The relationship between prenatal nutrition and offspring autism related outcomes, and its socioeconomic context

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Abstract

Background. Prenatal nutrition may be an aetiological factor in the development of offspring autism. However, findings are inconsistent, possibly because of methodological limitations, such as small sample size and retrospective study design. Additionally, understanding the socioeconomic context of prenatal diet-autism relationships may inform public health. The overall aim was to draw on causally informed approaches to investigate the association between prenatal nutrition and autism and measure the extent to which inequalities in autism may be explain by a 'healthy' prenatal dietary pattern (HPDP).

Methods. The research problem is introduced in Chapters 1-3, and the research aims were address in Chapters 4-7, which are in journal format. Chapter 4: in a systematic review and meta-analysis, I synthesised evidence on the association between prenatal multivitamin supplements and autism diagnosis and evidence of triangulation. Chapter 5: I measured the association between HPDP and offspring autism diagnosis and autism-associated traits in the Norwegian Mother, Father, and Child Cohort (MoBa). Results related to autism-associated traits were triangulated by replicating the analysis in the Avon Longitudinal Study of Parents and Children (ALSPAC). Chapter 6: Mendelian randomisation was applied to measure the relationship between the genetic instrument for HPDP and autism-associated traits in MoBa. Chapter 7: I measured the controlled direct effects of maternal socioeconomic deprivation on autism diagnosis and autism-associated traits when eliminating the proportion of the total effects which are attributed to HPDP.

Results. The probability of autism diagnoses reduced in relation to prenatal multivitamin supplement use compared to no/low use. In MoBa and ALSPAC, the probability of autism diagnosis and autism-associated traits reduced if mothers had a high adherence to HPDP compared to low adherence. Causally informed approaches considered in Chapters 4-7 strengthened the interpretation of results, particularly the cross-context comparison. However, no clear evidence of association emerged from the Mendelian randomisation analysis, though there was insufficient power to detect moderate to large associations. The controlled direct effects analysis demonstrated that a modest proportion of socioeconomic disparities in each outcome were explained by HPDP.

Conclusion. This thesis addressed some key limitations of the existing literature and applied causally informed approaches. Although these analyses provide stronger evidence, testing of causation was inconclusive. Future studies should continue to assess whether the associations between HPDP and autism diagnosis and autism-associated traits are causal. If causality were established, HPDP may be a target for intervention to reduce inequalities in the outcomes but when part of a larger strategy.

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Publications and other outputs

Below is a list of research activities directly related to the research conducted in fulfilment of this thesis. See 'Author's declaration for detailed description of my contribution to the thesis.

Publications

Friel C, Leyland AH, Anderson JJ, Havdahl A, Borge T, Shimonovich M, Dundas R. Prenatal Vitamins and the Risk of Offspring Autism Spectrum Disorder: Systematic Review and Meta-Analysis. Nutrients. 2021 Jul 26;13(8):2558. doi: 10.3390/nu13082558.

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Friel C, Leyland A, Anderson J, et al OP92 Does maternal folic acid supplements in pregnancy influence autism spectrum disorder in children? A systematic review and meta-analysis J Epidemiol Community Health 2020;74:A43. doi: 10.1136/jech-2020-SSMabstracts.90

British Dietetic Association Research symposium, 2020. Prenatal diet quality and the risk of autism (invited oral presentation (virtual)). Available at: https://www.bda.uk.com/events/calendar/bda-research-symposium-2020.html [Accessed 29th Oct. 2023]

British Dietetic Association Research symposium, 2020. An aetiological systematic review and meta-analysis on the association between prenatal multivitamins and autism in children (oral presentation (virtual)). Available at:https://www.bda.uk.com/events/calendar/bda-research-symposium-2020.html [Accessed 29th Oct. 2023]

Columbia University, Psychiatric Epidemiology Training Program, 2020. Does prenatal nutritional status alter the child's risk of autistic spectrum disorder, and are health inequalities evident? A systematic review and meta-analysis (invited oral presentation, seminar series (virtual))

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Author's Declaration

I declare that I am the sole author of this thesis, except where the contribution of others has been acknowledged, as below. The work in this thesis has not been submitted in any form for another degree or professional qualification at the University of Glasgow or any other institution. This thesis is presented in journal format, and results chapters, either published or submitted for publication, are presented in their published or submitted form. In the case of each of these papers I conceived the idea, was the primary author, wrote the first draft of each manuscript, and revised each manuscript based on feedback from co-authors. Co-authors are listed in each of the relevant chapters and their specific contributions are detailed below.

Contribution statements

The four papers were conducted in collaboration with researchers from the Norwegian Institute of Public Health and the University of Bristol as well as the University of Glasgow. Their details are below (excluding details of my supervisors). Chapters 4-7 relate to the four papers and use 'we' instead of 'I' to reflect the collaborative nature of this work, however I have led on all papers and my contribution to each paper is described below.

1. Alexandra Havdahl, affiliated with the Centre for Genetic Epidemiology and Mental Health, Norwegian Institute of Public Health, Norway; Nic Waals Institute, Lovisenberg Diaconal Hospital, Norway; Promenta Research Center, Department of Psychology, University of Oslo, Norway.

2. Anne Lise Brantsæter affiliated with the Department of Food Safety and Centre for Sustainable Diets, Norwegian Institute of Public Health, Norway.

3. Robyn E. Wootton affiliated with the Nic Waals Institute, Lovisenberg Diaconal Hospital, Norway; Medical Research Council Integrative Epidemiology Unit & the School and Psychology, University of Bristol, Bristol, England.

4. Tiril Borge, affiliated with Division for Health Services, Cluster of Reviews and Health Technology Assessments, Norwegian Institute of Public Health, Norway.

5. Michal Shimonovich affiliated with the Medical Research Council/Chief Science Office Social and Public Health Sciences Unit, University of Glasgow, Scotland.

Specific contributions to Chapters 4-7.

Chapter 4. Prenatal Vitamins, and the likelihood of Offspring Autism Spectrum Disorder: Systematic Review and Meta-Analysis.

Supervisory team: Ruth Dundas, Alastair Leyland, and Jana Anderson. Norwegian collaborators: Alexandra Havdahl and Tiril Borge. UK collaborator: Michal Shimonovich.

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I conceptualised the review questions and design, and led on all stages of the review process, including methodology, formal analysis, and all stages of the writing process, and was lead author of the review. Additionally, Ruth Dundas, Alastair Leyland and Jana Anderson provided supervision of all activities, from conceptualisation, methodology and formal analysis and writing, review, and editing. They also reviewed and critically revised the article for intellectual content. Furthermore, Jana Anderson contributed to the formal analysis of data by acting as adjudicator to resolve disputes of study inclusion. Alexandra Havdahl contributed towards the methodological approach and review, and editing. Tiril Borge and Michal Shimonovich assisted in the screening of titles/abstracts, full text review and quality assessment, and review, and editing.

Chapter 5. Associations between prenatal dietary pattern and offspring autism diagnosis and autism-associated traits in the Norwegian Mother, Father, and Child Cohort Study (MoBa) and the Avon Longitudinal Study of Parents and Children (ALSPAC).

Supervisory team: Ruth Dundas, Alastair Leyland, and Jana Anderson. Norwegian collaborators: Alexandra Havdahl and Anne Lise Brantsæter.

Submitted to The International Journal of Epidemiology

I led on all aspects of Chapter 5, including the conceptualisation of the research question, methodological approach, and researched the datasets and led on the application for data access. All data cleaning and analysis was conducted by me, and I led on the interpretation of findings and wrote the draft and revised manuscripts. The conceptualisation and formulation of the study was supported by all authors, as was the interpretation, and critical review of Chapter 5. Ruth Dundas, Alastair Leyland, Jana Anderson, and I led on the acquisition of project funding.

Chapter 6. Maternal polygenic score for a healthy prenatal dietary pattern is not associated with autism-associated traits in Mendelian randomisation analysis.

Supervisory team: Ruth Dundas, Alastair Leyland, Jana Anderson. Norwegian collaborators: Alexandra Havdahl and Robyn Wootton (also affiliated with the University of Bristol).

I led on all aspects of Chapter 6, from conceptualisation of the research question and study design, data analysis, interpretation of results, and each draft and revision of Chapter 6. Alexandra Havdahl led on the data acquisition: and myself, Alexandra Havdahl, Ruth Dundas, Alastair Leyland, Jana Anderson led on the acquisition of project funding. I performed the data analysis, supported by Robyn Wootton. All authors provided input into the methodological approach, interpretation of results and critically reviewed Chapter 6.

Chapter 7. The role of a healthy prenatal dietary pattern in explaining the associations between maternal socioeconomic indicators of deprivation and offspring autism diagnosis and autism-associated traits.

Supervisory team: Ruth Dundas, Alastair Leyland, and Jana Anderson. Norwegian collaborator: Anne Lise Brantsæter.

For Chapter 7, I lead on all aspects of the study. I identified suitable datasets, sought access, conceptualised, and formulated the study design, conducted the analysis, interpreted the results, and wrote the draft and revised manuscripts. All authors contributed to the interpretation of the results, critically reviewed the manuscript, and contributed to the scientific content of the manuscript. The conceptualisation and formulation of the study was supported by all authors. Ruth Dundas, Alastair Leyland, Jana Anderson, and I led on the acquisition of project funding.

List of abbreviations

ALSPAC	Avon Longitudinal Study of Parents and Children
BMI	Body mass index
CDE	Controlled direct effects
CI	Confidence intervals
DAG	Directed acyclic graph
DNA	Deoxyribonucleic acid
DOHaD	Developmental origins of health and disease
DSM	Diagnostic and statistical manual of mental disorders
EARLI	Early autism risk longitudinal investigation
FFQ	Food frequency questionnaire
GRADE	Grading of recommendations assessment, development, and evaluation
GWAS	Genome-wide association study
HOME	The Health outcomes and measures of the environment Study
HPDP	'Healthy' prenatal dietary pattern
HR	Hazard ratio
ICD	International classification of disease
INMA	INfancia y medio ambiente (Children and environment)
IPW	Inverse probability weight
IQ	Intellegence quotient
IV	Instrumental variable
MARBLES	Markers of autism risk in babies-learning early signs
MICE	Multivariate imputation by chained equations
MoBa	Norwegian mother, mather, and child cohort
MR	Mendelian randomisation
MTHFR	Methylenetetrahydrofolate reductase
NEST	Newborn epigenetics study
NHSII	The Nurse's health study II
NOK	Norwegian krone
OR	Odds ratios
PGS	Polygenic score
PUFA	Polyunsaturated fatty acid
RCT	Randomised controlled trial
RR	Relative risks

SCDC	Social and communication disorder checklist
SCQ-RRB	Social communication questionnaire, restrictive and repetative behaviours and interests domain
SCQ-SOC	Social communication questionnaire, social communication and language domain
SD	Standard deviation
SIMD	Scottish index of multiple deprivation
SNP	Single nucleotide polymorphism
SRS	Social responsiveness scale

Inclusive language and neurodiversity

Preference on the use of language in reference to autism varies across the autistic community (Kenny et al., 2016). In research, autism is often considered a ‘disorder’ of the brain and an ‘abnormality’. However, increasingly there are calls from the autistic community and researchers to reframe how autism is conceptualised and, accordingly, the language used (Pellicano and den Houting, 2022). In this thesis, I consider autism as part of a neurodiverse paradigm. When referring directly to autism I minimise the use of language that may frame autism as an ‘abnormality’, including the use of ‘exposure’ and ‘risk’. Hence, throughout the thesis I refer to ‘autism spectrum disorder’ as autism. Additionally, I use language related to ‘male’ and ‘female’ to refer to sex.

1 Chapter 1. Background

1.1 Chapter overview

The aetiology of autism is poorly understood, yet current consensus implicates genetic and environmental factors, of which maternal nutrition is of particular interest (Shin et al., 2018). The conceptualisation of autism has evolved over time with increased knowledge, awareness, and cultural acceptance. Subsequently, the prevalence of autism has increased (Fombonne, 2003), however, environmental factors may also contribute. Firstly, I summarise the prevalence of autism, the core features of autism and characteristics of autistic individuals. Secondly, I discuss the Developmental Origins of Health and Disease (DOHaD) as the broad context this thesis sits within. Thirdly, I introduce some of the potential genetic and environmental factors related to the aetiology of autism. The fourth section will summarise the existing literature that has investigated the relationship between prenatal nutrition and autism. The fifth section discussed inequalities in autism and the potential advantages of measuring the extent to which prenatal diet may explain inequalities in autism. Lastly, I highlight the benefits of using a causal framework in nutritional epidemiology.

1.2 An introduction to autism

When autism was first described in 1943 by Kanner (Kanner, 1971), and in 1944 by Asperger (Asperger, 1991) it was believed to be a rare condition. A review that summarised the earliest estimated prevalence of autism diagnosis from 32 surveys conducted across 13 countries. Between 1966-1991 the estimated prevalence of autism diagnosis was 4.4 per 10,000, rising to 12.7 per 10,000 between 1992-2001 (Fombonne, 2003). The prevalence has continued to rise, as autism diagnosis has become increasingly broadly defined, and awareness and recognition have increased (Lord et al., 2018). More recently, an estimated 1.76% of school children in the UK have an autism diagnosis (Roman-Urrestarazu et al., 2021). Although the estimated prevalence varies depending on the study design and population, the rising trends are consistently observed (Elsabbagh et al., 2012, O'Nions et al., 2023, Russell et al., 2022).

Autism ranges from mild to severe forms and is now regarded as a heterogeneous but related cluster of neurodevelopmental conditions that are lifelong (Lord et al., 2018). Due to the heterogeneity of autism, it can be challenging to diagnose, and so despite signs

emerging in some children before two years of age, the median age of autism diagnosis is estimated to be three to 6.8 years (Daniels and Mandell, 2014). But in more recent years (2000-2018) the median has been estimated to be 8.6 years in males and 10.8 years in females, possibly due to the extension of autism to milder presentations (O'Nions et al., 2023). The core traits that unite autism are persistent difficulties and differences in reciprocal social communication and restricted and repetitive behaviours and interests (World Health Organisation, 2020). Throughout the thesis, for brevity, restrictive and repetitive behaviours and interests will be referred to as restrictive and repetitive behaviours.

The binary diagnosis of autism has been criticised because the neurodiversity associated with autism is dimensional, with continuous interindividual variation that extend into non-autistic populations (Tang et al., 2020). The core traits of autism occur in non-autistic populations and are popularly termed ‘the broader autism phenotype’ and have varying proximity to diagnostic thresholds. Moreover, autism-associated traits are also present in other neurodevelopmental and mental health conditions and difficulties (Bora et al., 2017, Jouravlev et al., 2020, Kellerman et al., 2019, Moody et al., 2017). Furthermore, research suggests that the core traits are phenotypically and genetically dissociable (Warrier et al., 2019), and so may have different aetiological origins. Hence, it may be advantageous to aetiological understanding to measure both autism diagnosis, autism-associated traits, and separately measure social communication skills, and restrictive and repetitive behaviours.

Dimorphism in the autism phenotype is well documented. Most notable is the male preponderance which is reported in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) to be 4:1 males to females (American Psychiatric Association, 2013). This difference may be driven by sex-differences in genetic factors, sex hormones, and immune function (Ferri et al., 2018). However, underdiagnosis of autism in females may also occur. In countries with national screening the male to female ratio dropped to 3:1 (Loomes et al., 2017) and others suggest it may be even lower (McFayden et al., 2023). Historically autism was considered a male condition and our understanding of autistic traits is male-centric, and derived from research dominated by male samples (Lai et al., 2015). Contemporary evidence suggests autistic females have less restrictive and repetitive behaviours but more internalising behaviours compared to autistic males. Furthermore, gender-typical socialisation may lead to females being more motivated to socialise and camouflage their difficulties which contributes to the delayed detection of autism, especially when coupled with high verbal ability (Rea et al., 2022). On closer

investigation, females display atypical social behaviours such as overemphasised facial expressions and gestures, and socially normative special interests that are less intense than males. However, they have higher scores than males on other aspects of restrictive and repetitive behaviours. Unfortunately, current perceptions, practices, and research, are evolving and females are thought to be currently underdiagnosed (McFayden et al., 2023).

The all-cause mortality rate has been estimated to be almost 2.4 times higher in populations with an autism diagnosis compared to the general population (Catalá-López et al., 2022). Increased mortality may relate to autistic behaviours, a higher burden of co-occurring conditions and secondary effects of socioeconomic deprivation (Catalá-López et al., 2022). However, increased mortality is not consistently observed, possibly due to differences in cohorts, such as age (Smith et al., 2021). Similar to other neurodevelopmental conditions, autism commonly co-occurs with neurological conditions (for example, anxiety and depression, attention deficit hyperactivity disorder, epilepsy, motor difficulties) and physical health complaints (for example, gastrointestinal disturbance, immune dysregulation). For instance, attention deficit hyperactivity disorder co-occurs in an estimated 28.2% of people with autism. Co-occurring intellectual disability (intelligence quotient (IQ) <70) estimates vary between 11-65% due to variation in sample selection (Lord et al., 2022). Yet, autistic people have many strengths, for example, some display enhanced performance on visuo-perceptual tasks, whilst others may have heightened tolerance to repetitive tasks (Pellicano and den Houting, 2022). Despite these strengths, life-long support is necessary for many people with autism and carries a high financial cost, as support extends across domains, such as social, economic, educational, physical, and mental healthcare (Lord et al., 2022).

Interest in the aetiology of autism has risen alongside the rising prevalence of an autism diagnosis. There is debate about whether the rise in prevalence may also relate to a greater prevalence of aetiological factors (Lyall et al., 2017, Taylor et al., 2020). The aetiological origin of autism lies in a combination of genetic and environmental factors. Yet, no clear genes or environmental factors have been discovered for most cases of autism, perhaps due to the heterogeneity of autism (Lipkin et al., 2023). Evidence suggests that the prenatal period may be especially important in the development of autism. For example, signs of autism can be detected in infancy, and evidence suggests there may be early brain overgrowth (Lyall et al., 2017). Hence, prenatal environmental factors, including prenatal nutrition may contribute to the aetiology of autism (Hertz-Picciotto et al., 2018, Lyall et al., 2017).

1.3 Developmental origins of health and disease

The Developmental Origins of Health and Disease (DOHaD) research has uncovered valuable insights on the importance of maternal nutritional status and foetal development. In 1986, a ground-breaking report by British epidemiologist, David Barker, gave rise to the DOHaD concept (Barker and Osmond, 1986). Barker postulated that intrauterine signals or insults during a critical of sensitive period of development 'program' foetal development, which have lasting impacts on health in childhood and adulthood. Empirical evidence to support this concept was provided by Barker and colleagues (Barker, 1995, Barker, 1998, Lucas, 1994) and built on earlier observations by researchers in the 1970's (Stein et al., 1975, Widdowson and McCance, 1975). These investigations played a pivotal role in the development of Barker's 'foetal programming' theory, later termed the DOHaD. The current concept of DOHaD has expanded greatly since the 1980's, thanks to several fields of research. The principles of a life course approach and DOHaD can be integrated (Fleming et al., 2018, Suzuki, 2018). A life course approach suggests that postnatal factors can further influence the development of a trait, although some traits are fixed during foetal development (Kuh and Ben-Shlomo, 1997, Kuh et al., 2003).

Much of the initial DOHaD research was focused on physical health outcomes but has since expanded to neurodevelopment. A vast range of prenatal nutritional factors have been related to offspring neurodevelopment (Clare et al., 2019, Fleming et al., 2018, Hoffman et al., 2017). Here I provide a few examples. The Dutch 'hunger winter' of 1944 – 1945 provided a natural experiment in which intrauterine undernutrition (1004 kcal) was related to a greater risk of schizophrenic traits in offspring (Susser and Lin, 1992). Other prenatal nutrients have been attributed to neural development in offspring. For example, folate controls a key step in the synthesis of basic brain structures and closure of the neural tube at 28 days. Randomised controlled trials (RCT) demonstrated a reduced risk of neural tube defects if mothers took folic acid supplements pre-pregnancy and into pregnancy (Czeizel and Dudás, 1992, MRC Vitamin Study Research Group, 1991). Subsequently, in many countries, 400 µg/day folic acid supplement is recommended around two months prior to conception and throughout the first trimester (Barry et al., 2023, Dwyer et al., 2022, Lamers et al., 2018, National Institute for Health and Care Excellence, 2008). Furthermore, several countries around the world implemented mandatory fortification, as folate intakes are generally below 400ug/day in early pregnancy (Barry et al., 2023, Kancherla et al., 2022, UK Government, 2021). Additionally, worldwide, severe iodine deficiency is a major preventable cause of neurological damage which leads to conditions

such as cretinism (Pearce and Zimmermann, 2023). And as a final example, polyunsaturated fatty acids (PUFAs) have an established beneficial role in neuronal development. Foetal uptake of PUFAs occurs throughout pregnancy, especially docosahexaenoic acid (omega-3 PUFA) in the third trimester, which leads to abundant levels in the brain. A systematic review commissioned by the Scientific Advisory Committee on Dietary Guidelines for Americans concluded there was moderate to consistent evidence that high prenatal fish intakes reduced the probability of child neurocognitive developmental issues (Hibbeln et al., 2019).

1.4 Aetiology of autism

Autism is highly heritable. The heritability of autism is estimated at 80.5% across five countries (Demark, Sweden, Finland, Israel, Australia), though country specific estimates ranged from 50.9% (Finland) to 86.8% (Israel) (Bai et al., 2019). These estimates are consistent with previous studies (Tick et al., 2016). With such high heritability estimates, why investigate prenatal nutrition? Heritability estimates the proportion of a phenotype that is attributable to genetic variability. But it is a misconception that heritability partitions genes and environment into mutually exclusive causes. Indeed, contemporary debates focus on how and to what extent genes and environment may influence each other (Lipkin et al., 2023).

The genetic aetiology of autism is attributed to a combination of common genetic variants and de novo mutations. An exception is syndromic autism which can be attributed to a single genetic mutation in a small proportion of cases of autism (Lee et al., 2019b). For example, Rett syndrome which has now been removed from the autism diagnostic criteria in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and International Classification of Diseases (ICD-11) (American Psychiatric Association, 2013, World Health Organisation, 2020). Genetic vulnerability to autism, neurodevelopmental, and mental illness share many common genes (Grove et al., 2019, Lee et al., 2019b, Thomas et al., 2022, Warrier et al., 2022, Xie et al., 2020). These genes have been implicated in neurogenesis, immune regulation, de novo mutations, and epigenetic processes. This broad genetic vulnerability is reflected in the co-occurrence of other neurological conditions and autism, and stronger family history of psychiatric conditions (Thomas et al., 2022, Warrier et al., 2022, Xie et al., 2020). No gene is specific to autism, and implicated genes do not always cause autism (Lipkin et al., 2023). Instead, with exception of a few cases, numerous genes are involved and may act additively (Warrier et al., 2022).

Genetic studies have associated autism with common and rare single nucleotide polymorphisms (SNPs), and de novo mutations (Grove et al., 2019, Thomas et al., 2022, Warrier et al., 2022). Common SNPs represent genetic variation that is common in the population. De novo mutations are new gene mutations that can occur during or after foetal development or can be passed to children from parents' germ cells (reproductive cells). Common SNPs generally have very small effects but collectively are thought to be the most significant genetic factor in autism aetiology, whereas de novo mutations independently exert more influence than common SNPs, but overall explain a smaller proportion of the genetic variability in the autistic population. There is considerable genetic heterogeneity in autism, which is likely to relate to autism being a cluster of related conditions rather than a single condition (Warrier et al., 2022).

Several environmental factors have been associated with autism or autism-associated traits, and especially in the preconception and prenatal period (Hertz-Picciotto et al., 2018). For example, an increased likelihood of autism and/or autism-associated traits has been observed in relation to inadequate/suboptimal nutritional status, high pollutant, and toxin exposure, maternal cardiometabolic conditions, increased parental age at conception, maternal smoking, alcohol use, prescription/illicit drug use, immune activation, and the gut-microbiome (Hertz-Picciotto et al., 2018, Lyall et al., 2017). Notably many environmental factors impact each other and are thought to interact with genes. For example, through gene-environment interactions, through environmental factors triggering de novo mutations in deoxyribonucleic acid (DNA) or epigenetic changes (Cheroni et al., 2020, Lyall et al., 2017, Pugsley et al., 2022). Epigenetics can alter the expression of genes and imprinted genetic markers that can be conferred through generations without alteration to DNA structure (Lyall et al., 2017). In summary, the DOHaD literature and genetic investigations provide promising evidence that prenatal nutritional status is a plausible aetiological factor in the development of autism.

1.5 Prenatal nutrition and autism

Over the past two decades nutritional epidemiology expanded from a reductionist approach, focused on discrete nutrients or foods, to the analysis of whole dietary patterns (Schulz et al., 2021). Diet contains numerous nutrients and other bioactive components that have synergistic and antagonistic effects that can be cumulative and/or latent. Consequently, there are a multitude of correlated and complex pathways (Hu, 2002). The independent effects of discrete nutrients or foods are challenging to isolate and interpret

without contextual knowledge of diet (Tapsell et al., 2016, Zhao et al., 2021). Hence, dietary patterns capture the summation of all bioactive components from diet and their interactions, and model how foods and nutrients are consumed in the population (Tapsell et al., 2016). However, contrary to the field of nutritional epidemiology more broadly, neurodevelopment (Borge et al., 2017) and autism related research has focused on a limited range of prenatal nutritional indicators; mainly, discrete biochemical indicators of nutritional status, food groups, and prenatal nutritional supplements (Zhong et al., 2020).

In the following sections, I firstly provide a detailed discussion of the evidence in relation to whole dietary patterns and food groups as this relates most closely to the thesis aim. Evidence on biochemical indicators of prenatal nutrients will be summarised more briefly. The literature in relation to prenatal multivitamin supplements is discussed in Chapter 4, which is a systematic review and meta-analysis of the relationship between prenatal multivitamin supplements and offspring autism diagnosis.

1.5.1 Prenatal whole dietary intakes

Results observed across five previous cohorts (4 publications) are conflicting (Geetha et al., 2018, House et al., 2018, Li et al., 2018, Vecchione et al., 2022). In the first two prospective cohorts, neither autism diagnosis nor autism-associated traits were related to prenatal dietary patterns. The Early Autism Risk Longitudinal Investigation (EARLI) and The Nurse's Health Study II (NHSII) are American cohorts and were investigated by Vecchione et al (Vecchione et al., 2022). EARLI is a prospective cohort study based on the siblings of children with an autism diagnosis. A total of 154 mother-child dyads (children aged three years) were investigated, of which 32 children had an autism diagnosis based on the Autism diagnostic observation schedule. The NHSII was a prospective nested case-control study of 727 mother-child dyads (children aged 4-18 years). Autism diagnosis was based on maternal report and identified 106 children who were matched to 621 controls. Autism-associated traits were measured on a continuous scale with the social responsiveness scale (SRS) (high score indicates more traits) in EARLI and NHSII.

Dietary assessment was conducted using a food frequency questionnaire (FFQ), collected at 20-weeks' gestation in EARLI, but collected four yearly in NHSII which may increase the risk of misclassification bias. A range of dietary patterns were derived which were: empirical dietary inflammatory pattern, alternative healthy eating index and the alternative healthy eating index for pregnancy which excludes items not recommended in pregnancy

but includes key nutrients (iron, folate and calcium), adapted Mediterranean diet score, and the Western and prudent dietary patterns that were measured by principal component analyses. The SRS score increased in association with a high adherence to a Western dietary pattern but attenuated with adjusted for total energy intake (Vecchione et al., 2022). This may indicate an effect of gestational weight gain rather than nutritional composition. In each analysis, there was considerable uncertainty in the point estimates due to small sample size and low number of children with an autism diagnosis. Thus, there is uncertainty as to whether the results reflect a true null.

The last three cohort studies also had small sample sizes, and additional methodological limitations. All three cohorts observed a lower probability of autism diagnosis (Geetha et al., 2018, Li et al., 2018) or autism-associated traits (House et al., 2018) in association with high quality prenatal diet. The first case-control study on 374 people with an autism diagnosis and 354 non-autistic controls was based in China. The study compared a 'balanced' dietary pattern with a dietary pattern that consisted of 'mostly meat' and one of 'mostly vegetables'. It found that the two latter diets were associated with a four-fold and two-fold increase, respectively, in the probability of autism diagnosis. (Li et al., 2018). A second case-control study of 55 people with an autism diagnosis and 55 non-autistic controls based in India found that 'poor maternal nutrition' was associated with an increased risk of autism diagnosis but due to poor reporting the estimates were difficult to interpret (Geetha et al., 2018). However, there were long delays of 3-12 years between the pregnancy and data collection (Geetha et al., 2018, Li et al., 2018). Diet is complex and challenging to accurately recall several years after pregnancy. Additional recall bias may be introduced due to the retrospective study design. Lastly, the dietary assessment method and estimation of dietary pattern were poorly described in both studies, which limits interpretation and generalisability.

Lastly, an American based prospective cohort, the Newborn epigenetics study (NEST), observed 65% lower odds of autism-associated traits in association with high adherence to a Mediterranean prenatal dietary pattern, compared to low adherence to the latter dietary pattern (House et al., 2018). A Mediterranean dietary pattern was derived from the FFQ, administered to 325 mother-child dyads in the first trimester and reflected dietary intake around the time of conception (House et al., 2018). Additionally, the authors identified differential methylation patterns in relation to the Mediterranean dietary pattern but could not conduct a mediation analysis due to low sample size. The authors measured autism-

associated traits at 12-24 months with an adapted version of the brief infant toddler social and emotional assessment as per Kruizinga et al (Kruizinga et al., 2014).

Due to the inconsistent findings, it is uncertain whether prenatal dietary patterns associate with autism related outcomes. Methodological limitations are a contributing factor, particularly small sample sizes which can increase the risk of type 1 and type 2 errors. Although the number of autism diagnosis was higher in one cohort (Li et al., 2018), the retrospective study design and poor reporting limits confidence in the findings. Moreover, self-reported dietary intake measures, such as FFQ, are prone to systematic and random error due to recall bias which could be another source of heterogeneity between the studies (Burrows et al., 2019). The risk of recall bias may be especially high in two studies due to the 3–12-year delay in data collection (Geetha et al., 2018, Li et al., 2018). In nutritional epidemiology, different dietary patterns have been observed to produce qualitatively similar results, however, some inconsistencies can still arise (George et al., 2014, Milà-Villarroel et al., 2011, Thorpe et al., 2016). However, this did not appear to be a clear source of inconsistent results in the studies discussed here (Geetha et al., 2018, House et al., 2018, Li et al., 2018, Vecchione et al., 2022). Indeed, the studies which observed a lower likelihood of autism diagnosis or autism-associated traits had different dietary patterns (Geetha et al., 2018, House et al., 2018, Li et al., 2018), whereas Vecchione et al's investigation into EARLI and NHSII observed no clear evidence of association across six dietary pattern measures (Vecchione et al., 2022). Additionally, screening tools for autism were used to measure autism-associated traits but can be less reliable in children under four years, compared to children over four years (Chesnut et al., 2016). This may be a source of between-study heterogeneity but is difficult to assess in the context of other limitations. Larger prospective cohort studies would be an advantage to address this important question.

1.5.2 Prenatal dietary intake of food and nutrients

I begin this section with a discussion of the non-fish food components that have been investigated. There was difficulty interpreting this body of evidence due to the heterogeneity of the exposure measures and low number of studies which investigated each food group, which has been noted in earlier publications (Zhong et al., 2020). Fish and PUFA intakes will then be discussed, before moving to biochemical indicators. I highlight that the methodological limitations observed in measures of whole dietary patterns, described above, are also observed in studies of food groups. Some studies measure

multiple food components in one publication, or the same cohort study has been used for multiple publications.

1.5.2.1 Food groups, excluding fish

Another investigation into EARLI (mother-child dyads = 127) and NHSII (mother-child dyads = 713) applied Bayesian methods to simultaneously model a mixture of prenatal intakes of food groups and nutrients (Lyall et al., 2023). The nutrients measured were n-3 PUFAs, n-6 PUFAs, iron, zinc, vitamin D, folate, vitamin B_{12}, vitamin B_6, choline and betaine. Food groups measured in EARLI were: red meat, fish, fruit, leafy green vegetables, processed meat, nuts, legumes, wholegrains, tomatoes, and high energy drinks. In NHSII the food groups were: fish, yellow vegetables, other vegetables, cruciferous vegetables, nuts, processed meats, wholegrains, fruit, leafy green vegetables, and high energy drinks. The food groups and nutrients were generally not clearly associated with SRS (Lyall et al., 2023). The exception was an increase in SRS score (more autism-associated traits) in association with increasing intakes of green leafy vegetables in NHSII, but this was not observed in EARLI. Another study investigated 256 mother-child dyads from EARLI and did not observe clear evidence of association between total fruit and vegetable intakes and SRS, whilst adjusting for other foods (Joyce et al., 2022). In summary, in EARLI and NHSII, there was no clear evidence of association with whole dietary patterns (Vecchione et al., 2022), total fruits and vegetables (Joyce et al., 2022) or the food components described (Lyall et al., 2023). However, there was a high degree of uncertainty in the estimates.

Two further studies measured fruit (Gao et al., 2016) and cereal intakes (Gerges et al., 2020) and observed an association with autism diagnosis. Hence, these findings contrast with the results from EARLI and NHSII into autism-associated traits and fruit and vegetables (Joyce et al., 2022, Lyall et al., 2023) and wholegrains (Lyall et al., 2023). A Chinese retrospective case control study based on 108 children with an autism diagnosis and 108 controls measured maternal fruit intakes via FFQ (Gao et al., 2016). The odds of autism diagnosis increased approximately 2.4-fold in relation to low maternal fruit intake. Yet, there was limited adjustment for potential confounders and other food groups. The second case-control study in Lebanon measured maternal cereal intakes in 100 children with an autism diagnosis and 100 controls (Gerges et al., 2020). High maternal intake of cereals associated with an 79% lower probability of an autism diagnosis, compared to low cereal intakes. A narrow range of covariates were adjusted for, and although prenatal

nutritional supplement use was adjusted, other pertinent potential confounders were not. For example, other food groups, socioeconomic indicators, and pre-pregnancy body mass index (BMI) (Gerges et al., 2020).

Collectively, evidence from EARLI and NHSII contrasts with the other cohorts. The evidence from each cohort, except EARLI and NHSII, suggests a healthier prenatal dietary pattern or food group is associated with a lower likelihood of autism related outcomes. Overall, autism diagnosis was more consistently associated with prenatal dietary pattern (Geetha et al., 2018, Li et al., 2018) and food groups (Gao et al., 2016, Gerges et al., 2020), compared to autism-associated traits. However, autism-associated traits were only measured in three cohorts: EARLI, NHSII, and NEST (House et al., 2018, Joyce et al., 2022, Lyall et al., 2023, Vecchione et al., 2022). Thus, further studies would be advantageous to assess potentially differential associations between autism diagnosis and autism-associated traits.

The limitations observed in the investigations into food groups (excluding fish) were similar to studies into dietary patterns. And were: retrospective study design and high risk of recall bias (Gao et al., 2016, Geetha et al., 2018, Gerges et al., 2020, Li et al., 2018), small sample sizes and risk of measurement error in food groups and the autism-associated traits (Gao et al., 2016, Geetha et al., 2018, Gerges et al., 2020, Joyce et al., 2022, Li et al., 2018, Lyall et al., 2023). Moreover, three of four retrospective investigations had a high risk of confounding bias as there was limited adjustment for potential confounders (Gao et al., 2016, Geetha et al., 2018, Gerges et al., 2020). This may explain the especially large estimates of association (Gao et al., 2016, Geetha et al., 2018, Gerges et al., 2020). From the studies investigating food groups, only investigations into EARLI and NHSII adjusted for other dietary components (Joyce et al., 2022, Lyall et al., 2023). Additionally, when measuring food groups there can be substitution effects; for example high fruit and vegetable intakes may have a beneficial association with health outcomes because they displace other foods, for example those high in refined sugar (Tapsell et al., 2016).

1.5.2.2 Fish intakes

Fish are a key source of PUFA and especially omega-3 fatty acids which previous research has related to neurodevelopment (Hibbeln et al., 2019). However, there has been much concern regarding neurotoxic compounds in seafood which counteract beneficial effects of fish. Several of these toxins accumulate in fish and have been associated with an increased

probability of autism, such as heavy metals, microplastics, dioxins and dioxin-like polychlorinated biphenyls, and brominated and chlorinated flame retardants are lipophilic compounds. Hence, they accumulate in lipids and so are found in higher amounts in oily-fish compared to non-oily fish (Caspersen et al., 2013, European Food Safety Authority, 2012). Fish is also a major source of per- and poly-fluorinated alkyl substances (Skogheim et al., 2021). Several researchers have highlighted concerns that toxins and pollutants may increase the probability of autism and are found in a range of foods (Brown et al., 2018, Cheroni et al., 2020, Granillo et al., 2019, Skogheim et al., 2021, Ye et al., 2017). Levels of these toxins vary by fish type and investigations into the consumption of these toxins in fish have largely focused on mercury levels. Mercury accumulates up the food chain and is highest in large predatory species and can be found in non-oily and oily fish. Studies focused on methylmercury in fish have not found clear evidence that methylmercury associated with an increased probability of autism or other neurodevelopmental outcomes (Daniels et al., 2004, Golding et al., 2018, McKean et al., 2015, van Wijngaarden et al., 2013). Yet, there is further research required to understand the range of pollutants in different types of fish.

Autism related outcomes were not clearly associated with prenatal fish intakes. While high prenatal fish intakes were associated with a lower likelihood of autism diagnosis (Gao et al., 2016) and autism-associated traits (Julvez et al., 2016) in two cohorts, in the other four cohorts (three publications) there was no clear evidence of association with autism diagnosis (Lyall et al., 2013) or autism-associated traits (Steenweg-De Graaff et al., 2016, Vecchione et al., 2021). Gao et al, observed a lower probability of autism diagnosis in relation to prenatal fish intakes (same publication which measured prenatal fruit intake described above) (Gao et al., 2016). The limitations of this paper were previously described and included a lack of adjustment for other dietary components. These findings conflicted with an investigation from the NHSII based on 18,045 mother-child dyads (317 children with autism diagnosis) (Lyall et al., 2013). Autism diagnosis was not associated with total fish intakes or subtype (canned, shellfish, dark meat fish, other fish). This was a higher quality study (Lyall et al., 2013), compared to Gao et al (Gao et al., 2016). Lyall et al (Lyall et al., 2013) had a prospective study design, clearer reporting, and adjusted for a range of potential confounders, though other foods were not adjusted for.

There was one further investigation that used data from EARLI as well as the Health Outcomes and Measures of the Environment (HOME), which included 426 mother-child dyads and collected fish intake information via maternal interview (Vecchione et al.,

2021). The authors observed no clear evidence of association between SRS and total fish intakes in the first or second trimester or subtype (salmon, fatty fish, shellfish (both cohorts), and fried fish (EARLI only)). There was considerable uncertainty in the estimates, possibly due to small sample size. Additionally, SRS was measured in Lyall et al's investigation into EARLI and NHSII (described under food groups, excluding fish), and the authors also observed no clear evidence of association (Lyall et al., 2023). Similarly, a larger Dutch cohort, Generation R, measured prenatal fish intakes in 3802 pregnancies and child SRS at age six years (Steenweg-De Graaff et al., 2016) and found that total maternal fish intakes (measured with FFQ at 14-weeks' gestation) were not related to SRS. Conversely, another prospective cohort (1589 mother-child dyads) observed an inverse association between high prenatal fish intakes and childhood Asperger syndrome test score measured at five years of age (high score indicates more traits). The study used data from the Spanish Childhood and Environment (Infancia y Medio Ambiente) Project (INMA) (Julvez et al., 2016) and measured prenatal fish intakes via FFQ administered at 10-13 weeks' and 28-32 weeks' gestation (Julvez et al., 2016).

Unlike studies which investigate whole dietary intake and other food groups, investigations into maternal fish intakes included somewhat larger sample sizes. Yet, there is still little consistency in the associations observed. In two cohorts the analyses of fish intakes were a secondary aim of the study and fish were modelled simply as total intakes (Lyall et al., 2013, Steenweg-De Graaff et al., 2016). There was some evidence to suggest the associations may vary by quantity of fish consumed and type of fish consumed for example, oily versus non-oily fish (Gao et al., 2016, Julvez et al., 2016, Vecchione et al., 2021). Additionally, further investigation of a wider range of pollutants in fish may be advantageous. Moreover, only Lyall et al (Lyall et al., 2023) adjusted for other food groups, as this was not done in any of the other investigations discussed and may alter the associations observed (Gao et al., 2016, Julvez et al., 2016, Lyall et al., 2013, Steenweg-De Graaff et al., 2016, Vecchione et al., 2021). There is no clear evidence of association with prenatal fish and autism, yet more research may be necessary.

1.5.2.3 Polyunsaturated fatty acid intakes

PUFAs are often considered alongside evidence on fish intakes, as fish are an important source of PUFAs, especially some omega-3 fatty acids. There are two essential fatty acids, linoleic acid (an omega-6) and alpha-linolenic acid (an omega-3) as they cannot be synthesised in the body (Kaur et al., 2014). The three main omega-3 fatty acids are alpha-

linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. Key dietary sources of alpha-linolenic acid include nuts, seeds, and their oils, and in smaller amounts, soya oil and green-leafy vegetables. Alpha-linolenic acid is required to synthesis eicosapentaenoic acid and docosahexaenoic acid, but the conversion rate is generally low, and therefore dietary sources of fish, especially oily fish are regarded as a main dietary source (Kaur et al., 2014). However, fish also contain a variety of nutrients, for example, protein, iodine, calcium, phosphorous, iron, and vitamin A, D, and E. Additionally, there are many types of PUFAs, and they can be derived from various dietary sources other than fish which also contain other nutrients, such as fried foods and crisps which are often cooked in vegetable oils rich in omega-6 fatty acids. Hence, it is helpful to consider nutrients such as PUFAs in the context of their different food sources, not just fish, albeit this is true of all nutrients.

Results in relation to PUFA intakes are especially conflicting. In addition to measuring fish intakes in the NHSII, Lyall et al also measured maternal intake of a range of dietary fats and autism diagnosis (Lyall et al., 2013). Only, high maternal intakes of total omega-6 and linoleic acid related to a 34% lower likelihood of autism diagnosis. Hence, neither fish nor omega-3 intakes associated with autism diagnosis. Yet, in a second study the probability of autism diagnosis reduced as prenatal total omega-3 intakes increased, but no association was observed with omega-6 fatty acids (Huang et al., 2020). The Markers of Autism Risk in Babies-Learning Early Signs (MARBLES) study is based on the siblings of children with an autism diagnosis (Huang et al., 2020). The American based cohort study included 258 mother-child dyads (autism diagnosis = 57, non-typical development = 62, and typical development = 139). Dietary intakes assessed via FFQ in the first and second trimester, and maternal serum was collected in the third trimester (Huang et al., 2020). Lastly, the later publication by Lyall et al into the EARLI and NHSII (described in Chapter 1.5.2) observed no clear evidence of association with maternal omega-3 and omega-6 PUFA intakes and SRS (Lyall et al., 2023). Single nutrients have small effects which may be difficult to detect and can be sourced from a variety of foods, hence, isolating independent effects can be challenging as nutrients covary (Ioannidis, 2013). Although both authors adjusted for certain other nutrients and food groups (Huang et al., 2020, Lyall et al., 2013, Lyall et al., 2023), there may be residual and unmeasured correlations with other nutrients. Alternatively, the inconsistent associations may reflect chance findings.

1.5.3 Biochemical indicators of prenatal nutrition

Biochemical indicators are often viewed as superior measures as they are objective, compared to measures of food and nutrient intakes. Yet, biochemical indicators of nutrition can be affected by many factors, not just dietary intake, and are therefore not a direct reflection of dietary intake. For example, by an individual's genetics and metabolism, balance of other nutrients, stage of pregnancy, and homeostatistic mechanisms (Hollis and Wagner, 2017, Picó et al., 2019). Several methodological considerations were noted in studies that measure prenatal biochemical indicators of nutrients and autism. Nutrients typically have a U-shaped relationship with health, whereby deficient and excessive levels may confer harm, relative to sufficient levels. Cut-offs levels can be applied to biochemical indicators of nutrients to define deficiency, insufficiency, and excess but varied between studies. Some studies modelled nutrient biochemical measures linearly which can also impact the results observed. Biochemical indicators of nutrients often reflect a shorter time-frame compared to dietary measures (Kirkpatrick et al., 2019). Repeated measures of biochemical indicators over time may be required to more accurately reflect nutrient status may but was not conducted by any of the studies discussed below. Moreover, similar to single nutrient intakes, the effect of biochemical indicators may be small and correlated to other nutrients and so challenging to measure (Ioannidis, 2013, Ioannidis, 2018). Only one study simultaneously adjusted for a wide range of other biochemical indicators of nutrient levels (Egorova et al., 2020). Therefore, it is with these methodological considerations that I discuss evidence from prenatal biochemical indicators and autism.

1.5.3.1 Polyunsaturated fatty acids

Each study measured a range of maternal PUFA serum measures (Huang et al., 2020, Lyall et al., 2021, Steenweg-De Graaff et al., 2016). Maternal serum levels of PUFA were generally not associated with autism diagnosis when measured in the third trimester (Huang et al., 2020), or second trimester (Lyall et al., 2021). However, in the Generation R cohort, low omega-3:omega-6 fatty acid ratio, high total omega-6, and linoleic acid associated with an increased SRS (measured age six years) (mother-child dyads = 4,624) (Steenweg-De Graaff et al., 2016). But there was no clear evidence of association with maternal omega-3 fatty acids status in children with an intellectual disability or without intellectual disability when measured separately (mother-child dyads = 6,611) (Steenweg-De Graaff et al., 2016). This is contrary to Lyall et al who observed increased odds of autism diagnosis with co-occurring intellectual disability if mothers had extremely low omega-3 serum levels (lowest decile compared to top) (Lyall et al., 2021). In all other

analysis conducted there was no clear evidence of association in the main analysis with autism diagnosis (Lyall et al., 2021).

1.5.3.2 Vitamin D

Vitamin D is an essential nutrient although the major source is synthesis from ultraviolet beta radiation from sunlight. Serum vitamin D concentrations increase during pregnancy, especially early pregnancy when a 2-3-fold increase occurs. Vitamin D is critical for the healthy development and maintenance of neurological and immune function. Moreover, prevention of maternal foetal rejection in early pregnancy may occur through vitamin Ds effect on immune regulation (Hollis and Wagner, 2017). Vitamin D is challenging to consumed in adequate amounts through diet alone. Thus, in addition to sunlight and diet, vitamin D containing supplements are important for populations residing in high altitudes (Itkonen et al., 2021, Scientific Advisory Committee on Nutrition (SACN), 2016).

Several studies observed an increased probability of autism diagnosis in association with deficient or insufficient prenatal and/or neonatal vitamin D serum levels, compared to sufficient levels (Chen et al., 2016, Fernell et al., 2015, Lee et al., 2019a, Schmidt et al., 2019b, Sourander et al., 2021, Vinkhuyzen et al., 2017, Wu et al., 2018). However, two studies observed no clear association between vitamin D levels and autism diagnosis (Egorova et al., 2020, Windham et al., 2019b). Furthermore, greater autism-associated traits related to low prenatal vitamin D levels compared to sufficient or high prenatal vitamin D levels (Vinkhuyzen et al., 2018), although the associations may vary by trait (Lopez-Vicente et al., 2019, Madley-Dowd et al., 2022, Whitehouse et al., 2013). Moreover, a Mendelian randomisation (MR) study did not observe clear evidence of association between autism-associated traits and prenatal gestational vitamin D when modelled linearly. This was also observed in the phenotype analysis where vitamin D modelled linearly was not associated with autism-associated traits, whereas in the non-linear phenotype analysis it was. Furthermore, the authors cautioned that there was low statistical power to detect an association should one exist (Madley-Dowd et al., 2022). Overall, there is reasonably consistent evidence of an increased probability of autism diagnosis and autism-associated traits if mothers had deficient or insufficient gestational vitamin D serum levels compared to sufficient gestational vitamin D levels.

1.5.3.3 Iodine

Iodine is essential for the synthesis of thyroid hormones which regulate metabolic function and regulate foetal neurodevelopment through many processes, including neuronal migration and proliferation, myelination, synaptic transmission, and neuronal plasticity. The foetus is entirely dependent on maternal thyroid hormones and iodine until around mid-trimester (18-22 weeks gestation) when foetal thyroid function initiates. Maternal demand for thyroxine increases by 50% in pregnancy which is achieved through various adaptive changes. Iodine demands increase and are ideally achieved through physiological adaptations which maintain adequate thyroid function during pregnancy (Dosiou and Medici, 2017). However, insufficient iodine status in pregnancy is common (Bath et al., 2014, Brantsaeter et al., 2022).

There are few studies which specifically measured prenatal iodine status and offspring autism-associated traits and no studies measured autism diagnosis. A recent collaborative study utilised three prospective cohorts, Generation R (Netherlands), Avon Longitudinal Study of Parents and Children (ALSPAC) (England) and INMA (Spain) (Levie et al., 2020). The authors measured the relationship between offspring autism-associated traits and prenatal urinary iodine to creatinine ratio (< 150 μg/g compared to ≥ 150 μg/g) at ≤ 14 weeks' gestation, ≤ 18 weeks' gestation, and any point in pregnancy (Levie et al., 2020). Overall, results were inconsistent, there was no clear evidence of association observed in ALSPAC or INMA, however, a reduced odds of autism-associated traits were observed in the Generation R cohort in relation to lower urinary iodine to creatinine ratio at all three time points. Yet, there was no clear evidence of association in their meta-analysis of individual participant data from the three cohorts (Levie et al., 2020). Thus, I did not observe consistent evidence across the three cohorts. However, thyroid disfunction has been associated with autism diagnosis (Ge et al., 2020) and autism-associated traits (Levie et al., 2018). There may be alternative pathways that link thyroid disfunction and autism, beside insufficient iodine status. Therefore, further investigations into prenatal iodine status and autism-associated traits would be informative, as well as studies that look at autism diagnosis.

1.5.3.4 Folate and other B-vitamins

Folate is also described as vitamin B_9, and its synthetic form is folic acid. In addition to folate's role in the closure of the neural tube at 28 days gestation, folate and other B vitamins play a key role in one-carbon metabolism and methylation. This process is

considered important for the synthesis of DNA and DNA methylation. DNA methylation consists of epigenetic mechanism that alters gene expression and has been related to the development of offspring autism diagnosis (Zhu et al., 2020)

Five studies measured folate serum levels in pregnancy (Braun et al., 2014, Egorova et al., 2020, Raghavan et al., 2018, Steenweg-de Graaff et al., 2015). The results were conflicting but may relate to how folate was modelled. No clear evidence of association was observed between SRS and prenatal serum folate concentrations (Braun et al., 2014, Steenweg-de Graaff et al., 2015). However, folate concentrations were modelled linearly in these three investigations. Another two studies observed an increased probability of an autism diagnosis in association with high prenatal folate concentrations (Egorova et al., 2020, Raghavan et al., 2018). Raghavan et al measured prenatal folate concentration categorically and observed the clearest associations with prenatal plasma folate levels $\geq 90^{th}$ percentile), compared to the middle 80^{th} percentile ($>10^{th}$ and $< 90^{th}$) (Raghavan et al., 2018).

Similar trends were observed with prenatal serum vitamin B_{12} concentrations (Raghavan et al., 2018, Sourander et al., 2023). The probability of an autism diagnosis increased in association with high prenatal vitamin B_{12} serum concentration defined as $\geq 90^{th}$ percentile (Raghavan et al., 2018) and $\geq 80^{th}$ percentile (Sourander et al., 2023), compared to the middle 80^{th} percentile and 40-60^{th} percentile, respectively. Results on low maternal serum levels of folate and vitamin B_{12} were indicative of an increased likelihood of autism diagnosis but due to a high degree of uncertainty in the estimates, these associations were less clear (Raghavan et al., 2018, Sourander et al., 2023). However, another study observed no clear associations with vitamin B_{12} and autism diagnosis when vitamin B_{12} was modelled linearly (Egorova et al., 2020). From this limited number of studies there is some evidence of an increased likelihood of autism diagnosis in association with high maternal vitamin B_{12} and folate serum levels.

1.5.3.5 Other biochemical indicators of prenatal nutrition

Other biochemical indicators of prenatal nutrition were less frequently measured and include serum magnesium (Bakian et al., 2018), selenium (Lee et al., 2021a), anaemia (Wiegersma et al., 2019) and Egorova et al measured 62 biomarkers, including a range of amino acids and vitamins (Egorova et al., 2020). The probability of an autism diagnosis increased in relation to increasing prenatal selenium levels (Lee et al., 2021a), and

maternal anaemia before 30-weeks' gestation (Wiegersma et al., 2019). No clear evidence of associations was observed between magnesium and autism diagnosis (Bakian et al., 2018), and Egorova et al did not detect any associations with autism diagnosis, except folate (previously discussed) (Egorova et al., 2020).

1.5.4 Section summary

Overall, the evidence of an association between autism diagnosis and whole dietary patterns, food groups and intake of single nutrients is conflicting. Across the measures of biochemical indicators of prenatal nutrition, excess folate, vitamin B_{12} and deficient or insufficient vitamin D serum concentrations were associated with an increased likelihood of autism. Heterogeneity in approach and methodological limitations will contribute to the inconsistent results from the studies I have discussed. Resounding methodological limitations are small sample size and retrospective study design which were frequently encountered. In addition, there were only a small number of studies that investigated each prenatal nutritional factor. Additional sources of heterogeneity include: the measure of autism-associated traits and potentially the child's age at measurement, whether other food groups or nutrients were adjusted for, measurement error in dietary patterns and approach to model the nutritional indicator.

1.6 Autism and health inequalities

Upstream determinants of health are the macro-level factors such as political, economic, social, and environmental aspects that are beyond individual's control (Galobardes et al., 2006a, Galobardes et al., 2006b). Downward causation organises the determinants of health into a hierarchical structure whereby upstream determinants impact on intermediate factors which shape individual-level risk factors (Lakerveld and Mackenbach, 2017). Prenatal diet is an individual-level factor which is socially patterned, since diet quality improves as the level of deprivation an individual is exposed to decreases. As the determinants of diet quality relate to systematic differences in the wider determinants of health which are beyond individual control, the adverse effect prenatal diet has on offspring developmental outcomes is considered inequitable (Barker et al., 2017). Hence, a mother's exposure to deprivation can unfairly affect her diet quality and alter the child's foetal development and ability to prosper in later life. The DOHaD concept supported the argument that the distribution of health is inequitable, because, at least to an extent, health is formed prior to individual accountability (Baird et al., 2017).

Evidence of disparities in nutritional quality of diet is well-documented (Darmon and Drewnowski, 2015, Gete et al., 2022, Sawyer et al., 2021, Scottish Government, 2018). For example, the Scottish based maternal and infant nutrition survey (2016-2017) highlighted disparities in several nutrition related behaviours important for healthy foetal development (Scottish Government, 2018). Nutrition related behaviours were compared across the Scottish index of multiple deprivation (SIMD) (most deprived - 1, least deprived - 5). Five portions of fruit and vegetables were consumed at least 3 or more days per week by 83% of respondents in SIMD 5 compared to 69% in SIMD 1. Similarly, 41% of respondents in SIMD 1 reported taking folic acid supplements before pregnancy, compared to 67% of respondents residing in SIMD 5 (Scottish Government, 2018).

Proponents of DOHaD and a life course approach have long argued that investigations in the field should incorporate health inequalities (Baird et al., 2017). Observational studies can investigate differential associations with socioeconomic deprivation to provide contextual knowledge to inform interventions and aid interpretation of findings (Callander and McDermott, 2017). Moreover, prenatal diet is amenable to intervention, and so, it could be informative to understand the extent to which intervening on prenatal diet may lower the probability of autism, and therefore, may be able to reduce health inequalities in autism.

1.6.1 Socioeconomic deprivation and diagnostic bias

The relationship between social deprivation and autism diagnosis needs to be unpicked. Deprivation is associated with an increased risk of numerous physical and mental health conditions, yet observations on autism are inconsistent. In European countries, prenatal deprivation was more consistently associated with an increased prevalence of autism diagnosis (Kelly et al., 2019, Larsson et al., 2005, Lehti et al., 2013, Li et al., 2014, Rai et al., 2012, Russell et al., 2014). Conversely, in countries without universal health care and/or non-European countries, the direction of association is less consistent (Bhasin and Schendel, 2007, Burd et al., 1999, Croen et al., 2002, Dodds et al., 2011, King and Bearman, 2011, Segev et al., 2019, Yu et al., 2021). These findings have been scrutinised in further research, and there is now consensus that diagnostic bias can influence the socioeconomic patterning of autism diagnosis (Aylward et al., 2021, Durkin et al., 2010, Maye et al., 2022).

To illustrate, sociodemographic associations with autism were described based on 7,047,238 school children in England, aged 5 - 19 years (Roman-Urrestarazu et al., 2021). The authors observed wide disparities in the prevalence of autism diagnosis across England (range 0.6% to 3.4%) which related to several sociodemographic factors, such as deprivation, ethnicity, child sex, and local authority district. For example, deprivation was indicated by eligibility for free school meals, which was associated with a 61% increased likelihood of autism diagnosis compared to non-eligibility (Roman-Urrestarazu et al., 2021). However, autism-associated traits are often measured with autism screening tools, and do not face the same barriers to attainment as an autism diagnosis. Studies from Europe (Russell et al., 2011), Asia (Fujiwara, 2014, Guo et al., 2019b, Lung et al., 2018, Tsai et al., 2017), and America (Moody et al., 2017, Reyes et al., 2021, Rosenberg et al., 2018) observed a greater level of parent-reported autism-associated traits in children from families with greater levels of deprivation. Though research confirmed that, at least to an extent, the greater levels of autism-associated traits were driven by greater behavioural issues (Moody et al., 2017, Rosenberg et al., 2018).

Families with greater awareness, educational attainment, and resources may better navigate healthcare systems to obtain a diagnosis for their child. The acquisition of an autism diagnosis can be an arduous process, extended over years. An average of 4.6 years (standard deviation (SD) 4.4 years) between first parental concern and diagnosis has been observed, yet delays were largely attributed to the progression through health services (Crane et al., 2016). Between first contact with a healthcare professional and confirmed autism diagnosis, there was an average 3.6-year delay with wide variability (SD 4.1 years) and approximately half of parents were dissatisfied with the process (Crane et al., 2016). Numerous factors obscure the recognition of autism symptoms. For example, autism has a heterogeneous presentation, and there is often low performance of screening tools in distinguishing autistic and non-autism populations (Chesnut et al., 2016, Moody et al., 2017, Surén et al., 2019). Co-morbidities can distract from the identification of autism symptoms, and the conceptualisation of autism diagnosis is dynamic. Recognition of symptoms is influenced by cultural norms and stigma, and individual symptom profile evolves with age and quality of support. Subsequently, autism is a challenging condition to identify, and parental awareness and resources can influence the acquisition of an autism diagnosis (Maye et al., 2022, Smith et al., 2020). Hence, barriers to access healthcare are perceived to contribute to the higher risk of autism diagnosis in children from affluent families that has been observed in some studies, particularly American studies (Aylward et al., 2021, Durkin et al., 2010, Maye et al., 2022).

Briefly, I will discuss the proposition that genetics drives the increased prevalence of autism diagnosis associated with affluence. People with an autism diagnosis have more genetic variants associated with increased intelligence and educational attainment, compared to the general population (Grove et al., 2019). This underlying genetic structure may contribute towards the increased prevalence of high parental educational attainment and offspring autism diagnosis. However, a greater polygenic score (PGS) for IQ (greater genetic liability) and educational attainment in people with autism may not directly translate to higher educational attainment in parents. Transmission disequilibrium refers to a deviation from the expected transmission of a genetic variant from parents to offspring. Each parent has a variant of the same gene, and on average, offspring have a 50:50 probability of inheriting either parent's genetic variant. Genetic variants associated with educational attainment are over inherited in people with autism diagnosis (Weiner et al., 2017). I am not aware of any studies that measured the extent to which transmission disequilibrium explains the higher average PGS for educational attainment and IQ.

Few studies measure intelligence in parents of autistic children, and most have small sample sizes with conflicting results (Folstein et al., 1999, Fombonne et al., 1997, Gizzonio et al., 2014, Schmidt et al., 2008). I identified one large register-based cohort study of 360,151 based on fathers conscripted to the Swedish military. Parental intelligence had a J-shaped relationship with offspring autism diagnosis. The likelihood of autism diagnosis increased in association with parental IQ in the bottom 50% of the population (IQ <100) and top 5% (IQ >126) (Gardner et al., 2020). If the relationship with maternal education followed a similar trend, an average, the associations with low parental education may be stronger compared to high parental education. Together, the body of evidence suggests that in European countries, deprivation is related to an increased likelihood of autism diagnosis.

1.7 Causally informed approaches in nutritional epidemiology

There are sceptics and advocates of aetiological investigations in nutritional epidemiology. Diet and nutrition are notoriously challenging to investigate due to the complexity of diet (Ioannidis, 2013). The human diet is composed of a vast range of foods and fluids, with different compositions of bioactive components, and consumed in various quantities, all of which varies day-to-day. Moreover, large-scale cohort studies tend to rely on self-reported dietary assessment methods which are prone to error and bias, especially recall bias and

social desirability bias (Meltzer et al., 2008). The utility of RCTs in investigations of diet is reduced due to several issues such as compliance, substitution effects, practicalities, and financial implications (Hébert et al., 2016).

Researchers have argued that aetiological investigations in nutritional epidemiology should move beyond the dichotomous interpretation based on study design, whereby, only RCTs can measure 'effect' and observational studies can only measure 'association' (Brown et al., 2023). Causal inference requires critical thought in the context of study strengths and limitations, regardless of methodology (Hernán, 2018a). Other researchers call for radical reform of nutritional epidemiology (Ioannidis, 2018). Ioannidis argued that many estimates of nutrition, particularly single nutrients, produced implausibly large effect estimates that are better attributed to bias (Ioannidis, 2013). Indeed, the conditions necessary for causal inference more broadly are greatly debated (Cofield et al., 2010, Davey Smith et al., 2020, Hébert et al., 2016, Hernán, 2018a, Mukamal et al., 2020, Yeh et al., 2018).

Even the language of causal estimation is contentious (Cofield et al., 2010, Hernán, 2018b). Cofield et al judged causal language to be incongruous with nutritional observational study designs and discredited its use in all observational investigations (Cofield et al., 2010). In opposition to this, Hernan argues that without explicit reference to scientific questions of causality, researchers resort to implicit language which can lead to ambiguous interpretations (Hernán, 2018b). Instead, he suggests that researchers can explicitly discuss the causal context their research sits within, which may encourage clear discussion of the potential disparity between the causal effect and measure of association (Hernán, 2018b, Parra et al., 2021). Therefore, throughout this thesis I will make explicit the aetiological context that the investigations sit within. However, I have taken care to draw a distinction between causal effect and measures of associations and use language in accordance with this.

Causal inference encompasses multiple methods, yet in some fields of research the terms 'causal methods' and 'causal inference' have become aligned with a narrow set of approaches, for example, the potential outcomes framework and counterfactuals, and directed acyclic graphs (DAG) (Greenland et al., 1999, Hernán and Robins, 2006). These approaches provide a framework to support the conceptualisation of causal pathways and sources of bias which subsequently may bring clarity to the analytic strategy and interpretation of research. Additionally, strategies such as triangulation can also enhance causal assessment. Triangulation contrasts different approaches that are used to investigate

a similar underlying causal question but critically, have different key sources of bias (Lawlor et al., 2016). For example, prospective cohort studies can be triangulated with Mendelian randomisation. Prospective cohort studies have a high risk of confounding bias by socioeconomic and lifestyle factors, whereas this source of confounding bias is low in Mendelian randomisation. The rationale being that consistent findings across contrasting approaches are less likely to be due to bias and may indicate a causal relationship. I will refer to these approaches collectively as causally informed approaches and will discuss each in more detail in Chapter 3.

Each causally informed approach may help enhance the interpretation of findings compared to conventional approaches to measure association. However, they will not establish causality independently and results should be interpreted in the context of methodological limitations. Thus, causally informed approaches may further the contribution research findings make to ongoing investigations into the aetiology of autism.

1.8 Conclusion

Prenatal diet is a modifiable target for intervention which could potentially reduce the likelihood of the development of autism, if established as a causal factor. However, the aetiology of autism remains largely unknown, and although prenatal nutrition may be a factor, evidence of association is unclear. Whole dietary patterns were inconsistently associated with autism, but methodological limitations were evident, particularly small sample sizes and retrospective study designs. Establishing whether there is consistent evidence of association between prenatal dietary patterns and autism can make an important contribution towards understanding the aetiology of autism. Thus, large prospective cohort studies are required to measure the association between prenatal dietary pattern and autism. Additionally, this evidence may be strengthened through causally informed approaches, which may improve the causal interpretation. Lastly, aetiological understanding and public health practice, may be supported by knowledge of the extent to which prenatal dietary patterns may explain the association between maternal deprivation and autism.

2 Chapter 2. Thesis aims and objectives

2.1 Thesis aim

This thesis aims to understand the relationship between prenatal nutrition in pregnancy and offspring autism diagnosis and autism-associated traits, and the extent to which a 'healthy' prenatal dietary pattern explains the relationship between maternal deprivation and each outcome.

2.2 Thesis objectives

Objective 1. Chapter 4: To synthesise evidence of the association between prenatal multivitamin supplement use and autism diagnosis in offspring.

a. Synthesise evidence of the measured association between prenatal multivitamin supplement use and offspring autism diagnosis.

b. Synthesise evidence of triangulation within the included studies and the utility of these approaches to enhance causal assessment.

Objective 2. Chapter 5: Within a causal framework, measure the association between a 'healthy' prenatal dietary pattern (HPDP) and offspring autism diagnosis and autism-associated traits.

a. To measure the magnitude of association between HPDP and each of the following outcomes in the Norwegian Mother, Father, and Child cohort (MoBa).

    I. Offspring autism diagnosis.

    II. Social communication questionnaire score at age three and eight.

    III. Two subdomains of the social communication questionnaire at age three and eight years: the social communication score, and restrictive and repetitive behaviours score.

b. To measure the magnitude of association between HPDP and offspring social communication disorders checklist score at age eight years in ALSPAC.

Objective 3. Chapter 6: Within a causal framework, measure the association between maternal polygenic score (PGS) for HPDP and autism-associated traits using Mendelian randomisation (MR).

a. To measure the association between maternal PGS and each of the following outcomes in MoBa.

    I. Social communication questionnaire score at age three and eight.

    II. Two subdomains of the social communication questionnaire at age three and eight years: the social communication score, and restrictive and repetitive behaviours score.

Objective 4. Chapter 7: Within a causal framework, measure the extent to which HPDP may explain the association between maternal deprivation and autism diagnosis and autism-associated traits.

a. To measure the association between maternal deprivation, as indicated by either maternal education and parental income, and each of the following outcomes in MoBa.

    I. Offspring autism diagnosis.

    II. Social communication questionnaire score at age three and eight.

    III. Two subdomains of the social communication questionnaire at age three and eight years: the social communication score, and restrictive and repetitive behaviours score.

b. To measure the association between maternal deprivation, as indicated by either maternal education or Townsend deprivation index, and social communication disorders checklist score in ALSPAC.

3 Chapter 3. Background to the methods

3.1 Chapter overview

Each empirical paper includes the full description of technical aspects of the methodology. The background to the methods will be used as an opportunity to elaborate on the rationale for each of the main methodological approaches. Firstly, I provide a description of the datasets and key variables used in Chapters 5-7, with a detailed discussion of prenatal dietary pattern. Following this, I discuss the rationale for the main methodologies applied in this thesis in the following order: multiple imputation, causally informed approaches and marginal structural model as applied in Chapter 5, MR (Chapter 6), and the controlled direct effects (CDE) (Chapter 7).

3.2 Rationale for the systematic review and meta-analysis

All relevant details of the systematic review and meta-analysis methodology are included in Chapter 4. To avoid duplication, methodological details will not be provided here. The systematic review and meta-analysis were conducted on prenatal multivitamin supplements because there was an insufficient number of studies identified that investigated prenatal diet and autism. Indeed, at the time the systematic review and meta-analysis protocol was developed most studies that investigated prenatal nutrition and autism diagnosis focused on prenatal multivitamin supplements. By focusing on prenatal multivitamin supplements, I was able to apply a systematic approach to identify and critically evaluate the existing literature, including how triangulation informed a specific causal query.

3.3 Description of the cohorts and key methodological aspects of the data

I investigated two cohorts to assess the reproducibility of findings across two contexts with complementary features. ALSPAC and MoBa were established to investigate child health and development (Boyd et al., 2013, Magnus et al., 2006). In ALSPAC (Boyd et al., 2013) and MoBa (Magnus et al., 2006) recruitment was initiated in pregnancy in recognition of the early origins of child health and development. The cohorts addressed a gap in large longitudinal birth cohorts that had a comprehensive range of data, a richness which has been enhanced through linkage to routinely collected health and educational records.

Several large prospective UK birth cohorts were considered for my thesis (Connelly and Platt, 2014, Inskip et al., 2006, Wright et al., 2013), however, ALSPAC was one of few large prospective cohort studies that sought detailed prenatal dietary information from their full cohort (Emmett, 2009). Additionally, MoBa is the largest prospective birth cohort in Norway and also obtained detailed prenatal dietary information (Magnus et al., 2006). Moreover, both have detailed measurements of child neurodevelopment, and a range of socioeconomic indicators, behavioural characteristics, and health outcomes. Furthermore, there are established processes to link each cohort to health records using unique identifiers which allows long term follow-up. In MoBa, a large sub-study was conducted called The Autism Study, formally known as the Autism Birth Cohort Study (Surén et al., 2014). The sub-study began in 2003 in collaboration with Columbia University, New York, until 2018. The Autism Study continues today and aims to characterise people with autism. The study includes potential aetiological factors in autism, the natural course of autism, and the utilisation of health, education, and social services in the delivery of care. Scientific outputs from the Autism Study have been used throughout this thesis.

3.3.1 The Norwegian Mother, Father, and Child Cohort (MoBa)

MoBa recruited 95,669 mothers, 75,618 fathers, and 114,622 children between July 1999 and December 2009. There were no exclusion criteria, however, the questionnaires were in Norwegian which may restrict non-Norwegian speaking populations. A total of 277,702 pregnancies were invited to participate, of which 112,908 pregnancies were recruited (41%). Pregnant women were invited to participate when at their routine ultrasound examination (Magnus et al., 2016, Magnus et al., 2006).

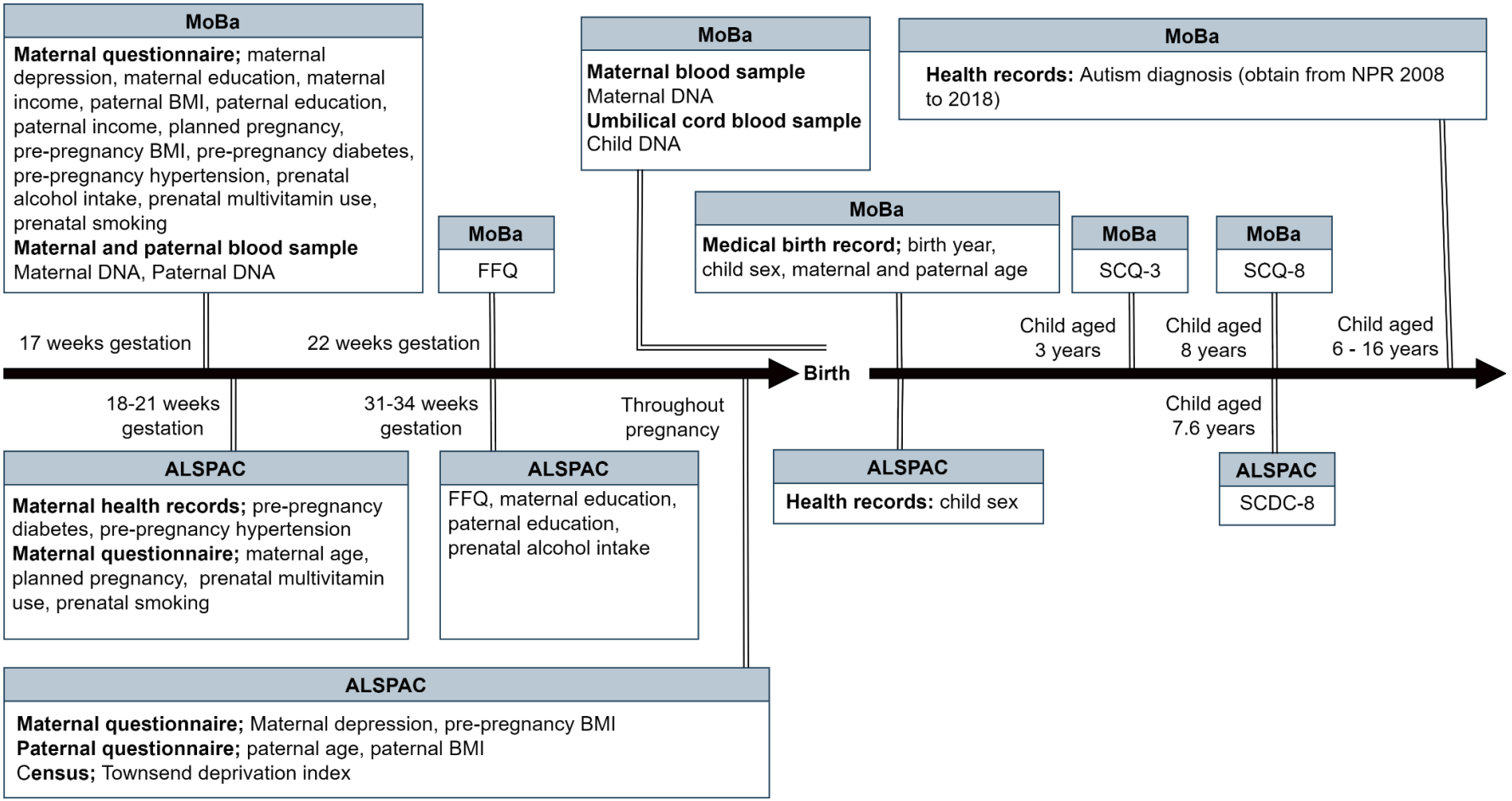
A wide range of information has been collected in MoBa and here I focus on the data sources used in this thesis. Four postal questionnaires were distributed at 17-, 22-, and 30-weeks' gestation and when the child was three and eight years old. Further information was obtained from health records (Magnus et al., 2016, Magnus et al., 2006). DNA was extracted from blood samples obtained from the mother and father at approximately 17-weeks' gestation and from the mother soon after birth. Children's DNA were extracted from umbilical cord blood samples (Corfield et al., 2022). Figure 1 shows a timeline of data collection and source for the information used in Chapters 5-7.

3.3.2 The Avon Longitudinal Study of Parents and Children cohort (ALSPAC)

ALSPAC recruited 14,541 participants (71.8% of 20,248 eligible pregnancies) for their core sample. Eligible women resided in the former county of Avon, Southwest England and had an estimated delivery date between the 1st of April 1991 and 31st of December 1992 (Boyd et al., 2013). Women migrating into this area were eligible but those migrating out of the catchment areas were not eligible unless the third trimester questionnaire was completed. Women were recruited via routine antenatal visits, media information and recruitment staff at across community locations (Boyd et al., 2013).

I used four postal questionnaires from ALSPAC, distributed throughout pregnancy, at 18-21 weeks gestation, 31-34 weeks gestation, and 91 months (child's age) (Iles-Caven et al., 2020). A 'catch-up' questionnaire was sent to women who missed earlier questionnaires and the timing is described as 'throughout pregnancy' as per Iles-Caven (Iles-Caven et al., 2020). Two reminder letters were sent if there was no response: one after seven days and a second after a further 10 days. After one month of non-response, contact was made via telephone or home visit by a researcher to encourage/assist with questionnaire completion (Iles-Caven et al., 2020). The last postal questionnaire was aimed at 91 months (7-8 years) and contained the social and communication disorder checklist (SCDC). No DNA data was used for the analysis of ALSPAC in this thesis. Further information on the timeline of data collection can be found online (University of Bristol., 2021).

Figure 1. Timeline and source of data collection in MoBa and ALSPAC.



ALSPAC, Avon Longitudinal Study of Parents and Children; DNA, Deoxyribonucleic acid; FFQ, food frequency questionnaire; MoBa, Norwegian Mother, Father, and Child Cohort; NPR, Norwegian patient register; SCDC, social communication disorders checklist; SCQ, social questionnaire All information was obtained from the questionnaires at the time point specified unless stated otherwise in Figure 1.

3.3.3 Outcome measures

3.3.3.1 Autism (MoBa)

The two major official diagnostic criteria are the Diagnostic and Statistical Manual of Mental Disorders (DSM) and International Classification of Diseases (ICD). The ICD is the official diagnostic criteria used by Norwegian health services, and published by the World Health Organisation which sets internationally relevant criteria (World Health Organisation, 2020). The DSM is published by the American Psychiatric Association and, though aimed at a North American population, is used by other countries (American Psychiatric Association, 2000). In 2013, the DSM-IV was superseded by the DSM-5, whereas the ICD-10 was not updated to the ICD-11 until 2022. The major change in DSM-5 and ICD-11 was the removal of the autism subtypes due to poor reproducibility. Secondly, the updated diagnostic criteria combined autism into two major dimensions instead of three: social and communication difficulties, and restrictive and repetitive behaviours. The social and communication subdomains were combined as they showed close agreement, but restrictive and repetitive behaviours had low correlation.

