, CR did not predict weight gain [51]. It also supports other studies reporting increases in CR in response to weight gain [138].

The inverse association between CR and weight amongst overweight individuals in **Chapter 4** requires further research. However, it may indicate that the abandonment of cognitive control over food intake in this group might facilitate weight gain or the maintenance of obesity. The results also show that high levels of CR attenuate the association between genetic predisposition to obesity and realised weight status. This suggests that CR is protective against obesity, at least amongst genetically vulnerable individuals. Together, these results support mounting evidence that CR does not lead to obesity but instead may be beneficial for controlling weight [51].

Heritability

EB is widely considered to be heritable and twin studies have suggested a substantial role for genetics in the determination of the adult EB traits examined in this thesis (EE, UE and CR) [66]. Here, the findings confirmed that UE is a partially heritable trait (**Chapter 7**). However, no evidence to support a substantive genetic basis for EE or CR was found. The GWAS analysis was underpowered due to low sample size and it is possible that larger studies will identify a genetic basis for these traits in the future. It is also interesting to note that UE has the largest number of contributing items in the TFEQ-R18 questionnaire (9 items, versus 3 items for EE and 6 items for CR), suggesting that it may be better specified than the other two phenotypes. However, the notion that environmental influences are likely to play a substantial role in the determination of EB traits is important. It suggests that future research could be designed to identify modifiable, environmental determinants.

8.3.3 Future research

The work contained within this thesis points to several specific opportunities for future research. These have been mentioned in the previous sections and are elaborated here.

8.3.3.1 Focus on implications for obesity prevention

As highlighted in **Section 8.3.1**, replicating and building upon the results of the analyses with the most immediate potential for obesity prevention is of paramount importance to the translation of this work into actionable evidence. Foremost, the findings amongst both infants and adults suggest that EB traits could represent targets for obesity prevention. The finding that CR modifies genetic predisposition to obesity requires replication, particularly in light of the fact that this effect was not identified amongst EDEN women. If this result is replicated with consistency, it will be important to determine the factors that differentiate CR from dieting and which make it a better tool for the prevention of weight gain. Further, the longitudinal associations between CR and BMI, across the BMI spectrum should also be examined in greater detail [88]. Given the finding that EB traits may not be substantively heritable, aetiological environmental factors that are amenable to change are of particular interest to future research for all EB traits. Longitudinal data is now available across a number of cohorts which could be exploited to identify these factors. Moreover, further research is needed in respect to the maternal attitudes studied in **Chapter 5**. As a first step, replication of our findings is required in order to build an evidence base for targeting these maternal attitudes in obesity prevention interventions.

8.3.3.2 Increase the sample size for eating behaviour gene discovery

The work contained within this thesis is cross-sectional and relied upon the informed assumption that EB should be modelled as the cause, rather than the consequence of BMI. However, the results of the MR analyses in **Chapter 7** indicate that BMI may be aetiologically involved in EB traits. Whilst this is just one interpretation of the findings and, in the absence of genetic predictors of EB, merely indicates that EB and BMI occur on a shared pathway, other studies have also suggested this direction of association [399]. It was hoped that the results of the GWAS reported in **Chapter 7** could be used to further interrogate the direction of causality using bi-directional MR, as well as to explore the biological pathways involved in EB. However, the GWAS was under-powered to detect any specific variants and thus it was not possible to fulfil this aim in full. Future research should aim to increase the sample size of EB GWAS such that specific variants can be identified. Ideally, these efforts would include the collection of EB data in large-scale epidemiological studies using validated questionnaires. Further, sample sizes can be increased through collaboration and consolidation between existing studies. Different EB traits are measured in infants and

children. Similar sample size issues limit the ability to conduct GWAS of these traits. This should be addressed in future studies.

8.3.3.3 Refine measures of behavioural traits

The range of measures used to assess EB, with little clarity as to how they are related, is a major obstacle to the consolidation of findings in EB research. As highlighted in **Chapter 7** this limits the sample sizes available for studies requiring large amounts of data to detect small associations. Further, it restricts meaningful comparisons between the findings of studies based on different questionnaires, as well as impeding longitudinal research, as different traits are typically measured in childhood and adulthood. Uncovering the biological basis of EB traits can be used to highlight points of differentiation and overlap between separate measures which will be important to consolidating the literature.

Beyond EB, **Chapter 6** suggests that the crude measures of behavioural traits sometimes used in large cohort studies may not accurately reflect biological reality. For example, we identified multiple pathways underlying the link between risk-taking propensity and BMI. These pathways may represent different, biologically distinct aspects of the risk-taking with divergent associations to health. As such, crude measures could mask true associations between aspects of risk-taking propensity and traits of interest, including obesity, leading to erroneous conclusions regarding the health implications of behaviour. Future work should aim to isolate specific pathways, or aspects of behaviour, as well as identifying how these may be more accurately measured.

8.4 Strengths and limitations

The results and conclusions of this work must be interpreted in the context of its strengths and limitations. Considerations specific to particular studies are presented in the relevant chapters. Here, over-arching considerations are discussed.

8.4.1 Bias

Bias describes systematic errors in the design, conduct or analysis of studies that may result in a distortion of the relationship between exposures and outcomes [400]. In contrast to random error, which is inversely related to the sample size of a study, biases are not reduced by increasing sample size. Here, the potential impact of both information and selection bias is considered with reference to this thesis.

8.4.1.1 Selection bias and the representativeness of the sample

Selection bias arises when study participants differ from the population from which they are drawn in systematic ways. This may result from sampling, attrition or non-response bias. Whilst the findings of studies subject to these biases may be internally valid, there are limitations to their generalisability. Pre-defined exclusion criteria limit external validity in a known capacity. For example, the Baby Milk Trial recruited only healthy, term, formula-fed infants of normal birthweight [257]. As such, results from this study should not be considered applicable to other groups, notably those who are breastfed or born prematurely. The genetic work in this thesis also has limited external validity. In order to avoid population stratification, the GWAS analyses described in **Chapters 6** and **7** were restricted to individuals of European ancestry. Replication of the findings in different ancestry groups is required before the results can be generalised.

The known limitations of generalisability are made clear within specific chapters. However, unspecified differences between participants and the general population may also exist. Of particular concern is the healthy volunteer effect, a phenomenon whereby individuals who respond to requests to participate in research are healthier than the general population from which they are drawn [401]. The work described in this thesis relies primarily upon data from UKB, the Fenland study and the Baby Milk Trial. In the case of UKB there is direct evidence for the healthy volunteer effect. The response rate for requests to participate in the UKB study was 5.5% [402]. Relative to the general population, participants were less likely to be obese, smoke, drink alcohol on daily basis or live in a socio-economically deprived area than the general population [402]. Participants also reported fewer health conditions and experienced lower all-cause mortality at 70-74 years than the general population [402]. No formal comparisons of either the Fenland or Baby Milk Trial participants with the general

population are available. The response rate for Fenland was ~ 27%. Reflecting the census population of the area, participants primarily reported white ethnicity [403] and, in keeping with UK population norms, the majority were overweight [14]. In the Baby Milk Trial, 31% of those assessed for inclusion were randomised (699/2133) [259]. In the absence of evidence to the contrary, it is reasonable to assume that these studies may be subject to the healthy volunteer effect.

Overall, the study findings should be generalised with caution, particularly bearing in mind known exclusion criteria. These exclusion criteria, as well as the characteristics of the study participants are detailed in each study to make clear the limits of external validity. Replication of the findings in other participant groups will increase confidence in the generalisability of the results.

8.4.1.2 Information bias and the measurement of eating behaviour

Throughout this thesis, EB traits were measured by questionnaire. The tools used are validated and have been shown to be reliable. However, they are not objective measures. They rely on parent- or self-report and, as such, depend upon the insight of participants into their own behaviour, or those of their child, as well as the intention to report them accurately. These issues are discussed in detail in **Chapter 1**. In particular, systematic bias in the reporting of EB on the basis of BMI due to perceptions of social desirability is of concern. Whilst it was not possible to validate EB measures specifically in our studies, the strong, positive associations between the appetitive EB traits and BMI (or body weight) in this thesis, as well as the wider literature, is reassuring. **Chapter 4** also reports a novel quadratic relationship between CR and BMI, with a negative correlation between weight and CR being observed amongst the most obese participants. Together these results suggest that, on average, overweight individuals did not report high levels of restraint and low levels of EE and UE, as would be anticipated if social desirability bias systematically influenced reporting amongst this group.

The BEBQ relies on parent-report. This presents challenges for the measurement of EB beyond self-report measures and it is possible that parental factors, including EB or concerns regarding their child's eating, influence parental responses. We were unable to test correlations between maternal and infant EB in our study, as data on maternal EB was not collected. However, other studies suggest that such correlations exist, at least with regard to eating-related traits, such as food intake [404]. Whether these associations are true or the result of mother's conflating their characteristics with those of their children remains unknown. In a recent study, mother's who reported concern about their child becoming underweight reported higher levels of SR in their child, whilst mothers concerned that their child might become overweight reported higher levels of FR in their child [319]. This study was cross-sectional and the direction of causality could not be determined. Prospective

associations between appetite and weight gain suggest that appetite is linked to growth and might be the cause, rather than the consequence, of maternal concern [143].

On balance, the use of validated questionnaire-based measures for EB is a strength of this work, particularly in light of the need to analyse and interpret data from multiple cohorts together in **Chapters 4** and **7**. As m-Health technologies that are able to objectively measure typical EB in free-living populations are developed (e.g. [405, 406]), the validation of questionnaire-based measures of EB traits should be continued and results should be replicated using objectively measured data.

8.4.2 Error

Random error refers to non-systematic mistakes in the measurement of variables of interest to a study. It is, by definition, random, such that error in the measurement of one variable is not dependent upon the level of any other variable. Error acts to diminish the ability of a study to detect differences between groups, increasing the probability of Type II errors (failures to reject a false null hypothesis). Where true associations are correctly identified, random error adversely affects the precision of effect estimates. Unlike bias, the impact of random error on a study is inversely related to the sample size. Thus a clear strength of this research was the large sample sizes used throughout.

8.4.3 Chance

Throughout this thesis, effect estimates are presented alongside *p*-values and 95% confidence intervals (CIs). The *p*-value quantifies the probability of the observed data under the null hypothesis, thus specifying the probability that a significant result is the consequence of chance. The 95% CIs compliment the *p*-value, providing a range within which, on 95% of occasions with re-sampling from a given population, the true population parameter is expected to fall [309]. A standard *p*-value threshold of 0.05 has been applied to identify significant associations throughout this work, alongside Bonferroni correction to maintain a family wise error rate of 5% in instances of multiple testing [407].

The standard threshold of $p < 5 \times 10^{-8}$ was also applied to assess the significance of the GWAS results in **Chapters 6** and **7**. This threshold was established in 2005, alongside the earliest GWAS studies, and is based on an estimate of the number of common, independent genetic variants distinguishing haplotypes across the entire genome [408]. However, as new imputation panels have increased the number of haplotypes that can be distinguished, the correction has become approximate and outdated for use alongside the imputation panels included in this thesis. Although recent studies have suggested that $p < 5 \times 10^{-8}$ remains appropriate for testing common genetic variation (MAF >5%) in European populations [408], there remains a possibility that the results reported in **Chapter 6** result from

chance and replication is required. Overall, clear communication of *p*-values and 95% CIs throughout the thesis, alongside the application of appropriate thresholds for significance and correction for multiple testing, is a clear strength of the research.

Replication is one approach used to provide reassurance that chance does not account for the results. In the case of mediation of the BMI-GRS to BMI association by appetitive traits reported in **Chapter 4**, the consistency in our results, alongside their plausibility and replication in other studies make a strong case for a true finding. In GWAS studies, it is common practice to replicate genetic associations from discovery-stage analyses in independent, ancestry-matched cohorts [409]. In the case of the risk-taking analysis the lack of access intersecting risk-taking and genetic data from any independent samples made this impossible. However, indirect confirmation of results for the related *Ever Smoking* phenotype strengthens the case in favour of the reported associations reflecting truth. All novel findings reported in this thesis require replication.

