The effect of maternal obesity on offspring neurodevelopmental outcomes

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Note

Acknowledgement

بِسْمِ اللهِ الرَّحْمٰنِ الرَّحِيْمِ

In the name of God, the Gracious, the Most Merciful.

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Finally, to my children Sunna, Jenna and Elias, you have been with me throughout this journey, I hope you see my ambition to contribute to knowledge as an inspiration to pursue your own future endeavours. You kept me grounded during this PhD. I thank you for your patience and all the love you were so generous to afford me while I watched you grow during these 4 years. I dedicate this thesis to you.

Declaration of originality

I certify the work included in this thesis is original and conducted by me. Any contribution from others within has been disclosed below.

Chapter 1,2 and 3 are written by me.

Chapter 4 and 5
All data related to the UPBEAT study and 3-year follow-up was collected by research midwives/assistants between July 2009 and October 2017 under the supervision of Dr Annette Briley and Claire Singh, and curated by the UPBEAT research team. The contribution of collaborators involved in the biochemical analyses performed is detailed in the main study (Poston et al., 2015) (https://doi.org/10.1016/S2213-8587(15)00227-2) and referenced further in the chapters.

In the work included in these chapters, Dr Sara White and Dr Angela Flynn curated and provided the biomarkers and dietary variables and provided advice. Dr Kathryn Dalrymple supported the curation of the 3-year follow-up data and provided the anthropometric measures.

In these two chapters I designed the studies under the guidance of my supervisors Prof Mary Rutherford and Prof Lucilla Poston, formulated and carried out the statistical plan. Interpretation of findings was discussed with my supervisors and co-authors as appropriate. Chapter 4 is presented as the content of the manuscript and the supplementary material submitted for publication.

Chatper 6 and Chapter 7

Collection of neonatal neuroimaging data was done through the developing Human Connectome Project and the research team at St Thomas' hospital. Pre-processing of structural and diffusion data was according to the dHCP pipeline, which is referenced in the chapters. Preparation of the diffusion data involved Dr Alexandra Bonthrone, Dr Daan Christiaens and Dr Dafnis Batalle. Otherwise, I conceptualised the studies, performed data cleaning, devised and implemented the tractography protocols, performed the FBA and statistical analysis. Dr Max Pietsch and Dr Jacques-Donald Tournier provided support with the software implementation of the tractography and of the FBA pipeline and in the interpretation of findings along with my supervisors.

No content within this thesis has been submitted to obtain any other qualification.

Julie Sif Nihouarn Sigurdardottir
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Communication of the included work

2019 Poster at the British Maternal & Fetal Medicine Society 21st Annual Conference (Edinburgh) Sigurdardóttir J., Rutherford M., Poston L., and the UPBEAT consortium. *Investigating the causal pathways to neurodevelopmental impairment in offspring exposed to maternal obesity and gestational diabetes in utero.*

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Publications completed during candidature

- Murray, L., Richards, M. P. M., & **Nihouarn-Sigurdardottir, J.** (2019). Mothering. In M. H. Bornstein (Ed.), Handbook of parenting: Being and becoming a parent (pp. 36–63). Routledge/Taylor & Francis Group. https://doi.org/10.4324/9780429433214-2
- Lautarescu, A., Pecheva, D., Nosarti, C., **Nihouarn, J.**, Zhang, H., Victor, S., Craig, M.C., Edwards, A. D. & Counsell, S. J. (2020). Maternal prenatal stress is associated with altered uncinate fasciculus microstructure in premature neonates. Biological Psychiatry, 87(6), 559-569. doi.org/10.1016/j.biopsych.2019.08.010
- Wilson, C. A., Seed, P., Flynn, A. C., Howard, L. M., Molyneaux, E., **Sigurdardottir, J.**, & Poston, L. (2020). Is There an Association Between Diet, Physical Activity and Depressive Symptoms in the Perinatal Period? An Analysis of the UPBEAT Cohort of Obese Pregnant Women. Maternal and child health journal, 24(12), 1482-1493.
- Lloyd, W. K., Morriss, J., Macdonald, B., Joanknecht, K., **Nihouarn, J.**, & Van Reekum, C. M. (2021). Longitudinal change in executive function is associated with impaired top-down frontolimbic regulation during reappraisal in older adults. NeuroImage, 225, 117488.
- **Sigurdardottir, J.**, White, S.L., Flynn, A., Rutherford, M., Poston, L. and on behalf of the UPBEAT Consortium (2022). Longitudinal phenotyping of maternal antenatal depression in obese pregnant women supports multiple-hit hypothesis for fetal brain development, a secondary analysis of the UPBEAT study. EClinicalMedicine, 50, 101512.

Public Engagement derived from the thesis

October 2021 ***In Utero*** | Science Museum (London)

A collaboration between myself and Sarah Schrimpf, Wushuang Tong, Stiliyana Minkovska, three artists from the Royal College of Art and Design, under the *Art X Science* initiative. This was instigated by the School of Biomedical Engineering and Imaging Science and the RCA in the summer of 2021 which led to our participation in the Great Exhibition Road Festival in October 2021. Our installation presented the research topic of this thesis in the form of art to the wider public at the Medicine gallery of the Science Museum in London. Through our installation we presented the cutting edge methods in perinatal imaging which are used to enquire about health in pregnancy. More importantly, we were also keen to educate on neurodevelopment and explore with our audience the wider topic of maternal physical and mental health in pregnancy and the importance of supporting new parents in this process.

COVID Impact Statement

A string of conceptual and methodological explorations forms the work contained in this thesis, relying on data collected from the UPBEAT study and the dHCP. The content of this thesis laid a strong foundation for my design of a prospective study which was specifically aimed to answer the question of the role of gestational diabetes in pregnancy and offspring brain development. This thesis should have included some of the output from **"My Baby's brain and Me"** (myBBM) which obtained ethical approval in April 2020. However, recruitment was repeatedly halted and further limited by the pandemic and there wasn't sufficient time left within the timeframe of the PhD to carry it out. Nevertheless, the protocol of myBBM is included in **Appendix B**.

Further, the pandemic disrupted the output of the PhD due to my parental responsibilities which implied shifting to a full-time caring and home-schooling schedule during the entirety of the two lockdowns. The PhD timeline was therefore delayed and the analysis of other available data, such as the 18-month follow-up from the dHCP was not possible.

Abbreviations

Abbreviation	Explanation
11-HSD-1	11-hydroxysteroid dehydrogenase Type 1
11-HSD-2	11-hydroxysteroid dehydrogenase Type 2
AAL	Anatomical Automatic Labelling
ACTH	Adrenocorticotrophic hormone
AD	Axial Diffusivity
ADHD	Attention Deficit Hyperactivity Disorder
AFD	Apparent fibre density
ANS	Autonomous Nervous System
ANTs	Advanced Normalisation Tools
BAT	Brown adipose tissue
BBB	Blood-brain-barrier
BMI	Body Mass Index
BOLD	Blood Oxygenation Level Dependent (effect)
CNS	Central nervous system
CRH	Corticotrophin-releasing hormone
CRP	C-reactive Protein
CSD	Constrained Spherical Deconvolution
dHCP	Developing Human Connectome Project
dMRI	Diffusion MRI
DTI	Diffusion Tensor Imaging
EPDS	Edinburgh Postnatal Depression Scale
FA	Fractional Anisotropy
FBA	Fixel-Based Analysis
FC	Fibre Cross-section
FD	Fibre Density
FDC	Fibre Density and Cross-section
FFA	Free fatty acid
FOD	Fibre Orientation Distribution
GA	Gestational Age
GM	Grey Matter
HARDI	High Angular Resolution Diffusion Imaging

(continued)

Abbreviation	Explanation
HbA1c	Haemoglobin A1c
HDL	High-density lipoprotein
HFD	High-fat diet
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HPA	Hypothalamic Pituitary Adrenal (axis)
IASPSG	International Association of Diabetes and Pregnancy Study Groups
IL-6	Interleukin-6
MAR	Missing at random
MD	Mean Diffusivity
MIA	Maternal Immune Activation
MRI	Magnetic Resonance Imaging
MSMT-CSD	Multi-Shell Multi-Tissue Constrained Spherical Deconvolution
MVM	Maternal vascular malperfusion
NAFLD	Non-alcoholic fatty liver disease
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit
ODF	Orientation Distribution Function
OGTT	Oral glucose tolerance test
PlGF	Placental Growth Factor
PMA	Postmenstrual Age
PNS	Peripheral Nervous System
PNV	Paraventricular nucleus
RD	Radial Diffusivity
RF	Radio Frequency
ROI	Region of Interest
SDQ	Strength and Difficulty Questionnaire
SES	Socioeconomic Status
SNR	Signal-to-Noise Ratio
SSRIs	Selective serotonin reuptake inhibitors
TE	Echo Time
TNF-alpha	Tumour necrosis factor-alpha
TR	Repetition Time
UPBEAT	UK Better Eating and Activity Trial
VF	Visceral fat
WAT	White adipose tissue
WHO	World Health Organisation
WM	White Matter

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Abstract

Background and aims

The incidence of obesity in pregnancy is growing worldwide and has become a large concern to public health due to the higher risks of adverse outcomes for both the woman and her offspring. The causal pathways associating maternal obesity in pregnancy and higher risks of neurodevelopmental disorders in the child and the roles of maternal depression and gestational diabetes (GDM) in these associations are not well understood. Further, these children are also at higher risk of metabolic disorders and obesity. The etiology of these disorders may have a uterine origin through the disruption of neurodevelopment, however which brain networks are implicated remains to be clarified and these have not been explored in the neonatal brain where postnatal effects are reduced. The availability of multidimensional datasets which have longitudinal measures of health through pregnancy and the advances of neuroimaging in the neonate offer unprecedented opportunities to study the associations above. When several aspects of health and antenatal exposures are better understood, the potential for early detection of vulnerabilities improves and the strategies for intervention become better targeted.

This thesis addresses multiple concepts in the relationship between maternal obesity and child health outcomes and employs various methods within the structural equation modeling toolbox which support the conceptual framework of the Developmental Origins of Health and Disease (DOHaD). It contains several aims:

Aim 1) Explore the concomitant exposures potentially adverse to normal neurodevelopment in relation to maternal antenatal depressive symptoms in pregnancies with obesity.
Aim 2) Estimate the risks of psychopathology in 3-year old children born of obese mothers and the relationship between their psychological phenotypes and adiposity.
Aim 3) Formulate causal models for the relationships between antenatal exposures and risks of psychopathology in the offspring of obese mothers.
Aim 4) Identify the potential neurological underpinning for adverse child psychological and metabolic outcomes by studying the neonatal brain using MRI.

Methods

The thesis starts by exploring and reviewing the multiple facets of the obese pregnancy through physiology and maternal mental health (Chapter 1). Then, the potential pathways to how these facets may impact on neurodevelopment are described in Chapter 2, along with an epidemiological framework. Chapter 3 introduces the methodology applied in the subsequent chapters/studies which answer the aims of the thesis.

The study in chapter 4 involves data collected from the UPBEAT study of 1554 pregnant women with obesity. Latent class growth modeling of antenatal depressive symptom was performed based on scores from the Edinburgh Postnatal Depression Scale (EPDS). Classes were then compared (3-step approach) on maternal diet, blood biomarkers, infection rate, pregnancy and birth outcomes (Aim 1).

In chapter 5, latent class analysis based on the the Strength and Difficulty Questionnaire (SDQ) subscales was used to assess the psychological outcomes in a sample of 462 3-year old offspring of women from UPBEAT. Psychological phenotypes were compared on anthropometric measurements (Aim 2). The third aim was addressed by employing structural equation models (SEMs) using the Bayesian statistical framework to characterize the likely contribution of maternal antenatal GDM/glycaemic status, adiposity, inflammation and depression towards psychological outcomes in the children of UPBEAT participants.

Chapter 6 and 7 fulfill the fourth aim and relied on structural and diffusion MRI data acquired through the developing Human Connectome Project (dHCP). First, it involved segmenting the neonatal hypothalamus, nucleus accumbens and ventral tegmental area (Chapter 6). Then, tractography protocols for white-matter bundles were developed to generate 7 tracts implicated in energy homeostasis, the reward and limbic systems, in neonates of 37 to 44 weeks postmenstrual age. This was followed by a fixel-based analysis and SEMs to understand the development of tract-wise macro- and microstructure after normal-weight pregnancy. Subsequently, neonates born of normal-weight mothers (n=137) were compared to obesity-exposed newborns (n=28).

Results

In Chapter 4, results suggest there were four latent depressive symptom trajectories present in the UPBEAT cohort. Compared to women with very low risk of depression, the women with the highest longitudinal depressive profile had worse diet, higher rates of infection, higher inflammation (interleukin-6 and glycoprotein acteyls), lower placental growth factor, and 3 times the risk of preterm birth. There were further heterogeneities found across the other three classes on these measures. Infection rate was the lowest in the Not depressed class. There was no difference in the incidence of preeclampsia or GDM diagnoses between the classes but the Depressed class was more likely to have missed their OGTT and had higher glucose levels in the last trimester. There were socio-economic disparities and ethnic differences between the classes.

In Chapter 5, 11.3% (52/462) of the children in UPBEAT were at risk of psychopathology based on the total SDQ score. The LCA retrieved 3 classes: internalising phenotype (n=19,4.1%), externalising phenotype (n=37, 8%) and a well-adjusted phenotype (n=406, 87.9%). Compared to the well-adjusted children, those presenting the externalising profile were of higher BMI-for-age and heavier-for age (by World Health Organisation standards) and with larger waist circumferences. SEMs based statistics provided evidence that maternal antenatal depression is a significant predictor for risk of psychopathology at 3 years which was independent of GDM, maternal adiposity and postnatal depressive symptoms.

Chapter 6 demonstrated that the quality of the reconstructed structural data from the dHCP enabled the neonatal hypothalamus, nucleus accumbens and ventral tegmental areas to be segmented following established protocols in the adult literature.

Chapter 7 presents, to my knowledge, the first time in the human neonate, tractography of the stria terminalis, medial forebrain bundle, dorsal longitudinal fasciculus, amygdalo-accumbens fasciculus and ventral amygdalofugal pathway. Results indicate that there is heterogeneity in the development of these white-

matter tracts. Fiber density increases across time in the short period of 37 to 44 weeks postmenstrual age but not equally across the bundles. Furthermore, male neonate show larger cross-section than female in the bundles associated with the temporal pole and amygdala. There was an interaction of exposure group with PMA on the fibre density in the bilateral amygdaloaccumbens fasciculus and right uncinate fasciculus so that obesity-exposed neonate did not show the same increase in fibre density with increasing age as the controls.

Conclusion

In conclusion, the study of associations between maternal obesity and child health outcomes need to take a holistic approach which encompasses the multiple exposures potentially adverse to the fetus *in utero*. Although a genetic contribution was beyond the scope of this thesis, the role of maternal mental health, socio-economic disparities and medical determinants are likely to be significant determinants in explaining health outcomes and some aspects in the transgenerational transfer of disease risk. The prioritization of pre-conceptual health advocated through the DOHaD framework should promote strong policies addressing not only metabolism but also mental health and social disparities into any future initiatives.

Chapter 1

The pregnancy with obesity: the physiological and psychological facets

1.1 Introduction

The adoption of a more sedentary lifestyle, high-calorie food consumption and urbanization across the globe has resulted in a dramatic increase in the prevalence of overweight and obesity and their associated co-morbidities. A substantial corpus of evidence now promotes the necessity of a continuous monitoring of both population trends towards obesity and of the presence of a cumulative impact pre-pregnancy obesity presents for the health care of women and the subsequent generations. This emerges from the mounting evidence for a uterine origin for obesity, metabolic and cardiovascular disorders and more recently the observation that the incidence of neurodevelopmental disorders, which have likewise been associated with antenatal exposure to obesity, are increasing in parallel (Rivera et al., 2015; Li et al., 2016a; Catalano and Shankar, 2017). In light of these trends and a potential for intergenerational transfer of disease risks, the field of lifecourse epidemiology largely advocates the period of pre-conception as the window of opportunity for intervention on this effect of *fetal programming* to advance the agenda of improving population health worldwide (Stephenson et al., 2018).

Obesity is defined by the World Health Organisation (WHO) as a value of Body Mass Index (BMI) equal or above 30kg/m^2, with overweight defined as 25 to 29.9kg/m^2, normal-weight between 18.5 to 24.9kg/m^2 and less than 18.5kg/m^2 as underweight. The estimated rates of obesity among females in the world has steadily increased in the last two decades reaching over 35% in some countries. In the period 2005-2014, the number of overweight or obese *pregnant* women has shown a small increase for countries with high population rates such as the United States (3.8%) or Mexico (5.3%), but elsewhere such as Nigeria it increased by 55.4% and in Tanzania by 59.3%. To illustrate this trend, Figure 1.1 represents female obesity rates for the top 20 countries with the highest burden of obesity in pregnancy, collected from WHO, the World Bank and the Food and Agricultural Organization (Chen et al., 2018).

This phenomenon has not escaped England where it is estimated that 30% of women are obese and the distribution across those aged 16 to 44 years considered of child-bearing age ranges from 20% to 28%. If

considering overweight *and* obesity, this proportion reaches 60%, illustrated in Figure 1.2 for the year of 2017 (National Health Service Digital, 2017) and these values are comparable in 2021 (National Health Service Digital, 2021). In comparison to normal-weight pregnancies, obese pregnancies present significantly higher risks of adverse perinatal and long-term outcomes for both the women and their children (Ma et al., 2016), summarised in Table 1.1. Public health bodies are concerned that the global steady rise in rates of obesity will inadvertently further promote such burden (NCD Risk Factor Collaboration, 2016; Stephenson et al., 2018) and inadvertently its financial ramifications. The mean costs to the UK National Health Service (NHS) associated with caring for overweight and obese women during their pregnancies and 2 months post-partum can amount to 23% (£4244) and 37% (£4718) more, respectively, than their normal-weight peers (£3546) (Morgan et al., 2014). However, the impact of obesity rates being compounded across generations should not only to be measured along economical projections or cost-effectiveness of necessary interventions (Jacklin et al., 2017) but also through the societal implications for individuals meeting barriers to reaching their own potential as they experience lower quality of life and higher mortality.

Table 1.1: *Maternal and offspring outcomes associated with obesity in pregnancy.*

Maternal adverse outcomes		**Offspring adverse outcomes**	
Short-term	Long-term	Short-term	Long-term
Gestational Diabetes Mellitus (GDM)	Type-2 Diabetes	Preterm birth (<37 weeks)	Obesity
Preeclampsia	Pregnancy Weight Retention	Macrosomia (>4kg birthweight)	Type-2 diabetes
Miscarriage		Congenital malformation	Cardiovascular disease
Infection (urinary, genital, wound)		Hypoglycemia	Non-alcoholic fatty liver disease
Stillbirth		NICU admission	Autism
Postpartum hemorrhage			Attention-deficit Hyperactivity Disorder
Induction of labour			
Excessive Gestational Weight Gain (GWG)			
C-section			

Note:
Outcomes are compared against women of normal pre-pregnancy weight (BMI 20-25) from the following sources: Alberico et al. (2014), Persson et al. (2017), Callaway et al. (2006), Cremona et al. (2020), Sebire et al. (2001), Ornoy et al. (2016), Krakowiak et al. (2012a), Li et al. (2016c), Lahti-Pulkkinen et al. (2019). NICU: Neonatal Intensive Care Unit.