In Chapter 4, I synthesised evidence from published literature that defined autism based on different versions of the ICD and DSM. However, in Chapters 5 and 7, I used the ICD-10 to define an autism diagnosis (World Health Organisation, 1993). Autism diagnosis was obtained from the Norwegian patient registry via linkage to MoBa using a unique identification number (Surén et al., 2014). This captures all children with a diagnosis of autism between 2008 to 2018 based on the F84 codes from the ICD-10. I avoided use of the ICD-10 subtypes of autism and excluded Rett syndrome (F84.2) in accordance with the ICD-11 criteria.

3.3.3.2 Autism-associated traits (MoBa)

The Social Communication Questionnaire (SCQ) is a 40-item parent reported screening tool for autism (Rutter, 2003). The SCQ is commonly used to score the degree of autism-associated traits and has been based on the Autism Diagnostic Interview-Revised. The first item identifies the presence of phased speech, and the other 39 questions measure social and communication skills, language skills and restrictive and repetitive behaviours and interests. Seven items relate to phased speech and may be less relevant to children who have not developed phased speech. Hence, children with phased speech are scored out of 39 and children without phased speech are scored out of 32. A cut-off of $\geq 93^{rd}$ percentile

was applied and corresponds to a score of 11 at age three and at age eight. To avoid underscoring children without phased speech, I applied an adjustment to children without phased speech as per Eaves (Eaves et al., 2006) and shown in Equation 1. Additionally, I included children with over 50% response rate out of 32 or 39 questions (Surén et al., 2019). The SCQ was completed by mothers about their children at three and eight years old and are referred to as SCQ-3 and SCQ-8. Additionally, the SCQ was split into 26 items related to social communication and language (SCQ-SOC-3 and SCQ-SOC-8), and 13 items related to restrictive and repetitive behaviours (SCQ-RRB-3 and SCQ-RRB-8). In sensitivity analyses I repeated the scoring of each SCQ measure but without the adjustment for phased speech.

Equation 1. Adjustment to the social communication questionnaire as per Eaves et al (Eaves et al., 2006)

$$SCQ = \frac{sum\ of\ responses * 39}{(39 - number\ of\ missing\ responses)}$$

SCQ, social communication questionnaire

The sensitivity and specificity of the SCQ-3 has been measured in MoBa as part of the Autism study (Surén et al., 2019). The SCQ-3 was validated against an autism diagnosis and based on a cut-off of 11. The sensitivity was 42% (95% confidence interval (CI) 37, 47) and specificity was 89% (89, 90). The SCQ-8 was not validated in MoBa as part of the Autism study. Poor performance of the SCQ in children under four is a known challenge, and improves in children over the age of four years (Chesnut et al., 2016). A meta-analysis on the validity of the SCQ against diagnosed autism found the sensitivity ranged from 0.47 to 0.96 and specificity 0.52 to 0.99, with higher sensitivity and specificity in older children (Chesnut et al., 2016). I re-estimated the sensitivity and specificity based on the sample of MoBa used in this thesis and autism diagnosis defined as described above (Table 1).

Table 1. Sensitivity and specificity of the social communication questionnaire at age three and eight years in MoBa.

Outcome	**Score**	**No autism diagnosis** (number of children)	**Autism diagnosis** (number of children)	**Sensitivity** (%)	**Specificity** (%)
SCQ-8 (%)	Low	36606	180		
	High	3255	213	54.2	91.8
SCQ-8-RR (%)	Low	34104	133		
	High	5757	260	66.2	85.6
SCQ-8-COM (%)	Low	36964	254		
	High	2897	139	35.4	92.7
SCQ-3 (%)	Low	45789	365		
	High	5209	199	35.3	89.8
SCQ-3-RR (%)	Low	46587	491		
	High	4411	73	12.9	91.4
SCQ-3-COM (%)	Low	46143	338		
	High	4855	226	40.1	90.5

SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCQ-SOC, communication skills subdomain of SCQ. The number following each outcome denotes the approximate age of the child in years when the measure was obtained. i.e. SCQ-SOC-8; social communication subdomain measured at age 8 years. The presence of the outcome from each questionnaire was indicated by a high score.

3.3.3.3 Autism-associated traits (ASLAPC)

The Social and communication disorders checklist (SCDC) was the main outcome in ALSPAC as only 31 people had a diagnosed autism cases were obtained via linkage to health records (Williams et al., 2008). The SCDC is a 12-item questionnaire that was designed to screen for autism (Skuse et al., 2009, Skuse et al., 2005). The primary carer completed the SCDC when the child was approximately 7.6 years old and will be referred to as SCDC-8. The SCDC-8 measures autism-associated traits that related to social and communication skills. However, the other major dimension of autism, restricted and repetitive behaviours are not measured, nor is early developmental and language delays which are predictive of autism (Skuse et al., 2005).

The SCDC was not validated directly in ALSPAC or the general population. Instead, a range of approaches to assess concurrent validity, discriminant validity, and criterion validity were applied based on 230 children recruited from a social communication disorders clinic, 53 children recruited from a mental health clinic and 118 from a control group of the general population (Skuse et al., 2005). The sensitivity and specificity were 0.90 and 0.69, respectively. However, ‘autism’ was defined from a computer-generated

algorithm based on a parental autism interview. Additionally, the use of clinical populations may inflate the SCDC performance. Based on all measures, the authors concluded that the SCDC performed well in discriminating autism (Skuse et al., 2005). A cut-off of 9 out of a possible score of 24 is recommended in the SCDC-8 which represents the 93rd percentile of SCDC scores distribution in ALSPAC.

3.3.4 Prenatal dietary intake assessment method

Food frequency questionnaires (FFQ) are widely used across large scale epidemiological investigations to measure usual dietary intake. Advantages in large epidemiological studies compared to other dietary assessment methods are the low cost, low researcher, and participant burden, and FFQ can reflect habitual dietary intake. However, it is well accepted that FFQ are prone to error (Meltzer et al., 2008). Error is present in biological and physical measurements in every branch of science, albeit to varying degrees. FFQ quantify intake by ranking diet in relative terms and therefore cannot estimate dietary intake with absolute precision. Yet, FFQ have been demonstrated to be sufficient to examine the relationship between diet and many health outcomes. This is because FFQ rank diet in varying degrees of low to high intakes and can provide a relative indication of dietary intake at a population level (Satija et al., 2015). However, it is important to consider the validity of the FFQ used in MoBa and ALSPAC.

MoBa's 255-item semi-quantitative self-reported FFQ was distributed at 22-weeks' gestation, and women were asked to report their average dietary intake since conception. The FFQ was developed specifically for pregnant women in MoBa and was not distributed in the MoBa study until 2002, and so the FFQ responses are available for recruitment years between 2002-2008. Questions relate to commonly consumed foods and beverages, dietary supplement use, meal patterning and dieting behaviours (Meltzer et al., 2008). Daily nutrient intakes were estimated using FoodCalc (Lauritsen, 1998) and the Norwegian food composition database (Norwegian Food Safety Authority, 2005).

MoBa FFQ was validated in a subgroup of 119 participants using several reference methods: a four day weighed food record that covered three weekdays and one weekend day; motion sensors for assessment of energy expenditure; and multiple biochemical nutritional indicators (Brantsaeter et al., 2008, Brantsaeter et al., 2009, Brantsaeter et al., 2007, Brantsaeter et al., 2010). The absolute correlation (r) between FFQ and a food diary for nutrients when averaged was r = 0.36 with a range of r = 0.13 (vitamin E) to r = 0.55

(dietary fibre). However, according to Hankin et al (Hankin et al., 1991), at this strength of correlation the reliability of the FFQ can be considered fair. For the food items, 10 out of 16 had a reliability of moderate to very strong. The average correlation was 0.48 and ranged from 0.33 to 0.80 and so can be considered 'good' (Hankin et al., 1991). The correlations between biological measures and the FFQ are weak; protein intakes and urinary nitrogen (r = 0.27), vitamin D intake and plasma 25-hydroxycholecalciferol (r = 0.32), folate intakes and serum folate (r = 0.26), iodine intake and urinary iodine (r = 0.38).

All self-reported dietary assessment methods are prone to error, and therefore the strength of correlation is impacted by error in the FFQ as well as by error in the four-day weighed food record and biochemical measures. Additionally, biochemical nutritional indicators are indirectly related to habitual dietary intake for various reasons, as discussed in Chapter 1.5.3 (Kirkpatrick et al., 2019). For example, biochemical nutritional indicators were measured, on average, 24 days (±12 days (SD) prior to the FFQ. Overall, the MoBa FFQ is considered a fair approximation of dietary intake (Brantsaeter et al., 2008).

In ALSPAC a 103-item self-reported FFQ was distributed at 31-34 weeks gestation (Emmett, 2009, Rogers et al., 1998). Women were asked to recall their dietary intake 'nowadays' in relation to 43 food groups but no further clarity on the timeframe is provided. The questions related to food preparation, weekly frequency of commonly consumed foods and drinks, and nutritional supplements. The FFQ was unquantified and so nutrient intakes were estimated based on average portion sizes and McCance and Widdowson's food composition tables (Holland et al., 1991). Estimated nutrient intakes excluded alcohol and nutritional supplements (Rogers et al., 1998). Averaging portion sizes is less accurate than measured portion sizes, especially for women who consume large amounts of a limited range of foods as it will underestimate nutrient intakes, and vice versa, women who consume small amounts of a large variety of foods will have overestimated nutrient intakes (Rogers et al., 1998).

Unfortunately, ALSPAC's full FFQ was not validated against alternative dietary assessment measures due to limited funding and time restrictions (Emmett, 2009). However, seafood consumption questions were validated against blood levels of docosahexaenoic acid which showed reasonable agreement (Williams et al., 2001). Additionally, Rogers et al (Rogers et al., 1998) descriptively compared ALSPAC nutrient intakes to the UK average intakes for women aged 16-64 years. All nutrient intakes compared well with exception of folate, vitamin C, sugar and calcium which were

considerably higher and vitamin A was considerably lower in ALSPAC women. The differences in estimated nutrient intakes may relate to dietary changes in pregnancy (Rogers et al., 1998). Lastly, numerous studies have demonstrated that the characteristics and diet-disease associations observed in ALSPAC are consistent with theoretical expectations and existing empirical studies (Emmett et al., 2015).

3.3.5 Dietary pattern

Several statistical methods exist to derive dietary patterns and can be broadly defined as investigator-driven, data-driven, and hybrid methods. Each method has strengths and limitations which vary depending on the context they are applied to (Schulz et al., 2021, Zhao et al., 2021). I selected *a posteriori* approach because important dietary factors for the potential aetiology of autism diagnosis and autism-associated traits are not established. Additionally, many dietary indices require measurement of food items in grams or portions per day which were unavailable in ALSPAC. Exploratory factor analysis is widely used to derive dietary patterns based on the data at hand (Schwedhelm et al., 2018, Varraso et al., 2012). Factor analysis is a data reduction method that reduces a range of dietary items by simultaneously measuring their correlations to derive factors which represent an unobserved latent variable. The latent variable is the dietary pattern which is interpreted and labelled based on the factor loadings of each dietary variable and interpretability of the factor. The factor score is used as a measure of adherence to the dietary pattern derived (Hu, 2002). I derived a 'healthy' prenatal dietary pattern (HPDP) in both cohorts. The HPDP was characterised by a range of food groups and soft drinks (described in Chapter 5). The pattern was denoted 'healthy' as food items with high factors scores represent foods that are encouraged according to dietary guidelines worldwide and nationally (Nordic Council of Ministers, 2023, UK Government, 2023, Willett et al., 2019).

Reported energy intakes can reflect random and systematic error and are widely conditioned on to correct for error in dietary measurements (Ejima et al., 2019, Jessri et al., 2016). Nutritional requirements are met through foods and drinks which contain macronutrients and surmount to total energy intake. The challenge arises due to diversity in nutritional requirements between individuals due to many factors such as body mass and composition, metabolic activity, and physical activity. Hence, there is variability in absolute dietary intakes that relates to requirements and is not related to dietary composition or the concept of a 'healthy' diet (Willett et al., 1997). The random error created through individual nutritional requirements can limit comparison of raw dietary

patterns. Hence, conditioning on total energy intake can improve between-person comparisons (Ejima et al., 2019, Jessri et al., 2016).

Information bias is a source of measurement error in FFQ that may lead to under and over estimation of dietary intake, although underestimation is more common (Burrows et al., 2019, Kesmodel, 2018, Svensson et al., 2014). Recall bias is common in FFQ due to memory difficulties and/or social desirability bias. The latter occurs when a participant skews their dietary intake to be more in fitting with what they perceive to be socially desirable. However, there are many sources of error, for example, variation in the number of items on the questionnaire, length of reporting period, language used in questions, accuracy of the estimation of portion sizes, recorder error or use of food databases used to calculate nutrient intakes (Althubaiti, 2016, Burrows et al., 2019, Kesmodel, 2018). Individual characteristics have been shown to predict systematic error in reported energy intake and can bias the estimates obtained (McGowan and McAuliffe, 2012, Moran et al., 2018, Thomas et al., 2016). Conditioning on total energy intakes can, to an extent, correct for systematic error (Willett et al., 2022, Willett et al., 1997).

I applied the residuals method to statistically adjust the HPDP for total estimated energy intake. In linear regression the linear factor score is modelled as the dependant variable and estimated total energy intakes is the independent variable. The residuals are then extracted and used as the dietary pattern measure (Willett et al., 1997). There are debates on how best to manage total estimated energy in nutritional epidemiology (Banna et al., 2017, Dhurandhar et al., 2015, McCullough and Byrd, 2022, Willett et al., 2022). No estimation method has been demonstrated to be superior, each has strengths and limitations, and none will eliminate all bias (Ejima et al., 2019, Jessri et al., 2016). I selected the residuals method in this thesis as it avoided energy intake to energy expenditure ratios, which assume weight stability (Black, 2000a, Black, 2000b, Goldberg et al., 1991) and are unrealistic in pregnant populations. Lastly, I aimed to avoid non-individualised cut-offs which may increase the risk of misclassification bias and selection bias (Banna et al., 2017). However, the MoBa dietary data contained especially implausible intakes which created outliers in the factor analysis. Thus, as applied by MoBa's nutritional epidemiology researchers, I used cut-offs (<4.5 and >20 Mega joules) to exclude extreme estimated energy intakes (Meltzer et al., 2008). The potential increase in selection bias introduced is likely to be small as I applied wide cut-offs and led to the exclusion of only 1.6% of the sample. Overall, empirical methodological studies generally suggest that there is a lower

risk of bias and results are more concordant with theoretical expectation when corrected by total estimated energy intake, including cut-offs (Jessri et al., 2016).

3.3.6 Measures for mediation analysis

In Chapter 7, I first measured the association between maternal indicators of deprivation and each outcome, and then estimated the CDE. The indicators of maternal deprivation are described in this section of the thesis, and description of the analytical approach is described in section Chapter 3.7. The maternal indicators were maternal education (MoBa and ALSPAC), maternal and paternal income (MoBa), and Townsend deprivation (ALSPAC).

Socioeconomic position is a complex concept with many indicators that measure different but related and overlapping facets (Galobardes et al., 2006a, Galobardes et al., 2006b). In general, there is no superior indicator as the pathways, and so the relevance of each depends on the exposure and health outcome, and what point in the life course the exposure acts (Galobardes et al., 2006a, Galobardes et al., 2006b). Hence, I measured a range of socioeconomic indicators. In both MoBa and ALSPAC, I analysed maternal education which was defined as the highest educational qualification achieved, measured as categories. In MoBa maternal education was obtained from the maternal questionnaire distributed at 17-weeks' gestation, and in ALSPAC maternal education was obtained from the questionnaire 31-34 weeks' gestation.

Ideally, the financial resources available to the mother would be measured in this thesis; however, based on the available data, income may be imprecisely measured. In MoBa I analysed maternal and paternal income obtained from the maternal questionnaire at 17-weeks' gestation. However, in ALSPAC income was not obtained until the child was four years old and so I did not use this indicator due to a risk of reverse causality. This is because caring for a children with autism and other neurodevelopmental conditions may place a greater demand on parents and reduce earning potential (Cidav et al., 2012, Montes and Halterman, 2008). Maternal and paternal yearly gross income (inclusive of benefits) are available in MoBa. Yet, income was coded categorically based on ranges of income and so this was challenging to combine. Thus, I measured parental income, and maternal and paternal income separately.

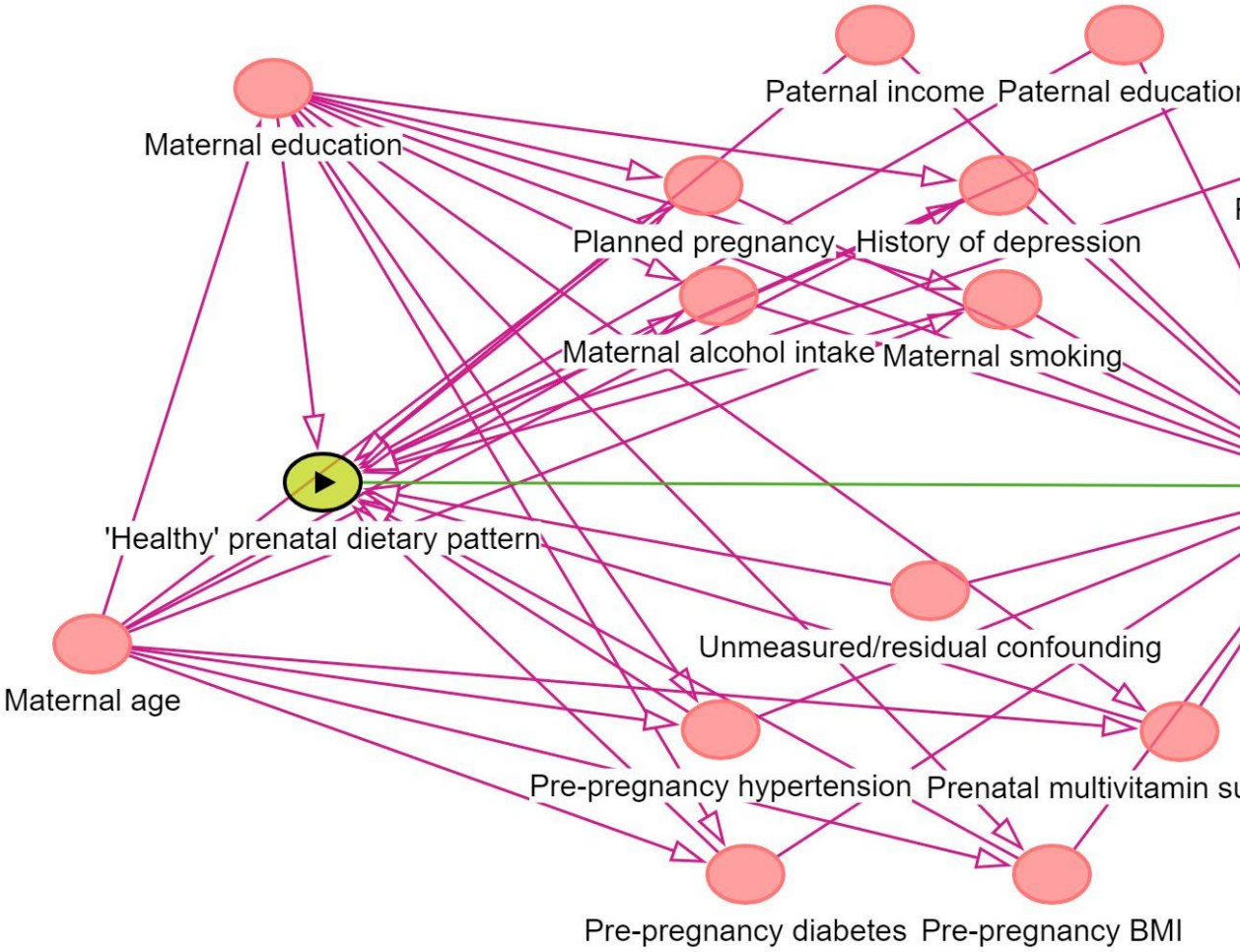
Area-based deprivation is another dimension of deprivation of particular importance to dietary intake (Gilham et al., 2020). Area-based deprivation (the Townsend deprivation score) was available in ALSPAC but in MoBa there was no area-based indicator of maternal deprivation available in pregnancy. The Townsend deprivation score is a well-

used area-based measure of deprivation in England in the 1990s (Townsend et al., 1988). The score is derived from the proportion of homes within the area (ward) that are: not owner-occupied, without car access, occupied by one or more persons per room and occupied by unemployed but economically active individuals. The absolute scores are standardised using z-score and equal weights are applied to each of the four components. Townsend deprivation index applied at baseline (pregnancy) was estimated based on wards at the 1991 census. Additionally, the score can be linked to postcode and applied to individuals, and although the Townsend deprivation index has been criticised when used as an indicator of individual deprivation, in this thesis it is the deprivation within the individual's local area I aimed to measure as this relates to their food environment.

3.4 Multiple imputation

Missing data is a pervasive problem in epidemiological investigations and can bias the estimates obtained (Lee et al., 2021b). MoBa and ALSPAC contain missing items within their questionnaires. There are different approaches to managing missing data in epidemiological studies depending on the mechanism of missingness. I created one imputation model to be used for analyses in Chapters 5 and 7. I applied multivariate imputation by chained equations (MICE) based on the assumption, 'missing at random' (Azur et al., 2011). Missing completely at random assumes the pattern of missingness is random but is rare in survey data (Lee et al., 2021b), such as MoBa and ALSPAC. Missing not at random occurs when the missing items depend on unmeasured factors and is the most challenging to address in epidemiologic investigation. Missing at random assumes the missingness mechanism is non-random but the probability of missingness can be predicted based on other measured variables (Lee et al., 2021b).

MICE is advantageous as it assumes the distribution for each variable rather than the whole dataset (Azur et al., 2011). The imputation model included all variables used in the analyses from Chapters 5 and 7 and auxiliary variables predictive of missingness (Table 2). ALSPAC and MoBa were inspected for the proportion and pattern of missingness, and auxiliary variables predictive of missingness were identified using logistic regression. The descriptive characteristics of mothers lost to follow-up post pregnancy are presented in Supplementary figure 1. Directed Acyclic Graph (DAG) of the relationship between 'healthy' prenatal dietary pattern and autism related outcomes.



Abbreviations: BMI, body mass index

Supplementary table 1-3. The number of imputations was arrived at using von Hippel's approach (von Hippel, 2020). To allow assessment of potential interactions with HPDP, I stratified the sample by level of adherence to HPDP (low, medium, high) and imputed each strata separately (Tilling et al., 2016). The models were run in each imputed dataset before the estimates and standard errors were pooled using Rubin's rule (Campion and Rubin, 1989).

Table 2. Variables used to predict missingness in imputation models.

MoBa, variables	**Method**	**ALSPAC, variables**	**Method**
birthweight	Predictive mean matching	birthweight	Predictive mean matching
autism diagnosis	nil missing	child sex	nil missing
birthyear	polyreg	gestational hypertension	logreg
child sex	logreg	'healthy' prenatal dietary pattern	nil missing
'healthy' prenatal dietary pattern	nil missing	history of depression	logreg
history of depression	logreg	hospitalisation before six years (child)	logreg
maternal age	nil missing	household income (child age 4 years)	polyreg
maternal education	polyreg	maternal age	Predictive mean matching
maternal income	polyreg	maternal education	polyreg
paternal age	polyreg	maternal social class	polyreg
paternal BMI	Predictive mean matching	parity	logreg
paternal education	polyreg	paternal age	polyreg
paternal income	polyreg	paternal BMI	Predictive mean matching
planned pregnancy	logreg	planned pregnancy	logreg
prenatal alcohol intake	logreg	prenatal alcohol intake	logreg
prenatal multivitamin supplement use	logreg	prenatal multivitamin supplement use	logreg
prenatal smoking	logreg	prenatal smoking	logreg
pre-pregnancy BMI	Predictive mean matching	pre-pregnancy BMI	Predictive mean matching
pre-pregnancy diabetes	logreg	pre-pregnancy diabetes	logreg
pre-pregnancy hypertension	logreg	pre-pregnancy hypertension	logreg
SCQ-3	logreg	SCDC-8	logreg
SCQ-8	logreg	severe vomiting	logreg
total energy intake	nil missing	total energy intake	nil missing
		Townsend deprivation	polyreg

logreg, logistic regression, polyreg, Bayesian polytomous regression. ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index; MoBa, The Norwegian Mother, Father, and

Child Cohort Study; SCQ, social communication questionnaire; SCDC, social communication disorders checklist

3.5 Causally informed approaches

Causally informed approaches can support aetiological investigations in observational studies. Approaches include the potential outcomes framework and counterfactuals and causal diagrams such as DAGs (Greenland et al., 1999, Hernán and Robins, 2016, Hernán and Robins, 2006, Neyman, 1923, Robins et al., 2000). These contemporary approaches provide a framework to support the formation of causal questions and analytic strategy, as well as the interpretation of bias. Triangulation can also be applied to further the assessment of bias and causal interpretations (Lawlor et al., 2016, Pearce and Lawlor, 2017, Vandenbroucke et al., 2016), and is used in this thesis. I discuss the potential outcomes framework and counterfactuals and DAGs in more detail within the context of Chapter 5 analyses strategy but continue to draw on these principles for Chapters 6 and 7.

3.5.1 The target causal quantity for the main analysis

The formulation of a well-defined estimand can improve the relevance of findings to public health. The estimand is the conceptual quantity that most closely aligns with the estimation model. Hence, ambiguity in the estimand creates vagueness in the interpretation of the estimate (Hernán and Taubman, 2008, VanderWeele, 2016a). Ideally, we ask a clear causal question that can form a precise and quantifiable target causal quantity in order to form a well-defined estimand (Petersen and van der Laan, 2014). Conceptualisation of a hypothetical intervention has been suggested as a strategy to help sharpen causal queries (Hernán and Taubman, 2008, VanderWeele, 2016a). However, this has also raised much controversy. Some argue the estimand sought should reflect a precisely defined hypothetical intervention (Hernán and Taubman, 2008). Whilst other argue this is too restrictive as it limits the types of questions asked (Krieger and Davey Smith, 2016, Vandenbroucke et al., 2016). A precise target causal quantity in nutritional epidemiology is challenging to define within a counterfactual framework. Prenatal dietary intake is a complex and imprecisely measured with FFQ. Thus, reduction to a precise single uniform definition is unrealistic. I, along with other researchers, believe consideration of the target causal quantity as an intervention is an informative thought experiment but should not limit causal queries (Glass et al., 2013, Schwartz et al., 2016, VanderWeele, 2016a).

The estimand sought in this thesis is the average treatment effect, as the ultimate aim is to improve the entire populations' diet (Barker et al., 2018). The average treatment effect estimates the differences between the average potential outcome had the entire population

received one level of diet quality, compared to the average potential outcome had the entire population received another level of diet quality (Imbens, 2000, Lopez and Gutman, 2017). Hence, I estimated the average risk of autism had the entire sample had a high adherence to HPDP, compared to the average risk of autism had the entire sample received a low or medium adherence HPDP. As I have three levels of adherence, low, medium, and high, I have three average treatment effects which can be estimated based on pairwise comparisons.

3.5.2 Marginal structural model

I used inverse probability weights (IPW) in a marginal structural model to estimate the associations in Chapters 5 and 7. Firstly, the probability of the exposure level conditional on the covariates is estimated and the balancing constraint applied (IPW). Secondly, a marginal structural model is weighted by the IPW to adjust for potential confounders when estimating the association between HPDP and each autism related outcome. Marginal structural models and IPW are preferred here because it: encourages review of the positivity assumption, formalises review of covariate balance and model specification, and separates the design and analysis stage (Ali et al., 2016). Each of these benefits will be discussed in more detail.

3.5.3 Assumptions

Causal inference from these methods is dependent on three main assumptions. Three assumptions were set out: exchangeability, positivity, and the consistency assumption (Imbens, 2000, Robins et al., 2000). Exchangeability refers to no measured or unmeasured confounding. Positivity means each participant should be a non-zero probability of having each level of adherence to HPDP. Consistency assumption has two dimensions: there should be no interference between each level of adherence to HPDP and the effect of HPDP should be constant across all individuals.

The key stages of the analysis are now discussed and are:

- Covariate selection
- Estimation of the inverse probability weighting
- Assessment of covariate balance
- Marginal structural model

3.5.4 Covariate selection

I aimed to achieve exchangeability through statistical adjustment for a range of potential confounders. Potential confounders were identified using a DAG and evidence of potential causal pathways identified in the existing literature (Chapter 1 and (Hertz-Picciotto et al., 2018, Lyall et al., 2017)). A DAG can support clarity in the conceptualisation of potential causal pathways, and help distinguish confounders, mediators, and colliders (Greenland et al., 1999, Tennant et al., 2021). My assumptions on potential causal pathways are transparently depicted in a DAG. Additionally, I avoided data-driven approaches as they may add ambiguity to the estimand sought or introduce bias such as collider bias or over adjustment.

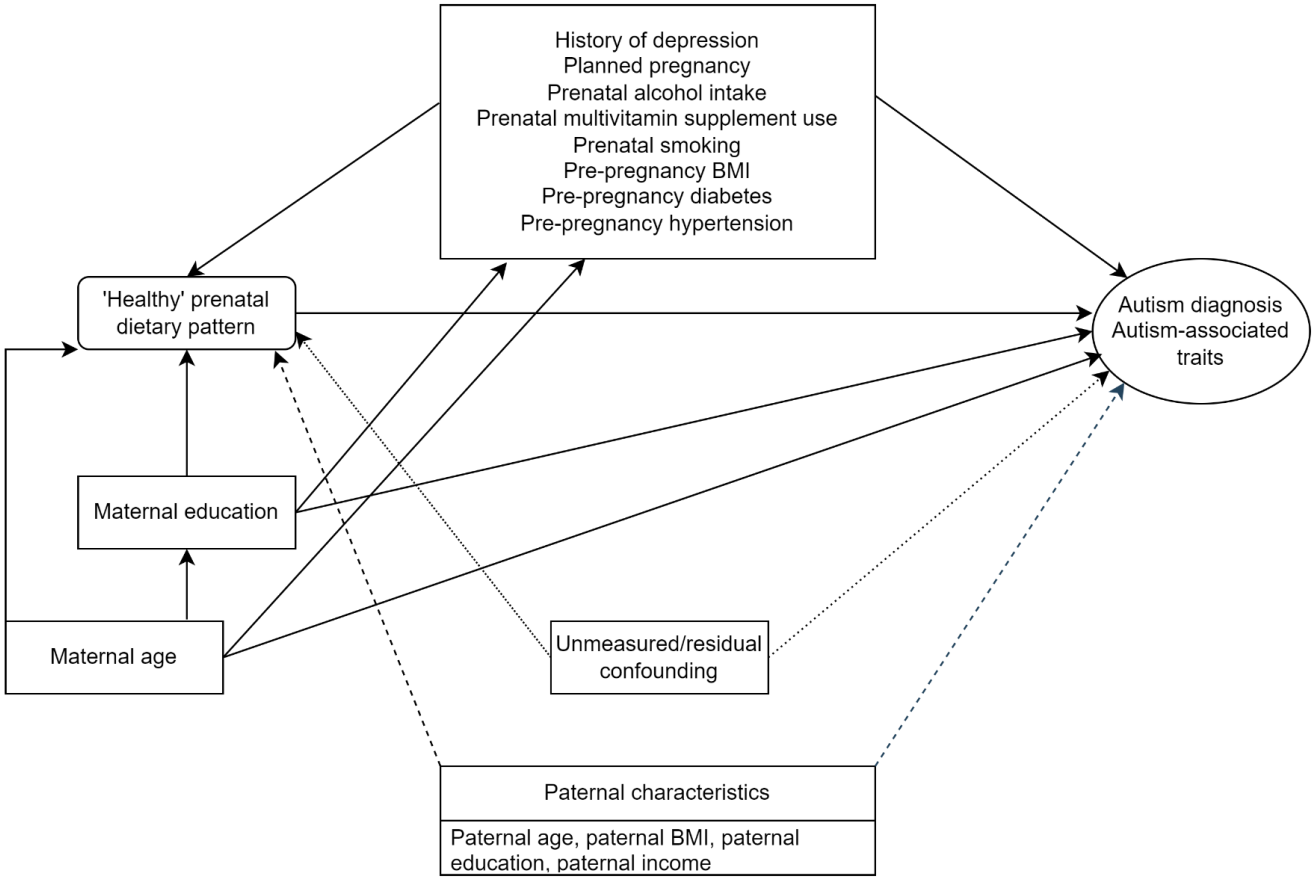
Throughout the thesis I use the term confounder, defined as a common cause of the exposure and the outcome that is not mediated through the exposure (Tennant et al., 2021). Confounders can lead to a spurious association if they are left unadjusted. A mediator is an intermediate factor between the exposure and the outcome through which the causal pathway moves. When measuring the total effect, a mediators should not be adjusted for as its part of the desired effect. A collider is a factor which is independently caused both the exposure and the outcome. Conditioning on a collider may bias in the measured association in either direction (Tennant et al., 2021).

I adjusted for a range of potential confounders most proximal to the mother, which may be stronger confounders of the association between HPDP and autism diagnosis or autism-associated traits. However, the assumption has been queried (Sharp et al., 2018, Suren et al., 2014) and so I additionally adjusted for a range of paternal characteristics in sensitivity analysis. Furthermore, children diagnosed with autism in later birthyears will be younger and may have more severe autism. If so, adjustment for birthyear could introduce bias and so it was adjusted in a sensitivity analysis only. This can help restrict the analyses to a minimally sufficient adjustment set whilst providing an indication of the robustness of results to additional, albeit potentially weaker, sources of potential confounding.

Figure 2 depicts potential confounders of the relationship between HPDP and autism diagnosis or autism-associated traits. Maternal age may cause maternal education, and both are potential causes of each downstream node (downstream to another variable), HPDP and autism diagnosis or autism-associated traits. Therefore, I adjusted for maternal education, maternal age, pre-pregnancy BMI, planned pregnancy, maternal depression,

prenatal multivitamin use, prenatal alcohol intake, prenatal smoking, pre-pregnancy hypertension, and pre-pregnancy diabetes. The sensitivity analyses additionally adjusted for the following paternal characteristics: paternal education, paternal income (MoBa only), paternal BMI, paternal age, and birthyear (MoBa only). The ‘unmeasured/residual confounding’ recognises the limitations of our study design, in that I cannot be certain all potential confounders have been adjusted for.

Figure 2. Representation of potential confounding pathways of the relationship between ‘healthy’ prenatal dietary pattern and autism related outcomes.



The diagram is a representation of the directed acyclic graph presented in Supplementary figure 1. Solid line represents pathways between covariates included in the main analysis. Wide-dashed line indicates potential pathways with parental characteristics adjusted in sensitivity and close-dotted line indicates unmeasured and/or residual confounding.

3.5.5 Inverse probability weighting

The propensity score is the probability of the exposure (Rosenbaum and Rubin, 1983, Rubin, 1972). The true propensity score is unknown in observation studies but can be estimated based on a set of covariates. As the estimated propensity score is a function of the covariates, Rosenbaum and Rubin (Rosenbaum and Rubin, 1983) highlighted that conditioning on the propensity score means the distribution of the covariates are independent across the exposed and unexposed. Hence, differences in the potential outcomes are then attributable to variability in the exposure levels, rather than variability in the covariates that determined them (Austin and Stuart, 2015). Granted, this relies on meeting the assumptions described above and requires a method to balance the probability scores.

The theoretical framework for propensity scores in ordinal exposures was developed by Imbens (Imbens, 2000), and extended to IPW by Robins and colleagues (Robins et al., 2000). The IPW for the average treatment effect are the inverse probability of having the level of adherence to HPDP that was observed, so that there are three sets of weights, one for each level of adherence to HPDP. I chose IPW over other balancing methods because they readily estimate the average treatment effect, limit the need to exclude participants, and can facilitate doubly robust estimation if required (Austin and Stuart, 2015, McCaffrey et al., 2013). Additionally, IPW are readily extended to the controlled direct effects (VanderWeele, 2009) which are measured in Chapter 7. However, all balancing methods have strengths and limitations. For example, IPW may be sensitive to extreme weights and poor covariate balance (Ali et al., 2016).

I estimated the inverse probability weights using entropy balancing to account for imbalance in the covariates between each level of adherence to HPDP. ‘First generation’ methods separate the estimation of propensity scores and balancing method into two stages (Robins et al., 2000). Entropy balancing is a generalisation of propensity scores and combines the covariate balancing constraints into the weighting process (Hainmueller, 2012). Entropy balancing calibrates the weights which best balance the known systematic and random error from the prespecified covariates against a set of base weights which best retain information (Hainmueller, 2012). Advantages over traditional approaches, such as logistic regression, are that extreme weights and poor covariate balance are minimised (Li and Li, 2021). Covariate balance was assessed as described in Chapter 5. I also reviewed the distribution of covariates across each level of HPDP to assess for potential positivity

violation. I then estimated the association between HPDP and each autism related outcome weighted by the IPW to account for potential confounding. Details of the marginal structural model are provided in Chapter 5.

3.6 Mendelian randomisation

MR is an instrumental variable (IV) analysis which utilises genetic instruments as IV. An IV should robustly relate to the exposure and only relate to the outcome through the exposure (Davey Smith and Ebrahim, 2003). General applications of IV have been around since the 1920s (Gagliano Taliun and Evans, 2021), however, MR was popularised following Davey Smith and Ebrahim's seminal article (Davey Smith and Ebrahim, 2003). Based on Mendel's first and second law, the inheritance of genetic variants from parents to children is random at conception (Sanderson et al., 2022). Mendel's first law, the law of segregation, suggests that offspring randomly inherit one allele from each parent's autosomal genome. Mendel's second law, the law of independent assortment, suggests the inherited alleles are independent of each other (with some exceptions) (Davey Smith and Ebrahim, 2003). Genes are also largely fixed at conception and so unaltered by later life exposures. Hence, genetic variants explain the small proportion of the variability of an exposure that is largely independent of socioeconomic and lifestyle confounding structures (Sanderson et al., 2022). Therefore, at a population level MR is less affected by confounding structures that are typically of concern in other observational study designs (Gagliano Taliun and Evans, 2021). A causal association between the exposure-outcome can be indicated through estimating the association between genetic IV and outcome, dependant on satisfying assumptions (Sanderson et al., 2022).

3.6.1 Assumptions

The three core MR assumptions listed below:

1. Relevance: the genetic IV is robustly associated with the exposure.

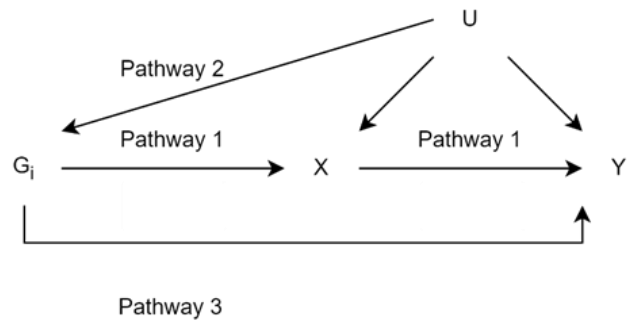
2. Independence: the genetic IV and outcome do not share a common cause (no confounding).

3. Exclusion restriction: the genetic IV is only associated with the outcome through the exposure.

Provided the assumptions are met I can test the presence of a causal association (see Figure 3). Assumption one, relevance, stipulates that the genetic IV is robustly associated with the exposure. The second assumption, independence, means the genetic instrument should not

be related to the confounders of the exposure-outcome relationship (pathway 2). The last assumption, exclusion restriction, means the genetic IV should only relate to the outcome through the exposure (pathway 3). Hence, I may reliably estimate the relationship between the genetic IV and the outcome (pathway 1) when pathways 2 and 3 are closed. The core assumptions for MR, 'conditions for valid instrumental variable', must be met to determine if an association is causal and are the same assumptions required for IV analysis. Yet at least one further assumption is required to derive an effect estimate of the magnitude of association between the exposure and outcome. The conditions are collectively described as 'the point-estimate-identifying conditions' and are an area of debate and methodological development (Sanderson et al., 2022). Furthermore, they all require strong assumptions (Gagliano Taliun and Evans, 2021) and will not be applied in this thesis.

Figure 3. Directed acyclic graph of the three core assumptions of Mendelian randomisation.



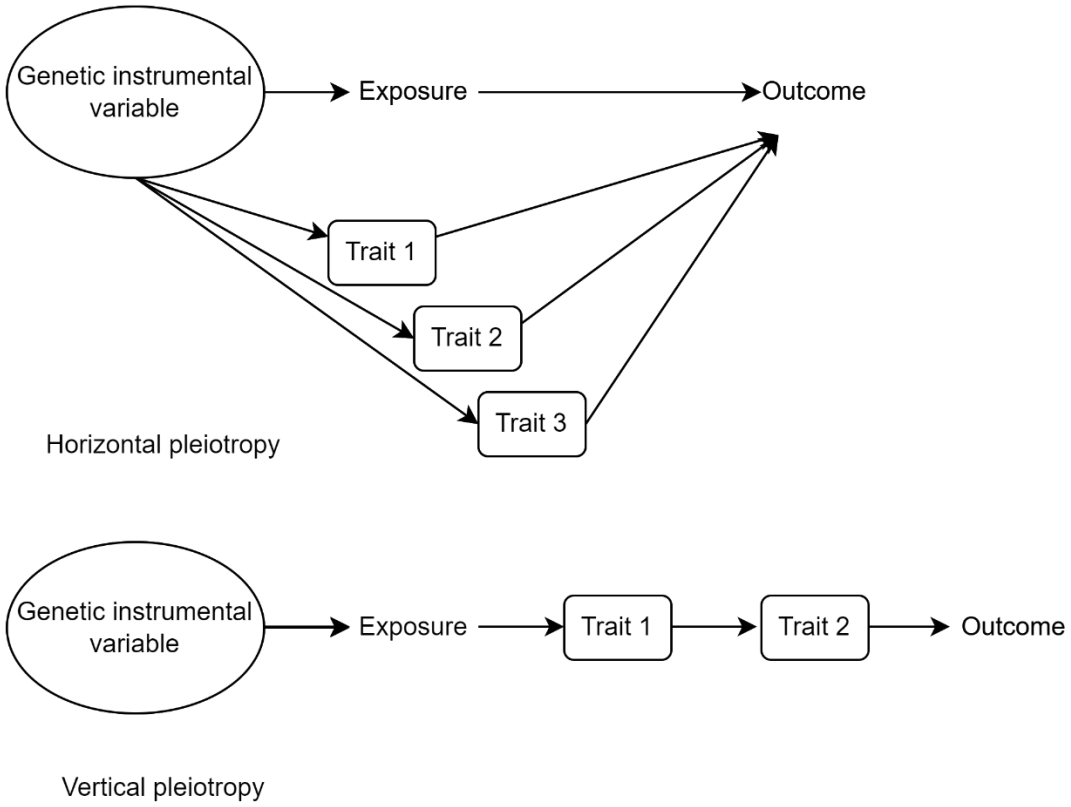
MR requires the genetic IV (G_i) is related to the exposure (X) and has no other pathway to the outcome (Y), except through the exposure (pathway 1). Confounders (U) of the exposure-outcome relationship are independent of the genetic IV. Pathway 3 indicates a violation of the exclusion-restriction assumption.

3.6.2 Horizontal and vertical pleiotropy

Assumptions two and three can be violated through horizontal pleiotropy (Figure 4) (Sanderson et al., 2022). Horizontal pleiotropy occurs when the genetic IV has independent effects on multiple traits. This can lead to an alternative pathway to the outcome and violates the exclusion restriction assumption. Horizontal pleiotropy can also lead to an association with confounders of the exposure-outcome relationship and violate the independence assumption. I tested for horizontal pleiotropy by applying different statistical methods to estimate the genetic IV-outcome association, each of which make different assumptions about horizontal pleiotropy. Horizontal pleiotropy would lead to inconsistent results across the statistical methods. Vertical pleiotropy occurs when the genetic IV

relates to another trait as well as the exposure, but the other trait is on the causal pathway to the outcome and does not invalidate the core MR assumptions.

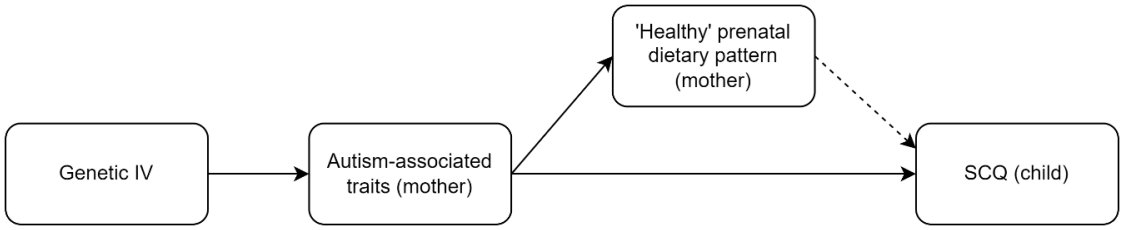
Figure 4. Horizontal and vertical pleiotropy.



3.6.3 Reverse causality

Genetic variants are fixed at conception, yet reverse causality can still occur in MR (Burgess et al., 2021). For example, genes identified in Genome-wide association study (GWAS) of the exposure, may only associate with the exposure because of the outcome. In other words, the outcome caused the exposure, and the genes related to the outcome are then picked-up in the exposure GWAS (Figure 5). I can test for the presence of reverse causality in two-sample MR using Steiger filtering and bidirectional MR. Steiger filtering measures the proportion of variance in the exposure and the outcome that is explained by the genetic IV. It is assumed that valid genetic IV explain more variance in the exposure than the outcome (Hemani et al., 2017). Alternatively, two-sample MR can be reversed by modelling the genetic IV-exposure associations as the outcome and model the genetic variant-outcome associations as the exposure.

Figure 5. Reverse causality in Mendelian randomisation.



IV, instrumental variable: SCQ, social communication questionnaire.
The Genetic IV may associated with a 'healthy' prenatal dietary pattern because of its effect on autism-associated traits in the mother. Subsequently, I can observe an association between maternal genetic IV and child SCQ. Reverse causality can be conceptualised as a form of confounding as presented here. However, because the genetic IV associated with multiple traits there is horizontal pleiotropy. The dashed line indicates a spurious association and the solid line indicates a potential causal pathway.

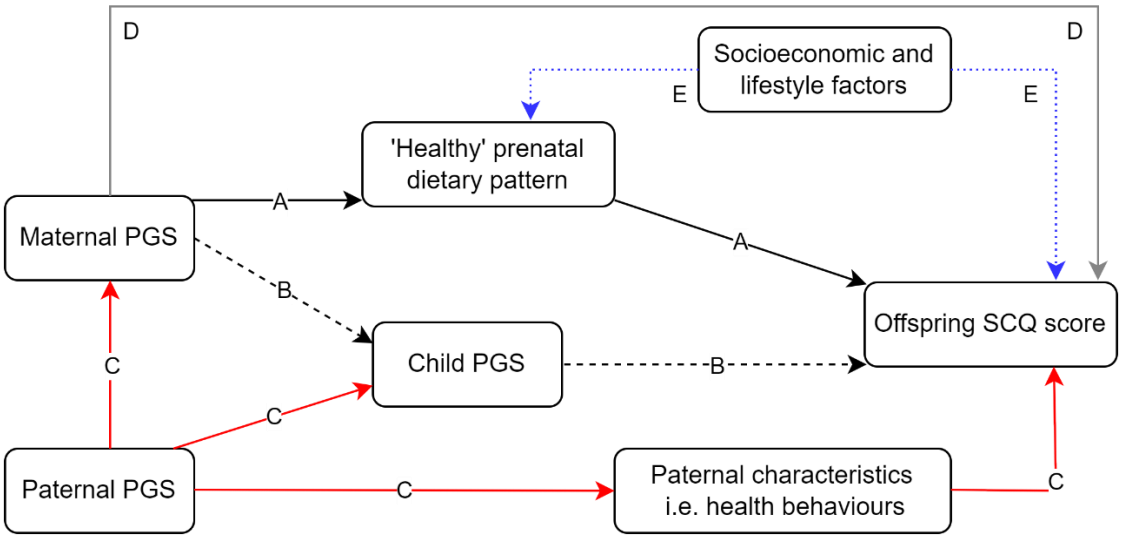
3.6.4 Mendelian randomisation and a 'healthy' prenatal dietary pattern

The application of MR to intrauterine exposures presents unique considerations (Figure 6) (Diemer et al., 2021). The unit of analyses are mother-child pairs, as the exposure is measured in the mother and the outcome in the child. Subsequently, a pathway is open between the mother's genotype and child's outcome, via the child's genotype. This occurs because, on average, 50% of maternal genotype is conferred to the child and so the measured association may be through the child's genotype that is shared with maternal genetic IV (Lawlor et al., 2017). There have been several approaches proposed to address this issue (Evans et al., 2019a, Lawlor et al., 2017), and in this thesis I will adjust for child and paternal genotype. It is necessary to adjust for paternal genotype because adjustment for the child's genotype alone can induce M-bias, a form of collider bias. Collider bias can induce a spurious relationship between the mother and father's genotype (Lawlor et al., 2017).

Two-sample MR measures the genetic variant-exposure association and the genetic variant-outcome association in two non-overlapping samples (Sanderson et al., 2022). One-sample MR measures the associations in the same sample, but external weights can be used to avoid overfitting which inflates the type 1 error rate. A PGS is formed based on external weights and this is used as the genetic IV. In the context of MR, a PGS is a single value estimate of genetic liability of a trait and uses genome-wide significant SNPs only. A PGS can be estimated as the sum of the individuals risk alleles, weighted by the effect estimate for each risk allele obtained from a genome-wide association study (Dudbridge,

2021). Because the weights are obtained from a second sample, the risk of overfitting is minimised in one-sample PGS-MR. In the absence of bias, the association between maternal diet PGS and each outcome implies there is an effect of maternal diet on the child's outcome at some point in the mother's life. Therefore, in MoBa I conducted MR analyses to measure the association between maternal genetic IV for HPDP and offspring SCQ, using data from mother-father-child trios. I used a PGS to genetically predict the likelihood of HPDP based on several genetic variants. Full details of the application of PGS-MR are provided in Chapter 6.

Figure 6. Directed acyclic graph of key potential causal pathways in Mendelian randomisation measuring maternal diet polygenic score on offspring social communication questionnaire score.



The directed acyclic graph (DAG) depicts the key potential pathways in polygenic score Mendelian randomisation (PGS-MR).
Pathway A (solid black line) shows the relationship maternal diet PGS has with a 'healthy' prenatal dietary pattern and potentially relates to offspring social communication questionnaire score (SCQ). This is the pathway I aim to isolate in PGS-MR.
Pathway B (black dashed line) depicts a potential violation of the exclusion restriction criteria, because, on average, 50% of genetic variants used in maternal diet PGS are inherited by offspring. The shared genetic variants may impact offspring SCQ score via the child and hence, I have not isolated pathway A.
Pathway C (solid red line) is potentially opened when I condition on child diet PGS to block pathway B, as child diet PGS is a collider. Hence adjustment can create a spurious relationship between maternal and paternal diet PGS (assumption 3: exclusion restriction). Additionally, assortative mating between maternal and paternal diet PGS could also lead to pathway C (assumption 2: independence).
Pathway D indicates an alternative pathway via horizontal pleiotropy (assumption 3: exclusion restriction)
Pathway E indicates that pathway A should be independent to socioeconomic and lifestyle factors (blue dotted line) that can confound observational studies that directly measure a 'healthy' prenatal dietary pattern (assumption 2: independence).
Pathway B and C can be blocked by adjusting for paternal and child diet PGS, and the other pathways can be assessed through sensitivity analysis.

3.7 Controlled direct effects

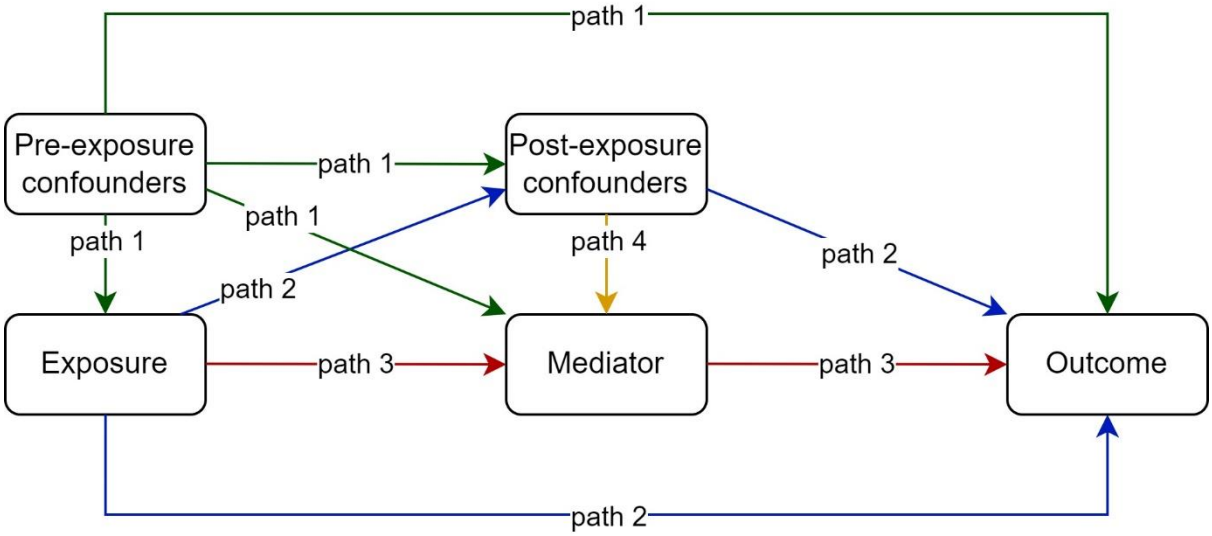
There is now a growing body of literature on causally informed approaches to mediation analyses (VanderWeele, 2016b), of which I applied the controlled direct effect (CDE) (VanderWeele, 2009). Robins & Greenland (Robins and Greenland, 1992) discussed the estimation of direct and indirect effects within a potential outcomes framework and Pearl (Pearl, 2001) later framed one approach as the 'controlled direct effects'. However, it was VanderWeele (VanderWeele, 2009) who proposed the estimation strategy applied in this thesis. This estimation approach applies marginal structural models to estimate the CDE and has advantages over other mediation methods.

The CDE has advantages over traditional mediation methods as it advances the conceptualisation and modelling of mediator-outcome confounders, mediator-outcome confounders that are caused by the exposure, and exposure-mediator interaction. Traditional mediation methods, for example, estimate the exposure-outcome relationship with and without adjustment for the mediator (indirect and direct effects, respectively) (VanderWeele, 2009). The indirect effects refer to the proportion of the exposure-outcome relationship that acts via the mediator, and the remaining alternative exposure-outcome pathway is the direct effect. These combined give the total effect. However, adjustment for the mediator in this way can lead to collider bias and over estimation of the indirect effect (Richiardi et al., 2013).

To elaborate, I first define exposure-outcome confounders and mediator-outcome confounders (a detailed explanation can be found accompanying Figure 7 in the caption). Exposure-outcome confounders are a common cause of the exposure, potentially the mediator, and the outcome. Mediator-outcome confounders are a common cause of the mediator and the outcome and may be caused by the exposure or exposure-outcome confounders (VanderWeele, 2009). In traditional mediation analyses, the presence of unmeasured or residual mediator-outcome confounders can induce collider bias due to conditioning on the mediator (collider). However, adjustment for mediator-outcome confounders may over adjust the indirect effects if mediator-outcome confounders are caused by the exposure. This is because part of the exposure-outcome relationship is via the mediator-outcome confounders (intermediate confounders), and therefore part of the desired effect (Richiardi et al., 2013). The CDE allows us to conceptualises and model the pre-exposure-outcome and pre-exposure-outcome confounders separately (see Figure 8 for

covariates used in this thesis), which overcomes the aforementioned sources of biases (VanderWeele, 2009).

Figure 7. Directed acyclic graph of pre-exposure outcome and post-exposure outcome confounders.



The directed acyclic graph is a simplification of key pathways to be considered when estimating the controlled direct effects.
I firstly estimated the total effects of the exposure-outcome relationship. Hence, I block confounders of the exposure-outcome but leave open the pathways from the exposure to the outcome. The total effects can be estimated when pathway 1 (green) is blocked but the remaining pathways from the exposure-outcome are open which are pathway 2 (blue), pathway 3 (red), and pathway 4 (yellow).
The controlled direct effects may be estimated when the exposure-outcome pathways are open, including the exposure-outcome pathway that is via the post-exposure confounders (pathway 2 (blue)). As with the total effects, I block the pre-exposure confounder pathway (pathway 1 (green)). However, in the controlled direct effects I also block the proportion of the exposure-outcome that is directly via the mediator (pathway 3 (red)) and indirectly via the mediator through the post-exposure confounders (pathway 4 (yellow)). The controlled direct effects separate the exposure/post-exposure confounders/outcome pathway that is part of the effect, from the exposure/post-exposure confounders/mediator/outcome pathway and not part of the effect.

A final consideration is exposure-mediator interaction which creates variable exposure-outcome estimates depending on the level of the mediator (Richiardi et al., 2013). In the presence of exposure-mediator interaction, CDE can even have advantages over other causally informed mediation analysis, such as the natural direct and indirect effects. CDE have relatively weaker assumptions, yet maybe of greater relevance to public health (Naimi et al., 2014). The CDE sets the mediator to a fixed value which reflects a hypothetical ‘intervention’ and models the exposure-mediator interaction, whereas the natural direct and indirect effects do not. Hence, in the presence of an exposure-mediator interaction, there is a distinction between the CDE and the natural direct effects, otherwise they are equivalent. As such, the proportion eliminated better reflects the observed change

I may achieve through 'intervention' and may be more relevant to public health (Naimi et al., 2014). Due to the exposure-mediator interaction, the CDE compared to the total effects (TE) estimates the 'proportion eliminated' rather than the proportion mediated (VanderWeele, 2009). Notably, the CDE is described in the literature as a causally informed mediation analysis, although strictly speaking it measures the proportion eliminated rather than mediated. Thus, in Chapter 7, the CDE reflects the TE of socioeconomic deprivation on autism diagnosis and autism-associated traits when the proportion estimated to be attributable to HPDP is eliminated.

3.7.1 Assumptions

Estimation of the CDE relies on the following identifiability assumptions.

Assumption 1. No exposure-outcome confounding

> Implies that conditional on a set of covariates, there is no unmeasured confounding of the exposure-outcome relationship.

Assumption 2. No mediator-outcome confounding

> Implies that conditional on a set of covariates, there is no unmeasured confounding of the mediator-outcome relationship.

3.7.2 Estimation of the controlled direct effects

Two sets of weights were initially estimated and the average used in the CDE model (Equation 2). The weights are calculated as per VanderWeele (VanderWeele, 2009). The first set of IPW were calculated as the probability of each maternal socioeconomic indicator divided by the probability of each maternal socioeconomic indicator, conditional on exposure-outcome confounders, denoted as W^A in Equation 2. These IPW were used in the marginal structural model to estimate the TE of maternal socioeconomic indicators on each outcome. The prenatal diet weights are calculated by dividing the probability of HPDP by the probability of HPDP, conditional on the exposure, and exposure- and mediator-outcome confounders, denoted as W^Z in Equation 2. The average IPW is W^A multiplied by W^Z and adjusts for the pre-exposure and post-exposure confounders without blocking the exposure-outcome path via the mediator-outcome confounders.

Equation 2. Estimation of the inverse probability weights

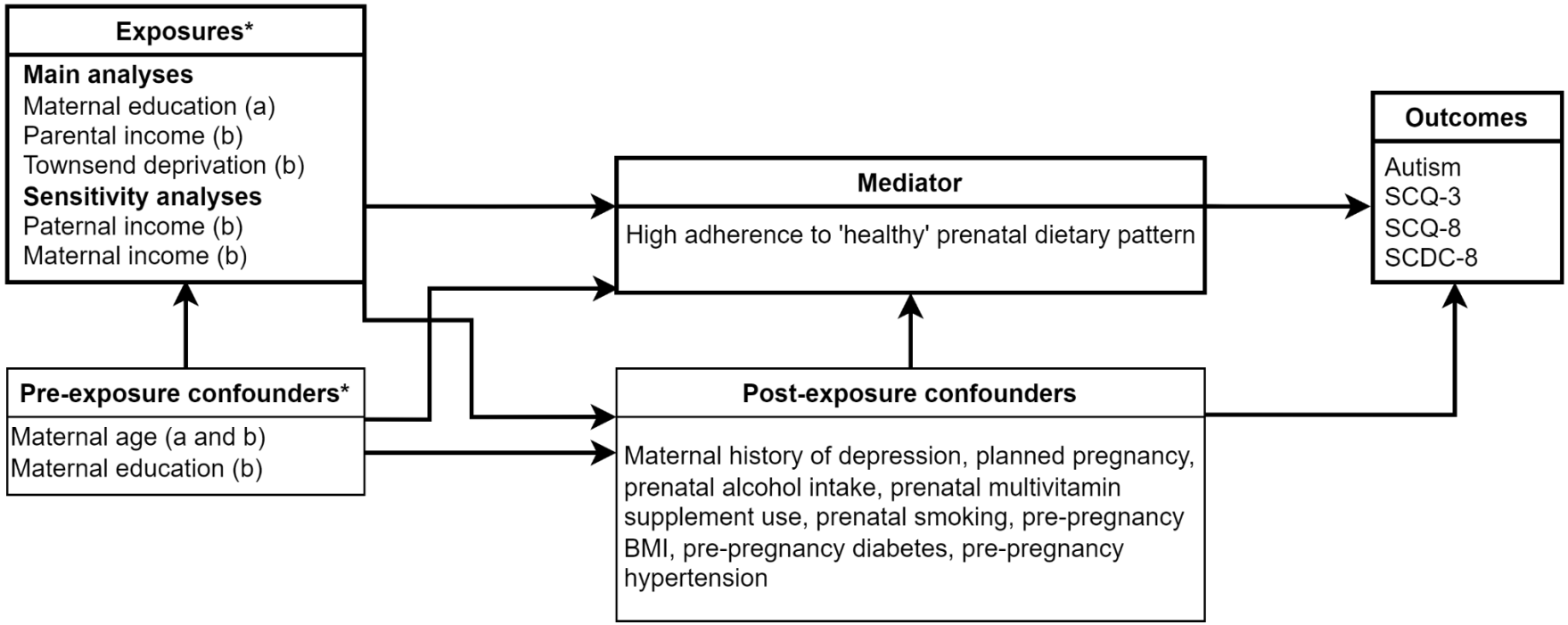
$$W^A = P(A)/P(A|X)$$

Multiplied by

$$W^Z = P(Z \mid A)/P(Z \mid A, X, W)$$

P, probability; Z, mediator; A exposure; X, exposure-outcome confounder(s); W, mediator-exposure confounders.
The exposure inverse probability weights (W^A) are calculated as the probability of the exposure divided by the probability of the exposure conditional on the exposure-outcome confounders. The mediator inverse probability weights (W^Z) are calculated as the probability of the mediator conditional on the exposure, divided by the probability of the mediator conditional on the exposure, exposure-outcome confounders, and mediator-outcome confounders. The average weights are calculated by multiplying the W^A and W^Z.

Figure 8. Directed acyclic graph of the potential causal pathways relating to the total effects and controlled direct effect of maternal socioeconomic indicators and autism diagnosis and autism-associated traits.



*The exposure IPW estimation included the pre-exposure confounders. In model a, exposure - maternal education was adjusted for maternal age, and in models b, each socioeconomic indicator (parental income and Townsend deprivation) was adjusted for both maternal age and maternal education as I conceptualised maternal education to be a cause of parental income and Townsend deprivation.
The mediator IPW estimation included all pre-exposure confounders, the exposure, post-exposure confounders.

3.8 Triangulation

Triangulation is a term used to describe the comparison of results that address a broadly similar question but use different approaches with unrelated key sources of bias (Lawlor et al., 2016). Triangulation is used in many scientific fields, however discussion of triangulation here is in the context of aetiological epidemiology.

Causal reasoning can be strengthened through triangulation when coupled with critical evaluation of the strength of evidence in the context of potential biases (Lawlor et al., 2016, Pearce and Lawlor, 2017, Vandenbroucke et al., 2016). Triangulation may 'test' the consistency of results given different and ideally unrelated key sources of bias. The premise of this idea is that consistency across approaches may reflect a true association because different and unrelated sources of bias create inconsistent results. However, bias in the same direction can lead to consistent results and should be considered. Hence, triangulation is unlikely to confirm a causal association on its own but can be a useful strategy to enhance our critical evaluation of the strength of evidence (Lawlor et al., 2016).

In this thesis I will triangulate using the following three approaches: marginal structural models (Chapter 5), cross context comparison of MoBa and ALSPAC (Chapter 5), and Mendelian randomisation (Chapter 6). The major sources of bias and the expected direction of bias were considered when selecting the approaches and can be viewed in Table 3.

Table 3. Key sources of bias and expected direction of bias in the marginal structural model, cross context comparison, and Mendelian randomisation.

Approach	Rationale	Key potential source of limitations and assumed direction	Exposure window
Marginal structural model			
Statistical approach to measure association between HPDP and autism diagnosis and autism-associated traits.	Ideally, the results obtained are unaffected by confounding, selection bias, and systematic error in measured exposures and outcomes.	Unmeasured or residual confounding by socioeconomic and lifestyle characteristics. May exaggerate any potential causal association. Selection bias due to self-selected study participation. May attenuate any potential causal association, but if extreme the direction of association can reverse. Measurement error in prenatal dietary pattern. Random and systematic error may bias towards the null. Measurement error in each outcome. Direction of bias is likely to vary.	MoBa – prenatal dietary intake was measured around 22-weeks' gestation and reflected diet since conception. ALSPAC – prenatal dietary intake was measured around 32-weeks' gestation and the time frame questions related to was unspecified.
Cross context comparison			
Compared the association between social communication difficulties and HPDP, cross MoBa and ALSPAC.	Comparisons are made between two populations that may have different key sources of bias to 'test' the consistency of results. Differences in country and time period. MoBa, Norway based cohort recruited 2002 – 2008. ALSPAC, English based cohort recruited 1990 – 1992.	Same limitations as in the individual analysis described above. Cross-context comparisons may have bias is in the same direction. For example, confounding bias, selection bias, measurement error in exposure and outcome.	There are differences in the exposure period between each cohort as described above.

Polygenic score-Mendelian randomisation			
In MoBa, the association between maternal PGS for HPDP and each SCQ outcome was measured.	Ideally, bias may be less affected by socioeconomic and lifestyle characteristics.	Confounding through for example assortative mating, population stratification, may exaggerate any potential causal association. Horizontal pleiotropy may bias in either direction. Weak instrument bias can exaggerate potential causal association. Selection bias may occur through self-selected recruitment and retention, restriction to mother-father-child trios that are Caucasian European populations and have genotyped data. May attenuate any potentially causal association, but if extreme the direction of association can reverse. Measurement error of prenatal dietary pattern. Random error is more likely and may bias towards the null. Measurement error of each outcome Direction of bias is likely to vary.	Maternal PGS for HPDP may reflect any point in time.

ALSPAC, Avon Longitudinal Study of Parents and Children; HPDP, 'healthy' prenatal dietary pattern; MoBa, Norwegian Mother, Father, and Child Cohort; PGS, polygenic score; SCQ, Social communication questionnaire.
Potential sources of bias are focused only on key sources of bias and discussed at a high level, and the potential direction of bias is assumed. Fuller discussion of potential bias and impact on the measured associations from the thesis are discussed in each Chapters 4-7 and the thesis discussion (Chapter 7).
If there is a true causal association, a high adherence to HPDP is expected to lower the likelihood of autism diagnosis and autism-associated traits. Hence, reference to bias exaggerating the potential causal association would further reduce the likelihood of autism diagnosis and autism-associated traits. Bias that reverses the direction of association would lead to an increased likelihood of autism diagnosis or autism-associated traits in association with high adherence to HPDP.

4 Chapter 4. Prenatal Vitamins and the likelihood of Offspring Autism Spectrum Disorder: Systematic Review and Meta-Analysis

Chapter 4 takes the form of the article 'Prenatal Vitamins and the Risk of Offspring Autism Spectrum Disorder: systematic review and meta-analysis. The article was published in July 2021 in the peer reviewed academic journal, Nutrients. It is reproduced here under the terms of a Creative Commons CC BY licence. In comparison to the published article, the supplementary information has been integrated into the main body of the paper. The language has been modified to be more inclusive and sensitive to the preferences of the autistic community and formatting and terminology such as 'alternative approaches' has been changed to 'causally informed approaches' for consistency across the thesis.

Friel C[1], Leyland AH[1], Anderson JJ[2], Havdahl A[3,4,5], Borge T[6], Shimonovich M[1], Dundas R[1]. Prenatal Vitamins and the Risk of Offspring Autism Spectrum Disorder: Systematic Review and Meta-Analysis. Nutrients. 2021 Jul 26;13(8):2558. doi: 10.3390/nu13082558.

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Declarations

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Conflict of interests

The authors declare they have no conflicts of interests.

4.1 Abstract

Background

Prenatal nutrition is associated with offspring autism spectrum disorder (herein referred to as autism), yet it remains unknown if the association is causal. Triangulation may improve causal inference by integrating the results of conventional multivariate regression with several alternative approaches that have unrelated sources of bias. We systematically reviewed the literature on the relationship between prenatal multivitamin supplements and offspring autism diagnosis, and evidence for the causally informed approaches applied.

Methods

Six databases were searched up to 8 June 2020, by which time we had screened 1309 titles/abstracts and retained 12 articles. Quality assessment was guided using Newcastle–Ottawa in individual studies, and the Grading of Recommendations Assessment, Development and Evaluation (GRADE) for the body of evidence. The effect estimates from multivariate regression were meta-analysed in a random effects model and causally informed approaches were narratively synthesised.

Results

The meta-analysis of prenatal multivitamin supplements involved 904,947 children (8159 cases), and the overall analysis showed no robust association with offspring autism diagnosis: however, a reduced likelihood was observed in the subgroup of high-quality observational studies (RR 0.77, ((95% CI) 0.62, 0.96), I^2 = 62.4%), early pregnancy (RR 0.76, (0.58; 0.99), I^2 = 79.8%) and prospective studies (RR 0.69, (0.48, 1.00), I^2 = 95.9%).

Conclusion

The quality of evidence was very low, and triangulation was of limited utility because causally informed approaches methods were used infrequently and often not robustly applied.

4.2 Introduction

Autism Spectrum Disorder, hereafter referred to as autism, is a neurodevelopmental condition characterised by early-onset impairment in social communication and restricted and repetitive behaviours (World Health Organisation, 2020). Prenatal nutrition may be a modifiable factor, which creates a potential target for prevention strategies and may reduce the significant public health implications of this condition. Autism is estimated to cost £27 billion (€29.8 billion) annually in the UK due to health, education, social care and lost productivity (Knapp et al., 2009), despite the modest prevalence of around 1.5% (Lyall et al., 2017).

Previous systematic reviews and meta-analysis reported a reduced likelihood of autism in offspring in association with prenatal folic acid and/or multivitamins supplements (Guo et al., 2019a, Li et al., 2019). Yet, as the conventional rhetoric states, 'correlation does not imply causation'. What conditions are necessary to estimate causality are greatly debated. Although randomised controlled trials are the gold-standard to estimate causality, their utility in nutritional epidemiology can be limited due to ethical, financial, and practical barriers. For example, as the prevalence of autism diagnosis is only 1.5%, a large sample size is necessary for adequate statistical power but financially burdensome (Kendall, 2003). Conversely, causal inference from non-experimental studies is problematic, largely due to bias, especially confounding (Lawlor et al., 2016). Confounders cause the exposure and outcome and can create a spurious association. Moreover, individuals may overreport compliance with prenatal nutritional supplements, leading to misclassification bias.

Aetiological triangulation recognises that all approaches have bias and then exploits unrelated sources of bias to 'test' the consistency of results (Lawlor et al., 2016). Causal inferences are strengthened if we have consistent results across multiple approaches with different sources of bias. Bias may vary across different study designs, methods, and analytical approaches. Some examples include conventional multivariate regression, gene-nutrient interaction studies, discordant sibling studies, cross context comparisons and negative controls (see Lawlor et al for an overview of approaches in triangulation (Lawlor et al., 2016)). We use the term 'causally informed approaches" to refer to the approaches mentioned above that are alternative to conventional multivariate regression and can be used in a triangulation framework.

Evidence on causally informed approaches is unsystematically synthesised in previous reviews, if at all, which limits transparency. As causally informed approaches are increasingly applied within studies and may significantly alter our causal reasoning, this evidence should be integrated in an explicit and scientifically rigorous process. This aligns with guidance from Cochrane (Higgins et al., 2019) and a recent guideline on aetiological systematic reviews of observational studies (Dekkers et al., 2019). We firstly reviewed the overall evidence from studies using conventional multivariate regression to investigate the association between prenatal nutritional status and autism diagnosis in offspring. Secondly, we narratively synthesised the causally informed approaches applied. Lastly, we updated the search and addressed limitations of previous reviews such as double counting individual studies (Guo et al., 2019a), and/or use of the DerSimonian Laird estimator which can underestimate uncertainty (Guo et al., 2019a, Li et al., 2019).

4.3 Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses were followed (Moher et al., 2010). The review protocol was registered on the Prospective Register of Systematic Reviews, accessed at: https://www.crd.york.ac.uk/prospero, registration number: CRD42019154613.

4.3.1 Inclusion and exclusion criteria

Included study designs were: trials, cohort, case-control and cross-sectional studies of any duration. We focused on folic acid/multivitamin supplement use in women during preconception and prenatal periods because folic acid is generally sourced from a multivitamin supplement (Li et al., 2019). We herein refer to this simply as a prenatal multivitamin supplement. Comparators were high versus low intakes or no supplement intakes. The outcome was offspring autism diagnosis based recognised criteria such as the DSM, ICD, and from health registers. There were no date limitations, but non-English language and animal studies were excluded.

4.3.2 Study identification and selection

The search strategy and selection of databases were guided by an information scientist with expertise in systematic review. Search strategies were adapted to each database. The following databases were searched from the earliest date to 08/06/2020; MEDLINE (OVID), EMBASE (OVID), PsycINFO (EBSCO), Web of Science core collection, Open Grey, and BioRix. See Table 4 for MEDLINE search strategy.

Table 4. Search strategy for MEDLINE (OVID).

Search string	Search terms
Population	(Pregnancy OR Fetal development OR Prenatal).af. OR Fetal development/ OR "f?etal development".af. OR "f?etal programming".af OR "f?etal programing".af
Exposure	NUTRITIONAL PHYSIOLOGICAL PHENOMENA OR Prenatal Nutritional Physiological Phenomena/ OR Maternal nutritional physiological phenomena/ OR DIET OR nutri* OR vitamin OR diet* OR mineral OR Nutritional Status
Outcome	ASPERGER SYNDROME/ OR Autism/ OR Autistic disorder/ OR Autism Spectrum Disorder/ OR "pervasive developmental disorder*".mp.

Titles and abstracts were screened, full text articles reviewed, and quality assessment were completed twice for each study, independently by CF, TB, and MS. Disagreements were resolved through discussion and adjudicated, by JA. Data were extracted by CF using a standardised form comprised of, author, year of publication, country and cohort, study design, sample size, age of participants, nutritional indicator, measure of autism diagnosis, covariates, results, and causally informed approach.

4.3.3 Quality assessment

The Newcastle Ottawa Scale guided quality assessment of each observational study. Scores range from 0-9, a score of 7-9 was considered high quality in the subgroup analysis and is consistent with similar previous reviews (Guo et al., 2019a, Wang et al., 2017). GRADE approach was used to rate the body of evidence based on the degree of certainty in the result observed (Higgins et al., 2019).

4.3.4 Data synthesis and analysis

We narratively synthesised studies that were inappropriate to meta-analyse. Additionally, we summarised the causally informed approaches applied in table format.

4.3.5 Meta-analysis

We meta-analysed the fully adjusted effect estimates in a random effects model using the Hartung-Knapp-Sidik-Jonkman estimator (Higgins et al., 2019). Analyses of nutritional supplements were pooled if the exposure was categorical, with no or low supplement intakes as the reference category compared against supplement use. Autism is a rare outcome and so we assumed the odds ratios (OR) and hazard ratio (HR) were directly comparable to relative risks (RR) (McKenzie and Thomas, 2020). The heterogeneity was measured with the Cochrane's Q-statistic and I^2 statistics. The interpretation of heterogeneity (I^2) was guided by Cochrane's reference ranges (Higgins et al., 2019). Prediction intervals estimate the range of effect estimates that may be expected in individual settings which could improve the application of research findings. They are distinct from the summary effect and 95% confidence intervals (CI) which estimate the average effect of the exposure (IntHout et al., 2016, Riley et al., 2011) (Higgins et al., 2019). The R version 3.6.3 packages used were 'meta' and 'forestplots' (Gordon and Lumley, 2020). Statistical tests of significance were 2-sided with α of 0.05.