8.4.4 Confounding

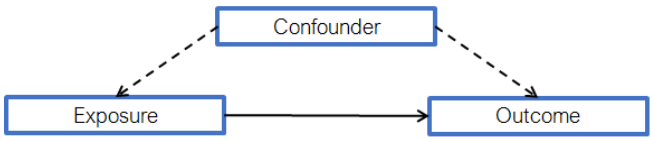


Figure 8.5 Confounding. The confounding variable is related to both the exposure and the outcome of interest but does not lie on the causal pathway between them.

A confounding factor is an extraneous variable that is independently associated with both the exposure and the outcome but does not lie on the causal pathway between them. The presence of confounding threatens the internal validity of a study and is a major concern in observational research, such as that reported in this thesis. As elaborated in **Section 6.3.2.9**, the random assortment of alleles such that traits are inherited independently, facilitates the controlling of confounding through using genotype as a proxy for an exposure of interest [410]. Throughout this thesis, wherever possible, MR was used to support any assertions made. Beyond the MR analyses, attempts were made to account for confounding through the inclusion of variables considered to be potential confounders in statistical models. Sensitivity analyses were also used, adjusting for a wider range of potential confounders than the main analyses. For example, throughout **Chapter 5** all models in the main analyses were adjusted for both infant sex and age. Other covariates (maternal BMI, education and ethnicity) were added to these models as sensitivity analyses. The same approach was taken to the exploration of confounding in **Chapter 4**. Age, sex and study centre (where appropriate) were included in models, and sensitivity analyses, adjusting for a more comprehensive list of potential confounders, were reported for the EB to BMI associations. Whilst it remains

possible that unmeasured, and unconsidered confounders, are important to the reported associations in these studies, reasonable methods to account for confounding were taken.

In gene discovery efforts, such as those described in **Chapters 6** and **7**, systematic differences in allele frequencies between sub-groups of a population, for example on the basis of ancestry, can confound associations between genetic variants and phenotypes. This is known as population stratification. As such, we restricted our analyses to individuals of self-reported European ancestry and further accounted for this potential source of confounding through the use of BOLT-LMM or adjustment for principal components.

8.4.5 Reverse causality

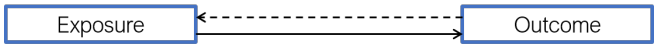


Figure 8.6 Reverse causality. Reverse causality occurs when the variable assumed to be the outcome in an association, exerts an influence on the variable assumed to be the exposure. This may wholly, or partially, account for the observed association.

The work described in this thesis was designed to illuminate the association between EB and obesity. In particular, we planned to use genetic variants to infer the direction of causation between EB and BMI through MR (**Section 6.3.2.9**). In observational research, associations between variables can result from reverse causality, whereby the variable assumed to be the exposure is, in reality, the outcome [28]. The fact that an individual's genotype is fixed at conception eliminates the potential for reverse causality when genetic instruments are used as a proxy for an exposure. However, given that genetic variants for EB traits that did not violate the assumptions of MR were not available, reverse causality is a central concern. Informed assumptions were made regarding the direction of causation between EB and BMI. In particular, **Chapters 4** and **5** rely upon the assumption that BMI and body weight are the consequence, rather than the cause, of EB. This was based on consistent evidence from longitudinal studies suggesting an association between EB and weight change.

The finding that BMI-related genetic variants are associated with EB, reported in **Chapter 7**, does not necessarily contradict this conclusion. In the absence of any genetic variants that robustly predict EB, bi-directional MR was not possible and all that can be concluded is that BMI and EB lie on the same pathway. Continued research is needed to definitively establish the direction of causality. Whilst the existing evidence supports a role for EB in weight gain, it is possible that the association is bidirectional or, as suggested by associations between CR and BMI, varies by BMI.

8.5 Conclusion

Together, the studies that comprise this thesis demonstrate diverse pathways underlying the relationship between EB and obesity and suggest broad consistency between the role of EB traits in infancy and adulthood. In adulthood, appetitive EB traits lie on the causal pathway between genetics and obesity, partially mediating the effect of known genetic variants on BMI. Conversely, restraint over eating modifies the impact of genetic susceptibility to obesity on realised BMI. These findings isolate EE, UE and CR as targets for obesity prevention with the potential to benefit those genetically predisposed to obesity. The cognitive nature of CR may make this trait a particularly attractive target. Amongst infants, the results indicate that appetitive EB traits are associated with both both milk intake and weight. Further, their associations with these outcomes are modified by positive maternal attitudes to following healthy infant feeding guidelines. Longitudinal research is needed. However, this indicates that the effects of infant EB traits can be attenuated, and healthy consumption and weight promoted, during a critical period of development through the targeting of maternal attitudes to healthy feeding guidelines. The findings also support the utility of GWAS studies in elucidating the relationships between behaviour and obesity. Novel insights into the genetic determinants and biology of risk-taking propensity are described and 26 risk-associated genetic variants are identified. Together, these suggest shared biological pathways with BMI as well as a positive, causal association with UE. GWAS also identified a genetic basis for UE, which partially overlaps with that of BMI.

In sum, the pathways linking behaviour to obesity are complex and manifold. Future obesity research should not be limited to the study of overtly obesogenic behavioural traits but should reflect the diverse mechanisms through which behaviour and obesity are linked. This includes investigating opportunities to reduce the impact of innate, or as yet unmodifiable, determinants of weight through targeting the modifiable influences with which they interact. Overall the studies contribute to a deeper understanding of the relationship between EB and the aetiology of obesity, isolating specific behavioural traits as potential targets for obesity prevention.

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APPENDIX A

SUPPLEMENTARY TABLES

A.1 Chapter 3

Table A.1 Self-reported ethnicity of the Fenland study participants (n=9242)

Self-reported ethnicity	Count	Percent (%)
White British	8,526	92.3
Other white	534	5.8
White Irish	77	0.8
Indian	30	0.3
White and Asian	20	0.2
Mixed race other	19	0.2
Any Asian	14	0.2
Any other	7	0.1
Pakistani	4	<0.1
White and black Caribbean	4	<0.1
African	3	<0.1
White and black African	3	<0.1
Bangladeshi	1	<0.1
Any Black	0	0
Caribbean	0	0
Chinese	0	0

Table A.2 Sensitivity analysis amongst men using a sex-specific BMI-GRS

	Sex-combined score		Male-specific only		
	Beta (SE)[a]	*p*-value	Beta (SE)[a]	*p*-value	% change in Beta
Weight	0.91 (0.10)	2.24E-21*	0.90 (0.10)	2.24E-20*	-1.4
WC	0.91 (0.09)	9.62E-22*	0.89 (0.10)	1.98E-20*	-2.1
HC	0.91 (0.10)	4.08E-21*	0.91 (0.10)	9.28E-21*	0.3
WHR	0.61 (0.09)	5.33E-11*	0.58 (0.09)	7.79E-10*	-5.2
Height	-0.02 (0.10)	0.85	-0.04 (0.10)	0.65	–
BF%	0.74 (0.09)	9.09E-15*	0.73 (0.10)	2.90E-14*	-0.8
Total fat	0.90 (0.09)	3.75E-21*	0.89 (0.10)	3.02E-20*	-1.2
Trunk fat	0.88 (0.09)	1.28E-20*	0.87 (0.10)	1.31E-19*	-1.5
Android fat	0.87 (0.09)	3.23E-20*	0.85 (0.10)	4.63E-19*	-2.0
Gynoid fat	0.87 (0.10)	1.84E-19*	0.86 (0.10)	9.47E-19*	-0.8
Legs fat	0.80 (0.10)	7.20E-17*	0.80 (0.10)	2.76E-16*	-0.8
SAT	0.98 (0.10)	2.23E-24*	0.97 (0.10)	1.58E-23*	-0.7
VAT	0.62 (0.09)	1.85E-11*	0.60 (0.09)	1.14E-10*	-2.9
VAT/SAT	0.06 (0.09)	0.54	0.04 (0.09)	0.66	–
Total lean	0.68 (0.10)	1.91E-12*	0.67 (0.10)	7.09E-12*	-1.5
Trunk lean	0.65 (0.10)	1.57E-11*	0.65 (0.10)	2.36E-11*	0.3
Android lean	0.63 (0.10)	4.30E-11*	0.63 (0.10)	1.15E-10*	-1.1
Gynoid lean	0.59 (0.10)	6.85E-10*	0.58 (0.10)	2.14E-09*	-1.8
Append. lean	0.65 (0.10)	9.63E-12*	0.63 (0.10)	7.07E-11*	-3.2
Total bone	0.39 (0.10)	5.69E-05*	0.36 (0.10)	2.11E-04*	-6.9
Trunk bone	0.48 (0.10)	6.04E-07*	0.46 (0.10)	2.11E-06*	-3.8
Android bone	0.29 (0.10)	2.74E-03	0.30 (0.10)	2.25E-03	–
Gynoid bone	0.32 (0.10)	9.66E-04*	0.30 (0.10)	2.11E-03	-5.7
Legs bone	0.36 (0.10)	1.60E-04*	0.33 (0.10)	7.26E-04*	-9.4

Standard error (SE); waist circumference (WC); hip circumference (HC), waist-to-hip ratio (WHR); body fat percentage (BF%); subcutaneous adipose tissue (SAT); visceral adipose tissue (VAT); Appendicular (Append.)

[a] Effect estimates (Beta) are the age-adjusted SD change in the body composition variable per unit increase in the BMI-GRS from the age-adjusted linear regression of the BMI-GRS on body composition *z*-score. The BMI-GRS used in the sex-combined score analysis weights the SNPs included in the score by their European-only, sex-combined effect estimates from Locke et al. [149]. The BMI-GRS used in the male-specific analysis weights the SNPs included in the score by their European-only, male-only effect estimates from Locke et al. [149]

– Not applicable

* $p < 1.03 \times 10^{-3}$

Table A.3 Sensitivity analysis amongst women using a sex-specific BMI-GRS

	Sex-combined score		Female-specific only		
	Beta (SE)[a]	*p*-value	Beta (SE)[a]	*p*-value	% change in Beta
Weight	1.01 (0.10)	9.93E-24*	1.01 (0.10)	1.82E-24*	-0.3
WC	0.89 (0.10)	6.23E-19*	0.88 (0.10)	2.2E-19*	-0.7
HC	0.96 (0.10)	1.63E-21*	0.95 (0.10)	3.2E-22*	-0.2
WHR	0.41 (0.10)	4.24E-05*	0.40 (0.10)	4.14E-05*	-1.8
Height	0.10 (0.10)	0.31	0.09 (0.10)	0.36	–
BF%	0.80 (0.10)	4.14E-16*	0.80 (0.10)	8.09E-17*	0.4
Total fat	0.96 (0.10)	6.12E-22*	0.96 (0.10)	8.00E-23*	0.2
Trunk fat	0.93 (0.10)	1.00E-20*	0.93 (0.10)	1.86E-21*	-0.1
Android fat	0.92 (0.10)	2.47E-20*	0.92 (0.10)	5.47E-21*	-0.3
Gynoid fat	0.90 (0.10)	3.74E-19*	0.90 (0.10)	7.89E-20*	-0.1
Legs fat	0.89 (0.10)	8.58E-19*	0.90 (0.10)	1.01E-19*	0.7
SAT	0.92 (0.10)	3.66E-19*	0.91 (0.10)	1.49E-19*	-0.8
VAT	0.70 (0.10)	2.16E-12*	0.69 (0.10)	1.61E-12*	-1.3
VAT/SAT	0.28 (0.10)	4.29E-03	0.28 (0.10)	3.77E-03	–
Total lean	0.85 (0.10)	2.66E-17*	0.83 (0.10)	2.04E-17*	-1.6
Trunk lean	0.75 (0.10)	7.25E-14*	0.74 (0.10)	6.85E-14*	-1.8
Android lean	0.77 (0.10)	2.49E-14*	0.74 (0.10)	5.80E-14*	-3.3
Gynoid lean	0.81 (0.10)	2.99E-16*	0.80 (0.10)	3.43E-16*	-2.1
Append. lean	0.88 (0.10)	1.14E-18*	0.87 (0.10)	7.24E-19*	-1.3
Total bone	0.45 (0.10)	3.96E-06*	0.44 (0.10)	4.32E-06*	-2.3
Trunk bone	0.60 (0.10)	1.41E-09*	0.58 (0.10)	1.95E-09*	-2.8
Android bone	0.42 (0.10)	2.83E-05*	0.41 (0.10)	3.03E-05*	-2.3
Gynoid bone	0.56 (0.10)	2.20E-08*	0.55 (0.10)	2.02E-08*	-1.7
Legs bone	0.51 (0.10)	3.44E-07*	0.50 (0.10)	2.77E-07*	-1.2

Standard error (SE); waist circumference (WC); hip circumference (HC), waist-to-hip ratio (WHR); body fat percentage (BF%); subcutaneous adipose tissue (SAT); visceral adipose tissue (VAT); Appendicular (Append.)