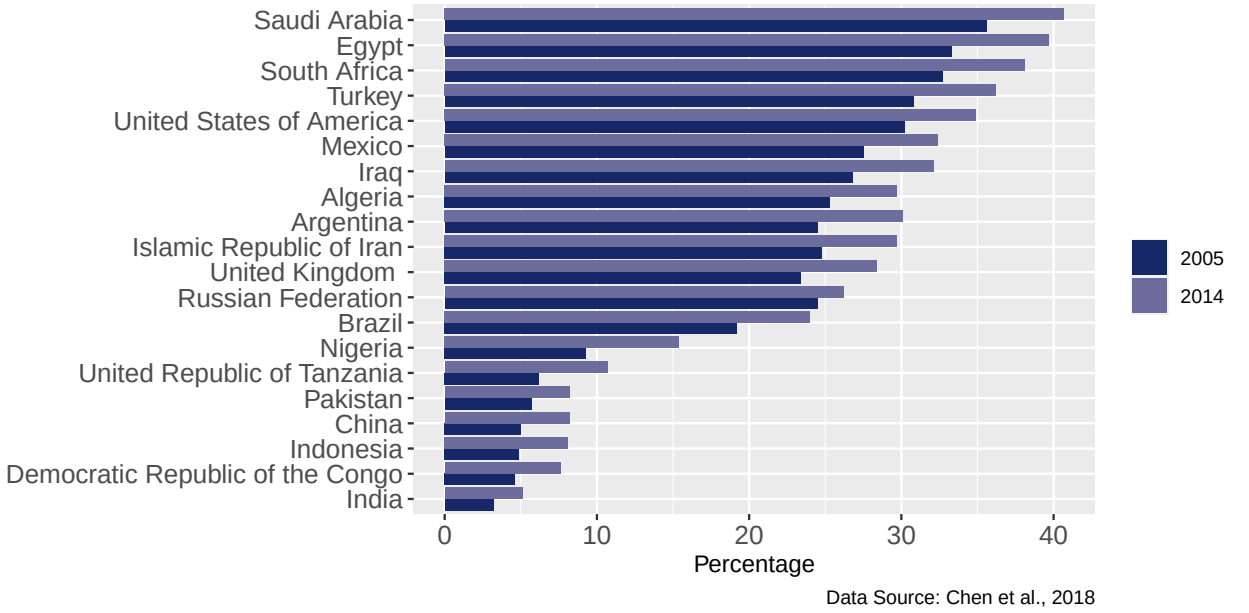

Figure 1.1: *Rates of female obesity across top 20 countries with the highest burden of overweight and obesity among pregnant women in the world.*

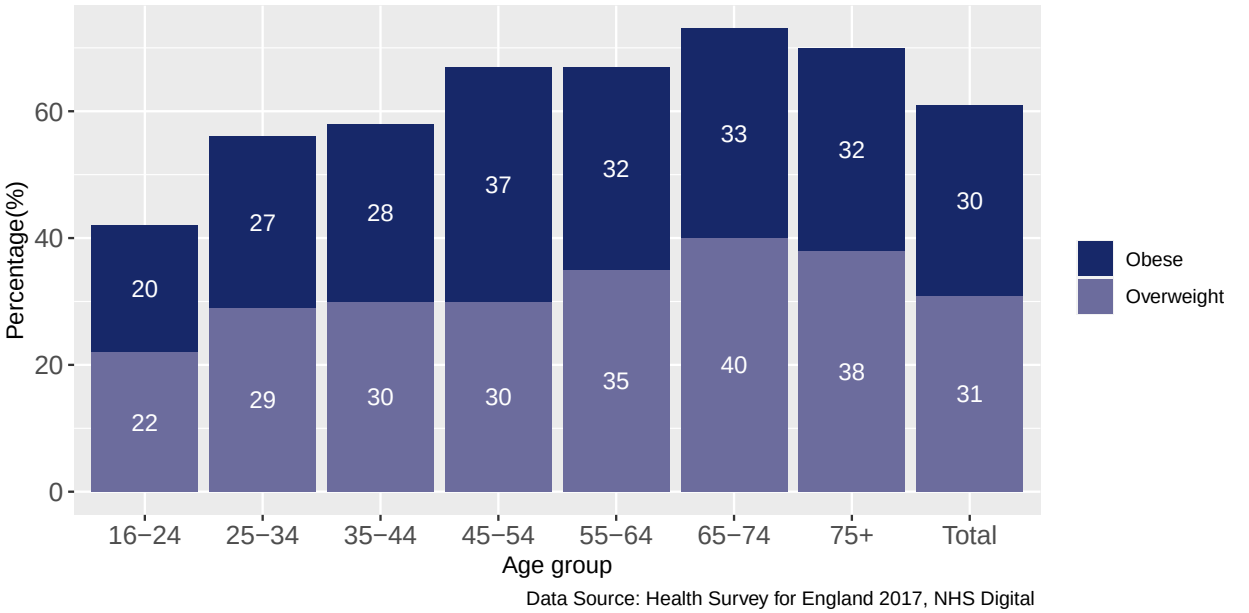

Figure 1.2: *Distribution of overweight and obesity among females in England in 2017.*

Evidence for a transgenerational transfer of disease risk have supported the theory of *fetal programming* coined by Barker and then later conceptualised under the overarching framework of the *Developmental Origins of Health and Disease* (DOHaD). In 1993, Barker associated population-wide shared events (i.e. famines/viral infections) to long-term health outcomes in the subsequent generations. Barker's historical cohort study found associations between low-birth weight, growth restriction and pre-term births on rates

of hypertension, coronary heart disease and diabetes in middle life (Barker et al., 1993). At the same time others inferred maternal glucocorticoids were associated with neonatal low birthweight and the same year had established in rats that uterine exposure to high levels of maternal glucocorticoids due to an impaired placental conversion of cortisol to cortisone could explain hypertension in the adult offspring (Edwards et al., 1993). These first clues paved the way for in-depth modelling of what is now an intense field of research and the emergence of animal models to study the effect of early nutrient deprivation, overnutrition and various metabolic and stress paradigms. These models would circumvent the limitations of retrospective observational human cohort studies in terms of confounding effects such as those of socio-economical status and common genetic factors in associations with offspring outcomes such as adult offspring body-size (Prentice, 2005).

While it is clear that maternal obesity and its associated comorbidities can have distinct etiologies (e.g., genetic, lifestyle) and the outcomes of the offspring are very heterogeneous, the causal pathways therefore to adverse offspring outcomes, including neurodevelopmental, are likewise complex. In this thesis, gestational diabetes mellitus (GDM), a type of diabetes first diagnosed in pregnancy, and depression are the two facets of obese pregnancies which are explored as additional exposures and as possible moderators or confounders to the current reports on maternal obesity-to-offspring neurodevelopment associations. This is given the high rate of diagnosis of GDM in obese pregnancies (up to 30%) and the burden of depression in obesity in the wider population. Importantly, depression in the antenatal period correlates with postnatal depression (Milgrom et al., 2008) and thus has a longer timeline of possible influence on offspring health (Coelho et al., 2011; Wolford et al., 2017). Importantly, the lack of appreciation for overlapping exposures and outcomes in pregnancies complicated by depression and diabetes is apparent in the current literature and presents a real challenge in identifying and disentangling associations when obesity is a common denominating milieu.

This chapter will introduce important concepts relating to the physiological profile of obesity and the maternal milieu and its potential for influencing fetal brain development as the first empirical studies in the thesis presents a large array of fetal exposures and causal models which necessitate this introduction. Although the focal outcome under scrutiny in this thesis is offspring brain development in utero, the place of the postnatal period in the context of these outcomes is also mentioned.

1.2 Obesity and adiposity

The extent to which fat cells expand and localise, especially in the context of calorie excess, can reflect a susceptibility or predisposition determined by early antenatal exposures, genetics, sex or postnatal factors (Eder et al., 2016). Obesity, characterised as surplus adiposity, occurs commonly in a state of a positive energy balance caused by a high energy intake and low energy expenditure i.e. by low physical activity and a sedentary lifestyle. It can have an early (childhood) or late onset and its etiology is considered multi-factorial with both environmental factors involved in addition to a genetic susceptibility which is considered heterogenous (Loos and Janssens, 2017). The current understanding is that the gene-environment interaction is likely to explain most of the variations in BMI, through epigenetic modulation (Rohde et al., 2019). Metabolism and energy homeostasis are regulated by the central nervous system (CNS) and particularly the brain region of the hypothalamus, further described in Chapters 6 and 7.

As will be further contextualised in the subsequent sections and chapters, it should be noted that BMI is a proxy measure of adiposity and body morphology which does not discriminate between fat and lean/muscle

mass and is mostly established from Caucasian populations, similar to genetic studies in the field (Locke et al., 2015). Therefore it may not be surprising to observe that some women classified "normal-weight" by BMI (18 to 25 kg/m^2) who have a high total body fat percentage (reclassified as "normal-weight obese") also display high risks of metabolic abnormalities associated with surplus adiposity such as adverse cardiovascular events and diabetes. Conversely not all women of BMI $\geq$ 30kg/m^2 will experience these events (De Lorenzo et al., 2013).

Adiposity is distinguished by its location and distribution while adipocytes (adipose cells) are also distinguished by type. Generally, subcutaneous adipose tissue (SAT) is viewed as "healthy" fat in so far that in energy surplus excess lipids are stored within it in the form of triglycerides. However, in the event of saturation of this expansion, stress or an inability thereof (due to genetic predisposition) lipids deposit onto internal organ such as the heart, liver, pancreas and kidneys as ectopic fat and in the abdominal cavity. Such visceral adipose tissue (VAT) is viewed as unhealthy due to its impact on internal function (González-Muniesa et al., 2017). Of note, in the presence of surplus energy intake resulting in increased adipose tissue accretion, women tend to have a higher number of adipocytes in SAT. SAT accretion occurs through *hyperplasia* (increase in cell number) whereas VAT deposition occurs via *hypertrophy* (expansion of cell size) in both sexes (Tchernof and Després, 2013).

1.2.1 Adipocytes

Adipocytes are of several types: white, brown and beige and are mostly differentiated by their thermogenic properties and lineage. *White adipocytes* are responsible for fat storage of lipid throughout the body and *brown adipocytes*, dense in mitochondria and capillaries, emit energy in form of heat from intracellular triglycerides, a process which also requires glucose uptake (Bartelt and Heeren, 2014). Adult brown adipose tissue (BAT) is distributed largely in the supraclavicular, cervical and axillar regions (Leitner et al., 2017). BAT is activated by cold exposure via the sympathetic arm of the Autonomic nervous system (ANS) and release of catecholamines i.e. adrenaline, noreadrenaline and dopamine produced by the adrenal glands, or by pharmacological injection. Importantly, BAT has a positive effect on glucose tolerance and insulin activity (Stanford et al., 2013). Obesity has been associated with decreased BAT activity (Liu et al., 2013; Bartelt and Heeren, 2014) and it has been recently shown that BAT presence (vs absence) is associated with lower odds of type-2 diabetes, coronary heart disease, dyslipidemia and hypertension in more than 50 000 individuals (Becher et al., 2021). *Beige adipocytes* share the thermogenic properties of brown adipocytes but are scattered within white adipose tissue and derive from the same cell lineage, whereas brown cells share precursors with skeletal muscle cells.

1.2.2 Adipokines

Adipose tissue is an organ which secretes many type of molecules and hormones (adipokines) which can regulate feeding behaviour through satiety and hunger signalling, inflammation, homeostasis, blood pressure and insulin resistance (Blüher, 2014).

Leptin is a hormone which is secreted by adipocytes and has receptors in the hypothalamus in the brain, relaying information on fat storage in the body and thus regulates metabolism and energy homeostasis as it acts as a satiety hormone and hunger inhibitor (Blüher, 2014). Leptin levels are proportional to body fat mass (Amitani et al., 2013) and counteract the function of *ghrelin* which is denoted as the "hunger" hormone.

However, the inability of leptin to act appropriately in obese individuals with high circulating leptin led to research into *leptin resistance*. Although leptin transfer into the brain has not been entirely clarified, so far it appears that in order to play its role in satiety it must be maintained as a narrow range of concentration to cross the blood-brain-barrier (Izquierdo et al., 2019).

Adiponectin is another hormone secreted by adipocytes with a circulating concentration inversely proportional to adiposity (especially adipocyte size) and is considered insulin sensitizing. In contrast to leptin, adiponectin has an anti-inflammatory role (González-Muniesa et al., 2017) and its receptors are also in the brain such as the cortex, hippocampus, hypothalamus, brain stem and pituitary (Thundyil et al., 2012; Bloemer et al., 2018).

Although not exclusively secreted either by adipocytes, other adipokines include the pro-inflammatory cytokines *Tumour necrosis factor-alpha* (TNF-alpha), *Interleukin-6* (IL-6), which decrease insulin sensitivity, and MCP-1 which is a chemoattractant for macrophage infiltration (Blüher, 2014). These components of adipose tissue are further described in section 1.5 below.

1.3 Adipogenesis

It is understood that *adipogenesis* (the initiation of adipocyte from stem cell precursors) happens in late fetal and early postnatal life and the number of adipocytes is determined early on in childhood, at a time when adipose tissue expansion is, unlike in adulthood, therefore more likely through hyperplasia (Entringer et al., 2012; Chait and Hartigh, 2020). However, BAT mass is at maximum at the time of birth and their thermoregulatory property is activated by catecholamines and thus is essential for neonatal survival. Interestingly catecholamine release is increased by cord cutting (Padbury et al., 1981) but catecholamines are significantly lower in plasma of babies delivered by pre-labour C-section, indicating the role of labour in this process. Those neonates subsequently have significantly lower body temperature than newborns delivered vaginally (Hyde et al., 2012).

1.4 Comorbidities and complications

Along with changes in lifestyles, the main factors which relate obesity to adverse health outcomes in the individual stem from the dysregulation excess adiposity confers not only on metabolic function but also on the endocrine and adaptive immune systems. As alluded above, the location and size of the adipocytes is what is thought to determine the impact of surplus adiposity on risks of cardiovascular diseases, type-2 diabetes and immunological disturbances. The known sex-differences in those diseases is partly linked to the differences in fat storage between the sexes and to some extend the protective effect of oestrogens given that women tend to store fat subcutaneously and men as visceral fat i.e. within the cavity of the abdomen (SantaCruz-Calvo et al., 2021).

Insulin resistance is often a precursor or the underlying background to obesity-induced type-2 diabetes and metabolic dysregulation and occurs when the tissues of the body no longer respond to insulin, the hormone which stimulates glucose absorption. It is measured clinically and in research by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), also a surrogate measure for the function of beta-cells, the cells secreting insulin in the pancreas.

A mechanism towards cardiovascular disease and diabetes in obesity relates to the adipocyte size. Small and large (white) adipocytes have functional differences. O'Connell et al. (2010) studied severely obese patients of BMI ranging 40-71 undergoing bariatric surgery and showed that the size of VAT cells but not SAT was positively associated with insulin resistance (HOMA-IR, correlation r=0.73), triglyceride/high-density lipoprotein (HDL)-cholesterol ratio and HbA1c (a glycaemic marker). In culture, adipocyte size is positively associated with pro-inflammatory cytokine secretion (IL-6, IL-8) and leptin. Conversely, it is negatively associated with secretion of anti-inflammatory factors such as IL-1a, IL-10 and with adipocyte insulin sensitivity, reflected by lower adiponectin concentration (Skurk et al., 2007).

In clinical settings, high blood triglyceride and low HDL-cholesterol are considered hallmarks of visceral obesity and is described more commonly in the literature as the *dyslipidemic state*. These features and along with elevated waist circumference, high blood pressure and increased fasting glucose are the five criteria which are pooled around this pathophysiological state referred to as the *metabolic syndrome*. In 2009, a harmonised criteria for the metabolic syndrome was presented where individuals who meet three of the five criteria would be considered to have the metabolic syndrome (Alberti et al., 2009).

Adiposity can also be associated with alterations in brain structure, both grey and white matter (GM,WM). A study in non-diabetic Caucasian males and females (24 obese and 36 lean) using voxel-based morphometry showed in the obese group lower GM density in the middle frontal gyrus, putamen, frontal operculum, postcentral gyrus, regions associated with behavioural control and reward (Pannacciulli et al., 2006). A more recent report from the Cardiff Ageing and Risk of Dementia Study (CARDS) demonstrated using MRI in a sample of 166 asymptomatic individuals aged 38 to 71 years that visceral fat area fraction and waist-to-hip ratio correlated negatively with a measure of white-matter microstructure in the fornix. The fornix is a white-matter tract and part of the limbic system linkings the mammilary bodies in the hypothalamus to the hippocampus and is affected in Late Onset Alzheimer's disease. However, BMI and subcutaneous fat did not correlate to white-matter microstructure (Metzler-Baddeley et al., 2019). This effect was mediated by the leptin/adiponectin (i.e. pro/anti-inflammatory adipokines) ratio in men whereas age mediated this in women who generally presented less VAT in this sample. Further description on brain structures involved in obesity and in studies following exposures to obesity in utero will follow in subsequent sections.

1.5 Chronic inflammation

A key factor in the pathogenesis of obesity which was alluded to above is the chronic low-grade inflammation associated with it. The place of dietary and lifestyle habits, pollution and other environmental factors are strong antecedants to chronic inflammation and explain most of the inflammatory signature of individuals, as has been observed in studies on twins (Brodin et al., 2015).

In line with the above and the description of adipocytes, it is the expansion adipose tissue which is thought to provoke the production of inflammatory cytokines e.g. TNF-alpha, IL-6 and IL-beta. Overall hypertrophy is generally considered a larger issue in these events then hyperplasia.

This is first explained by the observation that, in this expansion and intracellular triglyceride accumulation, adipose tissue undergoes structural remodelling, especially through hypertrophy which disrupts large array of signals. This can also promote tissue inflammation due to increased stress on cellular function, adipocyte apoptosis and hypoxia (Reilly and Saltiel, 2017). This process is thought to also result from the lipotoxicity

induced from glycerol and free-fatty acids (FFA) (Luo and Liu, 2016), the by-products of triglycerides. The developing hypoxia is understood to be partly a result of the oxygen demands and/or low perfusion within the growing adipose tissue. Under normal growth conditions, angiogenesis promotes tissue oxygenation and requires a highly coordinated partnership with other cells types including endothelial cells (Zatterale et al., 2020). If this process is inadequate due to accelerated expansion, cellular oxygen requirements will not be met.