4.3.6 Sensitivity analysis

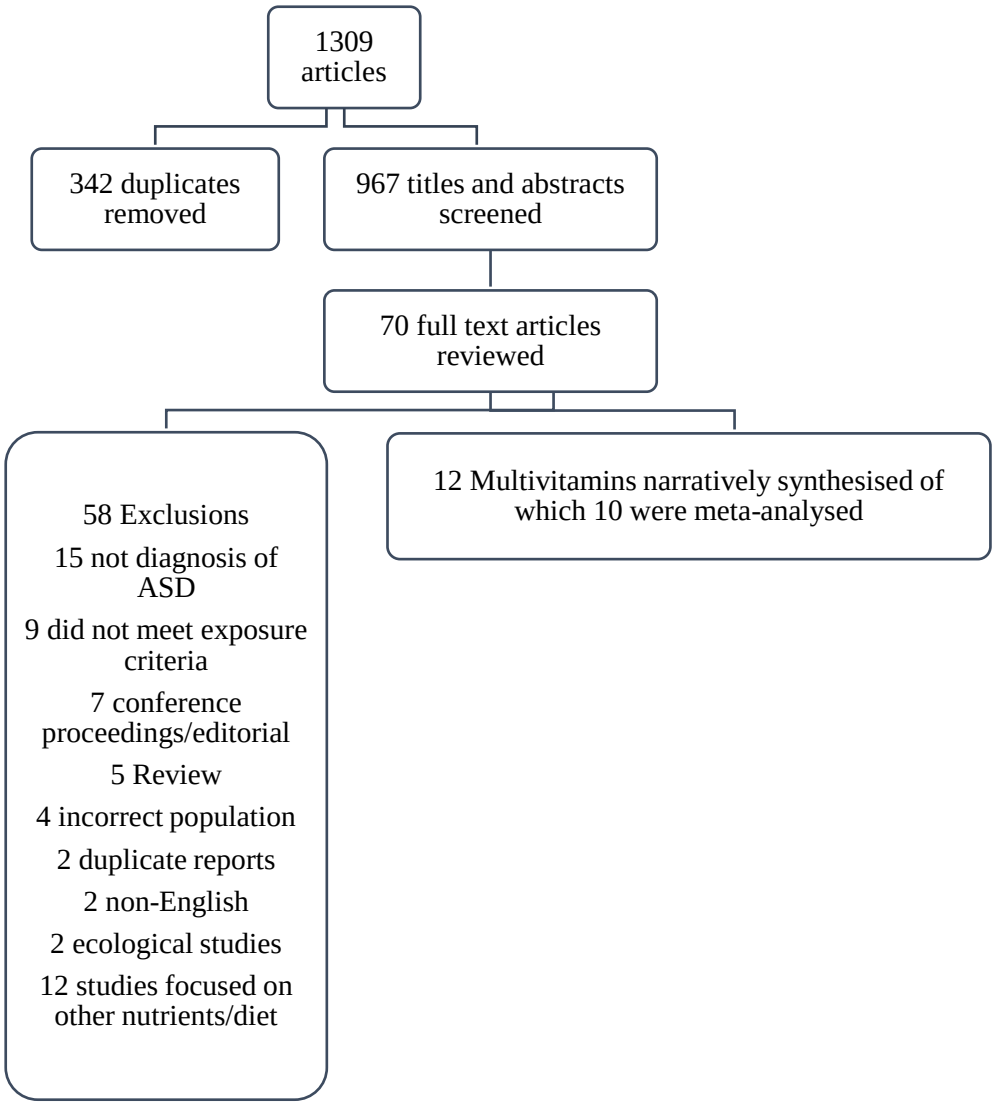
Sources of heterogeneity were explored through identification of outliers, leave-one-out analysis, and subgroup analysis if there were ≥10 studies. A random effects model was used to estimate between and within subgroup effects. Pre-defined subgroups were: study quality, study design (prospective/retrospective), region, mandatory fortification (yes/no), stage of pregnancy defined as early pregnancy (first trimester) and any point in pregnancy if the exposure period was undefined or after the first trimester. Between subgroup differences were measured with Q statistic and significance indicated by p <0.1 (2-sided). Small study publication bias was assessed through inspection of funnel plot and Egger's test (Duval and Tweedie, 2000). The DerSimonian-Laird estimator is widely used yet may underestimate uncertainty (Higgins et al., 2019). To facilitate comparison to previous research we applied the DerSimonian-Laird estimator in sensitivity analyses.

4.4 Results

4.4.1 Identification of studies

A total of 1309 titles were identified with 342 duplicates, leaving 967 titles and abstracts to be screened (Figure 9). 897 titles were excluded based on title and abstract review, leaving 70 titles for full text review, of which 13 met inclusion criteria. However, two reports were duplicated (Strom et al., 2018, Virk et al., 2016), and so the larger cohort was retained (Strom et al., 2018) leaving 12 studies in the final review, 10 of which were meta-analysed. The other two were narratively synthesised as the reference category was not low or no supplement use (Table 5) (Raghavan et al., 2018, Tan et al., 2020).

Figure 9. PRISMA flow chart of study selection.



ASD, Autism spectrum disorder; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Table 5. Study characteristics and results from individual studies.

Author/ study design	Country/ cohort/ sample	Exposure measure(s)	Outcome measure	Covariates[a]	Results Effect size estimate and 95% CI
Desoto & Hitlan (Desoto and Hitlan, 2012)/ case-control.	USA 256 cases and 752 controls, age range 6-12 years	Self-reported folic acid or multivitamin supplement use obtained from health records.	ICD-9	Child: anaemia, pica, gender, birth weight, birth order, year of birth, breast feeding, gender and folic acid interaction. Maternal; age, poverty ratio, adequacy of prenatal care, cholesterol screen, pap smear, prenatal alcohol use, prenatal viral infections, lead exposure.	Reference, no supplement use Folic acid/multivitamin HR 2.34 1.14, 4.82
DeVilbiss et al (DeVilbiss et al., 2017)/ prospective cohort & sibling case control.	Sweden/Stockholm youth cohort 273,107/1064 cases age range 4-15 years	Self-reported multivitamin supplement use recorded by midwife at first booking.	DSM-IV or ICD-10	Child: sex, birth year, and years of residence in Stockholm County. Maternal: country of birth, maternal education, disposable family income, age, parity, smoking, BMI at first antenatal visit, neurologic or psychiatric conditions before the child's birth (anxiety disorders, autism, bipolar disorder, depression, intellectual disability, non-affective psychosis, stress related disorders, epilepsy), antiepileptics and antidepressants medication. Sibling analysis child sex and birth year, maternal parity.	Reference, no supplement use Multivitamin OR 0.89 0.82, 0.97 Sibling analysis, multivitamin OR 0.95 0.81, 1.13 Propensity score matching OR 0.86 0.78, 0.95

Levine et al (Levine et al., 2018)/ nested case-control	Israel/ Meuhedet population based register. 572 cases and 45,300 controls average age 10 years (SD 1.4).	Pharmacy records, Multivitamins and/or folic acid supplements.	ICD-9	Child: sex, birth year. Maternal: parity, socioeconomic status (high vs low), psychiatric diagnosis by childbirth, age. Paternal: psychiatric diagnosis, age.	Reference, no supplement use	
					F/multivitamins	
					RR 0.27	0.22, 0.33
					Negative control	
					Reference, no supplement use	
					Multivitamin use 2 years prior to pregnancy	
					0.12	0.07, 0.20
Li et al (Li et al., 2018)/case control	China/ Autism clinical and environmental database. 354 cases and 374 controls, average age 4.5 years.	Self-reported folic acid supplements.	DSM-IV-TR	Child: age, premature birth, gender. Maternal: pre-pregnancy BMI preconception and predelivery, mode of delivery. Parental: age, education Dietary patterns were additionally adjusted for other dietary patterns. Folic acid supplements were additionally adjusted for other supplements.	Reference, no supplement use	
					Folic acid	
					OR 0.64	0.41, 1.00
Moser et al (Moser et al., 2019)/ nested case-control	Israeli/ Maccabi Healthcare Services. 2009 cases and 19,886	Dispensing records for folic acid with or without multivitamins supplements.	DSM, version unreported	Child: sex, birth year, birth order. Maternal: age, region of residence, poverty index, number of physician visits, diabetes mellitus, hypertension, cardiovascular disease, cancer, subfertility, epilepsy, antifolate	Reference, ≤0.2mg/day	
					Folic acid supplement	
					0.2-<0.4mg/day	
					OR 1.15	0.98, 1.24
					0.4 - <1mg/day	
					OR 1.10	0.98, 1.24
					1 <3mg/day	

	controls.			medication (proguanil, methotrexate, sulfasalazine, sulphamethoxazole and trimethoprim, pyrimethamine, valproate, carbamazepine, phenytoin and phenobarbital). Final model adjusted for; maternal age, subfertility, number of physician visits, birth order, parity.	OR 1.14	0.98, 1.34
					≥3mg/day	
					OR 1.01	0.60, 1.70
Raghavan et al (Raghavan et al., 2018) / prospective cohort.	USA/Boston Medical Centre. 1257/86 cases, aged up to 9 year.	Self-reported maternal multivitamin supplement use.	ICD-9	Child; sex, gestational age birth year. Maternal; homocysteine, race, age, smoking status, diabetes, reduction, parity, MTHFR 677 genotype, BMI.	Reference, multivitamin 2 – 5 times/week	
					First trimester ≤2/week	
					HR 3.4	1.6, 7.2
					>5/week	
					HR 2.3	1.2, 3.9
					Second trimester ≤2/week	
					HR 3.8	1.8, 8.0
					>5/week	
					HR 2.1	1.2, 3.6
					Third trimester	
					≤2/week	
					HR 3.5	1.7, 7.4
					>5/week	
					HR 2.1	1.2, 3.6
Schmidt et al, 2019 (Schmidt et al., 2019a)/ prospective cohort	USA/Markers of Autism Risk in Babies: Learning Early Signs	Self-reported multivitamin supplement use obtained through three telephone interviews.	Autism Diagnostic Observation Schedule and DSM-5	Child: birthplace, sex, year of birth. Maternal: education, age, pre-pregnancy BMI, planned pregnancy, race/ethnicity, home ownership, insurance delivery type.	Reference, no supplement use	
					Multivitamin	
					RR 0.50	0.30, 0.81

	332/55 cases, average age 36.5 months			Paternal: age. Covariates in final model, maternal characteristics (education, age, insurance delivery type) and child characteristics (race).		
Schmidt et al 2012 (Schmidt et al., 2012)/ case control.	USA/Childhood autism risks from genetics and environment 429 cases and 278 controls age range 24-60 months	Self-reported obtained folic acid intake based on supplements, including multivitamin supplements, and fortified breakfast cereals, shakes and bars obtained via telephone interview.	Health records and Autism Diagnostic Interview–Revised and the Autism Diagnostic Observation Schedule–Generic.	Child: birth year, sex, race. Maternal: race, age, education, pre-pregnancy BMI, birthplace, residing with a smoker, smoking status, alcohol consumption, other nutrients intakes (calcium, iron, vitamin A, B_6, B_{12}, C, D and E). Preeclampsia, type of delivery, vaginal bleeding during pregnancy, induced labour. Paternal: age Covariates in final model; childbirth year and maternal education.	Reference, no supplement use	
					All strata combined. Folic acid/multivitamin	
					OR 0.62	0.42, 0.92
					Strata by C/T genotypes	
					maternal CC	
					OR 1.20	0.61, 2.34
					maternal CT/TT	
					OR 0.46	0.25, 0.85
					child CC	
					OR 1.15	0.55, 2.38
					child CT/TT	
					OR 0.48	0.27, 0.88
					both mother & child	
					OR 1.29	0.54, 3.10
					either mother or child CT/TT	
					OR 0.49	0.16, 1.50
					both mother and child CT/TT	
					OR 0.30	0.10, 0.90

Suren et al, 2013 (Suren et al., 2013)/prospective cohort	Norway/ Norwegian mother, father, and child cohort (MoBa) 85176/114 cases, mean age of 6.4 years.	Self-reported questionnaire responses, multivitamin and mineral supplement, folic acid supplements.	DSM-IV or ICD-10	Child: birth year Maternal: planned pregnancy, smoking, BMI, parity, weight gain at 18 and 30 weeks. Parental: education, age. Covariates retained in final model: birthyear, parity, and maternal education.	Reference, no supplement use	
					Folic acid/multivitamins	
					OR 0.61	0.41, 0.90
					Negative control	
					Reference, no fish oils supplements	
					Fish oil supplements	
					OR 1.29	0.88, 1.89
Strom et al, 2017 (Strom et al., 2018)/ prospective cohort	Denmark/Danish National Birth Cohort 87210/ 1234 cases, age range 11-17 years	Self-reported folic acid, vitamin and mineral supplement use, reported during GP interview.	ICD-10	Child: sex. Maternal: age, parity, smoking, education, socioeconomic status (based on occupation and education), planned pregnancy, pre-pregnancy BMI. Paternal: age.	Reference, no supplement use	
					Folic acid/multivitamins	
					HR 1.06	0.94, 1.19
Tan et al 2020 (Tan et al., 2020)/ case-control	China 416 cases and 201 control mean age 4.68 years cases, 4.47 years controls.	Self-reported folic acid and/or micronutrient supplements.	DSM-5	Child: age, sex, gestational age, birth weight. Maternal: residence (rural/urban), labour mode. Paternal: age. Household: income.	Reference, supplement use	
					No folic acid supplements	
					1.91	1.24, 2.93
					No micronutrient supplements	
					1.72	1.20, 2.47
Nilsen et al, 2013 (Nilsen et al., 2013)/	Norway/Norwegian registry 507, 856/2072 cases,	Self-reported folic acid intake obtained via maternal health	ICD-10	Child; birth year Maternal; age, marital status, parity, hospital size.	Reference, no supplement use	
					Folic acid	
					OR 0.86	0.78, 0.95

prospective cohort	mean age 7 years.	records.	Paternal; age

[a]All covariates initially considered relevant by the authors are listed as well as the final selection.
Acronyms: BMI, Body Mass Index; DSM, Diagnostic and Statistical Manual of Mental Disorders; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders Text Revision; GDM, gestational diabetes mellitus; GWG, gestational weight gain; ICD, International Classification of Disease; SD standard deviation; MTHFR 677, Methlyenetetrahydrofolate reductase 677 genotype; CT/TT/CC, are variants of the genotype; Q, Quintile; SNP, single-nucleotide polymorphism; USA, United States of America

4.4.2 Quality assessment

Based on Newcastle Ottawa Scale, five (DeVilbiss et al., 2017, Nilsen et al., 2013, Raghavan et al., 2018, Schmidt et al., 2019a, Suren et al., 2013) of seven cohort studies (DeVilbiss et al., 2017, Levine et al., 2018, Nilsen R.M. et al., 2010, Raghavan et al., 2018, Schmidt et al., 2019a, Strom et al., 2018, Suren et al., 2013) were of high quality, and two (Moser et al., 2019, Schmidt et al., 2012) of five case-control studies (Desoto and Hitlan, 2012, Li et al., 2018, Moser et al., 2019, Schmidt et al., 2012, Tan et al., 2020) were of high quality (Table 6-7). The quality of the body of evidence based on GRADE was very low. The details for the evidence profile and rationale for GRADE rating are provided in Table 8-9.

Table 6. Quality assessment using the Newcastle Ottawa Scale: case-control.

Authors	Selection	Comparability	Exposure	Total
DeSoto & Hitlan 2013	2	1	1	4
Li et al 2018	2	1	1	4
Moser et al 2019	4	2	2	8
Schmidt et al 2012	3	2	2	7
Tan et al 2020	2	1	1	4

Scores ranged from 0-9, where 9 indicates the highest quality.

Table 7. Quality assessment using the Newcastle Ottawa Scale: cohort.

Authors	Selection	Comparability	Outcome	Total
DeVilbiss et al 2017	3	2	3	8
Levine et al 2018	3	2	1	6
Raghavan et al 2018	3	2	3	8
Schmidt et al 2019	3	2	2	7
Strom et al 2017	2	1	2	5
Suren et al 2013	3	2	3	8
Nilsen et al 2013	3	1	3	7

Scores ranged from 0-9, where 9 indicates the highest quality.

Table 8. GRADE evidence profile.

Quality assessment							Summary of findings		
Number of studies	**Limitations**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Upgrade**	**Sample size cases/total sample**	**RR (95% CI)**	**Quality of evidence GRADE**
12	no serious limitations	[a]serious inconsistency	no serious indirectness	no serious imprecision	no serious publication bias	not upgraded	8761/ 1,025,534	0.74 (0.53, 1.04)	very low

GRADE; Grading of Recommendations Assessment, Development and Evaluation. [a] Considerable heterogeneity only partially explained by study quality. Two studies had harmful effects that were distinctly different from the body of evidence which was unexplained. Information in table 9 supported the assessment of the risk of bias.

Table 9. Optimal information size.

RR	**20%**	**25%**	**30%**
Population 1	0.01	0.01	0.01
Population 2	0.012	0.0125	0.013
Sample size for each group	42,693	27,937	19,827

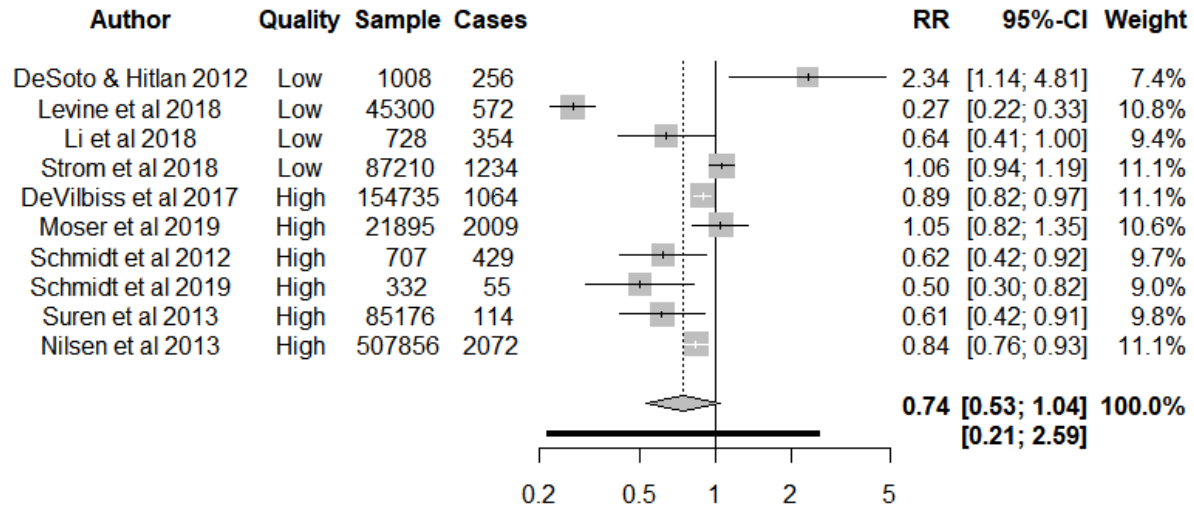
Based on 1% baseline risk, alpha = 0.05, power = .80

4.4.3 Meta-analysis

A total of 904,947 children including 8159 cases from six countries were included in the meta-analysis. All studies measured nutritional supplements and one also included fortified foods.

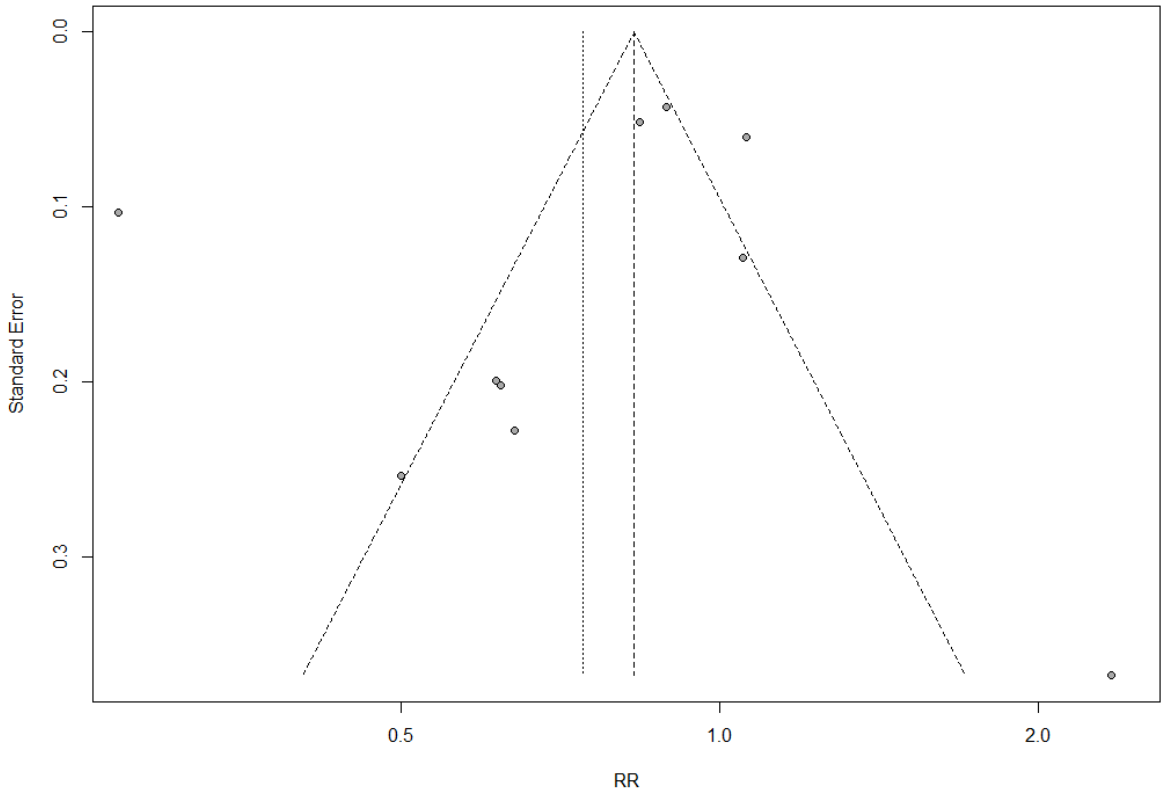
In the overall meta-analysis, there was no robust evidence that taking prenatal multivitamins was associated with autism diagnosis, compared to no/low intakes (RR 0.74, 95% CI: 0.53, 1.04) (Figure 10). However, the CI were wide and there was considerable heterogeneity (I^2 = 94.3%, p <0.001). Egger's test (p = 0.44) and inspection of the funnel plot suggests no evidence of funnel plot asymmetry (Figure 11). Precision increased when the Dersimonian Laird estimator was applied compared to the Hartung-Knapp-Sidik-Jonkman estimator (RR 0.74, 95% CI: 0.57, 0.95, I^2 = 94.3%). Upon removal of the outlier, DeSoto and Hitlan (Desoto and Hitlan, 2012), there was a 32% reduced likelihood of autism diagnosis (RR 0.68, 95% CI: 0.51, 0.91, I^2 = 94.7%, p <0.001), though heterogeneity remained considerable. There were no other influential studies (Table 10). The 95% prediction interval indicated the dispersion in the distribution of effect estimates was large and ranged from reduced to increased likelihood of autism diagnosis (RR 0.21, 2.59).

Figure 10. Forest plot of prenatal multivitamin supplements and the likelihood of offspring autism diagnosis.



CI, confidence interval; RR, relative risk. Reference is no/low prenatal multivitamin supplement use.

Figure 11. Prenatal multivitamins: funnel plot.



RR, relative risk.

Table 10. Influential study analysis.

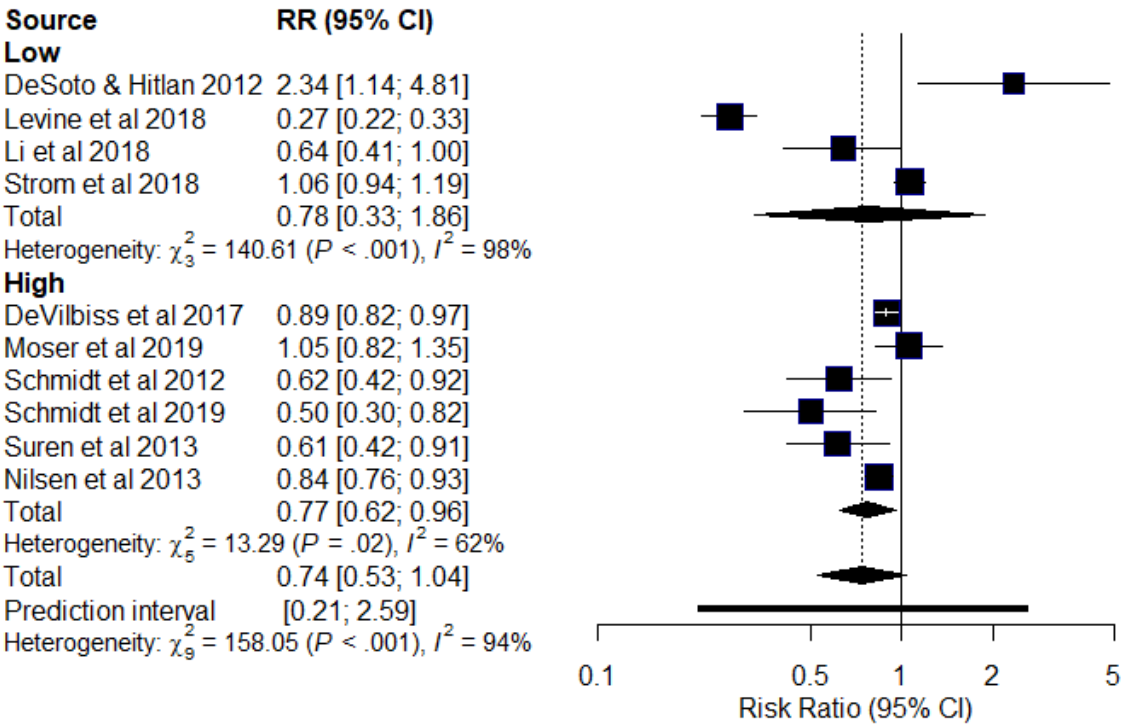
Author omitted	**RR**	**95% CI**	**I^2**
Levine et al 2018	0.84	0.64, 1.10	75.1%
Schmidt et al 2019	0.77	0.54, 1.11	94.8%
Suren et al 2013	0.76	0.53, 1.10	94.9%
Schmidt et al 2012	0.76	0.52, 1.10	94.9%
Li et al 2018	0.76	0.52, 1.10	94.9%
Nilsen et al 2013	0.73	0.50, 1.07	94.9%
DeVilbiss et al 2017	0.73	0.50, 1.06	94.8%
Moser et al 2019	0.71	0.49, 1.03	94.8%
Strom et al 2018	0.71	0.49, 1.03	94.2%
DeSoto & Hitlan 2012	0.68	0.51, 0.91	94.7%
Summary estimate	0.74	0.53, 1.04	94.3%

RR, relative risk.

4.4.4 Subgroup analysis

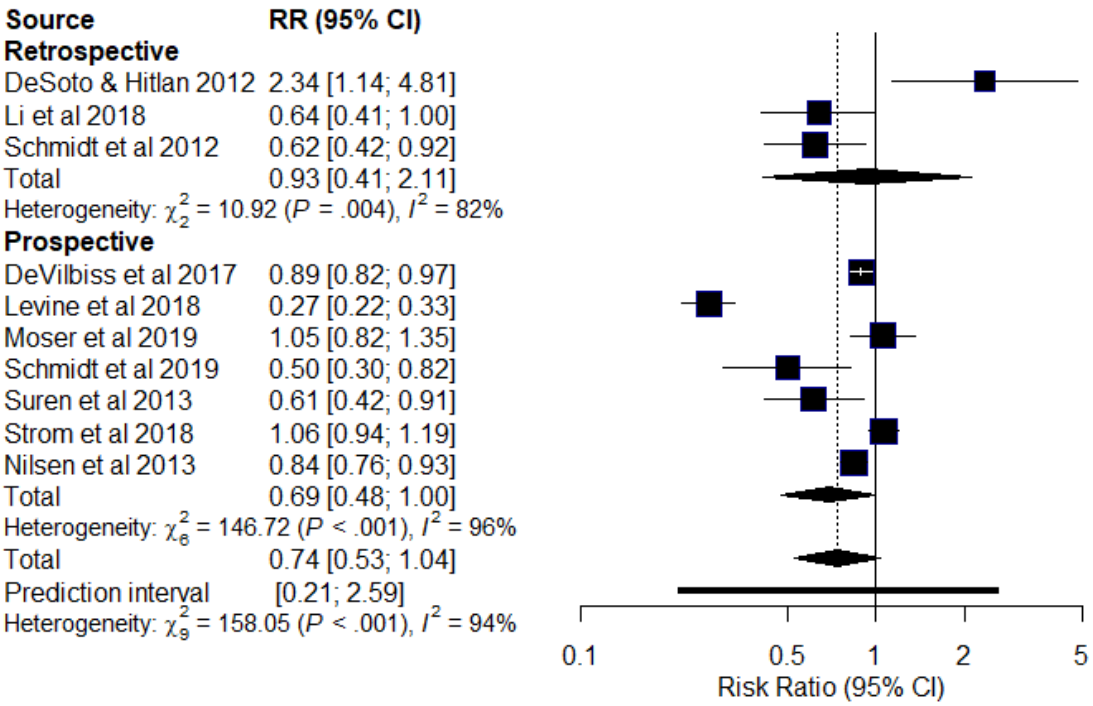
See Figure 12-15. In high quality observational studies there was a 23% reduced likelihood of autism diagnosis in association with prenatal multivitamin use, and heterogeneity reduced from considerable to substantial (RR 0.77, 95% CI: 0.62, 0.96, I^2 = 62.4%). No association was observed in low quality studies (RR 0.78, 95% CI: 0.33, 1.86, I^2 = 97.9%), and there were no between subgroup differences detected (Q = 0.00, *p* = 0.98). Weak evidence of association was observed in prospective studies (RR 0.69, 95% CI: 0.48, 1.00, I^2 = 95.9%), whilst no association was evident in the retrospective studies (RR 0.93, 95% CI: 0.41, 2.11, I^2 = 81.7%). There were no differences between subgroups for study design (Q = 0.42, *p* = 0.51). Subgroup analysis of regions reduced heterogeneity from considerable in Nordic studies (RR 0.87, 95% CI: 0.72, 1.06, I^2 = 76.8%) and American studies (RR 0.87, 95% CI: 0.5, 2.15, I^2 = 84.5%). Studies from Asian countries had the largest estimated association but there was considerable heterogeneity present (RR 0.56, 95% CI: 0.26, 1.23, I^2 = 97.1%). No differences between subgroups were detected (Q= 1.14, *p* = 0.57). Subgroup analysis of stage of pregnancy showed similar estimated associations in each group (early pregnancy, RR 0.76, 95% CI: 0.58, 0.99, I^2 = 79.8%; and any stage of pregnancy, RR 0.78, 95% CI: 0.40, 1.53, I^2 = 96.6%), and there were no between subgroup differences (Q = 0.01, *p* = 0.93). Regions without mandatory fortification produced a stronger reduction in the probability of autism diagnosis (RR 0.71, CI 95%: 0.50, 1.02, I^2 = 96%), compared to regions with mandatory fortification (RR 0.87, CI 95%: 0.35, 2.15, I^2 = 84%). There were no between subgroup differences detected (Q = 1.14, *p* = 0.57).

Figure 12. Forest plot of prenatal multivitamin supplements and the likelihood of offspring autism diagnosis: subgroup analysis by study quality.



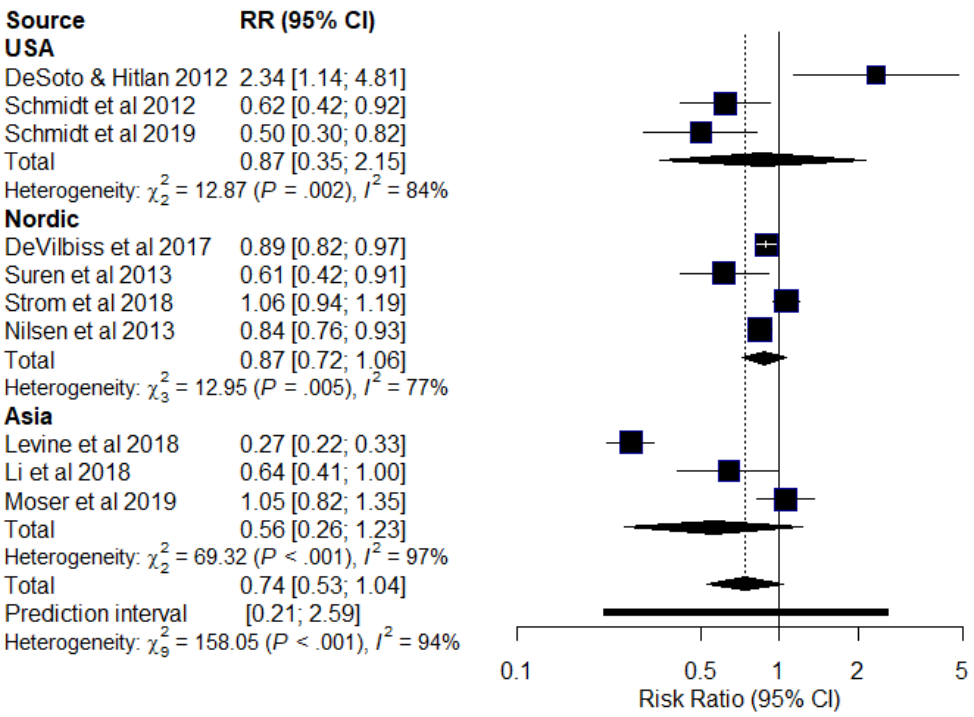
CI, confidence intervals; RR, relative risk. Reference is low/no prenatal multivitamin supplement use.

Figure 13. Forest plot of prenatal multivitamin supplements and the likelihood of offspring autism diagnosis: subgroup analysis by study design.



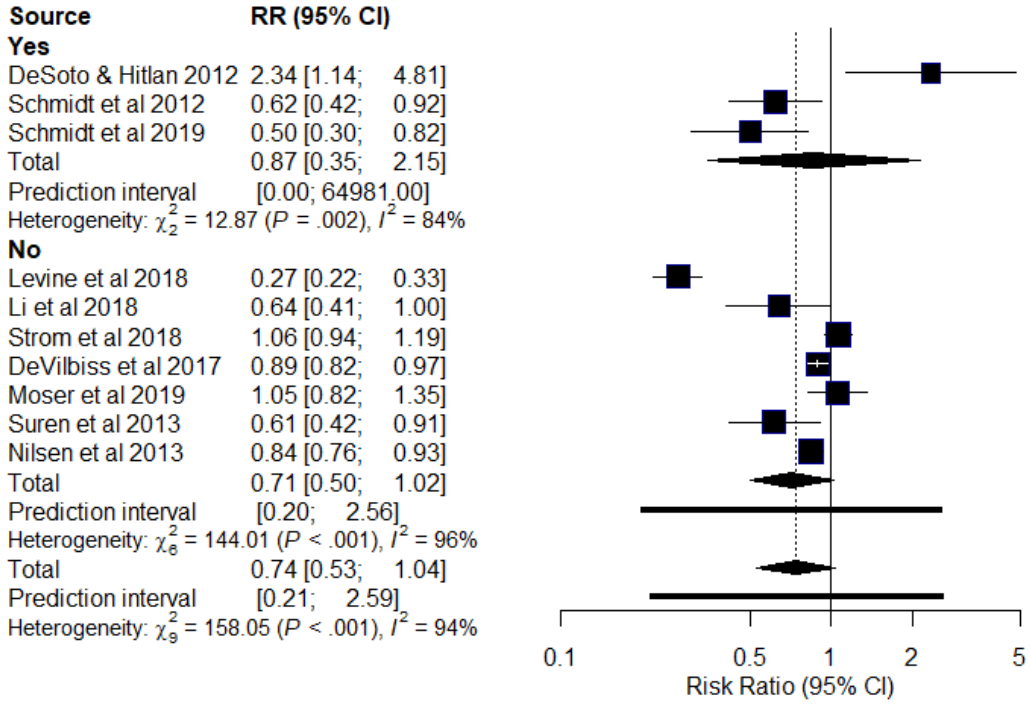
CI, confidence intervals; RR, relative risk. Reference is low/no prenatal multivitamin supplement use.

Figure 14. Forest plot of prenatal multivitamin supplements and the likelihood of offspring autism diagnosis: subgroup analysis by region.



CI, confidence intervals; RR, relative risk. Reference is low/no prenatal multivitamin supplement use.

Figure 15. Forest plot of prenatal multivitamin supplements and the likelihood of offspring autism diagnosis: subgroup analysis by mandatory fortification.



CI, confidence intervals; RR, relative risk. Yes, the country has mandatory fortification. No, the country does not have mandatory fortification.

4.4.5 Causally informed approaches

All studies (Fernell et al., 2015, Traglia et al., 2020) measured the association between prenatal multivitamins and autism diagnosis using conventional multivariate regression. Alternative approaches were infrequently applied (Table 11). The following approaches were identified: one discordant sibling analysis (DeVilbiss et al., 2017), two negative controls studies (Levine et al., 2018, Suren et al., 2013), and one genetic interaction study (Schmidt et al., 2012). When applied, these approaches were generally used as a secondary analysis to conventional multivariate regression. A detailed summary of the key sources of bias in each approach can be read from Table 12.

Table 11. Summary of approaches which demonstrates multivariate regression was commonly applied whilst all other approaches were infrequently used.

Study	Design	Sample size cases/controls or children (cases)	Multivariate regression	Causal Diagram	Propensity score	Sibling study	Negative control	Genetic studies
Multivitamins								
Desoto & Hitlan, 2012 (taking supplements)	Case-control	256/ 752	▼					
DeVilbiss et al, 2017 (taking supplements)	Cohort	273,107 (1064)	▲		▲	△		
Levine et al, 2018 (taking supplements)	Cohort	45,300 (572)	▲				▲[b]	
Li et al, 2018 (taking supplements)	Case-control	354/ 374	▲					
Moser et al, 2019 (taking supplements)	Case-control	2009/19,886	△					
Nilsen et al, 2013 (taking supplements)	Cohort	507,856 (2072)	▲					
Raghavan et al, 2018 (taking supplements < twice/week or taking supplements >five times/week)	Cohort	1257 (86)	▼					
Schmidt et al, 2019 (taking supplements)	Cohort	241 (55)	▲					
Schmidt et al 2012 (taking supplements)	Case-control	429/ 278	▲					▲[c]
Suren et al, 2013 (taking supplements)	Cohort	85,176 (114)	▲				▽[d]	
Strom et al, 2017 (taking supplements)	Cohort	87,210 (1234)	△					
Tan et al, 2020 (not taking supplements)	Case-control	416/201	▲					

Effect direction: ▲ = positive health impact, ▼ = negative health impact. Significance association = black arrow, no association = unshaded arrow. The nutrient source is supplements/fortified foods (reference group: no/low intake). [a] Raghavan et al 2018 reference group is 3-5/week compared to low and high supplements intakes. [b] negative control was two years pre-pregnancy and had a stronger association with autism diagnosis. [c] Beneficial effects of folic acid supplements were only detected if the child or mother had at least one T allele which reduces the efficiency of folate metabolism, [d] negative control was fish oils which show no association with autism diagnosis.

Table 12. Summary of the key sources of bias for each causally informed approach.

Approach	Generic description of approach and assumptions.	Description of approach included in the reviewed studies.	Key sources and direction of bias in relation to the main assumptions of each approach for included studies.
Prenatal multivitamin			
Multivariate regression	Confounding bias may be reduced through statistical adjustment for multiple covariates. Key assumptions are no residual confounding from either poorly measured or unmeasured covariates.	1,025,534 children including 8,761 people with an autism diagnosis were synthesised, two studies were narratively synthesised and 10 studies meta-analysed.	Generally, there was good adjustment for the main known confounders except planned pregnancy which may exaggerate the magnitude of association. Causes of autism are not fully known and so there remains potential for unobserved confounding which can bias the associations in either direction. Inappropriate covariate selection was evident due to adjustment for mediators and/or conditions co-morbid with the outcome or restriction of the study sample based on potential mediators. The associations may be biased in either direction.

Discordant sibling analysis	The approach compares siblings who are discordant for the exposure to adjust for shared familial confounding. Key assumptions are that time-varying confounding between siblings is minimised and there is minimal random error.	One discordant sibling study (DeVilbiss et al., 2017) found an association in the general population multivariate regression which was attenuated in the discordant sibling analysis. The study sample included 3066 mothers discordant for multivitamins supplement use and 7122 children discordant for an autism diagnosis.	The authors had a large sample size, but it was substantially smaller than that required based on our power calculation in Table 9 which may bias the association towards the null. Time-varying covariates that were adjusted were child sex, birthyear and parity. However, other potential confounders, such as maternal age and planned pregnancy were unadjusted. Maternal age increases the likelihood of autism and planned pregnancy may reduce the likelihood of autism. The overall impact of unmeasured confounding is unclear.
Negative control	Negative controls have similar covariate structure to the exposure-outcome relationship but are not related to the outcome. Key assumptions are that the major sources of bias for the exposure and negative control are similar. They should be scaled similarly, especially when the negative control is the exposure taken at a less biologically plausible period.	One study (Suren et al., 2013) measured 85,176 children, of which 114 had an autism diagnosis. A lower likelihood of autism diagnosis was observed in association with multivitamin supplement use but not the negative control (fish oils supplements). The second study (Levine et al., 2018) measured 45,300 children and 572 people with autism. The authors found stronger association between multivitamin supplements and autism diagnosis 'two years prior to pregnancy' than 'during pregnancy', and a similar magnitude of association with 'pre-pregnancy and pregnancy'.	In the first study (Suren et al., 2013), fish oils may reduce the likelihood of autism and so may have a relationship with autism diagnosis, which may invalidate the negative control. The second study found counter intuitive results and may reflect different sources of bias between the negative control and exposure, as well as the negative control having a relationship with autism diagnosis (Levine et al., 2018). Thus, their results are difficult to interpret.

Gene-nutrient interaction	The interaction between genotype and nutrient are measured. Key assumption is that there is no population stratification which occurs when groups of individuals have systematic differences in their genetic ancestry and the phenotype. This may create a spurious association.	One study (Schmidt et al., 2012) measured 332 children of which, 55 children had an autism diagnosis. An interaction between folate supplements/fortified foods and the methylenetetrahydrofolate reductase 677 genotype was observed. A reduced likelihood of autism diagnosis in association with prenatal folic acid supplements was only identified if the mother or infant had the C>T allele which is associated with less efficient folate metabolism.	The main limitations are that the study has a small sample size and is yet to be replicated. We cannot be certain of the direction of bias this could confer. The authors tested for population stratification and reported no evidence of population stratification based on the expected Hardy-Weinberg equilibrium proportions (p = > 0.05).

4.5 Discussion

Prenatal multivitamins supplement use was not robustly associated with autism diagnosis in the overall meta-analysis. However, a reduced likelihood of autism diagnosis was observed in high quality studies, prospective studies, early pregnancy, and following removal of an outlier. In contrast with previous meta-analyses, we did not observe any strong evidence of association in our main results (using all studies) which was sensitive to the estimator (Hartung-Knapp-Sidik-Jonkman estimator, rather than DerSimonian-Laird) which underestimates uncertainty and was applied in previous meta-analysis (Guo et al., 2019a, Iglesias Vazquez et al., 2019, Li et al., 2019). We also identified an additional two studies (Moser et al., 2019, Tan et al., 2020). However, although some associations were identified, based on GRADE the certainty of evidence is of very low-quality owing to the inherent risk of bias in observational study designs, considerable heterogeneity, and unexplained inconsistency in the direction of effect. As GRADE does not easily incorporate causally informed approaches (Guyatt et al., 2011), we have structured the discussion to firstly consider the limitations identified through the application of GRADE. Secondly, we evaluate whether the causally informed approaches have been of value to the interpretation of causality.

Regional variation in baseline nutritional status and genotype (Guo et al., 2019a, Virk et al., 2018) may be major contributors to both heterogeneity and inconsistency in the direction of effect. Nutrients confer a benefit to health up until physiological requirements are satisfied, thereafter we observe a plateau effect, and when intakes are extreme, toxicity or deficiency may occur and cause harm (European Food Safety Authority, 2018). To illustrate this potential U-shaped relationship, we will consider baseline folate status. Studies in this review from Nordic countries generally showed effect estimates close to the null, except Norway (Nilsen et al., 2013, Suren et al., 2013). The associations measured in Nordic countries correlate with rates of plasma folate deficiency/insufficiency which are reported to be 0.7% in Denmark (Milman et al., 2006), 4% in Sweden (Ohrvik et al., 2018), but 24.9% in a subsample of the Norwegian Mother, Father and Child cohort (Nilsen et al., 2013). However, the comparisons between supplement use and plasma folate levels are drawn from different populations, and population heterogeneity and confounding may vary at the individual level. Future studies should consider the response to nutritional supplements in relation to baseline nutritional status.

Out of the reviewed studies, only the studies conducted in America (Desoto and Hitlan, 2012, Raghavan et al., 2018, Schmidt et al., 2019a, Schmidt et al., 2012) were subject to mandatory fortification of diet with folic acid and could be expected to have higher baseline folic acid intakes. Less than 1% of the American population has deficient plasma folate levels since the introduction of mandatory fortification in 1998 (Tsang, 2016) which may reduce the benefits from supplements as physiological requirements are already met. However, folate insufficiency remains common and has been estimated to be 18.8% in the general population of America based on the National Health and Nutrition Examination Survey (2011-2016) (Pfeiffer et al., 2019).

The two American studies found a reduced probability of autism diagnosis in association with multivitamin supplement use (Schmidt et al., 2019a, Schmidt et al., 2012), and two studies observed an increased likelihood of autism diagnosis (Desoto and Hitlan, 2012, Raghavan et al., 2018). There is uncertainty and debate that mandatory fortification with folic acid, coupled with folic acid supplements may result in toxicity, depending on the individuals total intakes and genotype (Wiens and DeSoto, 2017). Wiens & DeSoto (Wiens and DeSoto, 2017) argue that excessive intakes saturate metabolic pathways, leading to an accumulation of unmetabolized folic acid which may cause autism. Furthermore, folic acid is absorbed more readily than the folate form and is used in supplements and fortified foods which the authors argue will further exacerbate the excessive intakes. However, other research groups have found inconclusive evidence to support such claims, yet limitations in available evidence were acknowledged, and the debate continues (Scientific Advisory Committee on Nutrition (SACN), 2017).

Indeed, the two studies in this review that observed a reduced likelihood of autism diagnosis, had folic acid intakes well in excess of the recommended 400ug/day (Schmidt et al., 2019a, Schmidt et al., 2012). Yet, these two studies focused on early pregnancy when, it is speculated, folic acid requirements and so tolerance to high doses is greater. ‘Birth order bias’ is an alternative explanation for the observed increased probability of autism diagnosis and was considered by Moser et al (Moser et al., 2019). Autism and supplement use can be independently positively correlated, as supplement use is usually greater in first pregnancies, and families affected by autism have fewer children. An increased probability of autism diagnosis in Moser et al was attenuated to the null with restriction to first born boys (Moser et al., 2019).

An additional source of heterogeneity is variation in the stage of pregnancy that the multivitamin supplement is taken. Taking multivitamin supplements in early pregnancy was associated with a reduced probability of autism diagnosis and lower heterogeneity. Periconception is an established critical period in which folic acid can reduce the incidence of neural tube defects by up to 70% (Stephenson et al., 2018). Furthermore, folic acid in periconception may reduce the risk of; low birth weight, small for gestational age, stillbirth, neonatal mortality, preeclampsia and miscarriage (Stephenson et al., 2018).

Lastly, autism is a heterogeneous condition and specific features might have differential causal pathways (Whitehouse and Stanley, 2013) which may contribute to variability in results. All studies that stratified by severity, observed a greater magnitude of association with more severe forms of autism diagnosis (DeVilbiss et al., 2017, Levine et al., 2018, Suren et al., 2013, Tan et al., 2020) as defined by minimally verbal status at age three years (Suren et al., 2013), low intelligence quotient (DeVilbiss et al., 2017, Levine et al., 2018), or high autism symptom severity (Tan et al., 2020).

4.5.1 Causally informed approaches

4.5.1.1 Multivariate regression

Causally informed approaches were infrequently applied, and the application was often not robust, and therefore limited our ability to triangulate the findings. All studies used multivariate regression, a key assumption of which, is no residual confounding (Lawlor et al., 2016). However, there is a risk of residual confounding in this review as studies adjusted for many but seldom all key confounders. The included studies commonly adjusted for: maternal age, physical and/or mental health, socioeconomic status, parity, planned pregnancy, pre-pregnancy BMI, and health behaviours. Due to the risk of residual confounding we attempted to triangulate the results from the multivariate regression with alternative approaches that have different sources of bias. The gene-nutrient interaction analysis provided the stronger evidence.

4.5.1.2 Gene-nutrient interaction

In Schmidt et al, an association between folate use and autism diagnosis was only observed if the mother/child had the methylenetetrahydrofolate reductase (MTHFR) 677 C>T genotype which is less likely due to confounding by socioeconomic and lifestyle characteristics (Schmidt et al., 2012). MTHFR 677 C>T genotype codes for a less efficient

enzyme that metabolises folate. Hence, larger folate doses may be necessary to overcome inefficient enzymatic function (Schmidt et al., 2012). Although these findings are yet to be replicated on larger samples, there is consistency in the wider literature. A recent meta-analysis identified an 86% increased likelihood of autism in association with the less efficient genotype (TT genotype versus CC genotype: OR 1.86, 95% CI: 1.12, 2.18) (Pu et al., 2013). However, this association was not evident in countries that had higher intakes of folic acid secondary to mandatory fortification with folic acid. Collectively, this may imply genotype influences the response to supplements and/or mandatory fortification of folic acid.

4.5.1.3 Discordant sibling analysis

Discordant sibling analyses may overcome shared unmeasured confounding, albeit there are several key methodological considerations. In DeVilbiss et al's discordant sibling analysis, the reduced likelihood of autism diagnosis observed in their main analysis was attenuated in the discordant sibling analysis (DeVilbiss et al., 2017). Sibling comparison studies are a quasi-experimental study design intended to remove shared familial confounding by matching siblings discordant for the outcome. However, this includes shared genetic risk factors as siblings share, on average, 50% of their genetic material (Lawlor et al., 2016). As indicated in Schmidt et al's study (Schmidt et al., 2012), it may be the combination of MTHFR 677 C>T genotype and no folic acid supplements that lead to an increased probability of autism diagnosis. Thus, the sibling comparison may adjust for a causal component. Furthermore, there is a high type II error rate as only siblings discordant for the exposure and outcome contribute to the effect estimate in conditional logistic regression models (Frisell et al., 2012). DeVilbiss et al's discordant sibling analysis may be underpowered as the point estimate was consistent with the main analysis, but the confidence intervals were wider (DeVilbiss et al., 2017). Furthermore, random error can be amplified in discordant sibling analysis and bias towards the null. Conversely, confounding not shared by siblings, such as time varying confounders, should be adjusted as matching on siblings does not control for this (Frisell et al., 2012). Thus, the null association observed here should be interpreted with caution as it could reflect a type II error or adjustment for a causal component, MTHFR genotype.

4.5.1.4 Negative control

Two studies applied negative controls. Negative controls act as ‘mock’ exposures or outcomes that indicate the presence of bias (Shi et al., 2020). Negative controls do not rely on the assumption of no unmeasured confounding. Instead, they assume confounding, and sometimes other biases, are similar in the exposure and negative control analyses. A further assumption is that the negative control has no plausible relationship with the outcome. Therefore, the exposure-outcome relationship can be distinguished from bias by comparing the strength of association with the negative control analysis (Lawlor et al., 2016). However, the assumptions of negative control analysis are empirically untestable. Thus, we are limited to a subjective interpretation based on subject knowledge (Shi et al., 2020), which is presented here.

Suren et al observed an association with prenatal multivitamin supplements, but not fish oils (Suren et al., 2013). However, fish oils are a rich source of polyunsaturated fatty acids, especially omega-3s, and cod liver oil is a rich source of vitamin D, all of which have been associated with positive neurodevelopmental outcomes (Chen et al., 2016, Lyall et al., 2013, Vinkhuyzen et al., 2017, Wu, 2018). Conversely, cod liver oil is a rich source of vitamin A which is teratogenic and may be harmful to foetal development at high doses (World Health Organization, 2016). Thus, this negative control violates the assumption of no plausible relationship with the outcome which confuses the interpretation. Similarly, Levine et al’s (Levine et al., 2018) negative control may have a relationship with the exposure. The authors compared mutually exclusive groups for prenatal multivitamins; two years prior to pregnancy; two years prior to pregnancy and during pregnancy; during pregnancy only. All groups were associated with a reduced likelihood of autism diagnosis, yet the association was strongest and most similar were ‘two years prior to pregnancy’ and ‘two years prior to pregnancy and during pregnancy’. No ‘wash-out’ period was described and so two years prior to pregnancy may have included the potentially critical period, preconception. Furthermore, the assumption of similar bias may not be met either. As women who discontinue supplements during pregnancy may have different characteristics to women who adhere to health advise and take the supplements during pregnancy. Thus, we lack confidence in the utility of this negative control.

4.5.1.5 Triangulation

Collectively, triangulation as a strategy to further causal interpretation was limited, mainly due to the infrequent utilisation, and limitations in the application of alternative approaches. The multivariate regression and gene-nutrient interaction (Schmidt et al., 2012) findings suggest there could be a causal association and we feel this warrants further investigation. However, within the context of this systematic review and meta-analysis, the discordant sibling analysis and negative control analyses (Levine et al., 2018) were of limited utility. Discordant sibling analyses need to be conducted on larger samples and with adjustment for a range of time-varying confounders, and there should be careful consideration given to the choice of negative control.

4.5.2 Strengths and limitations

This review has several strengths. Numerous steps to reduce bias were taken, such as: a search of grey literature sources, application of GRADE guidelines, calculation of prediction intervals, and use of Hartung-Knapp-Sidik-Jonkman estimator. Yet, the strongest advance was a formal narrative synthesis of the range of causal approaches. As an explicit approach it provides transparent evidence of the approaches applied, their findings and outlines areas for future studies to improve on. Nonetheless, there are weaknesses, mainly the heterogeneity and inconsistency observed across studies and low study numbers. The prediction intervals indicated if there was an effect it could range from negative to positive in individual settings.

4.5.3 Conclusion

At present, the evidence is inconclusive, and we are unable to confirm a causal association between prenatal multivitamin supplement use and autism diagnosis in offspring. Future studies should improve the study design and data analyses through adequately powered prospective birth cohorts. Measurement of nutritional supplements could be improved through reporting the nutrient composition of supplements, dose, compliance and duration and timing of use, which are all known to impact on biological response but were rarely considered in the studies reviewed. Furthermore, there should be greater consideration of the complexity of nutrition by modelling U-shaped relationships and considering how the response to nutrients is altered by variation in baseline requirements, and whether this is affected by recent nutrient intakes, changes to physiological demand in early pregnancy or

genetic variation. Lastly, we recommend the considered application of causally informed approaches within a triangulation framework to better gauge causality.

5 Chapter 5. Associations between prenatal dietary pattern and offspring autism diagnosis and autism-associated traits in the Norwegian Mother, Father, and Child Cohort Study (MoBa) and the Avon Longitudinal Study of Parents and Children (ALSPAC)

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Declarations

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Conflicts of Interest

The authors declare no conflicts of interest.

5.1 Abstract

Background

Prenatal diet is associated with autism, yet findings are conflicting and based on small samples. We investigated the association between a 'healthy' prenatal dietary pattern (HPDP) and autism diagnosis and autism-associated traits in two large prospective cohorts, The Norwegian Mother, Father, and Child Cohort (MoBa), and The Avon Longitudinal Study of Parents and Children (ALSPAC).

Methods

We focused on singleton pregnancies with self-reported food frequency questionnaire responses, which included 84,548 pregnancies recruited between 2002-2008 in MoBa, and 11,760 pregnancies recruited between 1990-1992 in ALSPAC. In MoBa, the offspring outcomes were autism diagnosis, elevated social communication questionnaire score at age three (SCQ-3) and eight years (SCQ-8), with further analysis of the subdomains, social communication difficulties (SCQ-SOC) and restrictive and repetitive behaviours (SCQ-RRB); and in ALSPAC, elevated social communication difficulties checklist score at age eight years (SCDC-8). A 'healthy' prenatal dietary pattern was derived using factor analysis and modelled as low-, medium- and high- adherence in marginal structural models. We adjusted for potential confounders and conducted various sensitivity analyses.

Results

High adherence to HPDP was associated with reduced odds of autism diagnosis (OR 0.78, 95% CI 0.66, 0.92), and social communication difficulties (SCQ-SOC-3: OR 0.76 (95% CI 0.70, 0.82); SCDC-8: OR 0.74 (95% CI 0.55, 0.98)). There was no consistent evidence of association with the other outcomes.

Conclusions

A HPDP was associated with lower odds of autism diagnosis and social communication difficulties but not restrictive and repetitive behaviours.

Key messages

- A HPDP was associated with a lower likelihood of offspring autism diagnosis in MoBa.

- There was some evidence that HPDP was associated with a lower likelihood of social communication difficulties, but there was no consistent evidence of association with restrictive and repetitive behaviours.

- The association between HPDP and social communication difficulties at age eight years may be greater in females compared to males, however further research is required to substantiate these findings.

5.2 Introduction

The prevalence of Autism Spectrum Disorder diagnosis is estimated to be 1-2% in the general population (Lyall et al., 2017, Roman-Urrestarazu et al., 2021). In this article, we use "autism", in response to the preferences of the autistic community (Kenny et al., 2016, Pellicano and den Houting, 2022). Autism diagnosis reflects a heterogeneous spectrum of neurodevelopmental conditions characterised by persistent difficulties and differences in reciprocal social communication and restricted and repetitive behaviours and interests (World Health Organisation, 2020). These autism-associated traits extend to subclinical manifestations commonly referred to as 'the broader autism phenotype' (Kellerman et al., 2019) and are observed in autistic and non-autistic populations (Moody et al., 2017). Furthermore, the relationship between social communication difficulties and restrictive and repetitive behaviour are phenotypically and genetically dissociable (Thomas et al., 2022). Therefore, it may be advantageous to aetiological understanding to measure autism diagnosis and autism-associated traits in the population, including the subdomains: social communication difficulties, and restrictive and repetitive behaviours.

Prenatal dietary patterns are emerging as a plausible causal factor in the complex aetiology of autism, yet this evidence base is limited. Previous studies have largely focused on discrete facets of prenatal nutrition, and found autism diagnosis and autism-associated traits were inversely related to prenatal multivitamin and/or folic acid supplement use, adequate vitamin D and folate status (Zhong et al., 2020). Yet, nutrients have synergistic and antagonistic effects, the summation of which can be measured through prenatal dietary patterns which could broaden our aetiological perspective and compliment investigations of discrete nutrients and prenatal multi-nutrient supplements (Tapsell et al., 2016).

Only four studies have investigated the relationship between prenatal dietary patterns and autism diagnosis or autism-associated traits (Vecchione et al., 2022, House et al., 2018, Geetha et al., 2018, Li et al., 2018). However, the sample sizes were small and in two studies the results may be affected by recall bias (Geetha et al., 2018, Li et al., 2018). Small sample sizes can increase the risk of type I and type II errors and inflated effect estimates. Furthermore, imprecise measures of diet and autism-associated traits increase random error, which requires larger sample sizes to detect an association, should one exist. Thus, while each study had strengths, their limitations may create heterogeneous results. Therefore, we sought to build on this evidence and measured the associations between high

adherence to HPDP compared with low adherence, with autism diagnosis and autism-associated traits in two large prospective cohort studies, MoBa, and ALSPAC.

5.3 Methods

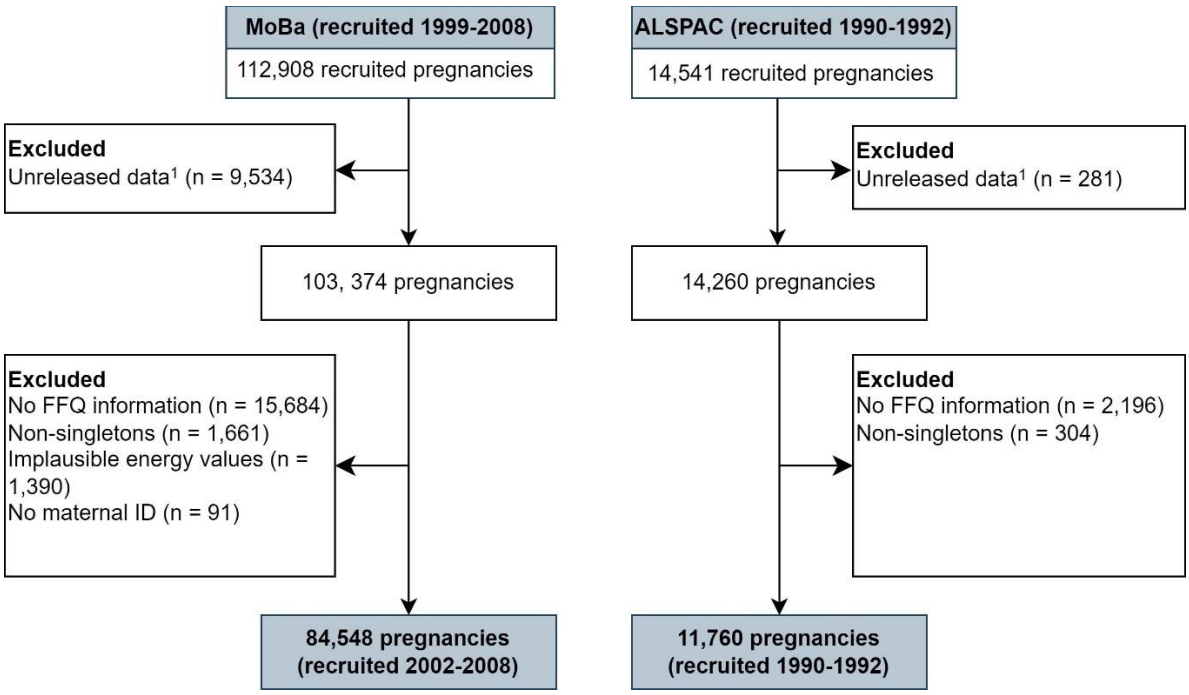
5.3.1 Study population

We separately analysed ALSPAC and MoBa to test the consistency of results across contexts although to ease comparability, where possible, harmonised the analytical approach used in each cohort. MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (Magnus et al., 2016). Participants were recruited from all over Norway from 1999-2008. The women consented to participation in 41% pregnancies. The cohort includes approximately 114,500 children, 95,200 mothers and 75,200 fathers. The current study is based on version 12 of the quality-assured data files released for research in January 2019. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is currently regulated by the Norwegian Health Registry Act. The current study was approved by The Regional Committees for Medical and Health Research Ethics (2013/201).

ALSPAC is a prospective cohort primarily of pregnant women and their offspring. Pregnant women resident in the Southwest of England and with expected dates of delivery between 1st April 1991 and 31st December 1992. 71.8% of the target population participated in ALSPAC resulting in 14,541 pregnancies initially enrolled. For further details see (Boyd et al., 2013). The study website contains details of all the data that is available through a fully searchable data dictionary (www.bristol.ac.uk/alspac/researchers/our-data). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

In MoBa and ALSPAC we restricted the samples to individuals with plausible FFQ responses and singleton pregnancies, which left 84,548 and 11,760 mothers-child dyads in ALSPAC and MoBa respectively (Figure 16). In MoBa FFQ responses were only available for recruitment years 2002 to 2008. Figure 1 shows the source and timeline of all data collection.

Figure 16. Flow chart of sample selection in MoBa and ALSPAC.



[1]The rationale for unreleased data is detailed in the cohort profiles (Boyd et al., 2013, Fraser et al., 2013, Magnus et al., 2016). FFQ, food frequency questionnaire; ID, identifier.

5.3.2 Outcome

5.3.2.1 Autism diagnosis

The Norwegian Patient Registry was linked to all MoBa children via unique identification number, capturing all diagnoses of autism made in the public specialist health care system from 2008 to 2018. Hence children were up to 16 years of age at diagnosis. Autism diagnosis is recorded using the ICD-10 criteria (International Classification of disease, 10th edition), and we included all F84 codes except Rett syndrome (F84.2) (World Health Organisation, 1993).

5.3.2.2 Autism-associated traits

From MoBa, we used the 40-item SCQ about autism-associated SCQ-SOC and SCQ-RRB (Rutter, 2003). The SCQ was completed by the mother about their child at two time points, aged three and eight years. Item one of the questionnaire screens for phrase-speech and is not scored, and we restricted to children who completed >50% of the SCQ (Surén et al., 2019). We applied an adjustment for absence of phrase-speech as per Eaves et al, as children without phased-speech may be underscored due to omission of inapplicable items (Eaves et al., 2006). As secondary outcomes, we analysed the subdomains, social communication, and restrictive and repetitive behaviours subdomains. From ALSPAC we used the 12-item Social and communication disorders checklist (SCDC-8), which was completed by the primary care giver about their child at approximately 7.6 years. The SCDC is a questionnaire that measures difficulties with social and communication skills. High scores indicated greater autism-associated traits and were defined as ≥93rd percentile for SCDC-8, SCQ at age three years (SCQ-3), and eight years (SCQ-8), and was guided by previous validation studies for MoBa (Surén et al., 2019) and ALSPAC (Skuse et al., 2005).

5.3.3 Dietary assessment and dietary pattern

Both MoBa and ALSPAC used a self-reported FFQ for which detailed methods have been previously published. In MoBa, pregnant women were asked to report their average diet since conception in a validated 255-item semi-quantitative FFQ (Brantsaeter et al., 2008, Brantsaeter et al., 2009, Brantsaeter et al., 2007, Brantsaeter et al., 2010). ALSPAC applied a non-quantitative FFQ, which asked pregnant women to report their food intake ‘nowadays’ in relation to 43 food groups (Emmett, 2009, Rogers et al., 1998). MoBa and

ALSPAC collected information on commonly consumed foods and beverages, and dietary supplement use. Food intake was expressed as frequency of consumption in ALSPAC, and grams per day in MoBa.

We matched the food and beverage items used in ALSPAC and MoBa and derived one 'healthy' dietary pattern from each cohort, using exploratory factor analysis with varimax rotation (all factor analysis and nutrient intakes are presented in Supplementary table 4-6). The derived factors were selected based on interpretability and Scree plot with the eigenvalues >1.5. The linear factor score was adjusted for total energy intake using the residuals method (Willett et al., 1997), and categorised into terciles: high-, medium-, and low-adherence to HPDP.

5.3.4 Covariates

The Medical Birth Registry is a national health registry containing information about all births in Norway and was used in the Norwegian Mother, Father, and Child Cohort (MoBa) to obtain information on child sex, maternal age, and paternal age. All other covariates were obtained from the self-completed questionnaires. Maternal covariates from MoBa were: highest level of maternal education (9-year secondary school/ 1–2-year high school/ technical high school/ 3-year high school general studies, junior college/ regional technical college, 4-year university degree (Bachelor's degree, nurse, teacher, engineer)/ university, technical college, more than 4 years (Master's degree, medical doctor, PhD)), maternal age (years), pre-pregnancy BMI (kg/m^2), pre-pregnancy depression (yes/no), planned pregnancy (yes/no), continuous use of multivitamin or folic acid supplement use from four weeks preconception to at least 12 weeks' gestation (yes/no) (referred to a prenatal multivitamin use), alcohol intake (yes/no), pre-pregnancy diabetes (yes/no), maternal smoking (yes/no), pre-pregnancy hypertension (yes/no). Paternal covariates are: paternal BMI (kg/m^2), paternal age (years), and paternal income (no income / under 150,000 Norwegian Krone (NOK) / 151,000-199,999 NOK / 200,000-299,999 NOK / 300,000-399,999 NOK / 400,000-499,999 NOK / over 500,000 NOK). Paternal covariates were all located from the maternal questionnaires as they were more complete.

Health records were used to obtain information on maternal diabetes, hypertension, and child sex. All other covariates were collected via self-completed questionnaires. Maternal covariates from ALSPAC were: maternal education (certificate of secondary education/none, vocational, ordinary level, advanced level, degree), maternal age (years),

maternal pre-pregnancy BMI (kg/m^2), history of depression (yes/no), planned pregnancy (yes/no), any multivitamin or folic acid supplement use up to 18-weeks' gestation (yes/no), alcohol intake (yes/no), smoking (yes/no), pre-pregnancy diabetes (yes/no), and pre-pregnancy hypertension (yes/no). The paternal questionnaire was used to obtain paternal covariates on paternal age (years) and paternal BMI (kg/m^2), which were only available from the paternal questionnaire, but paternal education (certificate of secondary education/none, vocational, ordinary level, advanced level, degree) was obtained from the maternal questionnaire.

5.3.5 Statistical methods

The analyses were conducted separately in ALSPAC and MoBa, however the same analytic approach was applied. Descriptive statistics were presented for both the cohorts overall and by terciles of HPDP.

The estimand sought is a theoretical parameter which in our analysis is the average treatment effect conditional on a set of covariates (Imbens, 2000). If all assumptions held (positivity, consistency, exchangeability), the average treatment effect would reflect the causal effect of HPDP on each outcome (MoBa: autism diagnosis, SCQ-3, and SCQ-8; ALSPAC: SCDC-8), had everyone 'received' a high (or medium) adherence to HPDP compared to the potential outcome if everyone had a low adherence to HPDP. In truth, our statistical estimate is subject to systematic and random error (Imbens, 2000). Potential confounders were identified with a DAG (Greenland et al., 1999) (Figure 2) and existing literature (Lyall et al., 2017). Covariates were maternal age, maternal education, maternal depression, pre-pregnancy BMI, planned pregnancy, prenatal multivitamin use, prenatal alcohol intake, prenatal smoking, pre-pregnancy hypertension, and pre-pregnancy diabetes. The minimally sufficient adjustment set was adjusted for by using inverse probability weights estimates through entropy balancing (Hainmueller, 2012). Weights and covariate balance were checked (approximately <0.1 may suggests negligible covariate imbalance) (Austin, 2009, Chesnaye et al., 2021).

Within a marginal structural model, we clustered on the mother to account for the intercorrelation between siblings. Robust standard errors were estimated using the Horvitz–Thompson variance estimator to account for the inverse probability weights and clustering (Horvitz and Thompson, 1952, Lumley and Scott, 2017). R-studio, version 4.0.3 (2020-10-10) (MoBa) and 4.2.2 (2022-10-31) (ALSPAC) was used to conduct analyses (R

Core Team, 2022), along with key packages (Lumley, 2020, Pishgar et al., 2021, van Buuren and Groothuis-Oudshoorn, 2011).

Data were assumed to be missing at random and imputed using multivariate imputation by chained equations (van Buuren and Groothuis-Oudshoorn, 2011). We included all variables in the models and a range of auxiliary variables (Table 2). We used 115 (ALSPAC) and 156 (MoBa) imputations which were selected based on von Hippel's approach (von Hippel, 2020), and imputed each level of HPDP separately to facilitate the testing of interaction terms (Tilling et al., 2016). The weights and marginal structural models were estimated for each imputed dataset (Granger et al., 2019) before combining them using Rubin's rule to produce the single and final estimate of association (Campion and Rubin, 1989).

5.3.6 Sensitivity analysis

We repeated the main analyses, making changes to the confounding, outcome, and exposure measurements. For confounding, we additionally adjusted for paternal education, paternal income (MoBa only), paternal BMI, paternal age, and birthyear, and assessed the estimates without adjustment for prenatal multivitamin supplements use. We recalculated the SCQ-3 and SCQ-8 scores based on fully complete responses to the SCQ without adjustment for phased speech. All SCQ scores were analysed as binary outcomes with a cut-off at the ≥93rd percentile to indicate a high level of autism-associated traits compared to a low level or no autism-associated traits.

The precise composition of HPDP will vary across individuals and it is plausible that different facets of diet have a different association with the outcome. Therefore, we tested the consistency of our HPDP by deconstructing the HPDP into three food groups: plant-based, fish-based, and an 'unhealthy'. We repeated the main analyses but exchanged HPDP for each food group and used the same covariates. The factor analyses were repeated using the same procedures as the main analyses but with different food items taken from the HPDP (Supplementary table 5). The categories were plant-based (included all fruit and vegetables, nuts, and granary/high fibre/wholemeal cereals and bread items in factor analyses), fish-based (included all fish items in factor analyses), and an 'unhealthy' foods (included soft drinks, fries, white bread, processed meat, meat (nonpoultry)). However, discrete dietary subgroups can be weaker measures with greater variability (Hu,

2002) and so the interpretation focused on marked differences in the overall trend to avoid over interpretation.

Interactions were measured on a multiplicative scale in logistic regression models adjusted for the same covariates as the main analyses, using the Wald-test extended to imputed data using the D_2-statistic (Allison, 2002, Grund et al., 2016). Interactions tested were between HPDP and: pre-pregnancy BMI (<25kg/m^2 / ≥25kg/m^2), child's sex (male / female), prenatal multivitamin supplement use (yes / no) and maternal education (<degree / ≥ degree). The main models in MoBa and ALSPAC were repeated and further adjusted for the following paternal characteristics BMI (kg/m^2) and age (years), and in MoBa only, paternal income, which was not available in ALSPAC during pregnancy. Lastly, we repeated all main analysis based on complete cases in MoBa only, due to low counts in ALSPAC.

5.4 Results

The event rate for each outcome (autism diagnosis, SCQ-3, SCQ-8, SCDC-8), by HPDP adherence were, respectively, low (1.4%, 6.7%, 4.2%, 5.4%), medium (1.0%, 6.2%, 4.2%, 4.1%), and high (1.0%, 6.4%, 3.9%, 4.4%) (Table 13).

The sociodemographic characteristics varied across cohorts, and within cohorts (Table 14-15). Compared to ALSPAC, MoBa mothers were older at recruitment, had higher educational levels, were more likely to have planned their pregnancy, abstain from alcohol, and take a multivitamin supplement. MoBa and ALSPAC mothers with high HPDP versus low, were more likely to be older, with high educational attainment, take prenatal multivitamin supplements, and be non-smokers. In ALSPAC only, high HPDP related to greater prevalence of alcohol consumption in pregnancy, lower prevalence of a planned pregnancy and a history of depression. Good covariate balance was achieved and there were no extreme weights (

Supplementary figure 2upplementary figure 2-3).

Table 13. Autism diagnosis and autism-associated traits by adherence to a 'healthy' prenatal dietary pattern in ALSPAC and MoBa.

		Adherence to a 'healthy' prenatal dietary pattern			
Outcome		**Overall**	**Low**	**Medium**	**High**
MoBa					
Number of participants		84,548	28,183	28,182	28,183
Autism diagnosis (%)	No	83606 (98.9)	27791 (98.6)	27901 (99.0)	27914 (99.0)
	Yes	942 (1.1)	392 (1.4)	281 (1.0)	269 (1.0)
	Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SCQ-3 (%)	No	46154 (54.6)	15017 (53.3)	15645 (55.5)	15492 (55.0)
	Yes	5408 (6.4)	1879 (6.7)	1736 (6.2)	1793 (6.4)
	Missing	32986 (39.0)	11287 (40.0)	10801 (38.3)	10898 (38.7)
SCQ-RRB-3 (%)	No	47078 (55.7)	15456 (54.8)	15978 (56.7)	15644 (55.5)
	Yes	4484 (5.3)	1440 (5.1)	1403 (5.0)	1641 (5.8)
	Missing	32986 (39.0)	11287 (40.0)	10801 (38.3)	10898 (38.7)
SCQ-SOC-3 (%)	No	46481 (55.0)	14961 (53.1)	15678 (55.6)	15842 (56.2)
	Yes	5081 (6.0)	1935 (6.9)	1703 (6.0)	1443 (5.1)
	Missing	32986 (39.0)	11287 (40.0)	10801 (38.3)	10898 (38.7)
SCQ-8 (%)	No	36786 (43.5)	11677 (41.4)	12460 (44.2)	12649 (44.9)
	Yes	3468 (4.1)	1196 (4.2)	1184 (4.2)	1088 (3.9)
	Missing	44294 (52.4)	15310 (54.3)	14538 (51.6)	14446 (51.3)
SCQ-RRB-8 (%)	No	34237 (40.5)	10864 (38.5)	11662 (41.4)	11711 (41.6)
	Yes	6017 (7.1)	2009 (7.1)	1982 (7.0)	2026 (7.2)
	Missing	44294 (52.4)	15310 (54.3)	14538 (51.6)	14446 (51.3)
SCQ-SOC-8 (%)	No	37218 (44.0)	11829 (42.0)	12598 (44.7)	12791 (45.4)
	Yes	3036 (3.6)	1044 (3.7)	1046 (3.7)	946 (3.4)
	Missing	44294 (52.4)	15310 (54.3)	14538 (51.6)	14446 (51.3)
ALSPAC					
Number of participants		11,760	3,920	3,920	3,920
SCDC-8 (%)	No	6735 (57.3)	1865 (47.6)	2274 (58.0)	2596 (66.2)
	Yes	544 (4.6)	210 (5.4)	160 (4.1)	174 (4.4)
	Missing	4481 (38.1)	1845 (47.1)	1486 (37.9)	1150 (29.3)

ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index; MoBa, Norwegian Mother, Father, and Child Cohort; n, number of mother-child dyads; SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCDC, social communication disorders checklist; SCQ-SOC, communication skills subdomain of SCQ. The number following each outcome denotes the approximate age of the child in years when the measure was obtained. i.e. SCQ-SOC-8; social communication subdomain measured at age 8 years. The presence of the outcome from each questionnaire was indicated by a high score.

Table 14. Sociodemographic characteristics by adherence to a ‘healthy’ prenatal dietary pattern in MoBa and ALSPAC.