[a] Effect estimates (Beta) are the age-adjusted SD change in the body composition variable per unit increase in the BMI-GRS from the age-adjusted linear regression of the BMI-GRS on body composition *z*-score. The BMI-GRS used in the sex-combined score analysis weights the SNPs included in the score by their European-only, sex-combined effect estimates from Locke et al. [149]. The BMI-GRS used in the male-specific analysis weights the SNPs included in the score by their European-only, female-only effect estimates from Locke et al. [149]

– Not applicable

* $p < 1.03 \times 10^{-3}$

Table A.4 Sensitivity analysis amongst men reporting white ethnicity

	Whole cohort		White only		
	Beta (SE)[a]	*p*-value	Beta (SE)[a]	*p*-value	% change in Beta
Weight	0.91 (0.10)	2.24E-21*	0.93 (0.10)	1.10E-20*	2.2
WC	0.91 (0.09)	9.62E-22*	0.92 (0.10)	7.62E-21*	1.1
HC	0.91 (0.10)	4.08E-21*	0.93 (0.10)	8.59E-21*	2.3
WHR	0.61 (0.09)	5.33E-11*	0.60 (0.10)	1.92E-10*	-1.0
Height	-0.02 (0.10)	0.85	-0.05 (0.10)	0.60	–
BF%	0.74 (0.09)	9.09E-15*	0.78 (0.10)	2.57E-15*	6.3
Total fat	0.90 (0.09)	3.75E-21*	0.94 (0.10)	3.17E-21*	4.4
Trunk fat	0.88 (0.09)	1.28E-20*	0.93 (0.10)	9.14E-21*	4.9
Android fat	0.87 (0.09)	3.23E-20*	0.92 (0.10)	1.37E-20*	5.4
Gynoid fat	0.87 (0.10)	1.84E-19*	0.91 (0.10)	9.77E-20*	4.7
Legs fat	0.80 (0.10)	7.20E-17*	0.83 (0.10)	4.85E-17*	3.7
SAT	0.98 (0.10)	2.23E-24*	1.01 (0.10)	2.83E-24*	3.8
VAT	0.62 (0.09)	1.85E-11*	0.66 (0.10)	5.78E-12*	7.1
VAT/SAT	0.06 (0.09)	0.54	0.09 (0.10)	0.37	–
Total lean	0.68 (0.10)	1.91E-12*	0.66 (0.10)	2.44E-11*	-1.8
Trunk lean	0.65 (0.10)	1.57E-11*	0.65 (0.10)	8.24E-11*	-0.4
Android lean	0.63 (0.10)	4.30E-11*	0.62 (0.10)	4.42E-10*	-2.1
Gynoid lean	0.59 (0.10)	6.85E-10*	0.58 (0.10)	3.71E-09*	-0.9
Append. lean	0.65 (0.10)	9.63E-12*	0.63 (0.10)	1.52E-10*	-2.6
Total bone	0.39 (0.10)	5.69E-05*	0.35 (0.10)	4.60E-04*	-9.7
Trunk bone	0.48 (0.10)	6.04E-07*	0.46 (0.10)	3.86E-06*	-3.5
Android bone	0.29 (0.10)	2.74E-03	0.27 (0.10)	8.03E-03	–
Gynoid bone	0.32 (0.10)	9.66E-04*	0.29 (0.10)	4.24E-03	-10.1
Legs bone	0.36 (0.10)	1.60E-04*	0.33 (0.10)	9.09E-04*	-8.9

Standard error (SE); waist circumference (WC); hip circumference (HC), waist-to-hip ratio (WHR); body fat percentage (BF%); subcutaneous adipose tissue (SAT); visceral adipose tissue (VAT); Appendicular (Append.)

[a] Effect estimates (Beta) are the age-adjusted SD change in the body composition variable per unit increase in the BMI-GRS from the age-adjusted linear regression of the BMI-GRS on body composition *z*-score. In both analyses, the BMI-GRS is weighted using the European-only effect estimates from Locke et al. [149]. However, the white-only analysis includes only men who reported their ethnicity as white

– Not applicable

* $p < 1.03 \times 10^{-3}$

Table A.5 Sensitivity analysis amongst women reporting white ethnicity

	Whole cohort		White only		
	Beta (SE)[a]	*p*-value	Beta (SE)[a]	*p*-value	% change in Beta
Weight	1.01 (0.10)	9.93E-24*	1.04 (0.10)	7.58E-24*	3.2
WC	0.89 (0.10)	6.23E-19*	0.92 (0.10)	3.74E-19*	3.5
HC	0.96 (0.10)	1.63E-21*	0.98 (0.10)	2.18E-21*	2.3
WHR	0.41 (0.10)	4.24E-05*	0.44 (0.10)	2.00E-05*	7.2
Height	0.10 (0.10)	0.31	0.13 (0.10)	0.19	–
BF%	0.80 (0.10)	4.14E-16*	0.80 (0.10)	2.65E15*	-0.1
Total fat	0.96 (0.10)	6.12E-22*	0.98 (0.10)	1.53E-21*	2.0
Trunk fat	0.93 (0.10)	1.00E-20*	0.95 (0.10)	2.07E-20*	2.3
Android fat	0.92 (0.10)	2.47E-20*	0.94 (0.10)	4.2E-20*	2.5
Gynoid fat	0.90 (0.10)	3.74E-19*	0.91 (0.10)	1.99E-18*	0.9
Legs fat	0.89 (0.10)	8.58E-19*	0.90 (0.10)	2.59E-18*	1.3
SAT	0.92 (0.10)	3.66E-19*	0.94 (0.11)	4.63E-19*	2.7
VAT	0.70 (0.10)	2.16E-12*	0.70 (0.10)	9.07E-12*	0.3
VAT/SAT	0.28 (0.10)	4.29E-03	0.28 (0.10)	0.01	–
Total lean	0.85 (0.10)	2.66E-17*	0.89 (0.10)	3.37E-18*	5.3
Trunk lean	0.75 (0.10)	7.25E-14*	0.79 (0.10)	2.01E-14*	4.5
Android lean	0.77 (0.10)	2.49E-14*	0.79 (0.10)	1.75E-14*	2.8
Gynoid lean	0.81 (0.10)	2.99E-16*	0.84 (0.10)	1.28E-16*	3.8
Append. lean	0.88 (0.10)	1.14E-18*	0.93 (0.10)	1.04E-19*	5.6
Total bone	0.45 (0.10)	3.96E-06*	0.49 (0.10)	1.36E-06*	7.5
Trunk bone	0.60 (0.10)	1.41E-09*	0.61 (0.10)	1.52E-09*	2.7
Android bone	0.42 (0.10)	2.83E-05*	0.43 (0.10)	2.94E-05*	2.9
Gynoid bone	0.56 (0.10)	2.20E-08*	0.59 (0.10)	1.27E-08*	4.6
Legs bone	0.51 (0.10)	3.44E-07*	0.55 (0.10)	8.62E-08*	7.9

Standard error (SE); waist circumference (WC); hip circumference (HC), waist-to-hip ratio (WHR); body fat percentage (BF%); subcutaneous adipose tissue (SAT); visceral adipose tissue (VAT); Appendicular (Append.)

[a] Effect estimates (Beta) are the age-adjusted SD change in the body composition variable per unit increase in the BMI-GRS from the age-adjusted linear regression of the BMI-GRS on body composition *z*-score. In both analyses, the BMI-GRS is weighted using the European-only effect estimates from Locke et al. [149]. However, the white-only analysis includes only women who reported their ethnicity as white

– Not applicable

* $p < 1.03 \times 10^{-3}$

Table A.6 Heat map results

		Fat mass				Lean mass				Bone mass			
SNP	Nearest gene	Beta	SE	*p*-val.	*z*-statistic*	Beta	SE	*p*-val.	*z*-statistic*	Beta	SE	*p*-val.	*z*-statistic*
rs657452	*AGBL4*	1.41	0.63	0.03	2.24	0.82	0.39	0.03	2.11	0.40	0.43	0.35	0.94
rs3101336	*NEGR1*	0.98	0.44	0.03	2.20	0.63	0.27	0.02	2.33	0.27	0.30	0.37	0.89
rs17024393	*GNAT2*	1.85	0.71	0.01	2.62	0.94	0.43	0.03	2.17	1.28	0.48	0.01	2.66
rs543874	*SEC16B*	1.00	0.37	0.01	2.72	0.36	0.23	0.11	1.60	0.43	0.25	0.09	1.72
rs13021737	*TMEM18*	0.91	0.31	3.55E-03	2.92	0.75	0.19	8.22E-05	3.94	0.69	0.21	1.05E-03	3.28
rs10182181	*ADCY3*	1.52	0.46	1.04E-03	3.28	-0.03	0.28	0.90	-0.12	-0.07	0.31	0.82	-0.22
rs11126666	*KCNK3*	1.45	0.69	0.04	2.10	0.52	0.42	0.22	1.23	0.04	0.47	0.93	0.09
rs11688816	*EHBP1*	2.14	0.84	0.01	2.56	0.99	0.51	0.05	1.94	0.59	0.57	0.30	1.04
rs13078960	*CADM2*	1.30	0.60	0.03	2.17	0.86	0.37	0.02	2.35	0.76	0.41	0.06	1.87
rs16851483	*RASA2*	1.52	0.58	0.01	2.63	0.42	0.35	0.24	1.18	0.11	0.39	0.79	0.27
rs1516725	*ETV5*	0.85	0.46	0.07	1.84	0.41	0.28	0.15	1.45	0.03	0.31	0.93	0.08
rs10938397	*GNPDA2*	0.70	0.36	0.05	1.93	0.25	0.22	0.27	1.11	-0.03	0.24	0.91	-0.11
rs205262	*C6orf106*	1.71	0.73	0.02	2.35	1.36	0.44	0.00	3.05	1.12	0.49	0.02	2.27
rs1167827	*HIP1*	1.07	0.73	0.14	1.47	0.50	0.45	0.26	1.12	-0.10	0.50	0.85	-0.20
rs16907751	*ZBTB10*	1.59	0.69	0.02	2.31	0.76	0.42	0.07	1.82	0.40	0.46	0.39	0.85
rs11191560	*NT5C2*	1.76	0.49	3.66E-04	3.56	-0.34	0.30	0.26	-1.13	-0.43	0.33	0.20	-1.27
rs3817334	*MTCH2*	0.85	0.56	0.13	1.52	-0.26	0.34	0.45	-0.75	-0.78	0.38	0.04	-2.06
rs12286929	*CADM1*	1.28	0.65	0.05	1.98	0.22	0.40	0.58	0.55	0.30	0.44	0.49	0.68
rs7138803	*BCDIN3D*	1.73	0.45	1.08E-04	3.87	0.61	0.27	0.03	2.24	0.62	0.30	0.04	2.05
rs9540493	*MIR548X2*	1.17	0.68	0.09	1.71	0.14	0.42	0.73	0.34	0.08	0.46	0.86	0.18
rs7164727	*LOC100287559*	1.90	1.02	0.06	1.87	0.23	0.62	0.72	0.36	-0.58	0.69	0.40	-0.84
rs2080454	*CBLN1*	2.74	0.87	1.62E-03	3.15	1.60	0.53	2.60E-03	3.01	1.39	0.59	0.02	2.36
rs1558902	*FTO*	0.91	0.18	2.09E-07	5.19	0.45	0.11	3.21E-05	4.16	0.20	0.12	0.09	1.68
rs12940622	*RPTOR*	1.66	0.80	0.04	2.08	0.83	0.49	0.09	1.71	0.58	0.54	0.28	1.08
rs1808579	*C18orf8*	1.82	0.72	0.01	2.54	-0.25	0.44	0.58	-0.56	-0.07	0.49	0.88	-0.15
rs7239883	*LOC284260*	2.08	0.92	0.02	2.27	1.29	0.56	0.02	2.29	1.47	0.62	0.02	2.37
rs6567160	*MC4R*	1.24	0.30	3.06E-05	4.17	0.78	0.18	1.58E-05	4.32	0.84	0.20	2.88E-05	4.18
rs6091540	*ZFP64*	2.28	0.82	0.01	2.77	0.59	0.50	0.24	1.18	-0.22	0.56	0.69	-0.40