Additional pathways to inflammation relate to macrophages, the first responder immune cells residing in the adipose tissue, which increase in number upon its expansion. They take a pro-inflammatory state (Lumeng et al., 2007) where they become M1-polarized, a switch from their M2 “monitoring” state, which otherwise induces an anti-inflammmatory pathway and is insulin-sensitising to the adipocytes (Reilly and Saltiel, 2017). This shifting to secretion of inflammatory cytokines and inflammation instigates insulin resistance via a cascade of events which disrupt cellular function in the metabolic organs such as the liver, pancreas, brain and adipose tissue itself (Hotamisligil, 2017). It was mentioned that VAT expansion is through hypertrophy and so to this extent, VAT is considered more pro-inflammatory than the SAT compartment (Alvehus et al., 2010) and coincidentally releases these cytokines but also a higher fraction of FFA in the portal circulation of the liver (Item and Konrad, 2012).

Intracellular triggers to meta-inflammation include mitochondrial dysfunction and endoplasmic reticulum stress (Zatterale et al., 2020). The mechanical stress imposed by increased storage of triglycerides in cells surrounded by extra-cellular matrix are also proposed as proinflammatory (Reilly and Saltiel, 2017).

More distally, inflammation could be a mediated by the gut microbiome (Cani and Jordan, 2018) or a consequence of increased leaking through the gut with subsequent increased blood concentrations of lipopolysaccharide (Cani et al., 2007), an endotoxin and a molecule present on the surface of bacteria.

1.6 Diet and inflammation

Dietary lipids are also thought to promote inflammation through the effect of FFA on the infiltration of macrophages (Reilly and Saltiel, 2017). However, it is very interesting to note that the type of FA ingested seems to also be important as it was shown in a murine model of high-fat-diet (HFD) that ingestion of saturated FA (as found in palm oil) induced hypertrophic adipose expansion, inflammatory secretion of IL-beta and insulin resistance. However, ingestion of mono-unsaturated FA induced hyperplastic adipose expansion and partially protects against insulin resistance (Finucane et al., 2015).

1.7 Maternal immune modulation in pregnancy

The potential background of low-grade inflammation in the pregnant obese woman described above is important to establish because a pregnancy which is “successful” depends on a well-balanced modulation of the maternal immune system. This is especially true in the prevention of fetal loss in early gestation, and key immunological processes are implicated and shifted from the time of placentation until birth. This is thoroughly discussed in a recent review by Wang et al. (2020) but are briefly mentioned here.

The immune response involves a pro-inflammatory response in the defense of intracellular pathogens, bacteria and viruses and involve the cells of the T-helper pathway 1 (Th1) while the Th2 pathway is triggered by

extracellular cues and favors repair and an anti-inflammatory profile. Following conception and during the first 7 weeks a successful embryonic implantation requires the Th1 response to allow for the remodelling for uterine vasculature (Germain et al., 2007) but also prevent an uncontrolled invasion of the trophoblast of the newly formed embryo into the maternal uterine wall (Jiang et al., 2014). This is then shifted to a Th2 immune response, which dominates thereafter, to support both fetal and placental development. This process involves the peripheral M1 macrophages engaging in the early stage of uterine vascular remodelling and the M2 macrophages immunosuppresive state once the placenta is formed (Nagamatsu and Schust, 2010).

Although the Th1/Th2 is now considered an oversimplistic explanation of the regulatory dynamic of the immune system (St-Germain et al., 2020) it has shaped the current understanding of the etiologies of spontaneous abortion and obstetric disorders such as preeclampsia and the discovery of Th17 and Treg cells in these pathways (Eghbal-Fard et al., 2019; St-Germain et al., 2020). Overall, although a large cascade of cytokines are involved the main premise of acquiring an adequate maternal immunological/immunosuppresive balance is to sustain the tolerance of a semiallogeneic fetus (Jiang et al., 2014).

In obese pregnancies, the higher prevalance of background metabolic disturbance and meta-inflammation is understood to contribute to the increased risks of disruption in the maternal-fetal tolerance with resulting impairment in placentation, obstetric complications such as preeclampsia and GDM and poorer pregnancy outcomes (Pollheimer et al., 2018). For example, placental histological abnormalities are more frequent with obesity and include thrombi and marginal rather than central umbilical cord insertion (Luo and Liu, 2016). Obesity in pregnancy implicates placental function possibly through a higher lipotoxic placental environment which promote placental inflammation (Saben et al., 2014), although placental vascular dysfunction is primarily associated with preeclampsia. This is further described in Section 2.12 of the next chapter.

1.8 Acute immune responses

The inflammatory cascade and low-grade inflammation described earlier also has implication for the maternal response to infection. Metainflammation is thought to be metabolically activated and is reflected also in the macrophage surface marker which is distinct from those markers expressed during active infection.

Hence the long-winding process towards meta-inflammation and its chronicity should be differentiated from the crucial role of the short-lived acute inflammatory response. The acute response also requires energy but subserves the maintenance of tissue integrity and their remodelling through local repair and renewal (Furman et al., 2019). The acute response implicates several cytokines released from immune cells such as IL-6, TNF-alpha, IL-1beta, and C-reactive protein (CRP). In the clinic, a CRP >10 mg/L indicates infection. In the context of infection therefore, a well regulated metabolic homeostasis system is also required to support this acute immune response.

A chronic activated state which could interfere with the health and function of organs and tissues implicated in metabolism (e.g. of lipids by liver and pancreas) would therefore impede or delay the success in reestablishing homeostasis after infection in cells and therefore an organism (Hotamisligil, 2017). In pregnancy with obesity, risks of infections are higher. In a UK study of 287 213 singleton pregnancies, including 31 276 obese and 176 923 normal weight women Sebire et al. (2001) reported the following odds ratio (OR, 95%CI) after adjusting for demographic factors, pre/gestational diabetes and hypertensive disorders: urinary tract infection = 1.30 (1.07–1.56), genital tract infection =1.30 (1.07–1.56) and wound infection = 2.24 (1.91–2.64).

The implication to the fetus will be discussed further in coming sections.

1.9 Glucose homeostasis in pregnancy

Glucose is the main fuel of the fetus and adequate maternal supply and transfer through the placenta is essential for fetal growth. Glucose homeostasis can be assessed through several blood biomarkers beside glucose such as haemoglobin A1c (HbA1c), insulin and c-peptide. C-peptide is produced along insulin by pancreatic beta-cells but is not absorbed or bound by receptors in other tissues in the body so is a more stable marker of insulin secretion. HbA1c is a marker of glucose attached to hemoglobin which offers an average glucose level for the period of 2-3 months.

In pregnancy, maternal metabolism goes through adaptation stages so that early pregnancy is characterised by an anabolic, energy storing, stage which switches to a catabolic, energy-releasing stage, to increase nutrient utilisation by the placenta. In normal pregnancy, insulin resistance rises by the late second trimester which is reflected in a 50% lowering of liver and peripheral tissue sensitivity to insulin which therefore reduces their glucose absorption (Catalano et al., 1991). This increases blood glucose availability in the mother and is thus physiologically favourable to fetal nutrient access. At the same time, adequate pancreatic beta-cells compensation supports an increase in insulin secretion to maintain maternal normoglycemic status, reaching 2-3 fold increase. Figure 1.3 illustrates the metabolic pathway to insulin release. However, pregnant women with obesity have a characteristically lower pancreatic insulin response compared to lean women (Catalano et al., 2009).

Although glucose is a major requirement for optimal progression of the pregnancy and fetal growth, other metabolites are also fluctuating to address maternal and fetal needs, including amino acids and lipids. Their role in fetal brain growth will carry on in subsequent sections and their trends in maternal circulation during gestation described in Chapter 4 for three time points.

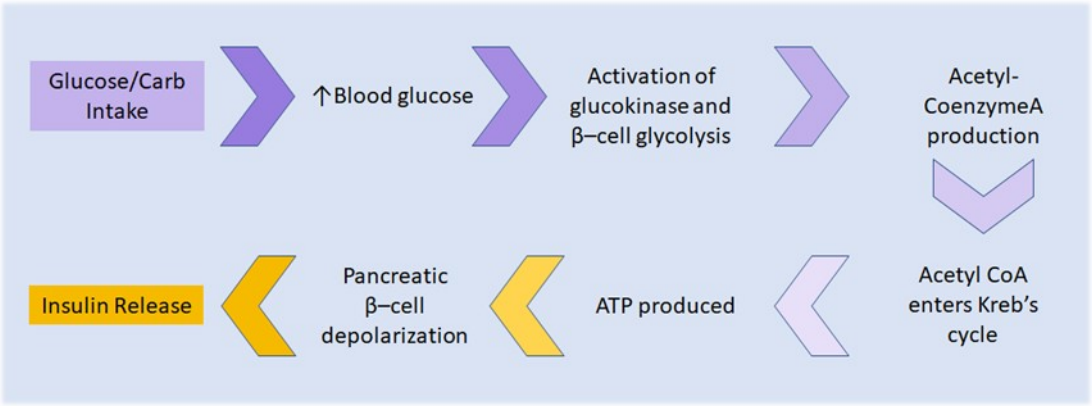

Figure 1.3: *Metabolic pathway to insulin reactivity in the pancreas following glucose/carbohydrate intake.*

1.10 Gestational Diabetes Mellitus

GDM is a type of diabetes which is defined as first arising or is first diagnosed during pregnancy. GDM is one of the most common pregnancy complications, especially among ethnic non-white minorities and is three

times more likely to be diagnosed if the woman is obese than lean (Torloni et al., 2009). It represents 87.5% of diabetes in pregnancy and Type-1 represents 7.5% and Type-2 accounts for 5% (Magon and Chauhan, 2012). GDM often resolves postpartum but is associated with the emergence of Type-2 diabetes for the mother in subsequent years (Bellamy et al., 2009).

The change in glucose metabolism in normal pregnancy was mentioned above. GDM is thought to reflect a failure in the compensatory insulin-secretory mechanism to retrieve maternal normoglycemia (Catalano et al., 1999). It is also characterized by the coexistence of abnormalities in hormones (including leptin and adiponectin), cytokines, triglycerides, free fatty acids, vitamin D and endothelial function observed from either blood, adipose tissue or the placenta (Abell et al., 2015).

As mentioned, obesity itself is associated with “metainflammation”, which is reflected by the higher systemic concentrations of pro-inflammatory immune signaling molecules (cytokines). The role of visceral fat in this pro-inflammatory profile was also described. Metainflammation is thought to promote insulin resistance in the non-pregnant state by affecting pancreatic and liver function and may be at play in the obese pregnancies which develop GDM. However, it should be noted that although pregnancy-induced metabolic changes can trigger GDM, its detection first in pregnancy does not exclude either an undetected pre-conceptual hyperglycemia or a subthreshold hyperglycemia which is tipped over by pregnancy.

However, GDM in pregnancies of lean/normal-weight women where metainflammation is considered less prominent is viewed as a suboptimal secretory response but the etiology has been more complex to establish. Given the description on VAT above, it is not unlikely that women considered lean by BMI could have a substantial VAT mass, such as caused by stress or a genetic predisposition. This possibility has gained more attention in the past few years, especially in describing etiological heterogeneity in GDM but also in the development of clinical biomarkers. It may be then unsurprising that simply by performing ultrasonography in the first-trimester, it was found that odds of GDM in an obese or lean pregnancy increased with higher pregestational VAT volume but not SAT volume (De Souza et al., 2016).

1.10.1 GDM diagnosis and management

GDM is often clinically detected through a 75g oral glucose tolerance test (OGTT) usually performed at 24-28 weeks gestation. During antenatal booking appointments in the UK, women who have any of the following screening characteristics are offered an OGTT:

- BMI $\geq$ 30kg/m^2
- previous birth of macrosomic neonate (>4.5 kg)
- previous GDM pregnancy
- first degree relative with diabetes
- ethnicity with high prevalence (non-white)

It follows that many women who could be at risks are not offered an OGTT, including primiparous women, white women and those classified as overweight by BMI. Often, late screening through an OGTT would be indicated in the presence of an large for gestational age (LGA) fetus measured by sonography or fundal height by which point the mother and the fetus may have been overexposed to an undiagnosed hyperglycemia for a long period.

Although screening and diagnostic criteria can vary across countries, in the UK screening women at risk of GDM, GDM diagnosis and management follow the National Institute for Health and Care Excellence (NICE) guidelines (National Institute for Health and Care Excellence, 2015). Another GDM diagnostic criteria broadly implemented internationally follows the guideline from International Association of Diabetes and Pregnancy Study Groups (IADPSG) (International Association of Diabetes and Pregnancy Study Groups Consensus Panel, 2010) which is also adopted by WHO. The IADPSG criteria are the ones followed in the next chapters. Table 1.2 presents the glucose threshold values compared between NICE and IADPSG guidelines.

Intervention for GDM aims to improve maternal and child outcomes, especially in delivery to reduce the need for c-sections and complications from delivering macrosomic neonates but also neonatal hypoglycemia and asphyxia. Antenatal treatment involves firstly a referral to a dietician to aid dietary changes and promote exercise to decrease glycemic load. Women are asked to self-monitor their glucose and are followed every two weeks by a clinician. If glucose cannot be maintained through diet and exercise within 1-2 weeks women are then prescribed metformin and/or insulin as the last measure (National Institute for Health and Care Excellence, 2015).

Current NHS screening guidelines are viewed as cost-effective to universal screening (Jacklin et al., 2017) and beside promoting normoglycemia, a meta-analysis suggests that these treatments have shown some benefit in reducing macrosomia, shoulder dystocia and preeclampsia but little benefit in other adverse events such as NICU admission, c-section and small-for-gestational age neonates (Hartling et al., 2013).

Table 1.2: *Diagnostic criteria for GDM according to NICE and IADPSG*

OGTT timepoint	IADPSG	NICE
fasting	>= 5.1 mmol/L	>=5.6 mmol/L
1h	>= 10.0 mmol/L	–
2h	>= 8.5 mmol/L	>= 7.8 mmol/L

Note:
IADPSG: International Association of Diabetes and Pregnancy Study Groups; NICE: National Institute for Health and Care Excellence; OGTT: Oral Glucose Tolerance Test.

1.11 Stress and glucocorticoids

The distribution of adipose tissue has been described above but it should be noted that beyond sex differences, other factors influence this process. The action of the glucocorticoid hormone cortisol (in humans, *corticosterone* in rodents) is relevant because it has been largely investigated in pregnancy to characterise the association between maternal depression and stress exposure *in utero* and brain development.

However, in the context of pregnancy, cortisol is not commonly referred to for its association with adiposity although outside pregnancy, the place of glucocorticoids is well characterised. Indeed, the described distribution in adipose tissue accretion can be modulated by other factors such as chronic stress. Glucocorticoids act on the energy homeostatic system because the normal stress response generates cortisol release and

cortisol acts to mobilise energy in the form of glucose generation through *gluconeogenesis* within the liver and the release of free fatty acids via *lypolysis*, i.e. the cleavage of triglycerides bonds. This release of energy enables the organism to respond to the stressful stimuli. This mechanism is then understood to promote VAT accretion in humans under (chronic) stress (Rebuffé-Scrive et al., 1992) via a state of mild hypercortisolemia and sustained sympathetic nervous system activation (Tchernof and Després, 2013).

The release of cortisol follows a well-known multisystemic activation (Kloet and Herman, 2018). Briefly, under a psychological (e.g. perceived threat) or physiological stress (e.g. energy depletion), corticotrophin-releasing hormone (CRH) and other peptides such as arginine vasopressin are released through the median eminence into the hypophysial portal vasculature from neurons in the paraventricular nucleus (PVN) of the hypothalamus. These molecules travel into the portal vasculature of the anterior pituitary which initiates the secretion of the adrenocorticotrophic hormone (ACTH). Thereupon ACTH can enter the peripheral circulation targeting the adrenal cortex where secretion of glucocorticoids occurs. A negative feedback loop promotes the return to system homeostasis whereby activation of the HPA-axis and further cortisol secretion by the adrenal glands is inhibited by the binding of glucocorticoids to receptors in the brain. This describes a rather slow cascade of events which follows and contrasts to the stress response implicating the almost immediate activation of the autonomic nervous system (ANS) and the release of adrenaline from the adrenal glands. Adrenaline promotes rising of blood glucose through gluconeogenesis and glycolysis but also lypolysis and thermogenesis, meeting immediate demands of energy as this axis is primarily implicated in the flight-fight response to stress and threat (Cryer, 1993; Charmandari et al., 2005).

Nevertheless, the negative-feedback system of the HPA-axis system has been recently hypothesized to implicate adipocytes via several mechanisms. This includes signals from adipocyte through CRH in the PVN in the hypothalamus or from the FFA released by the glucocorticoid-enhanced glycolysis, a signaling which may occur via the vagus nerve (Kloet and Herman, 2018). The vagus nerve is the most important fibre connecting the visceral organs, including the gut and heart, to the brain. It was suggested by Kloet and Herman (2018) that in this circumstance FFA may then be utilised both as fuel to respond to the stress situation and also as a inhibitor to the glucorticoid secretion.

1.12 Glucocorticoids in adipose tissue

Glucocorticoids are not only involved in the stress response and glucose regulation but further dynamic interplay exist between psychobiological states, adiposity and metabolism. In fact, adipose tissue expressed glucocorticoid receptors (GR) abundantly. Adipose tissue synthesizes cortisol via *type 1 11-hydroxysteroid dehydrogenase* (11-HSD-1). Cortisol and glucose levels are associated with adipose function as it has been shown in seminal work that 11-HSD-1 knock out mice resisted hyperglycemia under stress or diet-induced obesity (Kotelevtsev et al., 1997). Adipose GR are important in the negative feedback loop following induction of the stress response by the HPA as it has been shown that mice with localised adipose GR deletion showed blunted inhibition of the HPA-axis response but also resisted diet-induced obesity (Kloet et al., 2015).

Therefore, stress cues drive a surge of glucose and alertness necessary for immediate and successful response but glucocorticoids are also relevant factors in the interplay with adiposity and energy homeostasis through their involvement in metabolic changes induced by chronic-stress and depression. In turn, as will be described later, depression is also associated with maternal cortisol in pregnancy and subsequent offspring temperament (Davis et al., 2007).

The above brings the subject of maternal depression into the context of this thesis as well as hypothalamic function and the ANS. While the previous sections present the likely maternal physiological milieu, the suggestion in this thesis is that the systems which may be disregulated in the endocrine and especially ANS and CNS homeostasis systems in the *mother* are implicated in the phenotypes of the *offspring*.

1.13 Maternal Depression

The domain of obstetrics views maternal obesity as a primary common denominator and physiological predisposition to complications such as GDM, gestational hypertension or in more extreme cases preeclampsia. And so in the clinic, physicians and health care allies have as a priority to help the woman maintain essential biomakers (glucose, blood pressure) within the normal range, which motivates screening and a monitoring afforded throughout pregnancy at each antenatal visit. However, the obese female population is multifaceted and includes large between-individual variation in pregnancy, birth and long-term outcomes. *Maternal psychopathology* is one such facet and is unfortunately less commonly accounted for when exploring the links between obesity and/or GDM with offspring neurodevelopmental outcomes. This is despite the strong interactions between obesity, diabetes and depression in the general population. Depression is a significant risk factor for T2DM in the general population (Mezuk et al., 2008) and has been shown it could predict GDM (Hinkle et al., 2016). Important to the remit of this thesis, it is quite significant that antenatal depression confers overlapping labour and neurodevelopmental outcomes in the child as seen in antenatal obesity, i.e. preterm birth and ADHD/autism (O'Donnell and Meaney, 2017; Chan et al., 2018).