MoBa		**Adherence to a 'healthy' prenatal dietary pattern**			**ALSPAC**		**Adherence to a 'healthy' prenatal dietary pattern**		
Variable	Overall	Low	Medium	High	Variable	Overall	Low	Medium	High
Number of pregnancies									
	84,548	28,183	28,182	28,183		11,760	3,920	3,920	3,920
Child sex (%)									
Male	43277 (51.2)	14465 (51.3)	14443 (51.2)	14369 (51.0)	Male	6034 (51.3)	2017 (51.5)	1998 (51.0)	2019 (51.5)
Female	41206 (48.7)	13690 (48.6)	13717 (48.7)	13799 (49.0)	Female	5725 (48.7)	1902 (48.5)	1922 (49.0)	1901 (48.5)
Missing	65 (0.1)	28 (0.1)	22 (0.1)	15 (0.1)	Missing	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)
History of depression (%)									
No	76340 (90.3)	25331 (89.9)	25604 (90.9)	25405 (90.1)	No	10263 (87.3)	3261 (83.2)	3470 (88.5)	3532 (90.1)
Yes	6474 (7.7)	2262 (8.0)	2021 (7.2)	2191 (7.8)	Yes	956 (8.1)	391 (10.0)	282 (7.2)	283 (7.2)
Missing	1734 (2.1)	590 (2.1)	557 (2.0)	587 (2.1)	Missing	541 (4.6)	268 (6.8)	168 (4.3)	105 (2.7)
Maternal age (years) (mean (SD))									
	30.2 (4.6)	29.1 (4.6)	30.4 (4.4)	31.3 (4.5)		27.9 (4.9)	26.0 (4.7)	27.9 (4.64)	29.8 (4.4)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	Missing	250 (2.1)	131 (3.4)	75 (1.9)	44 (1.1)
Maternal education (%)									
9-year elementary	1960 (2.3)	974 (3.5)	546 (1.9)	440 (1.6)	Certificate of secondary education/none	2324 (19.8)	1311 (33.4)	698 (17.8)	315 (8.0)
1-2 years further education	3736 (4.4)	1851 (6.6)	1057 (3.8)	828 (2.9)	Vocational	1155 (9.8)	522 (13.3)	405 (10.3)	228 (5.8)
Technical high school	10253 (12.1)	4651 (16.5)	3271 (11.6)	2331 (8.3)	Ordinary level	4063 (34.5)	1432 (36.5)	1533 (39.1)	1098 (28.0)
3-year high school general studies, junior college	11267 (13.3)	4651 (16.5)	3538 (12.6)	3078 (10.9)	Advanced level	2645 (22.5)	498 (12.7)	887 (22.6)	1260 (32.1)

Regional technical college, 4-year university degree (Bachelor's degree, nurse, teacher, engineer)	33190 (39.3)	10617 (37.7)	11698 (41.5)	10875 (38.6)	Degree	1503 (12.8)	119 (3.0)	377 (9.6)	1007 (25.7)
University, technical college, more than 4 years (Master's degree, medical doctor, PhD)	19319 (22.8)	3822 (13.6)	6550 (23.2)	8947 (31.7)					
Missing	4823 (5.7)	1617 (5.7)	1522 (5.4)	1684 (6.0)	Missing	70 (0.6)	38 (1.0)	20 (0.5)	12 (0.3)
Planned pregnancy (%)									
No	15512 (18.3)	5642 (20.0)	4819 (17.1)	5051 (17.9)	No	8113 (69.0)	2504 (63.9)	2731 (69.7)	2878 (73.4)
Yes	67461 (79.8)	22036 (78.2)	22884 (81.2)	22541 (80.0)	Yes	3356 (28.5)	1271 (32.4)	1099 (28.0)	986 (25.2)
Missing	1575 (1.9)	505 (1.8)	479 (1.7)	591 (2.1)	Missing	291 (2.5)	145 (3.7)	90 (2.3)	56 (1.4)
Prenatal alcohol intake (%)									
No	64040 (75.7)	21874 (77.6)	21386 (75.9)	20780 (73.7)	No	4524 (38.5)	1627 (41.5)	1539 (39.3)	1358 (34.6)
Yes	8861 (10.5)	2577 (9.1)	3045 (10.8)	3239 (11.5)	Yes	2130 (18.1)	538 (13.7)	704 (18.0)	888 (22.7)
Missing	11647 (13.8)	3732 (13.2)	3751 (13.3)	4164 (14.8)	Missing	5106 (43.4)	1755 (44.8)	1677 (42.8)	1674 (42.7)
Prenatal multivitamin supplement use (%)									
No	55706 (65.9)	20282 (72.0)	18268 (64.8)	17156 (60.9)	No	8877 (75.5)	3067 (78.2)	3009 (76.8)	2801 (71.5)
Yes	24632 (29.1)	6506 (23.1)	8551 (30.3)	9575 (34.0)	Yes	2568 (21.8)	683 (17.4)	823 (21.0)	1062 (27.1)
Missing	4210 (5.0)	1395 (4.9)	1363 (4.8)	1452 (5.2)	Missing	315 (2.7)	170 (4.3)	88 (2.2)	57 (1.5)
Prenatal smoking (%)									
No	70442 (83.3)	21843 (77.5)	23815 (84.5)	24784 (87.9)	No	8821 (75.0)	2426 (61.9)	3033 (77.4)	3362 (85.8)

Yes	6275 (7.4)	3333 (11.8)	1752 (6.2)	1190 (4.2)	Yes	2709 (23.0)	1368 (34.9)	820 (20.9)	521 (13.3)
Missing	7831 (9.3)	3007 (10.7)	2615 (9.3)	2209 (7.8)	Missing	230 (2.0)	126 (3.2)	67 (1.7)	37 (0.9)
Pre-pregnancy BMI (kg/m^2) (mean (SD))									
	24.1 (4.3)	24.5 (4.6)	24.1 (4.2)	23.5 (4.0)		22.9 (3.8)	23.3 (4.3)	23.1 (3.79)	22.4 (3.3)
Missing	7831 (9.3)	2959 (10.5)	2613 (9.3)	2259 (8.0)	Missing	1343 (12.9)	584 (14.9)	405 (10.3)	309 (7.9)
Pre-pregnancy diabetes (%)									
No	82401 (97.5)	27501 (97.6)	27484 (97.5)	27416 (97.3)	No	10706 (91.0)	3454 (88.1)	3592 (91.6)	3660 (93.4)
Yes	413 (0.5)	92 (0.3)	141 (0.5)	180 (0.6)	Yes	44 (0.4)	13 (0.3)	15 (0.4)	16 (0.4)
Missing	1734 (2.1)	590 (2.1)	557 (2.0)	587 (2.1)	Missing	1010 (8.6)	453 (11.6)	313 (8.0)	244 (6.2)
Pre-pregnancy hypertension (%)									
No	82216 (97.2)	27411 (97.3)	27455 (97.4)	27350 (97.0)	No	11663 (99.2)	3883 (99.1)	3889 (99.2)	3891 (99.3)
Yes	882 (1.0)	309 (1.1)	281 (1.0)	292 (1.0)	Yes	28 (0.2)	9 (0.2)	7 (0.2)	12 (0.3)
Missing	1450 (1.7)	463 (1.6)	446 (1.6)	541 (1.9)	Missing	69 (0.6)	28 (0.7)	24 (0.6)	17 (0.4)

ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index; MoBa, Norwegian Mother, Father, and Child Cohort; SD, standard deviation

Table 15. Descriptive statistics for additional sociodemographic characteristics and outcomes used in sensitivity analyses.

MoBa					
Characteristic		**Overall**	**Low**	**Medium**	**High**
	Number of pregnancies	84,548	28,183	28,182	28,183
Interactions					
Child sex (%)	Male	43277 (51.2)	14465 (51.3)	14443 (51.2)	14369 (51.0)
	Female	41206 (48.7)	13690 (48.6)	13717 (48.7)	13799 (49.0)
	Missing	65 (0.1)	28 (0.1)	22 (0.1)	15 (0.1)
Maternal education category (%)	No	52509 (62.1)	14439 (51.2)	18248 (64.8)	19822 (70.3)
	Yes	27216 (32.2)	12127 (43.0)	8412 (29.8)	6677 (23.7)
	Missing	4823 (5.7)	1617 (5.7)	1522 (5.4)	1684 (6.0)
Pre-pregnancy BMI category kg/m^2 (%)	<25	56032 (66.3)	17430 (61.8)	18511 (65.7)	20091 (71.3)
	≥ 25	25708 (30.4)	9818 (34.8)	8769 (31.1)	7121 (25.3)
	Missing	2808 (3.3)	935 (3.3)	902 (3.2)	971 (3.4)
Prenatal multivitamin supplement use (%)	No	55706 (65.9)	20282 (72.0)	18268 (64.8)	17156 (60.9)
	Yes	24632 (29.1)	6506 (23.1)	8551 (30.3)	9575 (34.0)
	Missing	4210 (5.0)	1395 (4.9)	1363 (4.8)	1452 (5.2)
Additional adjustment for Birthyear (%)					
	2002	4364 (5.2)	2017 (7.2)	1377 (4.9)	970 (3.4)
	2003	11463 (13.6)	4934 (17.5)	3614 (12.8)	2915 (10.3)
	2004	12127 (14.3)	4914 (17.4)	3934 (14.0)	3279 (11.6)
	2005	13264 (15.7)	4757 (16.9)	4405 (15.6)	4102 (14.6)
	2006	15116 (17.9)	4591 (16.3)	5203 (18.5)	5322 (18.9)
	2007	13753 (16.3)	3608 (12.8)	4800 (17.0)	5345 (19.0)
	2008	11454 (13.5)	2758 (9.8)	3889 (13.8)	4807 (17.1)
	2009	2848 (3.4)	555 (2.0)	922 (3.3)	1371 (4.9)
	Missing	159 (0.2)	49 (0.2)	38 (0.1)	72 (0.3)
Additional adjustment for paternal characteristics					
Paternal age (%)	<25 years	3803 (4.5)	2003 (7.1)	1069 (3.8)	731 (2.6)

	25-34 years	51800 (61.3)	18354 (65.1)	17456 (61.9)	15990 (56.7)
	35-44 years	26291 (31.1)	7241 (25.7)	8861 (31.4)	10189 (36.2)
	45-54 years	2063 (2.4)	427 (1.5)	636 (2.3)	1000 (3.5)
	≥ 55 years	164 (0.2)	29 (0.1)	35 (0.1)	100 (0.4)
	Missing	427 (0.5)	129 (0.5)	125 (0.4)	173 (0.6)
Paternal BMI kg/m^2 (mean (SD))		25.8 (3.3)	26.1 (3.5)	25.9 (3.3)	25.5 (3.1)
	Missing	4289 (5.1)	1400 (5.0)	1367 (4.9)	1522 (5.4)
Paternal income (%)	No income	712 (0.8)	245 (0.9)	235 (0.8)	232 (0.8)
	Less than 150,000 NOK	4302 (5.1)	1563 (5.5)	1310 (4.6)	1429 (5.1)
	150-199,999 NOK	3243 (3.8)	1283 (4.6)	993 (3.5)	967 (3.4)
	200-299,999 NOK	16862 (19.9)	6939 (24.6)	5414 (19.2)	4509 (16.0)
	300-399,999 NOK	26214 (31.0)	9084 (32.2)	8890 (31.5)	8240 (29.2)
	400-499,999 NOK	13593 (16.1)	3751 (13.3)	4831 (17.1)	5011 (17.8)
	over 500,000 NOK	13346 (15.8)	3022 (10.7)	4554 (16.2)	5770 (20.5)
	Missing	6276 (7.4)	2296 (8.1)	1955 (6.9)	2025 (7.2)
Paternal education (%)	9-year elementary	3575 (4.2)	1640 (5.8)	1086 (3.9)	849 (3.0)
	1-2 years further education	4430 (5.2)	2096 (7.4)	1336 (4.7)	998 (3.5)
	Technical high school	19673 (23.3)	8300 (29.5)	6417 (22.8)	4956 (17.6)
	3-year high school general studies, junior college	9337 (11.0)	3505 (12.4)	3116 (11.1)	2716 (9.6)
	Regional technical college, 4-year university degree (Bachelor's degree, nurse, teacher, engineer)	21106 (25.0)	6172 (21.9)	7378 (26.2)	7556 (26.8)
	University, technical college, more than 4 years (Master's degree, medical doctor, PhD)	18376 (21.7)	3785 (13.4)	6263 (22.2)	8328 (29.5)
	Missing	8051 (9.5)	2685 (9.5)	2586 (9.2)	2780 (9.9)
ALSPAC					
Characteristic		Overall	Low	Medium	High
	Number of pregnancies	11,760	3920	3920	3920
Interactions					
Child sex (%)	Male	6034 (51.3)	2017 (51.5)	1998 (51.0)	2019 (51.5)
	Female	5725 (48.7)	1902 (48.5)	1922 (49.0)	1901 (48.5)
	Missing	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)

Maternal education category (%)	High	4148 (35.3)	617 (15.7)	1264 (32.2)	2267 (57.8)
	Low	7542 (64.1)	3265 (83.3)	2636 (67.2)	1641 (41.9)
	Missing	70 (0.6)	38 (1.0)	20 (0.5)	12 (0.3)
ALSPAC					
Pre-pregnancy BMI category kg/m^2 (%)	<25	8275 (70.4)	2501 (63.8)	2693 (68.7)	3081 (78.6)
	≥ 25	2142 (18.2)	835 (21.3)	777 (19.8)	530 (13.5)
	Missing	1343 (11.4)	584 (14.9)	450 (11.5)	309 (7.9)
Prenatal multivitamin supplement use (%)	No	8877 (75.5)	3067 (78.2)	3009 (76.8)	2801 (71.5)
	Yes	2568 (21.8)	683 (17.4)	823 (21.0)	1062 (27.1)
	Missing	315 (2.7)	170 (4.3)	88 (2.2)	57 (1.5)
Additional adjustment for paternal characteristics					
Paternal age category (%)	<25 years	827 (7.0)	447 (11.4)	267 (6.8)	113 (2.9)
	25-29 years	2526 (21.5)	934 (23.8)	872 (22.2)	720 (18.4)
	29-34 years	2559 (21.8)	607 (15.5)	889 (22.7)	1063 (27.1)
	35-39 years	1071 (9.1)	217 (5.5)	327 (8.3)	527 (13.4)
	≥ 40 years	426 (3.6)	74 (1.9)	147 (3.8)	205 (5.2)
	Missing	4351 (37.0)	1641 (41.9)	1418 (36.2)	1292 (33.0)
Paternal BMI kg/m^2 (mean (SD))		25.2 (3.3)	25.4 (3.7)	25.2 (3.3)	24.9 (2.9)
	Missing	4089 (34.8)	1576 (40.2)	1333 (34.0)	1180 (30.1)
Paternal education (%)	Certificate of secondary education/none	2884 (24.5)	1445 (36.9)	926 (23.6)	513 (13.1)
	Vocational	958 (8.1)	381 (9.7)	362 (9.2)	215 (5.5)
	Ordinary level	2389 (20.3)	824 (21.0)	874 (22.3)	691 (17.6)
	Advanced level	2970 (25.3)	822 (21.0)	1041 (26.6)	1107 (28.2)
	Degree	2039 (17.3)	183 (4.7)	561 (14.3)	1295 (33.0)
	Missing	520 (4.4)	265 (6.8)	156 (4.0)	99 (2.5)

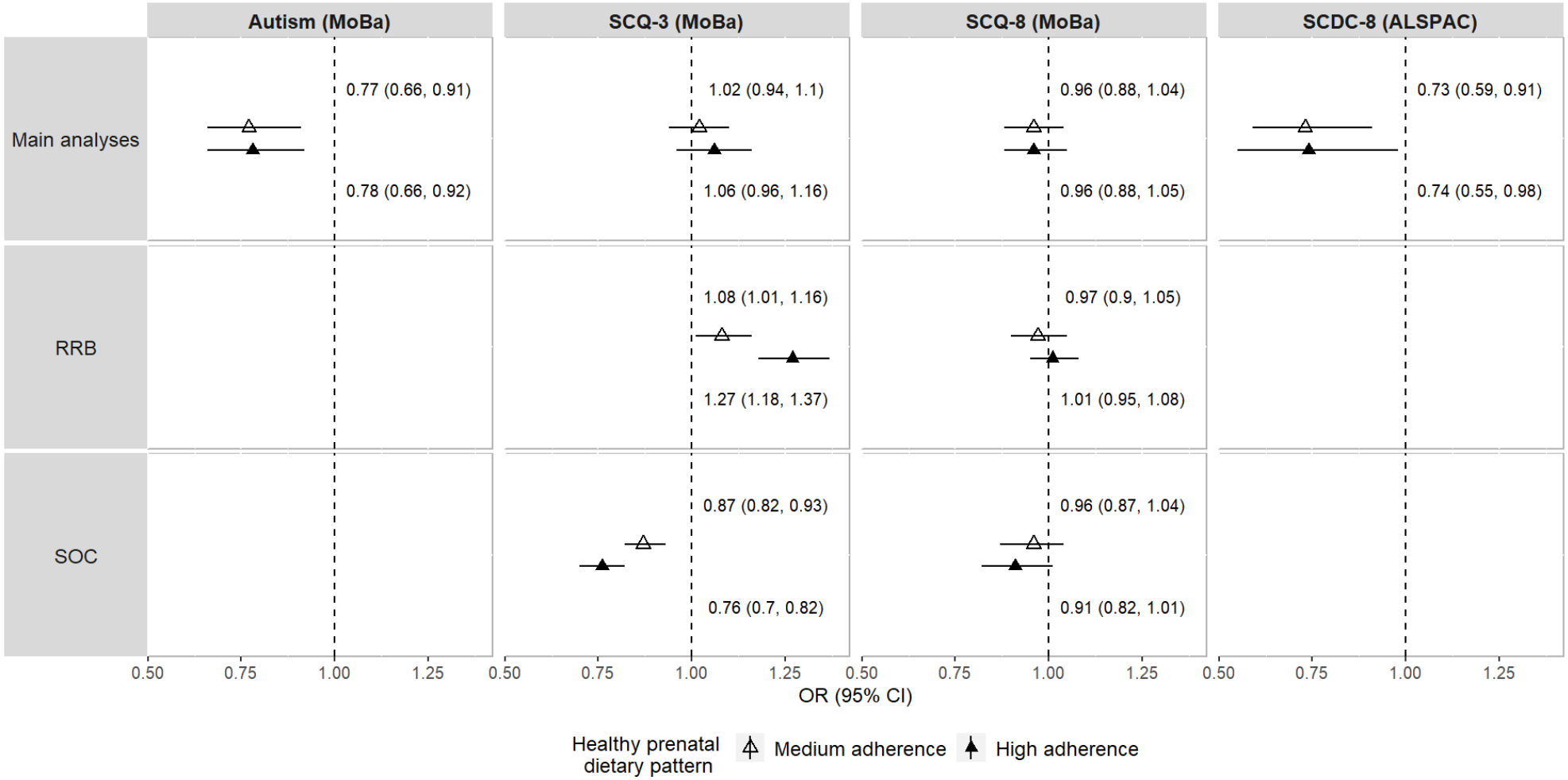
ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index; NOK, Norwegian Krone, MoBa, Norwegian Mother, Father, and Child Cohort; SD, standard deviation

5.4.1 Main results and sensitivity analysis

Higher HPDP were associated with a lower likelihood of autism diagnosis (MoBa), and SCDC-8 (ALSPAC), yet no clear evidence of association was observed for SCQ-3 or SCQ-8 (MoBa). In MoBa a higher HPDP was associated with a lower likelihood of SCQ-SOC-3 but higher likelihood of SCQ-RRB-3 (Figure 17 for results and Table 16). Results were robust to additional adjustment for paternal characteristics, birthyear, and prenatal multivitamin use (Table 17).

Further sensitivity analysis indicated that generally the clearest associations related to HPDP compared to the subgroups of food groups analysed here. The likelihood of SCQ-8 (0.92 ((95% CI) 0.86, 0.99)), and SCQ-SOC-8 (0.89 (0.82, 0.96)) reduced in relation to a plant-based food group, and the likelihood of SCQ-RRB-8 (0.93 (0.88, 0.99)) reduced in relation to a fish-based food group (Table 16). Evidence for interaction was unclear and/or inconsistent across MoBa and ALSPAC, except between HPDP and child sex (SCQ-SOC-3, SCQ-SOC-8, SCDC-8) (Table 18). A greater magnitude of association was observed in females in relation to SCQ-SOC-8 and SCDC-8 and for males in relation to SCQ-SOC-3. The complete case analyses in MoBa produced similar results to the main analyses (Table 19).

Figure 17. The associations between adherence to a ‘healthy’ prenatal dietary pattern and autism diagnosis and autism-associated traits in MoBa and ALSPAC.



ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father, and Child Cohort; SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCDC, social communication disorders checklist; SCQ-SOC, communication skills subdomain of SCQ. The number following each outcome denotes the approximate age of the child in years when the measure was obtained i.e. SCQ-8 social communication questionnaire measured at age 8 years. The presence of the outcome from each questionnaire was indicated by a high score.
Autism was measured binary (yes/no), and the other outcomes represents a high level of autism-associated traits. The reference category is low adherence to a 'healthy' prenatal dietary pattern.
Adjusted covariates in each model; child sex, history of depression, maternal age, maternal education, planned pregnancy, prenatal alcohol intake, prenatal multivitamin supplement use, prenatal smoking, pre-pregnancy BMI, pre-pregnancy diabetes, pre-pregnancy hypertension.

Table 16. Average treatment effect of adherence to a ‘healthy’ prenatal dietary pattern and dietary subgroups on autism diagnosis and autism-associated traits in ALSPAC and MoBa.

Outcome	Level of adherence	'Healthy' prenatal dietary pattern OR (95% CI)	Plant-based food group OR (95% CI)	Fish-based food group OR (95% CI)	'Unhealthy' food group OR (95% CI)
Autism diagnosis (MoBa)	Medium	0.77 (0.66, 0.91)	0.94 (0.80, 1.10)	0.90 (0.77, 1.06)	0.89 (0.75, 1.05)
Autism diagnosis (MoBa)	High	0.78 (0.66, 0.92)	0.91 (0.77, 1.08)	0.98 (0.83, 1.14)	1.05 (0.89, 1.24)
SCQ-3 (MoBa)	Medium	1.02 (0.94, 1.10)	1.00 (0.94, 1.07)	0.95 (0.89, 1.01)	0.93 (0.86, 1.00)
SCQ-3 (MoBa)	High	1.06 (0.96, 1.16)	1.07 (1.00, 1.15)	0.94 (0.88, 1.01)	0.94 (0.88, 1.00)
SCQ-RRB-3 (MoBa)	Medium	1.08 (1.01, 1.16)	1.07 (0.99, 1.15)	0.95 (0.89, 1.02)	1.09 (1.00, 1.18)
SCQ-RRB-3 (MoBa)	High	1.27 (1.18, 1.37)	1.26 (1.15, 1.37)	0.94 (0.87, 1.01)	1.11 (1.03, 1.20)
SCQ-SOC-3 (MoBa)	Medium	0.87 (0.82, 0.93)	0.86 (0.80, 0.92)	0.92 (0.86, 0.98)	1.09 (1.00, 1.18)
SCQ-SOC-3 (MoBa)	High	0.76 (0.70, 0.82)	0.80 (0.74, 0.86)	0.95 (0.89, 1.02)	1.11 (1.03, 1.20)
SCQ-8 (MoBa)	Medium	0.96 (0.88, 1.04)	0.92 (0.86, 0.98)	0.93 (0.85, 1.01)	0.95 (0.87, 1.02)
SCQ-8 (MoBa)	High	0.96 (0.88, 1.05)	0.92 (0.86, 0.99)	0.95 (0.89, 1.02)	0.97 (0.89, 1.06)
SCQ-RRB-8 (MoBa)	Medium	0.97 (0.90, 1.05)	0.98 (0.92, 1.04)	0.94 (0.89, 1.00)	0.94 (0.89, 1.00)
SCQ-RRB-8 (MoBa)	High	1.01 (0.95, 1.08)	1.03 (0.97, 1.09)	0.93 (0.88, 0.99)	1.00 (0.95, 1.06)
SCQ-SOC-8 (MoBa)	Medium	0.96 (0.87, 1.04)	0.93 (0.86, 1.00)	0.96 (0.88, 1.05)	1.00 (0.91, 1.09)
SCQ-SOC-8 (MoBa)	High	0.91 (0.82, 1.01)	0.89 (0.82, 0.96)	0.98 (0.88, 1.08)	1.01 (0.93, 1.10)
SCDC-8 (ALSPAC)	Medium	0.73 (0.59, 0.91)	0.76 (0.62, 0.94)	0.87 (0.74, 1.04)	1.10 (0.89, 1.36)
SCDC-8 (ALSPAC)	High	0.74 (0.55, 0.98)	0.83 (0.66, 1.04)	0.87 (0.68, 1.10)	1.21 (0.99, 1.49)

CI, confidence intervals; OR, odds ratio; SCDC, social communication disorders checklist; SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCDC, social communication disorders checklist; SCQ-SOC, communication skills subdomain of SCQ: SCQ, social communication questionnaire. The number following each outcome denotes the child’s age in years when the measure was obtained.

Autism was measured binary (yes/no), and the other outcomes represents a high level of autism-associated traits. The reference category is low adherence to a ‘healthy’ prenatal dietary pattern. High SCQ and SCDC score indicates greater difficulties. Adjusted covariates in each model; child sex, history of depression, maternal age, maternal education, planned pregnancy, prenatal alcohol intake, prenatal multivitamin supplement use, prenatal smoking, pre-pregnancy BMI, pre-pregnancy BMI, pre-

pregnancy diabetes, pre-pregnancy hypertension. Each of the dietary subgroups (plant-based, fish-based, ‘unhealthy’) were simultaneously adjusted for each other. The between correlation for each subgroup of a ‘healthy’ dietary pattern as a continuous score was below 0.50.

Table 17. Average treatment effect of adherence to a 'healthy' prenatal dietary pattern on autism diagnosis and autism-associated traits in MoBa and ALSPAC with a different adjustment set of covariates.

		[a]Paternal characteristics	[b]Birthyear	[c]SCQ uncorrected	[d]Unadjusted for prenatal multivitamin supplements
Outcome	**Adherence to 'healthy' prenatal dietary pattern**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Autism (MoBa)	Medium	0.77 (0.65, 0.90)	0.80 (0.68, 0.95)		0.78 (0.66, 0.92)
Autism (MoBa)	High	0.77 (0.65, 0.91)	0.84 (0.71, 1.00)		0.79 (0.66, 0.93)
SCQ-3 (MoBa)	Medium	1.02 (0.94, 1.10)	1.01 (0.93, 1.09)	0.95 (0.85, 1.07)	1.01 (0.93, 1.09)
SCQ-3 (MoBa)	High	1.05 (0.95, 1.16)	1.05 (0.96, 1.16)	1.08 (0.98, 1.20)	1.05 (0.96, 1.15)
SCQ-RRB-3 (MoBa)	Medium	1.08 (1.01, 1.16)	1.07 (1.00, 1.15)	1.00 (0.89, 1.12)	1.08 (1.01, 1.15)
SCQ-RRB-3 (MoBa)	High	1.26 (1.17, 1.36)	1.26 (1.16, 1.36)	1.37 (1.24, 1.51)	1.26 (1.17, 1.36)
SCQ-SOC-3 (MoBa)	Medium	0.87 (0.82, 0.93)	0.88 (0.82, 0.93)	0.95 (0.85, 1.05)	0.87 (0.82, 0.92)
SCQ-SOC-3 (MoBa)	High	0.76 (0.70, 0.82)	0.76 (0.70, 0.83)	1.07 (0.98, 1.17)	0.76 (0.70, 0.82)
SCQ-8 (MoBa)	Medium	0.96 (0.88, 1.04)	0.95 (0.87, 1.04)	0.92 (0.83, 1.02)	0.95 (0.87, 1.03)
SCQ-8 (MoBa)	High	0.96 (0.87, 1.05)	0.96 (0.88, 1.05)	0.96 (0.84, 1.09)	0.95 (0.87, 1.04)
SCQ-RRB-8 (MoBa)	Medium	0.97 (0.90, 1.05)	0.96 (0.89, 1.04)	0.90 (0.80, 1.02)	0.97 (0.90, 1.05)
SCQ-RRB-8 (MoBa)	High	1.01 (0.94, 1.08)	1.00 (0.92, 1.07)	1.07 (0.93, 1.24)	1.01 (0.95, 1.08)
SCQ-SOC-8 (MoBa)	Medium	0.95 (0.87, 1.04)	0.95 (0.87, 1.05)	0.94 (0.78, 1.13)	0.95 (0.87, 1.03)
SCQ-SOC-8 (MoBa)	High	0.91 (0.82, 1.01)	0.92 (0.83, 1.01)	0.98 (0.83, 1.17)	0.90 (0.81, 1.00)
SCDC-8 (ALSPAC)	Medium	0.75 (0.60, 0.94)			0.80 (0.63, 1.03)
SCDC-8 (ALSPAC)	High	0.77 (0.57, 1.03)			0.81 (0.61, 1.06)

ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index; MoBa, Norwegian Mother, Father, and Child Cohort; CI, confidence intervals; OR, odds ratio; SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCDC, social communication disorders checklist; SCQ-SOC, communication skills subdomain of SCQ. The number following each outcome denotes the approximate age of the child in years when the measure was obtained. i.e. SCQ-SOC-8; social communication subdomain measured at age 8 years. The presence of the outcome from each questionnaire was indicated by a high score.

Autism was measured binary (yes/no), and the other outcomes represents a high level of autism-associated traits. The reference category is low adherence to a ‘healthy’ prenatal dietary pattern.
Model c adjusted for the same covariates as the main analysis which are: child sex, history of depression, maternal age, maternal education, planned pregnancy, prenatal alcohol intake, prenatal multivitamin supplement use, prenatal smoking, pre-pregnancy BMI, pre-pregnancy BMI, pre-pregnancy diabetes, pre-pregnancy hypertension. Model a was additionally adjusted for paternal age, paternal BMI, paternal education, paternal income (MoBa only). Model b adjusted for model c covariates and was additionally adjusted for birthyear and model d adjusted for model c covariates but excluded prenatal multivitamin supplements.

Table 18. Average treatment effect of adherence to a 'healthy' prenatal dietary pattern on autism diagnosis and autism-associated traits in MoBa and ALSPAC, analyses are stratified by prenatal multivitamin supplement use, pre-pregnancy BMI, child sex and maternal education.

	Maternal education		**Prenatal supplement use**		**Pre-pregnancy BMI**		**Child sex**	
Outcome	Subgroup	OR (95% CI)	Subgroup	OR (95% CI)	Subgroup	OR (95% CI)	Subgroup	OR (95% CI)
Autism (MoBa)	p = 0.93, D^2-statistic = 0.07		p = 0.83, D^2-statistic = 0.18		p = 0.47, D^2-statistic = 0.76		p = 0.11, D^2-statistic = 2.22	
Medium	< Degree	0.78 (0.59, 1.03)	Yes	0.74 (0.55, 0.99)	< 25 kg/m^2	0.78 (0.63, 0.96)	Male	0.73 (0.61, 0.87)
High	< Degree	0.77 (0.60, 1.00)	Yes	0.76 (0.57, 1.02)	< 25 kg/m^2	0.82 (0.66, 1.01)	Male	0.78 (0.65, 0.94)
Medium	≥ Degree	0.77 (0.60, 0.99)	No	0.80 (0.66, 0.96)	≥ 25 kg/m^2	0.78 (0.61, 1.00)	Female	0.99 (0.71, 1.40)
High	≥ Degree	0.77 (0.61, 0.97)	No	0.77 (0.63, 0.95)	≥ 25 kg/m^2	0.68 (0.51, 0.90)	Female	0.71 (0.48, 1.05)
SCQ-3 (MoBa)	p = 0.011, D^2-statistic = 4.80		p = 0.012, D^2-statistic = 4.60		p = 0.78, D^2-statistic = 0.25		p = 0.007, D^2-statistic = 5.47	
Medium	< Degree	0.95 (0.85, 1.05)	Yes	0.90 (0.78, 1.05)	< 25 kg/m^2	0.98 (0.90, 1.07)	Male	0.96 (0.87, 1.05)
High	< Degree	0.96 (0.85, 1.08)	Yes	1.14 (0.98, 1.33)	< 25 kg/m^2	1.07 (0.98, 1.16)	Male	0.96 (0.87, 1.06)
Medium	≥ Degree	1.04 (0.94, 1.16)	No	1.02 (0.94, 1.10)	≥ 25 kg/m^2	1.00 (0.89, 1.13)	Female	1.04 (0.91, 1.19)
High	≥ Degree	1.17 (1.03, 1.33)	No	1.03 (0.95, 1.12)	≥ 25 kg/m^2	1.05 (0.91, 1.22)	Female	1.26 (1.09, 1.46)
SCQ-RRB-3 (MoBa)	p = 0.51, D^2-statistic = 0.68		p = 0.13, D^2-statistic = 2.05		p = 0.52, D^2-statistic = 0.66		p = 0.20, D^2-statistic = 1.64	
Medium	< Degree	1.08 (0.94, 1.23)	Yes	0.98 (0.83, 1.15)	< 25 kg/m^2	1.07 (0.98, 1.17)	Male	1.06 (0.96, 1.17)
High	< Degree	1.26 (1.09, 1.44)	Yes	1.33 (1.16, 1.53)	< 25 kg/m^2	1.32 (1.20, 1.45)	Male	1.22 (1.12, 1.33)
Medium	≥ Degree	1.04 (0.94, 1.15)	No	1.08 (1.00, 1.18)	≥ 25 kg/m^2	1.04 (0.91, 1.18)	Female	1.06 (0.94, 1.19)
High	≥ Degree	1.32 (1.17, 1.48)	No	1.26 (1.16, 1.38)	≥ 25 kg/m^2	1.21 (1.06, 1.39)	Female	1.39 (1.21, 1.61)
SCQ-SOC-3 (MoBa)	p = 0.34, D^2-statistic = 1.09		p = 0.73, D^2-statistic = 0.32		p = 0.16, D^2-statistic = 1.85		p = 0.001, D^2-statistic = 7.86	
Medium	< Degree	0.81 (0.73, 0.90)	Yes	0.86 (0.75, 0.97)	< 25 kg/m^2	0.82 (0.77, 0.88)	Male	0.83 (0.77, 0.91)
High	< Degree	0.72 (0.63, 0.81)	Yes	0.77 (0.68, 0.89)	< 25 kg/m^2	0.73 (0.67, 0.80)	Male	0.67 (0.62, 0.73)
Medium	≥ Degree	0.90 (0.82, 0.99)	No	0.87 (0.81, 0.93)	≥ 25 kg/m^2	0.94 (0.84, 1.06)	Female	0.92 (0.83, 1.01)
High	≥ Degree	0.77 (0.70, 0.86)	No	0.74 (0.68, 0.81)	≥ 25 kg/m^2	0.77 (0.69, 0.87)	Female	0.90 (0.79, 1.02)
SCQ-8 (MoBa)	p = 0.006, D^2-statistic = 5.49		p = 0.93, D^2-statistic = 0.08		p = 0.25, D^2-statistic = 1.42		p = 0.05, D^2-statistic = 3.16	

	Maternal education		Prenatal supplement use		Pre-pregnancy BMI		Child sex	
Outcome	Subgroup	OR (95% CI)	Subgroup	OR (95% CI)	Subgroup	OR (95% CI)	Subgroup	OR (95% CI)
Medium	< Degree	0.85 (0.74, 0.97)	Yes	0.96 (0.83, 1.12)	< 25 kg/m^2	1.00 (0.90, 1.11)	Male	0.90 (0.81, 0.99)
High	< Degree	0.95 (0.84, 1.08)	Yes	0.95 (0.80, 1.14)	< 25 kg/m^2	0.95 (0.85, 1.06)	Male	0.95 (0.85, 1.07)
Medium	≥ Degree	1.04 (0.94, 1.15)	No	0.95 (0.85, 1.06)	≥ 25 kg/m^2	0.87 (0.75, 1.01)	Female	1.07 (0.93, 1.24)
High	≥ Degree	0.91 (0.81, 1.01)	No	0.92 (0.83, 1.02)	≥ 25 kg/m^2	0.90 (0.79, 1.04)	Female	0.88 (0.72, 1.07)
SCQ-RRB-8 (MoBa)	p = 0.08, D^2-statistic = 2.75		p = 0.40, D^2-statistic = 0.94		p = 0.27, D^2-statistic = 1.36		p = 0.11, D^2-statistic = 2.26	
Medium	< Degree	0.90 (0.79, 1.01)	Yes	0.97 (0.86, 1.10)	< 25 kg/m^2	0.94 (0.87, 1.02)	Male	0.95 (0.85, 1.07)
High	< Degree	0.93 (0.81, 1.06)	Yes	1.04 (0.91, 1.19)	< 25 kg/m^2	1.03 (0.95, 1.12)	Male	0.95 (0.86, 1.04)
Medium	≥ Degree	1.00 (0.90, 1.12)	No	0.95 (0.86, 1.04)	≥ 25 kg/m^2	0.96 (0.83, 1.12)	Female	0.95 (0.86, 1.04)
High	≥ Degree	1.06 (0.96, 1.17)	No	0.98 (0.92, 1.06)	≥ 25 kg/m^2	0.93 (0.85, 1.03)	Female	1.08 (0.98, 1.20)
SCQ-SOC-8 (MoBa)	p = 0.27, D^2-statistic = 1.35		p = 0.34, D^2-statistic = 1.11		p = 0.024, D^2-statistic = 3.97		p = 0.016, D^2-statistic = 4.42	
Medium	< Degree	0.88 (0.77, 1.01)	Yes	1.03 (0.82, 1.29)	< 25 kg/m^2	1.03 (0.91, 1.16)	Male	0.90 (0.82, 0.99)
High	< Degree	0.88 (0.76, 1.02)	Yes	1.01 (0.81, 1.27)	< 25 kg/m^2	0.91 (0.79, 1.04)	Male	0.92 (0.82, 1.04)
Medium	≥ Degree	0.99 (0.88, 1.12)	No	0.93 (0.84, 1.03)	≥ 25 kg/m^2	0.81 (0.71, 0.92)	Female	1.04 (0.90, 1.21)
High	≥ Degree	0.88 (0.78, 0.99)	No	0.85 (0.76, 0.94)	≥ 25 kg/m^2	0.86 (0.73, 1.01)	Female	0.82 (0.70, 0.96)
SCDC-8 (ALSPAC)	p = 0.21, D^2-statistic = 1.57		p = 0.35, D^2-statistic = 1.07		p = 0.19, D^2-statistic = 1.69		p = 0.040, D^2-statistic = 3.36	
Medium	≥ A levels	0.57 (0.38, 0.87)	No	0.57 (0.38, 0.87)	< 25 kg/m^2	0.57 (0.38, 0.87)	Male	0.81 (0.61, 1.06)
High	≥ A levels	0.59 (0.36, 0.96)	No	0.59 (0.36, 0.96)	< 25 kg/m^2	0.59 (0.36, 0.96)	Male	0.89 (0.67, 1.19)
Medium	< A levels	0.86 (0.66, 1.12)	Yes	0.86 (0.66, 1.12)	≥ 25 kg/m^2	0.86 (0.66, 1.12)	Female	0.71 (0.48, 1.06)
High	< A levels	0.82 (0.62, 1.08)	Yes	0.82 (0.62, 1.08)	≥ 25 kg/m^2	0.82 (0.62, 1.08)	Female	0.48 (0.33, 0.70)

CI, confidence intervals; OR, odds ratio; p, p-value; SCDC, social communication disorders checklist; SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCDC, social communication disorders checklist; SCQ-SOC, communication skills subdomain of SCQ: SCQ, social communication questionnaire. The number following each outcome denotes the child's age in years when the measure was obtained.
Autism was measured binary (yes/no), and the other outcomes represents a high level of autism-associated traits. The reference category is low adherence to a 'healthy' prenatal dietary pattern.

High SCQ and SCDC score indicates greater difficulties. Adjusted covariates in each model; child sex, history of depression, maternal age, maternal education, planned pregnancy, prenatal alcohol intake, prenatal multivitamin supplement use, prenatal smoking, pre-pregnancy BMI, pre-pregnancy BMI, pre-pregnancy diabetes, pre-pregnancy hypertension.

Table 19. Complete case analyses measuring the associations between adherence to a 'healthy' prenatal dietary pattern and autism diagnosis and autism-associated traits in MoBa.

Outcome	Total sample (cases)	Adherence to 'healthy' prenatal dietary pattern	OR (95% CI)
Autism diagnosis (MoBa)	58553 (393)	Medium	0.80 (0.62, 1.02)
Autism diagnosis (MoBa)	58553 (393)	High	0.74 (0.57, 0.95)
High SCQ-3 score (MoBa)	32362 (2023)	Medium	1.03 (0.92, 1.16)
High SCQ-3 score (MoBa)	32362 (2023)	High	1.16 (1.03, 1.31)
High SCQ-RRB- 3 score (MoBa)	32341 (1590)	Medium	1.03 (0.90, 1.19)
High SCQ-RRB- 3 score (MoBa)	32341 (1590)	High	1.45 (1.26, 1.66)
High SCQ-SOC- 3 score (MoBa)	32296 (1924)	Medium	0.89 (0.80, 1.01)
High SCQ-SOC- 3 score (MoBa)	32296 (1924)	High	0.72 (0.63, 0.82)
High SCQ-8 score (MoBa)	26215 (1304)	Medium	1.03 (0.90, 1.19)
High SCQ-8 score (MoBa)	26215 (1304)	High	0.88 (0.76, 1.03)
High SCQ-RRB- 8 score (MoBa)	26215 (1833)	Medium	0.98 (0.82, 1.18)
High SCQ-RRB- 8 score (MoBa)	26215 (1833)	High	1.14 (0.95, 1.37)
High SCQ-SOC- 8 score (MoBa)	26215 (1054)	Medium	1.10 (0.94, 1.28)
High SCQ-SOC- 8 score (MoBa)	26215 (1054)	High	0.90 (0.76, 1.07)

CI, confidence intervals; OR, odds ratio; SCDC, social communication disorders checklist; SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCDC, social communication disorders checklist; SCQ-SOC, communication skills subdomain of SCQ: SCQ, social communication questionnaire. The number following each outcome denotes the child's age in years when the measure was obtained.
Autism was measured binary (yes/no), and the other outcomes represents a high level of autism-associated traits. The reference category is low adherence to a 'healthy' prenatal dietary pattern. High SCQ and SCDC score indicates greater difficulties. All analyses were adjusted for; maternal education, maternal age, maternal pre-pregnancy BMI, history of depression, planned pregnancy, multivitamin/folic acid supplement use in early pregnancy, alcohol intake.

5.5 Discussion

Our study found a lower likelihood of autism diagnosis and autism-associated traits in relation to HPDP, albeit inconsistently for measures of autism-associated traits. This inconsistency related to differential associations with the subdomains and at different ages. Overall, there was more consistent evidence of an association with social communication difficulties. Lastly, there was weak evidence of effect modification by child sex, but evidence of an interaction between HPDP the other characteristics was inconsistent (maternal education, pre-pregnancy BMI, and prenatal supplement use).

Our investigation into HPDP complements the wider literature on prenatal nutrition and autism related outcomes. Previous investigations have largely focused on multi-nutrient supplements, discrete nutrients, or fish intakes (Zhong et al., 2020). However, diet makes the largest contribution to overall nutrient intakes and captures the totality of complex nutrient interactions (Tapsell et al., 2016). Furthermore, our large prospective investigation addressed some key limitations observed in the existing literature. Retrospective studies have found a 'healthy' prenatal diet was associated with a lower likelihood of autism diagnosis (Geetha et al., 2018, Li et al., 2018), but a prospective investigation did not replicate their findings (Vecchione et al., 2022). However, the retrospective investigations had a high risk of bias, especially recall bias (Geetha et al., 2018, Li et al., 2018), and most studies included less than 100 children with autism (Geetha et al., 2018, Vecchione et al., 2022). Hence, our results are more robust to bias from small sample size and recall bias.

We only identified two previous prospective investigations into autism-associated traits (measured linearly), and their results were conflicting. The first investigated two cohorts with 727 and 154 mother-child dyads, and measured offspring social responsiveness score between 4-18 years and 36-months, respectively (Vecchione et al., 2022). Of the six prenatal dietary patterns investigated, only a Western prenatal dietary pattern was positively related to increased autism-associated traits, yet, this association may reflect gestational weight gain as it was only evident when unadjusted for total energy. The second prospective study of 325 mother-child dyads observed an inverse relationship between Mediterranean prenatal dietary pattern and greater offspring autism-associated traits (House et al., 2018). Autism-associated traits were measured at 12-24 months using a subcomponent of the Infant Toddler Social and Emotional Assessment questions. We obtained more precise estimates, yet our main results were also conflicting.

The variable findings on autism-associated traits may relate to the performance of the SCQ-3, SCQ-8, and SCDC-8. In addition to autism diagnosis, the SCQ and SCDC detect non-autistic children with greater social communication difficulties or inflexible behaviours and interests, which do not necessarily constitute a diagnosable condition (Skuse et al., 2005, Surén et al., 2019). This is particularly true in under four year olds, especially for restrictive and repetitive behaviours which are a common feature of typical development (Lewis and Kim, 2009). A validation study in MoBa confirmed the SCQ-3-RRB did not discriminate between autistic and non-autistic children, whereas the SCQ-SOC-3 performed fairly well (Surén et al., 2019). Additionally, we considered the full SCQ (39 items) and SCDC to be part of our main analysis as they are designed to screen for autism (Skuse et al., 2005, Surén et al., 2019), yet the SCDC only measures social communication skills (Skuse et al., 2005). Overall, social communication difficulties related more consistently to HPDP.

We observed weak evidence that females in comparison to males, had a greater reduction in the likelihood of social communication difficulties at approximately eight years old, if mothers had consumed a HPDP. This was replicated across MoBa and ALSPAC but only in relation to the highest adherence to HPDP and not at age three years. Despite considerable interest in a 'female protective effect', few investigations test for effect modification by child sex (Ferri et al., 2018). The studies that do so, observed a greater magnitude of association in females, compared to males (Levine et al., 2018, Schmidt et al., 2019a, Schmidt et al., 2019b), or no clear differences, although their main results were also null (Lyall et al., 2021, Schmidt et al., 2014, Vecchione et al., 2021, Vecchione et al., 2022).

The male preponderance of autism diagnosis contributes to debates of a female protective effect. Several mechanisms have been proposed and relate to an extreme male brain, such as sex hormones, genetics, and immune function (Ferri et al., 2018). However, misclassification bias and sex differences in developmental trajectories may impact results (McFayden et al., 2023). The conceptualisation of autism has been predominantly based on males which has led to underdiagnosis in females and can impact the performance of autism-screening tools. Additionally, many features of communication develop earlier in girls compared to boys (McFayden et al., 2023), and may further explain our conflicting results between age three and eight years. Overall, social communication skills may relate more strongly to HPDP in females, but the mechanisms remain to be determined.

Lastly, we measured the subgroups because, although nutrients are distributed across multiple foods, there are key food sources of specific nutrients, such as omega-3 fatty acids in fish. In most subgroup analysis we observed small variations in the point estimates and precision which could reflect differences in random error and bias, rather than nutritional composition. In MoBa, and to a lesser extent in ALSPAC, plant-based foods, compared to fish-based and 'unhealthy' foods, had clear associations with social communication difficulties. There are several explanations for the variation across food groups, such as: different bias structures, larger number of food items, or a true effect of plant-based foods. We identified one previous study which directly compared food groups whilst simultaneously adjusting for other foods, however the results were inconclusive, possibly due to limited statistical power to detect an association (Lyall et al., 2023). Further studies are required to clarify if plant-based foods have clearer associations with social communication difficulties.

Whether the associations observed are causal remain to be established. The aetiology of autism has been linked to several pathways such as genetics, maternal immune activation, sex hormones, the microbiome, and environmental factors (Ferri et al., 2018). It is hypothesised that prenatal diet may alter DNA methylation patterns (House et al., 2018), regulate immune processes, or interact with toxins (Zhong et al., 2020).

5.5.1 Strengths and limitations

Our study had many strengths such as the large prospective design, cross context comparison, analyses of subdomains of autism-associated traits, dietary subgroups, and interactions. We carefully considered and adjusted for a wide range of potential confounders, including paternal characteristics, and further 'tested' this through sensitivity analysis. However, unmeasured and residual confounding can still occur, for example, genetic confounding, parental care giving or childhood diet. Furthermore, autism-associated traits and prenatal diet are complex measures which rely on self-reported information, and FFQ can only provide an imprecise approximation of habitual dietary intake (Brantsaeter et al., 2008, Brantsaeter et al., 2009, Brantsaeter et al., 2007, Brantsaeter et al., 2010). We adjusted for a proportion of error through the residuals method (Willett et al., 1997). Even autism diagnosis is broadly defined, and diagnostic criteria and practices have changed over time (Reed et al., 2019, Russell et al., 2022). These factors increase heterogeneity, reduce precision, and increase bias which may have affected our results. Selection bias may impact our findings through the inevitable

exclusion of non-viable pregnancies, systematic differences in recruitment and attrition, especially in the autism-associated traits analysis due to the high attrition rate (Boyd et al., 2013, Magnus et al., 2016). Selection bias may have biased our results towards the null and reduced generalisability to non-Caucasian European ethnic minorities and deprived groups who were underrepresented in MoBa and ALSPAC (Boyd et al., 2013, Magnus et al., 2016). Multiple imputation can only partly ameliorate attrition bias.

5.5.2 Conclusions

The likelihood of autism diagnosis reduced by 22% in association with HPDP. With regard to autism-associated traits, we observed a somewhat consistent association between social communication difficulties and higher HPDP, but inconsistent associations with restrictive and repetitive behaviours. Generally, the associations were clearest when measuring HPDP, rather than discrete dietary subgroups. Lastly, females may have a greater magnitude of association with social communication difficulties at age eight years. At present, we remain uncertain as to whether the associations observed are causal.

5.5.3 Recommendations

Further research should substantiate our findings, especially given the inconsistencies in the previous literature and across our measures of autism-associated traits. It would be advantageous to measure the subdomains separately as well as combined and to improve the precision of measures of prenatal diet and autism associated traits. Additionally, representative recruitment and retention of samples representative of the general population of pregnant women is required in prospective cohort studies. Future studies could assess whether plant-based foods have a stronger association with autism-associated traits, especially, when considering the approach to define the food groups, adjust for other foods, or any additional differences in bias, such as confounding structures. Advanced approaches to dietary pattern analysis may estimate the contribution food groups make in the context of overall diet (Lyall et al., 2023, Zhao et al., 2021). Lastly, triangulation with alternative study designs and exploration of potential mediators may improve causal interpretation of the associations observed in our study.

6 Chapter 6. Maternal polygenic score for a 'healthy' prenatal dietary pattern is not associated with autism-associated traits in Mendelian randomisation analysis.

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Declarations

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Conflicts of Interest

The authors declare no conflicts of interest.

6.1 Abstract

Background

A ‘healthy’ prenatal dietary pattern has been associated with a reduced probability of autism-associated traits in prospective cohort studies and case control studies. Whether the associations are causal is unknown. We conducted a one-sample Mendelian randomisation to investigate the relationship between a maternal polygenetic score (PGS) for a ‘healthy’ prenatal dietary pattern (HPDP) and offspring autism-associated traits.

Methods

We investigated 16,569 genotyped mother-father-child trios from The Norwegian Mother, Father, and Child Cohort (MoBa) and The Avon Longitudinal Study of Parents and Children (ALSPAC). Maternal PGS for a HPDP was estimated using an independent genome-wide association study and formed the genetic instrumental variable. Outcome measures were social communication questionnaire score measured at age three (SCQ-3) and eight years (SCQ-8), and the subdomains: restrictive and repetitive behaviours (SCQ-RRB); and social communication difficulties (SCQ-SOC). High scores indicate greater autism-associated traits. In multivariate linear regression, we adjusted for child and paternal PGS, and repeated the analyses, stratified by child sex. A range of sensitivity analyses were conducted to test the robustness of findings.

Results

In the fully adjusted models, there was no clear evidence of an association between maternal PGS and each offspring outcome (SCQ-3, beta 0.03 ((95% CI) -0.04, 0.09); SCQ-RRB-3, beta 0.02 (-0.03, 0.07); SCQ-SOC-3, beta 0.01 (-0.03, 0.04); SCQ-8 beta -0.04 (-0.10, 0.02); SCQ-RRB-8, beta -0.02 (-0.05, 0.00); SCQ-SOC-8, beta -0.02 (-0.07, 0.04)), including the child sex stratified models. Sensitivity analyses indicated the strength of the genetic instrument was acceptable, and that we had sufficient power to detect moderate to large associations.

Conclusion

Larger studies are required to assess whether small to moderate associations exist between maternal PGS for HPDP and each offspring outcome.

Key messages

- We did not observe clear evidence of an association between maternal PGS for HPDP and offspring autism-associated traits.

- We only had sufficient power to detect moderate to large effects. Further studies are required to determine if there are small to moderate effects of maternal PGS for HPDP on offspring autism-associated traits.

6.2 Introduction

Autism is a neurodevelopmental condition characterised by an early onset of the core diagnostic traits; social communication difficulties, and restrictive and repetitive behaviours and interests (World Health Organisation, 2020). These autism-associated traits occur not only among people with autism diagnosis and relatives manifesting broader autism phenotypes, but are also distributed in the general population, (Kellerman et al., 2019, Moody et al., 2017). A complex combination of genetic and environmental factors influence the development of autism and autism-associated traits (Lyall et al., 2017). Although prenatal diet has been hypothesized to be involved, further research is required to establish causality.

The most frequently investigated prenatal nutritional indicators are multi-nutrient supplements and discrete nutrients (Zhong et al., 2020). However, dietary patterns make a larger contribution to overall nutrient intakes (Tapsell et al., 2016). Only four previous observational studies have investigated whole dietary patterns in association with autism related outcomes and, results are conflicting possibly due to study limitations (Geetha et al., 2018, House et al., 2018, Li et al., 2018, Lyall et al., 2023, Vecchione et al., 2022). We previously investigated two large prospective cohorts and identified a lower probability of autism diagnosis and social communication difficulties in association with a 'healthy' prenatal dietary pattern (Chapter 5). We were able to address some of the limitations of previous studies (Geetha et al., 2018, House et al., 2018, Li et al., 2018, Lyall et al., 2023, Vecchione et al., 2022) such as recall bias and small sample size. However, there is a high risk of confounding bias in prospective cohorts and case-control studies (Chapter 5, (Geetha et al., 2018, House et al., 2018, Li et al., 2018, Lyall et al., 2023, Vecchione et al., 2022)). Therefore, complementary study designs are required to support causal assessment.

Mendelian randomisation (MR) is a form of instrumental variable analysis that utilises genetic variants as instrumental variables (IV) to pursue causal queries. MR is less susceptible to confounding bias by socioeconomic and lifestyle factors compared to other observational study designs. This is because of the random allocation of alleles from parents to offspring at conception (Davey Smith and Ebrahim, 2003). In MR, the genetic variant-exposure association, and the genetic variant-outcome association can be measured in the same sample (one-sample MR) or in two non-overlapping samples (two-sample MR). Two-sample MR avoids overfitting which can occur in one-sample MR and biases away from the null. An exception is one-sample MR that uses polygenic scores (PGS)

weighted by the effect estimates obtained from an external genome-wide association study (Richardson et al., 2019).

The investigation of intrauterine exposures on offspring outcomes presents unique considerations (Lawlor et al., 2017). Firstly, the maternal IV and offspring outcome relationship is measured within mother-child pairs which can be achieved through one-sample MR. Secondly, on average, 50% of maternal genotype is conferred to their offspring. A pathway is then open between the maternal genotype and child outcome, via the child's genotype that is correlated with the maternal IV. The pathway can be blocked through adjustment for the child's genotype, yet this may introduce collider bias, therefore it is advantageous to adjust for paternal genotype (Lawlor et al., 2017).

In the current study, we investigated the relationship between a 'healthy' prenatal dietary pattern and offspring autism-associated traits at age three and eight years. We applied one-sample MR using genome-wide significant polygenic scores (PGS) associated with a 'healthy' prenatal dietary pattern as IV. We utilised within family genotyped mother-father-child trios to adjust for genetic confounding via the child and further adjusted for potential collider bias via the father. We applied the same methods to our secondary analysis, where we measured the subdomains of autism-associated traits: social communication difficulties, and restrictive and repetitive behaviours, and stratified by child sex to assess effect modification.

6.3 Methods

6.3.1 Sample population

MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (Magnus et al., 2016). Participants were recruited from all over Norway from 1999-2008. The women consented to participation in 41% of pregnancies. The cohort includes approximately 114,500 children, 95,200 mothers and 75,200 fathers. Blood samples were obtained from both parents during pregnancy and mothers and children (via umbilical cords) at birth (Paltiel et al., 2014). The current study is based on version 12 of the quality-assured data files released for research in January 2019. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is currently regulated by the Norwegian Health Registry Act. The current study was pre-approved by The Regional Committees for Medical and Health Research Ethics (2016/1702).

We restricted to unrelated mother-father-child trios to avoid bias due to intracluster correlations between related sets of trios. Trios were of European genetic ancestry and had offspring outcome data at age three (trios = 16569) and eight years (trios = 12600). Genotyping and quality control procedures are previously described (Corfield et al., 2022).

6.3.2 Outcome

The SCQ was completed by primary care giver about the child at approximately age three (SCQ-3) and eight years (SCQ-8). The SCQ is a 40-item questionnaire with a yes/no response format but only 39-items are scored as the first question identifies verbal and non-verbal children (Rutter, 2003). The SCQ focuses on social communication skills (22 items), and restrictive and repetitive behaviours (11 items). Children without phased speech may be underscored (Eaves et al., 2006), as a result those questions related to phased speech were excluded. We measured the total SCQ score and the two subdomains, social communication skills (SCQ-SOC-3 and SCQ-SOC-8) and restrictive and repetitive behaviours (SCQ-RRB-3 and SCQ-RRB-8). A high score represented more autism-associated traits.

6.3.3 'Healthy' prenatal dietary pattern

The genome-wide association study (GWAS) for a 'healthy' dietary pattern was based on 181,211 females of Caucasian European ancestry age 40-69 years in the UK Biobank (Niarchou et al., 2020). The GWAS detected 23 genome-wide significant ($p < 5x10^{-8}$) SNPs.

6.3.4 Assumptions

The minimum assumptions required to infer causality in MR are set out below. We do not seek to estimate the magnitude of effect which requires additional point-estimate-identifying conditions (Sanderson et al., 2022). See Figure 6 for DAG and explanation of the assumptions.

1. Relevance: the genetic IV is robustly associated with the exposure

2. Independence: the genetic IV and outcome do not share a common cause (no confounding)

3. Exclusion restriction: the genetic IV is only associated with the outcome through the exposure.

6.3.5 Statistical methods

The trio PGS-MR is the main analysis. Independent ($r^2 = <0.1$, 500 kb) genome-wide significant SNPs were used in PRSice (Euesden et al., 2015) to calculate the mother, father, and child PGS as the weighted sum of the individuals alleles associated with a HPDP. For brevity, the PGS for a HPDP will be referred to as diet PGS throughout and an increased diet PGS indicates greater adherence to a HPDP. In multivariate linear regression models, we estimated the association between maternal PGS and each offspring's outcome. To prevent a violation of assumption three (exclusion restriction) we adjusted for the child PGS, and then the father's PGS in the final adjusted main models. All models were adjusted for genotype batch and 10 principal components of population structure to adjust for population stratification (Burgess et al., 2019). There may be aetiological differences by child sex (Ferri et al., 2018) therefore, we also ran all models stratified by child sex.

6.3.6 Sensitivity analysis

In the MoBa trios, we measured the proportion of variance explained, and to assess assumption one (genetic IV robustly associated with exposure), we additionally measured the F-statistic, in relation to a HPDP (as derived in Chapter 5). The F-statistic from the regression of HPDP score on the maternal PGS for HPDP can indicate the strength of the genetic IV. A rule of thumb is that an F-statistic of ≥10% suggests the genetic instrument robustly predicts the exposure (Burgess and Thompson, 2011).

To assess the likelihood of violation of MR assumption two (independence), we measured the association between a range of potential confounders (see Table 20 for list of covariates) and maternal PGS in multivariate linear regression models, adjusted for genotype batch and 10 principal components of population structure, and then further adjusted for child and father PGS. We presented a range of sample characteristics (mean (SD) and proportions).

We performed two-sample MR to assess the likelihood of violation of assumptions of no horizontal pleiotropy and reverse causality because this is more readily assessed in two-sample MR (Hemani et al., 2018). Two-sample MR uses two independent samples to obtain summary level exposure-SNP and outcome-SNP associations. We used the same SNPs used to weight the PGS for the exposure-SNP effect estimates in the discovery sample (Niarchou et al., 2020), and in MoBa, we measured the SNP-outcome effect estimates using multivariate linear regression, adjusted for genotype batch and 10 principal components. To detect evidence of potential violation of MR assumption three (exclusion restriction), we applied different statistical estimation methods with different assumptions about the mode of pleiotropy; the inverse variance weighted (IVW) method, the weighted median, contamination mixture, and MR-Egger (Slob and Burgess, 2020). The I^2_{GX} was estimated to assess the robustness of MR-Egger analyses (Bowden et al., 2016). Outliers can also indicate horizontal pleiotropy, so we estimated the contribution each individual SNP makes to the Q-statistic (Bowden et al., 2018). Reverse causality was assessed through Steiger filtering (Hemani et al., 2017) and reverse MR, whereby SCQ-SNP effect estimates were modelled as a potential cause of the HPDP -SNP effect estimates. We used the genome-wide association study of each outcome from MoBa to identify SNPs at a relaxed threshold of $p = <5 \times 10^{-6}$.

Sample size calculation was conducted for the PGS-MR analyses (Brion et al., 2012). Analyses were conducted using R-studio version 4.0.3 (2020-10-10) (PGS-MR analyses) and 4.2.2 (2022-10-31) (two-sample MR sensitivity analyses) (R Core Team, 2022).

6.4 Results

See Table 20 for descriptive statistics on sample characteristics and Table 21 for breakdown of the sample size for each analysis.

Table 20. Socioeconomic and lifestyle characteristics across MoBa genotyped subsample.

Characteristic	Level	MoBa genotype
Number of pregnancies		16,569
Prenatal multivitamin supplement use (%)	No	9499 (57.3)
	Yes	4841 (29.2)
	Missing	2229 (13.5)
Maternal education (%)	9-year elementary	222 (1.3)
	1-2 years further education	504 (3.0)
	Vocational	1602 (9.7)
	Sixth form	1875 (11.3)
	University/ college ≤4 years	6243 (37.7)
	University/ college > 4 years	3686 (22.2)
	Missing	2437 (14.7)
Pre-pregnancy diabetes (%)	No	14549 (87.8)
	Yes	114 (0.7)
	Missing	1906 (11.5)
Pre-pregnancy BMI (mean (SD))		23.9 (4.0)
	Missing	0 (0)
Pre-pregnancy depression (%)	No	13528 (81.6)
	Yes	1135 (6.9)
	Missing	1906 (11.5)
Planned pregnancy (%)	No	2160 (13.0)
	Yes	12530 (75.6)
	Missing	1879 (11.3)
Prenatal smoking (%)	No	12536 (75.7)
	Yes	909 (5.5)
	Missing	3124 (18.9)
Prenatal alcohol intake (%)	No	11423 (68.9)
	Yes	1693 (10.2)
	Missing	3453 (20.8)
Child sex (%)	Male	8459 (51.1)
	Female	8110 (48.9)
	Missing	0 (0)
Pre-pregnancy hypertension (%)	No	14783 (89.2)
	Yes	140 (0.8)
	Missing	1646 (9.9)
SCQ-3 (mean (SD))		6.2 (3.3)

Characteristic	Level	MoBa genotype
	Missing	0 (0)
SCQ-RRB-3 (mean (SD))		3.8 (2.5)
	Missing	0 (0)
SCQ-SOC-3 (mean (SD))		2.3 (1.8)
	Missing	0 (0)
SCQ-8 (mean (SD))		3.3 (2.9)
	Missing	0 (0)
SCQ-RRB-8 (mean (SD))		0.6 (1.2)
	Missing	0 (0)
SCQ-SOC-8 (mean (SD))		2.6 (2.4)
	Missing	0 (0)
Autism diagnosis (%)	No	16,282 (98.3)
	Yes	287 (1.7)
	Missing	0 (0)

BMI, body mass index; MoBa, Norwegian Mother, Father, and Child Cohort; NOK, Norwegian Krone; SCQ, social communication questionnaire: SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviors and interests; SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years; SD, standard deviation.

Table 21. Number of trios in each analysis

Outcome	Trios	Trios - males	Trios - females
SCQ-3	16569	8459	8110
SCQ-RRB-3	16531	8442	8089
SCQ-SOC-3	16562	8453	8109
SCQ-8	12600	6467	6133
SCQ-RRB-8	12590	6461	6129
SCQ-SOC-8	12591	6463	6128

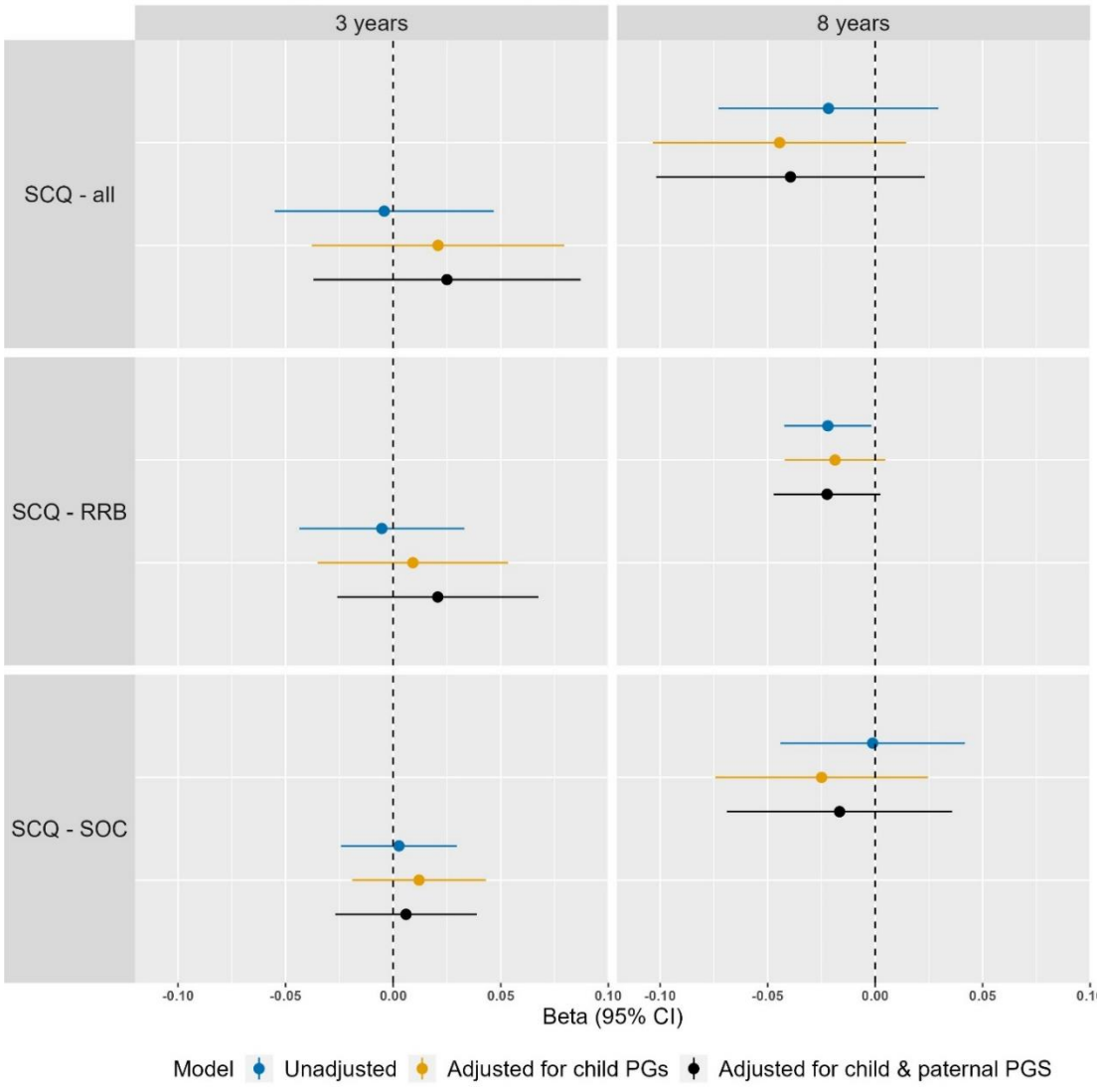
SCQ, social communication questionnaire; SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests: SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years.
Mother-father-child trios were used in the main models.

6.4.1 Main results, trio PGS-MR

6.4.1.1 Polygenic score analysis

Of the 23 SNPs associated with a HPDP in the discovery GWAS, 18 were available in the quality controlled MoBa data and included in the PGS. There was no clear evidence of association between maternal PGS and offspring outcomes in the main models (Figure 18 and Table 22) or sex stratified models (Figure 19 and Table 23).

Figure 18. The association between maternal polygenic score (PGS) for a 'healthy' prenatal dietary pattern and offspring social communication questionnaire outcomes.



SCQ, social communication questionnaire: SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests: SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years. Estimates are interpreted as the likelihood of each outcome per 1 SD increase in the polygenic score for a 'healthy' prenatal dietary pattern factor score. Higher outcome scores represent greater autism-associated traits. Higher maternal PGS relates to a higher factor score and indicates greater

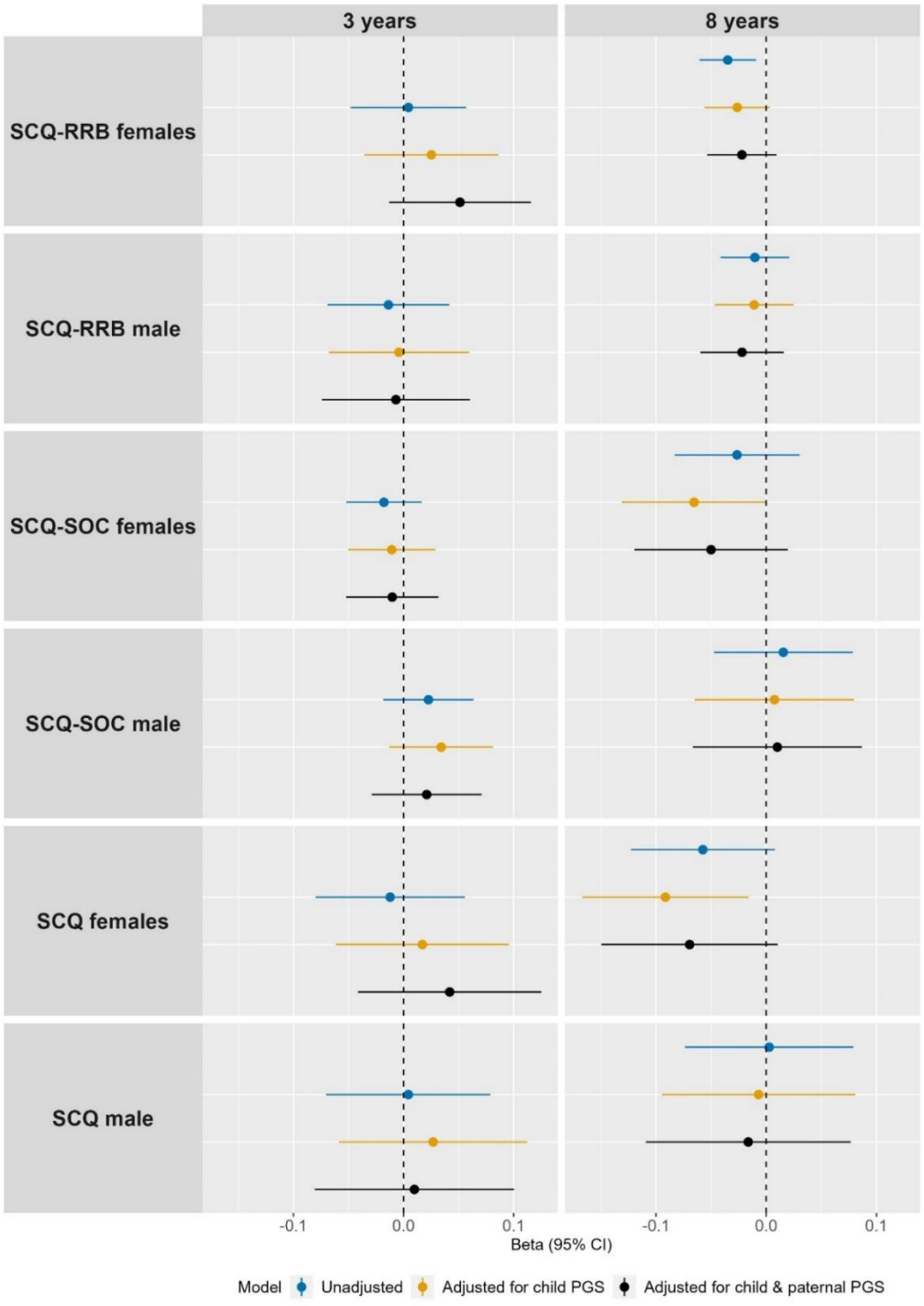
adherence to a ‘healthy’ prenatal dietary pattern. All results are adjusted for child and paternal diet PGS.

Table 22. The association between maternal polygenic score for a ‘healthy’ prenatal dietary pattern and offspring outcomes using Mendelian randomisation.

	Unadjusted for child & father PGS		**Adjusted for child PGS**		**Adjusted for child & father PGS**	
Outcome	Beta (95% CI)	P-value	Beta (95% CI)	P-value	Beta (95% CI)	P-value
Trios						
SCQ-3	-0.004 (-0.055, 0.047)	0.87	0.021 (-0.038, 0.080)	0.49	0.025 (-0.037, 0.087)	0.43
SCQ-RRB-3	-0.005 (-0.044, 0.033)	0.79	0.009 (-0.035, 0.053)	0.68	0.021 (-0.026, 0.068)	0.39
SCQ-SOC-3	0.003 (-0.024, 0.030)	0.84	0.012 (-0.019, 0.043)	0.45	0.006 (-0.027, 0.039)	0.72
SCQ-8	-0.022 (-0.073, 0.029)	0.41	-0.044 (-0.103, 0.014)	0.14	-0.039 (-0.102, 0.023)	0.22
SCQ-RRB-8	-0.022 (-0.042, -0.002)	0.034	-0.019 (-0.042, 0.005)	0.12	-0.022 (-0.047, 0.002)	0.08
SCQ-SOC-8	-0.001 (-0.044, 0.042)	0.96	-0.025 (-0.074, 0.024)	0.32	-0.017 (-0.069, 0.036)	0.54

CI, confidence interval; PGS, Polygenic score: SCQ, social communication questionnaire; SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests: SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years.
Estimates are interpreted as the likelihood of each outcome per 1 SD increase in the polygenic score for a ‘healthy’ prenatal dietary pattern factor score. Higher outcome scores represent greater autism-associated traits. Higher maternal diet PGS relates to a higher factor score and indicates greater adherence to a ‘healthy’ prenatal dietary pattern.

Figure 19. The association between maternal polygenic score (PGS) for a ‘healthy’ prenatal dietary pattern and offspring social communication questionnaire outcomes stratified by offspring sex.



SCQ, social communication questionnaire: SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests: SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years. Estimates are interpreted as the likelihood of each outcome per 1 SD increase in the polygenic score for a ‘healthy’ prenatal dietary pattern factor score. Higher outcome scores represent greater autism-associated traits. Higher maternal diet PGS relates to a higher factor score and indicates greater adherence to a ‘healthy’ prenatal dietary pattern.

Table 23. Sex stratified models for the association between maternal polygenic score for a ‘healthy’ prenatal dietary pattern and offspring outcomes using Mendelian randomisation.

		Unadjusted for child or father PGS		**Adjusted for child PGS**		**Adjusted for child & father PGS**	
Outcome	**Child sex**	Beta (95% CI)	P-value	Beta (95% CI)	P-value	Beta (95% CI)	P-value
Trios							
SCQ-3	Males	0.004 (-0.070, 0.079)	0.91	0.027 (-0.059, 0.112)	0.54	0.010 (-0.081, 0.100)	0.83
SCQ-3	Females	-0.012 (-0.080, 0.056)	0.73	0.017 (-0.061, 0.096)	0.67	0.042 (-0.041, 0.125)	0.32
SCQ-RRB-3	Males	-0.014 (-0.069, 0.042)	0.63	-0.004 (-0.068, 0.059)	0.90	-0.007 (-0.074, 0.060)	0.84
SCQ-RRB-3	Females	0.004 (-0.048, 0.057)	0.87	0.025 (-0.036, 0.086)	0.42	0.051 (-0.013, 0.116)	0.12
SCQ-SOC-3	Males	0.023 (-0.018, 0.064)	0.28	0.034 (-0.013, 0.081)	0.16	0.021 (-0.029, 0.071)	0.41
SCQ-SOC-3	Females	-0.018 (-0.052, 0.016)	0.31	-0.011 (-0.050, 0.029)	0.59	-0.010 (-0.052, 0.032)	0.63
SCQ-8	Males	0.003 (-0.074, 0.079)	0.95	-0.007 (-0.095, 0.081)	0.88	-0.016 (-0.109, 0.077)	0.73
SCQ-8	Females	-0.058 (-0.123, 0.008)	0.08	-0.092 (-0.167, -0.016)	0.018	-0.070 (-0.150, 0.011)	0.09
SCQ-RRB-8	Males	-0.010 (-0.041, 0.021)	0.52	-0.011 (-0.047, 0.025)	0.55	-0.022 (-0.060, 0.016)	0.26
SCQ-RRB-8	Females	-0.035 (-0.061, -0.009)	0.008	-0.026 (-0.056, 0.003)	0.08	-0.022 (-0.054, 0.009)	0.17
SCQ-SOC-8	Males	0.016 (-0.047, 0.079)	0.63	0.007 (-0.065, 0.080)	0.84	0.010 (-0.067, 0.087)	0.80
SCQ-SOC-8	Females	-0.027 (-0.083, 0.030)	0.36	-0.066 (-0.131, 0.000)	0.05	-0.050 (-0.120, 0.020)	0.16

CI, confidence interval; PGS, Polygenic score: SCQ, social communication questionnaire: SCQ-RRB, social communication questionnaire subdomain, restrictive and repetitive behaviours and interests: SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years.
Estimates are interpreted as the likelihood of each outcome per 1 SD increase in the polygenic score for a ‘healthy’ prenatal dietary pattern factor score. Higher outcome scores represent greater autism-associated traits. Higher maternal diet PGS relates to a higher factor score and indicates greater adherence to a ‘healthy’ prenatal dietary pattern.