Standard error (SE); Beta, SE, *p*-value and *z*-statistic refer to the results of the age and sex-adjusted linear regression of the specified SNP on the standardised body composition measure;
* *z*-statistic was calculated as Beta/SE

A.2 Chapter 4

Table A.7 EE adjusted for UE and UE adjusted for EE

	BMI-GRS to EB		EB to BMI		BMI-GRS to BMI		BMI-GRS to BMI			
	Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Sobel test *p*-value	Mediation ratio
Emotional eating (adjusted for UE)										
Fenland	0.00 (-0.02, 0.03)	0.84	1.35 (1.15, 1.55)	$<1x10^{-10}$	0.61 (0.46, 0.76)	$<1x10^{-10}$	–	–	–	–
EDEN	0.03 (-0.01, 0.06)	0.10	1.07 (0.85, 1.30)	$<1x10^{-10}$	0.62 (0.44, 0.80)	$<1x10^{-10}$	–	–	–	–
Uncontrolled eating (adjusted for EE)										
Fenland	0.03 (0.01, 0.06)	0.01	0.65 (0.45, 0.85)	$<1x10^{-10}$	0.63 (0.48, 0.78)	$<1x10^{-10}$	0.61 (0.46, 0.76)	$<1x10^{-10}$	0.02	3%
EDEN	0.01 (-0.02, 0.04)	0.59	0.22 (-0.00, 0.45)	0.05	0.62 (0.44, 0.80)	$<1x10^{-10}$	–	–	–	–

– Not applicable (the EB was not associated with both the BMI-GRS and BMI)
Eating behaviour (EB); body mass index (BMI); genetic risk score for BMI (BMI-GRS)
The BMI-GRS to EB, EB to BMI and BMI-GRS to BMI associations (Beta (95% CI) and *p*-values) were each derived from separate linear regression models. All models were adjusted for age, sex, the other EB (UE in models testing EE; EE in models testing UE) and, in EDEN, recruitment centre

Table A.8 The residual method to determine the independent mediating effects of EE and UE on the association between the BMI-GRS and BMI

	BMI-GRS to EB		EB to BMI		BMI-GRS to BMI		BMI-GRS to BMI (adj. for EB)			
	Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Sobel test *p*-val.	Mediation ratio (%)
Emotional eating (residual of the regression of UE on EE)										
Fenland	0.00 (-0.02, 0.03)	0.87	1.32 (1.11, 1.54)	$<1x10^{-10}$	0.70 (0.54, 0.85)	$<1x10^{-10}$	–	–	–	–
EDEN	0.03 (-0.01, 0.06)	0.10	1.08 (0.85, 1.31)	$<1x10^{-10}$	0.62 (0.44, 0.80)	$<1x10^{-10}$	–	–	–	–
Uncontrolled eating (residual of the regression of EE on UE)										
Fenland	0.03 (0.01, 0.06)	0.01	0.74 (0.53, 0.95)	$<1x10^{-10}$	0.70 (0.54, 0.85)	$<1x10^{-10}$	0.67 (0.52, 0.83)	$<1x10^{-10}$	0.01	4%
EDEN	0.01 (-0.02, 0.04)	0.59	0.22 (-0.02; 0.46)	0.07	0.62 (0.44, 0.80)	$<1x10^{-10}$	–	–	–	–

– Not applicable (the EB was not associated with both the BMI-GRS and BMI)
Eating behaviour (EB); body mass index (BMI); genetic risk score for BMI (BMI-GRS)
The BMI-GRS to EB, EB to BMI and BMI-GRS to BMI associations (Beta (95% CI) and *p*-values) were each derived from separate linear regression models. EE was represented in the models by the residuals from the regression of UE on EE, adjusted for age and sex. UE was represented in the models by the residuals from the regression of EE on UE, adjusted for age and sex. All models were adjusted for age, sex and, in EDEN, recruitment centre

Table A.9 Individual SNP to EB associations in the Fenland cohort

		EE		UE		CR	
SNP	Nearest gene	Beta	*p*-val.	Beta	*p*-val.	Beta	*p*-val.
rs1000940	*RABEP1*	0.05	0.03	0.03	0.17	0.04	0.15
rs10132280	*STXBP6*	-0.05	0.04	-0.05	0.05	-0.03	0.30
rs1016287	*FLJ30838*	0.03	0.24	0.03	0.27	-0.01	0.58
rs10182181	*ADCY3*	0.06	0.02	0.04	0.10	0.04	0.06
rs10733682	*LMX1B*	-0.02	0.35	0.00	0.91	-0.01	0.65
rs10938397	*GNPDA2*	0.01	0.58	0.01	0.75	0.00	0.87
rs10968576	*LINGO2*	0.01	0.65	0.04	0.09	-0.01	0.67
rs11030104	*BDNF*	-0.04	0.14	-0.04	0.21	0.03	0.33
rs11057405	*CLIP1*	-0.06	0.11	-0.03	0.38	-0.04	0.32
rs11126666	*KCNK3*	0.01	0.67	0.00	0.99	0.01	0.74
rs11165643	*PTBP2*	0.05	0.02	0.08	0.00	0.00	0.87
rs11191560	*NT5C2*	0.00	1.00	-0.01	0.90	-0.01	0.76
rs11583200	*ELAVL4*	0.03	0.18	0.02	0.43	0.02	0.45
rs1167827	*HIP1*	-0.01	0.65	0.01	0.58	-0.01	0.82
rs11688816	*EHBP1*	-0.01	0.75	0.00	0.97	0.02	0.37
rs11727676	*HHIP*	0.02	0.61	0.01	0.86	0.07	0.07
rs11847697	*PRKD1*	0.03	0.60	0.06	0.32	0.02	0.70
rs12286929	*CADM1*	0.01	0.66	0.00	0.92	-0.01	0.59
rs12401738	*FUBP1*	0.03	0.26	0.04	0.11	0.00	0.87
rs12429545	*OLFM4*	-0.02	0.64	-0.01	0.73	-0.04	0.30
rs12446632	*GPRC5B*	0.01	0.86	-0.02	0.49	-0.01	0.79
rs12566985	*FPGT-TNNI3K*	0.01	0.55	-0.01	0.63	0.04	0.06
rs12885454	*PRKD1*	0.00	0.91	-0.03	0.22	0.01	0.57
rs12940622	*RPTOR*	0.00	0.86	0.02	0.4	0.00	0.99
rs13021737	*TMEM18*	0.02	0.57	0.03	0.41	0.03	0.38
rs13078960	*CADM2*	0.06	0.05	0.09	0.00	0.01	0.73
rs13107325	*SLC39A8*	0.03	0.52	-0.02	0.63	0.02	0.59
rs13191362	*PARK2*	-0.03	0.32	0.01	0.74	-0.03	0.37
rs13201877	*IFNGR1*	-0.03	0.46	0.00	0.91	-0.05	0.12
rs1441264	*MIR548A2*	-0.03	0.20	0.00	0.93	0.00	0.84
rs1460676	*FIGN*	0.03	0.27	0.04	0.26	0.07	0.03
rs1516725	*ETV5*	0.01	0.79	0.01	0.68	0.03	0.37
rs1528435	*UBE2E3*	0.00	0.86	-0.01	0.77	0.02	0.51
rs1558902	*FTO*	0.02	0.49	0.03	0.26	0.03	0.19
rs16851483	*RASA2*	-0.07	0.13	-0.04	0.40	0.08	0.06
rs16907751	*ZBTB10*	-0.02	0.69	-0.03	0.51	0.03	0.48

		EE		UE		CR	
SNP	Nearest gene	Beta	*p*-val.	Beta	*p*-val.	Beta	*p*-val.
rs16951275	*MAP2K5*	-0.01	0.75	-0.03	0.28	-0.01	0.69
rs17001654	*SCARB2*	0.03	0.42	0.04	0.29	0.05	0.17
rs17024393	*GNAT2*	0.03	0.72	-0.03	0.66	0.05	0.54
rs17094222	*HIF1AN*	-0.03	0.23	0.01	0.7	0.02	0.51
rs17203016	*CREB1*	-0.01	0.73	0.05	0.1	-0.02	0.47
rs17405819	*HNF4G*	0.01	0.74	0.00	0.88	0.04	0.14
rs17724992	*PGPEP1*	-0.03	0.32	0.00	0.97	-0.02	0.47
rs1808579	*C18orf8*	0.00	0.93	-0.03	0.14	0.00	0.99
rs1928295	*TLR4*	-0.01	0.53	0.00	0.92	0.01	0.78
rs2033732	*RALYL*	0.01	0.78	0.01	0.66	0.09	0.00
rs205262	*C6orf106*	-0.03	0.25	-0.04	0.14	0	0.99
rs2075650	*TOMM40*	-0.01	0.83	0.05	0.16	-0.12	0.00
rs2080454	*CBLN1*	-0.01	0.70	0.01	0.56	0.01	0.71
rs2112347	*POC5*	-0.03	0.23	0.01	0.75	-0.02	0.37
rs2121279	*LRP1B*	0.04	0.27	-0.02	0.57	-0.02	0.50
rs2176040	*LOC646736*	-0.01	0.67	-0.01	0.60	0	0.85
rs2176598	*HSD17B12*	-0.03	0.33	0.01	0.63	0.04	0.12
rs2207139	*TFAP2B*	0.03	0.31	0.03	0.38	-0.02	0.47
rs2245368	*PMS2L11*	0.02	0.60	0.06	0.05	0.05	0.11
rs2287019	*QPCTL*	0.02	0.56	0.05	0.12	0.08	0.01
rs2365389	*FHIT*	0.01	0.69	0.03	0.18	0.01	0.75
rs2650492	*SBK1*	-0.01	0.82	0.01	0.78	-0.01	0.61
rs2820292	*NAV1*	0.01	0.80	0.03	0.27	0.01	0.76
rs2836754	*ETS2*	0.01	0.67	0.03	0.21	-0.03	0.20
rs29941	*KCTD15*	0.00	0.92	0.00	0.92	0.03	0.29
rs3101336	*NEGR1*	0.02	0.47	0.03	0.28	0.00	0.86
rs3736485	*DMXL2*	0.01	0.60	0.00	0.92	0.00	0.9
rs3810291	*ZC3H4*	0.06	0.02	0.04	0.08	0.02	0.53
rs3817334	*MTCH2*	0.03	0.21	-0.01	0.81	0.00	0.88
rs3849570	*GBE1*	-0.05	0.04	-0.02	0.49	0.03	0.22
rs3888190	*ATP2A1*	-0.01	0.75	0.01	0.79	-0.03	0.24
rs4256980	*TRIM66*	0.02	0.30	0.02	0.36	0.00	0.94
rs4740619	*C9orf93*	-0.05	0.03	-0.04	0.09	0.01	0.54
rs4787491	*INO80E*	0.00	0.95	-0.03	0.19	0.01	0.56
rs492400	*USP37*	0.01	0.63	-0.02	0.32	0.01	0.60
rs543874	*SEC16B*	0.08	0.01	0.11	0.00	-0.01	0.69
rs6091540	*ZFP64*	0.01	0.76	0.00	0.87	-0.02	0.49
rs6465468	*ASB4*	0.01	0.58	0.02	0.48	-0.01	0.83