Depression is conventionally diagnosed by golden standard following Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (DSM) or the Beck Depression Inventory (Beck et al., 1961). The current incidence in the pregnant UK population for mental health disorders assessed close after first booking appointment is 1 in 5 women. Mild/major depression prevalence is 11%, anxiety 15%, obsessive-compulsive disorder 0.8% and PTSD 2% (Howard et al., 2018). Social risks factors for antenatal depression include lack of partner support, domestic violence and low socio-economic status (Ogbo et al., 2018).

It is not inconceivable that women of high BMI may be particularly vulnerable to new emerging depressive or anxious symptoms in pregnancy. Obese pregnant women may feel stigmatised and under scrutiny given their BMI places them in the category of at-risk pregnancies under antenatal care guidelines and monitoring focuses intensively on decreasing odds of adverse outcomes for mother and fetus. This is echoed by women who have expressed the challenges in living with GDM (Draffin et al., 2016). Additionally, women may be more likely to stop, reduce or decline the use of anti-depressant medication for their low mood which increases their risks of experiencing mental health difficulties in pregnancy (Bonari et al., 2005).

Despite the robust evidence of depression and obesity being reciprocally predictive (Luppino et al., 2010), the detection, monitoring and accessibility to intervention for psychological/psychiatric problems within the antenatal period are not as straight forward and arguably not afforded the same swiftly available resources as "conventional" complications of pregnancy.

Unfortunately, this occurs in the context of the large body of evidence relating mental health problems to adverse fetal and maternal outcomes (Stein et al., 2014), including high maternal comorbities and mortality (Knight et al., 2018). To demonstrate, Easter et al. (2021) performed a linkage study to explore the

prevalance of life-threatening obstetric complications (near misses) among women with serious mental illness (SMI). Complications included renal failure, cardiac arrest, failure or infarction and embolism at birth and SMI was based on a history of attending secondary mental healthcare prior to delivery (n = 13 570). They found an adjusted risk of OR =1.6 [1.3–2.0] compared to women who did not have SMI.

Antenatal depression is also linked to other behaviours unfavourable to optimal pregnancy and fetal growth such as smoking, substance use, low motivation, poor diet and hygiene (Neggers et al., 2006; Connelly et al., 2013). Interestingly, the rates of maternal infection (of genital tract, urinary tract and wound) mentioned above (Sebire et al., 2001) did not assess the effects of mental health and health seeking behaviours in these outcomes.

The relationships between SES, obesity, ethnicity and maternal depression is likewise complex. Socio-economic deprivation during pregnancy and in the postpartum period are strongly associated with depression, especially among older women (Ban et al., 2012). While minority ethnic women are more vulnerable to low mood in the UK and Americas in the perinatal period (Liu and Tronick, 2014), SES can moderate these associations but does not account for low mood equally across ethnicities (e.g. Asian/Pacific Islander vs African American, Hispanic women; Liu and Tronick (2014)). In addition, it has been shown that the association between pregnancy weight and risks of postnatal depression also covaries with ethnicity but also does so in a heterogenous manner (Non-White Hispanic vs Hispanic but not among Black or Asian women) even after adjusting for socio-economic status (Green et al., 2021).

1.13.1 Physiological underpinning of Depression

It has been suggested that inflammation may be induced by depression (Capuron et al., 2008; Slavich and Irwin, 2014; Branchi et al., 2021) or could mediate the link between depression and obesity (Bremmer et al., 2008; Moazzami et al., 2019). In a review on many meta-analyses of depression, Milaneschi et al. (2020) suggest that depression exists largely with immunometabolic dysregulation (includes evidence from studies on insulin resistance, leptin dyregulation, inflammation and abdominal adiposity in this context) such as present in obesity. This also relates to shift in lipid profiles (i.e. lower blood HDL-cholesterol, higher low-density lipoprotein and triglycerides) reminiscent of the metabolic syndrome mentioned in Section 1.4. However it was noted in the above associations that the effect was larger if depression is rated on symptoms of atypical depression (increased eating [hyperphagia], hypersomnia, weight gain), also more prevalent among women than men, than the typical clinical presentation (hypophagia, insomnia). As alluded to above, inflammation could mediate the relationship between psychopathology and obesity. It has been shown that IL-6 can act as a feedforward regulator of the HPA-axis by stimulating cortisol release by the adrenal glands but also that adipose IL-6 can be modulated by glucocorticoids (Fried et al., 1998).

In pregnancy, using the DSM classification, depression (mostly treated by medication including Selective serotonin reuptake inhibitors [SSRIs]) was associated with higher IL-6 and TNF-alpha in maternal blood at 11-14 weeks gestation, after adjusting for BMI and maternal age and excluding pregnancies with chronic disorders such as diabetes or complicated by preeclampsia, placental abnormalities and fetal abnormalities (Haeri et al., 2013). Furthermore, it has also been proposed that an overreactive immunological response to infection could trigger sickness behaviour and thereafter depressive like symptoms through the neurotoxic effect downstream of the kynurenine metabolism pathway (Dantzer, 2017).

However, it is important to consider that in pregnancy a strong adaptive switch from the Th1 to Th2 profile is

involved and thus the directionality of depression to pro-inflammatory processes is difficult to establish and the inflammatory profile in early and late gestation remains to be clarified. In the context of the maternal immunological adaptation it was indeed reported in a sample excluded of GDM and adjusted for preeclampsia that mothers who were depressed in pregnancy or on anti-depressant presented a lower macrophage induced inflammatory profile than controls (Edvinsson et al., 2017) although not of the conventionally measured IL-6 and TNF-alpha.

1.13.2 Detection and monitoring of antenatal depression

Currently under the NICE guidelines in the UK, questions on mental wellbeing directed at the woman should occur at their booking appointment, twice thereafter in pregnancy. Women are asked by a GP or midwives the *Whooley* questions and give Yes/No answers:

- *"During the past month have you often been bothered by feeling down, depressed or hopeless?"*
- *"During the past month have you often been bothered by little interest or pleasure in doing things?"*

These questions are found to have high specificity (0.94) to detect a mental health disorder at the first appointment, not only depression, but have low sensitivity (0.23)(Howard et al., 2018).

An alternative screening tool is the Edinburgh Postnatal Depression Scale (EPDS; Murray and Cox (1990)) which will be further described in subsequent chapters of the thesis. It is composed of 10 questions but is not part of standard care although it presents higher sensitivity (0.59) and equal specificity (0.94)(Howard et al., 2018) to the 2-item Wooley questions. The EPDS conventional cutoff of 12/13 also shows improved performance at detecting major depression (likelihood ratio 9.8 vs 8.2, Howard et al. (2018)).

1.13.3 Challenges in the clinic

The current approach to detecting low mood in pregnancy meets several barriers. The Whooley screening questions may not be enough to reach women at risk of mental health problems in pregnancy as it is not uncommon that women are not able or willing to disclose about their wellbeing. They may also be deterred by the lack of continuous care and some evidence suggest health care allies are often untrained and are not consistent in asking the Whooley questions, which decreases acceptability to answer (Darwin et al., 2016). Detection can be limited also when assessed in pregnancy if medical history is not available and women misinterpret depressive symptoms for normal pregnancy symptoms. This can lead to a significant risk of underdetection and result in untreated depression (Kelly et al., 2001). Importantly, women of non-white ethnic minorities are also less likely to be asked about their mental health (Prady et al., 2016).

Beside the biological implication of depression/anxiety on fetal growth, negative affect could pose a challenge when antenatal clinical management relies on women interacting with health care allies such as midwives and attend regular checkup appointments. Studies show that mental health and social problems pose barriers to the attendance to an OGTT, beside being from an ethnic minority and high BMI (Lachmann et al., 2020) and this should not be overlooked. Non-attendance due to negative feelings associated with the diagnosis could preclude those who have history of diabetes as they may remember the scrutiny and being chastised by their partner and health professionals for not abiding or completing the aims of treatment plans (Evans

and O'Brien, 2005).

Given the likelihood of mental health issues experienced prior to pregnancy to carry on during and after the pregnancy (Milgrom et al., 2008), clinician should be encouraged to do a thourough check of maternal psychiatric history, including in previous pregnancies and be aware of the emergence of new mental health difficulties attached to the index pregnancy, which is of course not limited to depression.

1.13.4 Diet, mental state and eating behaviour

Mental health can present other avenues to influence fetal growth in the obese pregnancy such as through eating behaviour and food intake. It has been shown that women suffering from chronic stress and who have low cortisol stress reactivity are more likely to ingest more calories from chocolate and less from vegetable and had higher total body fat compared to women with low chronic stress with high stress cortisol response (Tryon et al., 2013). This latter point is important in regard to the nutrient quality reserved for the fetus during pregnancy, especially in the context of macronutrient insufficiencies and deficiencies. However, previously, high cortisol response following stressful tasks was associated with higher snack consumption (Epel et al., 2001) which suggest the baseline HPA activity and stress chronicity interact with food intake but mechanisms are yet to be clarified.

The effect of the Western diet (high fat and high calorie) on health outcomes has been extensively studied in animal models of high-fat, high-sugar diets. Here the effects of maternal diet is both associated with her own health, including effects on her brain. For example, in mice models, high-fat diet can induce suppression of adiponectin receptor and lead to brain neuroinflammation, microglial and astrocyte activation, brain insulin resistance and cognitive impairement reminiscent of Alzheimer pathogenesis (Bloemer et al., 2018). Western diet affect the woman's metabolism and organ function, including the placenta and indirectly the quality and quantity of nutrients transfer to the growing fetus.

1.13.5 Postnatal effect of depression

Antenatal depressive and anxiety disorders predict depression after delivery (Coelho et al., 2011). This suggest a continued knock-on effect of maternal mental health during important period of neurodevelopment of the offspring, which occurs both in the antenatal and postnatal periods. This carries significant weight on increasing the burden of potential psychopathology across generations. Furthermore, the literature is abundant on the impact of antenatal and postnatal depression and anxiety on mental health and cognitive development in the offspring (Murray and Cooper, 1997; Sutter-Dallay et al., 2011; Monk et al., 2019) and ADHD (Wolford et al., 2017). Therefore, characterising the impact of antenatal effects independent of maternal postnatal depressive symptoms is still ongoing and benefits from longitudinal designs rather than single measures at either side of birth. Nevertheless, this field of research also seems to focus largely on affective and neurodevelopmental outcomes in the child but this is further described in the next chapter.

1.14 Why is studying depression and diabetes in obese pregnancy relevant to offspring neurodevelopment?

All the previous sections have established some of the complex relations between many systems regulating metabolism, adiposity and psychological state which could present in obesity. To illustrate, cortisol can induce glucose availability in the context of both psychological and physiological stress (Kloet and Herman, 2018) and psychological stress can also increase VAT accretion (Rosmond et al., 1998; Incollingo Rodriguez et al., 2015) which was identified in previous sections as a risk for metabolic dysregulation and the emergence of GDM in pregnancy.

The relationship extends to the local catalyzation of inert cortisone to active cortisol by the enzyme Type 1 11-hydroxysteroid dehydrogenase (11-HSD-1) which is abundantly present in adipose tissue. It was noted previously that both local and systemic cortisol concentration could contribute to insulin resistance (Tchernof and Després, 2013). Importantly, prenatal exposure to high glucocorticoid levels or stress have shown to explain adverse psychiatric outcome, including hyperactivity and autism (Babenko et al., 2015), but also insulin resistance in the adult offspring (Chan et al., 2017; O'Donnell et al., 2014; O'Donnell et al., 2009; Entringer et al., 2015, 2014; Ronald et al., 2011).

Nevertheless, placental enzyme Type 2 11-hydroxysteroid dehydrogenase (11-HSD-2) is normally highly efficient in converting cortisol to inactive cortisone, thus minimizing exposure to the fetus. Despite that, in the environment of maternal inflammatory and metabolic dysregulation, the function of 11-HSD-2 may be compromised (Duthie and Reynolds, 2013). Evidence suggests that lower placental 11-HSD-2 expression occurs in pregnancies of high maternal anxiety and this is also associated with lower birth weights and shorter gestations (Kajantie et al., 2003, O'Donnell et al., 2012; Ding et al., 2014).

Finally it was related above that adiponectin is an adipose tissue-derived hormone which is insulin sensitizing, anti-inflammatory and inversly related to adipose cell size. However, evidence suggest its action is blocked by cortisol (Fallo et al., 2004). It is thus interesting to note that low adiponectin is correlated with high depressive behavior (Liu et al., 2012) but, when measured early in pregnancy, is also a predictor of GDM (Corcoran et al., 2018; Iliodromiti, 2016; White et al., 2016; William et al., 2004).

1.15 Other factors not included

What is deemed beyond the scope of this thesis are other factors associated with maternal obesity that could impact child outcomes (metabolic and neurodevelopmental). These include sleep disturbances during pregnancy (see Lavonius et al., 2020), given the sleep apnea is more frequent among obese than lean women (adjusted OR 13.23[6.25–28.01]; Rice et al. (2015)), and is associated with shorter gestation (Blair et al., 2015). Sleep disorders are more generally associated with the immune response and cardiovascular health (Irwin, 2015), inflammation (Motivala et al., 2005; Wang et al., 2019) and depression (Motivala et al., 2005). However, sleep in pregnancy was a measure in the proposed prospective study design described in Appendix B.

Additionally, autoimmune diseases in causative mechanisms to adverse offspring neurodevelopment should not be ignored in light of findings of their association with ASD risks. In their study, Chen et al. (2016) excluded specifically Type-1 diabetes, an auto-immune disease, to rule out hyperglycemia as a mechanism

and found a pooled OR, 1.34 (95% CI, 1.23-1.46) (Chen et al., 2016).

The higher incidence of c-section and the potential for differences in maternal and child gut microbiome is also beyond the scope of this thesis. Research on the role of the gut is emerging and a possibly exciting avenue explaining the continued effect of immunomodulation and the role of the gut-brain axis in subsequent neurodevelopment. For example, in the relationship between maternal and neonatal microbiome it has been found to be delivery-mode dependent and also influenced by maternal saturated and mono-unsaturated fatty acid intake (Selma-Royo et al., 2021). Additional factors such as the effect of obesity and diet on the initiation, duration and quality of breastmilk in the association with neurodevelopment (Gawlińska et al., 2021) is not explored.

1.16 Summary

This chapter hopefully characterises the close affinity among biomarkers which are often studied independently (glucose or cortisol) in the discourse on fetal programming following obesity, hyperglycemia or depression. It appears that the literature which reports relationships between maternal obesity/GDM and offspring psychological outcomes largely fails to account for interactions or confounding of maternal mental state and vice versa. Importantly, maternal behaviour (e.g., substance abuse, non-attendance to OGTT), infection and birth event can either confound or bias our current understanding of causal model of fetal programming.

Few studies have presented a framework which encompasses the facets of obese pregnancy described thus far both in such a comprehensive manner that is incorporates biological factors described within the metabolic, glycemic and inflammatory pathways but also the dietary and psychosocial factors which could potentially expand towards formulating new hypothesis and interventions. This was the background narrative of what is to come in Chapter 4 where maternal depressive state is an exposure and the physiological, dietary, birth and pregnancy outcomes are comprehensively described.

This current chapter also touched on the areas of the neuroendocrine system, brain and ANS which are incorporating and translating the various signals of the peripheral organs and although this was mentioned in the mother, these systems are also implicated in the *offspring* metabolism and psychological outcomes and so are the focus of the next chapter and of the content of Chapter 6 and 7 based on neonatal MRI data.

Overall, understanding contributors to brain alteration during development could improve the discovery of epigenetic programming pathways for which some evidence is building (e.g. Nardone and Elliott (2016); Braun et al. (2020)). Now that the maternal milieu in the obese pregnancy has been described, the next chapter focuses on the offspring brain and its development in an environment which could confer various vulnerabilities to abnormal growth and long-term health.

Chapter 2

Neurodevelopment and offspring outcomes

In the context of maternal metabolic dysregulation and obesity, there are gaps in our understanding of the key elements which could predispose a child to psychological deficits including neurodevelopmental disorders such as autism and ADHD (Kong et al., 2020). The brain develops both during pregnancy and into the early postnatal years. Obesity, GDM and depression/stress are risk factors for other pregnancy and labour complications and certain neonatal outcomes which could also be implicated in these long-term conditions (Rivera et al., 2015) or exacerbate a hereditary or a *in utero* derived predisposition. Indeed, estimates are that only 1 in 3 obese pregnancies is free of any obstetric and birth complications (Vieira et al., 2017). In the postnatal period offspring brain development could further be altered postnatally through unfavourable attachment or parenting strategies (Murray, 1992; Field, 2010).

At the same time, animal models of obesity, hyperglycemia and exposure to maternal high-fat diets have provided direct evidence of *in utero* modulation and demonstrated that offspring brain gene expression and neuronal development are altered (Rivera et al., 2015; Edlow et al., 2016) suggesting developmental "programming" of the fetal brain. In the human it should be noted that the genetic and epigenetic predisposition to obesity, hyperglycemia or psychopathology is not limited to the offspring but will also affect the mother herself.

This chapter will introduce the current epidemiological evidence for the association between maternal obesity and child psychological and neurodevelopmental outcomes, with GDM and depression as comorbidities. The specific roles of the GDM and depression remain uncertain because studies often fail to recognise and assess their confounding or interacting effects. Thereafter development of the brain *in utero* is described followed by how the maternal milieu addressed in Chapter 1 could explain the epidemiological observed relationships. Identification of the etiological pathways leading to neurodevelopmental disorders necessitates disentangling the antenatal and postnatal chain of events so as to unveil at which stage offspring brain abnormalities are most likely initiated.

These previously reported associations between maternal obesity/depression and childhood neurodevelopmental abnormalities informed the empirical studies described in Chapters 4 and 5. These chapters cover

in utero fetal exposures across antenatal longitudinal depressive symptom trajectories and the associations with 3 year postpartum psychological outcomes in the offspring. Chapters 6 and 7 focus on neonatal brain MRI data to inform whether neurological structural abnormalities might underpin the reported risks of adverse psychological outcomes arising from exposure to maternal obesity and related co-morbidities.

2.1 Offspring neurodevelopmental and psychological outcomes

To date the literature on the effects of maternal obesity, GDM and depression/stress have suggested independent associations between these antenatal exposures and childhood diagnosis of neurodevelopmental and psychologicial disorders, including autism, ADHD, affective disorders and cognitive deficits (Rivera et al., 2015; Contu and Hawkes, 2017; Kong et al., 2020).