6.4.2 Sensitivity analyses results

6.4.2.1 Consideration of Mendelian randomisation core assumptions

Assumption one. The strength of association a 'healthy' prenatal dietary pattern had with maternal PGS was acceptable and a small proportion of variance was explained by maternal PGS (F-statistic = 11.8, R^2 = 1.2%) (Supplementary table 7). An F-statistic ≥10 is regarded as acceptable (Burgess et al., 2011).

Assumption two and three. Maternal PGS was not associated with any potential confounding factors, except maternal income over 500,000 Norwegian Krone (Supplementary table 8). Additional tests for horizontal pleiotropy were assessed in two sample Mendelian randomisation. We estimated the R^2 for HPDP to be 0.34% in the discovery genome-wide association sample based on the 18 SNPs used in PGS-MR. Subsequently the adjusted I^2_{GX} statistic was zero and the MR-Egger estimates were unlikely to be reliable even with simulation extrapolation (Bowden et al., 2016). Hence, we removed the MR-Egger sensitivity analysis and results are presented in Supplementary table 9 for reference. Two-sample MR results were reasonable consistent across the inverse variance weighted, weighted median, contamination mixture (Supplementary table 10). There was no clear evidence of heterogeneity across all SNPs based on Q-statistic (Supplementary table 11). However, outliers were identified in the following outcome models; SCQ-3 (rs7098100), SCQ-RRB-3 (rs11209952), SCQ-SOC-3 (rs7098100) (Supplementary table 12-14 and Supplementary figure 4-9). Steiger filtering identified two potentially invalid SNPs from two separate outcome models (SCQ-RRB-3 (rs11209952) and SCQ-SOC-8 (rs150175599)). Removal of potentially invalid SNPs in the leave-one-out analysis led to broadly similar conclusions for each outcome, except SCQ-3 and SCQ-RRB-3 analysis (Supplementary figure 10-15). Inclusion of the potentially invalid SNP (rs7098100) biased towards the null in the SCQ-3 analysis. For the SCQ-RRB-3 analysis, the potentially invalid SNP (rs11209952) biased away from the null. Lastly, we were unable to conduct a reverse MR as no SCQ-SNP effect estimates met the threshold of $p = < 5x\ 10^{-6}$.

6.4.2.2 Sample size calculation

Using Brion et al's equations (Brion et al., 2012), and based on our sample size, we had 80% power to detect an effect of Beta = -0.35 (Supplementary table 15).

6.5 Discussion

We assessed the relationship between HPDP and SCQ score in offspring at ages three and eight years, using maternal diet PGS as a genetic instrumental variable. No clear evidence of association was observed in our fully adjusted main analyses or child sex stratified models. A range of sensitivity analyses were applied to assess the robustness of our findings to violations of the assumptions and other common limitations. We observed minimal evidence that the models related to age eight outcomes were biased by horizontal pleiotropy. However, the leave-one-out analysis indicated that removal of the potentially invalid SNPs in the SCQ-3 and SCQ-RRB-3 may alter the conclusions drawn. Thus, we have less confidence in PGS-MR results related to SCQ-3 and SCQ-RRB-3. Lastly, we had sufficient power to detect a moderate to large magnitude of association (-0.35 or larger).

We observed a modest strength of association between maternal diet PGS and HPDP. The modest strength of our genetic instrument is typical in complex traits (Mathieson, 2021). Many factors drive dietary choices and this variability increases in pregnancy due to, for example, increased health consciousness, morning sickness/ hyperemesis gravidarum, hormonal adaptations, changes in energy balance, and/or increased risk of illness during pregnancy (Poulain et al., 2021). The omnigenic model of complex traits posits that most heritability is dispersed across the human genome, with only a small number of 'core' genes that are used as IV (Boyle et al., 2017). Additionally, GWAS methods, measurement of SCQ, and dietary assessment methods, such as the single 24-hour recall used in discovery GWAS, are prone to systematic and random error (Burrows et al., 2019, Mathieson, 2021, Surén et al., 2019, Young et al., 2019). Thus, the maternal diet PGS detects a small facet of the variability of HPDP.

In the absence of MR and RCT, which measure the effect of prenatal diet on autism related outcomes, we considered studies of prenatal nutrition and neurodevelopmental outcomes more broadly. RCTs (Caffrey et al., 2021, D'souza et al., 2021, McNulty et al., 2019, Nazeri et al., 2021, Nevins et al., 2021, Sass et al., 2020, Srinivasan et al., 2020, Thomas et al., 2019, Zhu et al., 2023) and MR (Bonilla et al., 2012, Caramaschi et al., 2017, Lewis et al., 2014, Madley-Dowd et al., 2022, Steenweg–de Graaff et al., 2012) investigations produced null associations or limited evidence of an effect. Assessments of causality through analogy requires careful consideration due to different causal effects, assumptions, and bias structures (Schwingshackl et al., 2021). For example, severe prenatal iodine deficiency is an established cause of a congenital syndrome of intellectual and physical

disabilities but there is less clear evidence for less severe iodine deficiency and for other neurodevelopmental outcomes (Levie et al., 2018, Pearce and Zimmermann, 2023). Furthermore, previous investigations into child neurodevelopment focused on the IV of biochemical indicators of single nutrients in MR (Bonilla et al., 2012, Caramaschi et al., 2017, Lewis et al., 2014, Madley-Dowd et al., 2022, Steenweg–de Graaff et al., 2012), and RCTs focused on single and select nutrient supplements in the second and/or third trimester (Caffrey et al., 2021, D'souza et al., 2021, McNulty et al., 2019, Nazeri et al., 2021, Nevins et al., 2021, Sass et al., 2020, Srinivasan et al., 2020, Thomas et al., 2019, Zhu et al., 2023). However, dietary patterns measure whole dietary intakes and reflect the summation of nutrient interactions and their cumulative and latent effects (Tapsell et al., 2016). Hence, the potential causal effect estimate sought is different across previous studies and compared to the current investigation.

The lack of clear evidence of association in RCTs and MR may relate to a lack of causal effect. However, several factors may lead to these results and only a few key considerations are highlighted here. Response to nutritional supplements may be relative to baseline nutritional status which is often not considered in RCTs (Stephenson et al., 2018). Additionally, supplementation of certain nutrients in the second and third trimester may be insufficient to achieve sufficient biochemical nutrient levels within the critical phrase or even in the pregnancy as a whole. Indeed, researchers have highlighted that interventions in the preconception period may be most effective (Stephenson et al., 2018). Previous MR studies addressed some limitations as the IV reflects prolonged exposure to discrete biochemical indicators of nutrients. However, other complexities in nutrition are challenging to address. From example, non-linear effects can be challenging to model in MR and the extent to which an IV can reflect states of nutrient deficiency and excess is unclear (Wade et al., 2022). Hence, all MR investigations on prenatal nutrients and offspring neurodevelopment modelled linear effects and several authors acknowledged that the effects measured did not reflect extreme nutrient levels (Lewis et al., 2014, Madley-Dowd et al., 2022, Steenweg–de Graaff et al., 2012).

Triangulation, however, focuses on the comparison of results using different methodology to examine similar causal questions (Lawlor et al., 2016). Previous investigations identified that ‘healthy’ or balanced prenatal dietary patterns were associated with a lower likelihood of autism diagnosis in a prospective cohort study (Chapter 5) and case-control studies (Geetha et al., 2018, Li et al., 2018), although one study observed no clear evidence of

association (Vecchione et al., 2022). However, evidence in relation to autism-associated traits is more conflicting (Chapter 5, (House et al., 2018, Lyall et al., 2023, Vecchione et al., 2022). It is unclear whether methodological limitations led to conflicting results, such as confounding bias. To the best of our knowledge, our MR investigation is the first to use prenatal dietary patterns on neurodevelopmental outcomes. Our IV approach provides an alternative methodology which can address confounding bias through socioeconomic and lifestyle characteristics (Lawlor et al., 2017). We did not observe clear evidence of a causal effect of HPDP on autism-associated traits, however, our study was unpowered to detect small to moderate estimates of association.

6.5.1 Strengths, key considerations, and limitations

Our PGS-MR had many strengths. In this first study to investigate prenatal dietary patterns and offspring neurodevelopment. We directly related maternal diet PGS to HPDP and offspring outcomes and adjusted for chid and paternal diet PGS, which reduced the risk of a violation of the exclusion restriction criteria (Lawlor et al., 2017). Additionally, adjusting for paternal diet PGS reduced the risk of bias from cross-trait assortative mating. The evidence of association we observed between maternal diet PGS and SCQ outcomes was attenuated with adjustment for the child and paternal diet PGS. Hence, these associations may be biased by child diet PGS and/or collider bias. A low risk of confounding bias by socioeconomic and lifestyle characteristics was observed, and we had no clear evidence of reverse causality in the overall maternal diet PGS. Approaches to assess horizontal pleiotropy are subject to fallible assumptions (Burgess et al., 2019, Hemani et al., 2017), thus we applied a range of approaches that make different assumptions about the nature of pleiotropy. We observed some evidence of horizontal pleiotropy through outliers, Steiger filtering and univariate analysis. However, this did not appear to markedly alter the pooled estimates from all SNPs, except in the SCQ-3 and SCQ-RRB-3.

Furthermore, a consideration is whether the exposure period was pregnancy, as genetic variants are present from conception (Diemer et al., 2021). Maternal diet PGS may reflect post pregnancy diet and may impact the child's diet, through maternal provision of diet for the child (Wade et al., 2022). Additionally, early research suggests diet may even alter primordial oocytes (main female gametes present from birth) (Di Berardino et al., 2022), and environmental factors are hypothesised to alter the likelihood of offspring autism through epigenic changes in oocytes (Escher et al., 2022). Nonetheless, bias due to pre- or

post-pregnancy diet would be more likely to lead to an association with each outcome, yet our findings were null.

We were unable to assess directional pleiotropy through MR-Egger, due to severe violation of the 'no measurement error assumption'. Lastly, the discovery GWAS (UK Biobank) (Fry et al., 2017) and MoBa cohorts have systematic differences in sample characteristics compared to the general population (Magnus et al., 2016) and we observed systematic difference in characteristics associated with attrition. Selection bias in the maternal diet PGS-outcome relationships may occur, as demonstrated in several previous studies (Gkatzionis and Burgess, 2018, Munafò et al., 2017, Taylor et al., 2018), and in most real-life examples, bias was towards the null (Taylor et al., 2018). Furthermore, live birth bias also causes selection bias (Diemer et al., 2021), and can even reverse the direction of effect (Liew et al., 2015, Raz et al., 2018). Hence, our findings may not generalise to less affluent populations of non-European ancestry (Mathieson, 2021). Lastly, the proportion of variance in HPDP explained by maternal diet PGS was low and reduced our power to detect small-moderate causal effects (Brion et al., 2012).

6.5.2 Conclusion

We did not find convincing evidence of an association between HPDP and offspring autism-associated traits. Our results are unlikely to be explained by many potential limitations such as those discussed, however, we cannot exclude selection bias and we had insufficient statistical power to detect small to moderate effects, should one exist.

6.5.3 Recommendations for future studies

Larger genotyped within-family samples would be advantageous, especially intergeneration samples. Additionally, concerted efforts should be made to address potential selection bias at recruitment and retention phases. Robust dietary instruments are necessary but need to be underpinned by improvements to the dietary assessment methods applied in large cohort studies. Additionally, the field of nutritional epidemiology is progressing from basic linear models of diet and outcome relationships, to advanced statistical approaches to better model the complexity of diet and nutrients (Lyall et al., 2023, Morgenstern et al., 2021) and adjust for systematic and random error (McCullough and Byrd, 2022). These methodological approaches were not applied in the discovery genome-wide association study (Niarchou et al., 2020), and methodological developments

are required to identify how best to model the complexity of nutrition in MR (Wade et al., 2022). Methods such as MR have the potential to make a valuable contribution to causal investigations in nutritional epidemiology (Ohukainen et al., 2021). There should be continued efforts to improve causal investigations in nutritional epidemiology.

7 Chapter 7. The role of a 'healthy' prenatal dietary pattern in explaining associations between maternal socioeconomic indicators of deprivation and offspring autism diagnosis and autism-associated traits

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Declarations

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Conflicts of Interest

The authors declare no conflicts of interest.

7.1 Abstract

Background

Socioeconomic deprivation is associated with offspring autism diagnosis and autism-associated traits through multiple pathways. We previously observed a lower likelihood of autism diagnosis and autism-associated traits in relation to a 'healthy' prenatal dietary pattern (HPDP) in two large prospective cohorts, The Norwegian Mother, Father, and Child Cohort (MoBa), and The Avon Longitudinal Study of Parents and Children (ALSPAC). Here, we applied causally informed mediation analysis to measure the extent to which HPDP may explain the relationship between maternal deprivation and autism related outcomes.

Methods

We restricted to singleton pregnancies with food frequency questionnaire (FFQ) responses, which resulted in 84,548 pregnancies from MoBa (recruited 2002-2008), and 11,760 pregnancies from ALSPAC (recruited 1990-1992). The outcome measure in ALSPAC was high social communication disorders checklist score (SCDC-8), and in MoBa, were autism diagnosis, high social communication questionnaire score at age three years (SCQ-3) and eight years (SCQ-8), and the subdomains social communication difficulties, restrictive and repetitive behaviours. The mediator (HPDP) was derived through factor analysis. The total effect (TE) and controlled direct effect (CDE) of maternal education, parental income and Townsend deprivation on each outcome were estimated in marginal structural models, and adjusted for potential confounders.

Results

Generally, low maternal education and low parental income associated with an increased probability of each outcome with the exception of Townsend deprivation, which showed no clear association with SCDC-8. The CDE analysis indicated that HPDP explained a modest proportion of these associations, if at all.

Conclusion

Maternal education had the strongest associations with each outcome, although the proportion explained through HPDP was modest. Hence, if found to be causally associated, HPDP may reduce inequalities as part of a multifaceted intervention.

Key messages

- The probability of autism diagnosis and autism-associated traits increased in relation to indicators of high maternal deprivation.

- The probability of autism and autism associated traits in relation to indicators of high maternal deprivation partly reduced under the hypothetical scenario that everyone had a high adherence to a HPDP.

- The associations were strongest in relation to maternal education, as was the proportion eliminated through a high adherence to a HPDP.

7.2 Introduction

Autism spectrum disorder, herein referred to as autism (Kenny et al., 2016), is a complex cluster of conditions that are diagnosed based on difficulties with social communication along with restricted and repetitive behaviours. We use 'autism-associated traits' to refer to social communication difficulties and restricted and repetitive behaviors, that in addition to autism, also occur as part of many other neurodevelopmental trajectories and psychiatric conditions (Moody et al., 2017, Rosenberg et al., 2018).

Autism diagnosis and autism-associated traits have a complex aetiology related to genetic and environmental factors, which may include maternal socioeconomic deprivation (Lipkin et al., 2023). Socioeconomic deprivation has been associated with an increased likelihood of autism diagnosis (Rai et al., 2012, Roman-Urrestarazu et al., 2022) and autism-associated traits (Rosenberg et al., 2018). However, there are exceptions (Kelly et al., 2019, King and Bearman, 2011). It has been suggested that diagnostic bias increases the prevalence of autism diagnosis in children from affluent families, as they have greater resources with which to navigate health care systems (Lord, 2013, Sturm et al., 2021).

Several environmental factors, have been associated with autism (Villamor et al., 2022, Zhong et al., 2020) and are, in part, socioeconomically determined such as, prenatal nutrition (Sawyer et al., 2021). Suboptimal prenatal nutritional status is more prevalent in socially deprived groups (Sauder et al., 2021) who may also face more barriers to making healthy dietary changes during pregnancy (Gete et al., 2022). We previously demonstrated a lower likelihood of autism diagnosis and autism-associated traits in association with a medium and high adherence to HPDP (Chapter 5). Whilst we recognise aetiological understanding of autism diagnosis and autism-associated traits is evolving, if prenatal diet were to be causally related, evidence to support equitable intervention strategies would be beneficial (Adams et al., 2016).

The CDE is a causally informed mediation analysis (Pearl, 2001, Robins and Greenland, 1992, VanderWeele, 2009) of relevance to public health (Naimi et al., 2014, VanderWeele, 2009). The CDE represents the association between socioeconomic deprivation and autism related outcomes, under the hypothetical scenario that everyone received HPDP. Thereby simulating the potential impact of a dietary 'intervention' and providing mechanistic understanding to support aetiological knowledge. To provide context, the CDE is compared to the TE socioeconomic deprivation has on autism related outcomes. This

approach can overcome three main sources of bias observed in traditional mediation analyses (Chapter 3.7 and (Richiardi et al., 2013, VanderWeele, 2009)). Namely, the inaccurate conceptualisation and modelling of mediator-outcome confounding, mediator-outcome confounding affected by the exposure, and exposure-mediator interaction (VanderWeele, 2009).

Therefore, we sought to extend our previous work to improve the relevance of our findings to public health policy. We measured the TE and the CDE of a range of maternal socioeconomic indicators in relation to autism diagnosis and autism-associated traits in two large European cohorts with universal access to healthcare, the MoBa, and the ALSPAC. The aim of the present study was to examine the extent to which HPDP may explain the relationship between maternal deprivation and autism related outcomes.

7.3 Methods

7.3.1 Study population

We investigated the MoBa and ALSPAC cohorts independently of each other but where possible, the analytic approach was replicated.

MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (Magnus et al., 2016). Participants were recruited from all over Norway from 1999-2008. The women consented to participation in 41% of the pregnancies. The cohort includes approximately 114,500 children, 95,200 mothers and 75,200 fathers. The current study is based on version 12 of the quality-assured data files released for research in January 2019. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is currently regulated by the Norwegian Health Registry Act. The current study was approved by The Regional Committees for Medical and Health Research Ethics (2013/201). Postal questionnaires used in the present study were distributed at 15-, 22-, and 30-weeks' gestation and when the child was three and eight years old.

ALSPAC is a large prospective cohort study conducted in England with an estimated response rate to participate of 71.8% (Boyd et al., 2013). Pregnant women resident in the Southwest of England with expected dates of delivery between 1st April 1991 and 31st December 1992. The initial number of pregnancies enrolled was 14,541. For further details see (Boyd et al., 2013, Fraser et al., 2013). Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (www.bristol.ac.uk/alspac/researchers/our-data). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Postal questionnaires were distributed at 17-, 24-, and 32-weeks' gestation, and when the child was 7.6 years old.

We excluded pregnancies without FFQ responses (MoBa: 15,684, ALSPAC: 2196), non-singletons (MoBa: 11661, ALSPAC: 304), and implausible FFQ responses in MoBa (1393 (≤4.5 Mega joules (MJ) and ≥20 MJ (Meltzer et al., 2008)), as ALSPAC nutritional data was provided cleaned. Our final samples included 84,454 and 11,760 mother-child dyads in MoBa and ALSPAC, respectively (same sample as Chapter 5).

7.3.2 Outcome measures

Autism diagnosis was obtained from the Norwegian Patient Registry via unique identification numbers, which allows linkage of all MoBa children. The Norwegian Patient Registry records all diagnoses made in the public specialist health care system from 2008 to 2018. Autism diagnostic criteria was based on the ICD-10 and included all F84 codes, excluding Rett syndrome (F84.2) (World Health Organisation, 1993).

MoBa distributed the validated 40-item SCQ when the child was age three (SCQ-3) and eight years (SCQ-8) (Rutter, 2003). The SCQ gathers information on the child's social communication skills and restricted and repetitive behaviours. One question screens for the absence of phrased speech; this was not included in the total score. As per Eaves (Eaves et al., 2006), we made an adjustment for children without phrased speech, as some questions are of less relevance. High scores indicated more autism-associated traits and we applied a binary cut-off at the ≥93rd percentile for SCQ (39 items), which reflected a previous validation study (Surén et al., 2019).

In ALSPAC, the 12-item, social communication disorders checklist (SCDC-8) was completed by the primary carer about the child at 7.6 years of age. A high score indicated greater difficulties, and we applied a cut-off at the ≥93rd which reflected the validation study (Skuse et al., 2005).

7.3.3 Socioeconomic indicators

In MoBa, the socioeconomic indicators were measured via self-reported postal questionnaires distributed at approximately 15-weeks' gestation. Maternal education was categorised as equal to or below 3-year high school general studies, junior college, and equal to or more than regional technical college, 4-year university degree (Bachelor's degree, nurse, teacher, engineer). The binary categories were referred to as <degree or ≥degree. The binary categories were derived from the original variable to form <degree (9-year secondary school/ 1–2-year high school/ technical high school/ 3-year high school general studies, junior college) and ≥degree (regional technical college, 4-year university degree (Bachelor's degree, nurse, teacher, engineer)/ university, technical college, more than 4 years (Master's degree, medical doctor, PhD)). Maternal gross and paternal gross income were available as categorical variables. Hence, we measured parental income defined as maternal and paternal income less than 300,000 Norwegian Krone (NOK) /

either maternal and paternal 300,000 NOK and above. We additionally used maternal and paternal income separately as a sensitivity analysis (less than 300,000 NOK/ 300,000 NOK and above analyses). The original variable had seven categories (no income / under 150,000 NOK/ 151,000-199,999 NOK / 200,000-299,999 NOK / 300,000-399,999 NOK / 400,000-499,999 NOK / over 500,000 NOK). There was no indicator of area-based deprivation available in MoBa.

In ALSPAC, Maternal education was assessed at approximately 24 weeks gestation via self-completed postal questionnaires. Maternal education was coded as less than A level education or A level and above (A levels are the highest high school qualification attainable in the UK). Due to low cell count we did not use degree education as the cut point, and maternal education was derived from the original five level variable (certificate of secondary education or none/ vocational/ ordinary level/ advanced level/ degree level and above). Area-based material deprivation was measured using the Townsend Index based on wards at the 1991 census (Boyd et al., 2019). Participants in the most deprived area (60^{th} percentile and above) were compared against the remaining sample (less than 60^{th} percentile of deprivation). Income was not available during pregnancy in ALSPAC.

7.3.4 Dietary assessment and dietary pattern (mediator)

The dietary assessment was conducted using a FFQ in MoBa and ALSPAC, distributed at 22- and 32-weeks' gestation, respectively. In MoBa, a validated 255-item semi-quantitative FFQ collected information on commonly consumed foods, beverages, and dietary supplement use since the start of pregnancy (Brantsaeter et al., 2008, Brantsaeter et al., 2009, Brantsaeter et al., 2007, Brantsaeter et al., 2010). MoBa researchers applied standard portion sizes and expressed food intakes in grams per day. The Norwegian food composition database (Norwegian Food Safety Authority, 2005) and FoodCalc (Lauritsen, 1998) were used to derive daily nutrient intakes.

In ALSPAC, the non-quantitative FFQ was distributed at 32 weeks' gestation. Women reported their frequency of consumption 'nowadays' for 43 groups of foods and beverages, and nutritional supplements (Emmett, 2009, Rogers et al., 1998). The McCance and Widdowson's food composition tables and average portion sizes (Ministry of Agriculture and Food, 1991) were used to estimate daily nutrient intakes, which excluded alcohol and nutritional supplements (Rogers et al., 1998).

The dietary patterns used in MoBa and ALSPAC have been previously estimated (Chapter 5) but in short, they were derived using exploratory factor analysis with varimax rotation, and total daily energy intake was adjusted for using the residuals method (Willett et al., 1997). Factors were selected based on eigenvalues (≥1.5), Scree plots and interpretability, and derived HPDP which was categorised into terciles as low-, medium-, and high-adherence.

7.3.5 Covariates

Maternal covariates used in MoBa were obtained via postal questionnaires at approximately 15 weeks gestation. The maternal covariates were maternal age at delivery (< 25 years age, 25-29 years, 30-34 years, ≥35 years), maternal pre-pregnancy BMI (kg/m^2), planned pregnancy, prenatal multivitamin/folic acid supplement use 4 weeks prior to pregnancy until at least 12 weeks' gestation, maternal history of depression (yes/no), prenatal alcohol intake (yes/no), prenatal smoking (yes/no), pre-pregnancy diabetes (yes/no), pre-pregnancy hypertension (yes/no), maternal education (9-year secondary school/ 1–2-year high school/ technical high school/ 3-year high school general studies, junior college/ regional technical college, 4-year university degree (Bachelor's degree, nurse, teacher, engineer)/ University, technical college, more than 4 years (Master's degree, medical doctor, PhD)).

Maternal covariates in ALSPAC were collected from questionnaires distributed around 17-, 24-, and 32 weeks' gestation, and are maternal age (less than 25 years age, 25-29 years, 30-34 years, 35 years and above), maternal pre-pregnancy BMI (kg/m^2), maternal education (none/certificate of secondary education/ vocational /ordinary level/ advanced level/ degree), planned pregnancy (yes/no), multivitamin/folic acid supplement use at 17 weeks gestation (yes/no), prenatal alcohol intake (yes/no), maternal history of depression (yes/no). Pre-pregnancy diabetes and pre-pregnancy hypertension (yes/no) were obtained from maternity records.

7.3.6 Statistical methods

The covariates were presented as percentages and counts for categorical variables and mean and standard deviation for continuous variables. A directed acyclic graph (DAG) and prior literature informed covariate selection (Chapter 3, Figure 8). Propensity scores were estimated separately for the exposure and mediator and the IPW was calculated, from

which we derived the average inverse probability weights, as per VanderWeele (VanderWeele, 2009) (described in Chapter 3.7). The TE and CDE were estimated in marginal structural models weighted by the exposure weights and average weights, respectively, and an exposure-mediator interaction term was included in the CDE models. The change in estimates from the TE to CDE indicates the 'proportion eliminated' through HPDP. We clustered on the mothers to account for intracluster correlation between siblings, and estimated robust standard errors (Horvitz and Thompson, 1952, Lumley and Scott, 2017). TE is the estimated association that socioeconomic deprivation has with each outcome, whereas the CDE estimates the remaining association, if we 'eliminate' the proportion that is mediated through a HPDP and any socioeconomic-dietary pattern interaction effects. We checked for extreme weight and covariate balance was assessed using a standardised mean difference of <0.1 which may indicate negligible covariate imbalance (Austin, 2009, Chesnaye et al., 2021).

We used multivariate imputation by chained equations (MICE) assuming data to be missing at random (van Buuren and Groothuis-Oudshoorn, 2011). Variables included in the imputation model were the exposures, mediator, outcome, and variables potentially predictive of missingness (Table 2). The number of imputations were based on von Hippel's quadratic approach (von Hippel, 2020) and were 156 in MoBa and 115 in ALSPAC. Imputation model was stratified by level of HPDP to allow estimation of the interaction (Tilling et al., 2016). We estimated the IPW and marginal structural models for each imputation (Granger et al., 2019) before combining them using Rubin's rule (Campion and Rubin, 1989).

7.4 Results

See Table 24 for the distribution of maternal characteristics by socioeconomic indicators for both cohorts. Mothers with high maternal education, high parental income, and low levels of area-based deprivation (Townsend deprivation) were more likely to: have high adherence to HPDP, be older, more likely to consume alcohol in pregnancy, have a lower pre-pregnancy BMI, and were less likely to: smoke pre-pregnancy or have a history of depression. Mothers with high maternal education and high parental income were more likely to take prenatal multivitamin supplements, and in MoBa only, mothers with high education and parental income were more likely to plan their pregnancy. Child sex, pre-pregnancy diabetes, and pre-pregnancy hypertension did not vary by socioeconomic deprivation indicator.

The distribution of the outcomes across maternal indicators of deprivation are presented in Table 25. In MoBa, autism diagnosis, all SCQ-3 measures, and SCQ-8 were more frequent in children of parents with low maternal education. There were fewer clear differences for SCQ-SOC-8 and SCQ-RRB-8. There were no clear differences by socioeconomic indicator and SCDC-8 in ALSPAC, nor by parental income and for each outcome in MoBa. The exception was autism diagnosis, which was more frequent in low parental income compared to high.

Table 24. MoBa and ALSPAC cohort characteristics by indicator of socioeconomic deprivation.

Characteristic	Level	Overall (MoBa)	Maternal education		Parental income		Overall (ALSPAC)	Maternal education		Townsend deprivation	
			< Degree	≥ Degree	Low	High		< A level	≥ A level	High deprivation	Low deprivation
Number of pregnancies		84,548	27,216	52,509	20,398	59,476	11,760	7,542	4,148	2,722	4,223
Adherence to 'healthy' prenatal dietary pattern (%)	Low	28183 (33.3)	12127 (44.6)	14439 (27.5)	8628 (42.3)	17745 (29.8)	3920 (33.3)	3265 (43.3)	617 (14.9)	1084 (39.8)	1230 (29.1)
	Medium	28182 (33.3)	8412 (30.9)	18248 (34.8)	6362 (31.2)	20392 (34.3)	3920 (33.3)	2636 (35.0)	1264 (30.5)	870 (32.0)	1493 (35.4)
	High	28183 (33.3)	6677 (24.5)	19822 (37.7)	5408 (26.5)	21339 (35.9)	3920 (33.3)	1641 (21.8)	2267 (54.7)	768 (28.2)	1500 (35.5)
Child sex (%)	Male	43277 (51.2)	14034 (51.6)	26781 (51.0)	10421 (51.1)	30441 (51.2)	6034 (51.3)	3900 (51.7)	2098 (50.6)	1399 (51.4)	2160 (51.1)
	Female	41206 (48.7)	13161 (48.4)	25690 (48.9)	9955 (48.8)	28992 (48.7)	5725 (48.7)	3641 (48.3)	2050 (49.4)	1323 (48.6)	2063 (48.9)
	Missing	65 (0.1)	21 (0.1)	38 (0.1)	22 (0.1)	43 (0.1)	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Maternal history of depression (%)	No	76340 (90.3)	24095 (88.5)	48507 (92.4)	18108 (88.8)	54664 (91.9)	10263 (87.3)	6413 (85.0)	3791 (91.4)	2296 (84.3)	3804 (90.1)
	Yes	6474 (7.7)	2716 (10.0)	3342 (6.4)	2011 (9.9)	4048 (6.8)	956 (8.1)	691 (9.2)	261 (6.3)	268 (9.8)	279 (6.6)
	Missing	1734 (2.1)	405 (1.5)	660 (1.3)	279 (1.4)	764 (1.3)	541 (4.6)	438 (5.8)	96 (2.3)	158 (5.8)	140 (3.3)
Maternal age category (%)	< 25 years	8930 (10.6)	7327 (26.9)	1894 (3.6)	5314 (26.1)	3718 (6.3)	2719 (23.1)	2293 (30.4)	393 (9.5)	831 (30.5)	862 (20.4)

Characteristic	Level	Overall (MoBa)	Maternal education		Parental income		Overall (ALSPAC)	Maternal education		Townsend deprivation	
			< Degree	≥ Degree	Low	High		< A level	≥ A level	High deprivation	Low deprivation
	25-29 years	27805 (32.9)	8978 (33.0)	18727 (35.7)	8393 (41.1)	19610 (33.0)	4628 (39.4)	3047 (40.4)	1568 (37.8)	1041 (38.2)	1699 (40.2)
	30-34 years	32870 (38.9)	7312 (26.9)	23106 (44.0)	5036 (24.7)	25405 (42.7)	3111 (26.5)	1550 (20.6)	1547 (37.3)	589 (21.6)	1212 (28.7)
	≥ 35 years	14943 (17.7)	3599 (13.2)	8782 (16.7)	1655 (8.1)	10743 (18.1)	1052 (8.9)	456 (6.0)	592 (14.3)	217 (8.0)	404 (9.6)
	Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	250 (2.1)	196 (2.6)	48 (1.2)	44 (1.6)	46 (1.1)
Maternal education* (%)	1 (lowest)	1960 (2.3)			866 (4.2)	747 (1.3)	2324 (19.8)			717 (26.3)	676 (16.0)
	2	3736 (4.4)			1560 (7.6)	1738 (2.9)	1155 (9.8)			276 (10.1)	435 (10.3)
	3	10253 (12.1)			3860 (18.9)	5594 (9.4)	4063 (34.5)			861 (31.6)	1520 (36.0)
	4	11267 (13.3)			3822 (18.7)	6744 (11.3)	2645 (22.5)			530 (19.5)	1013 (24.0)
	5	33190 (39.3)			6973 (34.2)	25166 (42.3)	1503 (12.8)			317 (11.6)	564 (13.4)
	6	19319 (22.8)			1860 (9.1)	17087 (28.7)					
	Missing	4823 (5.7)			1457 (7.1)	2400 (4.0)	70 (0.6)			21 (0.8)	15 (0.4)
Planned pregnancy (%)	No	15512 (18.3)	7104 (26.1)	7248 (13.8)	5273 (25.9)	8819 (14.8)	8113 (69.0)	5001 (66.3)	3082 (74.3)	1681 (61.8)	2985 (70.7)
	Yes	67461 (79.8)	19738 (72.5)	44746 (85.2)	14860 (72.9)	50060 (84.2)	3356 (28.5)	2310 (30.6)	1012 (24.4)	985 (36.2)	1179 (27.9)

Characteristic	Level	Overall (MoBa)	Maternal education		Parental income		Overall (ALSPAC)	Maternal education		Townsend deprivation	
			< Degree	≥ Degree	Low	High		< A level	≥ A level	High deprivation	Low deprivation
	Missing	1575 (1.9)	374 (1.4)	515 (1.0)	265 (1.3)	597 (1.0)	291 (2.5)	231 (3.1)	54 (1.3)	56 (2.1)	59 (1.4)
Prenatal alcohol intake (%)	No	64040 (75.7)	20534 (75.4)	40359 (76.9)	15440 (75.7)	45733 (76.9)	4524 (38.5)	3038 (40.3)	1459 (35.2)	1062 (39.0)	1615 (38.2)
	Yes	8861 (10.5)	2298 (8.4)	6151 (11.7)	1758 (8.6)	6718 (11.3)	2130 (18.1)	1105 (14.7)	1016 (24.5)	423 (15.5)	830 (19.7)
	Missing	11647 (13.8)	4384 (16.1)	5999 (11.4)	3200 (15.7)	7025 (11.8)	5106 (43.4)	3399 (45.1)	1673 (40.3)	1237 (45.4)	1778 (42.1)
Prenatal multivitamin supplement use (%)	No	55706 (65.9)	20684 (76.0)	31979 (60.9)	15221 (74.6)	37427 (62.9)	8877 (75.5)	5899 (78.2)	2929 (70.6)	2074 (76.2)	3236 (76.6)
	Yes	24632 (29.1)	4954 (18.2)	18712 (35.6)	4156 (20.4)	19814 (33.3)	2568 (21.8)	1391 (18.4)	1164 (28.1)	577 (21.2)	927 (22.0)
	Missing	4210 (5.0)	1578 (5.8)	1818 (3.5)	1021 (5.0)	2235 (3.8)	315 (2.7)	252 (3.3)	55 (1.3)	71 (2.6)	60 (1.4)
Prenatal smoking (%)	No	70442 (83.3)	20802 (76.4)	46153 (87.9)	15760 (77.3)	51633 (86.8)	8821 (75.0)	5205 (69.0)	3576 (86.2)	1833 (67.3)	3331 (78.9)
	Yes	6275 (7.4)	4269 (15.7)	1685 (3.2)	2411 (11.8)	3224 (5.4)	2709 (23.0)	2151 (28.5)	534 (12.9)	851 (31.3)	853 (20.2)
	Missing	7831 (9.3)	2145 (7.9)	4671 (8.9)	2227 (10.9)	4619 (7.8)	230 (2.0)	186 (2.5)	38 (0.9)	38 (1.4)	39 (0.9)
Pre-pregnancy BMI (mean (SD))		24.1 (4.3)	24.9 (4.9)	23.7 (3.9)	24.5 (4.8)	23.9 (4.1)	22.9 (3.8)	23.19 (4.02)	22.49 (3.35)	23.03 (4.13)	22.78 (3.58)
Pre-pregnancy diabetes (%)	No	82401 (97.5)	26655 (97.9)	51602 (98.3)	20008 (98.1)	58434 (98.2)	10706 (91.0)	6760 (89.6)	3887 (93.7)	2446 (89.9)	3905 (92.5)

Characteristic	Level	Overall (MoBa)	Maternal education		Parental income		Overall (ALSPAC)	Maternal education		Townsend deprivation	
			< Degree	≥ Degree	Low	High		< A level	≥ A level	High deprivation	Low deprivation
	Yes	413 (0.5)	156 (0.6)	247 (0.5)	111 (0.5)	278 (0.5)	44 (0.4)	24 (0.3)	19 (0.5)	9 (0.3)	16 (0.4)
	Missing	1734 (2.1)	405 (1.5)	660 (1.3)	279 (1.4)	764 (1.3)	1010 (8.6)	758 (10.1)	242 (5.8)	267 (9.8)	302 (7.2)
Pre-pregnancy hypertension (%)	No	82216 (97.2)	26578 (97.7)	51548 (98.2)	19950 (97.8)	58326 (98.1)	11663 (99.2)	7480 (99.2)	4114 (99.2)	2706 (99.4)	4191 (99.2)
	Yes	882 (1.0)	317 (1.2)	513 (1.0)	224 (1.1)	627 (1.1)	28 (0.2)	17 (0.2)	11 (0.3)	7 (0.3)	12 (0.3)
	Missing	1450 (1.7)	321 (1.2)	448 (0.9)	224 (1.1)	523 (0.9)	69 (0.6)	45 (0.6)	23 (0.6)	9 (0.3)	20 (0.5)

ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, Body Mass Index; MoBa, Norwegian Mother, Father, and Child Cohort; n, number of mother-child dyad

*MoBa; 1: 9-year secondary school; 2: 1–2-year high school; 3: Technical high school; 4: 3-year high school general studies, junior college; 5: Regional technical college, 4-year university degree (Bachelor's degree, nurse, teacher, engineer); 6: University, technical college, more than 4 years (Master's degree, medical doctor, PhD)

*ALSPAC; 1: None/ Certificate of secondary education; 2: vocational; 3: Ordinary level; 4: Advanced level; 5: Degree)

Table 25. MoBa and ALSPAC outcomes by indicator of socioeconomic deprivation.

			Maternal education		**Parental income**	
Outcome	Level	Overall	< Degree	≥ Degree	Low	High
MoBa						
n		84,548	27,216	52,509	20,398	59,476
Autism (%)	No	83606 (98.9)	26810 (98.5)	52023 (99.1)	20075 (98.4)	58912 (99.1)
	Yes	942 (1.1)	400 (1.5)	485 (0.9)	319 (1.6)	562 (0.9)
	Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SCQ-3 (%)	No	46154 (54.6)	12526 (46.0)	31431 (59.9)	10457 (51.3)	33793 (56.8)
	Yes	5408 (6.4)	2163 (7.9)	2925 (5.6)	1529 (7.5)	3578 (6.0)
	Missing	32986 (39.0)	12527 (46.0)	18153 (34.6)	8412 (41.2)	22105 (37.2)
SCQ-RRB-3 (%)	No	47078 (55.7)	13017 (47.8)	31823 (60.6)	10756 (52.7)	34356 (57.8)
	Yes	4484 (5.3)	1672 (6.1)	2533 (4.8)	1230 (6.0)	3015 (5.1)
	Missing	32986 (39.0)	12527 (46.0)	18153 (34.6)	8412 (41.2)	22105 (37.2)
SCQ-SOC-3 (%)	No	46481 (55.0)	12729 (46.8)	31503 (60.0)	10666 (52.3)	33901 (57.0)
	Yes	5081 (6.0)	1960 (7.2)	2853 (5.4)	1320 (6.5)	3470 (5.8)
	Missing	32986 (39.0)	12527 (46.0)	18153 (34.6)	8412 (41.2)	22105 (37.2)
SCQ-8 (%)	No	36786 (43.5)	9243 (34.0)	25766 (49.1)	8176 (40.1)	27246 (45.8)
	Yes	3468 (4.1)	1365 (5.0)	1932 (3.7)	845 (4.1)	2426 (4.1)
	Missing	44294 (52.4)	16608 (61.0)	24811 (47.3)	11377 (55.8)	29804 (50.1)
SCQ-RRB-8 (%)	No	34237 (40.5)	8604 (31.6)	24017 (45.7)	7487 (36.7)	25504 (42.9)
	Yes	6017 (7.1)	2004 (7.4)	3681 (7.0)	1534 (7.5)	4168 (7.0)
	Missing	44294 (52.4)	16608 (61.0)	24811 (47.3)	11377 (55.8)	29804 (50.1)
SCQ-SOC-8 (%)	No	37218 (44.0)	9458 (34.8)	25951 (49.4)	8313 (40.8)	27493 (46.2)
	Yes	3036 (3.6)	1150 (4.2)	1747 (3.3)	708 (3.5)	2179 (3.7)
	Missing	44294 (52.4)	16608 (61.0)	24811 (47.3)	11377 (55.8)	29804 (50.1)
ALSPAC						
			Maternal education		**Townsend deprivation**	
Outcome	Level	Overall	< A level	≥ A level	High deprivation	Low deprivation

Number of pregnancies		11,760	7,542	4,148	4,223	2,722
SCDC-8 (%)	No	6735 (57.3)	3860 (51.2)	2852 (68.8)	2508 (59.4)	1366 (50.2)
	Yes	544 (4.6)	352 (4.7)	191 (4.6)	199 (4.7)	117 (4.3)
	Missing	4481 (38.1)	3330 (44.2)	1105 (26.6)	1516 (35.9)	1239 (45.5)

ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, Body Mass Index; MoBa, Norwegian Mother, Father, and Child Cohort; n, number of mother-child dyads

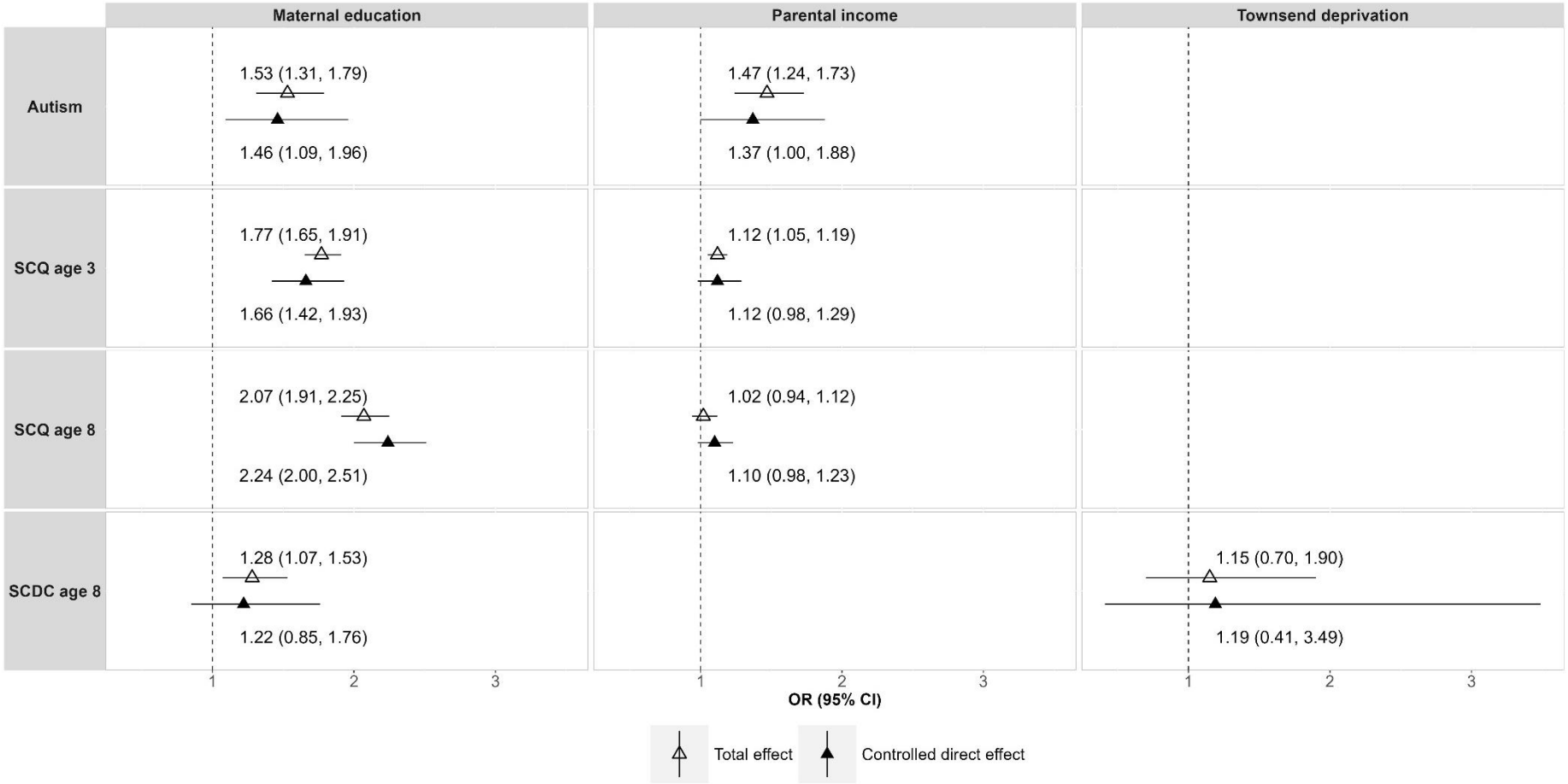
'Yes' indicates a high score for each measure which suggests more autism-associated traits. Autism diagnosis was measured binary (yes/no).

7.4.1 Main results

We generally achieved good covariate balance with a few exceptions in which the standardised difference was only slightly >0.1 or the socioeconomic indicator was adjusted in the CDE model. There were no extreme weights, except for maternal income in MoBa CDE model (see Supplementary material for Chapter 7 and Supplementary figure 16-27). See Figure 20 and Table 26 for the TE and CDE results. The TE of low maternal education associated with an increased probability of each outcome, whereas low parental income associated with an increased probability of autism diagnosis and SCQ-3, SCQ-RRB-3, SCQ-SOC-3, SCQ-RRB-8. However, the magnitude of association was small in each analysis, except autism diagnosis. There was no clear evidence of association with Townsend deprivation.

The CDE was reduced compared to the TE in the following analysis: low maternal education and autism diagnosis, SCQ-3, SCQ-SOC-3, SCQ-RRB-8, SCDC-8; low parental income and autism diagnosis and SCQ-RRB-8. The magnitude of association between low maternal education and SCQ-8 increased in the CDE, and there were no clear differences in the other main analysis. In sensitivity analysis, parental income and paternal income had the most similar associations, and maternal income was not associated with any outcome (TE or CDE).

Figure 20. Total and controlled direct effects of each socioeconomic indicator in association with autism related outcomes.



CI, confidence interval: OR, odds ratio; SCDC, social communication disorders checklist: SCQ, social communication questionnaire
Autism was measured binary (yes/no) and the other outcomes represents a high level of autism-associated traits. The reference category is low deprivation and the CDE is the association high deprivation has on each outcome when everyone has a high adherence to a 'healthy' prenatal dietary pattern.

Maternal education was adjusted for the average inverse probability weights based on: exposure inverse-probability weight based on maternal age; and mediator inverse probability weights based on maternal age, maternal education, pre-pregnancy BMI, maternal depression, planned pregnancy, prenatal multivitamin supplement use, prenatal alcohol intake, pre-pregnancy diabetes and pre-pregnancy hypertension.
Parental income was adjusted for the average inverse probability weights based on: exposure inverse-probability weights based on maternal age and maternal education; and mediator inverse probability weights based on maternal age, maternal education, pre-pregnancy BMI, maternal depression, parental income, planned pregnancy, prenatal multivitamin supplement use, prenatal alcohol intake, pre-pregnancy diabetes and pre-pregnancy hypertension.
Townsend deprivation was adjusted for the average inverse probability weights based on: exposure inverse-probability weights based on maternal age and maternal education; and mediator inverse probability weights based on Townsend deprivation, maternal age, maternal education, pre-pregnancy BMI, maternal depression, planned pregnancy, prenatal multivitamin supplement use, prenatal alcohol intake, pre-pregnancy diabetes and pre-pregnancy hypertension.

Table 26. Total effects versus the controlled direct effects of each socioeconomic indicator and outcome (including sensitivity analysis of maternal and paternal income).

	Total effect OR, (95% CI)	**Controlled direct effect OR, (95% CI)**
Autism		
Maternal education	1.53 (1.31, 1.79)	1.46 (1.09, 1.96)
Parental income	1.47 (1.24, 1.73)	1.37 (1.00, 1.88)
Paternal income	1.29 (1.09, 1.53)	1.25 (0.92, 1.70)
Maternal income	1.27 (0.88, 1.82)	1.63 (1.08, 2.48)
High SCQ score age three years		
Maternal education	1.77 (1.65, 1.91)	1.66 (1.42, 1.93)
Parental income	1.12 (1.05, 1.19)	1.12 (0.98, 1.29)
Paternal income	1.11 (1.05, 1.17)	1.11 (0.98, 1.25)
Maternal income	0.96 (0.87, 1.05)	1.01 (0.88, 1.17)
High SCQ-RRB score age three years		
Maternal education	1.52 (1.40, 1.65)	1.57 (1.38, 1.78)
Parental income	1.08 (1.01, 1.17)	1.11 (0.97, 1.27)
Paternal income	1.07 (1.00, 1.14)	1.09 (0.95, 1.25)
Maternal income	0.94 (0.87, 1.03)	1.05 (0.89, 1.24)
High SCQ-SOC score age three years		
Maternal education	1.69 (1.58, 1.80)	1.61 (1.42, 1.82)
Parental income	1.07 (1.00, 1.14)	1.10 (0.96, 1.26)
Paternal income	1.05 (0.99, 1.12)	1.06 (0.93, 1.20)
Maternal income	1.04 (0.96, 1.12)	1.05 (0.89, 1.24)
High SCQ score age eight years		
Maternal education	2.07 (1.91, 2.25)	2.24 (2.00, 2.51)
Parental income	1.02 (0.94, 1.12)	1.10 (0.98, 1.23)
Paternal income	1.01 (0.92, 1.10)	1.06 (0.94, 1.18)
Maternal income	0.97 (0.89, 1.07)	1.10 (0.89, 1.37)
High SCQ-RRB score age eight years		
Maternal education	1.53 (1.42, 1.65)	1.48 (1.32, 1.66)
Parental income	1.14 (1.06, 1.22)	1.00 (0.90, 1.12)
Paternal income	1.16 (1.09, 1.22)	1.02 (0.91, 1.13)
Maternal income	0.97 (0.91, 1.03)	1.06 (0.89, 1.27)
High SCQ-SOC score age eight years		
Maternal education	1.90 (1.75, 2.06)	1.90 (1.70, 2.13)
Parental income	0.98 (0.90, 1.07)	1.09 (0.94, 1.25)
Paternal income	0.95 (0.86, 1.05)	1.04 (0.90, 1.19)
Maternal income	0.98 (0.88, 1.09)	1.07 (0.85, 1.35)
High SCDC score age eight years		
Maternal education	1.28 (1.07, 1.53)	1.22 (0.85, 1.76)
Townsend deprivation	1.15 (0.70, 1.90)	1.19 (0.41, 3.49)

CDE, controlled direct effect; CI, confidence interval; OR, odds ratio; SCDC, social communication disorder checklist; SCQ, social communication questionnaire; TE, total effects Autism was measured binary (yes/no), and the other outcomes represents a high level of autism-associated traits. The reference category is low deprivation, and the CDE is the association high deprivation has on each outcome when everyone has a high adherence to a ‘healthy’ prenatal dietary pattern.

We considered parental income to be our primary analyses, however paternal and maternal income were analysed separately as sensitivity analyses and are provided for reference. Covariates adjustment set is the same as parental income.

Maternal education was adjusted for the average inverse probability weights based on: exposure inverse-probability weight based on maternal age; and mediator inverse probability weights based on maternal age, maternal education, pre-pregnancy BMI, maternal depression, planned pregnancy, prenatal multivitamin supplement use, prenatal alcohol intake, pre-pregnancy diabetes and pre-pregnancy hypertension.
Parental income was adjusted for the average inverse probability weights based on: exposure inverse-probability weights based on maternal age and maternal education; and mediator inverse probability weights based on maternal age, maternal education, pre-pregnancy BMI, maternal depression, parental income, planned pregnancy, prenatal multivitamin supplement use, prenatal alcohol intake, pre-pregnancy diabetes and pre-pregnancy hypertension.
Townsend deprivation was adjusted for the average inverse probability weights based on: exposure inverse-probability weights based on maternal age and maternal education; and mediator inverse probability weights based on Townsend deprivation, maternal age, maternal education, pre-pregnancy BMI, maternal depression, planned pregnancy, prenatal multivitamin supplement use, prenatal alcohol intake, pre-pregnancy diabetes and pre-pregnancy hypertension.

7.5 Discussion

Autism diagnosis and autism-associated traits were most strongly related to low maternal education, and to a lesser extent, low paternal income, when compared to area-based deprivation. Public health practice may be better informed by research from an intervention perspective (Pearce et al., 2019), therefore, we estimated the impact of a potential dietary 'intervention'. We observed a modest attenuation in the CDE, compared to TE for maternal education and autism diagnosis, SCQ-3, SCQ-SOC-3, SCQ-RRB-8, and SCDC-8; and for parental income and autism diagnosis and SCQ-RRB-8. In the other models (including the sensitivity analysis) there was generally no strong evidence of attenuation in the CDE. Thus, the hypothetical 'intervention' on HPDP resulted in a modest reduction in inequalities in some outcomes, particularly in relation to low maternal education.

An advantage of our study is that we conceptualised the pathways between socioeconomic deprivation and autism related outcomes within a causal framework and transparently reported them. This can reduce the risk of certain biases, such as collider bias or overadjustment, and can improve clarity in the interpretation of our findings when comparing to the existing literature. Thus, we focused on previous studies that investigated socioeconomic indicators measured around pregnancy and compared the covariate adjustment set most aligned with our conceptualisation of potential causal pathways.

Previous studies have observed an increased likelihood of autism diagnosis or autism-associated traits in relation to socioeconomic disadvantage (Dodds et al., 2011, Fujiwara, 2014, Goh et al., 2018, Larsson et al., 2005, Lehti et al., 2013, Li et al., 2014, Lung et al., 2018, Rai et al., 2012, Russell et al., 2014, Segev et al., 2019, Tsai et al., 2017). However, there were inconsistencies (Bhasin and Schendel, 2007, Burd et al., 1999, Croen et al., 2002, Kelly et al., 2019, King and Bearman, 2011, Yu et al., 2021). This may relate to ethnic and socio-cultural differences, as previously observed as well as diagnostic bias which is thought to affect American studies more (Aylward et al., 2021, Durkin et al., 2010, Maye et al., 2022). Similar to our findings, previous studies from European populations found that autism diagnosis and autism-associated traits were related to low parental education (Fujiwara, 2014, Goh et al., 2018), and low income (Larsson et al., 2005, Rai et al., 2012). In contrast with a previous study (Li et al., 2014), we observed a null association with area-based deprivation, however, there was considerable uncertainty in our estimate due to the high proportion of missing data. Additionally, in the Born in

Bradford cohort study an increased likelihood of autism diagnosis was observed if mothers had high educational attainment (Kelly et al., 2019). However, the distribution of other factors such as high levels of immigration may explain their findings (Morinaga et al., 2021).

Across the indicators of deprivation, maternal education had the largest magnitude of association with each outcome, especially autism-associated traits. Hence, other neurodevelopmental difficulties detected by the SCQ have a stronger relationship with maternal education than autism diagnosis. In the wider literature, maternal education is an especially strong predictor of child neurodevelopment, perhaps due to the proximal relationship to foetal and child development (Pearce et al., 2019). Moreover, as in our DAG, the implications of education can accumulate through the other major socioeconomic determinants, as maternal education predicts occupation and income.

Socioeconomic disadvantage increases the likelihood of numerous perinatal complications related to autism diagnosis and autism-associated traits, such as preterm birth or gestational diabetes. Additionally, postnatal deprivation can further impact child neurodevelopment through cumulative effects over the life course (Baird et al., 2017). For example, cognitive-verbally stimulating and socioemotionally supportive parenting behaviours are related to greater language skills and social skills in the child (Huang et al., 2022). We observed the strongest associations at age eight years, which may be due to the longer exposure period.

In the current study, HPDP generally explained a modest proportion of the association between socioeconomic indicator and each outcome, which indicates that there are alternative pathways. Other studies also observed a modest proportion of the association of parental socioeconomic deprivation on offspring neurodevelopment through downstream environmental factors (Marx et al., 2022, Thomson et al., 2023). Prenatal diet is perceived as more modifiable than the wider determinants of health inequalities, yet diet quality becomes socially patterned through a complex network of pathways. For example, education is an important predictor of access to health information and self-efficacy, which only partly explains the relationship with diet quality (McLeod et al., 2011). Adherence to dietary guidelines is estimated to cost more than the least ‘healthy’ diets and accounts for a greater proportion of income for low-income groups (Darmon and Drewnowski, 2015). Moreover, socioeconomic deprivation is strongly related to stress, which can trigger ‘unhealthy’ food choices as a coping strategy. Collectively, the socioeconomic drivers of diet are numerous, dynamic, and inter-related to the numerous additional challenges and

consequences of socioeconomic deprivation. Sawyer et al suggests that over time, ‘unhealthy’ food choices are normalised and ‘healthy’ eating is deprioritised (Sawyer et al., 2021).

Investigation of the differential effect of the major socioeconomic indicators has provided contextual knowledge which may aid aetiological interpretation and help shape interventions. Our results suggest that diet, along with other pathways may create disparities in autism related outcomes. Individual level interventions are often favoured, yet their efficacy and equitability are disputed (Adams et al., 2016). Intervention success is highly dependent on the individual’s psychosocial, environmental, and material resources, and strategies that fail to address this can increase health inequalities (Adams et al., 2016). Hence, if prenatal diet were causally associated with autism, a complex whole-system approach to address health inequalities is potentially required, which could include structures at the macro, meso, and micro level (Wilderink et al., 2022). Prenatal diet remains an important target for foetal health, as does pre-pregnancy due to the delay in establishing improvements in nutritional status (Stephenson et al., 2018). By viewing prenatal dietary patterns in the socioeconomic context, we shift the focus to population level factors, which have considerably more influence in comparison to individual level behavioural factors such as diet.

7.5.1 Strengths and limitations

We applied a causally informed approach with relevance to public health policy, clarified potential causal relationships and sources of bias using a DAG, investigated two large prospective cohort studies from different countries, adjusted for a range of confounders, and had complete data for autism diagnosis. Nonetheless, we encourage the reader to consider key limitations. An extreme weight was observed for maternal income in MoBa CDE model however this has not changed the our conclusions because the TE of maternal income was not associated with any outcome. We cannot exclude bias due to unmeasured and residual confounding, especially as the proportion eliminated was modest. The standardised mean difference was slightly greater than 0.1 for a small number of covariates and may suggest residual confounding, though there is no universally accepted cut-off (Austin, 2009). For example, socioeconomic deprivation is a latent concept, and each facet is correlated and imprecisely measured. Additionally, maternal deprivation may relate to pathways via the father or family unit (Antaki et al., 2022). Furthermore, genetic

confounding can occur, as autism diagnosis is associated with more genes for high educational attainment (Grove et al., 2019).

Deprived groups and ethnic minorities were underrepresented (Boyd et al., 2013, Magnus et al., 2016), and selection bias can occur and may attenuate the results, especially due to the high attrition at ages three and eight years. Hence, our results may generalise to Caucasian European populations with universal health care who are more affluent. Lastly, self-reported and imprecise measures of both exposures and outcomes may introduce random error and bias in either direction. These sources of bias may explain the increased magnitude of association observed between maternal education and SCQ-8 and SCQ-RRB-3.

7.5.2 Conclusion

Maternal deprivation was related to an increased likelihood of autism diagnosis and autism-associated traits. A modest proportion of these associations could be hypothetically eliminated if everyone had HPDP, if the associations were causal. Hence, our results suggest that prenatal dietary pattern may be an effective intervention target if part of a multifaceted strategy to reduce inequalities in the outcomes.

7.5.3 Recommendations for future studies

Further research is required to establish the aetiology of autism related outcomes and should consider the socioeconomic context. It is also advantageous to provide clear and transparent reasoning for the approach to modelling these relationships.

8 Chapter 8. Discussion

8.1 Chapter overview

The discussion will bring together the main contribution the findings of this thesis make to the existing literature. In summary, the results demonstrated a lower probability of autism diagnosis and social communication difficulties if mothers consumed HPDP. I strengthened the analytic strategy and interpretation of the thesis findings through the application of causally informed approaches. Triangulation helped strengthen the causal interpretation of the association between HPDP and social communication difficulties, which was observed in MoBa and ALSPAC. Yet, methodological limitations were a barrier to robust triangulation with Mendelian randomisation (MR). The principal limitations of MR included low statistical power to detect an association should one exist, exacerbation of the risk of selection bias, and potentially invalid SNPs in the SCQ-3 and SCQ-RRB-3 analysis. Additionally, I was unable to triangulate findings on autism diagnosis or restrictive and repetitive behaviours due to insufficient case numbers and lack of available data. Nonetheless, this thesis had many strengths in addressing gaps in the existing literature and provides insights which may inform future research.

Therefore, I initiate the discussion by arguing that this thesis has strengthened the body of evidence on the prenatal diet and autism diagnosis by addressing several key methodological limitations of previous studies. Next, the discussion will focus on autism-associated traits and key insights this thesis provides which may explain the heterogeneous results. In particular, the differential findings by subdomain are potentially important. Social communication difficulties and restrictive and repetitive behaviours are phenotypically and genetically dissociable and may have different aetiological origins. I finish the section on autism-associated traits by highlighting that only weak evidence of effect modification by child sex was observed. I further highlight that I cannot distinguish between sex differences in the performance of the SCQ from aetiological origins.

In the next section I focus on nutritional aspects. I will revisit an earlier discussion in Chapter 3, and argue that due to the complexity of diet, a truly precise estimand is challenging to define. Nonetheless, though measures of HPDP differed between MoBa and ALSPAC yet consistent results were still observed with social communication difficulties. This is possibly due to the wide dispersion of most nutrients across foods and the range of nutrients implicated in the aetiology of autism. Lastly, how the thesis findings relate to

existing evidence on food groups, nutritional supplements and biochemical indicators will be integrated into this discussion. I finish the section on the associations between HPDP and autism diagnosis and autism-associated traits by presenting some key potential mechanisms that may relate prenatal nutrition to the development of autism related outcomes.

The final sections begin with a focus on socioeconomic inequalities in autism diagnosis and autism-associated traits. I will describe only some of the many complex and interrelated pathways that may explain inequalities in the outcomes, including HPDP. I will further consider the importance of maternal diet as a strategy to reduce inequalities in the outcomes. Lastly, the discussion focuses on key methodological considerations emerging from the thesis, and implications for public health practice and future studies, before concluding the thesis discussion.

8.2 Autism diagnosis and autism-associated traits

In this thesis, I observed a 23% lower odds of autism diagnosis in children, if mothers adhered to HPDP. MoBa is a large prospective cohort study, and in Chapter 5, I was able to include 84,548 pregnancies of which 942 offspring had an autism diagnosis. The large sample size is advantageous because the general population prevalence of autism is estimated to be only 1-2% (Elsabbagh et al., 2012, O'Nions et al., 2023, Roman-Urrestarazu et al., 2021, Russell et al., 2022). Hence, a large sample is required to have a sufficient number of people with autism, and subsequently, sufficient statistical power to detect an association, should one exist. This challenge was evident in the previous prospective investigations, the EARLI (154 mother-child dyads (16-36 autism diagnosis)), and NHSII (106 autism diagnosis/ 621 non-autistic controls) (Vecchione et al., 2022). This was also true in Geetha et al's (Geetha et al., 2018) smaller retrospective case-control study with 55 people with an autism diagnosis and 55 non-autistic controls. Yet, when a larger study included 374 people with an autism diagnosis and 354 non-autistic controls was investigated by Li et al (Li et al., 2018), my confidence in these findings was still low due to the retrospective study design.

Large prospective cohorts, such as MoBa and ALSPAC, are necessary when the outcome is uncommon, but are also advantageous if the measures have a large degree of random error. For example, prenatal dietary pattern is subject to a high degree of random and systematic error (Burrows et al., 2019). Of course, large sample size does not overcome

systematic error, but random error can reduce in large cohorts because the estimates centralise around the best estimate, as the sample size increases (Khoury and Ioannidis, 2014). Another key advantage of our investigation is the prospective study design which confers a lower risk of recall bias, compared with the two retrospective study designs which measured prenatal diet 3-12 years after pregnancy (Geetha et al., 2018, Li et al., 2018). Thus, MoBa had greater statistical power to detect an association, compared to previous investigations, and lower likelihood of recall bias, and as such, MoBa has provided more robust evidence of an association.

In the existing literature, evidence of an association was more consistent with an autism diagnosis than autism-associated traits. I considered the consistency across studies into prenatal dietary pattern as well as prenatal intakes of food groups, but focused on cohorts to avoid double counting, as several investigations were conducted in NHSII and EARLI. Out of six cohorts, four observed a lower likelihood of autism diagnosis in relation to 'healthier' maternal dietary patterns or food groups (Gao et al., 2016, Geetha et al., 2018, Gerges et al., 2020, Li et al., 2018). In NHSII and EARLI, there was no clear evidence of association with prenatal dietary patterns and autism diagnosis (Vecchione et al., 2022). When incorporating the findings of this thesis, there was more consistent evidence of a lower probability of autism diagnosis in association with prenatal dietary patterns, as observed in Chapter 5 and previous studies (Geetha et al., 2018, Li et al., 2018), though not consistently (Vecchione et al., 2022).

In this thesis, I observed inconsistent evidence in relation to the overall measures of autism-associated traits (SCQ and SCDC). In previous research, across a range of prenatal dietary patterns, associations with autism-associated traits were inconsistent (House et al., 2018, Vecchione et al., 2022). Indeed, this trend was observed across studies that investigated food groups as well. In four out of six cohorts, there was no clear evidence of association between autism-associated traits and dietary patterns or food groups. The cohorts were, NHSII, EARLI, HOME, and Generation R (Joyce et al., 2022, Lyall et al., 2023, Steenweg-De Graaff et al., 2016, Vecchione et al., 2021, Vecchione et al., 2022). The other two cohorts (NEST and INMA) observed a lower likelihood of autism-associated traits in relation to 'healthier' dietary patterns and food groups (House et al., 2018, Julvez et al., 2016). Hence, as observed in previous research, this thesis also found inconsistent evidence of an association with autism-associated traits, despite the limitations I was able to address.

Autism diagnosis is assessed by specialists, based on established criteria, and must include both social communication difficulties and restrictive and repetitive behaviours (World Health Organisation, 2020). Measures of autism-associated traits are broadly defined, often parental reported, detect autism-associated traits in autistic and non-autistic populations, and the performance is impacted by other characteristics such as age, child sex, and social deprivation (Garcia-Primo et al., 2014, Moody et al., 2017, Reyes et al., 2021, Rosenberg et al., 2018, Shuster et al., 2014). Furthermore, heterogeneity may arise through the specific autism screening tool used to measure autism-associated traits, which varied across the studies (House et al., 2018, Joyce et al., 2022, Julvez et al., 2016, Lyall et al., 2023, Steenweg-De Graaff et al., 2016, Vecchione et al., 2021, Vecchione et al., 2022). Additionally, studies that obtained an autism diagnosis through record linkage (Gao et al., 2016, Geetha et al., 2018, Gerges et al., 2020, Li et al., 2018, Lyall et al., 2013, Vecchione et al., 2022) had a lower risk of lost to follow-up and selection bias than autism-associated traits (House et al., 2018, Joyce et al., 2022, Julvez et al., 2016, Lyall et al., 2023, Steenweg-De Graaff et al., 2016, Vecchione et al., 2021, Vecchione et al., 2022). In this thesis, the child's age at measurement and child sex were additional sources of heterogeneity in the associations with HPDP and were related to the autism-associated traits subdomain. The inconsistency in results related to prenatal diet and autism-associated traits could reflect the balance of traits measured by the different tools (for example, SCDC-8 and SCQ-8). Collectively, heterogeneity in the outcome measures and bias may lead to inconsistency in the associations measured with autism-associated traits and prenatal diet.

8.3 Social communication difficulties and restrictive and repetitive behaviours

In Chapter 5, the associations with HPDP appeared to vary depending on subdomain and child's age and child sex. Overall, I observed somewhat more consistent associations between social communication difficulties and HPDP across MoBa and ALSPAC: two cohorts from different decades and countries. The increased likelihood of restrictive and repetitive behaviours at age three in association with HPDP, attenuated to the null at age eight, though no clear evidence of association was observed with social communication difficulties in MoBa at age eight either. I did not have access to a comparable measure of restrictive and repetitive behaviours in ALSPAC. Selection bias may have led to an attenuation of the associations observed, as lost to follow-up increased at age eight in MoBa (discussed in Chapter 8.6.2). Hence, I cannot exclude that a HPDP may increase the

likelihood of restrictive and repetitive behaviours in older children. However, the performance of the SCQ-RRB-3 was especially poor and should be taken into consideration. In brief, the restrictive and repetitive behaviours subdomain of the SCQ does not discriminate autism from typical child development at three years in MoBa (Surén et al., 2019). Generally, the reliability of autism screening tools improves in children over four years, compared to younger children (Pierce et al., 2019). However, in previous studies into prenatal diet and autism-associated traits, no consistent patterns emerged in relation to the child's age. Although only two studies measured autism-associated traits in children under four years, and no study measured the subdomains (House et al., 2018, Vecchione et al., 2022). Further studies should consider how the child's stage of development impacts the association with prenatal diet and assess whether this depends on the type of autism-associated trait.

There are few studies which measured aspects of prenatal nutrition by subdomain of autism-associated traits, none of which measured prenatal diet. Social communication skills emerged as having somewhat clearer associations with prenatal nutrition (Lopez-Vicente et al., 2019, Madley-Dowd et al., 2022, Patti et al., 2021), compared to restrictive and repetitive behaviours. However, this was not entirely consistent (Whitehouse et al., 2013). The association between prenatal vitamin D serum levels and various autism-associated traits were measured in 7689 pregnancies from ALSPAC (52.4% of recruited pregnancies) (Madley-Dowd et al., 2022). A restricted cubic regression spline was used to model the non-linear relationship between prenatal vitamin D and each outcome: social communication (measured at age eight years), speech coherence (age nine years), repetitive behaviour (age five years), sociability temperament (age three years), and mean autism-associated traits score (combination of all traits). Results suggested a U-shaped relationship with each outcome, except restrictive and repetitive behaviours. However, there was considerable uncertainty, and only difficulties in social communication, speech coherence and mean autism traits score were clearly associated with low prenatal vitamin D serum levels (Madley-Dowd et al., 2022). In a second study (INMA cohort), maternal vitamin D sufficiency (≥30 ng/mL) was associated with over a two-fold increase in offspring social competence (measured at age five years), though there were no associations observed with the childhood autism spectrum test (age five years) (Lopez-Vicente et al., 2019). However, a study based on the Raine cohort, did not observe clearer associations with social communication traits in older offspring (measured at age 20 years). Low prenatal vitamin D serum levels associated with an increased likelihood of difficulty with attention switching in offspring, but not the other subdomains of the autism

quotient which included social communication (communication, social skills, imagination, and attention to detail) (Whitehouse et al., 2013). An investigation using EARLI (child aged three years) and HOME (children aged 3-8 years) measured the association between maternal caffeine intakes and social awareness, social cognition, social communication, social motivation, and autistic mannerisms (related to restrictive and repetitive behaviours) (Patti et al., 2021). In EARLI, as maternal caffeine intake increased, there were increased difficulties in social awareness, social cognition, and social communication but there was no clear evidence of association with autistic mannerisms (Patti et al., 2021).

In summary, limited evidence suggests that for autism-associated traits, social communication difficulties may have a more consistent relationship with aspects of prenatal nutrition. However, the distinction was not especially clear from this small body of evidence. Additionally, each of the two subdomains are composed of a range of traits which may introduce further heterogeneity (Lopez-Vicente et al., 2019, Madley-Dowd et al., 2022, Patti et al., 2021). Additionally, most studies investigated prenatal vitamin D serum levels, rather than prenatal diet which is the focus of this thesis. Thus, when the body of research is viewed in the context of the thesis findings, I cautiously interpret that the evidence is suggestive of a clearer relationship between prenatal nutrition and social communication traits, compared to restrictive and repetitive behaviours. Therefore, further research would be important to establish whether these findings are reproducible.

I considered the subdomains of autism (restrictive and repetitive behaviours, and social communication difficulties) separately because previous research demonstrated they are phenotypically and genetically dissociable (Shuster et al., 2014, Warrier et al., 2019). Indeed, evidence of dissociation into two broad subdomains influenced the reformulation of diagnostic criteria (ICD-11 and DSM-5) into the two-core diagnostic domains: restrictive and repetitive behaviours, and social communication difficulties (Shuster et al., 2014). Studies conducted factor analysis on phenotypic traits of autism and provided empirical evidence of the structure of traits by analysing the strength of relationship between them. In a review of 36 studies, support for a two-dimensional diagnostic criteria were identified based on data reduction methods, such as exploratory and confirmatory factor analysis, or principal component analysis (Shuster et al., 2014).

Genetic evidence further supports the phenotypic dissociation between the two subdomains (Thomas et al., 2022, Warrier et al., 2019, Yousaf et al., 2020). For example, in a sample of autistic individuals the PGS for systematising was correlated with restrictive and

repetitive behaviours but not social communication traits. In the general population, the PGS for systematising was weakly correlated with SCDC (Warrier et al., 2019). A further investigation measured six autistic traits based on the autism diagnostic interview revised, which were social interaction, joint attention, peer interaction, nonverbal communication, repetitive sensory-motor behaviour, and restricted interest. A genetic correlation was identified between the social communication related subdomains but not with restrictive and repetitive behaviours (Yousaf et al., 2020). Nonetheless, the genetic correlations were low to moderate at best (Warrier et al., 2019, Yousaf et al., 2020), and autism continues to be regarded as a multidimensional condition for which the best approach to characterisation continues to be pursued (Wittkopf et al., 2022). Therefore, there is biological plausibility that the aetiology of autism diagnosis and autism-associated traits may vary by subdomain. Potentially, HPDP related causal pathways are connected to social communication difficulties, rather than restrictive and repetitive behaviours. However, replication of the associations would be required in autistic and general population cohorts, as well as clearer understanding of nutrition related aetiological of autism diagnosis or autism-associated traits.

8.4 Child sex differences in restrictive and repetitive behaviours and social communication difficulties

I observed weak evidence that child sex may be an effect modifier of the relationship between HPDP and social communication difficulties at age eight. I considered this evidence to be weak because although it was consistent across MoBa and ALSPAC at age eight, at age three males had a greater magnitude of association. In Chapter 6, trends were suggestive of a greater magnitude of association in females at age eight years, however, I had insufficient statistical power to detect small to moderate associations, should they exist. Additionally, theoretical evidence on the biological plausibility of a ‘female protective effect’ relates to autism diagnosis and autism-associated traits, yet in the autism diagnosis analysis I did not observe clear evidence of effect modification by child sex. This may challenge theories of a female protective effect, at least in relation to HPDP, however autism diagnosis includes both restrictive and repetitive behaviours and social communication difficulties. Alternatively, the results of this thesis appear to be consistent when age and child sex are considered as predictors of the performance of the SCQ and SCDC.

There are few nutrition studies which measured child-sex differences in autism diagnosis or autism-associated traits. Nonetheless, from the available literature I observe a greater magnitude of association in females, compared to males, or no child-sex differences. Female offspring, compared to males, had a lower likelihood of autism diagnosis in association with prenatal multivitamin or folic acid supplement use (Levine et al., 2018, Schmidt et al., 2019a) or increased neonatal vitamin D levels (Schmidt et al., 2019b). An exception was neonatal magnesium concentrations which were positively associated with a higher likelihood of autism diagnosis in males compared to females (Bakian et al., 2018). No clear evidence of effect modification by child sex was observed between autism diagnosis and the following: prenatal iron supplements (Schmidt et al., 2014), biochemical measures of PUFA's (Lyall et al., 2021), maternal red blood cell selenium levels (Lee et al.), and maternal vitamin B_{12} serum levels (Sourander et al., 2023). In studies of offspring autism-associated traits, there was no clear evidence of effect modification by child sex in relation to high prenatal caffeine intakes (Patti et al., 2021), prenatal dietary patterns (Vecchione et al., 2022) or prenatal fish intakes (Vecchione et al., 2021). However, none of the investigations into effect modification by child sex had separately measured the autism subdomains (social communication difficulties, and restrictive and repetitive behaviours). Overall, evidence of effect modification by child sex is inconsistent, but studies are heterogeneous in terms of prenatal indicator of nutrition, and findings from this thesis suggest results may vary by subdomain.

Diagnostic tools and screening tools for autism are designed to identify deviations from developmental trajectories that are 'typical' in the general population and align with an autism presentation. Yet, typical developmental trajectories are different for males and females which may impact the results observed in this thesis. In the general population, females typically develop language and social communication skills at an earlier age than males, however, by eight years these differences are less notable (McFayden et al., 2023). Moreover, the performance of autism-screening tools improves in females and may explain why I observed a greater magnitude of association in females at age eight. As autism was historically believed to be a male condition, the conceptualisation and recognition of autism is formed from research largely on males (Lai et al., 2015). Contemporary evidence suggests there are sex differences in symptom profile which impacts the performance of autism diagnostic and screening tools (Rea et al., 2022). Females require more severe symptoms to be detected on screening tools, meaning that high scores for social communication difficulties are actually stronger predictors of autism in females than males

(Evans et al., 2019b, McFayden et al., 2023). However, I cannot exclude alternative explanations behind these associations, including biological mechanisms.