		EE		UE		CR	
SNP	Nearest gene	Beta	*p*-val.	Beta	*p*-val.	Beta	*p*-val.
rs6477694	*EPB41L4B*	-0.02	0.42	-0.01	0.57	0.00	0.93
rs6567160	*MC4R*	0.01	0.85	0.00	0.98	0.03	0.27
rs657452	*AGBL4*	0.06	0.02	0.04	0.13	0.01	0.83
rs6804842	*RARB*	0.05	0.05	0.06	0.01	0.00	0.86
rs7138803	*BCDIN3D*	0.03	0.15	0.03	0.24	-0.03	0.29
rs7141420	*NRXN3*	0.01	0.83	-0.01	0.74	-0.02	0.40
rs7164727	*LOC100287559*	0.02	0.41	0.02	0.43	0.00	0.90
rs7239883	*LOC284260*	0.00	0.93	0.02	0.40	-0.01	0.73
rs7243357	*GRP*	-0.04	0.23	0.01	0.67	0.04	0.21
rs758747	*NLRC3*	0.05	0.07	0.02	0.36	-0.01	0.67
rs7599312	*ERBB4*	0.02	0.54	0.00	0.95	-0.02	0.44
rs7715256	*GALNT10*	0.00	0.87	0.02	0.37	-0.02	0.29
rs7899106	*GRID1*	-0.09	0.07	-0.12	0.03	0.04	0.46
rs7903146	*TCF7L2*	0.01	0.65	0.03	0.23	0.00	0.91
rs9374842	*LOC285762*	-0.02	0.55	-0.05	0.08	-0.02	0.36
rs9400239	*FOXO3*	0.05	0.06	0.03	0.21	0.03	0.28
rs9540493	*MIR548X2*	-0.02	0.48	-0.04	0.09	-0.01	0.78
rs9581854	*MTIF3*	-0.03	0.27	0.00	0.98	-0.01	0.78
rs9641123	*CALCR*	0.04	0.10	0.04	0.14	0.04	0.14
rs977747	*TAL1*	0.02	0.30	0.04	0.11	0.07	0.00
rs9914578	*SMG6*	0.01	0.71	-0.03	0.29	-0.01	0.65
rs9925964	*KAT8*	-0.03	0.17	-0.06	0.01	-0.01	0.58

The table comprises each of the 96 SNPs used to construct the Fenland BMI-GRS and EB. Effect estimates (Beta) and *p*-values are taken from the sex and age adjusted regressions of each SNP on the specified EB trait

Table A.10 Individual SNP to EB associations in the EDEN cohort

		EE		**UE**		**CR**	
SNP	Nearest gene	Beta	*p*-val.	Beta	*p*-val.	Beta	*p*-val.
rs10146997	*NRXN3*	0.03	0.50	0.03	0.45	0.02	0.64
rs10838738	*MTCH2*	0.08	0.01	0.06	0.06	-0.01	0.69
rs10913469	*SEC16B*	-0.01	0.72	-0.01	0.78	0.03	0.40
rs11847697	*PRKD1*	0.00	0.96	-0.04	0.57	0.06	0.35
rs12016871	*MTIF3*	-0.04	0.36	-0.01	0.84	-0.01	0.82
rs13107325	*SLC39A8*	-0.15	0.01	-0.16	0.00	0.05	0.40
rs1514175	*TNNI3K*	-0.05	0.14	-0.06	0.05	0.00	0.95
rs1555543	*PTBP2*	-0.01	0.81	0.00	0.97	0.05	0.13
rs17782313	*MC4R*	0.03	0.39	0.04	0.28	0.00	0.95
rs206936	*NUDT3*	-0.07	0.05	-0.09	0.01	-0.01	0.87
rs2112347	*FLJ35779*	0.01	0.82	0.01	0.81	0.02	0.48
rs2241423	*MAP2K5*	0.07	0.06	0.00	0.92	0.05	0.18
rs2287019	*QPCTL*	-0.06	0.16	-0.01	0.74	0.03	0.40
rs2568958	*NEGR1*	0.00	1.00	0.02	0.52	0.04	0.23
rs2890652	*LRP1B*	-0.01	0.87	0.04	0.40	0.04	0.30
rs3810291	*TMEM160*	0.06	0.04	0.04	0.26	0.02	0.51
rs4836133	*ZNF608*	-0.02	0.46	-0.04	0.15	0.04	0.21
rs4929949	*RPL27A*	-0.01	0.80	0.00	0.89	-0.02	0.58
rs6548238	*TMEM18*	0.02	0.65	0.02	0.66	0.06	0.12
rs713586	*RBJ/POMC*	0.02	0.47	-0.01	0.79	0.00	0.93
rs7138803	*BCDIN3D*	0.06	0.08	0.04	0.26	-0.01	0.81
rs7640855	*CADM2*	0.05	0.17	0.03	0.48	0.02	0.68
rs7647305	*TRA2B*	0.05	0.20	0.06	0.10	0.10	0.01
rs887912	*FANCL*	0.04	0.25	0.01	0.85	-0.01	0.77
rs925946	*BDNF*	0.03	0.37	0.05	0.11	0.08	0.02
rs987237	*TFAP2B*	-0.01	0.80	-0.03	0.47	0.04	0.36
rs9941349	*FTO*	0.08	0.01	0.08	0.01	0.02	0.48

The table comprises the 27 SNPs used to construct the EDEN BMI-GRS. Effect estimates (Beta) and *p*-values are taken from the sex and age adjusted regressions of each SNP on the specified EB trait

Table A.11 Mediation analyses using self-reported pre-pregnant BMI in EDEN

BMI-GRS to EB		EB to BMI		BMI-GRS to BMI		BMI-GRS to BMI (adj. for EB)			
Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Beta (95%CI)	*p*-val.	Sobel test *p*-val.	Mediation ratio (%)
Emotional eating (n=2154)									
0.06 (0.01, 0.10)	0.01	1.13 (0.96, 1.29)	$<1x10^{-10}$	0.55 (0.38; 0.72)	$3x10^{-10}$	0.49 (0.33, 0.66)	$6x10^{-9}$	0.01	11%
Uncontrolled eating (n=2154)									
0.05 (0.01, 0.09)	0.03	0.83 (0.66. 1.00)	$<1x10^{-10}$	0.55 (0.38; 0.72)	$3x10^{-10}$	0.52 (0.35, 0.68)	$2x10^{-9}$	0.03	7%
CR linear term (n=1200)									
0.04 (0.04, 0.15)	<0.01	1.55 (1.31, 1.78)	$<1x10^{-10}$	0.67 (0.43; 0.91)	$8x10^{-8}$	0.53 (0.30, 0.75)	$8x10^{-6}$	<0.01	22%
CR quadratic term (n=1200)									
-0.01 (-0.08, 0.05)	0.70	-0.29 (-0.49, -0.10)	$4x10^{-3}$	0.67 (0.43; 0.91)	$8x10^{-8}$	–	–	–	–

EE and UE analyses included both men and women but replaced the women's BMI with pre-pregnant BMI. The CR analyses included only women and replaced women's BMI with pre-pregnant BMI

– Not applicable (the EB was not associated with both the BMI-GRS and BMI)

Models were adjusted for age, recruitment centre and, in the case of the EE and UE analyses, sex

A.3 Chapter 5

Table A.12 The maternal attitude questionnaire items loadings onto the identified factor

Questionnaire item	Factor 1	Uniqueness
Optimal growth	0.74	0.45
Good about yourself	0.64	0.59
Best for baby	0.77	0.40
Baby will stay hungry	0.57	0.67
Baby will wake at night	0.48	0.77
Confident with crying	0.51	0.74
Confident without friends	0.57	0.67
Difficult without family	-0.08	0.99
Difficult to follow	0.5584	0.69
Intend to follow guidelines	0.79	0.38
Try to follow guidelines	0.69	0.53

Table A.13 Modification analysis of infant EB traits and the MAS to infant milk intake by infant sex

	Beta MAS or infant EB (95% CI)	*p*-val.	Beta infant sex (95% CI)	*p*-val.	Beta-interaction	*p*-interaction
Maternal attitudes score	-92.2 (-134.8, -49.5)	<0.001	-91.4 (-289.6, 106.8)	0.37	42.2 (-14.7, 99.0)	0.15
Infant FR	56.5 (17.5, 95.5)	0.01	90.6 (-15.9, 197.2)	0.10	-22.3 (71.7, 27.1)	0.38
Infant SR	-35.7 (-70.4, -1.1)	0.04	71.8 (-47.8, 191.4)	0.24	-8.1 (-55.2, 39.0)	0.74

Food responsiveness (FR); Satiety responsiveness (SR); Maternal attitudes score (MAS)
Results are from the linear regression: (1) Milk intake ~ [MAS*infant sex] + MAS + infant sex + infant age or (2) Milk intake ~ [infant EB*infant sex] + infant EB + infant sex + infant age
Effect estimates are ml/day per unit change in MAS or infant EB

Table A.14 Modification analysis of infant EB traits and the MAS to infant weight SDS by infant sex

	Beta MAS or infant EB (95% CI)	*p*-val.	Beta infant sex (95% CI)	*p*-val.	Beta-interaction	*p*-interaction
Maternal attitude score	-0.1 (-0.3, 0.0)	0.16	0.1 (-0.7, 0.9)	0.81	-0.0 (-0.2, 0.2)	0.92
Infant FR	0.2 (0.1, 0.4)	0.01	-0.1 (-0.5, 0.3)	0.61	0.0 (-0.2, 0.2)	0.65
Infant SR	-0.1 (-0.3, 0.0)	0.08	0.27 (-0.2, 0.8)	0.28	-0.1 (-0.3, 0.1)	0.32

Food responsiveness (FR); Satiety responsiveness (SR); Maternal attitudes score (MAS)
Results are from the regression: (1) Weight SDS ~ [MAS*infant sex] + MAS + infant sex + infant age or (2) Weight SDS ~ [infant EB*infant sex] + infant EB + infant sex + infant age
Effect estimates are SDs of infant weight per unit change in MAS or infant EB

Table A.15 The association between infant food responsiveness and infant milk intake within tertiles of the MAS

Tertiles of MAS	Beta (95% CI) (ml/day)	*p*-value
Lowest tertile	66.8 (17.8, 115.8)	0.01
Middle tertile	24.1 (-12.5, 60.7)	0.20
Highest tertile	36.0 (-4.0, 76.1)	0.08

Maternal attitudes score (MAS); Food responsiveness (FR)
Effect estimates and *p*-values are from the regression: Milk intake ~ infant food responsiveness + infant sex + infant age. Effect estimates are: change in infant milk intake (ml/day) per unit increase FR
Tertiles are tertiles of the maternal attitudes score

Table A.16 The association between infant satiety responsiveness and infant weight SDS within tertiles of the maternal attitudes score

Tertiles of MAS	Beta (95% CI) (SDs of weight)	*p*-value
Lowest tertile	-0.28 (-0.47, -0.10)	0.003
Middle tertile	-0.20 (-0.35, -0.04)	0.01
Highest tertile	-0.02 (-0.19, 0.14)	0.77

Maternal attitudes score (MAS); Satiety responsiveness (SR)
Effect estimates and *p*-values are from the regression: Weight SDS ~ infant satiety responsiveness + infant sex + infant age. Effect estimates are: change in infant weight SDS (SDs) per unit increase SR
Tertiles are tertiles of the maternal attitudes score

Table A.17 The associations between the MAS and infant EB traits with infant milk intake and weight SDS

	Infant weight SDS [a]		Infant milk intake [b]	
	Beta (95% CI) (ml/day)	*p*-value	Beta (95% CI) (SDs of infant weight)	*p*-value
Infant eating behaviour				
Food responsiveness	41.0 (16.6, 65.4)	0.001	0.26 (0.16, 0.36)	<0.001
Satiety responsiveness	-43.2 (-67.1, -19.2)	<0.001	-0.19 (-0.29, -0.09)	<0.001
Maternal attitudes score	-71.9 (-100.7, -43.0)	<0.001	-0.12 (-0.24, -0.01)	0.04

Effect estimates and *p*-values are from the regression: [infant weight SDS OR infant milk intake] ~ [infant EB OR MAS] + infant age + infant sex + maternal BMI + maternal age + maternal education + maternal ethnic group

[a] Effect estimates are SD change in infant weight SDS per 1 point increase in infant EB or the MAS

[b] Effect estimates are change in infant milk intake (ml/day) per 1 point increase in infant EB or the MAS

Table A.18 Modification of the association of infant EB traits to infant milk intake and infant weight SDS