Findings from the two meta-analyses which take either obesity or depression as the maternal exposure are summarised in Table 2.1 below. Based on 25 studies Sanchez et al. (2018) determined that antenatal exposure to maternal obesity increases risks of *any* offspring neurodevelopmental outcomes by 50% (pooled OR: 1.51 [1.35–1.69]). Specified by outcome they reported an OR of 1.36 [1.08–1.70] for ASD, 1.62 [1.23–2.14] for ADHD, 1.58 [1.39–1.79] for cognitive or intellectual deficit and 1.42 [1.26–1.59] for emotional or behavioural problems. Across these studies, classification for ADHD and ASD varied from hospital diagnostic codes to studies using cut-off scores on subscales (e.g. hyperactivity) within screening questionnaires such as the Strength and Difficulty Questionnaire (SDQ).

In parallel, when maternal stress/depression is the antenatal exposure, Manzari et al. (2019) reported that offspring have a 60% higher risk for these neurodevelopmental disorders. Across studies, maternal states varied from clinical diagnoses of depression/anxiety, counts of depressive symptoms, experience of bereavement, stressful life events (e.g.natural disasters) which were mostly measured by questionnaires in pregnancy. Child outcomes also varied in their measurements e.g. for ASD authors used diagnoses from medical health records (such as the WHO International Classification of Diseases, Tenth Revision [ICD-10] or DSM-IV) or being inpatient or outpatients for autism services. Nevertheless, for ASD in 11 studies they reported an adjusted OR of 1.64 [1.15–2.34] and for ADHD in 12 studies an OR of 1.72 [1.27–2.34]. Not all studies provided adjustment for child sex effects or common confounders such as maternal weight, obstetric comorbidities or for the potential effects of anti-depressants.

It should be recognised that prospective studies on maternal anti-depressant use have shown associations with changes in newborn behaviour and autonomous responses which is primarily measured by tremors and startle (Zeskind and Stephens, 2004; Rampono et al., 2009) but also heart rate variability (Zeskind and Stephens, 2004). Additionally, the neonates may present with lower Apgar compared to newborns exposed to major maternal depression which was untreated (Casper et al., 2003). However, others have found the anti-depressant effect on long-term behavioural and internalising difficulties in childhood is no longer independent once maternal antenatal depression and her IQ are taken into account (Nulman et al., 2012).

Table 2.1: *Meta-analyses of maternal obesity and depression on neurodevelopmental outcomes and covariates.*

Meta-analysis	Primary Antenatal Exposure	n studies	Primary offspring outcome	Pooled OR [95%CI]	Heterogeneity (%)
Manzari et al. (2019)	Depression/Stress	15	ASD	1.64 [1.15–2.34]*	90
Manzari et al. (2019)	Depression/Stress	12	ADHD	1.72 [1.27–2.34]*	85
Sanchez et al. (2018)	Obesity	25	Any neurodevelopmental	1.51 [1.35–1.69]	80
Sanchez et al. (2018)	Obesity	11	ASD	1.36 [1.08–1.70]	61
Sanchez et al. (2018)	Obesity	7	ADHD	1.62 [1.23–2.14]	70
Sanchez et al. (2018)	Obesity	14	cognitive or intellectual delay	1.58 [1.39–1.79]	76
Sanchez et al. (2018)	Obesity	6	emotional or behavioural problems	1.42 [1.26–1.59]	88

Note:
Outcomes are compared against women of normal pre-pregnancy weight (BMI 20-25). *ORs are adjusted according to the authors of the analyses.
ASD: Autistic Spectrum Disorder; ADHD: Attention-Deficit Hyperactivity Disorder; BMI: Body Mass Index.

The lack of a holistic and comprehensive characterisation of the pregnancies associated with obesity, including whether complicated by GDM and/or depression makes causal inference and thus strategies for intervention difficult. For example, based on large observational cohort studies, prevalence of autistic conditions and attention-deficit/hyperactivity disorder (ADHD) is two to four times higher in the children born of obese women and diagnosis of GDM seems to exacerbate the contribution of obesity alone but these risks do not take maternal depression into account (Krakowiak et al., 2012b; Ornoy et al., 2015; Li et al., 2016a).

In a systematic review, Adane et al. (2016) assessed the effect of GDM on cognitive outcomes in the exposed offspring. They concluded that, overall, the results indicated a negative impact on offspring outcomes, especially in the language domain, and the included studies did report positive to negative associations and heterogeneous effect sizes. The authors exposed, from the contributing studies, a lack of standardization in both cognitive assessments (blind/unblind) and lack of universal screening for GDM across studies. Importantly, out of 14 reviewed studies, only 6 adjusted for maternal socioeconomic status (SES), 2 adjusted for maternal BMI and none adjusted for depression. They also noted many studies do not provide stratification by child sex. This may be problematic as both preclinical animal studies and population studies provide evidence for sex dimorphism with boys in particular being more susceptible to receive diagnoses of autism/ADHD, and that this could be explained by a physiological vulnerability during development (Ferri et al., 2018).

Issues with previous associations between maternal obesity and child psychological outcomes involve the lack of adjustment or reporting of several maternal factors: the possible superimposition of comorbidities e.g. GDM with depression but also risks of maternal infections, antenatal hospital admissions and adverse birth outcomes (see below). Similarly, the heterogeneity factor (or I^2, the proportion of variability attributed to non-random differences between studies) in the meta-analyses reported by Sanchez et al. (2018) and Manzari et al. (2019) in Table 2.1 is above 60% across the child outcomes which demonstrates the difficulty in comparing results between studies. To illustrate further the current limitations in the published literature in identifying etiological pathways to adverse offspring psychological outcomes following obese pregnancies, Table 2.2 was generated to show which covariates were included in the 27 studies reviewed by Kong et al. (2020). Here, only four basic covariates were chosen from the studies on the outcomes of ASD, ADHD and cognitive/intellectual outcomes.

Table 2.2: *Offspring psychological outcomes associated with obesity or diabetes in pregnancy and their covariates.*

Primary exposure	Primary outcome	Reference	Covariates			
			Child Sex	Mat. SES	Mat. Psychiatric disorders*	Obst. comorb. or birth complication
BMI during pregnancy	Cognitive Function/ Intellectual Disability	Craig et al. (2013)	1	0	0	0
Diabetes in pregnancy	Cognitive Function/ Intellectual Disability	Fraser et al. (2014)		0	0	0
Diabetes, hypertension, and obesity	ASD	Krakowiak et al. (2012b)	1	0	0	0
GDM	ASD	Lyall et al. (2012)	0	1	0	0
GDM	ADHD	Nomura et al. (2012)	1	0	1	0
GDM	Cognitive Function/ Intellectual Disability	Veena et al. (2010)	1	1	0	0
GDM, preexisting diabetes	Cognitive Function/ Intellectual Disability	Fraser et al. (2012)	1	1	0	0
Pre-pregnancy BMI	Neuropsychiatric disorders	Casas et al. (2017)	1	1	1	0
Pre-pregnancy BMI	Neuropsychiatric disorders	Casas et al. (2013)	1	1	0	0
Pre-pregnancy BMI	Neuropsychiatric disorders	Hinkle et al. (2012)	1	1	0	0
Pre-pregnancy BMI	ASD	Xiang et al. (2015)	1	1	0	1
Pre-pregnancy BMI	ASD	Suren et al. (2014)	0	0	0	0
Pre-pregnancy BMI	ADHD	Andersen et al. (2018)	0	1	1	0
Pre-pregnancy BMI	ADHD	Musser et al. (2017)	1	0	1	0
Pre-pregnancy BMI	ADHD	Chen et al. (2014)	1	0	0	0
Pre-pregnancy BMI	Cognitive Function/ Intellectual Disability	Huang et al. (2014)	1	1	0	0
Pre-pregnancy BMI	Cognitive Function/ Intellectual Disability	Bliddal et al. (2014)	1	0	0	0
Pre-pregnancy BMI	Cognitive Function/ Intellectual Disability	Tanda et al. (2013)	1	1	1	0
Pre-pregnancy BMI	Cognitive Function/ Intellectual Disability	Basatemur et al. (2013)	1	1	0	1
Pre-pregnancy BMI	Cognitive Function/ Intellectual Disability	Brion et al. (2011)	0	1	0	0
Pre-pregnancy BMI	Affective disorders	Robinson et al. (2013)	0	1	0	1
Pre-pregnancy BMI and GWG	Cognitive Function/ Intellectual Disability	Pugh et al. (2015)	1	1	1	0
Pre-pregnancy BMI and PGDM	Neuropsychiatric disorders	Kong et al. (2018)	1	0	1	1
Pre-pregnancy BMI, GWG	ASD	Gardner et al. (2015)	1	1	1	0
Pre-pregnancy BMI, GWG	Psychosis and schizophrenia	Mackay et al. (2017)	1	1	1	0
Pre-pregnancy obesity, GDM	ASD	Li et al. (2016a)	1	0	0	0
Pre-pregnancy weight and GWG	ASD	Dodds et al. (2011)	1	0	1	0

Note:
Twenty-seven studies addressing the associations between exposure to maternal obesity and diabetes in utero and child psychological outcomes were reviewed by Kong et al. (2020) where outcomes were compared against women of normal BMI (20-25kg/m2). Here four covariates/covariate groups of interest were selected and 1 marks the study included the covariate and 0 marks it was not. *Psychological covariates included psychiatric or neurological disorders or anomalies of the Central Nervous system or intelligence. ADHD: Attention-Deficit Hyperactivity Disorder; ASD: Autism Spectrum Disorder; BMI: Body Mass Index; GDM: Gestational Diabetes Mellitus; GWG: Gestational Weight Gain; PGDM: Pregestational DM; Mat.: Maternal; Obst.comorb.: Obstetric comorbidities; SES: Socio-economic status. Adapted from Kong et al. (2020).

Despite animals models advancing our understanding of the consequences of a high-fat diet (HFD) induced obesity and hyperglycemia *in utero* in controlled environments, population studies are harder to assess due to the genetic predisposition and postnatal exposures which are less commonly accounted for. However, sibling studies can explore the effect of in uterine exposures in siblings discordant for the exposure while sharing the same genetic background and familial environment. Fraser et al. (2014) found through their linkage study of Swedish registers that GDM (adjusted for pre-gestational BMI) was associated with a lower IQ score (-1.36 points (95% CI −2.12, −0.60)) and educational achievement in adult male offspring but found no associations within siblings which led them to conclude the effects were derived from external influences such as socio-economic status. However, this study did not look or adjust for maternal mental health in the pregnancies nor distinguish between pregestational and gestational diabetes. Future studies which can incorporate other discordant exposures between siblings, including treatments, infection and diet are necessary to further provide clarity on intrauterine effects.

2.2 Towards understanding effects of antenatal depression

Some methodological limitations present in the literature implicating maternal depression in the causal pathways are worth mentioning. Maternal depression is often measured from self-report questionnaires of symptoms rather than clinical diagnostic criteria, can incorporate anxiety or stress, or is referred interchangeably as “antenatal stress” (Manzari et al., 2019), all of which complicates comparisons between studies. Either separating depression from stress and anxiety or evaluating them as co-morbid events (Falah-Hassani et al., 2017), which can occur in 30% of cases(Austin et al., 2010), could clarify the antecedents for fetal risk of neurodevelopmental disorders and avenues for intervention on the mother.

Moreover, the dichotomy of depression/stress vs control can have consequences on the statistical analysis and the lack of adjustment for sociodemographic risk factors mentioned above confounding results is not trivial. For example, a meta-analysis of studies addressing antenatal depression and offspring outcomes has shown in the context of preterm birth and low birth weight that using categorical definition of depression vs continuous score moderated the association with increased odds of preterm birth and low-birthweight (Grote et al., 2010). However, the authors also identified the higher odds of depression in the mothers and the adverse neonatal outcomes in low socio-economic contexts.

Furthermore, parent-report of child outcome is often used, so researchers also need to consider the confounding effect of maternal current depression on her evaluation of the child. Nevertheless, while maternal depression, although not anxiety, induces some discrepancies in reporting internalising symptoms, the small bias has been deemed of little impact in general population research (Van Der Toorn et al., 2010).

2.3 Offspring metabolic outcomes

Despite suggestions that neurodevelopmental and psychological outcomes are more adverse in children born of obese, diabetic and depressed mothers, it is important to add that the earlier interest in the effects of maternal obesity were focused on child obesity and the related metabolic outcomes. The year-by-year increase in the proportion of children diagnosed as obese paves the way for long-term adverse health outcomes and the pertinent drive to conceptualise the etiology of population obesity. In the United States half of children who develop obesity between 5 and 14 years are already overweight when they enter kindergarten (Cunningham et al., 2014). Maternal obesity in pregnancy is not only a risk for offspring obesity but also offspring comorbidities associated with their obesity such as non-alcoholic fatty liver disease (Ayonrinde et al., 2017; Soderborg et al., 2018), type-2 diabetes (Elsakr and Gannon, 2017; Nicholas et al., 2020) and cardiovascular disease which also show sex dimorphic effects (Nicholas et al., 2020). Animal models of diet-induced maternal obesity show association with cardiac dysfunction (Blackmore and Ozanne, 2013) and hypertrophy in offspring mice in males (Loche et al., 2018) and hypertension was also more likely in these offspring. An additive effect of the antenatal exposure with post-weaning obesogenic diet was noted for severity of metabolic abnormalities (hyperinsulinemia and hyperleptinemia).

The concept of fetal programming leading to greater risk of obesity suggests that the acquired risk of an individual to develop obesity and metabolic disease is related to intrauterine processes, given that genetic influence alone appear to account for a modest effect (Willyard, 2014; Robinson et al., 2017). In many studies, the interest lies also in dissociating the intrauterine from postnatal effects especially through models of hypercalorific nutrition. Since diet is a modifiable factor it had underpin the development of several targeted

interventions for these adverse maternal-to-offspring health associations.

Others have proposed the novel hypothesis that childhood maltreatment sustained by a woman may predispose her offspring to obesity and metabolic dysregulation (Lindsay et al., 2020). Offspring metabolic and cardiac outcomes associated with maternal depression are however yet to be clarified. For example, when maternal depression symptoms are measured longitudinally through a child lifespan (3 months to 10 years of age) is has also been shown that offspring of chronically depressed mothers are not only more likely to be depressed at 9.5 years but following acute stress tasks show both a lower initial cortisol response and changes in their cardiac function, through higher initial blood pressure and cardiac output and stroke volume (Gump et al., 2009). These results are reproduced in more recent longitudinal studies (Choe et al., 2020). However, the associations and impact over that of antenatal mechanisms still needs stronger evidence given the heterogeneity in postnatal offspring behaviour following antenatal maternal mood disorders (Parsons et al., 2012; Kaydırak et al., 2021).

Dramatically, all cause mortality is increased in offspring born of obese mothers by 35% while there is 30% higher risk of hospital admission for cardiovascular events. This was demonstrated in a large linkage study of over 35 000 individuals which adjusted for important confounders such as maternal age at delivery, socioeconomic status, gestational age at birth, birthweight and sex (Reynolds et al., 2013).

2.4 Is there any overlap between offspring psychological and metabolic outcomes?

In the context of this thesis it is important to point out that conventionally, the literature on the offspring neurodevelopmental outcomes focus on the psychological functioning of the exposed offspring i.e. emotional, cognitive or diagnoses e.g. autism and ADHD. However, as mentioned above the arguably larger corpus of evidence related to maternal obesity in pregnancy refers to the child metabolic outcomes which have been mentioned. This body of work was undertaken in light of the burden of disease attributed to the globally growing prevalence in obesity in all age strata and in both sexes.

Nevertheless, obesity also has its roots in the brain, conceptualised by research on energy homeostasis, food response, satiety and reward/reinforcement processing, for which extensive and controlled animal studies provide pre-clinical evidence, including substantial literature suggesting “rewiring” of the central pathways involved through *in utero* exposure to maternal obesity (Bouret, 2009). In fact the US Endocrine Society has stated that rather than defining obesity as excess adiposity it should be regarded as a dysfunction of the energy homeostasis system (Schwartz et al., 2017). For this reason the subsequent sections present normal neurodevelopment and describes potential pathways which related maternal physiological factors to suboptimal fetal brain growth. This shapes further exploration of an empirical framework aiming to combine the offspring outcomes presented above, but underpinned by the same central pathways of neurological control, namely within the hypothalamus, including the reward and autonomic nervous system.

2.5 Brain development and morphology

The fetal brain goes through several periods of growth whereby important physiological processes and cytoarchitectural developments are defined within discrete time periods and are summarised in Figure 2.1.

By week 5 of gestation, the neural tube is formed and the subsequent period of pregnancy presents a large array of transformation, from cellular proliferation and migration through structural organisation and differentiation to apoptosis and myelination. These are often referred to as *critical periods* in neurodevelopment due to the extent, severity and knock-on effect any disruption within these periods can have on long-term functioning and therefore how these disruptions could define the future behavioural and psychological phenotype of an individual. However, the term-born neonatal brain (born >37 weeks gestation) has only reached 36% of adult size and 80% by the second year, highlighting the importance of the postnatal environment on its growth.

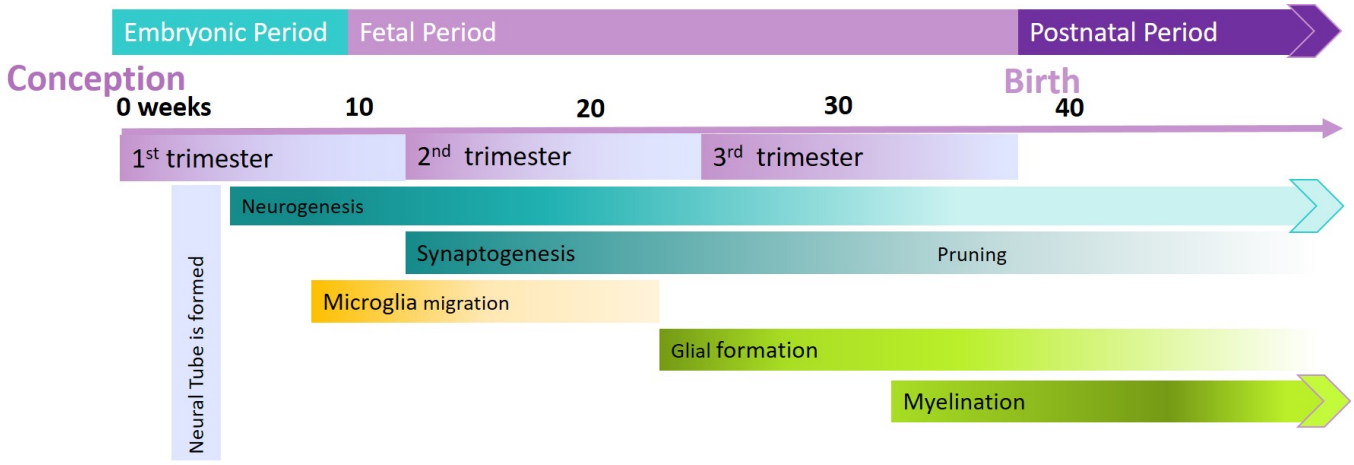

Figure 2.1: *Summarised ontology of brain development and critical windows. Neurons are produced mostly within the ventricular zone from embryonic day 42 to midgestation and, although their number is largely set by birth, neurogenesis continues to occur in adulthood in the dentate gyrus of the hippocampus and the olfactory bulb from progenitors in the subventricular and subgranular zones (Toda et al. (2019)). Fetal neuronal proliferation gives rise to the 6 cortical layers and their cells bodies remain in grey matter in the neocortex but also in the subcortical grey areas including the spine, cerebellum and midbrain structures.*

2.5.1 Grey matter (GM)

Referring to the layers of the brain which predominantly contain the cell bodies of neurons, GM is found in the cerebral cortex, eventually formed into 6 layers, the cerebellar cortex (3 layers) and as the “deep grey matter” in the medial/central areas (e.g., basal ganglia and the thalamus) and the brain stem. Cross-talk between neurons (synaptic transmission) within the GM support motor, sensory and cognitive function of an individual. These abilities emerge from a well-orchestrated process of cellular proliferation and migration in a first stage, followed by differentiation and myelination.