To explain sexual dimorphism in autism several mechanisms have been suggested, but evidence is fragmented, and a cohesive understanding is yet to be established. The male preponderance in autism diagnosis and autism-associated traits may relate to genetic factors, sex hormones, and immune function (Ferri et al., 2018). There is consistent evidence that autistic females have greater co-occurring developmental disabilities and lower IQ scores, which have been related to a higher load of putative functional de novo mutations, compared to males (Warrier et al., 2022). There is debate as to whether this relates to different genetic aetiology or diagnostic bias. Some authors consider this a multiple threshold liability effect, whereby females require a higher genetic load to express the autism phenotype and therefore have protection compared to males (Zhang et al., 2020). However, others argue there is a lack of consistent evidence to support the multiple threshold liability model as a mechanism for the female protective effect (Dougherty et al., 2022). Sex-mediated recurrence, called the Carter effect, is expected if females carry a higher genetic load that is heritable. There should be higher rates of recurrence within family members of autistic females, especially male siblings who develop autism at a lower genetic load. Yet, this is not consistently demonstrated (Dougherty et al., 2022). Estimates of sex-mediated familial recurrence may be affected by de novo mutations which occur more commonly in females and can reduce heritability estimates. Alternatively, females may be 'protected' from diagnosis rather than autism per se. The male preponderance of autism reduced in children with co-occurring intellectual disability is estimated to be 1-2:1 (Ferri et al., 2018). Subsequently, it is argued that females may require a more severe presentation to be diagnosed with autism which is why I observed a greater genetic load in females (Dougherty et al., 2022).

Several mechanisms for sex differences in autism have been proposed and may be inter-related. Arguably one of the most famous theories is the 'extreme male brain' proposed by Baron-Cohen (Baron-Cohen, 2002). The autistic brain, whether male or female, is purported to reflect an extreme masculinised brain due to testosterone exposure in utero. As males are on average naturally closer to this phenotype, they require less exposure to prenatal testosterone to develop autism (Baron-Cohen, 2002). Three recent systematic reviews showed an increased likelihood of autism diagnosis and autism-associated traits in relation to increased exposure to testosterone in utero. However, the quality of studies was low and replication by research groups independent of Baron-Cohen would strengthen

confidence (Amestoy et al., 2023, Coscini et al., 2021, Xiong et al., 2020). Furthermore, sex hormones in utero have many functions such as sexual differentiation of the brain and physical characteristics, immune function, neuroendocrine function, neurotransmitters, amongst many others (Ferri et al., 2018, Nelson et al., 2019). Whether autism can be characterised as an extreme male brain due to testosterone exposure in utero is being debated, but accumulating evidence from different fields of research suggests hormonal processes may relate to autism aetiology.

Other evidence suggests there are sex differences in the expression of genes related to hormonal and immune processes (Kissel and Werling, 2022). Animal models further suggested immune processes can be due to gene-environment interaction (Schaafsma et al., 2017). Putative sex-linked genes have also been considered, and although some differences in autism risk by XX and XY chromosomes have been identified, they are thought to account for 2% of the male preponderance of autism (Ferri et al., 2018). Other studies have identified sex dependant differences in brain structure and connectivity that relates to social and motor function in autistic people (Lombardo et al., 2020, Supekar and Menon, 2015). Hence, potentially sex differences in the aetiology of autism diagnosis relate to sex hormones, maternal immune activation, or genetics. However, many comparisons between male and females with autism are affected by diagnostic bias in the sample. Further research is required to establish whether prenatal diet may have different aetiological pathways to autism dependant on child sex.

8.5 Dietary patterns, food groups, prenatal nutritional supplements, and biochemical indicators. What do the findings of this thesis add?

In Chapter 3, I introduced some of the tensions between defining a precise estimand in a potential outcomes framework (Hernán and Taubman, 2008, VanderWeele, 2016a) and the marginalisation of causal pursuits (Lundberg, 2020, Schwartz et al., 2016). At the World Congress of Epidemiology in 2014, Hernan, a leading researcher of ‘causal inference’ stated (quoted by Broadbent, 2015, pp 72):

“Causal questions are well-defined as long as interventions are well-specified.”

The rationale being that causal questions aligned to an ‘ideal intervention’ are quantifiable and have more relevance to public health. It is further argued, a well-defined causal question is quantifiable, can improve methodological approach, identification of the causal

component, and support clearer aetiological interpretations (Glass et al., 2013, Hernán and Taubman, 2008, VanderWeele and Robinson, 2014). However, others criticize this aspect of the potential outcomes framework (Krieger and Davey Smith, 2016, Vandenbroucke et al., 2016). To achieve a well-defined estimand, we have to resort to the strict isolation of causal components that can be clearly defined and therefore narrows the focus of investigation. This can marginalize the types of causal questions asked and may neglect the interrelated pathways between causal components (Krieger and Davey Smith, 2016, Vandenbroucke et al., 2016). There is a distinction between investigations focused on what caused the outcome, and investigations of what happened to the outcome under a specific manipulation of the exposure ('ideal intervention'). Consideration of the estimand is a useful thought experiment, even for investigations focused on what caused the outcome, but need not restrict the causal question (Schwartz et al., 2016).

In the thesis, my interest was diet as a potential cause of autism diagnosis and autism-associated traits. I start this section by discussing why a 'healthy' diet is difficult to precisely defined in a potential outcome's framework and bring clarity to what a HPDP may reflect. The next section considers how HPDP may relate to the existing evidence on food groups, nutritional supplements, and biochemical indicators.

8.5.1 Dietary patterns

Truly precise measurement of whole dietary intake is challenging due to the complexity of diet and limitations of dietary assessment methods (discussed further in Chapter 3.3.4, 3.3.5 and Chapter 8.6.3). Self-reported measured of dietary intake such as FFQ can provide a reasonable approximation of dietary intake and lead to insights into diet-disease relationships. Thus, they remain an important focus of public health (Subar et al., 2015). As described by Byers (Byers, 2001, pp 1087):

"There is perhaps no other epidemiologic discipline that has attracted as much public attention and, at the same time, as much scientific criticism as has dietary epidemiology. That is because the exposure is both of immediate interest to the public and notoriously difficult to measure."

Here, I present only a few examples to illustrate the vast complexity of diet. In MoBa and ALSPAC I observed an upward trend in intakes of protein, fibre, vitamins, and minerals as adherence to HPDP increased. Although consistent with expectations, I directly measured

only 26 nutrients. Diet is estimated to contain over 26,000 distinct biochemicals, and the composition of bioactive components varies, as do their interactions and individual physiological response (Barabási et al., 2020). Bioavailability of nutrients varies dependant on numerous factors, including the presence of other bioactive components (Caudill, 2010). For instance, folate has lower bioavailability than folic acid which is a synthetic version used in fortified foods. Cooking method and other forms of food processing, and exposure to light or heat are just some of the factors which alter the nutrient content of foods (Caudill, 2010). Moreover, some bioactive components typical in 'healthy' diets are potentially harmful to health. For example, in pregnant populations, biochemical indicators of air pollutants, endocrine disrupting chemicals, pesticides, and heavy metals, such as mercury, and arsenic, are positively associated with intakes of fruits, vegetables, fish, grains, and 'healthy' dietary patterns (Golding et al., 2018, Lin et al., 2021, Marks et al., 2021, McKean et al., 2015, Papadopoulou et al., 2019). Other bioactive substances in foods include additives, such as preservatives, flavourings, stabilisers, colourants, and sweeteners. Some of these components are associated with an increased probability of neurodevelopmental conditions, including autism (Cheroni et al., 2020, Fowler et al., 2023). Thus, a HPDP reflects an average measure of the totality of a vast number of components with synergistic and antagonistic effects.

I observed measured differences in the composition of a HPDP between MoBa and ALSPAC. Differences in factor loadings and nutrient intakes were observed between MoBa and ALSPAC. Factor loadings are the correlation coefficients between the factor (HPDP) and food item which reflect the importance of foods within a HPDP, rather than quantity (Zhao et al., 2021). Additionally, unmeasured foods may correlate with HPDP (Ocké, 2013). In MoBa and ALSPAC, a HPDP loaded highly for fruits, vegetables, nuts, lean and oily fish, and low in fries and processed meats. However, high fibre bread loaded positively in MoBa but negatively in ALSPAC, and soft drinks loaded negatively in MoBa but were unimportant in ALSPAC.

I compared the factor loadings observed in MoBa and ALSPAC in the context of cultural trends in diet, as absolute food intakes were difficult to compare between studies. For example, MoBa measured food items in grams per day and ALSPAC in frequency of consumption. The results were consistent with dietary trends observed in the respective countries' nationally representative diet and nutrition survey (Lang et al., 2003, Similä et al., 2003). For example, between 1986-87 the UK population had especially low wholegrain intakes, at 2.5 serving per week, of which bread accounted for 48% (Lang et

al., 2003). Whereas, in 2002, 79% of Norwegian women consumed at least one to six serving of high fibre bread per week (Similä et al., 2003). The distribution of food intakes by quantity may be distinct for other items too. For instance, Norwegian women consumed an average of 0.3 serving of fish per day (Similä et al., 2003), whereas British women consumed an average of 0.3 serving per week (Whitton et al., 2011). Hence, the underlying diets are likely to be different between English and Norwegian populations, and when measured in different decades.

Despite variability in the composition of HPDP, the estimated association with social communication difficulties were reasonably consistent between MoBa and ALSPAC. It remains possible that numerous bioactive components vary between a HPDP measured in MoBa and ALSPAC. Within a potential outcomes framework, this tension in composite measures, such as HPDP, can be managed by interpreting the average (Cole and Frangakis, 2009, VanderWeele and Hernán, 2013). Hence, in this thesis I used the average dietary composition of a low, medium, or high adherence to HPDP, to reflect the net contribution of all bioactive components on the association with social communication difficulties which were at least qualitatively similar. Therefore, I considered a 'healthy' diet is difficult to precisely define within a potential outcomes framework because of the complexity in the dietary composition of bioactive components and regional differences in dietary intake as well as measurement error. These are only some of the factors which may make causal questions about diet somewhat vague (Meltzer et al., 2008). The potential outcomes framework suggests this creates a degree of ambiguity in the interpretation of results because we cannot identify the specific causal component, if there is one (VanderWeele, 2016a). Nonetheless, in this thesis I observed reasonable consistency in the association between HPDP and social communication difficulties between MoBa and ALSPAC.

Across the existing literature a wide range of approaches to derive dietary patterns were applied (Geetha et al., 2018, House et al., 2018, Li et al., 2018, Vecchione et al., 2022), but the dietary pattern measure did not emerge as a clear source of heterogeneity in the study findings. The first three studies observed evidence of association despite different dietary pattern measures, whereas the last study observed no clear evidence across six different dietary patterns. When Li et al (Li et al., 2018) and Geetha et al (Geetha et al., 2018) retrospectively investigated the association offspring autism diagnosis had with prenatal dietary intakes, there was insufficient description of their dietary assessment method or dietary pattern. Descriptions provided were 'poor nutrition' (Geetha et al., 2018) and unbalanced patterns: 'mostly vegetables' or 'mostly meat' (Li et al., 2018). House et al

(House et al., 2018) observed a lower likelihood of autism-associated traits in relation to high prenatal Mediterranean diet score. In contrast, Vecchione et al (Vecchione et al., 2022) observed no clear evidence of association with autism diagnosis or autism-associated traits with prenatal Mediterranean diet score or the other five dietary patterns measured. The dietary patterns were; empirical dietary inflammatory pattern, alternative healthy eating index and the alternative healthy eating index for pregnancy, adapted Mediterranean diet score, and the Western and prudent dietary patterns (Vecchione et al., 2022). Thus, across all studies, including my investigation in Chapter 5, different definitions of prenatal dietary pattern, from various countries have been associated with a lower likelihood of autism diagnosis (Geetha et al., 2018, Li et al., 2018) or autism-associated traits (House et al., 2018), though, not consistently (Vecchione et al., 2022).

At a population level, dietary patterns provide a broad approximation of a 'healthy' diet which averages across the rich aspects of diet, measured and unmeasured (Hu, 2002, Liese et al., 2015, Subar et al., 2015). When defined broadly, as I have in this thesis, I was able to detect reasonably consistent associations with social communication difficulties between MoBa and ALSPAC, and the findings were consistent with three other studies (Geetha et al., 2018, House et al., 2018, Li et al., 2018). Similar consistency in diet-outcome relationships is observed in other fields (Cena and Calder, 2020, Subar et al., 2015). It has been suggested that this may relate to broadly similar core components of a 'healthy' diet such as high fruits, vegetables, wholegrain intakes, and low intakes of animal products and highly processed foods (Schulze et al., 2018). Additionally, there is wide dispersion of nutrients and other bioactive components across a range of foods, coupled with multiple nutrients being suspected aetiological factors (Gusso et al., 2023). Other studies may complement this body of research by focusing on specific nutritional aspects.

8.5.2 Food groups

Dietary patterns reflect the holistic nature of diet and as such cannot identify key food groups (Zhao et al., 2021). Information on specific food groups may direct aetiological investigations and help tailor dietary advice. Although nutrients are distributed across a wide range of foods, some foods are an especially rich source of certain nutrients. For example, whole grains are a rich source of fibre, phytonutrients, and B vitamins (Belobrajdic and Bird, 2013), and fruit and vegetables are a rich source of flavonoids and carotenoids (Panche et al., 2016). In Chapter 5, I assessed whether specific food groups included in HPDP were more or less strongly related to each outcome. I interpreted the

results cautiously because this was a sensitivity analysis and because of the limitations to food group analysis (Tapsell et al., 2016). For example, correlation between foods, approach to measure food groups as they may be sensitive to the number of components I included in factor analysis, some foods are more and less sensitive to misreporting, and potentially have different confounding structures (Tapsell et al., 2019). Further studies would be required to confirm whether plant-based foods have stronger associations with social communication difficulties, compared to fish-based and 'unhealthy' foods.

As discussed in Chapter 1, there were conflicting results across the four previous studies that investigated maternal intakes of fruit and/or vegetables (Gao et al., 2011, Joyce et al., 2022, Lyall et al., 2023), cereals (Gerges et al., 2020), wholegrains, nuts, and legumes (Lyall et al., 2023). A lower likelihood of autism diagnosis was associated with high maternal fruit intakes and cereals, which is contrary to my findings on autism diagnosis (Gao et al., 2011, Gerges et al., 2020). However, both studies had a high risk of confounding from other food groups, whereas I adjusted for other food groups. Hence, this may partly explain why the association I observed was weaker. With regards to autism-associated traits, only one study directly compared food groups. Bayesian network analysis simultaneously models food groups in the context of overall diet and was applied when investigating the NHSII and EARLI (Lyall et al., 2023). High leafy green vegetable intakes associated with an increased SRS score (more autism-associated traits), whereas in EARLI there was weak evidence to suggest SRS score reduced as leafy green vegetable intakes increased. There were no other associations observed. Specific foods may have weak associations as analysed by Lyall et al (Lyall et al., 2023), however Joyce et al (Joyce et al., 2022) combined all prenatal fruit and vegetable intakes and also did not observe an association with SRS in EARLI. Lyall et al's (Lyall et al., 2023) investigation may be limited by low sample size. Furthermore, I observed the clearest associations with social communication difficulties, not SCQ which would be more comparable with SRS. This further highlights the need for future studies to analyse the subdomains of autism separately.

The authors suggested the increase in autism-associated traits in relation to higher intakes of certain vegetables could relate to pesticides (Lyall et al., 2023). Environmental toxins are increasingly queried as causally involved in autism related outcomes (Brown et al., 2018, Cheroni et al., 2020, Granillo et al., 2019, Skogheim et al., 2021, Ye et al., 2017). As discussed above, pollutants in diet are consumed with beneficial bioactive components, therefore, the net effect of pollutants with diet may vary compared to pollutants alone

(Bragg et al., 2022, Golding et al., 2018, Joyce et al., 2022). I also observed an increased likelihood of autism-associated traits in relation to plant-based foods which attenuated in the analysis of fish-based foods. However, these results were not consistent overall. The association was specific to SCQ-RRB-3, which did not differentiate between autistic and non-autistic children. In relation to evidence on pollutants, early studies indicate that nutrients may interact with pollutants and attenuate potential negative effects of pollutants on autism related outcomes (Bragg et al., 2022). Another EARLI study measured pesticide residue on fruit and vegetables and observed no clear evidence of association with SRS (Joyce et al., 2022), though direct measures of pollutants in NHSII would be preferred. Further research is required to assess pollutants on foods and their association with autism.

Previous investigations into food groups have largely focused on prenatal intakes of fish. Across six cohorts (five publications) (Gao et al., 2016, Julvez et al., 2016, Lyall et al., 2013, Steenweg-De Graaff et al., 2016, Vecchione et al., 2021), four observed no clear evidence of association with autism diagnosis or autism-associated traits (Lyall et al., 2013, Steenweg-De Graaff et al., 2016, Vecchione et al., 2021). Unlike autism studies on whole dietary patterns or other food groups, investigations into fish did include large prospective studies. In my investigation, I also did not observe any clear associations with fish-based foods. Yet again I would caution that as a sensitivity analysis, my approach to measure fish intakes may not be optimal. Notably, the other two large prospective cohorts that investigated maternal fish intakes, did so as a secondary or sensitivity analysis (Lyall et al., 2013, Steenweg-De Graaff et al., 2016). Thus, there is a need for large prospective cohort studies to investigate prenatal fish intakes in association with autism. Studies should consider different approaches to measure prenatal fish intakes, such as; timing in pregnancy, quantified fish intakes, maximum recommended fish intakes; they should also measure types of fish, for example, oily or non-oily; and finally fish intake in relation to toxins and pollutants. When two opposing forces, for instance, beneficial nutrients and harmful chemicals originate from the same foods, it is particularly difficult to account for this in estimates of the impact of diet (Budtz-Jørgensen et al., 2007, Choi et al., 2008, Ye et al., 2017). This will be true across all foods but as a potentially important aspect of diet it should remain an area for further investigation.

‘Unhealthy’ components generally had fewer clear associations with each outcome measured in this thesis. Only Vecchione et al (Vecchione et al., 2022) measured an ‘unhealthy’ dietary patterns in the form of a Western dietary pattern. The positive association observed between a Western dietary pattern and SRS score attenuated with

adjustment for total energy intakes, which indicated that gestational weight gain may have driven the associations. Although my findings are consistent with Vecchione et al (Vecchione et al., 2022), given the small number of investigations, further studies that investigate 'unhealthy' foods and autism related outcomes advantageous.

8.5.3 Prenatal nutritional supplementation

In Chapter 4, there was a 23% reduction in the probability of offspring autism diagnosis if mothers took a prenatal multivitamin supplement. I identified one study published after my systematic review. In Chapter 4, I highlighted that most studies did not adjust for prenatal dietary intake which may have overestimated the associations observed. However, in Chapter 5, adjustment for prenatal multivitamin supplement use, generally did not markedly alter the main results. Additionally, there was no clear evidence of an interaction between a HPDP and prenatal multivitamin supplement use. Thus, the magnitude of association between HPDP and autism diagnosis and social communication difficulties, was not altered by prenatal multivitamin supplement use as measured in this thesis. Therefore, prenatal multivitamin supplement use appeared to have an (at least partly) independent association with autism diagnosis.

Nutritional supplements make an important contribution to total nutrient intakes; hence an interaction with diet is biologically plausible (Brown and Wright, 2020). Nutritional demand for nutrients increases in pregnancy, and although maternal adaptation helps to achieve the increased demand, dietary intakes, even in a healthy diet, may fall short of certain nutrient requirements (Brown and Wright, 2020). Compared to each country's respective National Dietary Guidelines, ALSPAC had nutrient intakes below the reference nutrient intake for vitamin D, folate, iron, iodine, magnesium, and potassium. MoBa had intakes below the Nordic nutrient recommended intake for vitamin D, folate, iron, and low iodine status. This was even observed in participants with the highest adherence to HPDP. Furthermore, in MoBa, previous research demonstrated that prenatal supplement use made a substantial contribution to dietary intakes of; vitamin D, docosahexaenoic acid, eicosapentaenoic acid, vitamin B_6, folate, copper, and iron (Haugen et al., 2008). Unfortunately, there is no comparable data for ALSPAC, however, MoBa's findings are consistent with the wider literature (Brown and Wright, 2020). Hence, multivitamin supplements can provide additional nutrients that may not be attained through a 'healthy' diet alone.

There are several explanations for why I did not observe an interaction between HPDP and prenatal multivitamin supplements use in this thesis. Tests for interaction are sensitive to the scale of measurement. Supplements may still have an independent association with autism on an additive scale. On the other hand, the upper safe level of nutrient intakes is more likely to be exceeded through nutritional supplement use, though the likelihood remains low (Brown and Wright, 2020). For example, in MoBa, the upper safe level was exceeded by <1% of women for most nutrients, except retinol which was exceeded by 3.9% of women (3,000 μg/day), and zinc by 9.0% of women (25 mg/day)(Haugen et al., 2008). Nonetheless, in Chapter 3, I discussed debates around the safety of very high folic acid intakes in pregnancy. Moreover, in Chapter 1, high prenatal serum levels of folate and vitamin B_{12} were associated with an elevated probability of autism diagnosis, compared to adequate levels (Raghavan et al., 2018, Sourander et al., 2023). Many factors can influence the results in this thesis, such as compliance, measurement error, nutritional composition, dose, or critical period in pregnancy. Additionally, there can be a lag period between nutrient intakes and establishment of sufficient nutritional status (Brown and Wright, 2020). Several biological and methodological factors may alter the statistical interaction measured in this thesis. In summary, there was no clear evidence prenatal multivitamin supplements use versus non-use altered the magnitude of association between HPDP and each outcome.

8.5.4 Biochemical indicators of prenatal nutritional status

A HPDP captures the summation of discrete nutrients and their interactions, whereas nutritional biomarkers typically measure single nutrients. The existing literature on prenatal nutritional biomarkers and autism, has indicated nutrients that may be of importance. From the narrow range of prenatal nutritional biochemical indicators investigated, adequate vitamin D serum levels and adequate folate and vitamin B_{12} serum levels were associated with a lower probability of autism diagnosis, compared to insufficient or excessive vitamin D, vitamin B_{12}, and folate levels (Chen et al., 2016, Fernell et al., 2015, Lee et al., 2019a, Madley-Dowd et al., 2022, Schmidt et al., 2019b, Sourander et al., 2021, Vinkhuyzen et al., 2018, Vinkhuyzen et al., 2017, Whitehouse et al., 2013, Wu et al., 2018). However, associations between prenatal PUFAs serum levels and autism were especially inconsistent (Huang et al., 2020, Lyall et al., 2021, Steenweg-De Graaff et al., 2016). Investigation of a wider range of nutrients may help strengthen this evidence.

A ‘healthy’ diet may contribute towards intakes of vitamin D and folate but may not be sufficient to achieve optimal levels. As discussed, prenatal vitamin D, folate, iron, and iodine intakes were below recommended nutritional requirements even in a ‘healthy’ diet. Indeed, the main source of vitamin D is sunlight and national public health policies in Nordic countries, including Norway (Itkonen et al., 2021) and the UK (Scientific Advisory Committee on Nutrition (SACN), 2016), recommend vitamin D supplementation. Furthermore, Folic acid supplements are recommended in pregnancy (Dwyer et al., 2022), and the UK will soon introduce mandatory fortification with folic acid (UK Government, 2021). Many countries introduced mandatory fortification with folic acid as dietary intakes were insufficient to lower the risk of neural tube defects (Kancherla et al., 2022). If similar levels were required to lower the likelihood of autism, a ‘healthy’ diet would contribute importantly to intakes but may need to be enhanced through supplementation or fortification. Nonetheless, there may be several nutrient involved in the aetiology of autism, and many are attainable from a ‘healthy’ balanced diet.

8.5.5 Section summary on dietary patterns, food groups, nutritional supplements, and biochemical indicators of nutrients

In summary, any combination of the 26,000 plus bioactive compounds in diet may have led to a lower probability of autism diagnosis and social communication difficulties. Yet, the summation of all bioactive compounds still produced reasonably consistent associations between HPDP and social communication difficulties, and with autism diagnosis when compared to the wider literature. The primary focus of the thesis was whole dietary intake in pregnancy, yet knowledge of other facets of prenatal nutrition can complement the findings on prenatal diet. Plant-based foods may be particularly important, however, further research is required to clarify this and should consider whether pollutants modify the associations with autism, even though it is difficult to discern the effects of foods from toxins and pollutants (Budtz-Jørgensen et al., 2007, Choi et al., 2008). Nutritional supplements make a substantial contribution to nutrient intakes and have also been associated with a lower probability of autism. Lastly, research into biochemical indicators of prenatal nutrients point to adequate vitamin D and folate status as potentially important, which a HPDP can contribute towards achieving.

8.6 Potential mechanisms and nutritional aetiology of autism

The aetiology of autism is currently attributable to a combination of environmental and genetic factors(Hertz-Picciotto et al., 2018, Lyall et al., 2017). Several fields of research have contributed novel findings, yet these insights remain fragmented. Numerous factors have been implicated and the pathways may diverge depending on the cluster of autistic traits. At each stage of brain development, nutrients can have global and specific effects, which could be modified by the presence of genetics, other nutrients, risk factors, and progress in neural development thus far. Here, I describe some key insights into the potential nutritional aetiology of autism. These potential mechanisms are likely to be inter-related.

8.6.1 Critical, sensitive, and cumulative effects

The multiple threshold liability model suggests that the cumulative effects of an individual's genetic architecture and the totality of environmental exposures, the 'exposome', leads to autism (Georgieff et al., 2018). In addition to cumulative effects, there can be critical and sensitive periods. Critical periods refer to specific periods of time after which repair is not possible, whereas during sensitive periods brain development is more responsive to nutrition but a degree of neural plasticity remains (Georgieff et al., 2018). Foetal development is a process of rapid cellular growth, migration, and differentiation. During rapid growth, organs, including the brain, are more vulnerable to damage or 'programmed' effects (Georgieff et al., 2018). There is a high degree of neural plasticity until approximately two years of age, and so to an extent 'catch-up' periods may mitigate prior negative influences.

8.6.2 Genetics, epigenetics, and prenatal nutrition-gene interaction

Gene-nutrient interactions may occur through epigenetic changes or de novo mutations. Early evidence suggests that epigenetic modification may even increase the risk of de novo mutations (Pugsley et al., 2022). Several salient environmental factors associated with autism, including prenatal diet, have established epigenetic, mutagenic, and potentially genotoxic effects.

Gene-nutrient interactions can directly alter gene expression through histone modification and methylation, and these processes may be influenced by immune regulation, sex-hormones, microbiome, and oxidative stress (Lyall et al., 2017). In Chapter 4, I described a study by Schmidt et al (Schmidt et al., 2012) who identified evidence of a gene-folate interaction. MTHFR 677 C>T is a common polymorphism that less efficiently metabolises folate. Prenatal folic acid intakes were associated with a greater reduction in autism diagnosis if mothers or children had the C>T variant (Schmidt et al., 2012). The gene-folate interaction is thought to arise as folate has a critical role as a methyl donor during DNA and histone methylation in one-carbon metabolism. Other nutrients have also been related to epigenetic modification such as Choline and other B vitamins involved in one-carbon metabolism (Zhu et al., 2020), PUFAs in DNA methylation, and indirect effects of iron on methylation processes (Georgieff et al., 2018). In a two-step MR analyses, another study identified evidence that prenatal vitamin B_{12} altered DNA methylation patterns and appear to have a causal association with child IQ score (Caramaschi et al., 2017).

Putative de novo mutations are carried by an estimated 5 to 15% of individuals with an autism diagnosis. Environmental toxins can trigger de novo mutations through various mechanisms. For example, oxidative damage to DNA can lead to de novo mutations and several nutrients have antioxidant properties and protect DNA (Pugsley et al., 2022). Insufficient antioxidant availability and/or excessive production of oxidants such as free radicals, can increase the risk of oxidative damage to DNA (Pugsley et al., 2022). For example, increased exposure to pollutants and toxins, and cardiometabolic disorders may increase oxidative stress in the body, which antioxidant intakes can ameliorate, to an extent (Cheroni et al., 2020).

Evidence suggests the aetiology of autism may differ depending on the presence of co-occurring intellectual disability (Thomas et al., 2022, Warrier et al., 2022, Xie et al., 2020). In an autistic population, co-occurring intellectual disability is associated with more de novo mutations which lowered the estimated heritability (Warrier et al., 2022). Estimated heritability of autism with co-occurring intellectual disability was 33.4% compared to 64.6% in autism without co-occurring intellectual disability (Xie et al., 2020). Furthermore, autism without intellectual disability had a stronger family history of psychiatric conditions, suggesting a stronger heritable component (Xie et al., 2020). Several studies have found prenatal socioeconomic and lifestyle factors have a greater magnitude of association in autism with intellectual disability than without. In Chapter 4, in all studies that stratified autism diagnosis by severe and mild forms, there was a greater

reduction in severe autism in association with prenatal multivitamin supplement use (DeVilbiss et al., 2017, Schmidt et al., 2019a, Suren et al., 2013, Tan et al., 2020). Similar trends were observed in association with maternal anaemia $\leq 30^{th}$ week of pregnancy (Wiegersma et al., 2019) and prenatal serum polyunsaturated fatty acids (Lyall et al., 2021) but not neonatal vitamin D (Schmidt et al., 2019b, Windham et al., 2019a). Potentially, HPDP may have a greater role in the aetiology of autism with intellectual disability (Xie et al., 2020). However, this would need to be investigated in further studies.

8.6.3 Maternal immune activation

Maternal exposure to infection in pregnancy was first found to be associated with autism in epidemiological studies. Yet, it was subsequent animal models that demonstrated maternal immune activation affected neurodevelopment, rather than a direct response to the pathogen (Han et al., 2021a). Subsequent investigations have identified considerable evidence to support maternal immune activation as an aetiological factor in many neurodevelopmental conditions (Brown et al., 2014, Goines et al., 2011, Han et al., 2021b, Zerbo et al., 2016). Autism is associated with many dysregulated immune conditions and symptoms, such as autoimmune diseases, gastrointestinal dysfunction and food intolerances, atypical gut microbiome, and atopic skin complaints. Hence, immune dysregulation is a common feature in individuals with autism and may have early origins. Gene-immune interaction also occurs. For example, there is evidence that common genetic variants are associated with vulnerability to immune response (Mazina et al., 2015), and epigenic modification occurs secondary to immune processes (Zhu et al., 2020).

The placenta has numerous roles in pregnancy in addition to its primary function as the mechanism of exchange between mother and foetus, it also serves to pass substances such as nutrients and oxygen (Han et al., 2021a). The placenta has a role in maternal endocrine and vascular adaptation as well as foetal immune homeostasis. Foetal programming by maternal immune activation can occur through various pathways. In animal models, maternal immune activation is communicated to the foetus via processes related to immune, metabolic, endocrine, and stress. These processes can directly influence the function of the placenta or result in substrates that are transferred to the foetus. Collectively these processes can alter and ‘prime’ the foetal hypothalamic-pituitary-adrenal axis response and other foetal structures and processes. The hypothalamic-pituitary-adrenal axis is a complex system with many functions, including the structural and functional maturation of the central nervous system and regulation of inflammatory response.

Dysfunction of the hypothalamic-pituitary-adrenal axis has been linked to autism (Han et al., 2021a, Han et al., 2021b).

8.6.4 Maternal microbiome

It has been suggested that other factors such as diet, stress and depression which are more common in mothers of low socioeconomic status, may increase maternal gut permeability and increase transfer to placenta and foetal gut (Stiemsma and Michels, 2018). This may impact foetal immune function via foetal gut microbiome or by triggering epigenetic changes (Han et al., 2021b). Animal models have shown that maternal immune activation leads to changes in the offspring gut microbiome which contributes to behavioural abnormalities. Maternal gut microbiome may modulate the effects of maternal immune activation on offspring brain development. The mechanisms are unknown and require substantiation in human studies (Meyer, 2019). However, maternal diet may alter gut microbiome, particularly fibre which has been shown to affect substrates produced by the gut microbiome. Furthermore, the gut microbiome synthesises some vitamins and amino acids that are absorbed and used in the body. Yet, more research is required to understand the function of maternal gut microbiome in pregnancy, let alone any potential role in the development of autism in offspring. This evidence is yet to be established but is a mechanism of considerable interest (Meyer, 2019).

8.6.5 Summary of mechanisms and nutritional aetiology of autism

There are likely multiple pathways involved in the aetiology of autism, and each highlighted here may be interrelated. The role diet plays in each of these pathways is numerous (Schwarzenberg et al., 2018). Diet is required to provide essential nutrients to support foetal development including brain growth. There may be global and indirect effects, for example, a healthy diet promotes good general health, and reduces the risk of maternal immune activation. Each bioactive component may have multiple functions (Gusso et al., 2023). For example, flavonoids are a class of bioactive compounds with a phenolic structure and are naturally occurring in a range of fruits, vegetables, and some other plant-based foods, and certain drinks. Flavonoids are attributed to several beneficial effects through their antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties (Panche et al., 2016). Polyunsaturated fatty acids have an established role in neurodevelopment as key cellular components in the brain, regulators of inflammatory processes, and neuron growth (Schwarzenberg et al., 2018). Vitamin D also has a

particularly salient role in maternal immune activation and neuroinflammation (Hollis and Wagner, 2017). However, the effect of prenatal nutrition could be modified by other factors. Several studies observed an increased likelihood of autism in relation to prenatal pollutants and toxins exposure that was attenuated in the association with better prenatal nutrition (Bragg et al., 2022). This relationship may be further modified by genetic vulnerability (Cheroni et al., 2020). Potentially HPDP relates to the aetiology of autism through multiple pathways at critical and sensitive periods, and through accumulative effects.

8.7 Inequalities

Health inequalities in autism diagnosis and autism-associated traits were evident in this thesis, and other studies (Dodds et al., 2011, Fujiwara, 2014, Goh et al., 2018, Larsson et al., 2005, Lehti et al., 2013, Li et al., 2014, Lung et al., 2018, Rai et al., 2012, Russell et al., 2014, Segev et al., 2019, Tsai et al., 2017). However, there were inconsistencies which may relate to diagnostic bias and socio-cultural differences (Bhasin and Schendel, 2007, Burd et al., 1999, Croen et al., 2002, Kelly et al., 2019, King and Bearman, 2011, Yu et al., 2021). In Chapter 7, I observed a greater magnitude of association between each outcome and maternal education. Whereas parental income had relatively weaker associations with autism diagnosis and restrictive and repetitive behaviours, it was not associated with social communication difficulties.

The findings highlight how inequalities in maternal education and parental income can be perpetuated into the next generation. Inequalities in foetal and child health cast a long shadow over social and economic capacity, and subsequently health and quality of life in adulthood (McKerracher et al., 2020). Co-occurring conditions are common in autism, as are difficulties with socialisation, executive function, attention, and emotional regulation (White et al., 2016). These place additional challenges to maintaining further education and occupation. Educational attainment is lower compared to non-autistic populations (Keen et al., 2016, Paget et al., 2018, Toft et al., 2021). In an autistic population with average to high IQ, noncompletion rates for further education are higher than non-autistic populations (Toft et al., 2021). Moreover, an investigation into ALSPAC identified that children with social communication difficulties were around seven and half times more likely to be excluded from school (Paget et al., 2018). Subsequently, individuals with autism have lower educational attainment and lower employment rates compared to non-autistic populations (Keen et al., 2016, Toft et al., 2021). Hence, not only are there potential secondary effects of autism on socioeconomic deprivation (Catalá-López et al., 2022), this thesis found that people with autism were more likely to have mothers affected by deprivation during pregnancy. Overall, people with autism may have greater morbidity and mortality rates compared to the general population of non-autistic people (Hirvikoski et al., 2016, Schendel et al., 2016).

8.7.1 Potential pathways

In Chapter 7, maternal education had a stronger and more consistent relationship with each outcome, and in the wider literature is recognised as being especially important to child development (Pearce et al., 2019). The mechanisms through which indicators of socioeconomic deprivation relate to autism diagnosis or autism-associated traits is difficult to fully discern. This is due to the multiplicity of pathways and their dynamic inter-relatedness. Subsequently, maternal education is highly correlated to the other major dimensions of socioeconomic status as well as downstream factors. Hence, in Chapter 7, maternal education may also have a stronger relationship with each outcome as it is a determinant of other socioeconomic indicators, such as occupation, income, and area-based deprivation.

Several pathways may link maternal education and parental income to each outcome. For example, low maternal education is associated with poverty, single motherhood, unsupportive or even destructive relationships, stress, depression, and instability at home. A study observed that children had lower cognitive stimulation, for example, the provision of learning materials or activities, if mothers had low educational attainment (Hackman et al., 2010). Moreover, another study found that part of the association maternal education had with child cognitive development is mediated through household income, and a stable family structure which was defined as the mother cohabiting with the biological father (Jackson et al., 2017). Additionally, low levels of education have an especially strong relationship with maternal depression, which adversely affects child development (Jackson et al., 2017, Pearce et al., 2019).

The weaker associations observed with parental income compared to maternal education may relate to methodological approach. In the wider literature, income is recognised as a challenging socioeconomic indicator to measure (Benzeval and Judge, 2001, Shi et al., 2021, Subramanian and Kawachi, 2006). Parental income may not be a good proxy indicator of the financial resources available to the mother. Household income or expendable household income is often preferred, particularly for women who may not be the main earners of the household (Galobardes et al., 2006a, Galobardes et al., 2006b). However, only individual gross income was available and coded categorically, and so I derived parental income based on both parents' income category. It has been observed that female income is more sensitive to the definition of income, compared to men (Shi et al., 2021). Furthermore, I do not have knowledge of the distribution of financial resources

within the household. There is evidence to suggest mothers are more likely to prioritise distributing resources to their family before themselves (Sawyer et al., 2021). Furthermore, the sample of MoBa used in this thesis was recruited over 6 years, and inflation could not be accounted for. Hence, misclassification bias could impact our findings in relation to income. Lastly, parental income associated with autism diagnosis and restrictive and repetitive behaviours only which contrasts to maternal education. As highlighted by other researchers, paternal characteristics are important within the DOHaD literature (Sharp et al., 2018, Suren et al., 2014) and may confounding the association between parental income and autism diagnosis and restrictive and repetitive behaviours. The magnitude of association we observed with parental income may change in other studies based on different measures of income, especially if they more closely reflect the financial resources available to the mother.

8.7.2 Potential pathways related to a 'healthy' prenatal dietary pattern

The CDE estimates the extent to which HPDP may explain the relationship between maternal deprivation and each outcome. Generally, a modest proportion of maternal education was mediated through HPDP to each outcome or there was little to no change in the TE to the CDE. The association between parental income and autism diagnosis and restrictive and repetitive behaviours (age eight), was partly or completely attenuated through HPDP.

Potential mechanisms which link maternal education to diet and each outcome are numerous, and as discussed are correlated to the other indicators of deprivation. Here, I focus on a few examples to illustrate some potential pathways. Education is an important predictor of access to health information, and people with high educational attainment benefit more from health information campaigns (Adams et al., 2016). Studies have observed higher diet quality in pregnant women of higher educational attainment compared to low educational attainment (Gete et al., 2022, McLeod et al., 2011). Furthermore, nutritional knowledge was found to partly mediate the relationship between maternal education and diet quality (McLeod et al., 2011). Higher socioeconomic position is related to greater internal locus of control and self-efficacy, and women with these traits exhibited better dietary choices and health behaviours (Freed and Tompson, 2011). However, socioeconomic drivers of dietary intake are complex, and individual level aspects are overshadowed by determinants of knowledge and self-efficacy such as financial hardship,

instability, and higher levels of stress (Baird et al., 2017, Barker et al., 2017, McKerracher et al., 2019, McKerracher et al., 2020).

Several studies have related income to diet. Low-income groups, on average, spend a greater proportion of income on food which is of low nutritional value. For example, high income groups in America spent 11% of their income on food compared to 25% or more in low-income groups (Drewnowski and Specter, 2004). However, this does not fully capture the disparity. Despite spending a greater proportion of income on food, on average, the nutritional value is lower. This is because the absolute amount spent on food decreases in low-income groups, but as a proportion of income it increases. These cheaper foods are less nutritious and are typically higher in refined grains, added sugar and fats (Darmon and Drewnowski, 2015). Furthermore, simulation models have attempted to estimate the cost of consuming 'healthy' and 'unhealthy' diets. In women from the UK, compliance with national dietary guidelines was estimated to cost double that of the least 'healthy' diet at £6.63/day versus £3.29/day per person, respectively (Morris et al., 2014). Similarly, an Australian study estimated 40% of income would be spent in low-income groups if dietary guidelines were adhered to, compared to a 25% benchmark that was considered acceptable (Kettings et al., 2009). Yet, some have questioned if it is possible to make cheaper exchanges and still meet dietary guidelines. The results across the literature are mixed. Participants of some intervention trials have reported no change to food costs, and one randomised controlled trial reported this was similar between advantaged and disadvantaged groups (O'Brien et al., 2017). However, some simulation models demonstrated that although healthier diets are theoretically possible at lower costs, they may divert from social and cultural norms (Darmon and Drewnowski, 2015). Furthermore, increasing use of food banks or food pantries is related to low income and reasons cited for their use is inability to afford food, regardless of nutritional value (Garratt, 2020).

In a systems approach, Sawyer et al (Sawyer et al., 2021) mapped the dynamic and inter-related socioeconomic drivers of dietary choices of low-income groups. Income can impact the affordability of food and correlate with area-based deprivation. Low-income groups are more likely to reside in deprived areas compared to high income groups. Deprived areas often have a lower density of supermarkets and healthy food stores and higher density of unhealthy food stores which can necessitates travel to purchase healthier foods. However, low-income groups have a greater reliance on public transport which consumes more time and money, especially for carers of young children. Furthermore, bulk buys are more cost effective but impractical on public transport. Low-income groups also experience more

stress which may lead to unhealthy food choices and overeating. Over time these choices may normalise unhealthy food choices on an individual and cultural level, making unhealthy foods acceptable. Therefore, there may be a feedback loop whereby multiple inequitable influences drive demand to supply unhealthy food products to low-income groups (Sawyer et al., 2021).

8.7.3 Prenatal diet as an intervention target

In this thesis, the proportion of inequality related to each outcome was modestly reduced under the hypothetical 'intervention' of a HPDP, if at all. By measuring the extent to which HPDP explained the relationship between maternal deprivation and autism related outcomes, I have shown that a HPDP may be a target for intervention, but as part of a wider strategy to reduce health inequalities in the outcomes.

The structural determinants of health are thought to create the socially patterned distribution of proximal factors such as prenatal dietary pattern and barriers to intervention (Baird et al., 2017). As discussed, downstream determinants of diet, such as nutritional knowledge, are overshadowed by intermediate level of influence, such as depression, instability, financial hardship, and social and environmental factors (Barker et al., 2017). The intermediate level factors are ultimately related to higher level factors such as education, income, power, and status (Øversveen and Eikemo, 2018). Efforts to address diet quality through intervention may widen health inequalities, unless socially patterned drivers of diet quality are adequately addressed (Adams et al., 2016). Potentially, prenatal diet may be an effective intervention target to reduce inequalities when implemented as part of a complex intervention (Barker et al., 2017, McKerracher et al., 2020).

There is much debate about the most effective approach to address health inequalities (Mackenbach, 2016, Marmot, 2020, Øversveen and Eikemo, 2018, Wilderink et al., 2022). Inequalities continue to be a significant issue and current strategies need to improve, as evidenced in the persistence or even widening of health inequalities. For example, in England between 2014 to 2017 the downward trend in infant mortality within the first year of life reversed in deprived local authorities, but in affluent areas there was no clear change in trajectory. Subsequently, existing inequalities worsened by 52 more infant deaths per 100,000 in the most deprived local authorities compared to the least deprived (Taylor-Robinson et al., 2019). Moreover, in the UK infant death rates in the first year of life are

59% higher than Sweden, based on nationally representative cohorts (Zylbersztejn et al., 2018).

Health inequalities persist in many countries, despite different social strategies (Mackenbach, 2016, Mackenbach et al., 2008). Even in Norway, a highly egalitarian country, the persistence of relative health inequalities has been observed (Mackenbach et al., 2018). As a researcher and leader in the field of health inequalities, Marmot has had a central position in major reviews of the social determinants of health and is an open critic of the Conservative UK Government (Acheson et al., 1998, Marmot et al., 2020, Marmot et al., 2010). Marmot argues that the worsening of health inequalities in the UK is attributable to the UK Government's failure to adequately implement recommendations to redistribute public expenditure and address health inequalities (Marmot et al., 2020). Similar criticisms have been directed at Governments in other countries such as Denmark. Yet, an ambitious major public health strategy to reduce health inequalities was proposed in Norway in 2007 and supported by the Norwegian Government (Øversveen and Eikemo, 2018). The 10-year strategy was informed by health inequalities research and concentrated on long term strategies to address the structural determinants of health, using a social gradient approach. The Norwegian strategy targeted health across the population with increasing focus on the most deprived populations. Furthermore, the strategy targeted the distribution of resources at a structural level and individual level risk factors. For example, the strategy focused on education, poverty, child welfare, as well as behaviour and lifestyle factors. Yet, although indicators of health suggest Norwegians have amongst the best health outcomes across Europe, inequalities in the distribution of health persist.

The findings may highlight the scale and complexity of addressing health inequalities. Ultimately, there are many theories for the persistence of health inequalities (Mackenbach, 2016). It has been argued that not enough research focuses on 'the causes of the causes', or in other words, what causes the socioeconomic structures themselves (Øversveen and Eikemo, 2018). Advances may be achieved through a greater focus on how those with social and political power control the distribution of resources across a population and may therefore perpetuate health inequalities (Øversveen and Eikemo, 2018). Other researchers argue that a complex whole-system approach to address health inequalities is required and would include structures at the macro, meso, and micro levels (Wilderink et al., 2022). Each recognises the scale and complexity of addressing inequalities and the multifaceted approaches required. This thesis contributes to the literature on inequalities in autism diagnosis and autism-associated traits by identifying prenatal diet as potentially explaining

a modest proportion of inequalities in these outcomes. Thus, diet is one of many important factors that may mediate inequalities in each outcome.

8.8 Key methodological considerations and limitations

Causal inference requires a range of experimental and nonexperimental study designs, and can include lab and animal studies. Therefore, I positioned this thesis in the context of a body of evidence that contributes to the aetiological understanding of autism. In doing so, I do not purport the estimates to be causal effect estimates. Rather, I recognise that the methodology employed should realistically address the scientific question and the interpretation should rigorously respect the limits of knowledge. Hence, our estimates are measures of association. Causally informed approaches, such as DAGs, the potential outcomes framework, and triangulation support such interpretations by stipulating assumptions to evaluate our research against and identify bias (Lawlor et al., 2016, Petersen and van der Laan, 2014). Thus, in keeping with these principles, I now discuss the key methodological considerations and limitations of this thesis.

8.8.1 Exchangeability

The exchangeability assumption from the potential outcomes framework stipulates there should be equal probability of receiving each level of the exposure. Hence, confounding factors will violate the exchangeability assumption because they lead to an unequal probability of the exposure (Hernán and Robins, 2016). Large prospective cohort studies confer a high risk of confounding by socioeconomic and lifestyle characteristics due to the non-random allocation of exposure level. In this thesis I employed strategies to manage confounding bias, such as causally informed approaches to identify potential confounders, cross-context comparison between MoBa and ALSPAC, and further triangulation with PGS-MR. In Chapter 4 and Chapter 5, I adjusted for a range of covariates, including paternal characteristics, yet confidence that I have achieved exchangeability is limited from these studies alone. In the cross-context comparison, there was some consistency in the associations between HPDP and social communication difficulties observed across cohorts from different decades and countries. Additionally, some differences in the underlying confounding structures were observed. For example, MoBa women were older at recruitment and had a higher level of educational attainment and positive health behaviours compared to women in ALSPAC. Yet, in both cohorts the overall direction of confounding bias is expected to exaggerate any potential true association. Triangulation of the association between HPDP and SCQ outcomes measured in Chapter 5 and Chapter 7 was intended to provide clarity on potential confounding bias by socioeconomic and lifestyle characteristics. However, I remain uncertain as to whether the null results in the PGS-MR

are due to a lack of causal association or insufficient statistical power to detect an association should one exist.

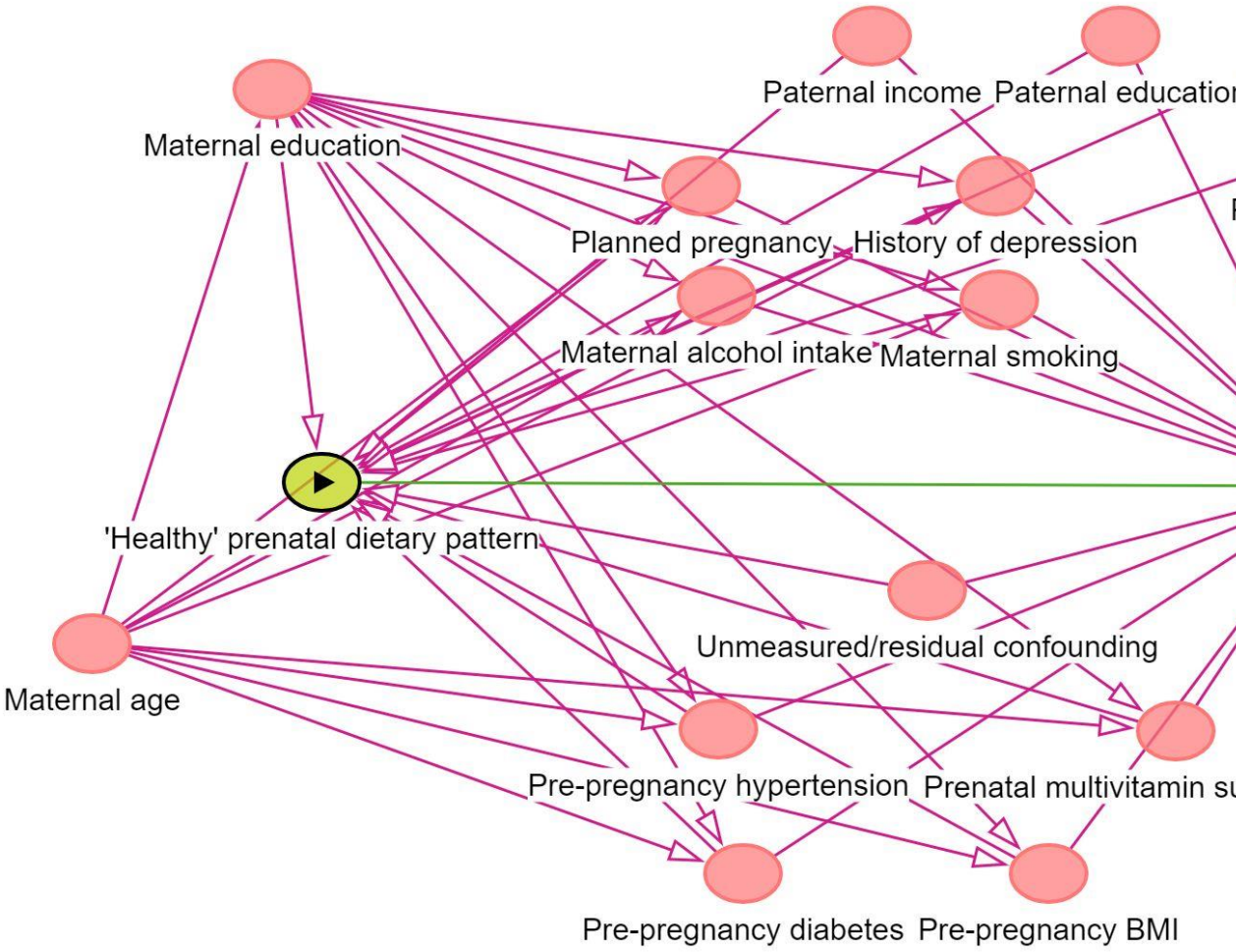
8.8.2 Selection bias

Selection bias may obscure the associations observed in Chapters 4-7, and the risk is potentially greatest in Chapter 6. Previous authors have demonstrated that selection bias can obscure the estimated association between a range of environmental and genetic exposure-outcome relationships (Biele et al., 2019, Howe et al., 2013, Munafò et al., 2017, Raz et al., 2018, Taylor et al., 2018). Several pathways create systematic differences in the cohort characteristics compared to the target population; for instance, the inevitable exclusion of non-viable pregnancies (Liew et al., 2015, Raz et al., 2018), self-selection into MoBa and ALSPAC, and systematic differences in the reason for attrition (Boyd et al., 2013, Magnus et al., 2016). Selection bias can occur through collider bias or effect modification. Effect modification occurs if study subgroups vary in participation rate and also vary in the magnitude of exposure-outcome association. Selection bias through collider bias can occur when both the exposure and outcome are the cause or related to the cause of study participation and/or retention (Munafò et al., 2017). For example, HPDP may improve maternal and child health which impacts study retention, and high autism-associated traits may increase the risk of attrition through the additional demand on families. Hence, selection into the study is inadvertently conditioned on and becomes a collider. Bias can be in either direction but it is usually in severe instances that the direction of association can reverse (Munafò et al., 2017).

A strength of this thesis was the ascertainment of autism diagnosis through deterministic linkage to medical records for all MoBa children. Attrition bias was minimised for analysis on autism diagnosis, although other sources of selection bias remain. ALSPAC recruited 71.8% of the target population (Boyd et al., 2013), whereas MoBa recruited only 41% (Magnus et al., 2016) leading to systematic differences in the sample characteristics. MoBa and ALSPAC recruited healthier women with more health behaviours, higher educational attainment, and had a smaller proportion of ethnic minorities, compared to the general population of women of reproductive age in Norway and England, respectively (Boyd et al., 2013, Magnus et al., 2016).

Hence, selection bias could impact each association measured in this thesis, though the risk may be higher in relation to autism-associated traits. There was a high proportion of

missing data on autism-associated traits (SCQ-3 (39.0%), SCQ-8 (52.4%), and SCDC-8 (38.1%)) (see Supplementary figure 1. Directed Acyclic Graph (DAG) of the relationship between 'healthy' prenatal dietary pattern and autism related outcomes.



Abbreviations: BMI, body mass index

Supplementary table 1-3). I observed greater attrition in mothers with the lowest adherence to a HPDP, lower educational attainment, poor health behaviours and health outcomes, and in children with an autism diagnosis and high SCQ-3 score and high SCQ-SOC-3. The opposite was observed in SCQ-RRB-3. High adherence to HPDP and high SCQ-RRB-3 traits where more likely to have missing information at age eight. Multiple imputation only partly ameliorates bias (Kontopantelis et al., 2017). Thus, potential bias may be towards the null, as missingness was greater in the lowest adherence to HPDP and in children with the outcome. In this thesis, selection bias could explain why no clear evidence of association was observed between HPDP and SCQ-SOC-8, when there was clear evidence of association with SCQ-SOC-3 and SCDC-8. Additionally, SCQ-RRB-8 may also be biased towards the null, however I had no complementary analysis in ALSPAC to assess this. Nonetheless, the true impact of selection bias is difficult to discern by virtue of missing data. Further research would be required to establish if selection bias impacted the measured associations.

Lastly, I applied the same outcome measures in Chapter 6 and therefore carried over the same potential selection bias. Yet, MoBa was further restricted to unrelated sets of trios with genetic data and subsequently included 19.6% of the analytic sample used in Chapter 5 and Chapter 7. Substantial selection bias in the PGS-outcome relationships can also occur (Biele et al., 2019, Gkatzionis and Burgess, 2018, Munafò et al., 2017, Taylor et al., 2018), and in most real-life examples this was towards the null (Taylor et al., 2018). Furthermore, the statistical power to detect an association, should one exist, was low in Chapter 6 and consequently, I was only likely to detect a moderate to large magnitude of association. Furthermore, disparities in the underlying ancestral diversity is a concern in MR studies (Mills and Rahal, 2019). The findings from a scientometric review showed the combined discovery and replication GWAS samples were based on an estimated 83.2% European, 12.4% Asian, 2.0% African American or Afro-Caribbean, 1.3% Hispanic or Latin American, and 0.9% from other or mixed ethnicities (Mills and Rahal, 2019). The narrow populations investigated may limit generalisability to other populations and contribute to health inequalities (Mills and Rahal, 2019).

8.8.3 Dietary measure

As discussed earlier, error is present in biological and physical measurements in every branch of science, albeit to varying degrees. It is widely accepted that the self-reported estimation of dietary intake is prone to error which may hinder advancements in

knowledge as systematic and random error obscure the measurement of true association (Subar et al., 2015). In MoBa (Brantsaeter et al., 2008, Brantsaeter et al., 2009, Brantsaeter et al., 2007, Brantsaeter et al., 2010) and ALSPAC (Emmett, 2009, Rogers et al., 1998) the FFQs were specifically designed for a pregnant population, and the validity was considered fair, as discussed in Chapter 3. Furthermore, our use of whole dietary patterns as the exposure measure may be less vulnerable to error than discrete FFQ components and specific nutrients (Hu, 2002). This is because our dietary pattern is an average measure of broadly defined adherence to HPDP.

Information bias is a source of error in FFQ that likely affected our analyses in Chapters 5-7. I applied the residuals method (Willett et al., 1997) to partly correct for random individual variability in energy requirements and systematic error in our measurement of HPDP. Characteristics associated with systematic misestimation have been studied in both pregnant and non-pregnant women. Underestimation of energy intake is associated with a BMI ≥25 kg/m^2 (McGowan and McAuliffe, 2012, Moran et al., 2018, Thomas et al., 2016), and lower educational attainment (McGowan and McAuliffe, 2012, Moran et al., 2018). Further characteristics associated with underestimation of energy intake in pregnancy were, body dissatisfaction and history of dieting behaviour (Moran et al., 2018), gestational diabetes, and increased birthweight (Thomas et al., 2016). Many of these predictors such as pre-pregnancy BMI ≥25km/m^2, gestation diabetes, birthweight, and educational attainment, have also been related to an increased likelihood of autism diagnosis and autism-associated traits (Hertz-Picciotto et al., 2018, Lyall et al., 2017). Hence, overreporting of 'healthy' foods may lead to misclassification into a high adherence to a HPDP more frequently for women with a greater likelihood of the outcomes. I adjusted for the predictors of misestimation of dietary intake that were considered confounders, which may partly reduce this bias. Overall, the associations observed in the highest quality diet may attenuate and explain why there was no consistent evidence of a dose response. However, this may also relate to confounding, such as genetic confounding by intelligence or educational attainment which is positively related to a healthy diet and had a J-shaped relationship with autism diagnosis (Gardner et al., 2020).

Differential misclassification of dietary intake is reported to affect genetic instruments less than phenotypic measures of dietary intake (Wade et al., 2022). Wade et al (Wade et al., 2022) reported that traits which predict differential misclassification, such as pre-pregnancy BMI, are not expected to predict the genetic IV. However, is an absence of methodological studies into nutritional measures in MR. Random misclassification of

dietary intake can reduce the precision of the estimated associations between maternal diet PGS and each outcome. Notably, total energy intake was not adjusted for in the GWAS (Niarchou et al., 2020) I derived the maternal diet PGS from which may have increased random error. Overall, maternal diet PGS may be less susceptible to systematic misclassification bias but is subject to random error.

Several researchers have promoted MR as a helpful methodology to overcome measurement error and confounding biases common in nutritional epidemiology (Bennett and Du, 2022, Carnegie et al., 2020, Ohukainen et al., 2022, Wade et al., 2022). However, identifying a robust genetic IV in MR can be challenging for several reasons. There are few genetic variants established in nutrition, and I did not identify any established genetic IV specifically for dietary patterns (Wade et al., 2022). Although maternal diet PGS and HPDP were robustly associated, cohorts replicating the genome-wide significant SNPs are desirable (Uffelmann et al., 2021). Currently, there is limited understanding of the biological mechanisms that underly genetic IV-exposure associations (Wade et al., 2022). Knowledge of the biological mechanisms may be advantageous to interpreting results, including confirming whether the potentially invalid SNPs I observed in Chapter 6 were indeed invalid.

There are many approaches to derive dietary patterns, all with different strengths and limitations, and each may capture a different facet of diet (Zhao et al., 2021). Factor analysis relies on subjective decisions and has received criticism as the reproducibility may be poor. In Chapter 5, I replicated the analytic decisions in both cohorts, and although the factor loadings were not identical, they were qualitatively similar with exception of bread intakes. Nonetheless, the measures of association with a HPDP were reproducible across MoBa and ALSPAC. In Chapter 6, I observed further evidence of the reproducibility of our dietary pattern derived by factor analysis. The maternal diet PGS was robustly associated with HPDP and was based on a GWAS from UK Biobank using factor analysis derived dietary pattern. This observation suggests that there is some reproducibility of dietary patterns derived by factor analysis when defined broadly. Several previous studies have now indicated there is some reproducibility of dietary patterns derived through data-driven methods such as factor analysis or principal component analysis (Zhong et al., 2020).

8.8.4 Outcome measures

8.8.4.1 Changing diagnostic criteria

The generalisability of the thesis findings on autism diagnosis may be impacted by the evolving concept of an autism diagnosis. The conceptualisation and assignment of an autism diagnosis has been dynamic over time (Reed et al., 2019). As discussed in Chapter 3.3, diagnostic criteria have changed overtime and subsequently, the incidence of autism diagnosis has increased over decades across the world. Indeed, this may explain the low prevalence of autism diagnosis in ALSPAC which is an older cohort. There has been considerable increase in awareness, cultural acceptance, and extension of autism diagnostic criteria (Russell et al., 2022), though aetiological factors may also contribute. Awareness and cultural shifts preceded changes to diagnostic criteria. For example, there has been more autism diagnoses based on milder traits (Arvidsson et al., 2018). Milder autism is often diagnosed later and may explain the increase in age of diagnosis (O'Nions et al., 2023, Russell et al., 2022). Earlier recognition and diagnosis of autism can improve long term outcomes. As a result, there has been a strong policy directive for the past 20 years to diagnose autism early (Lord et al., 2022). Moreover, UK legislation, the Autism Act 2009, stipulated that there should be availability of adult autism diagnostic services across the UK (UK Government, 2009). A UK study identified the adult autism diagnosis incidence was 1 per 100,000 in 1998 compared to 20 per 100,000 in 2018 (Russell et al., 2022). However, underdiagnosis of adults is predicted to be a prevailing issue (O'Nions et al., 2023). There has been greater awareness of underdiagnosis in females, who have accounted for a greater proportion of increased incidence of autism compared to males (Russell et al., 2022). Even within the same country and with the same diagnostic criteria for autism, the incidence vary by geographic region and individual characteristics such as ethnicity and level of socioeconomic deprivation (Roman-Urrestarazu et al., 2022).

Underdiagnosis in our autism outcome measure would result in misclassification bias particularly in milder cases of autism. Researchers have suggest severe autism diagnosis may have a greater magnitude of association with prenatal environmental factors (Xie et al., 2020), and our results in Chapter 4 supported these observations. I could not investigate autism diagnosis by severity in Chapters 5-7 with the available data. If severe autism diagnosis was more strongly associated with prenatal dietary pattern, then future studies that include a greater proportion of milder autism, may observe a lower magnitude of association compared to our findings in Chapters 5 and 7. However, future studies are

required to understand how the expansion of an autism diagnosis may impact the estimated association between prenatal environmental factors and autism diagnosis.

8.8.4.2 Autism-associated traits

Throughout this thesis the performance of the autism-associated trait measures may have impacted the results observed. The SCQ-RRB-3 was an especially poor predictor and did not discriminate autistic and non-autistic children. Restrictive and repetitive behaviours are common in children between two-seven years. For example, repeated kicking, rocking, hand flapping, and ritualistic behaviours such as bedtime routine, clothes, food preferences, albeit the frequency is greater in autistic children (Harrop et al., 2014, Lewis and Kim, 2009). Other challenges related to measures of autism-associated traits have previously been discussed and include increased risk of selection bias and greater heterogeneity due to several factors. For example, broader definition, child age and sex, parental socioeconomic deprivation.

8.9 Strengths

8.9.1 Advantages of 'big data'

To the best of my knowledge, this is the first large prospective investigation into prenatal dietary patterns and autism diagnosis and autism-associated traits. This was a gap in the existing literature as previous studies generally had low sample sizes (Geetha et al., 2018, House et al., 2018, Li et al., 2018, Vecchione et al., 2022). The introduction of large prospective cohort studies has expanded psychiatric investigations from small clinical studies (Larsson, 2021, Lipkin et al., 2023). Cohorts such as MoBa, ALSPAC and others (for example, INMA, Generation R, Millennium Cohort Study, NHSII, Childhood autism risks from genetics and environment, EARLI, HOME, Twins Early Development Study, Southampton Women's study) have provided insights into the potential causes, consequences, and the developmental course of neurodevelopmental conditions, including autism diagnosis and autism-associated traits. Large prospective cohorts, such as MoBa and ALSPAC are important to the investigation because of the low prevalence of autism. Furthermore, the large sample size can provide precise estimates which are important, especially given the imprecision of the exposure and outcomes measures. However, as discussed large sample sizes are still impacted by systematic bias. I also had greater power to detect smaller magnitudes of association and conducted subgroup analysis. Lastly, I was able to provide detailed description of participants characteristics, including those related

to attrition, and could use auxiliary variables to help predict missingness though multiple imputation.

Throughout the thesis I addressed several secondary outcomes of pertinence to the aetiological investigation of autism diagnosis and autism-associated traits. I analysed the subdomains of autism-associated traits, at age three and eight years, and identified that both age and subdomain were potential sources of variability in the measured associations. This was a potentially important finding, especially as few prenatal nutrition studies have analysed the subdomains separately. I further considered effect modification and interaction of several key characteristics, child sex, pre-pregnancy BMI, prenatal multivitamin supplement use, and maternal education. Lastly, I furthered our investigations from Chapters 5 and 6 by placing the findings into a public health context. In Chapter 7, I measured the potential implications an 'intervention' on prenatal diet could have on the relationship between maternal deprivation and each autism related outcome. In doing so, I highlighted the potentially modest impact of a HPDP, and the importance of aiming at the wider determinants of health.

8.9.2 Causally informed approaches

Causal inference is about working with errors, bias, and nuance to make balanced decisions that are substantiated with evidence and critical reasoning (Petersen and van der Laan, 2014). The potential outcomes framework stipulates the assumptions required for causal estimation and causal thinking encourages us to distinguish the process of causal inference from statistical estimation. In doing so, a high standard is established and provides a framework to support a causal evaluation of the robustness of findings to the assumptions (Petersen and van der Laan, 2014). Thus, I enhanced the conceptualisation and interpretation of the analysis through the application of different causally informed approaches.

The conceptualisation of causal structures relevant to the analyses was improved through the application of a DAG. The DAG provided a transparent representation of my assumptions and can support others research interpretation of the research conducted (Greenland et al., 1999, Hernán and Robins, 2006). Through the depiction of causal structures, I distinguished potential confounders from mediators and effect modifiers, and identified potential unmeasured confounders. I believe the framework has reduced the risk of introducing collider bias or overadjustment that may occur through inappropriate

covariate selection and statistical adjustment, albeit I cannot be certain this risk is eliminated. Additionally, DAGs provide clarity on complex concepts such as collider bias which supported a more accurate interpretation of selection bias (Tennant et al., 2021). Furthermore, in Chapter 7, a DAG was an especially useful tool to identify the pre- and post- HPDP confounders. This supported more accurate modelling of the TE and CDE, but also communication of the rationale for covariate selection.

A sharper causal question was considered through the formulation of the ideal hypothetical intervention and control (Hernán, 2004, Hernán and Taubman, 2008). Whether an exposure is well defined or ambiguous is subjective, however, I considered my measure of dietary pattern to be ambiguous. I was initially interested in a 'healthy' prenatal dietary pattern, yet a 'healthy' diet is not simple to conceptualise or quantify. Through causal reasoning I clarified that our measure of prenatal diet could reflect an average measure of a broadly defined 'healthy' prenatal diet. Additionally, consistent with previous literature, I further demonstrated that there was evidence of reproducibility across MoBa and ALSPAC, and MoBa and the GWAS sample from the UK Biobank. Subsequently, the formulation of each analysis was better informed and our interpretation of our results rigorously respected methodological limits.

Additionally, several approaches to mediation analyses can be applied but here I selected the CDE. The CDE is a causally informed 'mediation' analyses with greater relevance to public health compared to other causal mediation analysis, as the CDE estimates the proportion of the TE that is eliminated if we hypothetically 'intervene' on diet (VanderWeele, 2013). Furthermore, compared to naive approaches to mediation analyses, our CDE may reduce the risk of collider bias and/or overadjustment. I believe the application of causally informed mediation analyses has strengthened the findings of the thesis.

8.9.3 Triangulation

Triangulation can further support causal investigations, especially in observational epidemiology (Lawlor et al., 2016). Chapter 4 was a systematic review and meta-analysis of measures of association of prenatal multivitamin supplements and autism diagnosis. A secondary aim was to triangulate evidence from alternative approaches with different key sources of bias that measured the association between prenatal multivitamin supplement use and autism diagnosis. This allowed me to systematically evaluate the current

application of triangulation. I identified a small number of studies that applied the following alternative approaches, genetic-phenotype interaction, discordant sibling analysis, and negative controls. Across each study challenges in the robust application of triangulation were evident, and overall, the findings provided modest additional confidence that the associations measured may be causal.

To the best of my knowledge, the PGS-MR was the first study to measure prenatal dietary pattern and autism-associated traits. However, I experienced similar challenges to the robust application of triangulation with PGS-MR due to methodological limitations. However, the cross-context comparison between ALSPAC and MoBa was informative. In Chapter 5, I replicated our findings in relation to HPDP and social communication difficulties at age eight years. Reproducibility is integral to the scientific process and requires the replication of research results using the same methods and analytic techniques. As discussed, I observed the relationship across independent cohorts, from different countries and separated by over a decade in time. This is important as the current day relevance of dietary investigations from old cohorts such as ALSPAC has been questioned.

8.10 Implications for public health practice

Here, I consider the transferability of the associations I observed in Chapters 4-7 to public health practice, on the hypothetical bases they are found to be causal.

Maternal diet is central to current strategies to improve maternal and child health. This is reflected in numerous National and Global policies, guidelines, and strategic aims (Scottish Government, 2019, UK Government, 2022, World Health Organisation, 2014). For example, worldwide, the ‘First 1000 Days’ promotes the importance of maternal and child nutrition from conception to approximately two years (UK Government, 2022). These policies are aimed at improving the general health and development of children (Scottish Government, 2019, UK Government, 2022, World Health Organisation, 2014). Our results suggest that autism diagnosis and social communication difficulties may be another child outcome which is altered through prenatal diet. This adds weight to the pertinence of continued public health efforts to improve maternal and child health. Moreover, I observed evidence of health inequalities in autism diagnosis and autism-associated traits. Hence, potential strategies focused on the development of autism diagnosis or autism-associated traits should consider socioeconomic context.

Interventions targeting prenatal nutrition are limited by the high proportion of unplanned pregnancies (Bearak et al., 2022, Stephenson et al., 2018). Thus, interventions aimed at the general population are necessary, in addition to those specifically targeting maternal and child nutrition (Stephenson et al., 2018). Furthermore, interventions can be considered on a continuum between low and high agency. High-agency interventions are often favoured, yet concerns have been raised with regards to their efficacy and equitability (Adams et al., 2016, Barker et al., 2017). High-agency interventions offer advice, education, and encouragement to elicit behaviour change. However, intervention success is highly dependent on the individual psychosocial, environmental, and material resources which are socially patterned. Deprived groups who have fewer resources to draw on may benefit least from high-agency interventions, whilst affluent groups with greater resources benefit more, thereby increasing health inequalities (Adams et al., 2016). Furthermore, focus on the individual level can unduly place the 'burden' of responsibility on the mother who acts within the wider determinants of health (Barker et al., 2017). Hence, not only did deprivation relate to an increased likelihood of each outcome in Chapter 7, but interventions may also be less successful.

Eliciting change in dietary intake is complex. Several approaches have been proposed such as proportional universalism, whereby everyone is targeted but the scale and intensity is proportionate to need (Carey et al., 2015). A life course approach to intervention is also advocated for and reflects the establishment of good dietary habits from childhood with re-enforcement throughout adulthood (Stephenson et al., 2018). Multiple dietary interventions are likely required to achieve a healthy prenatal diet across the population in a way that reduces health inequalities (Adams et al., 2016). For example, sugar taxation (Rogers et al., 2023), 'Healthy Start' food and vitamin supplement voucher programme (Ohly et al., 2017), 'Best Start Foods' and free prenatal vitamins (Scottish Government, 2019), and voluntary and mandatory national dietary fortification programs, such as folic acid (UK Government, 2021). A focus of my thesis was to understand the relationship between prenatal dietary pattern and autism diagnosis and autism-associated traits within the socioeconomic context. In Chapter 7, findings suggest that inequalities in autism diagnosis and social communication difficulties may reduce to a modest extent in response to our hypothetical intervention on HPDP. There are more barriers to intervention in deprived groups. Even if I successfully transition the population to a 'healthy' prenatal dietary pattern, only a small proportion of inequalities in autism diagnosis or social communication difficulties will be moderated. Multiple pathways from maternal

deprivation may exist, and so prenatal diet may be a useful intervention target to reduce inequalities as part of a wider strategy.

Prominent researchers in the Developmental Origins of Health and Disease field have called for nutritional interventions targeting the preconception period (Stephenson et al., 2018). Nutritional status is established over time as maternal nutrient stores accumulate. A 'healthy' diet in the pre-conception phase is necessary to achieve optimal nutritional reserves from conception. In Chapter 4, I observed stronger evidence of a reduced probability of autism diagnosis in association with prenatal multivitamin use commenced in the preconception/first trimester compared to any point in pregnancy. This may indicate a critical period of the nutrient(s) or latency in achieving biologically optimal nutrient levels. Pregnancy is a time of dietary change and women are more receptive to health advice and more motivated to make changes. There are also dietary modifications secondary to the physiological process of pregnancy (Clarke et al., 2021). Direct measures of dietary change between preconception to pregnancy demonstrated the most consistent evidence for increased consumption of fruit and vegetables, increased consumption of dairy products and reduced caffeine intakes (Hillier and Olander, 2017) or specific nutrients (Hillier and Olander, 2017, Ishitsuka et al., 2020). However, a further investigation observed stability in dietary trajectories when measuring whole dietary patterns between preconception until the child was 8-9 years (Dalrymple et al., 2022). Dietary changes in discrete components may be insufficient to alter the measurement of overall dietary pattern when broadly categorised. Further studies would be helpful to understand differential effects between preconception and pregnancy diet on autism diagnosis and autism-associated traits.

Dietary trends have changed over time and can affect nutrient intakes. Physiological nutrient requirements are generally stable overtime, but there is evidence of fluctuations in dietary and nutrient intakes across time (Yau et al., 2019). Yet, these changes are modest and observed in discrete nutrients and foods or groups of foods. The National Diet and Nutrition Survey is a nationally representative sample in England, and since 2008 findings suggest that sweet confectionary and chocolate consumption and saturated fat intakes have increased, whilst red and processed meats have decreased. It is speculated that cultural trends have influenced dietary changes, for example, the increased awareness of global warming and benefits of sustainable diets, and the rising popularity of low-carbohydrate diets (Yau et al., 2019). Additionally, policies also impact diet and nutrient intakes, for example, the planned introduction of mandatory fortification of non-wholemeal wheat

flour in the UK (UK Government, 2021). Nonetheless, the findings of this thesis were consistent between MoBa and ALSPAC and have been observed in previous research (Geetha et al., 2018, House et al., 2018, Li et al., 2018, Vecchione et al., 2022), all of which had differences in dietary patterns.

8.11 Implications for future studies

Throughout this thesis I applied causally informed approaches which improved our analytic strategy and causal reasoning which may be advantageous to future studies. In Chapters 4-6 the utility of triangulation to support assessment of bias was reduced due to methodological limitations. Future studies should aim to establish larger cohorts with detailed phenotype and genotype information, particularly within families and if possible, across generations. In addition to understanding intergenerational effects, we can better manage potential genetic confounding and within family confounding.

Diet is notoriously challenging to measure but widely recognised as imperative to maternal and child health (Barker et al., 2018, Stephenson et al., 2018). Thus, efforts should continue to improve dietary assessment methods and dietary pattern modelling in large scale cohorts (Morgenstern et al., 2021). It would also be helpful to understand how different aspects of diet, such as plant-based foods, may differentially associate with autism. Advanced approaches that consider food groups in the context of overall diet could be helpful to address this question, such as that applied by Lyall et al (Lyall et al., 2023). Moreover, it would be informative to understand how toxins, pollutants, and potentially other bioactive components in foods may alter the associations observed with autism. Furthermore, methodological studies focused on dietary measures in MR should develop understanding of how best to measure dietary patterns for use in MR. Additionally, improvements in the measurement of multivitamin supplements could be achieved through measuring compliance, formulation, dose, timing, and control for potential effects from prenatal diet.

It would be advantageous to understand if the distinction in the association HPDP had with social communication difficulties and restrictive and repetitive behaviours could be replicated. Furthermore, I was not able to triangulate findings with autism diagnosis, and further large prospective studies are required to address this question. Additionally, it would be helpful to understand if there are differences in the magnitude of association with autism diagnosis, depending on the presence and severity of social communication

difficulties and restrictive and repetitive behaviours, as well as intellectual disability. This may be especially informative to understanding whether the broadening of autism diagnostic criteria impacts the strength of association. This may help further aetiological understanding of the potential role HPDP has in autism diagnosis and autism-associated traits. Child sex as a potential effect modifier should also be further explored, and future studies should aim to address sex biases in autism diagnosis and screening tools. This will support further research in establishing whether, and to what extent, the stronger associations are due to a female protective effect or diagnostic bias. It is important for future researchers to consider how the evolving conceptualisation of autism diagnosis impacts the findings observed.