	Beta infant EB (95% CI) [a]	*p*-val.	Beta MAS (95% CI) [b]	*p*-val.	Beta-interaction (95% CI) [c]	*p*-interaction
Infant milk intake						
Infant food responsiveness	164.5 (24.0, 304.9)	0.02	4.5 (-86.3, 95.2)	0.92	-37.8 (-78.2, 2.5)	0.07
Infant satiety responsiveness	-112.0 (-250.6, 26.6)	0.11	-124.5 (-220.6, -28.3)	0.01	19.5 (-19.4, 58.5)	0.33
Infant weight SDS						
Infant food responsiveness	0.7 (0.1, 1.3)	0.03	0.2 (-0.2, 0.5)	0.37	-0.1 (-0.3, 0.0)	0.15
Infant satiety responsiveness	-0.9 (-1.5, -0.3)	0.002	-0.6 (-1.0, -0.2)	0.003	0.2 (0.0, 0.4)	0.01

Eating behaviour (EB); Maternal attitudes score (MAS)
Results are from the regression: [Infant milk intake or weight SDS] ~ [MAS*infant EB] + infant EB + MAS + infant sex + infant age + maternal BMI + maternal age + maternal education + maternal ethnic group
[a] Effect estimates are change in infant milk intake (ml/day) or infant weight SDS (SDs) per unit change in infant EB
[b] Effect estimates are change in infant milk intake (ml/day) or infant weight SDS (SDs) per unit change in the maternal attitudes score
[c] Effect estimates are for the MAS*infant EB interaction term

A.4 Chapter 6

Table A.19 Association between the top 10 principal components and odds of risk-taking propensity

	Odds ratio	95% CI	*p*-value
PC1	1.008	(1.005, 1.011)	<0.001
PC2	0.995	(0.992, 0.998)	0.002
PC3	1.004	(1.000, 1.008)	0.036
PC4	0.99	(0.988, 0.992)	<0.001
PC5	1.012	(1.010, 1.013)	<0.001
PC6	1.004	(1.001, 1.008)	0.013
PC7	0.995	(0.992, 0.998)	<0.001
PC8	1.009	(1.006, 1.012)	<0.001
PC9	0.998	(0.996, 0.999)	0.004
PC10	1.003	(1.000, 1.006)	0.037

Principal component (PC); Confidence interval (CI)
Odds ratios, 95% CIs and *p*-values are from the logistic regression of each of the PCs on the odds of risk-taking, adjusted for age and sex

Table A.20 Look up of genome-wide significant SNPs for risk-taking in UK Biobank for *Ever smoking*

SNP	Chr	Base pair	Effect allele	Other allele	Effect allele frequency	Info.	SE	*p*-value
rs6762267	3	85513115	C	A	0.381262	0.998347	0.001051	3.40E-19
rs727644	7	114109349	G	A	0.595406	0.992547	0.001045	1.90E-01
rs62519827	8	65481947	T	C	0.887242	1	0.001616	4.60E-01
rs9841382	3	181408124	C	T	0.144996	0.992706	0.001453	4.70E-05
rs58560561	1	243537729	G	T	0.651824	0.985229	0.001078	9.40E-01
rs992493	4	106180264	T	C	0.188643	0.99873	0.001307	1.80E-04
rs6923811	6	27289776	T	C	0.678951	1	0.001097	9.90E-05
rs7817124	8	81404008	C	G	0.239069	0.997976	0.001199	5.60E-02
rs4801000	18	53456943	G	A	0.33539	0.997479	0.001083	3.40E-02
rs4653015	1	33776431	T	C	0.26086	0.994959	0.001164	2.50E-02
rs12476923	2	145830053	A	C	0.336033	0.999171	0.001078	5.20E-01
rs283914	3	17330649	T	C	0.531356	0.997244	0.001024	6.20E-02
rs4233093	1	73446245	A	G	0.516179	0.997724	0.001022	7.60E-07
rs7829912	8	33479228	T	C	0.555727	0.991336	0.001031	6.00E-02
rs3117340	6	29210596	G	T	0.622344	0.999729	0.001055	8.50E-07
rs1381287	14	98597552	T	C	0.456905	0.986444	0.001032	9.80E-07
rs28520003	22	46411969	G	A	0.68587	1	0.001102	1.60E-07
rs12115650	9	126367705	G	A	0.725133	0.986183	0.001151	1.10E-01
rs11226319	11	104221573	A	G	0.160313	0.994423	0.001394	2.50E-01
rs1358391	7	115111838	G	T	0.505524	0.986954	0.001027	8.80E-07
rs12617392	2	27336827	C	A	0.558239	0.992674	0.00103	7.00E-01
rs542883	2	45143382	C	G	0.559253	0.995628	0.001029	1.20E-14
rs10823791	10	73338334	T	A	0.398774	0.997238	0.001045	1.30E-01
rs34905321	6	109131107	T	C	0.567814	0.995805	0.001033	1.90E-01
rs891124	16	71440756	T	C	0.710131	0.988667	0.001132	1.20E-03
rs35914833	14	94182383	C	T	0.684169	0.978438	0.001111	3.80E-01

Single nucleotide polymorphism (SNP); Chromosome (Chr); Standard error (SE)

Info. refers to the imputation information value

Grey shading indicates nominal statistical significance in association with the ever smoking phenotype (p<0.05)

APPENDIX B

SUPPLEMENTARY FIGURES

B.1 Chapter 4

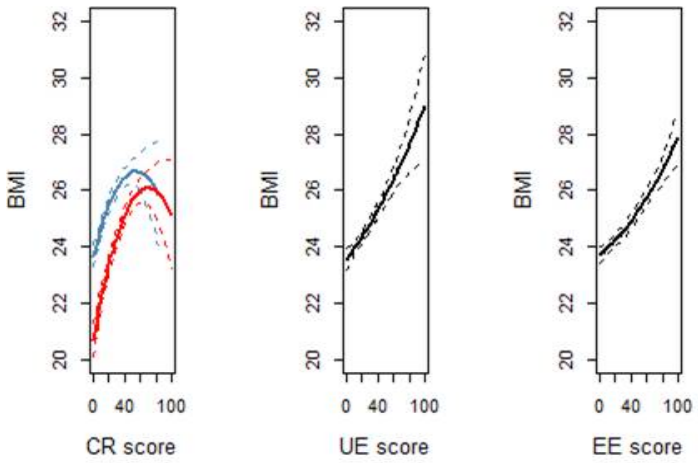


Figure B.1 The association between EB and BMI in the EDEN cohort. The graphs plot the scaled eating behaviour scores (0-100) on the x-axis against BMI (kg/m^2) on the y-axis. The association amongst women is shown in blue and the association amongst men is shown in red. The combined cohort is shown in black. The dotted lines mark the 95% CIs. Cognitive restraint (CR); Uncontrolled eating (UE); Emotional eating (EE).

B.2 Chapter 6

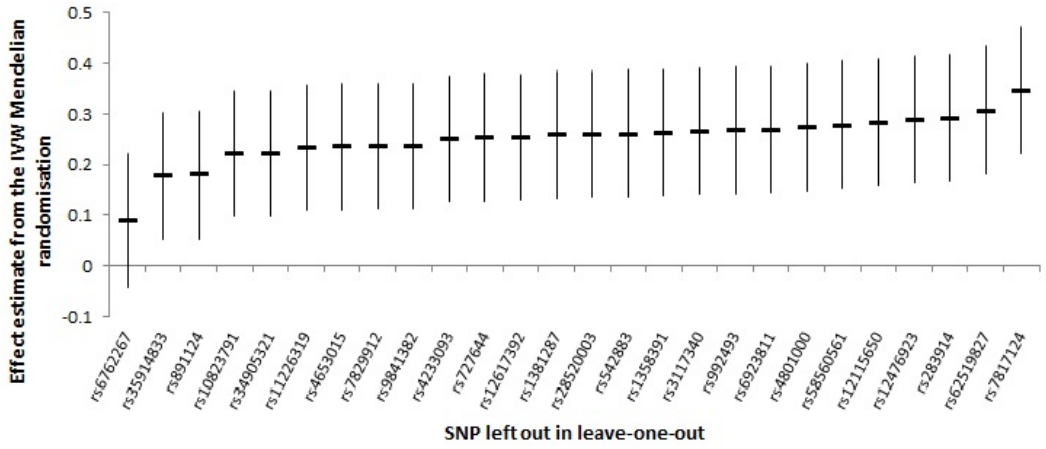


Figure B.2 Leave-one-out analysis. The figure plots the effect estimates and 95% confidence intervals from a series of inverse weighted variance (IVW) Mendelian randomisation analyses on the y-axis against the SNP removed from each analysis on the x-axis. A comparison of the effect estimates for the 4 SNPs that reached genome-wide significance for BMI (rs6762267, rs35914833, rs891124 and rs7817124) with the mean of the effect estimates from the sample with that SNP removed, showed a significant difference (p<0.05).

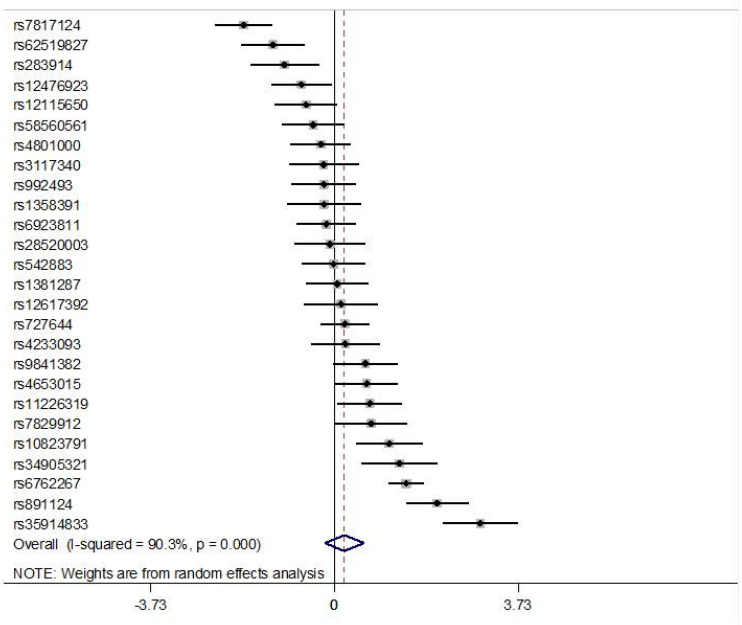


Figure B.3 Random effects inverse variance weighted MR analysis of risk-taking to BMI. This analysis combined the effect estimates ascertained when treating each of the genome-wide significant SNPs for risk-taking (displayed on the y-axis) as an individual instrument. Points indicate effect estimates, bars indicate 95% CIs.

B.3 Chapter 7

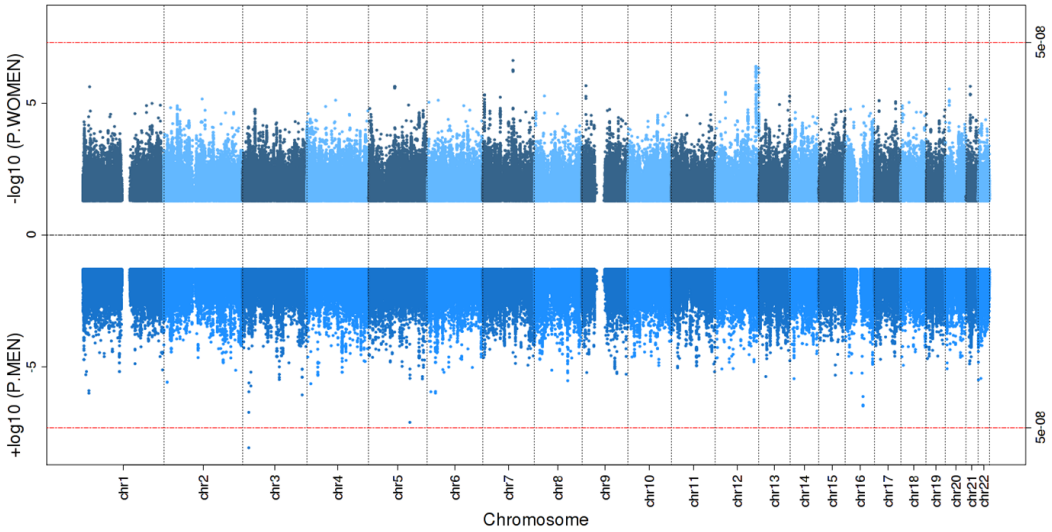


Figure B.4 Miami plot of the GWAS for emotional eating. The plot illustrates the results of the GWAS amongst 11,809 white European participants from the Fenland, FinnTwin, NHS and HPFS cohorts, stratified by sex. Each dot represents a genetic variant. The results for women (n=7382) and men (n=4427) are displayed on the top and bottom, respectively. Chromosomal position (x-axis) is plotted against the negative log-transformed p-values for each SNP for women (y-axis) and the positive log-transformed p-values for each SNP (y-axis) for men. The red dotted line indicates the threshold for statistical significance ($p<5\times10^{-8}$).