Neuronal migration is at its highest between 12 and 20 weeks’ gestation and largely completes by week 29. The accelerated thickening of the cortex in the third trimester is mostly due to the increased dendritic arborisation and synapse formation which peaks at 34 weeks (Tau and Peterson, 2010). Cortical folding and gyrification are essential to increase the surface area available for synaptic transmission and start around 15 weeks gestation but regionally the occipital and parietal lobes mature earlier than the frontal lobe which sees a fully developed just beyond adolescence (Tau and Peterson, 2010). Synaptic pruning and plasticity in the postnatal period remain a dynamic process involving both the regulatory developmental chronology but also

experience and thus maintain the brain's capacity to adapt but also to be impacted by specific insults. This has been demonstrated in the visual cortex which relies on postnatal sensory input at specific time period to form the necessary cortical connections that support normal visual function.

2.5.2 White matter (WM)

White matter is composed of the neural axons and their associated glial cells (microglia, astrocytes and oligodendrocytes), which support neuron growth and importantly their myelination. Oligodendrocytes are responsible for myelination and mature oligodendrocytes are present beyond 30 weeks gestation. Myelination occurs in a central-to-peripheral (subcortical-to-cortical) and posterior to anterior fashion (Barkovich et al., 1988) to support the similar pattern of axonal maturation and the prioritisation by functional necessity i.e. sensory and motor.

Myelin improves conduction of the action potential transmission along the axon of a neuron. As mentioned, in the term born neonate myelination is minimal which places a large importance on the postnatal environment in this process. However, diffusion MRI is acquired in the neonate with specific adaptation to the high water content and low myelination to study WM development in health or for example following acute brain injury (Tournier et al., 2020; Dubois et al., 2021). Disruption in the formation and maturation of premyelinating and myelinating oligodendrocytes have profound impacts on subsequent brain function especially such as in nutrient deficiency,prematurity (Guo et al., 2017), hypoxia (Agut et al., 2014) or congenital heart disease (Guo et al., 2019). While insular and thalamic projections show some myelin on histological samples in the second trimester, regions of the frontal lobe will first obtain myelin postnatally. By 36 weeks only 1% of the total brain volume is myelinated, reaching 5% by 40 weeks (Tau and Peterson, 2010). Myelination in development follows a non-linear growth from birth and is dependent on the dynamic of time and environment, including diet as in the normal adult brain axons are wrapped in myelin which is made of 70% lipids and 30% protein (Baumann and Pham-Dinh, 2001).

WM is conventionally categorised into three classes of neuronal (fibre) bundles: projection, commissural and association tracts. *Projection fibres*, such as the cortico-spinal tract and the fornix, denote those sending afference input into the brain or back to the periphery via efferent output (Jones, 2012). In contrast, *association fibres* (e.g. the long-range uncinate fasciculus and cingulum and the short-range U-fibres between cortical gyri) provide intra-hemispheric connections. *Commissural fibres* (e.g. corpus callosum, anterior commissure) relay information across the hemispheres.

2.6 The blood-brain barrier (BBB).

Development of the BBB is important in the context of this thesis given its crucial role in protecting the brain during the lifespan because it forms *in utero* the second barrier to exogenous exposures after the placenta. The BBB is a complex matrix of tightly knitted endothelial cells which allows essential molecules (glucose, amino acids etc) to be passed from the circulation but limit the passage of large molecules, water and free ions, by their low cellular proliferation and turn-over, high electrical resistance and lack of fenestration (Goasdoué et al., 2017).

Also important are the microglia which form a close contact with the BBB as the first barrier between the developing brain and CNS infiltration of pathogens. BBB development and changing permeability during

gestation and post-partum constitute both periods of vulnerability for the fetus and neonate but also provides opportunities for interventions as explored in relation to of hypoxia ischemia-related brain injury (Disdier and Stonestreet, 2020). Although the mature BBB functions to prevent, among others, pathogen infiltrations it is debated to what extent its full role has developed by birth as function continues to develop postpartum. Research in this field is restricted by the fact that these timelines are possibly species-dependent (Zhao et al., 2015).

2.6.1 Circumventricular organs

In the brain, including of the adult, there are windows of absent BBB which are worth considering. These areas have homeostatic roles due to the necessity to sense and respond to blood-derived signals from the periphery and also release agents such as neurohormones. These areas, named collectively as the *circumventricular organs* flank the third and fourth ventricles of the brain. They comprise the *organum vasculosum of lamina terminalis*, *the median eminence*, *the area posterma*, *subcommissural organ*, *the subfornical organ*, *neurohypophysis* (pituitary) and *the pineal gland*.

The hypothalamus is particularly vulnerable to the external circulation since it flanks the third ventricle bilaterally where the lamina terminalis forms the anterior delineation of the hypothalamus, the median eminence its inferior boundary and it has many of its neurons terminate and/or release molecules into the attached pituitary.

2.7 The role of nutrition in brain development

As mentioned above, development of the brain is particularly susceptible to the quality and availability of nutrients it receives. As a modifiable factor, nutrition during pregnancy plays a vital role as a regulator to brain growth not only during *in utero* development but also postnatally (Lindsay et al., 2019). A short description of the nutrient requirement follow, as Chapter 4 implicate the antenatal diet of the mother.

Normal development of the brain relies on a large variety of nutrients, the most prominent being listed in Table 2.3. Glucose is the source of energy for all fetal organs of the fetus while other components such as amino acids and fatty acids are also essential, with the balance of long-chain polyunsaturated fatty acid omega-6 to omega-3 intake (including docosahexaenoic acid [DHA]) being a focal point of research in fetal brain development (Cheatham, 2019; Basak et al., 2020). The role of animal models in clarifying associations between maternal nutrition and offspring brain development within the DOHaD framework has been reviewed by DeCapo et al. (2019). There, it alludes to the complexities in translational to humans particularly as a large portion of neurodevelopment in rodents, whose brain are more immature at birth, occurs after delivery.

In humans, the normal neurodevelopment relies on the timing of intake and concentrations of nutrients(Cheatham, 2019). Many have different potency and toxicity depending on their range of concentration (e.g. iron) and their presence or deficiency has a range of effects according to their necessity at a given stage of development (e.g., synaptogenesis, myelination). Nutrient needs can act globally or be localised within the developmental timeline of the various brain structures themselves. Early fetal nutrient deficiencies impact early cell proliferation (of all cells including neuronal and glial) whereas later nutrient insult could cause abnormal dendritic arborisation but also influence neurotransmitter availability (Georgieff, 2007). A more recent such example and a first hint of sex dimorphism is reflected in rodent models of maternal

low protein diet in pregnancy and lactation which showed that male offspring rather than female showed increased stressful behavioural response which was regulated by Neuropeptide-Y genes.

Table 2.3: *Nutrients involved in brain development and consequences of their deficiencies.*

Nutrients	Brain requirement	Circuit/Process affected by deficiency
Protein-energy	Cell proliferation, cell differentiation	Global
	Synaptogenesis	Cortex
	Growth factor synthesis	Hippocampus
Iron	Myelin	White matter
	Monoamine synthesis	Striatal-frontal
	Neuronal and glial energy metabolism	Hippocampal-frontal
Zinc	DNA synthesis	Autonomic nervous system
	Neurotransmitter release	Hippocampus, cerebellum
Copper	Neurotransmitter synthesis, neuronal and glial energy metabolism, antioxidant activity	Cerebellum
LC-PUFAs	Synaptogenesis	Eye
	Myelin	Cortex
Choline	Neurotransmitter synthesis	Global
	DNA methylation	Hippocampus
	Myelin synthesis	White matter

Note:
Adapted from Georgieff (2007). LC-PUFAs, long-chain polyunsaturated fatty acids.

2.8 Offspring outcomes in relation to fetal brain development in the obese pregnancy, potential mechanisms

Maternal obesity has been associated with a mild inflammatory state, reflected in an elevation of pro-inflammatory cytokines. In relation to fetal brain development, it has been hypothesized that maternal chronic transfer of pro-inflammatory cytokines may affect important fetal regulatory systems. This includes impairing serotonergic and dopaminergic circuit development and function, which underlie the emotion and reward regulatory networks implicated in many psychiatric conditions, ADHD and autism. Similarly, animal models have demonstrated that the central melanocortin system, which regulates energy intake and eating behaviour, is impacted by exposure to inflammatory cytokines (Scarlett et al., 2008; Rivera et al., 2015).

The combined effect of maternal inflammation on these systems may contribute to the increased prevalence of obesity in children with autism and ADHD when compared to their normally developing peers (Cortese et al., 2016; Zheng et al., 2017).

Another hypothesis suggests that obesity and diabetes may present an unfavourable environment which could antagonize the maternal immune response to infection. This could disrupt the protective capabilities of the placenta in an acute manner and alter fetal brain development directly by transfer of immune cells and molecules. This could also be achieved indirectly via fetal endogenous immune activation, as suggested by animal models (Money et al., 2018). This theory echoes the finding that maternal hospitalization during pregnancy due to infection in the first or second trimester incur higher risk of ASD diagnosis in the child (Atladóttir et al., 2010).

It should be noted that the obesity-related predispositions to infection and immune activation is regarded as a potential cause for extreme preterm birth (Goldenberg et al., 2008). This may lead to an activation or priming of fetal brain microglia. As microglia continue to proliferate postnatally, other insults (e.g. hypoxia, birth complications) could contribute to sustained impairement of microglial function in the brain through the lifespan. An example of this was demonstrated in murine models by Edlow et al. (2019). Following a high-fat diet (HFD) induced obesity of dams, placental and offspring brain cells of their offspring showed a higher proinflammatory cytokine secretion upon immunological stimulation than controls (3.8 fold in brain and 5.1 in placenta) but not when unstimulated (18 control and 16 obese pregnancies). Interestingly, this effect was more pronounced in the male offspring cells than female.

Maternal immune activation (MIA) has been extensively studied in animal models to inform on the severity of outcomes and symptom clusters based on the timing and type of infection during gestation. Samuelsson et al. (2006) showed that offspring of rats injected with human IL-6 in late gestation (days 16, 18, and 20) but not earlier (days 8, 10, and 12) were negatively impacted on spatial memory and learning. This was replicated by a PolyI:C protocol by Meyer et al. (2008b) who also demonstrated that early injection however led to disruption in sensorimotor gating and a reduction in dopamine receptors in the prefrontal cortex of the adult offspring. However, enhancing genetically an anti-inflammatory response (IL-10) of microglia can rescue offspring behavioural abnormalities arising from maternal viral infection (Meyer et al., 2008a). Studies such as these indicate that anti- and pro-inflammatory processes are intertwined in normal brain development and studies which have aimed to characterise the role of microglia during neurodevelopment and within MIA have clarified their implication in neuronal differentiation and maturation but also in synaptic pruning and the formation of the brain circuitry (Knuesel et al., 2014).

Murine models have also shown that exposure to both maternal diabetes and maternal immune activation *in utero* resulted in changes in the neurodevelopmental and inflammatory transcriptome in the fetus and are implicated a disruption in the dopaminergic neuron differentiation and the innate immune response (Money et al., 2018). In mouse models of diabetes, offspring circuits involved in feeding are altered within the hypothalamus. More specifically, adult offspring of diabetic dams showed lower fibre density although not number of neurons projecting to the paraventricular nucleus. Whereas leptin is required for the normal development of neurons enmanating from the arcuate nucleus of the hypothalamus, the authors reported offspring neonatal leptin resistance which was also present in adulthood (Steculorum and Bouret, 2011a). In another study of mice models of diabetes it was found that offspring had decreased cortical thickness, increased atrophy in the hippocampus and reduced neurogenesis in the subventricular zone and cortical layers which

persisted long-term and had impact on cognitive performance. However, intracerebroventricular insulin or nasal administration in the neonate rescued some of the central and cognitive effects (Ramos-Rodriguez et al., 2017).

2.9 Maternal depression/stress and brain development: potential mechanistic pathways

The description of the interplay between glucocorticoids, inflammatory cytokine and glucose in the mother were described in Chapter 1. However, maternal cortisol is so far extensively studied as a biomarker mediating maternal mental health and child health. This is due to its effect on the fetal HPA axis and its ubiquitous metabolic conversion to cortisone by placental 11beta-HSD-2, which reduces fetal cortisol exposure. The effects of obesity on placental structure already described could impact on this protective barrier resulting in high cortisol passage, impacting the fetal cortisol reactivity and the negative feedback loop of the offspring HPA axis (Van den Bergh et al., 2020).

Studies of maternal depression in humans have used electroencephalography and functional and diffusion MRI. Effects of maternal stress have focused on the offspring amygdala, which is implicated in stress reactivity, anxiety and emotion regulation. Decreased amygdala to thalamic connectivity has been observed in preterm neonates (Scheinost et al., 2016) and increased inverse amygdala-frontal connectivity in month-old infants exposed to maternal antenatal depression compared to non-exposed preterm born infant (Posner et al., 2016). Buss et al. (2012) studied bilateral amygdala and hippocampus in 35 girls and 30 boys aged 7 and showed that higher maternal cortisol at 15 weeks associated with larger amygdala volume in girls, which in turn was associated with higher affective symptoms. A systematic review suggest the role of antidepressant use in pregnancy in the neurodevelopmental outcomes in the children remains to be clarified given the large heterogeneity in these outcomes (Previti et al., 2014). Effects of treatment on cortical thinning vary according to whether effects are adjusted for postnatal depression and at which gestational age depression is measured (Van den Bergh et al., 2020).

Biological mechanisms implicated in the relationship between maternal antenatal depression/stress and offspring neurodevelopmental disorders are largely explored by measuring cortisol in maternal saliva or blood, but our understanding remains limited. For example, when cortisol is measured in the amniotic fluid at mean 17 weeks in pregnancy, it is associated with poorer child cognitive outcomes at 17 months, independent of demography, prenatal smoking, alcohol use, prescription medication and SES. However, this is only seen in dyads presenting poor postnatal attachment, suggesting a moderation of this relationship (Bergman et al., 2010). In this study an issue requiring further research and attention is that the amniotic fluid cortisol was not associated with maternal stress or depression which could indicate the importance of differentiating between the direct fetal cortisol level (endogenously produced or passed through the placenta) and the maternal salivary or blood cortisol. Alternatively this result could be related to the use of single-point measure in early pregnancy. However, bias due to maternal reporting of the child can be excluded as the child outcomes were measured by a blind assessor using a the highly standardized Bayley Scales of Infant Development—Second Edition (BSID-II). Nevertheless, when using maternal hair cortisol, less influenced by diurnal fluctuations, sexual dimorphic associations are seen with electrophysiological responses (Troller-Renfree et al., 2020; Freedman et al., 2021) and amygdala structure and connectivity (Stoye et al., 2020) of the newborn.

Osborne et al. (2018) performed a prospective study among 49 women with a diagnosis of Major Depressive Disorder (MDD) by DSM criteria and compared with 57 controls. They found that MDD women had higher inflammatory profiles (IL-6, IL-10, VEGF and TNF-alpha, not associated with BMI, at 25 weeks gestation) and salivary cortisol in pregnancy (32 weeks) and shorter gestation. Neonatal neurobehavioural outcomes at 6 days were poorer following maternal MDD exposure and child saliva cortisol reactivity to stress at 12 months (adjusting for maternal current depression) was higher. The last visit also included developmental assessment of the children which found no significant difference between the groups but was interpreted as underpowered given larger cohort studies measuring outcomes later have found decreased cognitive performance, IQ and delayed development (Deave et al., 2008; Tse et al., 2010).

Chapter 4 proposes that maternal depressive symptoms are associated with a wider range of metabolic and immunological phenomena which could confound previous studies which placed cortisol as the primary biomarker in the interaction between maternal mental health and child psychological outcomes.

2.10 Preeclampsia, GDM and offspring psychological outcomes

In GDM studies other comorbidities may be implicated in offspring psychological disorders i.e. whether diabetes is superimposed with hypertensive disorders, including preeclampsia, which is also prevalent with in pregnancies with obesity.

In their meta-analysis, Maher et al. (2018) pooled studies with hypertensive disorders as the maternal exposure, including over 1.3 million participants and showed an pooled adjusted OR for ADHD of 1.29[1.22 to 1.36] and among 770 000 participants an OR for autism of 1.35 [1.11 to 1.64]. However, only one study reporting an the association with ASD and one with ADHD adjusted for confounders of maternal age, socioeconomic status, ethnic origin, and family history of mental illness. In the former these authors adjusted for pregestational diabetes but did not report on GDM (Curran et al., 2018) and in the latter on ADHD outcomes there was no report on either pre-gestational or gestational diabetes (Getahun et al., 2013).

A large study on the associations between maternal GDM and ASD risk in the exposed offspring was reported by Xiang et al. (2015) which included 310 ASD children exposed to GDM. They controlled for maternal age, parity, education household income, race, history of comorbidity, child sex and preeclampsia. Notably, 1269/25035 (5.1%) of all GDM pregnancies had superimposed preeclampsia and 10282/290792 (3.5%) of non-GDM pregnancies had preeclampsia. Xiang et al. (2015) found that children who were exposed to GDM diagnosed prior to 26 weeks, but not after 26w, had a HR of 1.42 for ASD. Bivariate analysis showed the obesity was associated by HR 1.22(1.00-1.49) with ASD diagnosis but so was preeclampsia by HR: 1.44 (1.22-1.70) as well as maternal age, parity, education, income and race and male sex (5.10[4.62-5.62]).

2.11 Birth outcomes and psychological risks

The timing and recurrence of potential adverse exposures (and “insults”) to the offspring brain in the antenatal and peripartum remains uncertain and formulating etiological models of neurodevelopmental outcomes is therefore complicated. Nevertheless, when brain development occurs well into the first year, there is a possiblity of a “multiple hits” scenario which disrupt normal brain trajectories. Neonatal complications could in this way mediate or moderate the relationships between antenatal exposures and neurodevelopmental

disorders. Below, the examples of neonatal hypoglycemia and preterm birth are described.