Selection bias was a potential limitation in each of my analyses. Strategies to improve recruitment and retention in prospective cohorts is required. Furthermore, there was stark disparities in the ethnic diversity of the study populations, especially in MR investigations. Valuable insights could be gained by ensuring study samples are socioeconomically and ethnically diverse, and reflective of the target population. Lastly, I argued that further research highlighting the socioeconomic context that exposure-outcome associations sit within can encourage greater knowledge of socioeconomic inequalities and inform solutions.

8.12 Conclusion

In Chapter 4, I observed a 23% reduced likelihood of autism diagnosis in association with prenatal multivitamin use. However, triangulation with alternative approaches had limited utility in strengthening the evidence due to methodological limitations. But also because of the infrequent application of each approach.

I then measured the association between HPDP and autism diagnosis and autism-associated traits. HPDP was associated with a 23% reduced likelihood of autism diagnosis, 24% reduced likelihood of SCQ-SOC-3, and 26% reduced likelihood of SCDC-8. Although I also observed a 27% increased likelihood of SCQ-RRB-3, this may be due to measurement error. I also observed weak evidence that there is a greater magnitude of association between HPDP and social communication difficulties at age eight years in females, compared to males. The stronger association may be due to aetiological differences such as a female protective effect or it may reflect sex-bias in the performance of the autism screening tools.

In Chapter 6, I did not observe clear evidence of association between maternal PGS and each outcome in the fully adjusted models. The results may suggest that the associations measured in Chapter 5 are spurious. However, due to limited statistical power I caution that further MR investigations with a larger, more representative samples are required.

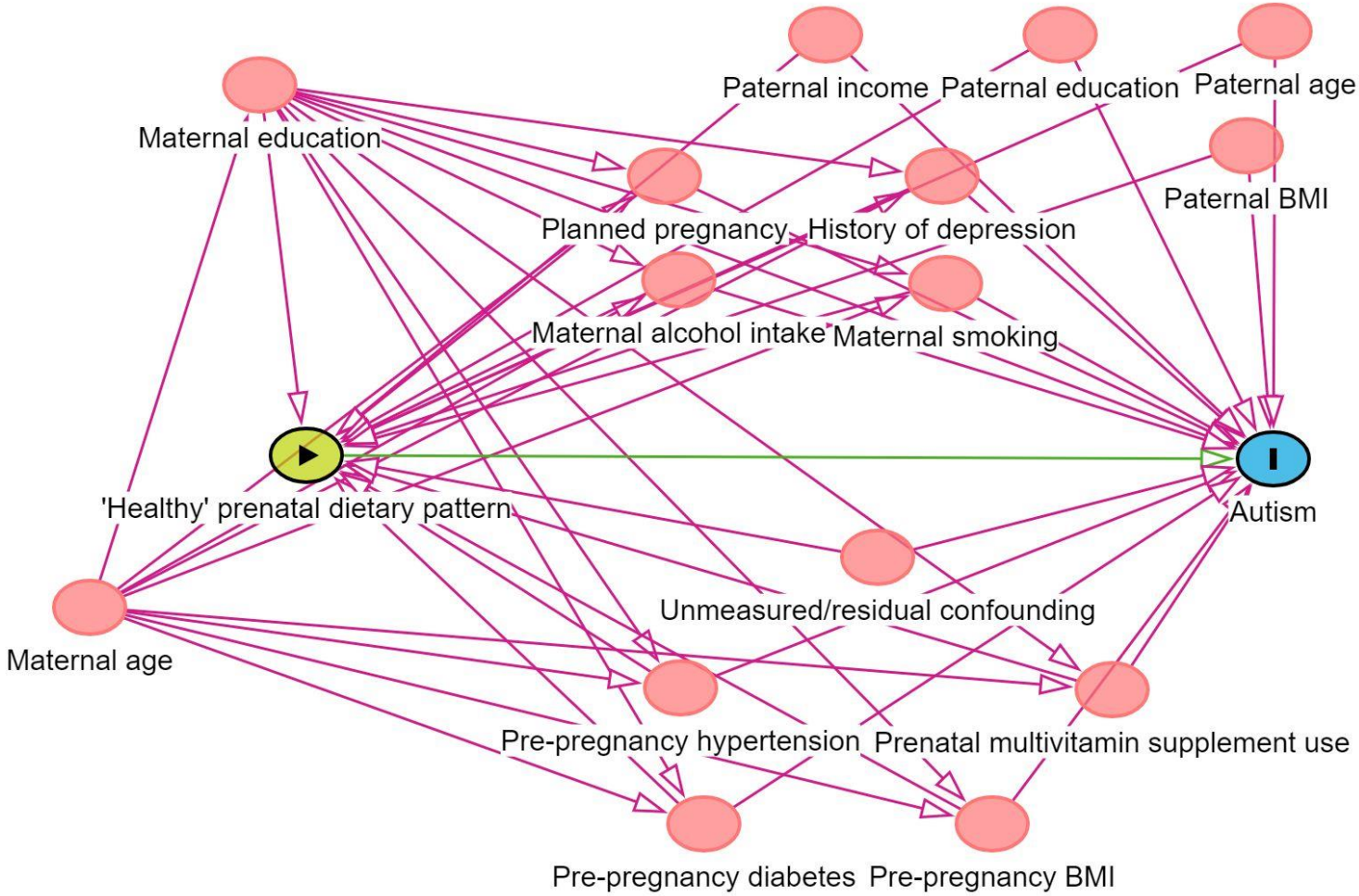
In Chapter 7, I observed that a modest proportion of the association between maternal indicator or deprivation and autism diagnosis and autism-associated traits was eliminated through high adherence to HPDP. The modest proportion eliminated highlights that HPDP only accounts for a small proportion of the pathways between maternal socioeconomic deprivation and each outcome. Thus, multiple targets for intervention would be required, and may be more efficiently addressed if targeted at upstream determinants.

In summary, I observed a lower likelihood of autism diagnosis and social communication difficulties in association with HPDP. Our results further suggest that if the associations were causal, then HPDP as an intervention target would only mitigate a modest proportion of the inequalities in the outcomes.

Appendix

Supplementary material for Chapter 3.

Supplementary figure 1. Directed Acyclic Graph (DAG) of the relationship between ‘healthy’ prenatal dietary pattern and autism related outcomes.



Abbreviations: BMI, body mass index

Supplementary table 1. Baseline characteristics of pregnancies with missing outcome data at age three in MoBa.

			Adherence to a 'healthy' prenatal dietary pattern (%)		
Characteristic	**Level**	**Overall**	**Low**	**Medium**	**High**
Number of pregnancies		32,986	11,287 (34.2)	10,801 (32.7)	10,898 (33.0)
Child sex (%)	Male	16975 (51.5)	5888 (52.2)	5508 (51.0)	5579 (51.2)
	Female	15946 (48.3)	5371 (47.6)	5271 (48.8)	5304 (48.7)
	Missing	65 (0.2)	28 (0.2)	22 (0.2)	15 (0.1)
Pre-pregnancy depression (%)	No	29210 (88.6)	9913 (87.8)	9649 (89.3)	9648 (88.5)
	Yes	2790 (8.5)	1047 (9.3)	827 (7.7)	916 (8.4)
	Missing	986 (3.0)	327 (2.9)	325 (3.0)	334 (3.1)
Maternal age (years) (mean (SD))		29.9 (4.7)	28.8 (4.8)	30.1 (4.6)	31.0 (4.6)
	Missing	0	0	0	0
Maternal education (%)	9-year elementary	1179 (3.6)	593 (5.3)	325 (3.0)	261 (2.4)
	1-2 years further education	1954 (5.9)	985 (8.7)	538 (5.0)	431 (4.0)
	Technical high school	4650 (14.1)	2116 (18.7)	1473 (13.6)	1061 (9.7)
	3-year high school general studies, junior college	4744 (14.4)	1917 (17.0)	1460 (13.5)	1367 (12.5)
	Regional technical college, 4-year university degree (Bachelor’s degree, nurse, teacher, engineer)	11692 (35.4)	3684 (32.6)	4075 (37.7)	3933 (36.1)
	University, technical college, more than 4 years (Master’s degree, medical doctor, PhD)	6461 (19.6)	1227 (10.9)	2204 (20.4)	3030 (27.8)
	Missing	2306 (7.0)	765 (6.8)	726 (6.7)	815 (7.5)
Planned pregnancy (%)	No	6983 (21.2)	2638 (23.4)	2109 (19.5)	2236 (20.5)
	Yes	25122 (76.2)	8375 (74.2)	8429 (78.0)	8318 (76.3)
	Missing	881 (2.7)	274 (2.4)	263 (2.4)	344 (3.2)
Prenatal alcohol intake (%)	No	24468 (74.2)	8617 (76.3)	8043 (74.5)	7808 (71.6)
	Yes	3215 (9.7)	977 (8.7)	1078 (10.0)	1160 (10.6)

Characteristic	Level	Overall	Adherence to a 'healthy' prenatal dietary pattern (%)		
			Low	Medium	High
	Missing	5303 (16.1)	1693 (15.0)	1680 (15.6)	1930 (17.7)
Prenatal multivitamin supplement use (%)	No	22747 (69.0)	8388 (74.3)	7329 (67.9)	7030 (64.5)
	Yes	8055 (24.4)	2149 (19.0)	2776 (25.7)	3130 (28.7)
	Missing	2184 (6.6)	750 (6.6)	696 (6.4)	738 (6.8)
Prenatal smoking (%)	No	26987 (81.8)	8573 (76.0)	8942 (82.8)	9472 (86.9)
	Yes	3089 (9.4)	1671 (14.8)	864 (8.0)	554 (5.1)
	Missing	2910 (8.8)	1043 (9.2)	995 (9.2)	872 (8.0)
Pre-pregnancy BMI kg/m^2 (mean (SD))		24.2 (4.5)	24.7 (4.8)	24.3 (4.4)	23.7 (4.2)
	Missing	1504 (4.6)	501 (4.4)	497 (4.6)	506 (4.6)
Pre-pregnancy diabetes (%)	No	31833 (96.5)	10919 (96.7)	10414 (96.4)	10500 (96.3)
	Yes	167 (0.5)	41 (0.4)	62 (0.6)	64 (0.6)
	Missing	986 (3.0)	327 (2.9)	325 (3.0)	334 (3.1)
Pre-pregnancy hypertension (%)	No	31805 (96.4)	10908 (96.6)	10440 (96.7)	10457 (96.0)
	Yes	356 (1.1)	125 (1.1)	112 (1.0)	119 (1.1)
	Missing	825 (2.5)	254 (2.3)	249 (2.3)	322 (3.0)
Autism diagnosis (%)	No	32608 (98.9)	11107 (98.4)	10705 (99.1)	10796 (99.1)
	Yes	378 (1.1)	180 (1.6)	96 (0.9)	102 (0.9)
SCQ-8 (%)	No	5861 (17.8)	1832 (16.2)	1945 (18.0)	2084 (19.1)
	Yes	643 (1.9)	231 (2.0)	201 (1.9)	211 (1.9)
	Missing	26482 (80.3)	9224 (81.7)	8655 (80.1)	8603 (78.9)
SCQ-8-RR (%)	No	5487 (16.6)	1734 (15.4)	1807 (16.7)	1946 (17.9)
	Yes	1017 (3.1)	329 (2.9)	339 (3.1)	349 (3.2)
	Missing	26482 (80.3)	9224 (81.7)	8655 (80.1)	8603 (78.9)
SCQ-8-COM (%)	No	5950 (18.0)	1861 (16.5)	1971 (18.2)	2118 (19.4)
	Yes	554 (1.7)	202 (1.8)	175 (1.6)	177 (1.6)
	Missing	26482 (80.3)	9224 (81.7)	8655 (80.1)	8603 (78.9)

Characteristic	Level	Overall	Adherence to a 'healthy' prenatal dietary pattern (%)		
			Low	Medium	High
Paternal age (%)	< 25 years	1921 (5.8)	1020 (9.0)	518 (4.8)	383 (3.5)
	25-34 years	19912 (60.4)	7123 (63.1)	6648 (61.5)	6141 (56.3)
	35-44 years	10074 (30.5)	2874 (25.5)	3309 (30.6)	3891 (35.7)
	45-54 years	781 (2.4)	174 (1.5)	244 (2.3)	363 (3.3)
	≥ 55 years	62 (0.2)	14 (0.1)	14 (0.1)	34 (0.3)
	Missing	236 (0.7)	82 (0.7)	68 (0.6)	86 (0.8)
Paternal BMI (mean (SD))		25.9 (3.3)	26.2 (3.5)	26.0 (3.3)	25.7 (3.2)
	Missing	2155 (6.5)	725 (6.4)	680 (6.3)	750 (6.9)
Paternal education (%)	9-year elementary	1707 (5.2)	770 (6.8)	492 (4.6)	445 (4.1)
	1-2 years further education	1984 (6.0)	951 (8.4)	581 (5.4)	452 (4.1)
	Technical high school	7951 (24.1)	3372 (29.9)	2542 (23.5)	2037 (18.7)
	3-year high school general studies, junior college	3793 (11.5)	1428 (12.7)	1251 (11.6)	1114 (10.2)
	Regional technical college, 4-year university degree (Bachelor’s degree, nurse, teacher, engineer)	7495 (22.7)	2229 (19.7)	2574 (23.8)	2692 (24.7)
	University, technical college, more than 4 years (Master’s degree, medical doctor, PhD)	6501 (19.7)	1291 (11.4)	2231 (20.7)	2979 (27.3)
	Missing	3555 (10.8)	1246 (11.0)	1130 (10.5)	1179 (10.8)
Maternal income category (%)	High	10933 (33.1)	2812 (24.9)	3714 (34.4)	4407 (40.4)
	Low	20319 (61.6)	7866 (69.7)	6548 (60.6)	5905 (54.2)
	Missing	1734 (5.3)	609 (5.4)	539 (5.0)	586 (5.4)
Paternal income category (%)	High	19842 (60.2)	6005 (53.2)	6782 (62.8)	7055 (64.7)
	Low	10048 (30.5)	4096 (36.3)	3080 (28.5)	2872 (26.4)
	Missing	3096 (9.4)	1186 (10.5)	939 (8.7)	971 (8.9)

BMI, body mass index; MoBa, Norwegian Mother, Father, and Child Cohort; SD, standard deviation; SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCQ-SOC, communication skills subdomain of SCQ. The number following each outcome denotes the approximate age of the child in years when the measure was obtained. i.e. SCQ-SOC-8; social communication subdomain measured at age 8 years. The presence of the outcome from each questionnaire was indicated by a high score.

Supplementary table 2. Baseline characteristics of pregnancies with missing outcome data at age eight in MoBa.

			Adherence to a 'healthy' prenatal dietary pattern (%)		
Characteristic	**Level**	**Overall**	**Low**	**Medium**	**High**
Number of pregnancies		44,294	15,310 (34.6)	14,538 (32.8)	14,446 (32.6)
Child sex (%)	Male	22774 (51.4)	7953 (51.9)	7426 (51.1)	7395 (51.2)
	Female	21455 (48.4)	7329 (47.9)	7090 (48.8)	7036 (48.7)
	Missing	65 (0.1)	28 (0.2)	22 (0.2)	15 (0.1)
Pre-pregnancy depression (%)	No	39462 (89.1)	13567 (88.6)	13022 (89.6)	12873 (89.1)
	Yes	3650 (8.2)	1349 (8.8)	1126 (7.7)	1175 (8.1)
	Missing	1182 (2.7)	394 (2.6)	390 (2.7)	398 (2.8)
Maternal age (years) (mean (SD))		29.9 (4.7)	28.7 (4.7)	30.0 (4.5)	30.9 (4.5)
	Missing	0	0	0	0
Maternal education (%)	9-year elementary	1435 (3.2)	726 (4.7)	380 (2.6)	329 (2.3)
	1-2 years further education	2494 (5.6)	1257 (8.2)	698 (4.8)	539 (3.7)
	Technical high school	6234 (14.1)	2853 (18.6)	1986 (13.7)	1395 (9.7)
	3-year high school general studies, junior college	6445 (14.6)	2667 (17.4)	2001 (13.8)	1777 (12.3)
	Regional technical college, 4-year university degree (Bachelor’s degree, nurse, teacher, engineer)	15884 (35.9)	5081 (33.2)	5554 (38.2)	5249 (36.3)
	University, technical college, more than 4 years (Master’s degree, medical doctor, PhD)	8927 (20.2)	1755 (11.5)	3026 (20.8)	4146 (28.7)
	Missing	2875 (6.5)	971 (6.3)	893 (6.1)	1011 (7.0)
Planned pregnancy (%)	No	8885 (20.1)	3355 (21.9)	2694 (18.5)	2836 (19.6)
	Yes	34370 (77.6)	11623 (75.9)	11542 (79.4)	11205 (77.6)
	Missing	1039 (2.3)	332 (2.2)	302 (2.1)	405 (2.8)
Prenatal alcohol intake (%)	No	33232 (75.0)	11791 (77.0)	11001 (75.7)	10440 (72.3)
	Yes	4228 (9.5)	1279 (8.4)	1419 (9.8)	1530 (10.6)

Characteristic	Level	Overall	Adherence to a 'healthy' prenatal dietary pattern (%)		
			Low	Medium	High
	Missing	6834 (15.4)	2240 (14.6)	2118 (14.6)	2476 (17.1)
Prenatal multivitamin supplement use (%)	No	30243 (68.3)	11360 (74.2)	9720 (66.9)	9163 (63.4)
	Yes	11383 (25.7)	3056 (20.0)	3968 (27.3)	4359 (30.2)
	Missing	2668 (6.0)	894 (5.8)	850 (5.8)	924 (6.4)
Prenatal smoking (%)	No	36318 (82.0)	11660 (76.2)	12084 (83.1)	12574 (87.0)
	Yes	4026 (9.1)	2189 (14.3)	1131 (7.8)	706 (4.9)
	Missing	3950 (8.9)	1461 (9.5)	1323 (9.1)	1166 (8.1)
Pre-pregnancy BMI kg/m^2 (mean (SD))		24.2 (4.5)	24.7 (4.7)	24.3 (4.4)	23.7 (4.2)
	Missing	1828 (4.1)	628 (4.1)	579 (4.0)	621 (4.3)
Pre-pregnancy diabetes (%)	No	42900 (96.9)	14857 (97.0)	14087 (96.9)	13956 (96.6)
	Yes	212 (0.5)	59 (0.4)	61 (0.4)	92 (0.6)
	Missing	1182 (2.7)	394 (2.6)	390 (2.7)	398 (2.8)
Pre-pregnancy hypertension (%)	No	42857 (96.8)	14843 (96.9)	14088 (96.9)	13926 (96.4)
	Yes	465 (1.0)	159 (1.0)	162 (1.1)	144 (1.0)
	Missing	972 (2.2)	308 (2.0)	288 (2.0)	376 (2.6)
Autism diagnosis (%)	No	43745 (98.8)	15061 (98.4)	14387 (99.0)	14297 (99.0)
	Yes	549 (1.2)	249 (1.6)	151 (1.0)	149 (1.0)
SCQ-3 (%)	No	15754 (35.6)	5337 (34.9)	5254 (36.1)	5163 (35.7)
	Yes	2058 (4.6)	749 (4.9)	629 (4.3)	680 (4.7)
	Missing	26482 (59.8)	9224 (60.2)	8655 (59.5)	8603 (59.6)
SCQ-3-RR (%)	No	16056 (36.2)	5518 (36.0)	5333 (36.7)	5205 (36.0)
	Yes	1756 (4.0)	568 (3.7)	550 (3.8)	638 (4.4)
	Missing	26482 (59.8)	9224 (60.2)	8655 (59.5)	8603 (59.6)
SCQ-3-COM (%)	No	16070 (36.3)	5376 (35.1)	5325 (36.6)	5369 (37.2)
	Yes	1742 (3.9)	710 (4.6)	558 (3.8)	474 (3.3)
	Missing	26482 (59.8)	9224 (60.2)	8655 (59.5)	8603 (59.6)

Characteristic	Level	Overall	Adherence to a 'healthy' prenatal dietary pattern (%)		
			Low	Medium	High
Paternal age (%)	< 25 years	2459 (5.6)	1304 (8.5)	680 (4.7)	475 (3.3)
	25-34 years	27315 (61.7)	9925 (64.8)	9036 (62.2)	8354 (57.8)
	35-44 years	13193 (29.8)	3761 (24.6)	4410 (30.3)	5022 (34.8)
	45-54 years	988 (2.2)	211 (1.4)	317 (2.2)	460 (3.2)
	≥ 55 years	78 (0.2)	16 (0.1)	20 (0.1)	42 (0.3)
	Missing	261 (0.6)	93 (0.6)	75 (0.5)	93 (0.6)
Paternal BMI kg/m^2 (mean (SD))		25.9 (3.4)	26.2 (3.6)	26.0 (3.3)	25.6 (3.2)
	Missing	2633 (5.9)	892 (5.8)	831 (5.7)	910 (6.3)
Paternal education (%)	9-year elementary	2222 (5.0)	1051 (6.9)	639 (4.4)	532 (3.7)
	1-2 years further education	2610 (5.9)	1252 (8.2)	769 (5.3)	589 (4.1)
	Technical high school	10900 (24.6)	4648 (30.4)	3489 (24.0)	2763 (19.1)
	3-year high school general studies, junior college	5148 (11.6)	1985 (13.0)	1701 (11.7)	1462 (10.1)
	Regional technical college, 4-year university degree (Bachelor’s degree, nurse, teacher, engineer)	10214 (23.1)	3025 (19.8)	3554 (24.4)	3635 (25.2)
	University, technical college, more than 4 years (Master’s degree, medical doctor, PhD)	8608 (19.4)	1770 (11.6)	2946 (20.3)	3892 (26.9)
	Missing	4592 (10.4)	1579 (10.3)	1440 (9.9)	1573 (10.9)
Maternal income category (%)	No	14900 (33.6)	3856 (25.2)	5063 (34.8)	5981 (41.4)
	Yes	27209 (61.4)	10691 (69.8)	8769 (60.3)	7749 (53.6)
	Missing	2185 (4.9)	763 (5.0)	706 (4.9)	716 (5.0)
Paternal income category (%)	No	26677 (60.2)	8213 (53.6)	9105 (62.6)	9359 (64.8)
	Yes	13690 (30.9)	5610 (36.6)	4233 (29.1)	3847 (26.6)
	Missing	3927 (8.9)	1487 (9.7)	1200 (8.3)	1240 (8.6)

BMI, body mass index; MoBa, Norwegian Mother, Father, and Child Cohort; SD, standard deviation; SCQ-RRB, restrictive and repetitive behaviours subdomain of SCQ; SCQ-SOC, communication skills subdomain of SCQ. The number following each outcome denotes the approximate age of the child in years when the measure was obtained. i.e. SCQ-SOC-8; social communication subdomain measured at age 8 years. The presence of the outcome from each questionnaire was indicated by a high score.

Supplementary table 3. Baseline characteristics of pregnancies with missing outcome data at age eight in ALSPAC.

			Adherence to a 'healthy' prenatal dietary pattern (%)		
Characteristic	**Level**	**Overall**	**Low**	**Medium**	**High**
Number of pregnancies		4,481	1,845 (41.2)	1,486 (33.2)	1,150 (25.7)
History of depression (%)	No	3604 (80.4)	1414 (76.6)	1217 (81.9)	973 (84.6)
	Yes	444 (9.9)	211 (11.4)	135 (9.1)	98 (8.5)
	Missing	433 (9.7)	220 (11.9)	134 (9.0)	79 (6.9)
Maternal age (years) (mean (SD))		26.7 (5.1)	25.06 (4.74)	26.96 (4.94)	28.81 (4.93)
	Missing	68 (1.5)	31 (1.7)	21 (1.4)	15 (1.3)
Maternal education (%)	Certificate of secondary education/none	1322 (29.5)	791 (42.9)	369 (24.8)	162 (14.1)
	Vocational	507 (11.3)	241 (13.1)	179 (12.0)	87 (7.6)
	Ordinary level	1501 (33.5)	574 (31.1)	558 (37.6)	369 (32.1)
	Advanced level	767 (17.1)	176 (9.5)	279 (18.8)	312 (27.1)
	Degree	338 (7.5)	32 (1.7)	91 (6.1)	215 (18.7)
	Missing	46 (1.0)	31 (1.7)	10 (0.7)	5 (0.4)
Planned pregnancy (%)	No	2750 (61.4)	1067 (57.8)	927 (62.4)	756 (65.7)
	Yes	1526 (34.1)	672 (36.4)	495 (33.3)	359 (31.2)
	Missing	205 (4.6)	106 (5.7)	64 (4.3)	35 (3.0)
Prenatal alcohol intake (%)	No	1739 (38.8)	774 (42.0)	576 (38.8)	389 (33.8)
	Yes	643 (14.3)	211 (11.4)	216 (14.5)	216 (18.8)
	Missing	2099 (46.8)	860 (46.6)	694 (46.7)	545 (47.4)
Prenatal multivitamin supplement use (%)	No	3333 (74.4)	1431 (77.6)	1100 (74.0)	802 (69.7)
	Yes	915 (20.4)	288 (15.6)	319 (21.5)	308 (26.8)
	Missing	233 (5.2)	126 (6.8)	67 (4.5)	40 (3.5)
Prenatal smoking (%)	No	2934 (65.5)	1002 (54.3)	1019 (68.6)	913 (79.4)
	Yes	1373 (30.6)	747 (40.5)	416 (28.0)	210 (18.3)
	Missing	174 (3.9)	96 (5.2)	51 (3.4)	27 (2.3)

Characteristic	Level	Overall	Adherence to a 'healthy' prenatal dietary pattern (%)		
			Low	Medium	High
Pre-pregnancy BMI (mean (SD))		23.1 (4.1)	23.33 (4.49)	23.27 (4.01)	22.52 (3.42)
	Missing	549 (1.2)	204 (1.1)	190 (1.3)	155 (1.4)
Pre-pregnancy diabetes (%)	Yes	18 (0.4)	7 (0.4)	<10*	<10*
	No	3839 (85.7)	1516 (82.2)	1297 (87.3)	1026 (89.2)
	Missing	624 (13.9)	322 (17.5)	*	*
Pre-pregnancy hypertension (%)	No	4438 (99.0)	1823 (98.8)	1474 (99.2)	1141 (99.2)
	Yes	<10*	<10*	<10*	<10*
	Missing	*	*	*	*
Paternal education (%)	Certificate of secondary education/none	1477 (33.0)	803 (43.5)	450 (30.3)	224 (19.5)
	Vocational	382 (8.5)	170 (9.2)	149 (10.0)	63 (5.5)
	Ordinary level	854 (19.1)	336 (18.2)	305 (20.5)	213 (18.5)
	Advanced level	963 (21.5)	330 (17.9)	340 (22.9)	293 (25.5)
	Degree	521 (11.6)	54 (2.9)	160 (10.8)	307 (26.7)
	Missing	284 (6.3)	152 (8.2)	82 (5.5)	50 (4.3)
Townsend deprivation (high) (%)	High	1516 (33.8)	540 (29.3)	526 (35.4)	450 (39.1)
	Low	1239 (27.7)	577 (31.3)	401 (27.0)	261 (22.7)
	Missing	1726 (38.5)	728 (39.5)	559 (37.6)	439 (38.2)
Paternal age category (%)	< 25 years	432 (9.6)	254 (13.8)	131 (8.8)	47 (4.1)
	25-29 years	855 (19.1)	368 (19.9)	292 (19.7)	195 (17.0)
	29-34 years	683 (15.2)	206 (11.2)	224 (15.1)	253 (22.0)
	35-39 years	272 (6.1)	71 (3.8)	86 (5.8)	115 (10.0)
	≥ 40 years	104 (2.3)	25 (1.4)	39 (2.6)	40 (3.5)
	Missing	2135 (47.6)	921 (49.9)	714 (48.0)	500 (43.5)

ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index; SD, standard deviation; SCDC-8, social communication disorders checklist. The number following each outcome denotes the approximate age of the child in years when the measure was obtained. *Exact values have been removed as low counts are potentially disclosive.

Supplementary material for Chapter 5.

Supplementary table 4. Food items and factor loadings representing a ‘healthy’ prenatal dietary pattern.

MoBa		**ALSPAC**	
Food item	Factor loading	Food item	Factor loading
Dried fruit	0.288	Fruit	0.51
Berries	0.192		
Traditional fruits	0.355		
Cooked vegetables	0.416	Greens	0.465
Leafy greens	0.356	Leafy greens	0.354
Raw vegetables	0.472	Salad	0.456
		Root vegetables	0.351
Nuts	0.191	Nuts	0.315
High fiber cereals	0.242	High fiber cereal	0.367
Wholemeal bread	0.327	Wholemeal bread	-0.458
		Granary bread	-0.352
Other fish	0.189		
Oily fish	0.3	Oily fish	0.471
Lean fish	0.246	Lean fish	0.29
Soft drinks (sugar sweetened)	-0.394	Soft drinks	0.057
Soft drinks (artificial sweeteners)	-0.402		
Fries	-0.2	Fries	-0.409
White bread	-0.35	White bread	0.531
Processed meat	-0.416	Processed meat	-0.279
Meat (nonpoultry)	-0.228	Meat (nonpoultry)	-0.043

ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, The Norwegian Mother, Father, and Child Cohort Study.
The food items selected have been matched across ALSPAC and MoBa where possible. Some categories were broken down to a greater extent in one dataset than the other, for example, fruit in MoBa had three broad categories but was one question in ALSPAC. ‘Other fish’ refers to fish in mixed dishes. To ensure the same food items were in each cohort we did not remove low factor loadings (<0.2). The proportion of variance explained by the factor is 10% in MoBa and 15% in ALSPAC. In ALSPAC, low factor loadings for wholemeal and granary bread could not be adjusted through rotation, and may reflect the low intakes in the general population of England around the early 1990s (Lang et al., 2003).

Supplementary table 5. Food items and factor loadings of the subgroups of a ‘healthy’ prenatal dietary pattern.

MoBa		**ALSPAC**	
Food item	Factor loading	Food item	Factor loading
Plant-based food group			
Dried fruit	0.277	Fruit	0.427
Berries	0.263		
Traditional fruits	0.446		
Cooked vegetables	0.546	Greens	0.678
Leafy greens	0.516	Leafy greens	0.56
Raw vegetables	0.653	Salad	0.402
		Root vegetables	0.467
Nuts	0.129	Nuts	0.276
High fiber cereals	0.197	High fiber cereal	0.297
Wholemeal bread	0.149	Wholemeal bread	-0.318
		Granary bread	-0.247
Fish-based food group			
Oily fish	0.196	Oily fish	0.626
Lean fish	0.679	Lean fish	0.626
Other fish	0.362		
'Unhealthy' food group			
Soft drinks (sugar sweetened)	0.911	Soft drinks	-0.107
Soft drinks (artificial sweeteners)	0.885		
Fries	0.185	Fries	0.59
White bread	0.167	White bread	-0.412
Processed meat	0.113	Processed meat	0.531
Meat (nonpoultry)	0.073	Meat (nonpoultry)	0.264

ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, The Norwegian Mother, Father, and Child Cohort Study.
‘Other fish’ refers to fish in mixed dishes.

Supplementary table 6. Estimated nutrient intakes in ALSPAC and MoBa by level of adherence to a 'healthy' prenatal dietary pattern.

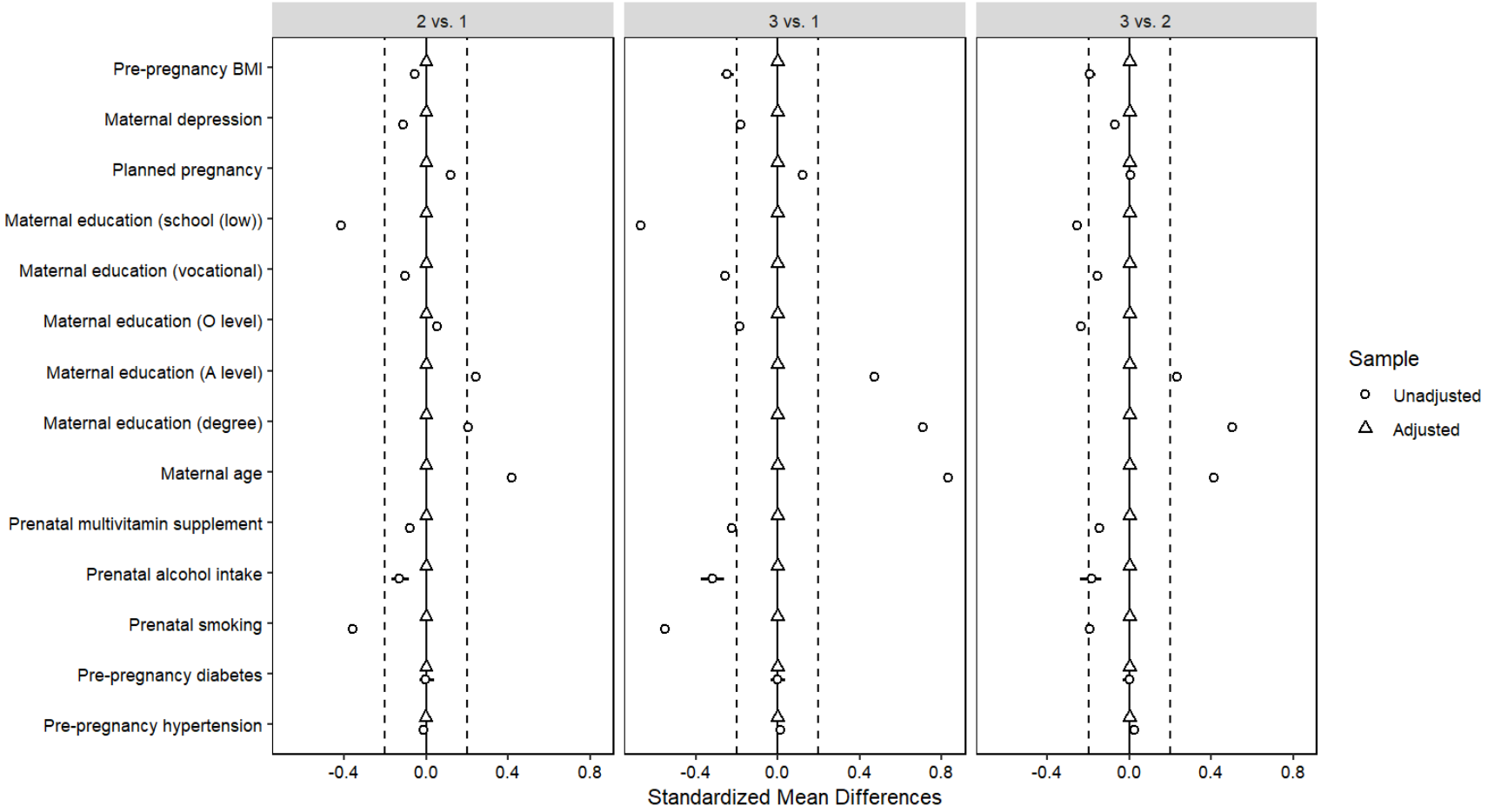
	MoBa (Median (interquartile range))				**ALSPAC (Median (interquartile range))**			
Nutrient	Overall	Low	Medium	High	Overall	Low	Medium	High
Total energy (kilocalories)	2,229 (1,880, 2,653)	2,256 (1,891, 2,694)	2,163 (1,825, 2,557)	2,276 (1,930, 2,709)	1,701 (1,404, 2,008)	1,694 (1,365, 2,029)	1,694 (1,404, 2,003)	1,712 (1,434, 1,997)
Carbohydrate (g)	295 (244, 358)	297 (243, 365)	284 (235, 342)	305 (254, 367)	207 (171, 248)	208 (166, 252)	205 (170, 247)	208 (175, 245)
Free sugars (g)	52 (36, 76)	64 (42, 95)	49 (34, 69)	47 (33, 66)	53 (37, 75)	60 (40, 89)	53 (37, 74)	48 (34, 64)
Fibre (g)	76 (63, 93)	79 (65, 96)	74 (62, 91)	76 (62, 93)	69 (55, 85)	71 (56, 88)	69 (56, 85)	68 (54, 82)
Total fat (g)	29 (24, 36)	30 (25, 37)	28 (23, 35)	28 (23, 35)	28 (21, 36)	30 (23, 39)	28 (22, 36)	26 (20, 33)
Saturated fat (g)	24 (20, 30)	25 (20, 30)	23 (19, 29)	24 (20, 31)	23 (18, 28)	24 (18, 30)	23 (18, 28)	23 (18, 28)
Monounsaturated fatty acid (mg)	14 (11, 18)	14 (11, 19)	13 (10, 17)	13 (10, 17)	11 (9, 15)	10 (8, 14)	11 (9, 15)	12 (10, 16)
Polyunsaturated fatty acid (g)	84 (72, 99)	81 (69, 96)	83 (71, 96)	89 (76, 103)	68 (56, 81)	62 (50, 74)	68 (56, 81)	74 (62, 87)
Protein (g)	2,044 (1,457, 3,237)	1,562 (1,177, 2,139)	1,967 (1,474, 2,899)	3,136 (2,056, 4,699)	1,996 (1,655, 2,274)	1,804 (837, 2,020)	1,999 (1,773, 2,213)	2,215 (1,973, 2,850)
Beta carotene (µg)	261 (209, 326)	225 (181, 279)	252 (208, 307)	311 (256, 379)	238 (193, 287)	202 (162, 244)	236 (197, 280)	277 (235, 323)
Folate (µg)	18 (16, 21)	17 (15, 20)	18 (16, 21)	20 (17, 23)	15 (12, 19)	13 (10, 16)	15 (12, 18)	17 (14, 21)
Niacin (mg)	1,108 (773, 1,586)	971 (684, 1,411)	1,059 (754, 1,504)	1,303 (930, 1,808)	626 (488, 845)	573 (424, 745)	624 (495, 837)	685 (543, 984)

	MoBa (Median (interquartile range))				ALSPAC (Median (interquartile range))			
Nutrient	Overall	Low	Medium	High	Overall	Low	Medium	High
Retinol equivalents (µg)	2,229 (1,880, 2,653)	2,256 (1,891, 2,694)	2,163 (1,825, 2,557)	2,276 (1,930, 2,709)	1,701 (1,404, 2,008)	1,694 (1,365, 2,029)	1,694 (1,404, 2,003)	1,712 (1,434, 1,997)
Riboflavin (mg)	295 (244, 358)	297 (243, 365)	284 (235, 342)	305 (254, 367)	207 (171, 248)	208 (166, 252)	205 (170, 247)	208 (175, 245)
Thiamin (mg)	1.5 (1.2, 1.8)	1.4 (1.2, 1.7)	1.4 (1.2, 1.7)	1.6 (1.3, 1.9)	1.4 (1.1, 1.7)	1.2 (1.0, 1.5)	1.4 (1.2, 1.7)	1.6 (1.3, 1.8)
Vitamin B_6 (mg)	1.5 (1.2, 1.8)	1.3 (1.1, 1.6)	1.4 (1.2, 1.7)	1.7 (1.5, 2.0)	1.9 (1.5, 2.2)	1.7 (1.4, 2.0)	1.8 (1.5, 2.2)	2.0 (1.7, 2.4)
Vitamin B_{12} (µg)	5.4 (4.1, 7.3)	5.1 (3.8, 7.0)	5.3 (4.0, 7.1)	5.8 (4.4, 7.7)	4.3 (3.1, 6.2)	3.6 (2.6, 4.8)	4.2 (3.1, 6.1)	5.6 (3.7, 7.1)
Vitamin C (mg)	146 (102, 206)	121 (81, 176)	137 (99, 189)	181 (134, 243)	74 (53, 100)	52 (39, 71)	74 (56, 95)	99 (77, 125)
Vitamin D (µg)	3.2 (2.1, 4.4)	3.0 (1.9, 4.1)	3.1 (2.1, 4.3)	3.5 (2.5, 4.8)	3.3 (2.3, 5.1)	2.7 (1.9, 3.6)	3.3 (2.4, 4.7)	4.7 (3.1, 6.3)
Vitamin E (mg)	10.0 (8.0, 12.8)	9.4 (7.4, 12.2)	9.6 (7.7, 12.0)	11.1 (9.0, 14.1)	7.7 (5.5, 10.8)	5.9 (4.3, 8.1)	7.9 (5.8, 10.8)	9.7 (7.2, 12.9)
Calcium (mg)	977 (753, 1,263)	936 (708, 1,234)	955 (738, 1,222)	1,039 (815, 1,322)	927 (745, 1,123)	875 (689, 1,084)	925 (741, 1,120)	978 (803, 1,160)
Magnesium (mg)	388 (321, 467)	355 (294, 427)	378 (317, 449)	433 (364, 514)	241 (195, 293)	203 (163, 244)	239 (199, 285)	283 (238, 333)
Phosphorus (µg)	1,640 (1,353, 1,984)	1,545 (1,270, 1,880)	1,605 (1,334, 1,923)	1,773 (1,484, 2,118)	1,234 (1,011, 1,470)	1,098 (888, 1,323)	1,231 (1,024, 1,457)	1,364 (1,159, 1,588)
Potassium (mg)	3,876 (3,225, 4,649)	3,515 (2,922, 4,218)	3,759 (3,180, 4,457)	4,370 (3,702, 5,155)	2,848 (2,387, 3,327)	2,668 (2,196, 3,171)	2,841 (2,395, 3,320)	3,007 (2,594, 3,466)
Selenium (µg)	53 (44, 62)	48 (40, 57)	52 (44, 61)	59 (50, 68)	67 (51, 85)	56 (43, 71)	66 (52, 83)	80 (63, 101)

	MoBa (Median (interquartile range))				ALSPAC (Median (interquartile range))			
Nutrient	Overall	Low	Medium	High	Overall	Low	Medium	High
Sodium (mg)	2,969 (2,516, 3,491)	2,985 (2,538, 3,505)	2,923 (2,479, 3,422)	3,004 (2,532, 3,546)	2,165 (1,750, 2,601)	2,069 (1,632, 2,526)	2,134 (1,748, 2,592)	2,273 (1,874, 2,685)
Zinc (mg)	11.0 (9.2, 13.0)	10.6 (8.9, 12.6)	10.8 (9.0, 12.7)	11.6 (9.7, 13.7)	8.0 (6.5, 9.7)	7.1 (5.7, 8.7)	8.0 (6.6, 9.6)	8.9 (7.4, 10.4)

ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father, and Child Cohort Study
In MoBa, daily nutrient intakes were estimated using FoodCalc (Lauritsen, 1998) and the Norwegian food composition database and excluded alcohol and nutritional supplements (Norwegian Food Safety Authority, 2005). In ALSPAC, daily nutrient intakes were estimated based on average portion sizes (Ministry of Agriculture and Food, 1991) using the McCance and Widdowson's 5th edition food composition tables and excluded alcohol and nutritional supplements (Rogers et al., 1998). Iodine intakes from diet were unavailable in our version of MoBa data, however further research from MoBa has confirmed iodine status is low as conformed by urinary iodine concentration and was unrelated to a 'healthy' diet (Brantsaeter et al., 2022)

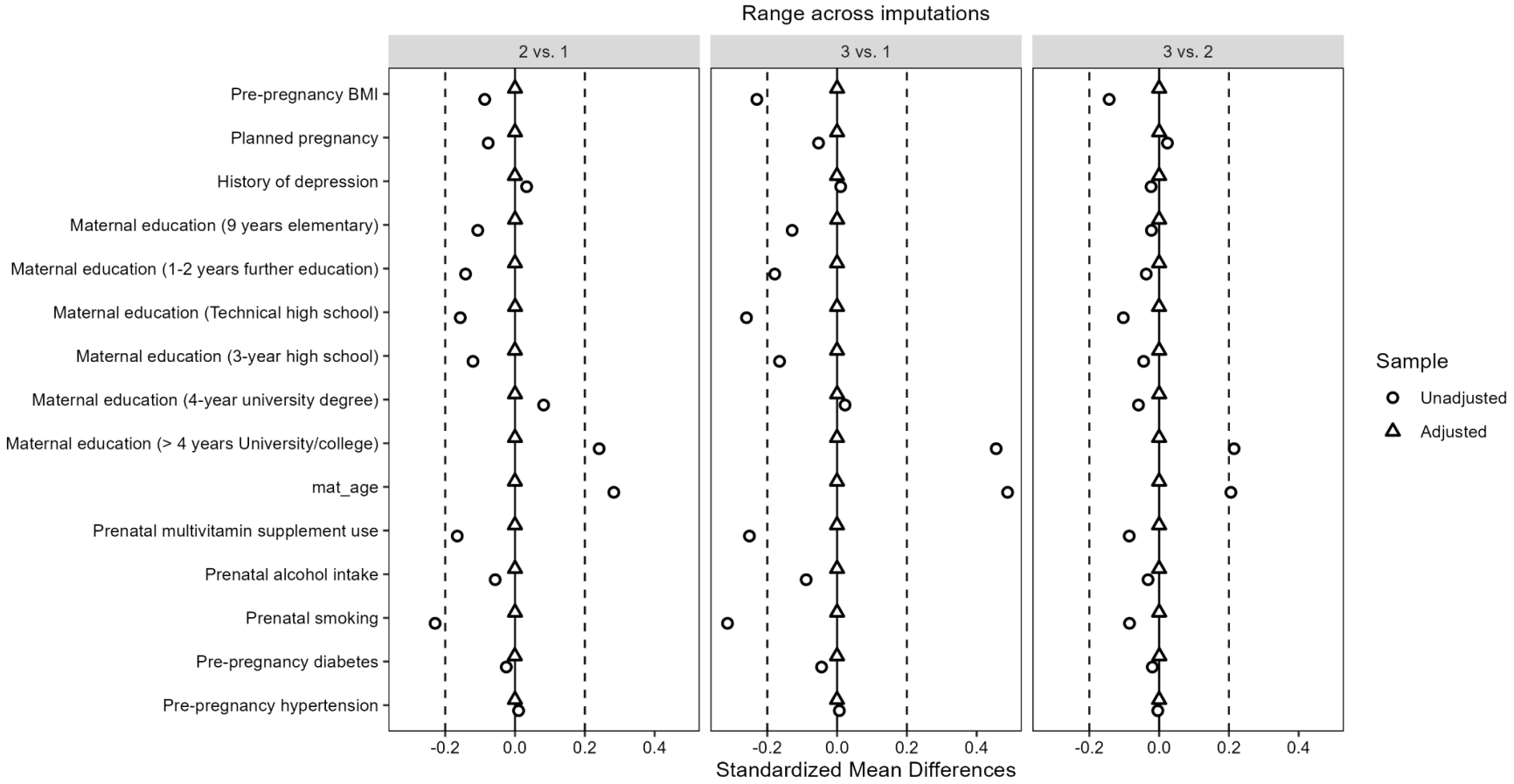
Supplementary figure 2. Covariate balance plot for adherence to a ‘healthy’ prenatal dietary pattern in ALSPAC (Chapter 5).



Good covariate balance was achieved. <0.1 standardised mean difference indicates a negligible difference between exposure groups. The numbers 1, 2, and 3 indicate low, medium, and high adherence to a ‘healthy’ prenatal dietary pattern, respectively. Similar covariate balance was achieved in all additional models used in sensitivity

analysis and there were no extreme weights. Details are available on request. Furthermore, we observed representation of each covariate across all levels of a HPDP (Chapter 5. Table 14-15). There were no extreme weights observed (maximum 7.35 and minimum 0.18). Entropy balancing calibrates the weights which best balance the measured systematic and random error of the covariates against a set of base weights which best retain information. propensity score estimation, entropy balancing combines covariate balancing constraints into the weighting process. This reduces extreme weights and avoids poor covariate balance (Hainmueller, 2012)

Supplementary figure 3. Covariate balance plot for adherence to a ‘healthy’ prenatal dietary pattern in MoBa (Chapter 5).



Good covariate balance was achieved. <0.1 standardised mean difference indicates a negligible difference between exposure groups and there were no extreme weights (Maximum 3.93 and minimum 0.25). The numbers 1, 2, and 3 indicate low, medium, and high adherence to a ‘healthy’ prenatal dietary pattern, respectively.

Similarcovariate balance was achieved in all additional models used in sensitivity analysis and there were no extreme weights. Details are available on request. Furthermore, we observed representation of each covariate across all levels of a HPDP (Chapter 5. Table 14-15).

Supplementary material for Chapter 6.

Due to the large volume of sensitivity analysis conducted in MR, I have present them in the supplementary materials.

Supplementary table 7. Strength of association (F-statistic) with a 'healthy' prenatal dietary pattern and the proportion of variance explained (R^2) by maternal polygenic score for a 'healthy' prenatal dietary pattern.

Subsample and exposure measure	**F-statistic**	**R^2**
Trios – 'Healthy' prenatal dietary pattern	11.8%	1.2%

F-statistic indicates the strength of association between the dietary pattern score and maternal diet polygenic score. The R^2 estimates the proportion of the dietary pattern explained by maternal diet polygenic score and is low.

Supplementary table 8. Univariate associations between maternal polygenic score for a ‘healthy’ prenatal dietary pattern and a range of potential confounders.

	Unadjusted for child & father PGS		**Adjusted for child & father PGS**	
Characteristic	Beta (95% CI)	P-value	Beta (95% CI)	P-value
Child sex (reference, male)				
Child sex (female)	0.011 (-0.045, 0.066)	0.71	0.011 (-0.045, 0.066)	0.71
History of depression (reference, no)				
History of depression (yes)	0.003 (-0.047, 0.052)	0.92	0.003 (-0.047, 0.052)	0.92
Maternal income (reference, no income)				
Maternal - under 150,000 NOK	0.091 (-0.050, 0.231)	0.21	0.086 (-0.029, 0.201)	0.14
Maternal - 151,000-199,999 NOK	0.072 (-0.070, 0.214)	0.32	0.064 (-0.052, 0.181)	0.28
Maternal - 200,000-299,999 NOK	0.093 (-0.043, 0.228)	0.18	0.094 (-0.016, 0.205)	0.09
Maternal - 300,000-399,999 NOK	0.089 (-0.047, 0.224)	0.20	0.093 (-0.018, 0.204)	0.10
Maternal - 400,000-499,999 NOK	0.049 (-0.094, 0.193)	0.50	0.085 (-0.033, 0.202)	0.16
Maternal - Over 500,000 NOK	0.125 (-0.027, 0.276)	0.11	0.143 (0.020, 0.267)	0.023
Maternal age (reference, more than 40 years)				
Under 25 years	0.084 (-0.068, 0.235)	0.28	0.042 (-0.081, 0.166)	0.50
26 - 30 years	0.091 (-0.056, 0.237)	0.23	0.054 (-0.066, 0.174)	0.37
31- 40 years	0.098 (-0.048, 0.244)	0.19	0.066 (-0.053, 0.186)	0.28
Maternal education (reference, 9-year secondary school)				
1–2-year high school	0.014 (-0.144, 0.173)	0.86	0.016 (-0.114, 0.145)	0.81
technical high school	0.031 (-0.109, 0.172)	0.66	0.040 (-0.075, 0.155)	0.50
3-year high school general studies, junior college	0.036 (-0.104, 0.176)	0.61	0.044 (-0.071, 0.158)	0.45
regional technical college, 4-year university degree (Bachelor’s degree, nurse, teacher, engineer)	0.078 (-0.056, 0.213)	0.25	0.085 (-0.025, 0.195)	0.13
University, technical college, more than 4 years (Master’s degree, medical doctor, PhD)	0.070 (-0.066, 0.207)	0.31	0.076 (-0.035, 0.187)	0.18
Paternal income (reference, no income)				
Paternal - under 150,000 NOK	0.085 (-0.150, 0.320)	0.48	0.121 (-0.070, 0.313)	0.21

	Unadjusted for child & father PGS		Adjusted for child & father PGS	
Characteristic	Beta (95% CI)	P-value	Beta (95% CI)	P-value
Paternal - 151,000-199,999 NOK	0.073 (-0.167, 0.314)	0.55	0.113 (-0.083, 0.310)	0.26
Paternal - 200,000-299,999 NOK	0.100 (-0.125, 0.326)	0.38	0.129 (-0.056, 0.313)	0.17
Paternal - 300,000-399,999 NOK	0.097 (-0.127, 0.322)	0.39	0.150 (-0.033, 0.333)	0.11
Paternal - 400,000-499,999 NOK	0.107 (-0.119, 0.332)	0.35	0.142 (-0.042, 0.326)	0.13
Paternal - Over 500,000 NOK	0.084 (-0.141, 0.310)	0.46	0.125 (-0.060, 0.310)	0.18
Planned pregnancy (reference, no)				
Planned pregnancy (yes)	-0.019 (-0.065, 0.027)	0.41	-0.019 (-0.065, 0.027)	0.41
Prenatal alcohol intake (reference, no)				
Prenatal alcohol intake (yes)	0.004 (-0.064, 0.071)	0.92	0.004 (-0.064, 0.071)	0.92
Prenatal multivitamin supplement use (reference, no)				
Prenatal multivitamin supplement use (yes)	-0.012 (-0.047, 0.022)	0.48	-0.012 (-0.047, 0.022)	0.48
Prenatal smoking (reference, no)				
Prenatal smoking (yes)	-0.011 (-0.049, 0.026)	0.56	-0.011 (-0.049, 0.026)	0.56
Pre-pregnancy BMI (linear)				
Pre-pregnancy BMI	-0.020 (-0.080, 0.041)	0.53	-0.020 (-0.080, 0.041)	0.53
Pre-pregnancy diabetes (reference, no)				
Pre-pregnancy diabetes (yes)	0.003 (0.000, 0.007)	0.05	0.003 (0.000, 0.007)	0.05
Pre-pregnancy hypertension (reference, no)				
Pre-pregnancy hypertension (yes)	-0.022 (-0.073, 0.030)	0.41	-0.022 (-0.073, 0.030)	0.41

BMI, body mass index (kg/m^2); CI, confidence interval; NOK, Norwegian Krone; PGS, polygenic score.

Supplementary table 9. MR-Egger results of sensitivity analysis were invalid and are presented for reference only.

Outcome	Beta (95% CI)	P-value	Mean instrument strength (mF)	Unweighted (I^2_{GX})	Weighted (I^2_{GX})
SCQ-3	-1.26 (-4.40, 1.87)	0.44	36.11	0.40	0.00
SCQ-8	-0.79 (-3.96, 2.37)	0.63	36.11	0.40	0.00
SCQ-RRB-3	0.38 (-2.03, 2.78)	0.76	36.11	0.40	0.00
SCQ-RRB-8	0.43 (-0.81, 1.68)	0.50	36.11	0.40	0.00
SCQ-SOC-3	-1.80 (-3.48, -0.13)	0.05	36.11	0.40	0.00
SCQ-SOC-8	-0.93 (-3.62, 1.76)	0.51	36.11	0.40	0.00

CI, confidence interval; PGS, Polygenic score; SCQ, social communication questionnaire; SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests; SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years.
Outcome-SNPs relate to higher outcome scores and represent greater autism-associated traits. Exposure-SNPs relate to a higher factor score and indicates greater adherence to a ‘healthy’ prenatal dietary pattern.

Supplementary table 10. Two-sample MR sensitivity analysis to assess for pleiotropy.

Method	Beta (95% CI)	P-value
SCQ-3		
Inverse variance weighted	-0.52 (-1.17, 0.14)	0.13
Weighted median	-0.98 (-1.85, -0.11)	0.027
Contamination Mixture	-0.97 (-1.98, -0.08)	0.032
SCQ-RRB-3		
Inverse variance weighted	-0.52 (-1.02, -0.02)	0.042
Weighted median	-0.43 (-1.10, 0.24)	0.21
Contamination Mixture	-0.16 (-1.91, 0.64)	0.57
SCQ-SOC-3		
Inverse variance weighted	-0.06 (-0.41, 0.29)	0.74
Weighted median	0.06 (-0.45, 0.58)	0.82
Contamination Mixture	-0.23 (-1.13, 0.89)	0.34
SCQ-8		
Inverse variance weighted	-0.60 (-1.27, 0.06)	0.08
Weighted median	-0.18 (-1.08, 0.72)	0.69
Contamination Mixture	-0.82 (-2.80, 0.86)	0.66
SCQ-RRB-8		
Inverse variance weighted	-0.11 (-0.37, 0.15)	0.42
Weighted median	0.00 (-0.35, 0.34)	0.98
Contamination Mixture	-0.08 (-0.77, 0.38)	0.60
SCQ-SOC-8		
Inverse variance weighted	-0.43 (-1.00, 0.13)	0.14
Weighted median	-0.16 (-0.93, 0.60)	0.68
Contamination Mixture	-0.50 (-1.43, 0.61)	0.29

CI, confidence interval; PGS, Polygenic score; SCQ, social communication questionnaire; SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests; SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years.
Outcome-SNPs relate to higher outcome scores and represent greater autism-associated traits. Exposure-SNPs relate to a higher factor score and indicates greater adherence to a ‘healthy’ prenatal dietary pattern.

Supplementary table 11. Measures heterogeneity based on Cochranes Q-statistic for each two-sample Mendelian randomisation analysis.

Outcome	Cochranes Q-statistic	P-value
SCQ-3	12.541	0.77
SCQ-8	12.219	0.79
SCQ-RRB-3	17.062	0.45
SCQ-RRB-8	5.571	1.00
SCQ-SOC-3	15.219	0.58
SCQ-SOC-8	12.835	0.75

SCQ, social communication questionnaire; SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests; SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years.

Supplementary table 12. Individual SNP contribution to Q-statistic in two-sample Mendelian randomisation analyses.

		Social communication questionnaire			Social communication questionnaire - restrictive & repetitive behaviours			Social communication questionnaire - social communication	
SNP	Outliers	Q-statistic	P-value	Outliers	Q-statistic	P-value	Outliers	Q-statistic	P-value
Outcomes obtained at approximately age three years									
rs11209952	Variant	1.185	0.28	**Outlier**	**3.874**	**0.049**	Variant	0.359	0.55
rs11581418	Variant	0.193	0.66	Variant	0.028	0.87	Variant	1.118	0.29
rs12285584	Variant	0.332	0.56	Variant	1.583	0.21	Variant	0.800	0.37
rs150175599	Variant	0.057	0.81	Variant	0.172	0.68	Variant	0.974	0.32
rs1799247	Variant	0.830	0.36	Variant	0.393	0.53	Variant	0.808	0.37
rs2189435	Variant	0.013	0.91	Variant	0.498	0.48	Variant	1.695	0.19
rs2393923	Variant	0.039	0.84	Variant	0.646	0.42	Variant	0.560	0.45
rs332040	Variant	0.022	0.88	Variant	0.021	0.89	Variant	0.077	0.78
rs33988101	Variant	0.393	0.53	Variant	0.039	0.84	Variant	1.956	0.16
rs35287743	Variant	0.292	0.59	Variant	0.002	0.97	Variant	0.994	0.32
rs4953150	Variant	0.431	0.51	Variant	0.930	0.33	Variant	0.020	0.89
rs56094641	Variant	0.818	0.37	Variant	2.971	0.08	Variant	0.656	0.42
rs56367474	Variant	1.255	0.26	Variant	1.666	0.20	Variant	0.097	0.76
rs5749330	Variant	0.125	0.72	Variant	0.229	0.63	Variant	0.021	0.89
rs615632	Variant	0.727	0.39	Variant	0.340	0.56	Variant	0.257	0.61
rs7098100	**Outlier**	**5.350**	**0.021**	Variant	2.216	0.14	**Outlier**	**4.309**	**0.038**
rs71276077	Variant	0.340	0.56	Variant	1.324	0.25	Variant	0.377	0.54
rs9316807	Variant	0.096	0.76	Variant	0.023	0.88	Variant	0.138	0.71
Outcomes obtained at approximately age eight years									
rs11209952	Variant	2.306	0.13	Variant	1.155	0.28	Variant	1.514	0.22
rs11581418	Variant	0.082	0.77	Variant	0.327	0.57	Variant	0.252	0.62
rs12285584	Variant	1.165	0.28	Variant	0.581	0.45	Variant	0.962	0.33
rs150175599	Variant	1.846	0.17	Variant	0.252	0.62	Variant	2.758	0.10
rs1799247	Variant	0.231	0.63	Variant	0.200	0.65	Variant	0.165	0.68
rs2189435	Variant	0.000	0.98	Variant	0.026	0.87	Variant	0.043	0.84
rs2393923	Variant	1.619	0.20	Variant	0.000	0.98	Variant	1.541	0.21
rs332040	Variant	1.017	0.31	Variant	0.351	0.55	Variant	0.380	0.54
rs33988101	Variant	0.187	0.67	Variant	0.358	0.55	Variant	0.032	0.86
rs35287743	Variant	0.243	0.62	Variant	0.727	0.39	Variant	0.099	0.75
rs4953150	Variant	0.076	0.78	Variant	0.157	0.69	Variant	0.088	0.77
rs56094641	Variant	0.357	0.55	Variant	0.012	0.91	Variant	0.544	0.46
rs56367474	Variant	0.980	0.32	Variant	0.073	0.79	Variant	1.413	0.23
rs5749330	Variant	0.015	0.90	Variant	0.280	0.60	Variant	0.023	0.88
rs615632	Variant	0.017	0.90	Variant	0.062	0.80	Variant	0.010	0.92
rs7098100	Variant	0.938	0.33	Variant	0.003	0.95	Variant	0.658	0.42
rs71276077	Variant	0.940	0.33	Variant	0.603	0.44	Variant	2.254	0.13
rs9316807	Variant	0.144	0.70	Variant	0.397	0.53	Variant	0.059	0.81

SNP, single nucleotide polymorphism

Supplementary table 13. Single SNP estimates from two-sample Mendelian randomisation for child outcomes at age three years.

Outcome	SNP	Beta (95% CI)	P-value
SCQ-3	rs11209952	-2.14 (-5.06, 0.78)	0.15
SCQ-3	rs11581418	-1.17 (-4.07, 1.74)	0.43
SCQ-3	rs12285584	-1.38 (-4.32, 1.56)	0.36
SCQ-3	rs150175599	-0.93 (-4.36, 2.49)	0.59
SCQ-3	rs1799247	-1.85 (-4.73, 1.02)	0.21
SCQ-3	rs2189435	-0.69 (-3.70, 2.32)	0.65
SCQ-3	rs2393923	-0.20 (-3.29, 2.88)	0.90
SCQ-3	rs332040	-0.32 (-2.90, 2.27)	0.81
SCQ-3	rs33988101	-1.38 (-4.06, 1.31)	0.32
SCQ-3	rs35287743	-1.13 (-3.35, 1.09)	0.32
SCQ-3	rs4953150	0.47 (-2.47, 3.41)	0.75
SCQ-3	rs56094641	-1.69 (-4.23, 0.85)	0.19
SCQ-3	rs56367474	0.93 (-1.60, 3.47)	0.47
SCQ-3	rs5749330	-1.06 (-4.07, 1.95)	0.49
SCQ-3	rs615632	0.67 (-2.05, 3.38)	0.63
SCQ-3	rs7098100	2.49 (-0.05, 5.04)	0.05
SCQ-3	rs71276077	0.43 (-2.73, 3.59)	0.79
SCQ-3	rs9316807	-1.00 (-4.05, 2.05)	0.52
SCQ-RRB-3	**rs11209952**	**-2.74 (-4.95, -0.53)**	**0.015***
SCQ-RRB-3	rs11581418	-0.33 (-2.53, 1.87)	0.77
SCQ-RRB-3	rs12285584	-1.95 (-4.18, 0.27)	0.09
SCQ-RRB-3	rs150175599	0.03 (-2.56, 2.62)	0.98
SCQ-RRB-3	rs1799247	-1.22 (-3.39, 0.96)	0.27
SCQ-RRB-3	rs2189435	-1.34 (-3.61, 0.93)	0.25
SCQ-RRB-3	rs2393923	0.44 (-1.89, 2.77)	0.71
SCQ-RRB-3	rs332040	-0.66 (-2.62, 1.29)	0.51
SCQ-RRB-3	rs33988101	-0.31 (-2.35, 1.72)	0.76
SCQ-RRB-3	rs35287743	-0.55 (-2.23, 1.13)	0.52
SCQ-RRB-3	rs4953150	0.58 (-1.64, 2.81)	0.61
SCQ-RRB-3	**rs56094641**	**-2.21 (-4.14, -0.29)**	**0.024**
SCQ-RRB-3	rs56367474	0.75 (-1.17, 2.66)	0.44
SCQ-RRB-3	rs5749330	-1.08 (-3.35, 1.20)	0.35
SCQ-RRB-3	rs615632	0.09 (-1.96, 2.14)	0.93
SCQ-RRB-3	rs7098100	0.95 (-0.98, 2.87)	0.33
SCQ-RRB-3	rs71276077	0.89 (-1.50, 3.28)	0.47
SCQ-RRB-3	rs9316807	-0.70 (-3.00, 1.61)	0.55
SCQ-SOC-3	rs11209952	0.42 (-1.14, 1.98)	0.60
SCQ-SOC-3	rs11581418	-0.90 (-2.45, 0.66)	0.26
SCQ-SOC-3	rs12285584	0.66 (-0.91, 2.23)	0.41
SCQ-SOC-3	rs150175599	-0.98 (-2.81, 0.85)	0.29
SCQ-SOC-3	rs1799247	-0.76 (-2.30, 0.77)	0.33
SCQ-SOC-3	rs2189435	1.01 (-0.60, 2.62)	0.22
SCQ-SOC-3	rs2393923	-0.69 (-2.33, 0.96)	0.41
SCQ-SOC-3	rs332040	0.14 (-1.25, 1.52)	0.85
SCQ-SOC-3	rs33988101	-1.08 (-2.52, 0.35)	0.14
SCQ-SOC-3	rs35287743	-0.66 (-1.85, 0.52)	0.27
SCQ-SOC-3	rs4953150	-0.17 (-1.74, 1.40)	0.83
SCQ-SOC-3	rs56094641	0.50 (-0.86, 1.86)	0.47
SCQ-SOC-3	rs56367474	0.16 (-1.20, 1.51)	0.82
SCQ-SOC-3	rs5749330	0.06 (-1.55, 1.67)	0.94
SCQ-SOC-3	rs615632	0.32 (-1.13, 1.76)	0.67
SCQ-SOC-3	**rs7098100**	**1.38 (0.02, 2.74)**	**0.047***

Outcome	SNP	Beta (95% CI)	P-value
SCQ-SOC-3	rs71276077	-0.59 (-2.28, 1.10)	0.49
SCQ-SOC-3	rs9316807	-0.37 (-2.00, 1.26)	0.66

CI, confidence interval; SCQ, social communication questionnaire; SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests; SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years; SNP, single nucleotide polymorphism.
*Identified as outliers in the radial plot based on the SNP contribution to Q-statistic.
Outcome-SNPs relate to higher outcome scores and represent greater autism-associated traits.
Exposure-SNPs relate to a higher factor score and indicates greater adherence to a 'healthy' prenatal dietary pattern.

Supplementary table 14. Single SNP estimates from two-sample Mendelian randomisation for child outcomes at age eight years.

Outcome	SNP	Beta (95% CI)	P-value
SCQ-8	rs11209952	-2.89 (-5.83, 0.06)	0.05
SCQ-8	rs11581418	-0.17 (-3.10, 2.75)	0.91
SCQ-8	rs12285584	-2.23 (-5.19, 0.72)	0.14
SCQ-8	rs150175599	-3.01 (-6.47, 0.46)	0.09
SCQ-8	rs1799247	0.11 (-2.79, 3.01)	0.94
SCQ-8	rs2189435	-0.57 (-3.61, 2.48)	0.71
SCQ-8	rs2393923	1.42 (-1.69, 4.53)	0.37
SCQ-8	rs332040	0.74 (-1.87, 3.35)	0.58
SCQ-8	rs33988101	0.00 (-2.72, 2.71)	1.00
SCQ-8	rs35287743	-0.04 (-2.28, 2.20)	0.97
SCQ-8	rs4953150	-0.18 (-3.16, 2.79)	0.90
SCQ-8	rs56094641	0.18 (-2.38, 2.75)	0.89
SCQ-8	rs56367474	-1.89 (-4.45, 0.66)	0.15
SCQ-8	rs5749330	-0.41 (-3.46, 2.64)	0.79
SCQ-8	rs615632	-0.42 (-3.16, 2.32)	0.76
SCQ-8	rs7098100	-1.87 (-4.44, 0.70)	0.15
SCQ-8	rs71276077	0.99 (-2.22, 4.20)	0.55
SCQ-8	rs9316807	-1.20 (-4.28, 1.88)	0.45
SCQ-RRB-8	rs11209952	-0.75 (-1.91, 0.42)	0.21
SCQ-RRB-8	rs11581418	-0.44 (-1.60, 0.71)	0.45
SCQ-RRB-8	rs12285584	-0.56 (-1.73, 0.60)	0.34
SCQ-RRB-8	rs150175599	0.24 (-1.12, 1.60)	0.73
SCQ-RRB-8	rs1799247	0.15 (-0.99, 1.30)	0.79
SCQ-RRB-8	rs2189435	-0.01 (-1.21, 1.19)	0.99
SCQ-RRB-8	rs2393923	-0.12 (-1.35, 1.10)	0.85
SCQ-RRB-8	rs332040	0.20 (-0.83, 1.23)	0.70
SCQ-RRB-8	rs33988101	0.22 (-0.85, 1.29)	0.69
SCQ-RRB-8	rs35287743	0.28 (-0.61, 1.16)	0.54
SCQ-RRB-8	rs4953150	0.13 (-1.05, 1.31)	0.83
SCQ-RRB-8	rs56094641	-0.17 (-1.18, 0.85)	0.75
SCQ-RRB-8	rs56367474	0.03 (-0.98, 1.04)	0.95
SCQ-RRB-8	rs5749330	-0.43 (-1.64, 0.77)	0.48
SCQ-RRB-8	rs615632	0.03 (-1.05, 1.11)	0.96
SCQ-RRB-8	rs7098100	-0.14 (-1.15, 0.88)	0.79
SCQ-RRB-8	rs71276077	-0.61 (-1.88, 0.66)	0.35
SCQ-RRB-8	rs9316807	-0.50 (-1.72, 0.72)	0.42
SCQ-SOC-8	rs11209952	-2.01 (-4.51, 0.50)	0.12

Outcome	SNP	Beta (95% CI)	P-value
SCQ-SOC-8	rs11581418	0.21 (-2.28, 2.69)	0.87
SCQ-SOC-8	rs12285584	-1.69 (-4.20, 0.82)	0.19
SCQ-SOC-8	rs150175599	-2.93 (-5.87, 0.01)	0.05
SCQ-SOC-8	rs1799247	0.08 (-2.39, 2.54)	0.95
SCQ-SOC-8	rs2189435	-0.16 (-2.75, 2.43)	0.90
SCQ-SOC-8	rs2393923	1.24 (-1.40, 3.89)	0.36
SCQ-SOC-8	rs332040	0.27 (-1.95, 2.48)	0.81
SCQ-SOC-8	rs33988101	-0.22 (-2.53, 2.09)	0.85
SCQ-SOC-8	rs35287743	-0.13 (-2.03, 1.78)	0.90
SCQ-SOC-8	rs4953150	-0.05 (-2.58, 2.48)	0.97
SCQ-SOC-8	rs56094641	0.39 (-1.79, 2.57)	0.73
SCQ-SOC-8	rs56367474	-1.75 (-3.92, 0.42)	0.11
SCQ-SOC-8	rs5749330	-0.23 (-2.82, 2.36)	0.86
SCQ-SOC-8	rs615632	-0.55 (-2.88, 1.78)	0.64
SCQ-SOC-8	rs7098100	-1.34 (-3.52, 0.85)	0.23
SCQ-SOC-8	rs71276077	1.66 (-1.07, 4.39)	0.23
SCQ-SOC-8	rs9316807	-0.76 (-3.38, 1.86)	0.57

CI, confidence interval; SCQ, social communication questionnaire; SCQ-RRB, social communication questionnaire subdomain restrictive and repetitive behaviours and interests; SCQ-SOC, social communication questionnaire subdomain social communication. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years; SNP, single nucleotide polymorphism.

Outcome-SNPs relate to higher outcome scores and represent greater autism-associated traits. Exposure-SNPs relate to a higher factor score and indicates greater adherence to a ‘healthy’ prenatal dietary pattern.

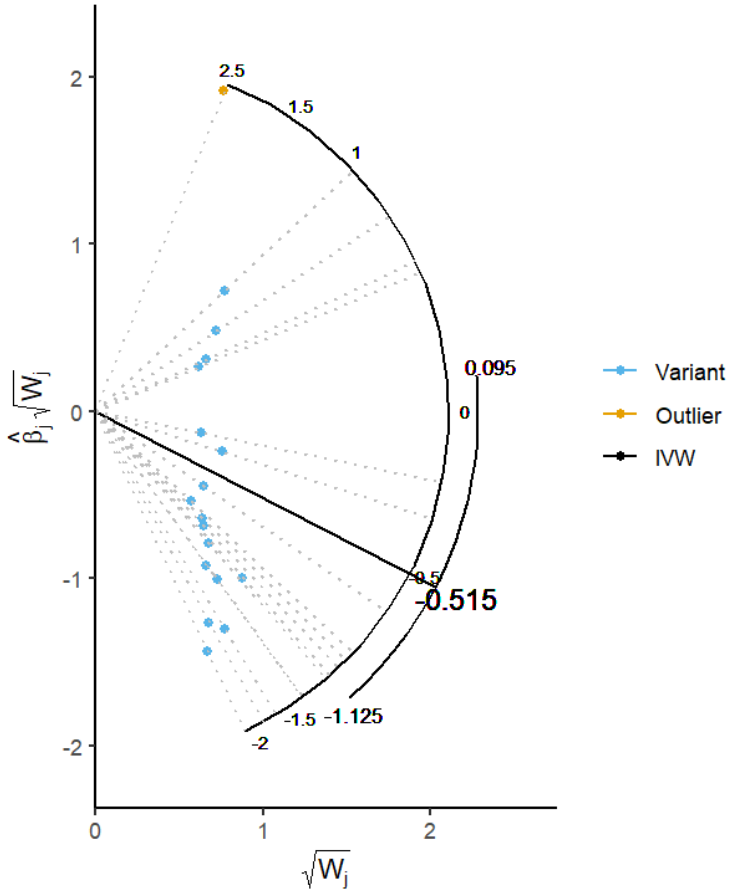
Radial plots based on two-sample Mendelian randomisation.

The radial plots below can indicate the potential presence of outlying SNPs which may signal pleiotropy. Each radial plot is structured as below (Bowden et al., 2018).

χ-axis; $\sqrt{W_j}$, square route of each SNP's weight in the inverse variance-weighted average method (IVW).

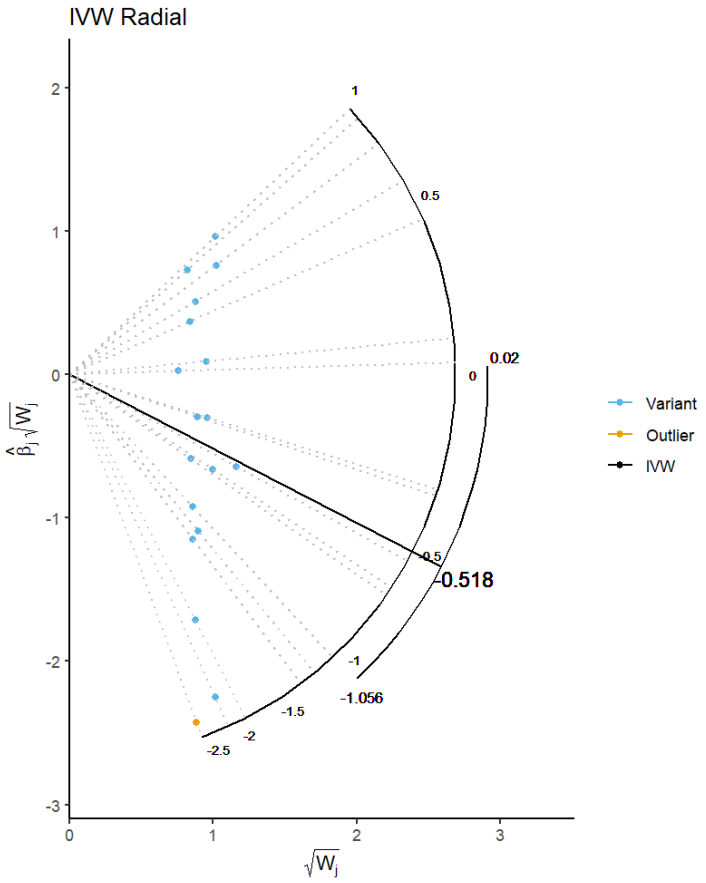
Y-axis; $\hat{\beta}_j \sqrt{W_j}$, each SNPs ratio estimate multiplied by the square-root of the corresponding weight

Supplementary figure 4. Radial plot, Social communication questionnaire age three.