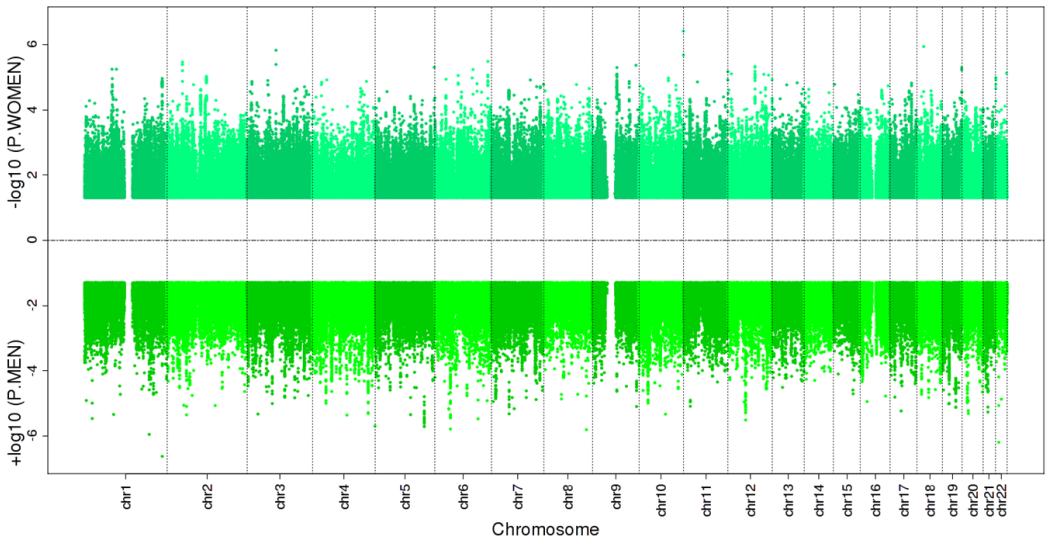


Figure B.5 Miami plot of the GWAS for uncontrolled eating. The plot illustrates the results of the GWAS amongst 11,827 white European participants from the Fenland, FinnTwin, NHS and HPFS cohorts, stratified by sex. Each dot represents a genetic variant. The results for women (n=7397) and men (n=4430) are displayed on the top and bottom, respectively. Chromosomal position (x-axis) is plotted against the negative log-transformed p-values for each SNP for women (y-axis) and the positive log-transformed p-values for each SNP (y-axis) for men. The red dotted line indicating the threshold for statistical significance ($p<5\times10^{-8}$) is not visible on this plot as no variants approach this threshold.

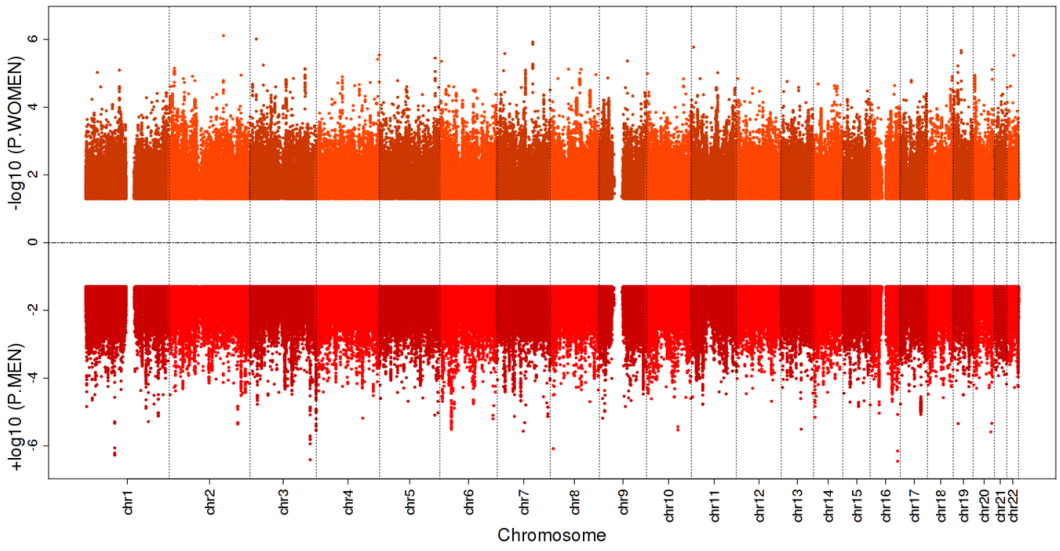
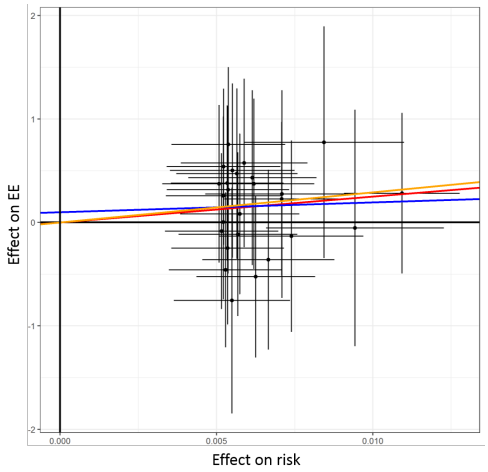
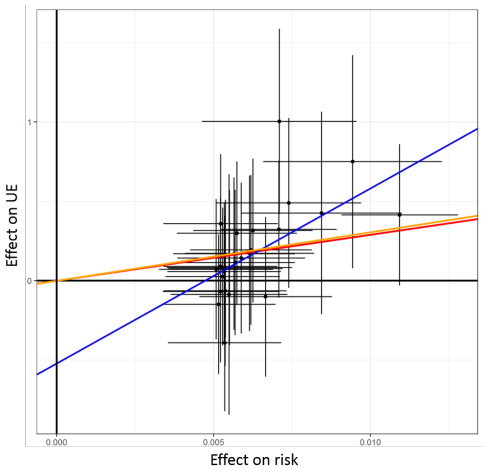


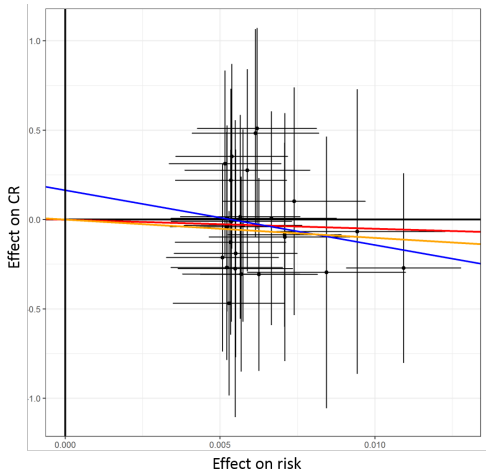
Figure B.6 Miami plot of the GWAS for cognitive restraint. The plot illustrates the results of the GWAS amongst 11,843 white European participants from the Fenland, FinnTwin, NHS and HPFS cohorts, stratified by sex. Each dot represents a genetic variant. The results for women (n=7408) and men (n=4435) are displayed on the top and bottom, respectively. Chromosomal position (x-axis) is plotted against the negative log-transformed p-values for each SNP for women (y-axis) and the positive log-transformed p-values for each SNP (y-axis) for men. The red dotted line indicating the threshold for statistical significance ($p<5\times10^{-8}$) is not visible on this plot as no variants approach this threshold.



(a) Emotional eating



(b) Uncontrolled eating



(c) Cognitive restraint

Figure B.7 Dosage plots showing the results of the MR analyses of risk-taking to EB. Each dot represents one of the 26 risk-associated SNPs, 95% CIs are represented by black lines. The effect of each SNP on risk-taking (x-axis) is plotted against its effect on EB (y-axis). The coloured lines represent the MR results. Red represents the IVW MR, blue represents the MR Egger, green represents the weighted median MR and orange represents the penalised weight median MR. The results of all MR analyses are displayed on each plot. However, where results overlap, some lines are not visible.

APPENDIX C

SUPPLEMENTARY INFORMATION

C.1 Chapter 1

The Three Factor Eating Questionnaire - Revised 18 item

The Three Factor Eating Questionnaire - Revised 18 item (TFEQ-R18)

Karlsson, J., Persson, L. O., Sjostrom, L. Sullivan, M. (2000) Psychometric properties and factor structure of the Three-Factor Eating Questionnaire (TFEQ) in obese men and women. Results from the Swedish Obese Subjects (SOS) study. *Int. J. Obes. Relat. Metab. Disord.* 24:1715–1725

Please read each statement and select from the multiple choice options the answer that indicates the frequency with which you find yourself feeling or experiencing what is being described in the statements below.

1. When I smell a delicious food, I find it very difficult to keep from eating, even if I have just finished a meal.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

2. I deliberately take small helpings as a means of controlling my weight.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

3. When I feel anxious, I find myself eating.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

4. Sometimes when I start eating, I just can't seem to stop.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

5. Being with someone who is eating often makes me hungry enough to eat also.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

6. When I feel blue, I often overeat.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

7. When I see a real delicacy, I often get so hungry that I have to eat right away.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

8. I get so hungry that my stomach often seems like a bottomless pit.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

9. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

10. When I feel lonely, I console myself by eating.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

11. I consciously hold back at meals in order not to weight gain.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

12. I do not eat some foods because they make me fat.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

13. I am always hungry enough to eat at any time.

Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

14. How often do you feel hungry?

Only at meal times (1)/ sometimes between meals (2)/ often between meals (3)/ almost always (4)

15. How frequently do you avoid “stocking up” on tempting foods?

Almost never (1)/ seldom (2)/ moderately likely (3)/ almost always (4)

16. How likely are you to consciously eat less than you want?

Unlikely (1)/ slightly likely (2)/ moderately likely (3)/ very likely (4)

17. Do you go on eating binges though you are not hungry?

Never (1)/ rarely (2)/ sometimes (3)/ at least once a week (4)

18. On a scale of 1 to 8, where 1 means no restraint in eating (eating whatever you want, whenever you want it) and 8 means total restraint (constantly limiting food intake and never “giving in”), what number would you give yourself?*

*For item 18, responses of 1 & 2 are coded 1; 3 & 4 are coded 2; 5 & 6 are coded 3; and 7 & 8 are coded 4.

Emotional eating (EE) is measured by items 3, 6 & 10; Uncontrolled eating (UE) is measured by items 1, 4, 5, 7, 8, 9, 13, 14 & 17; Cognitive restraint (CR) is measured by items 2, 11, 12, 15, 16 & 18.

The Baby Eating Behaviour Questionnaire (BEBQ)

The Baby Eating Behaviour Questionnaire (BEBQ) - retrospective version

Llewellyn, CH., van Jaarsveld, CHM., Johnson, L., Carnell, S. Wardle, J. (2011) Development and factor structure of the Baby Eating Behaviour Questionnaire in the Gemini birth cohort. *Appetite.* 57:388–396

These questions are about your baby's appetite over his/her first few months of life. We are specifically interested in the period during which your baby was fed milk only, i.e. no solid foods or pre-prepared baby food yet.

How would you describe your baby's feeding style at a typical daytime feed?