2.11.1 Neonatal hypoglycemia

Neonatal hypoglycemia can present as seizures in the first 48h of life, with or without overt injury to the brain on imaging studies, and has been associated with adverse outcomes on executive and visual motor function at 4.5 years (McKinlay et al., 2017). Additionally, neonatal hypoglycemia is also a risk factor for autism in term-born children (Buchmayer et al., 2009).

Of relevance to the hypotheses in this thesis, neonatal hypoglycemia is more likely to follow obese and GDM pregnancies even when GDM is treated (reported in 40% of cases, Kole et al. (2020)). It is more likely among macrosomic neonates than normal size neonates (Weissmann-Brenner et al., 2012) or in large for gestational age neonate (47%) but also small-for-gestational age infants (52%) (Harris et al., 2012) Neonatal hypoglycemia occurs of a sustained post-partum increase in insulin secretion which *in utero* compensates for raised maternal-fetal glucose transport. Another cause of neonatal hypoglycemia is when the infant's glycogen store within the liver is low due such as in the case of prematurity. .

2.11.2 Preterm birth

Preterm birth (<37 weeks gestation) is the leading risk factor for neurodevelopmental deficits and disorders (occurring in 30% of preterm born children) but also cardiovascular and metabolic disturbances (Luu et al., 2017). Each week shorter of term gestation increases odds of ADHD (Sucksdorff et al., 2015). However, the risk of preterm birth increases with BMI (Cnattingius et al., 2013) while shorter gestations and lower birth weights are themselves independent risk factors for autism and ADHD (Bhutta et al., 2002; Johnson and Marlow, 2011). It is possible preterm birth confounds some interpretation of relationships between maternal obesity and offspring neurodevelopment.

It should be noted that it is often underreported in the literature whether preterm birth was induced or spontaneous. This is important to distinguish when comparing outcomes against normal-weight pregnancies. Current reports fail to account for the implication of interventions and higher frequency of antenatal risks assessment and monitoring in the obese pregnancies. For example, following GDM diagnosis, clinical guidelines such as in the UK prescribe an induction of labour if no spontaneous labour occurs by 40 weeks gestation. Furthermore, more frequent maternal monitoring may call for preterm elective c-sections.

In this regard, in their meta-analysis of 84 studies McDonald et al. (2010) identified pooled risk of overall preterm birth before 37 weeks and of spontaneous birth was similar between normal-weight, overweight and obese pregnancies but taking into account publication bias overall preterm birth was more likely in obese women. However, *induced* preterm birth was more likely in obese (pooled RR 1.56 [1.42 to 1.71]) and very obese pregnancies (OR 1.71 [1.50 to 1.94]). Additionally, they found that preterm birth < 33 weeks was more likely in obese and very obese women RR 1.45[1.23 to 1.71] and 1.82 [1.48 to 2.24] respectively. Nevertheless the authors claimed the lack of adjustment for socioeconomic status was frequent and they did not report on whether maternal psychopathology was taken into account.

It should be noted that there could be antenatal factors which precipitate preterm birth which also are on the causal pathway to induce changes to fetal neurodevelopment. Currently, genetic predispositions to preterm birth within human observational studies of obstetric complications (infection, preeclampsia and

GDM) remains to be addressed. (Biggio et al., 2008). The birth complications such as low Apgar score, birth seizures and intracranial bleeding could also be important mediators between preterm birth (assuming shorter gestation as the main risk) and adverse psychological outcomes of ASD but are not readily reported (Buchmayer et al., 2009).

2.12 The Placenta

In the process of understanding the passage of potential epigenetic or environmental modulators to fetal brain growth it is necessary to contemplate the first mediator in these mechanisms which is the placenta. In a healthy pregnancy the placenta functions as the interface between the mother and fetus, allowing for exchange of necessary gases and nutrients, through the villous labyrinth at the fetal side. The placenta also provokes maternal hormones secretion to meet the demands of the pregnancy and the fetus, adapting its response to cues from both sides (Sandovici et al., 2012). The extent to which placental function is disrupted in obesity is an intense focus of research from initial placentation throughout pregnancy (Dimasuay et al., 2016).

2.12.1 Placental structure and inflammation

Disruption to the maternal physiological environment may be confer suboptimal placental growth from the time of placentation. Diet-induced obesity mouse models show decreasing microvessel density (Stuart et al., 2018). Placental tissue samples from human obese pregnancies show abnormal angiogenic regulation, increased oxidative stress and inflammation (Saben et al., 2014). Other findings include disruption in immunological balance sustaining the maternal-fetal tolerance which compounds the risk of impaired placentation and poorer pregnancy outcomes (Pollheimer et al., 2018).

Placental inflammation is regarded as a contributory factor to fetal brain injury through ischemic injury and/or the fetal immune response (Leviton et al., 2005; Dammann and O'Shea, 2008). Studies show that neonates born premature and in pregnancies showing histological evidence of placental chorioamnionitis had a widespread white-matter changes compared to unexposed premature infants (Anblagan et al., 2016) and higher risk of cerebral palsy (Freud et al., 2019).

While the placenta is considered an adaptable and effective barrier protecting the fetus from a harmful maternal environment, it is possible that a sustained and chronic exposure to a low-grade inflammation may facilitate trophoblast transfer of proinflammatory cytokines and other small molecules (Catalano et al., 2009; Denison et al., 2010) which to a disruption in fetal neurodevelopment (Rivera et al., 2015). Indeed, some have reported higher concentrations of proinflammatory cytokines IL-1beta, IL-6 and TNF-alpha in the placental tissue of obese human pregnancies (Challier et al., 2008). This does not exclude immune activation in the placental tissue itself such as induced by oxidative stress when vascularization and endothelial function may be further disrupted in such environment (Stewart et al., 2007; Hastie and Lappas, 2014). Additionally, placental secretion of these proinflammatory cytokines may be influenced by the hormones implicated in the pathogenesis of GDM such as leptin and adiponectin (Lappas et al., 2005).

2.12.2 The placenta and obstetric comorbidities

Obesity and GDM could have a direct effect on the rate, amount and composition of substrates (i.e. glucose, fatty acids, amino acids) passing through the placenta into the fetal circulation supplying the brain. Diabetes in pregnancy could have serious consequences for the perfusion efficiency of the highly vascularized placenta. The placenta of GDM pregnancies show more frequent villous immaturity and increased angiogenesis, increased placental weight, larger volume of the parenchymal tissue and more evidence for fibrinoid necrosis and choangiosis (Huynh et al., 2015).

Maternal vascular malperfusion (MVM) in the placenta is thought to develop or may contribute to hypoxic damage of the tissue as a consequence of perturbed remodelling of the spiral arterioles early in pregnancy and impairments of trophoblastic invasion. In one report, MVM was obsrved in 30.6% of GDM placentas and is associated with a two-fold increased risk of delivery of small-for-gestational age in overweight and obese women (Scifres et al., 2017). These effects play a role in underperfusion and chronic placental injury which could explain detrimental outcomes in the neonatal brain which relies on optimal oxygenation and nutrient transfer (Weiner et al., 2018).

2.13 Hypothalamus and the reward pathway

Previous sections have mentioned the role of the ANS and the hypothalamus in relaying or initiating important signals towards the periphery, receiving feedback modulatory information and as a central component of the HPA-axis. Beside neuropeptide release towards the pituitary, the hypothalamus integrates the vast array of sensory and motor afferents from the peripheral nervous system, the gut, heart and the central homeostasis system required under starvation, hypernutrition, hyper/hypothermia, psychological stress and the circadian cycle.

Hypothalamic function thus relies on the neuronal white-matter fibres which directly relay signals to and from the viscera which engages these homeostasis system of the CNS. The hypothalamus is an integral part of the limbic system via fibres reaching the frontal lobe, the amygdala and hippocampus which are involved in emotion regulation and cognitive function, as well as reinforcement and sensory processing. The hypothalamus mediates the WM fibers connecting the ventral tegmental area to the nucleus accumbens, the major pathway for reward and hedonistic sensitivity, included in models of addiction. These networks can, therefore, satisfy an interplay between behaviour, external stimulation and internal processing across many domains and have been implicated in neurodevelopmental disorders such as autism and ADHD but also eating behaviour. For example, comfort eating has been shown to dampen the stress response via the certain areas of the amygdala which can have dual excitatory and inhibitory effect on the stress vs reward/hedonic system (Ulrich-Lai et al., 2010). The relationship between anhedonia and the reward system has been explored in animal models which suggest that early life adversity is a risks for an aberrant reward structural and functionnal connectivity implicating the amygdala, medial PFC, ventral tegmental area and nucleus accumbens (Bolton et al., 2018).

These systems involving the hypothalamus and beyond into the limbic system are therefore good candidates for exploring the hypothesis that a structural abnormality of the CNS could explain phenotypes of both neurodevelopmental and metabolic disorders in the offspring following adverse maternal exposures, where these brain structures are implicated. The introductions to Chapter 6 and 7 will describe this in further detail

with emphasis on the neonatal hypothalamus and the reward network which has not been previously studied *in vivo* in human infants.

2.14 Conceptual framework

Building on the evidence above the framework which was developed during the process of the PhD is illustrated in Figure 2.2.

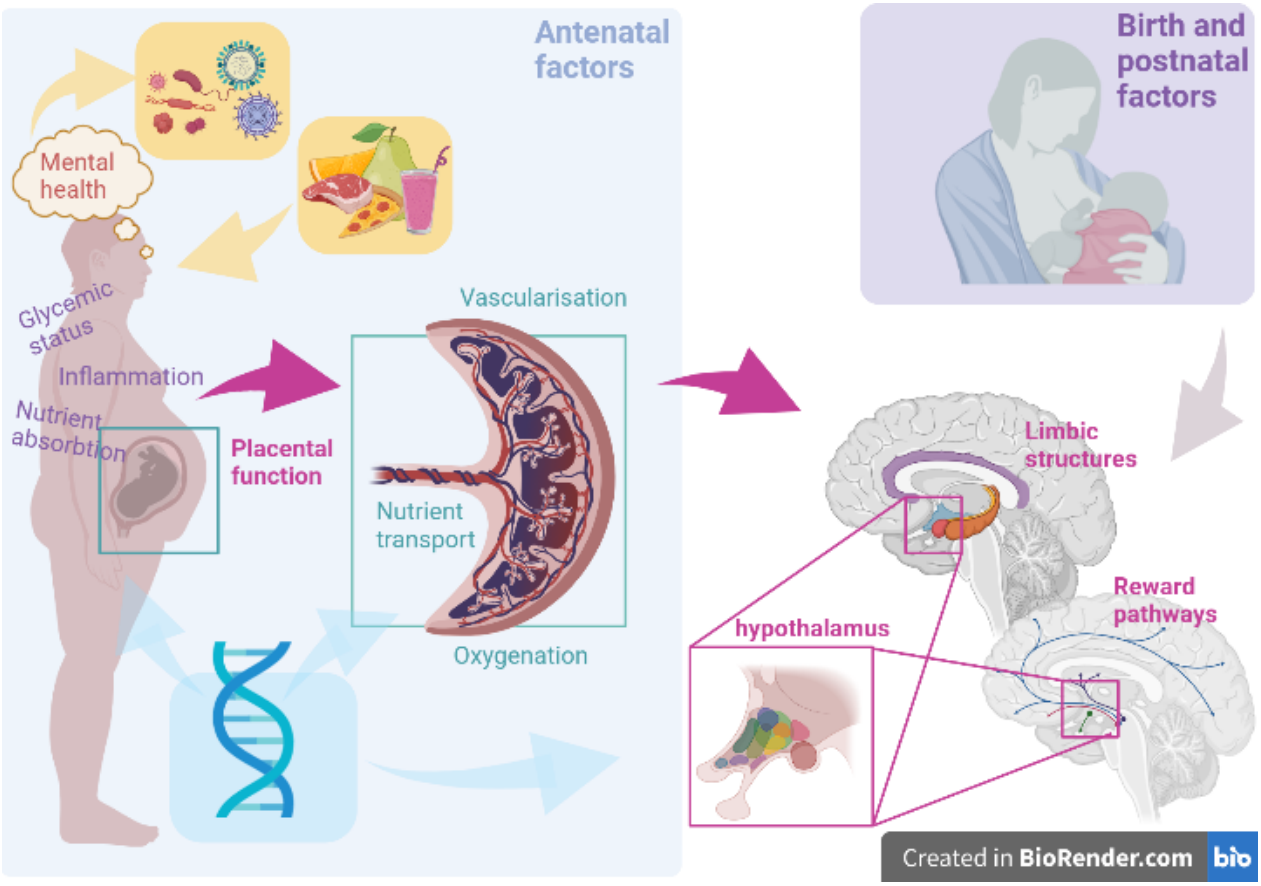

Figure 2.2: *Conceptual framework for the exploration of the effect of maternal obesity on offspring neurodevelopmental outcomes. In the antenatal period many maternal factors can influence fetal brain development such as inflammation, glycemic status, diet and exposure to pathogens. Placental function is associated with maternal and fetal feed-forward and feed-back loops which shapes its dynamic physiological adaptation. Other factors which could moderate long-term effects include birth events such as delivery mode, prematurity and neonatal intensive care admission and presence of hypoglycemia. Postnatal factors may impact neurodevelopment through indirect effects of mother-child attachment, nutrition and external stressors derived from the socio-economic context. The neural pathways of interest relate to the hypothalamic function and its relay between limbic structures and the wider reward network but also as the master regulator of the autonomic nervous system and energy homeostasis.*

Chapter 3

Methods

This chapter provides an overview of the methods employed in the studies described in Chapters 4 to 7, including the study samples involved and the statistical approaches. The latter relates to the structural equation modelling toolbox which supports an epidemiological framework for the enquiry of the causal pathways between maternal obesity and offspring neurodevelopment. Thereafter the perinatal MRI modalities employed to explore the hypotheses developed from the epidemiological background are described, namely diffusion and structural MRI of the brain.

3.1 Study samples

3.1.1 UPBEAT

Chapter 4 and 5 present studies using available data collected through the UK Better Eating and Activity Trial (UPBEAT) (Poston et al., 2015). UPBEAT was a randomised control trial aimed to prevent GDM and lower the incidence of large-for-gestational age newborns (>90th customized birthweight centile). The study was undertaken between 2009 and 2014 with approval from the National Health Service Research Ethics Committee (UK Integrated Research Application System; 09/H0802/5) and the trial results published in 2015. During this period, 1554 women of BMI $\geq$ 30 kg/m^2, age >16 years and meeting various inclusion criteria were consented to participate from 8 UK NHS trusts (Bradford, Glasgow, 3 London Centers, Manchester, Newcastle and Sunderland) (Poston et al., 2015). The intervention promoted diet substitutions in order to decrease saturated fat intake and glucose load, together with an incremental increase in physical activity.

Although the primary outcomes were not achieved, the intervention resulted in a reduction in gestational weight gain and some maternal anthropometric measurements in the third trimester, as well as self-reported saturated fat and glycaemic load intake in the intervention group. The protocol incorporated maternal blood sampling at three antenatal visits as well as food diaries, anthropometry measurement, health questionnaires and depression scales. Summaries of pregnancies and neonatal outcomes were collected from hospital records and in a subsample cord-blood data was available. The UPBEAT trial was subsequently extended into a 6-month and 3-year follow-up with extensive collection of maternal and infant health, anthropometric and lifestyle variables. The 3-year follow-up included a child psychobehavioural questionnaire (Strength and

Difficulty Questionnaire SDQ) and anthropometric measures which are included for the purpose of the thesis.

Given that the primary outcomes were not achieved but that the intervention had some effects on mothers and their offspring longer term, the analyses in this thesis take the randomisation arm into account in the outcomes under investigation. Additionally, only pregnancies which had confirmed live births in the dataset (1490/1554) were included and all details are provided in each relevant chapters.

3.1.2 Developing Human Connectome Project - dHCP

The developing Human Connectome project was a collaborative projects between King's College London, Imperial College London and Oxford University (http://www.developingconnectome.org). It received ethics approval (14/LO/1169) to model brain development from 20 weeks gestation until the first month of birth (up to ~ 44 weeks postmenstrual age) using MRI. Pregnant women and parents of newborn babies were invited for a scan of approximately 60 minutes and subsequently invited to attend a follow-up neurodevelopmental assessment between 18-24 months. The imaging dataset and processing pipelines comprises cross-sectional and longitudinal elements from over 250 pregnancies and over 500 newborns and includes structural, diffusion and functional imaging (Hughes et al., 2017; Makropoulos et al., 2018; Fitzgibbon et al., 2020). All scans were performed at St Thomas' Hospital using a bespoke neonatal system (Figure 3.1) using a 3T Pillips scanner under medical supervision and included state-of-the-art structural, diffusion and functional brain imaging acquisition. Further details are provided in relevant chapters.

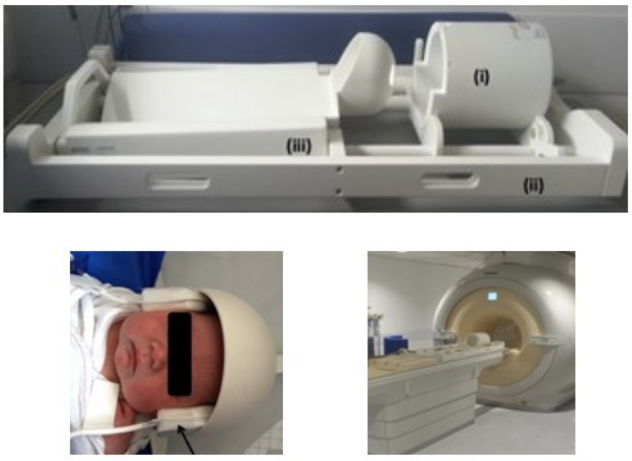

Figure 3.1: *The dHCP acquisition protocol includes a bespoke neonatal brain imaging system. Upper figures shows the head coil (i), the frame (ii) and the positioning shell (iii) which allows the neonate (lower left) to fit comfortably and snugly to minimise head movements for improved signal-to-noise. Adapted from Hughes et al. (2017).*

3.2 Statistics

3.2.1 Structural Equation modelling (SEM)

SEM and its applications refer to the tools which can incorporate, at the statistical level, a wide range of relationships to data in the study of both simple and increasingly complex phenomena. In science it addresses the interest of answering questions pertaining to the quality of *measurements* and the need for *prediction* (E. Kevin, 2015). In its causal modelling capacity (i.e. the "structural" model) of SEM it is unlike the common usage of multiple regression in that it incorporates more than one equation simultaneously. In SEMs, explanatory (exogenous) variables can be dependent variables (endogenous variables), such as when mediation is of interest. SEM models also allow for variables to correlate with each other on causal (regressive) paths. The whole process of model building is primarily informed by theory and thereby allows researchers to test whether a theory is supported by comparing empirical data to the theoretical pattern of associations between variables. The ability to iteratively build and compare models through SEM applications thus supports both hypothesis testing and but also exploratory designs.