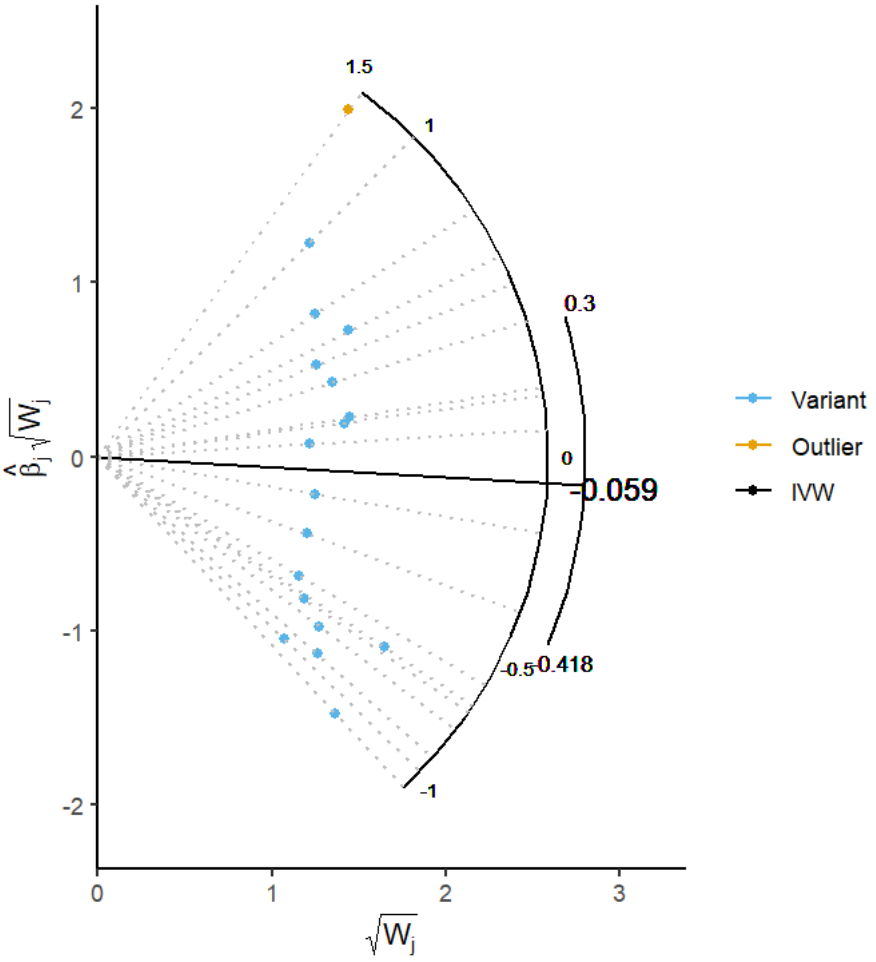
Outlier detected, SNP = rs7098100. Contribution to Q-statistic = 5.350, P-value = 0.021

Supplementary figure 5. Radial plot, Social communication questionnaire age three, restrictive and repetitive behaviours.



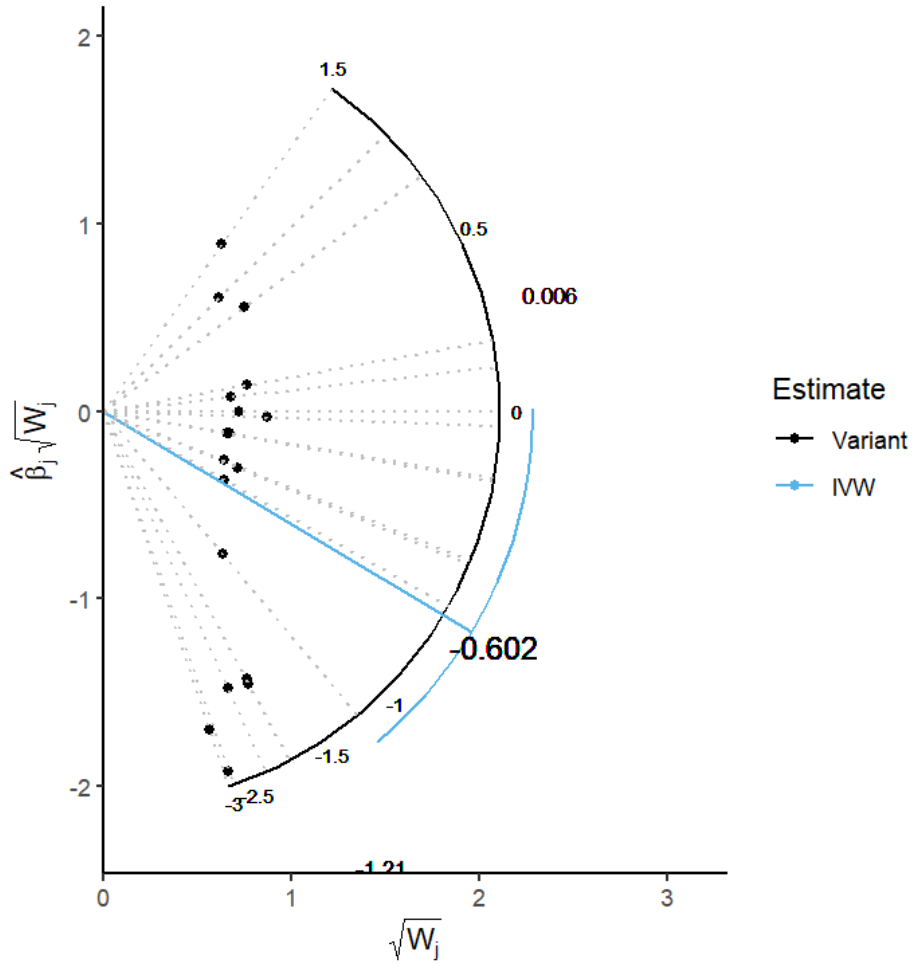
Outlier detected, SNP = rs11209952. Contribution to Q-statistic = 3.874, P-value = 0.049

Supplementary figure 6. Radial plot, Social communication questionnaire age three, social communication subdomain.



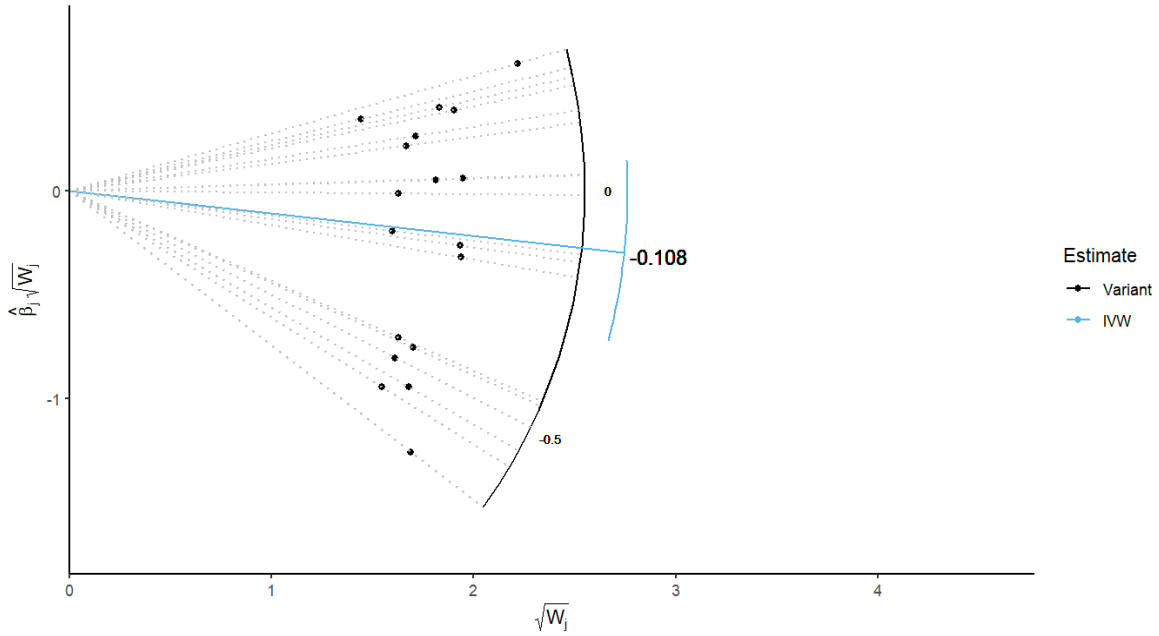
Outlier detected, SNP = rs7098100. Contribution to Q-statistic = 4.309, P-value = 0.038

Supplementary figure 7. Radial plot, Social communication questionnaire age eight.



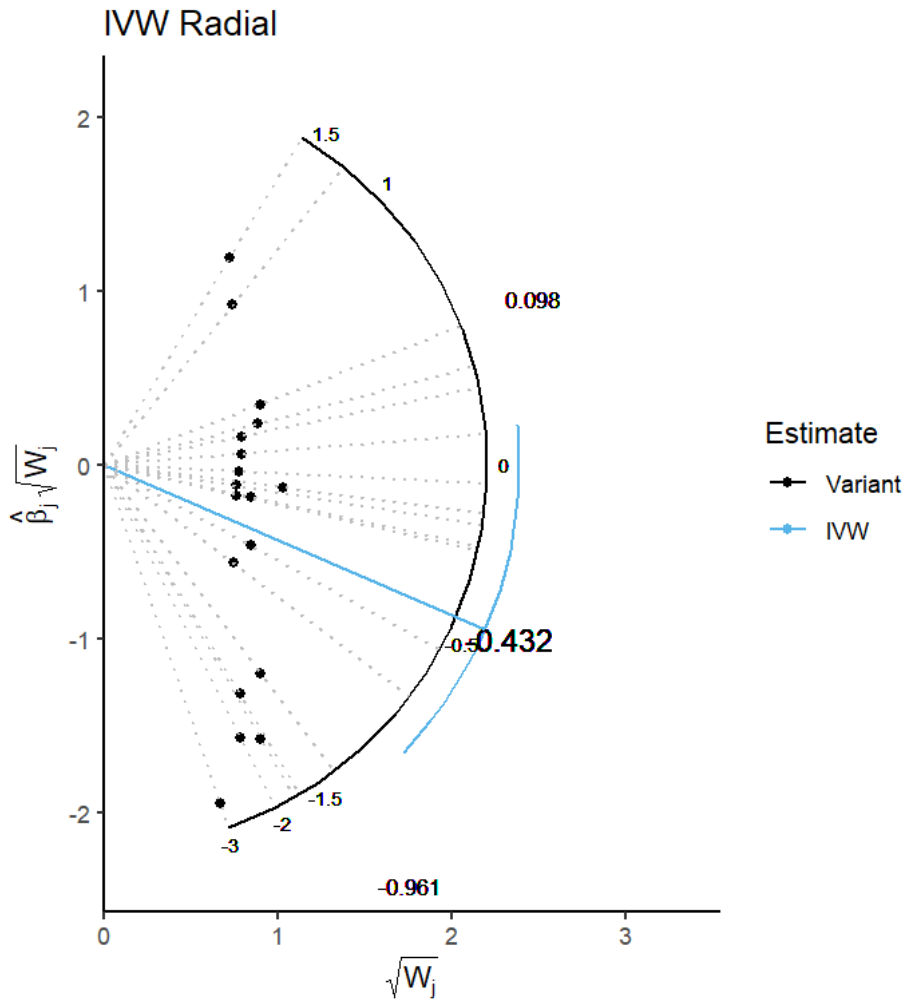
No outlier detected.

Supplementary figure 8. Radial plot, Social communication questionnaire age eight, restrictive and repetitive behaviours.



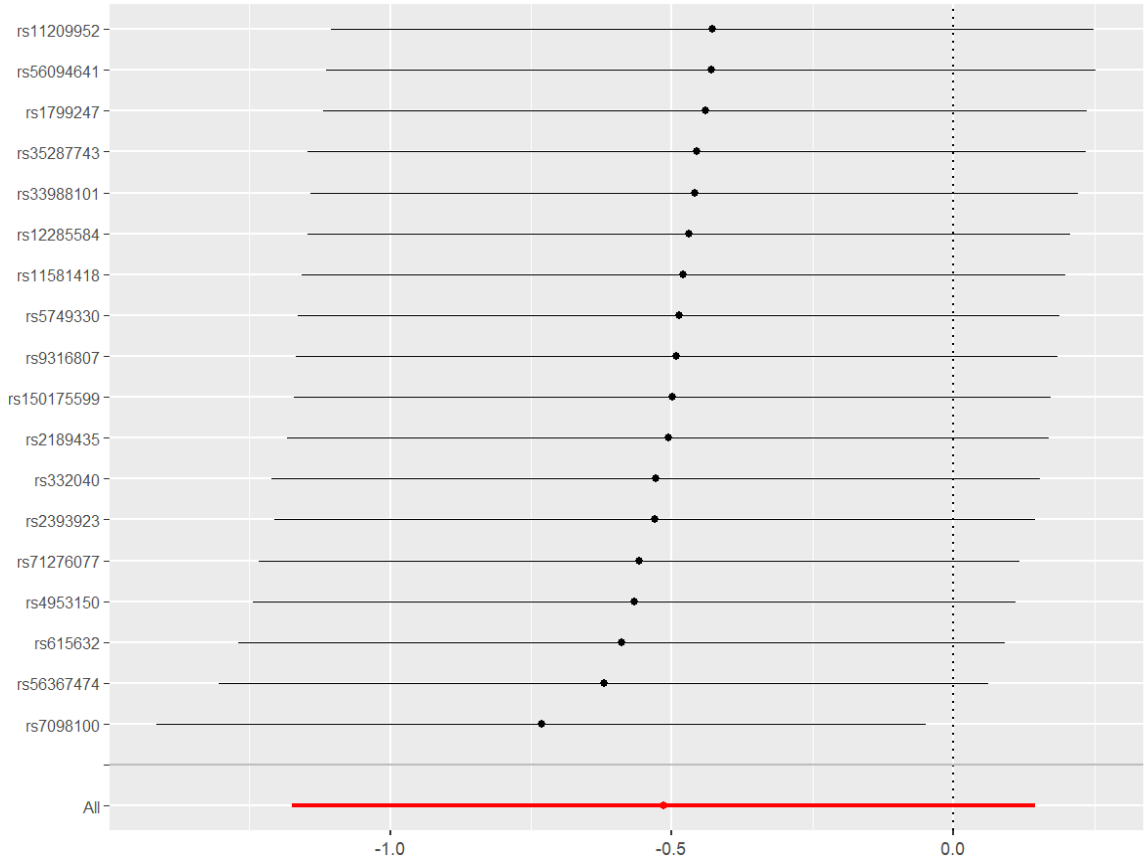
No outlier detected.

Supplementary figure 9. Radial plot Social communication questionnaire age eight, social communication.

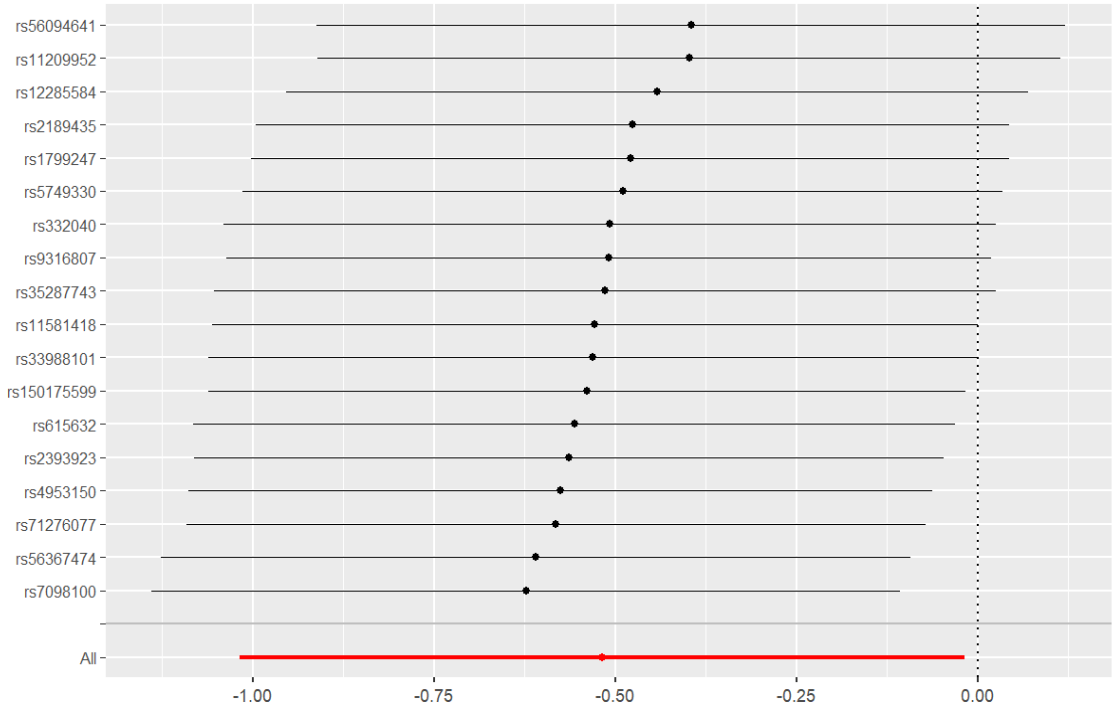


No outlier detected.

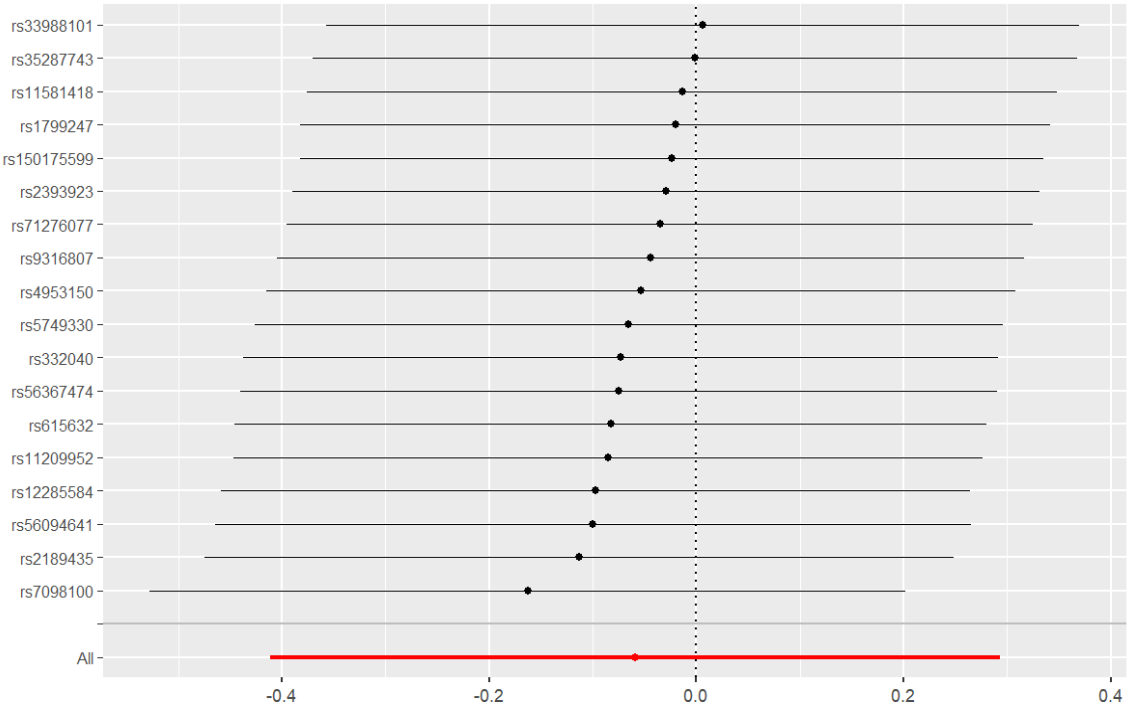
Supplementary figure 10. Leave-one-out analysis, Social communication questionnaire age three.



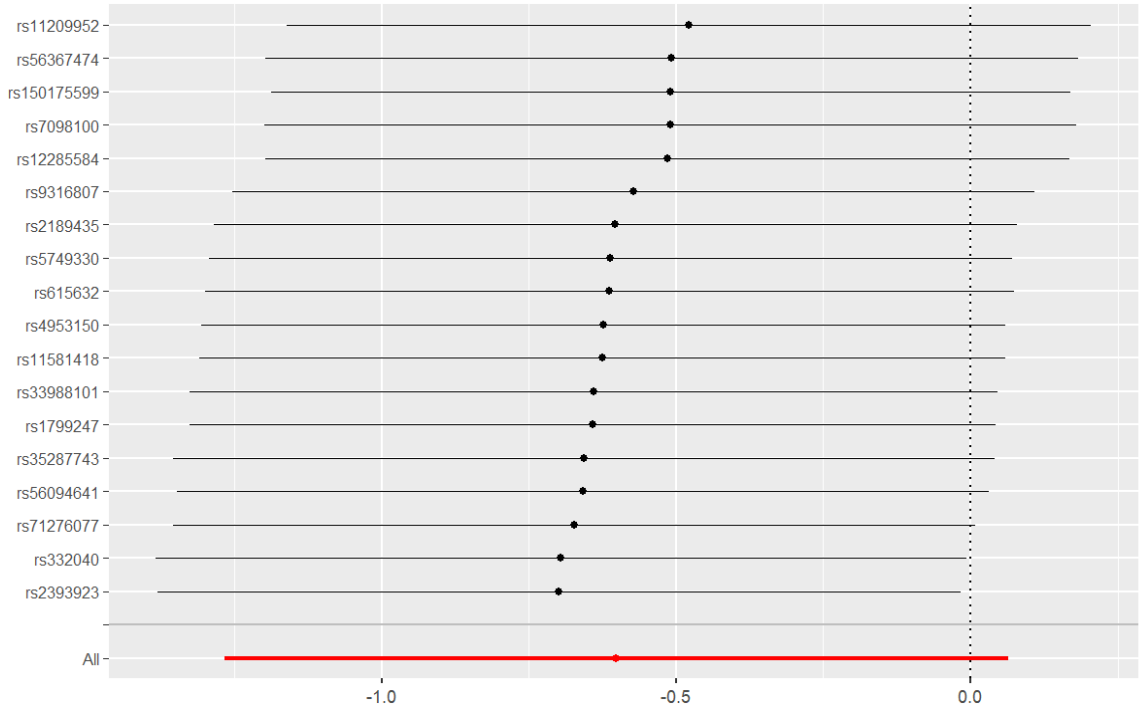
Supplementary figure 11. Leave-one-out analysis, Social communication questionnaire, restrictive and repetitive behaviours, age three.



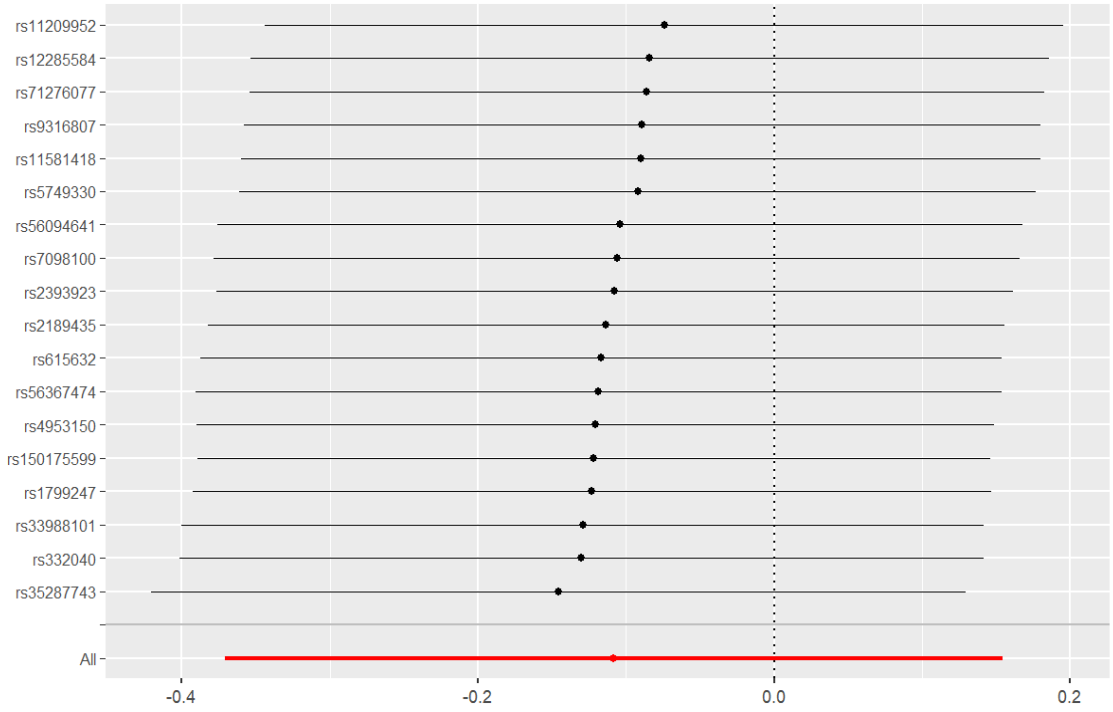
Supplementary figure 12. Leave-one-out analysis, Social communication questionnaire, social communication skills, age three.



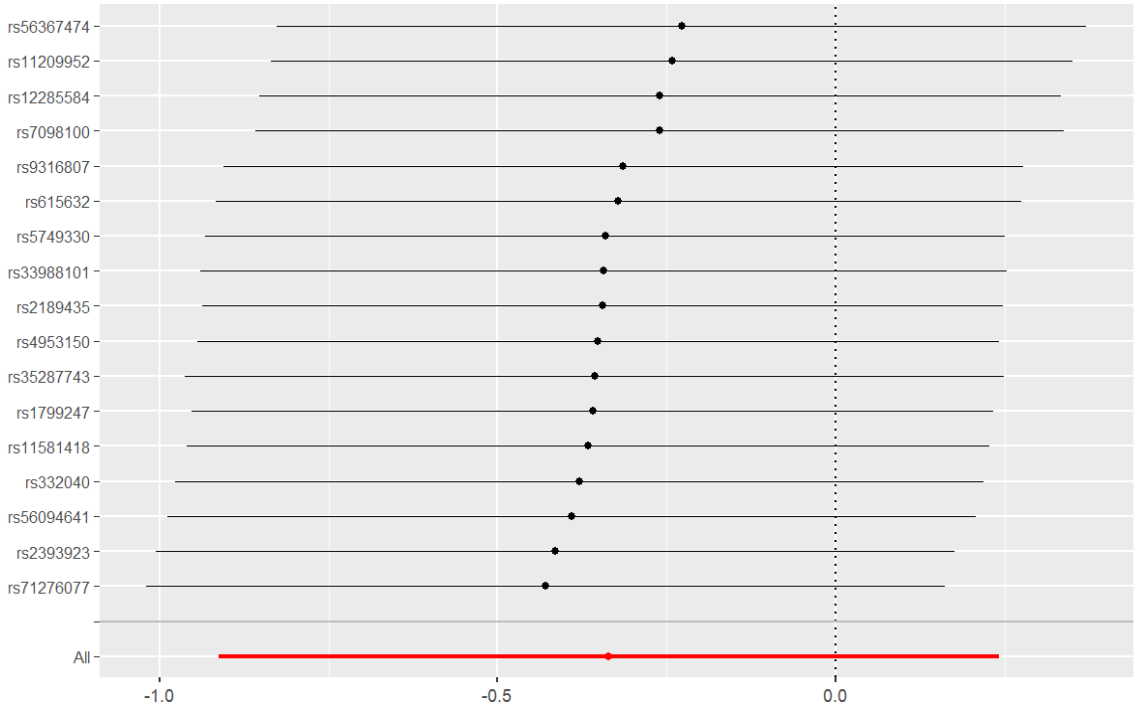
Supplementary figure 13. Leave-one-out analysis, Social communication questionnaire, age eight.



Supplementary figure 14. Leave-one-out analysis, Social communication questionnaire, restrictive and repetitive behaviours, age eight.



Supplementary figure 15. Leave-one-out analysis, Social communication questionnaire, social communication skills, age eight.



Supplementary table 15. Sample size calculation for the polygenic score-Mendelian randomisation.

Beta for the true causal effect a 'healthy' prenatal dietary pattern and SCQ	**Required sample size SCQ-3**	**Required sample size SCQ-8**
-0.10	190,129	220,551
-0.15	84,498	98,018
-0.25	30,414	35,281
-0.35	15,513	17,997

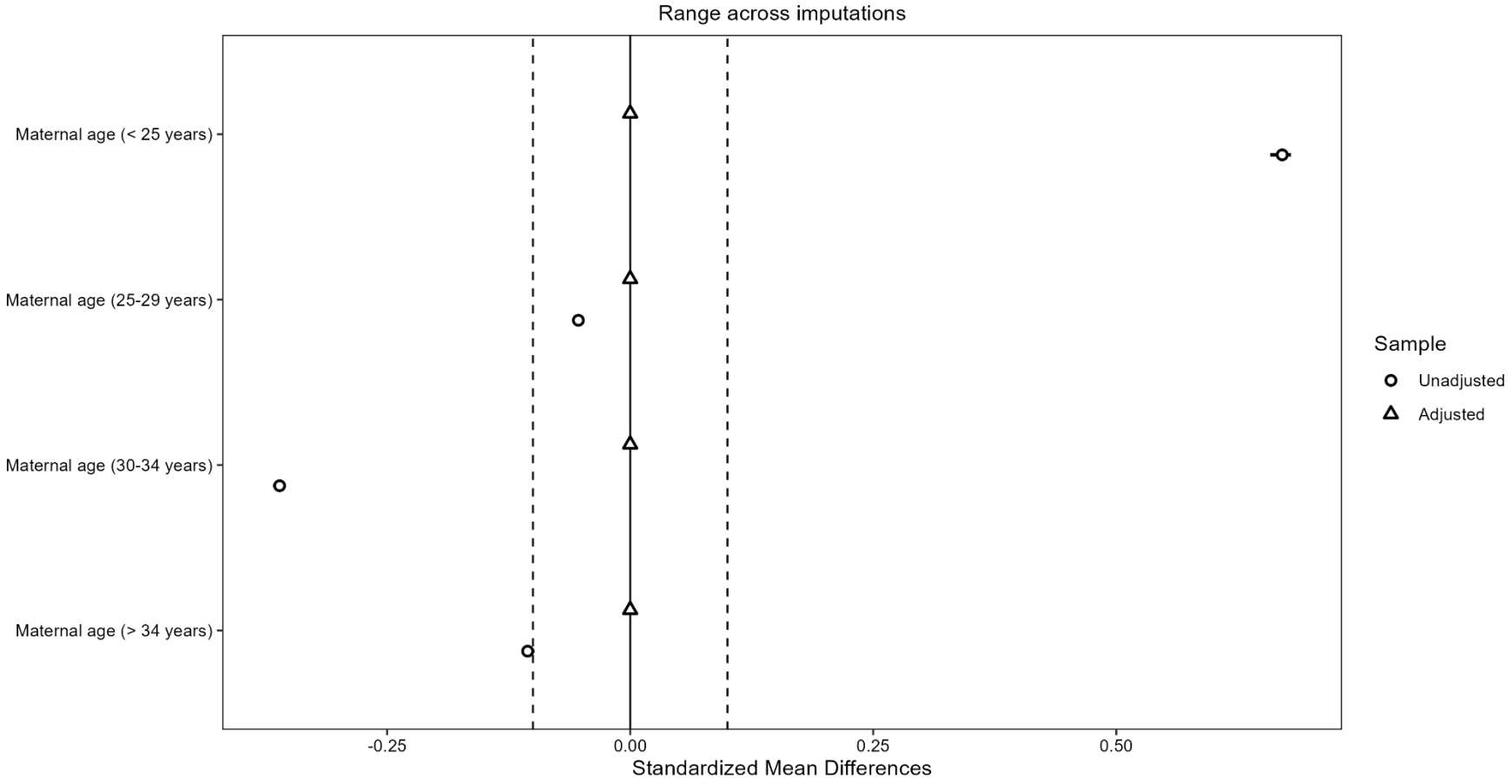
SCQ, social communication questionnaire. The number after each outcome represents the approximate age of measurement, i.e. SCQ-3 was measured at age three years.
Estimates are based on Brion et al (Brion et al., 2012). However, there is not sample size calculation specifically for intrauterine exposures and so we advise a cautious interpretation, as it may underestimate the required sample sizes.
Estimates are based on 80% power, alpha of 0.05, R^2 of 1.2%, and the standard deviation was extracted from the true values in MoBa and are SCQ-3 was 2.5, SCQ-8 was 2.9, and 'healthy' prenatal dietary pattern was 0.86. Hence, based on our sample size we had 80% power to detect an effect of -0.35.

Supplementary material for Chapter 7.

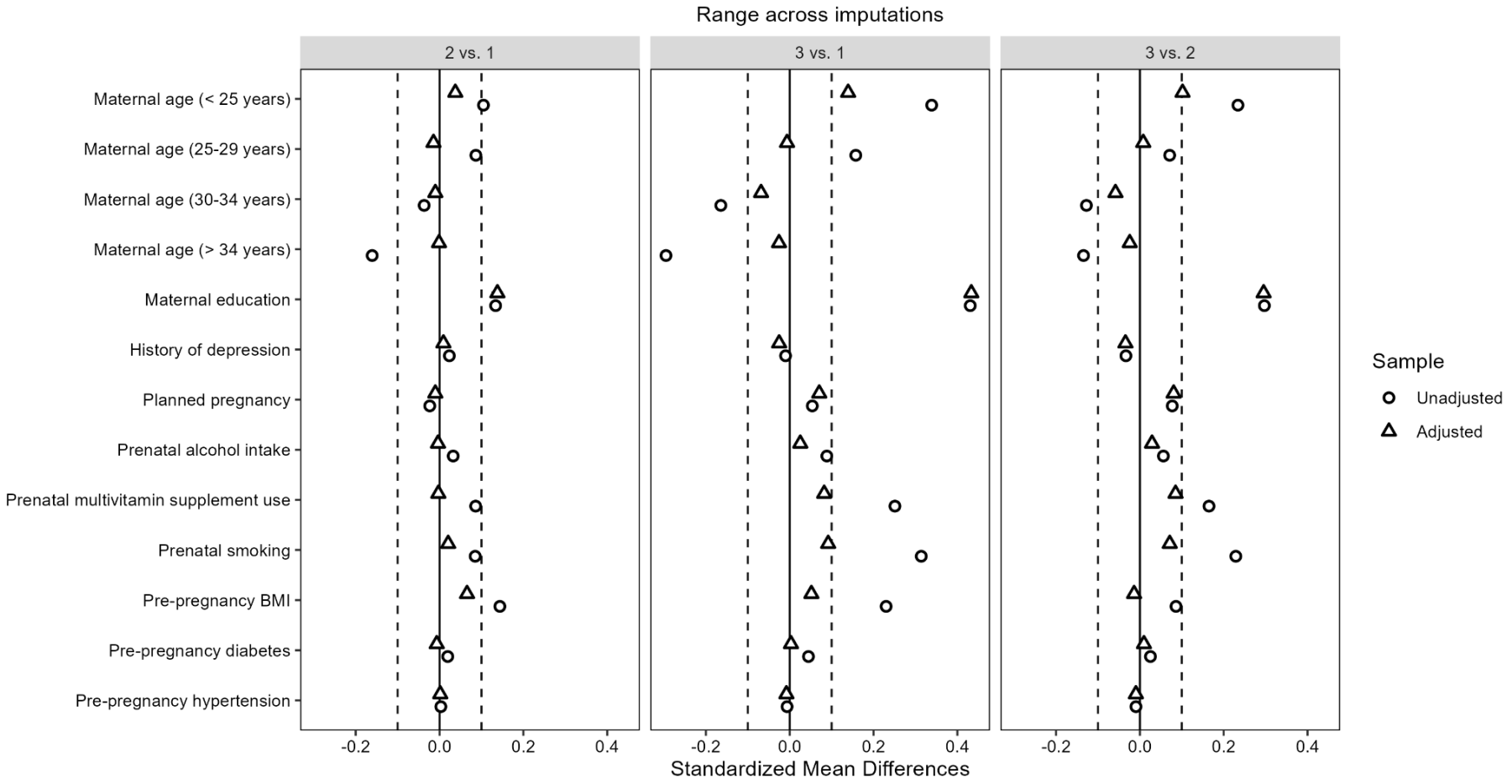
Covariate balance plots and weights

The covariate balance plots present the adjusted and unadjusted standardised mean differences for each covariate. The pre-confounder exposures are expected to balance based on exposure inverse probability weights, and the post-exposure confounders are expected to balance across the mediator inverse probability weights. Pairwise comparisons were made across each level of the exposure (high and low deprivation) and across each level of adherence to a 'healthy' prenatal dietary pattern (described in the plots as: 1 = high, 2 = medium, 3 = low). A standardised mean difference of approximately <0.1 may suggests negligible covariate imbalance (Chesnaye et al., 2021), and was generally observed in each model with a few exceptions. However, this is an arbitrary cut-off, applied as a rule of thumb (Austin, 2009) and in most instances, it only slightly exceeded 0.1, or in the case of the socioeconomic indicators they were additionally adjusted for in the CDE model. There were no extreme weights except for in the maternal income. The weights for each analysis are as follows: maternal education (MoBa), TE weights (maximum 3.0 and minimum 0.44) and average weights used in CDE (maximum 9.56 and minimum 0.23); parental income, TE weights (maximum and minimum) and average weights used in CDE (maximum and minimum); paternal income, TE weights (maximum 2.77 and minimum 0.43) and average weights used in CDE (maximum 11.47 and minimum 0.22); maternal income, TE weights (maximum 14.46 and minimum 0.48) and average weights used in CDE (maximum 56.3 and minimum 0.26); maternal education (ALSPAC), TE weights (maximum 9.45 and minimum 0.63) and average weights used in CDE (maximum 9.52 and minimum 0.31); Townsend deprivation (ALSPAC), TE weights (maximum 1.45 and minimum 0.68) and average weights used in CDE (maximum 9.76 and minimum 0.30).

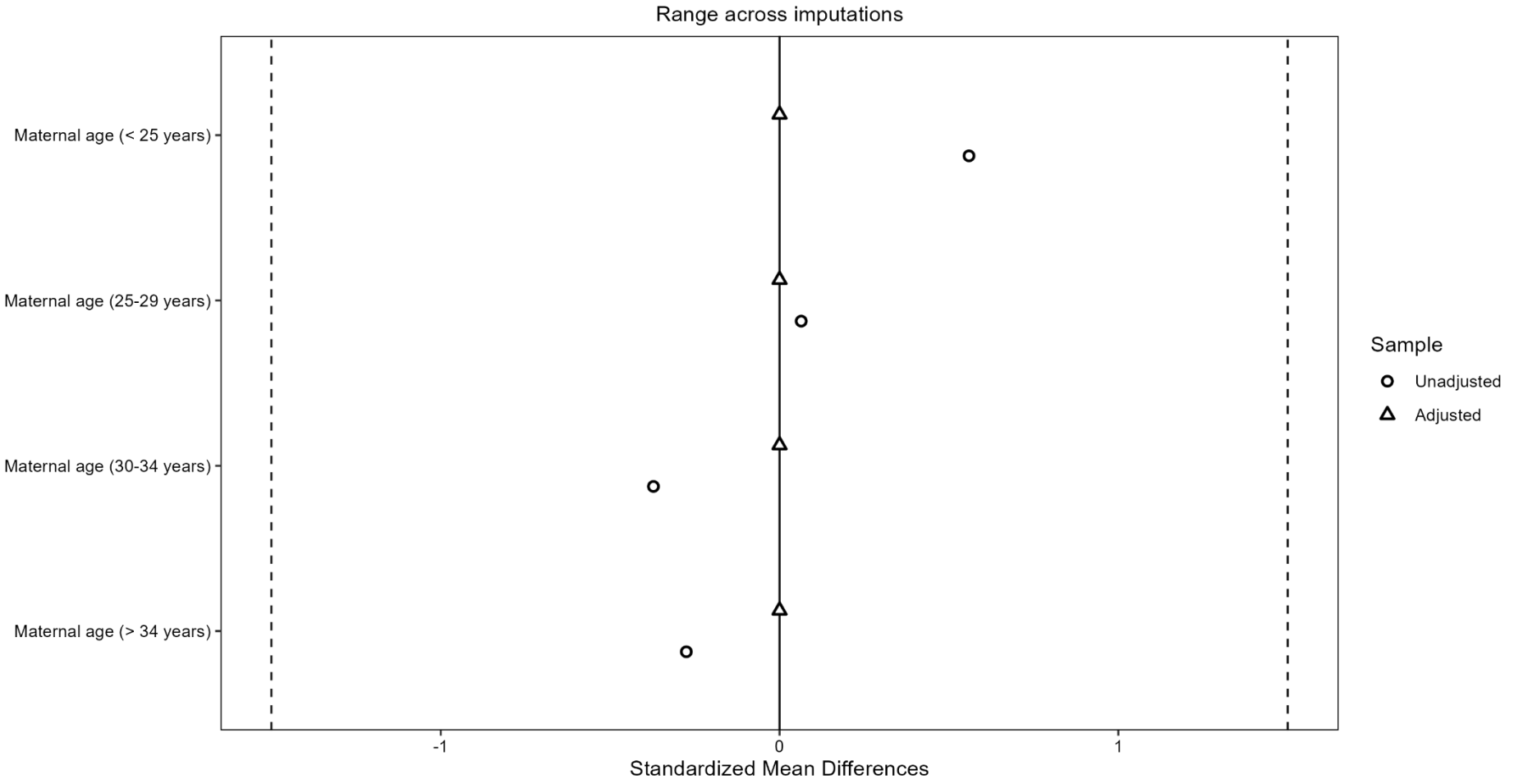
Supplementary figure 16. Covariate balance plot for maternal education covariates by exposure weights (MoBa) (Chapter 7).



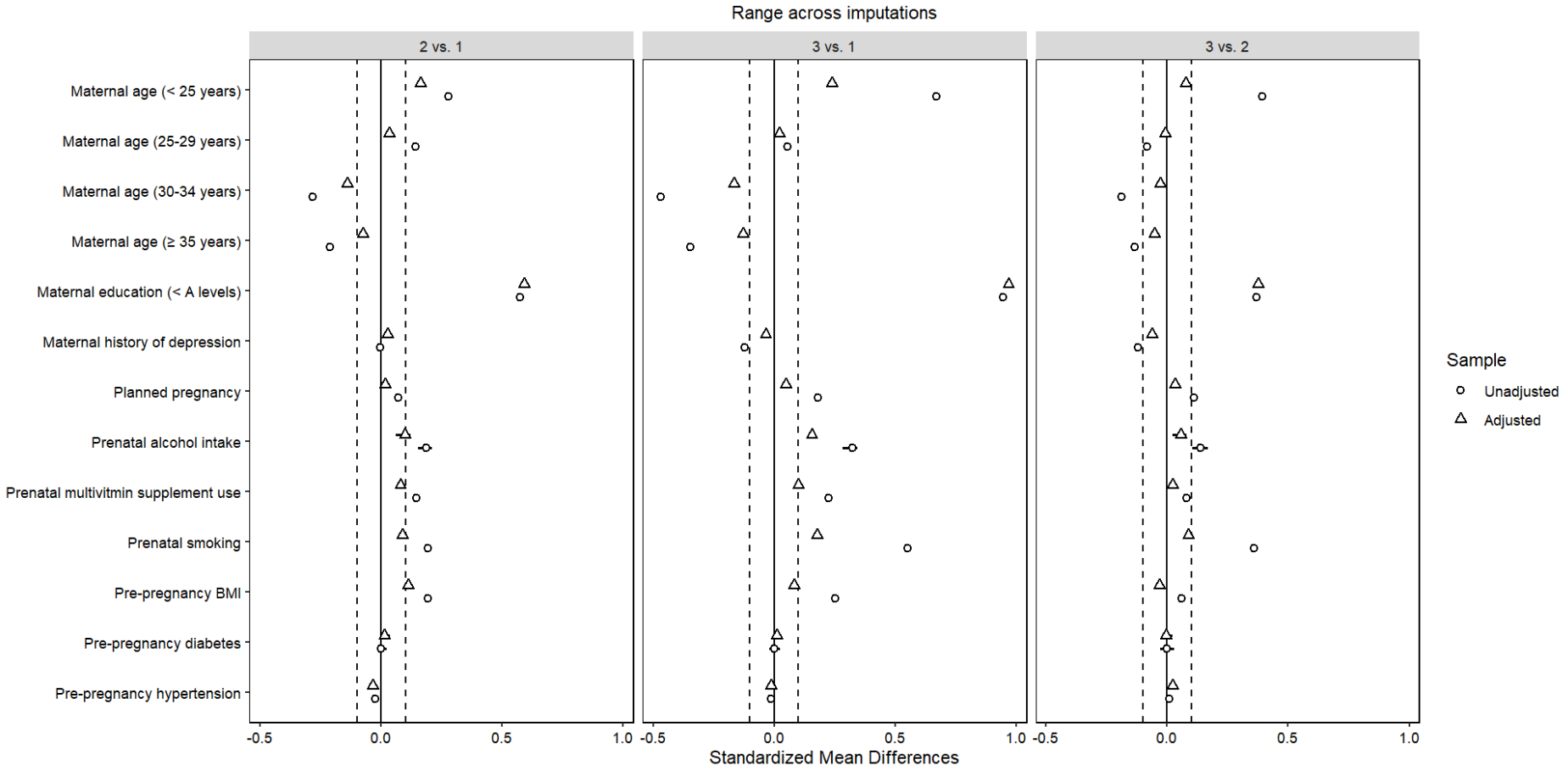
Supplementary figure 17. Covariate balance plot for maternal education covariates by mediator weights (MoBa) (Chapter 7).



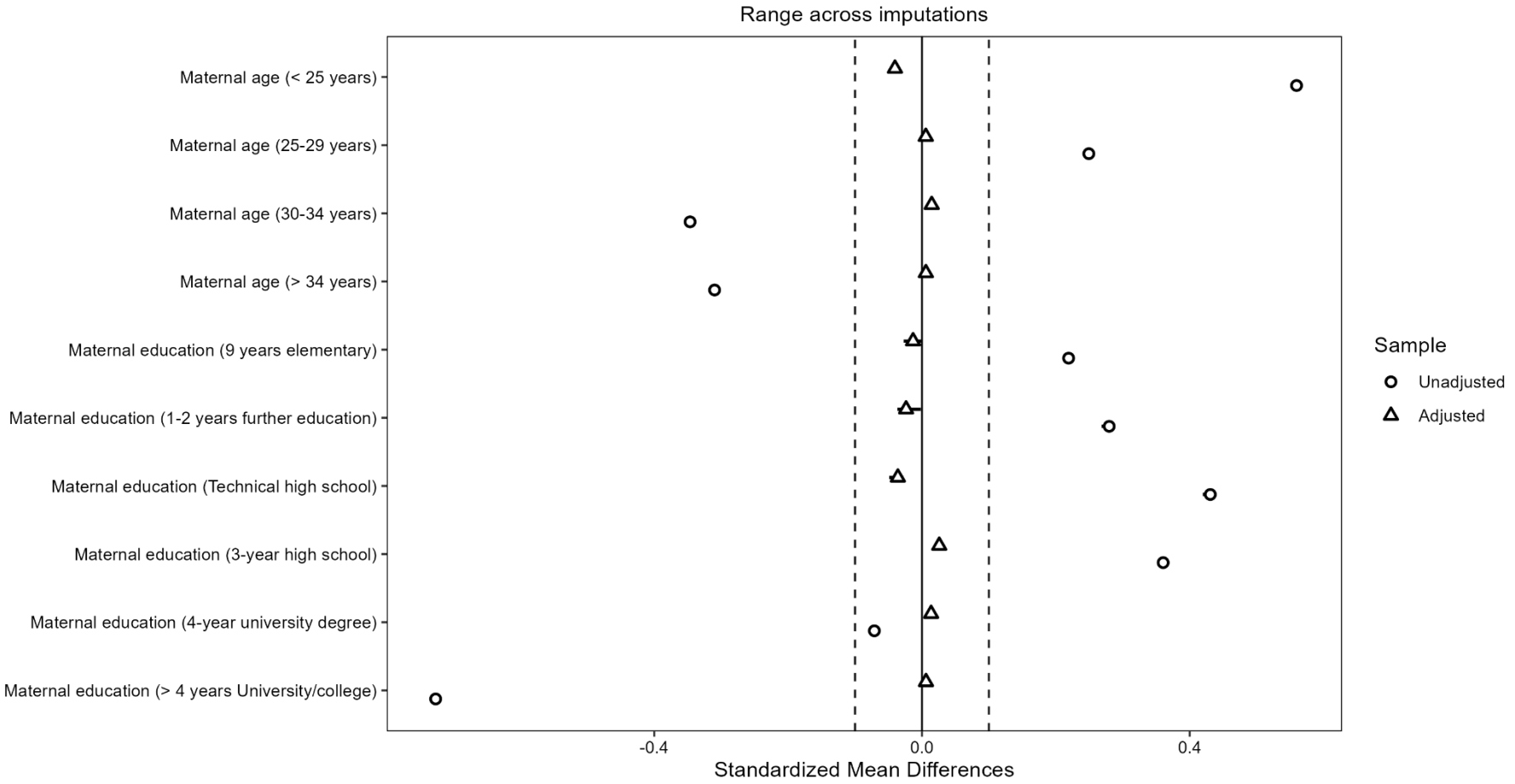
Supplementary figure 18. Covariate balance plot from maternal education covariates by exposure weights (ALSPAC) (Chapter 7).



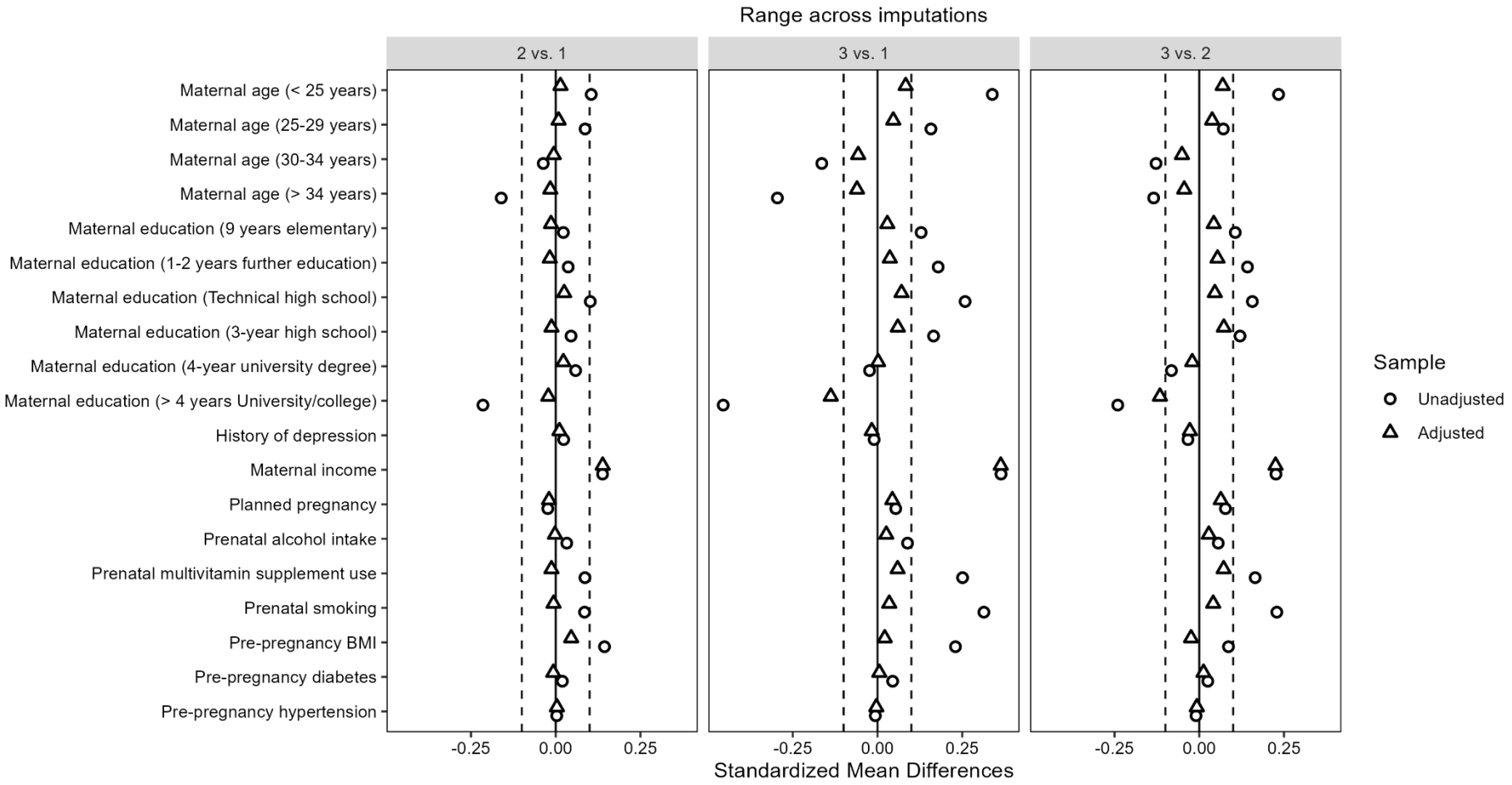
Supplementary figure 19. Covariate balance plot for maternal education covariates by mediator weights (ALSPAC) (Chapter 7).



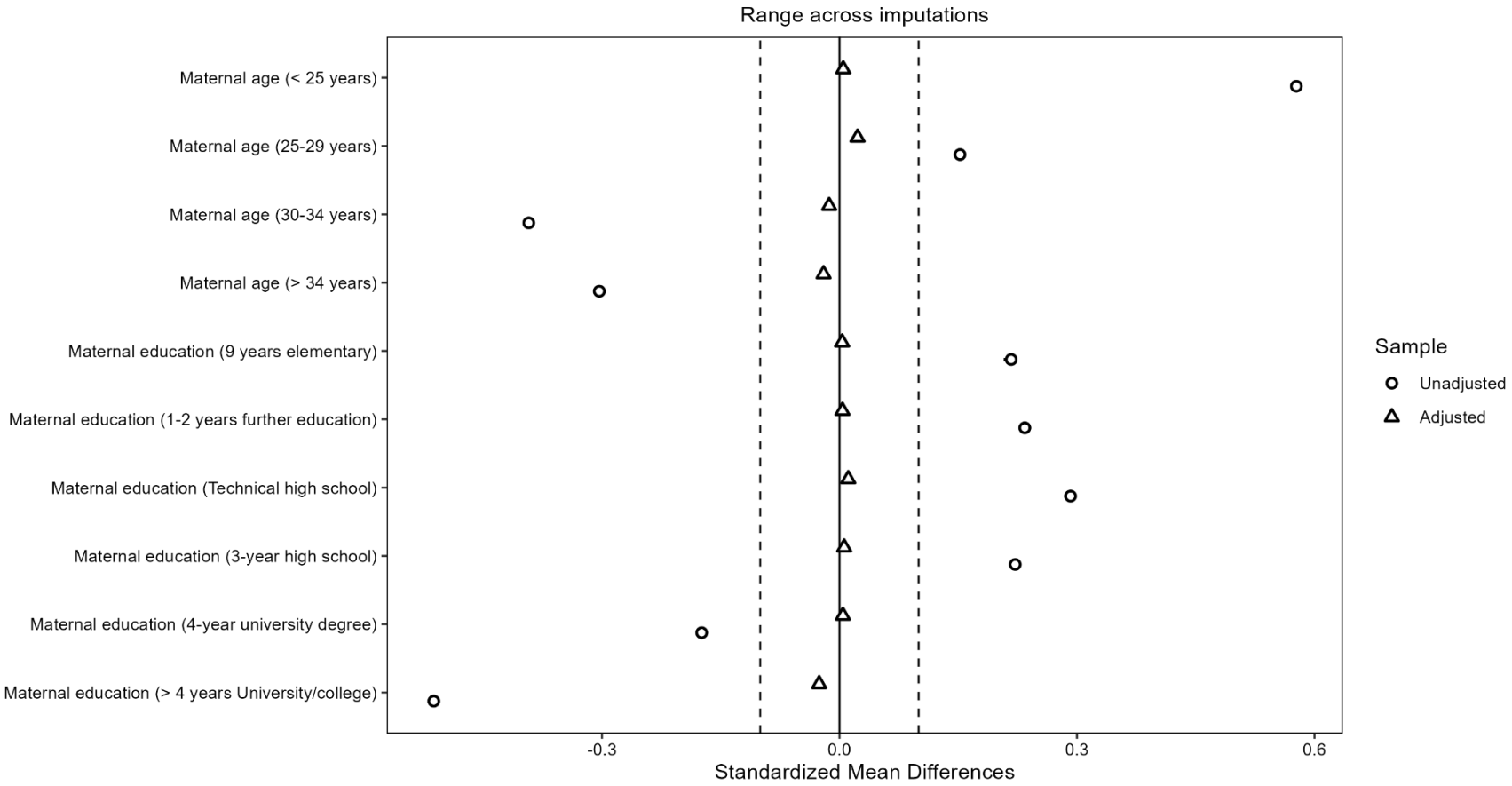
Supplementary figure 20. Covariate balance plot for maternal income covariates by exposure weights (MoBa) (Chapter 7).



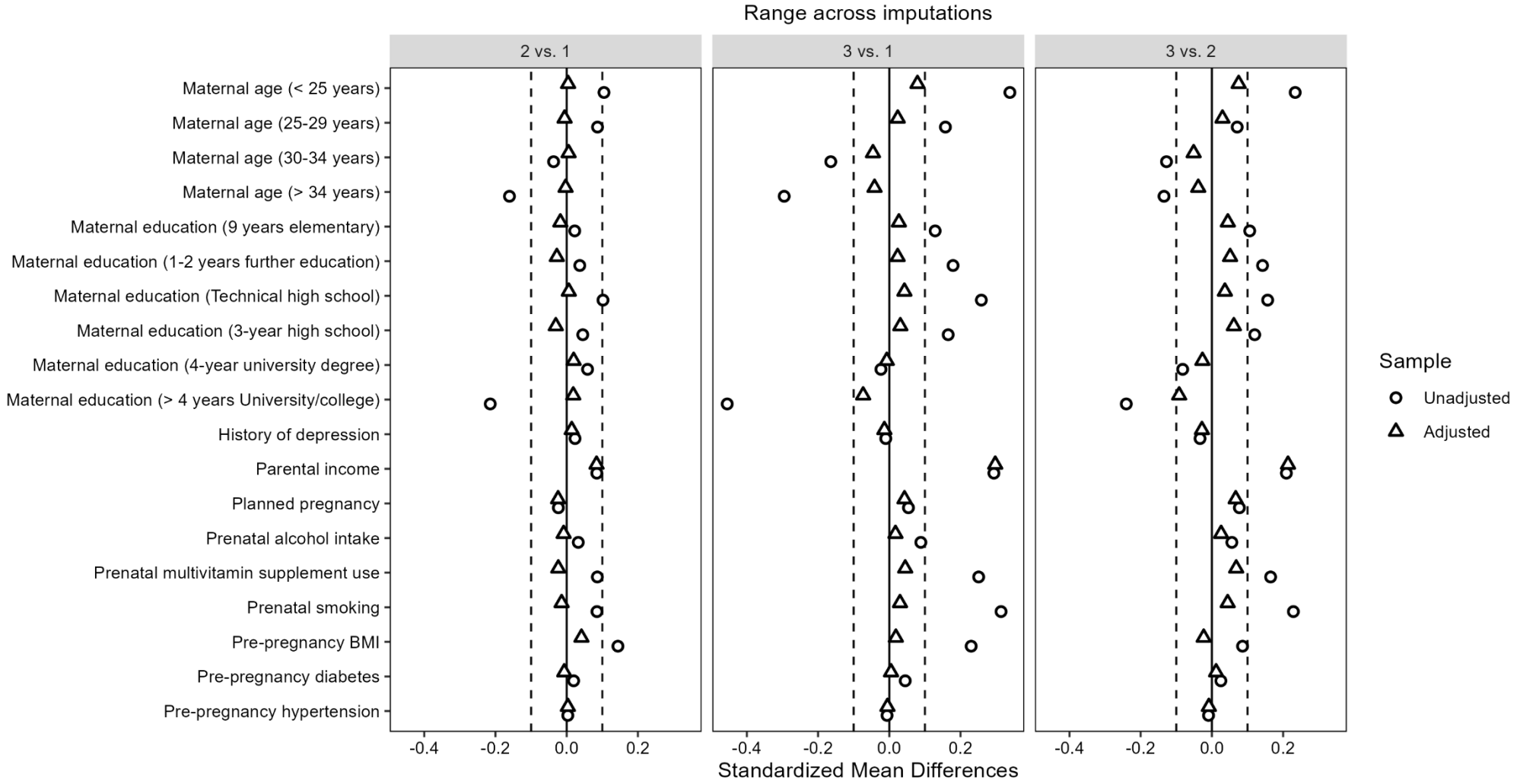
Supplementary figure 21. Covariate balance plots for maternal income covariates by mediator weights (MoBa) (Chapter 7).



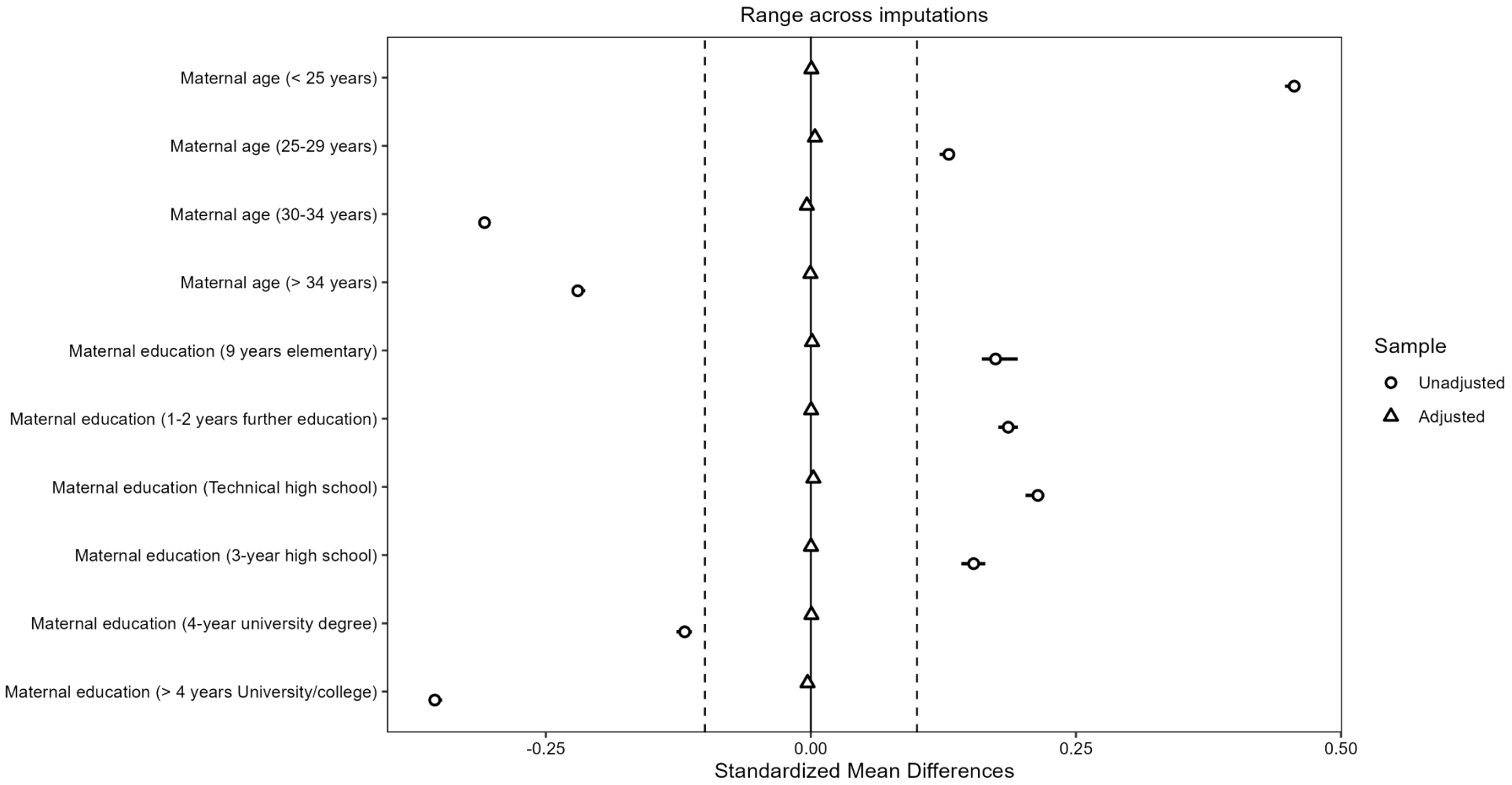
Supplementary figure 22. Covariate balance plot for parental income covariates by exposure weights (MoBa) (Chapter 7).



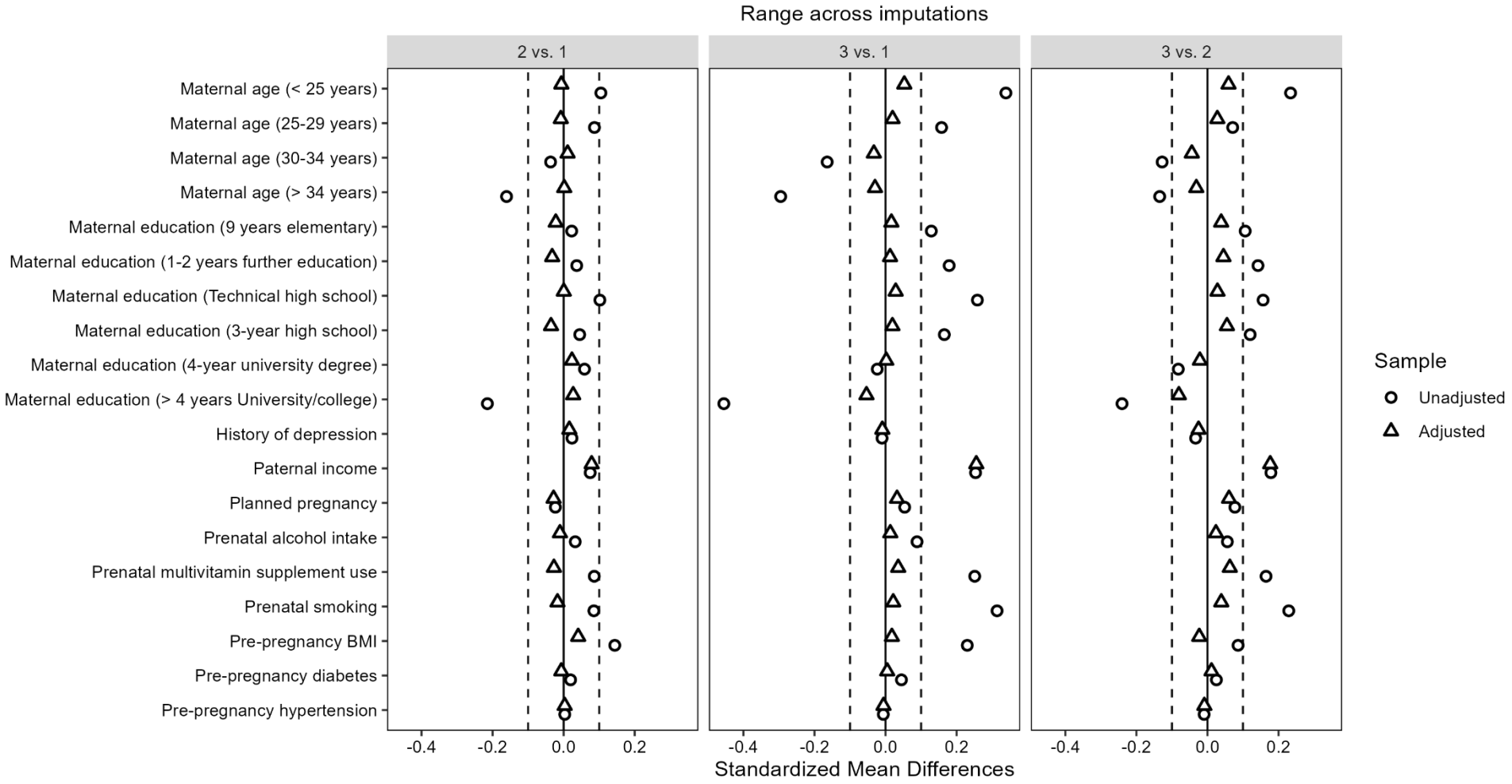
Supplementary figure 23. Covariate balance plot for parental income covariates by mediator weights (MoBa) (Chapter 7).



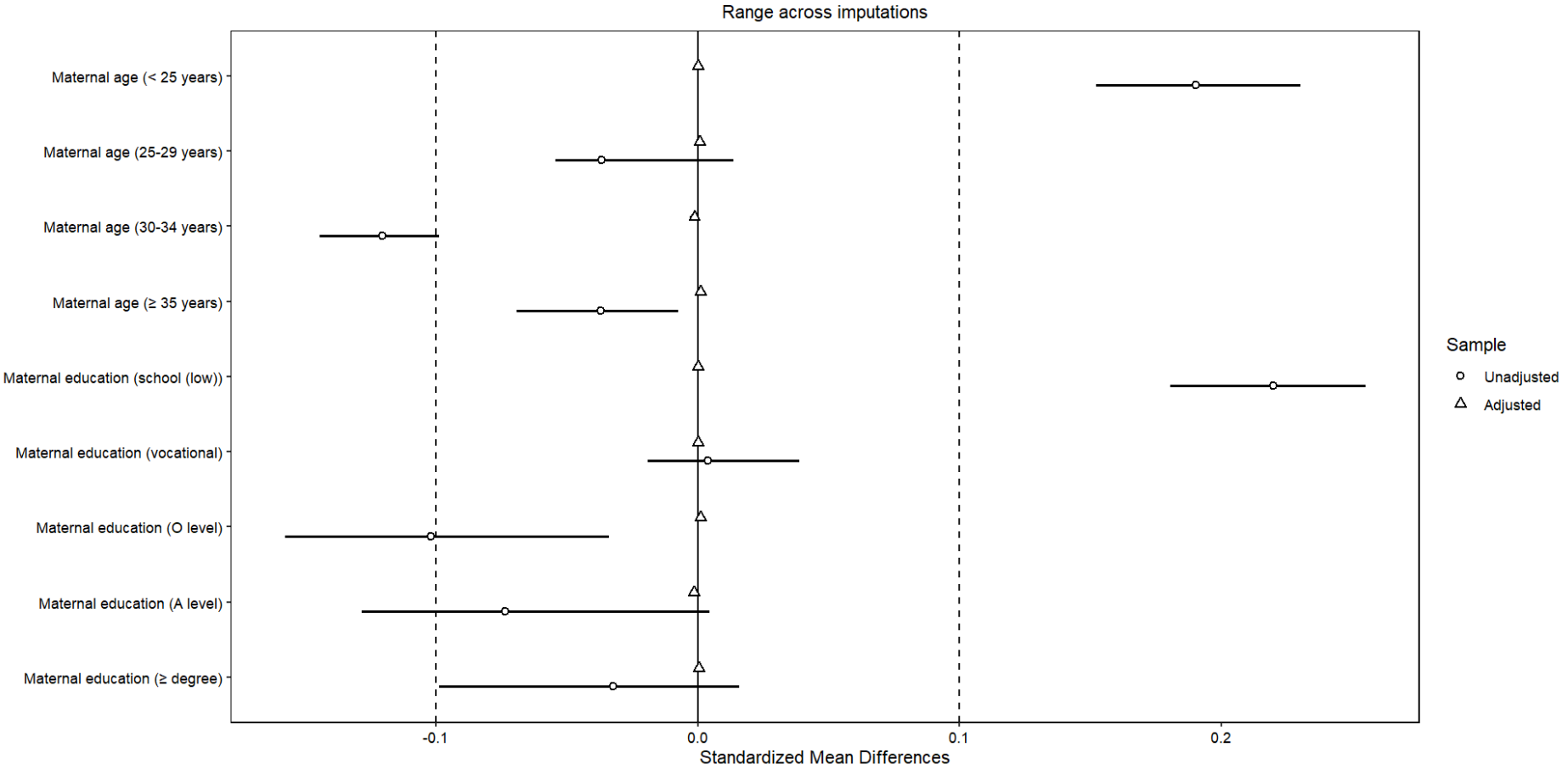
Supplementary figure 24. Covariate balance plots for paternal income covariates by exposure weights (MoBa) (Chapter 7).



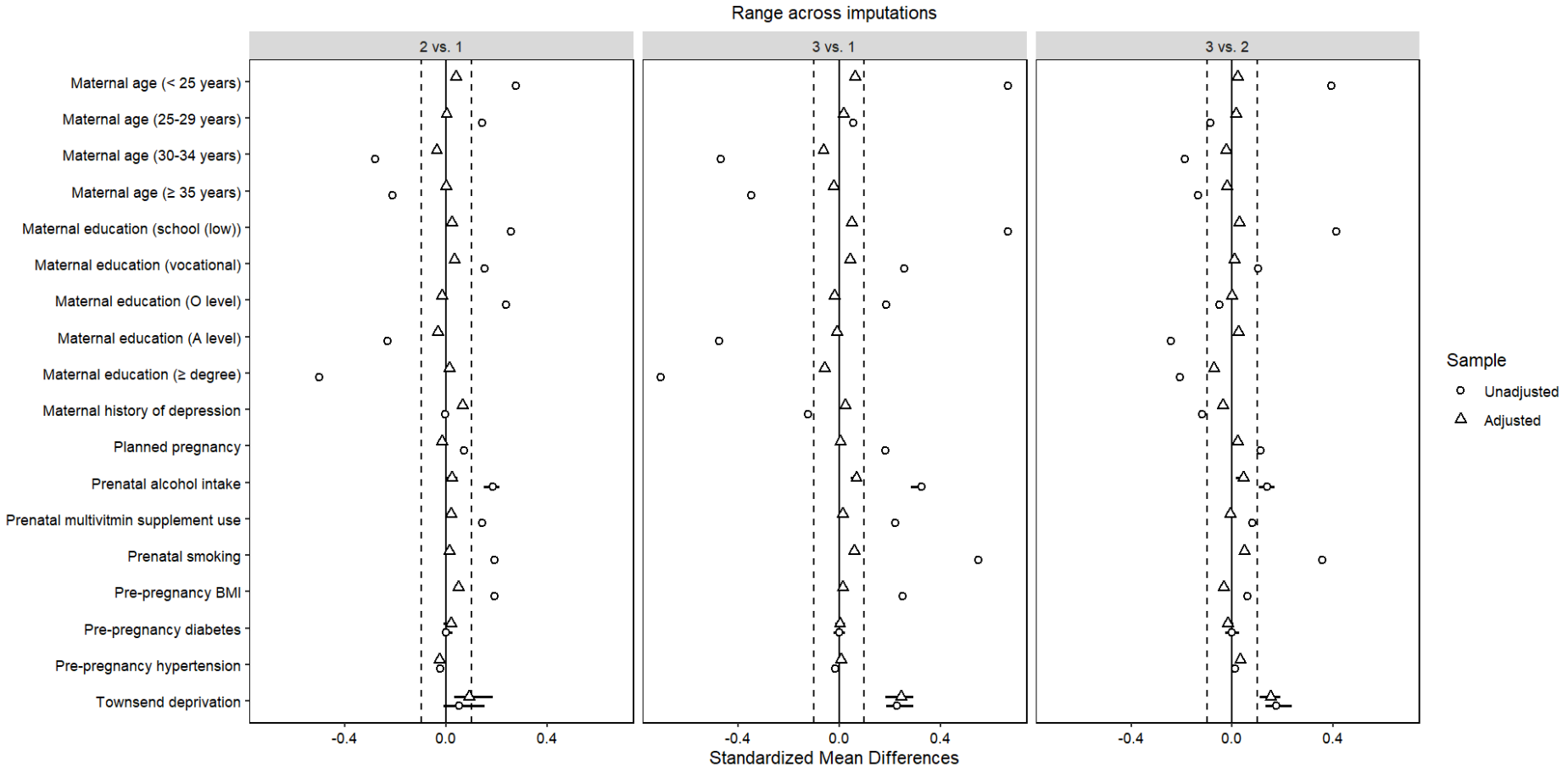
Supplementary figure 25. Covariate balance plot for paternal income covariates by mediator weights (MoBa) (Chapter 7).



Supplementary figure 26. Covariate balance plot for Townsend deprivation covariates by exposure weights (ALSPAC) (Chapter 7).



Supplementary figure 27. Covariate balance plot for Townsend deprivation covariates by mediator weights (ALSPAC) (Chapter 7).



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COVID-19 impacts

In March 2020 I was preparing to start data analysis, having completed a systematic review Jan 2020, however my access to secondary data was delayed. MoBa was due on April 2020 but delayed until Aug 2020 as the Norwegian Institute of Public Health reprioritised their workforce to COVID activities, and ALSPAC data was due April 2020 but delayed until Nov. 2020. Thus, my progress onto the data analysis was delayed.

As a part time dietitian in the NHS, I faced significant pressures due to rapid and unprecedented changes to clinical and professional guidelines, legislation, employment contracts, working practices, operational procedures, and increased staff meetings. Due to the volume of this workload, I undertook a proportion of outside of clinical hours which impacted progress on my PhD. Later, I increased my NHS hours and reduced the PhD to 50% FTE to assist with re-establishing routine care and delayed my PhD timeline.

Some skills/expertise are unavailable within my supervisory team but form a major aspect of my PhD such as Autism, Mendelian Randomisation, and the MoBa dataset. I strategically planned two academic visits to support me in these aspects of my PhD. The first to Columbia University for six weeks (in May 2020) and the Norwegian Institute of Public Health for one week (in April 2020) which were cancelled. I had been awarded the Early Careers Mobility Scheme grant to visit Columbia University which was withdrawn due to COVID. Fortunately, I was rewarded the grant in 2022 and although Columbia University were no longer participating in the scheme, I was able to visit McGill University. Overall, the disruptions due to COVID-19 destabilised my progress on the PhD and meant several opportunities to network and present at academic conferences were cancelled. I believe these opportunities would have enhanced my learning and subsequently my PhD. In particular, the opportunity to visit Ezra Susser at Columbia University and to have early in-person contact with the researchers in Norway. The greatest impact was the disruptions to my progress on the PhD.