1. My baby seemed contented while feeding.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

2. My baby frequently wanted more milk than I provided.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

3. My baby loved milk.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

4. My baby had a big appetite.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

5. My baby finished feeding quickly*.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

6. My baby became distressed while feeding*.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

7. My baby got full up easily.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

8. If allowed to, my baby would take too much milk.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

9. My baby took more than 30 minutes to finish feeding.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

10. My baby got full before taking all the milk I think he/she should have.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

11. My baby fed slowly.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

12. Even when my baby had just eaten well he/she was happy to feed again if offered.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

13. My baby found it difficult to manage a complete feed.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

14. My baby was always demanding a feed.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

15. My baby sucked more and more slowly during the course of a feed.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

16. If given the chance, my baby would always be feeding.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

17. My baby enjoyed feeding time.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

18. My baby could easily take a feed within 30 minutes of the last one.

Never (1)/ Rarely (2) / Sometimes (3)/ Often (4)/ Always (5)

* Items 5 and 6 need to be reversed for scoring.
Food responsiveness (FR) is measured by items 2, 8, 12, 14, 16 & 18; Satiety responsiveness (SR) is measured by items 7, 10 & 13; Enjoyment of food (EF) is measured by items 1, 3, 6 & 17; Slowness in eating (SiE) is measured by items 5, 9, 11 & 15; General appetite (GA) is measured by item 4.

C.2 Chapter 5

Maternal beliefs about following recommendations to reduce formula-milk feed quantities

Maternal beliefs about following recommendations to reduce formula-milk feed quantities

Lakshman, RR., Landsbaugh, JR., Schiff, A., Hardeman, W., Ong, KK. Griffin, SJ. (2011) Development of a questionnaire to assess maternal attitudes towards infant growth and milk feeding practices. *International Journal of Behavioural Nutrition and Physical Activity.* 8:35

1. If I follow the new feeding recommendation, my baby's growth will be optimal.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

2. If I follow the new feeding recommendation, I will feel good about myself.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

3. If I follow the new feeding recommendation, I will feel I do the best for my baby.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

4. If I follow the new feeding recommendation, my baby will remain hungry*.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

5. If I follow the new feeding recommendation, my baby will wake up frequently at night*.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

6. I am confident that I can follow the new feeding recommendation even if my baby cries between feeds.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

7. I am confident that I can follow the new feeding recommendation even if my friends do not follow the same recommendation.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

8. It would be difficult for me to follow the new feeding recommendation if my partner and family do not support me*.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

9. It would be difficult for me to follow the new feeding recommendation*.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

10. I intend to follow the new feeding recommendation.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

11. I will try to follow the new feeding recommendation.

Strongly disagree (1)/ Somewhat disagree (2)/ Neither agree nor disagree (3)/ Somewhat agree (4)/ Strongly agree (5)

* Reverse coded items.
Outcome expectancy (OE) is measured by items 1, 2, 3, 4 & 5; Self-efficacy (SE) is measured by items 6, 7, 8 & 9; Intention (I) is measured by items 10 & 11.

C.3 Chapter 7

Eating behaviour GWAS analysis plan

Eating behaviour GWAS analysis plan

Distributed 01.02.2017
If you have any queries please contact Emma Clifton (emma.clifton@mrc-epid.cam.ac.uk).

No genome-wide gene discovery studies have been conducted for human eating behaviour (EB). The aim of this project is to detect novel genetic signals by conducting a GWAS of the 3 subscales of EB measured by the three factor eating questionnaire (TFEQ). Both the revised 18-item version (TFEQ-R18) and revised 21-item version (TFEQ-R21) of the TFEQ are appropriate for this analysis. Both questionnaires are comprised of 3 subscales: uncontrolled eating, emotional eating and cognitive restraint. Each subscale measures a different aspect of EB. Uncontrolled eating refers to a tendency to overeat with loss of control over consumption, emotional eating describes a tendency to overeat in response to dysphoric emotional states and cognitive restraint refers to the intention to exert restrictive control over eating with the goal of influencing body shape or weight.

1 Phenotypes

We ask that you analyse the following 3 subscales:

1. Emotional eating
2. Uncontrolled eating
3. Cognitive restraint

These will be analysed in men and women separately, for a total of 6 final results files (assuming studies have both men and women).

1.1 Scaling the phenotypes

This study proposes to use data from both the 18-item (TFEQ-R18) and 21-item (TFEQ-R21) version of the TFEQ. Compared to the TFEQ-R18, the TFEQ-R21 has 3 additional items in the emotional eating scale. As such, we need to standardize between the phenotypes. Further, in order that the scores for each of the 3 EB subscales are scaled from 0 to 100, the following equation should be used for each participant in your study and for each of the subscales:

$$\text{Phenotype} = [((\text{raw score}–\text{lowest possible raw score})/\text{possible raw score range}) * 100]$$

The following definitions apply:

- ***Raw score***. The mean of the items for the eating behaviour subscale for each participant is taken (***Score mean***) and multiplied by the total number of items on the subscale. This step accounts for any missing data.
- ***Lowest possible raw score***. The lowest possible score a participant could receive for the subscale. As each item on the subscales is scored from 1 to 4, if there are 3 items on the scale, the lowest possible raw score would be 3.

- ***Possible raw score range***. The highest possible ***raw score*** (the number of items on the scale∗4) for the eating behaviour subscale minus the ***lowest possible raw score*** for the eating behaviour subscale.

This translates to the subscales as follows:

Emotional eating	EE raw score = EE mean*3	[((EE raw score – 3)/9)*100]
Uncontrolled eating	UE raw score = UE mean*9	[((UE raw score – 9)/27)*100]
Cognitive restraint	CR raw score = CR mean*6	[((CR raw score – 6)/18)*100

1.2 Exclusion criteria

Please exclude participants who fulfil the following criteria, if this information is available in your study:

- Clinically diagnosed eating disorder

2 Imputation

We request that all studies are imputed to the most up-to-date imputation panel. The following websites provide detailed instructions for the two alternative algorithms for imputation:

Minimac:
http://genome.sph.umich.edu/wiki/Minimac:_1000_Genomes _Imputation _Cookbook
IMPUTE2:
http://genome.sph.umich.edu/wiki/IMPUTE2:_1000_Genomes _Imputation _Cookbook

3 Quality control

Below are some standard quality control suggestions. Please let us know if substantially different procedures have been applied in your study.

3.1 SNP QC criteria

Pre-imputation
We assume that the following pre-imputation procedures have been applied to directly genotyped SNPs that have been used for imputation. Please state if otherwise.

- HWE (advised P>10E-06)
- SNP call rate (advised >95%)
- MAF (advised > 0.1%)

Post-imputation
We assume that the following post-imputation procedures have been applied in your study. Please state if otherwise.

- Imputed data is filtered on imputation quality only. Variants with imputation quality score <0.3 have been excluded.
- Mono-morphic SNPs have been excluded (these are likely to have missing values for Beta and standard errors (SEs) in results files).

- SNPs are not reported twice. Directly genotyped SNPs have been kept as they were genotyped and have not been substituted with imputed values.
- *P*-values and SE estimates have not been corrected for GC. This correction will be performed during the meta-analysis.

3.2 Sample level QC

We assume that your sample-level QC procedures are similar to those listed below. Please state if otherwise.

- Samples missing >5% genotypes have been excluded.
- Population clustering has been performed and used to identify and exclude samples demonstrating outlying ethnic ancestry.
- Samples exhibiting a high inbreeding coefficient or a heterozygote rate far from the median (indicating possible contamination) based on the distribution observed in the data have been identified and excluded.
- Samples showing gonosomal abnormalities have been excluded.
- Sex-mismatched samples have been excluded.
- Duplicate pairs have been identified. If not identical twins in cohorts with a twin design, one sample (the sample with less missing data) has been kept from duplicate pairs.
- Samples with unexpectedly high proportion IBD sharing have been excluded.
- Unexpected relatives, with consideration of family structure, based on high quality variants have been excluded.
- Indels have been retained for analysis alongside biallelic SNPs.

Known relatedness: Some of the contributing cohorts use family-based recruitment. In these cases, please take any usual steps to account for relatedness within the sample.

In all models: Assume additive genetic effect. Please do not impute missing phenotypes or omit true outliers (i.e. those which correspond to a real observation). Do not apply genomic correction to results, or filter results based on imputation quality; we can do so centrally.

4 Association Analyses

Conduct 6 separate genome-wide association studies: an analysis for each of the 3 EBs in each sex. Adjust for age and study specific covariates.

Separately, in each sex:

Emotional eating ~ Age, Study specific covariates
Uncontrolled eating ~ Age, Study specific covariates
Cognitive restraint ~ Age, Study specific covariates

Study specific covariates: Apply your normal approach to account for population structure (e.g. inclusion of genomic PCs or relationship matrix), take any usual steps to account for relatedness in your study and adjust for relevant study-specific covariates (e.g. study site if a multi-center study), as appropriate.

5 Results

5.1 Format

Results for each EB should be returned as a separate tab-delimited text file including both typed and imputed variants, one variant per line (multiple lines may be required for multi-allelic variants). For each variant, please return the variables listed in the table. The first line should contain the variables names as a header. Denote missing data using a full-stop/period (".").

Variable	Description	Format
snp _id	Unique SNP ID as rs number	rsID
chr	Chromosome number	Integer
pos	Position on NCBI build 37	Integer
strand	We request SNPs to be aligned to the forward (+) strand	+ or -
effect _allele	Allele to which the *Beta* estimate refers	String (**see below**)
other _allele	Alternative allele	String (**see below**)
eaf	Observed allele frequency for the *effect _allele* in the study cohort	Numeric
HWE _pval	Exact test Hardy-Weinberg equilibrium *p*-value (directly typed SNPs only)	Numeric
indel	Label "I" for insertion; "D" for deletion	I or D
Beta	Effect estimate for the *effect _allele*; 5 decimal places	Numeric
SE	Standard error of *Beta*; $\geq 5 decimal places$	Numeric
pval	*p*-value for *Beta*	Numeric
callrate	Genotyping call rate after exclusions	Numeric
n _total	Total sample with available phenotype and genotype for SNP	Integer
imputed	1/0 coding: 1=imputed SNP; 0=directly typed SNP	0 or 1
used _for _imp	1/0 coding: 1=used for imputation; 0=not used for imputation	0 or 1
oevar _imp	Imputation quality (observed divided by expected variance for imputed allele dosage). Report r2hat for minimac and proper _info for IMPUTE2	Numeric
imputation _prog	Indicate which program was used for imputation. 1=Minimac; 2=IMPUTE2; 3=OTHER (if other please describe in the GoogleDoc (Section 5.1). If directly genotyped, code as missing (".")	1, 2 or 3

Effect allele/Other allele: Please report the effect _allele as the allele referred to by the beta estimates and effect allele frequency. In the case of indels, please use the I/D coding, where I represents the longer of the two possible alleles, and D the shorter of the two.

5.2 File submission

The submitted data should be formatted as gzipped, tab-delimited text files.

File names should follow the rules for the file name as below:

PANEL _COHORTNAME _Eating _behaviour _SEX _DATE _INITIALS.txt

PANEL: Imputation panel (**1000G** or **HRC**)
COHORTNAME: Cohort name (e.g. **FENLAND**)
Eating _behaviour: **Emotional** or **Uncontrolled** or **Restraint**
Sex: **Men** or **Women**

Date: Analysis date (**DDMonthYYYY**)
Initials: Initials of person uploading the file

For example, for emotional eating amongst men and women in Fenland:

HRC _Fenland _Emotional _Men _09Feb2017 _EC.txt
HRC _Fenland _Emotional _Women _09Feb2017 _EC.txt

5.3 Data upload

An SFTP site hosted by the MRC Epidemiology Unit is provided for return of results.

Host: **[LINK]**
Username:
Password:

Unix and MacOS X: Use sftp or scp at the terminal.

Windows: We suggest using FileZilla (http://sourceforge.net/projects/filezilla). Connect your client to the host.

5.4 Google doc

Please provide the following information in the GoogleDoc via the link provided below:

[LINK PROVIDED]

Enter the following information for men and women on separate rows of the table:

General

The name of your cohort
The country of data collection
A brief description of your study
Named individuals with contact details, including: analysts and PIs
Acknowledgements for your study, including funding sources

Genotypes

The forms of genotyping QC conducted
The version of SNP Chip used
The imputation panel used
The imputation programme used

Analysis (participants included in the GWAS analysis only)

GWAS sample size
Mean age (standard deviation) and age range
Mean BMI (standard deviation) BMI and range
Mean eating behaviour scores (standard deviation) and range (these figures should refer to the scaled sub-scale scores. The scores should appear on a scale of 0-100 following use of the formula specified in **Section 1.1**).