SEM is related to path analysis in the way that it characterises predictive relationship and correlations between variables but has the distinction that SEM usually implies a measurement aspect is incorporated, i.e. through "latent" variables. *Latent* ("hidden") factors can represent a construct of interest which is otherwise *inferred* because it is *unobservable*, for which only one or several proxy observed measure(s) thereof are available. An example is the construct of *obesity* being often inferred by BMI from weight (kg) and height (m^2) only, even if weight and height do not intuitively represent the fat-to-lean mass distribution when one thinks of obesity. Such proxy measures of a construct, if introduced as observed factors in a regression as is done outside SEM, would inherently include measurement error because they are not the direct measures of the construct of interest.

An alternative to BMI would be to create a latent obesity factor which is "indicated" by not only weight and height but also anthropometric measurements, fat volume etc. In SEM such a construct would be characterised under a *measurement model* where the latent factor represents the overlap of variance of the proxy observed measures. In doing so the measurement error for the construct decreases and this provides more accurate coefficients in the equations. Said differently, bias due to measurement error of the factors in SEM reduces the estimation accuracy by increasing the unexplained variance. However, some observed factors can hold less bias than others and do not require latent factor modelling. Age is a good example which can be measured accurately albeit not infallible to random error if recording of dates is incorrect. Nevertheless, when including latent variables, the researcher is equally assessing the likely measurement error implied by observed measures (indicators) but also if these indicators related substantially and thus reliably, as hypothesized, with the latent variable.

Other applications of latent variables extend to mental health such as depression and other types of affect (Bollen and Noble, 2011) which are usually implied from questionnaires or scales using items relating to symptomology. Questionnaire items are assumed to measure the same construct and would have been previously validated through confirmatory factor analysis (CFA another SEM application) to ascertain each item contributes satisfactory variance to the latent factor. CFA is therefore used in the field of psychometrics to assess measurement quality, where validation of scales for unobservable latent constructs is of interest.

Multiple latent variables can be included in a SEM so that they are covariates, independent or dependent

variables just as observed variables. Importantly, one can also assess the likely associations between latent variables, especially when these are theoretically a combination of facets, such as is often the case in psychology with the example of executive function (Bollen and Noble, 2011). Therefore SEM-derived techniques allow for theoretically driven associations to be included in causal inference modeling, can integrate constructs with more fidelity and have the benefit therefore of reducing measurement error. Moreover, SEMs can include all types of variables (continuous, dichotomous, nominal, ordinal, censored) and applications include growth curve models, cross-lagged models and are used also in twin-studies and other nested (hierarchical) modelling studies.

SEMs unequivocally *supports* causal inference under an umbrella of need for description of phenomena and empirical testing. However, SEM is sometimes inappropriately promoted to infer *causality* whereas it actually implements associations reflecting the causal *assumptions*, data derived, rather than reality derived, made by the researcher/operator. In turn the researcher may have the eventual ambition to interpret well fitted models as more plausible with reality than ill-fitted ones.

The steps for building SEMs are often described as follows (Bollen and Noble, 2011; E. Kevin, 2015):

1. **model specification**: what does the research aim to evaluate? i.e. are all associations tested characterised (causal or correlated), latent measurement models specified and does it build upon the theory and/or the hypotheses of interest? Usually, the input is reflected by a path diagram, the visualisation of which enables the researcher to include these associations. To keep in mind is that directionality of associations and the absence or presence of associations also reflect assumption of directionality, absence or presence in the data, which should be justified. The model-implied moments are the means, variances and covariances being predicted by the model and if those align with the true population model then the specified model should hold and this should be reflected in the convergence (identification) and the model is said to fit the data (see below).

2. **identification**: A model is identified if a unique valued for all parameters requested (e.g. regression coefficients, variances, factor loadings) can be obtained. *Under-identified* models to not provide enough information for parameter estimation as there are more than one possible solution for the estimates (X + Y=10). *Just-identified* (“saturated”) models provide a perfect fit which will only provide one solution to reproduce the correlation matrix, e.g. conventional multiple regressions, despite potential inherent other sources of errors (in measurement) (E. Kevin, 2015). In *over-identified* models, there are more equations than unknowns so that, to the benefit of a researcher, multiple hypotheses can be tested (falsified) and they can equally conclude whether a model fit the data or not, unlike the just-identified models. Overidentification is obtained by fixing parameters, often to zero, which are not estimated, a process which should be guided by theory preferably but admittedly can also be forced by convergence issues.

3. **estimation**: The most prevalent estimator which utilises all the relationships inherent in the model (“full information” estimator for e.g. covariances, means, residuals) is the Maximum Likelihood, which assumes multivariate normality. However, in the software used in this thesis to implement SEM methods, *Mplus* implements the robust ML which can deal with non-normality. This software takes a first guess at the parameter estimates, calculates its implied covariance matrix and then compares with the actual data covariance matrix. In an iterative manner the estimates are adjusted until the fitting criterion set by the user or algorithm (e.g. Maximum Likelihood) complies with the criterion which minimises the a

loss function and reflects a very close similarity between the two matrices. The lower the fitting index, the closer the implied model matrix to the observed matrix.

4. **model fit**: As alluded above, the model fit is characterised by the difference between the sample covariances and the covariances predicted by the model. The smaller this difference the better the fit. The Chi^2 test is first reported by *Mplus* where a nonsignificant value indicates there is not evidence to reject the H0 hypothesis that the estimated and predicted covariance matrices differ. However, well known in the SEM literature that the Chi^2 test is bound to be significant as sample size increases (>200) and is not used to assess fit in large samples. Indices based on the residual and provided by *Mplus* include the Root mean square error of approximation (RMSEA; Steiger (1990)) and the standardized root mean square residual (SRMR). Steiger (1990) suggested a value below 0.10 to indicate good fit and under 0.05 as very good. Hu and Bentler (1999) suggested 0.06 as the cutoff for good fit using the RMSEA and 0.08 for SRMR. Other indices of fit relate to the Comparative fit, where the baseline null model (i.e. where variables in the composed model have no relationship specified) is compared to the researcher's model including the associations. The Comparative fit index (CFI) and the Tucker-Lewis index (TLI) of 0.95 and above are considered good fit to the data. Lastly, parsimonious fit refers to the cost-benefit trade off of fit and degrees of freedom (E. Kevin, 2015) and include the Aikaike information criterion (AIC), Bayesian information criterion (BIC) and sample size-adjusted BIC and are usually assessed when competing models are involved, with the lowest value as presenting a better fit.

5. **respecification**: Respecification relates to the researcher modifying the structural model: adding, removing paths between variables, usually in a iterative manner. Sometimes, modification indices are available but these should be used with caution since these suggestions will not necessarily have any sense in theory. This respecification of a model is more exploratory than confirmatory but again may be force by convergence issue. In the case of confirmatory analysis, the researcher is directly testing an hypothesis and would report on findings of fit for their research question or several research questions. In exploratory analyses the researcher is open to investigate potentially new causal/correlations and comparing fit between these nested models or testing consistency of fit between groups. Ideally, exploratory models could be validated in a new sample as to avoid overfitting and providing a poorly generalisable model.

3.3 What is a good model?

Deliberating on whether a model is "sound" and "acceptable" depends on many factors and is a topic of substantial discussions in the field. Considering the above model fit indices in itself does not equate "truth". Overfitting would be reflected in great goodness of fit but limits generalisability and replication, while the choice of parsimony over complexity is also central to the inference process (Preacher, 2006). As mentioned, modelling bio- or psychological processes requires a researcher to have a strong background in the theory relating both the external (environmental, behavioural etc) factors and the internal machinery of the human body. In human epidemiology, researchers should not ignore their interplay with the wider social context and how the dynamics and associations between these factors can be represented not only in a cause-effect directionality but also the place of mediators and moderators and confounders. This could lead the SEM user to experience several barriers in joining mathematics, technology and the empirical enquiry where they

meet overfitting, non-convergence and uninterpretable models which eventually prove to have none of the previously intended translational or hypothesis generating appeal.

3.4 Latent class Analysis and Latent Class Growth Analysis

In this thesis, Latent Class Analysis (LCA) and Latent Class Growth Analysis (LCGA) are used. Both LCA and LCGA are SEM mixture modelling techniques and allow for the exploration of hidden (latent) groups within a sample, based on individual values of observed variables. Classes are generated from the probabilities of individuals belonging to a classes. In this way the relationship between individuals, rather than between items in CFA, is the property of interest. Essentially, the assumption is that within a sample there are individuals who follow a similar pattern of responses/behaviour/qualities (as measured by e.g., a questionnaire) and who together can be separated from other individuals belonging to other classes.

The advantage of these methods is that classification of individuals is not only derived from one cut-off scores or diagnostic criteria which often means dichotomisation. Dichotomisation in itself pushes the notion that people who are borderline under or above a certain threshold are very different from one another. Dichotomisation also assumes that individuals below a threshold (aka “normal” or “controls”) are homogeneous which, in case this assumption is erroneous for the population, could introduce bias and attenuate the effect of other factors/covariates in statistical analyses (MacCallum et al., 2002).

LCGA differs from LCA in that a time factor is modelled in the patterns of observed variables over time (i.e. their trajectories on repeated measures). If heterogeneity in the population exists, individuals with similar trajectories form their respective classes, with their distinct intercept and slopes. Extending the benefit of LCA and LCGA is the modeling of covariates and comparisons of outcomes while accounting for the measurement error in the classification of the individuals. This is applied by the “three-step approach” (Asparouhov and Muthén, 2014) described and implemented in this thesis. This method is an improvement on the commonly used “classify-analyse” approach which only uses the most likely class membership, where the assignment is based on the individual’s highest probability to belong to a specific class (Vermunt, 2010).

Model choice (i.e. the number of classes accepted) should rely on fit indices and criteria (AIC, BIC, aBIC etc) although it is also at the discretion of the researcher to assess the interpretability of the classes obtained, parsimony and the classification in the wider research aims. Ideally, the classification should be validated via external measures which are expected to associate with the classes. In order to improve reliability and reproducibility, transparency in the reporting of the model enumeration and selection is necessary (Lanza and Cooper, 2016; Schoot et al., 2017a)

3.5 Adjustment for multiple comparisons

It should be noted that in this thesis, chapter 4 aims to describe comprehensively the offspring exposome in relation to maternal variables and birth. With the exploratory approach inherent to the LCGA and in the aim of providing hypothesis generating opotunities, the outcomes are presented unadjusted for multiple comparisons. In other analysis, results are presented unadjusted and adjusted for multiple comparisons using the False Discovery Rate (FDR) (Benjamini and Hochberg, 1995).

3.6 Basics of MRI

MRI relies on the magnetisation properties of hydrogen nuclei (single protons) in tissues of the body, primarily found in water and fat. A proton spins in a random fashion around its axis but this axis can be magnetized when a strong homogeneous magnetic field (B0, measured in Tesla units) is applied. The typical clinically used field strength is 1.5T and 3T. In the presence of a magnetic field, spins can exist in one of two states: parallel or antiparallel (in the counter direction) along the axis of the main B0 field of an MRI scanner. Approximately half the protons align in either direction, cancelling each other out, but a slight surplus of unmatched protons align parallel rather than antiparallel and this causes a net magnetic vector. At this stage, even if the protons are aligned along B0, they do not spin synchronously ("out-of-phase"). The number of mismatched protons is proportional to the strength of the magnetic field. The higher field produces a higher number of unmatched parallel protons.

The magnetisation can be modulated by applying electromagnetic radiofrequency (RF) waves by a transmitter coil at the same frequency as the spinning proton of interest (B1) and perpendicular to the B0 field. The unmatched protons absorb that energy, switch to an anti-parallel direction and rotate, or flip, their axis thus causing a deflection of the magnetic vector. At this point protons spin in synchrony ("in-phase"). Once the pulse is off, there is a return to resting state (along B0) which emits the energy back through another radio wave. The energy is received by the receiver RF coils placed on the body or head. Proton density differs in each tissue type and tissues will thus emit signal with different intensity of the MRI image. Fat, as found in myelin, is dense in hydrogen so has high signal intensity. This signal intensity is what is plotted in greyscale after applying a Fourier transformation to each location in the plane (the "k-space") to create the MR images. The Repetition Time (TR) is the time between successive RF excitation pulses applied within the same slice and the Echo Time (TE) is the time between the RF excitation pulse and the time when the signal is measured. This happens either when the signal is refocused to form an echo in a Spin Echo sequence or when the signal is read-out in a Gradient Echo.

The speed at which the molecules return to equilibrium after the application of the RF pulse, is measured in two types of relaxation times relevant in MRI, the recovery of longitudinal magnetisation and the decay of transverse magnetisation. Different tissue types (e.g. water vs fat) in the body are characterized by different relaxation times (T1 and T2) and this property allows for their differentiation and contrast on MR images. T1 relaxation (or longitudinal relaxation) is related to the time for protons to recover or realign with the external magnetic field. In T1-weighted images, cerebrospinal fluid (CSF) has a low signal intensity (it appears dark) because it has a short T1 time. T2 relaxation (transverse relaxation) is the characteristic time for the signal decay due to the loss of phase coherence of the individual spins (out-phase to in-phase) (Le Bihan, 2003) and helps differenciate between different tissue types because of their MR decay property. CSF is bright on a T2-weighted image.

The signal-to-noise ratio (SNR) can be determined by a number of acquisition parameters, including the TE, TR and resolution. Larger voxels (lower resolution) generally produce higher SNR as they contain more spines and more magnetisation. However, larger voxels can include several tissue types ("partial voluming") which makes their contribution to the signal difficult to disantangle. Several methods employed to achieve shorter acquisition times such as parallel imaging lead to a reduced SNR. Other parameters influencing the quality of the MR image are the thickness of each slice, the RF sequence chosen and artifacts caused by motion such as ghosting and signal dropout or distortion such as from eddy currents.

3.7 Perinatal imaging and MRI

Although fetal imaging is not included in this thesis it is important to note certain aspects which are relevant to the research population included in this thesis. Imaging in the antenatal period is well established clinically in the care pathway through ultrasonographic anomaly and growth screening. However, ultrasound is challenging in pregnancies of high BMI, which have a higher risks of fetal congenital anomalies, due to poorer visualisation. For example, in the first trimester screening, lower quality imaging hinders the ability to obtain clear fetal nuchal translucency for Down's syndrome screening, and impedes correct assessment of the neural tube and the heart (Maxwell and Glanc, 2011). Detection of such potential anomalies is moreover hampered by the reported missed return appointments when the screening was incomplete at the first ultrasound (Racusin et al., 2012) which can occur in up to 44% of women in the obesity class III (≥40.00 kg/m^2) against 10.2% in normal-weight (Eastwood et al., 2017).

This goes to illustrate that research which could both promote the participation of women with obesity, at risk of complications, and the investigation of their infants using methods capable of generating high-resolution and high-quality imaging is therefore warranted. MRI in women with obesity, including the uterine milieu such as the placenta and the fetal body, offers substantial benefit therefore over sonography for research purposes due to its enhanced coverage and decreased dependence between BMI and quality. This consideration motivated the design of a prospective study with antenatal MRI imaging as a key component. This study ("My Baby's Brain and Me") is described in Appendix B.

The following sections describe the complementary nature of MRI in studying neurodevelopment. Advancement in perinatal MRI acquisition, MR data pre- and post-processing and software development will be discussed and how they have enabled to push the frontiers of exploration of neurodevelopment in at-risk populations.

3.8 Structural neonatal MRI

Fetal and neonatal MRI have seen vast improvements in the data quality and granularity. However, it is important to remember some of the challenges and the resulting considerations. These pertain to the image acquisition (including resolution, Signal-to-Noise ratio (SNR), safety in smaller subjects), motion artefacts inherent to the neonate but also imaging contrast and brain anatomy (Batalle et al., 2018).

In neonatal MRI data included in this thesis was acquired through the dHCP study. T1 and T2-weighted images need different analysis solution compared to the adult brain. Imaging in the neonate requires high resolution given the smaller brain size which is approximately half the volume of an adult (Makropoulos et al., 2014). The contrast and boundaries between WM and GM in the neonate is much less pronounced due to the low lipid contain (i.e. low myelin) and so T2-weighted images, as opposed to T1 in the adult, are often used to visualise the brain and to create parcellation maps from brain atlases (Figure 3.2).

The dHCP provided several technological advances for this challenging population. This includes developing a bespoke neonatal positioning system (Hughes et al., 2017) as well as the development of imaging sequences and processing tools specific to the neonate population which address especially the effects of the increased motion (Cordero-Grande et al., 2018). Combining novel acquisition and analytical elements has allowed to reconstruct structural images in the dHCP at an excellent imaging quality of a high resolution

($0.5mm^3$).

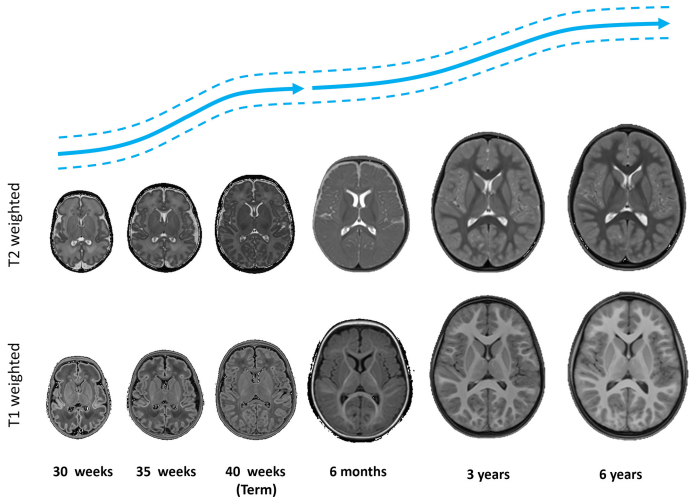

Figure 3.2: *As the brain grows, the T1 and T2 acquired signals present varying contrasts between grey and white matter structures. In the neonate T2 is usually chosen to visualise the brain rather than T1 in the adult population. Reproduced from Batalle et al. (2018).*

Anatomical differences to the adult brain are clear but these are firstly obvious across gestation itself and into the neonatal period. Anatomical segmentations of the brain requires fine tuning of parcellation propagation protocols sensitive to changes occurring at the cortical (gyri and sulci) and subcortical boundaries between weeks. However, even then another difficulties in applying single brain atlases relate to other features such as the *cavum septum pellucidum*, a cavity between the left and right anterior horns of the lateral ventricle encased in thin membrane, which does not disappear at the same time for all neonates albeit most likely around 6 weeks postpartum (Oishi et al., 2019).

To date, many subcortical/deep-grey matter areas such as the midbrain and diencephalon have been less studied during development but the availability of large and high-quality neonatal datasets offer ample opportunities for improvement and contributions. Within the aims of this thesis, an emphasis was put on the diencephalon primarily due to the interest in characterising the neonatal reward system and the hypothalamus in relation to outcomes of children exposed to obesity in gestation.

3.9 Diffusion MRI

Diffusion refers to the random motion of molecules due to thermal agitation (Brownian motion). This is often depicted as a spherical ellipsoid in a fully homogeneous medium where random motion of water in all direction is equally likely, termed *isotropic* diffusion. Diffusion MRI is sensitive to these displacements as the molecules experience hindrance by the biological microarchitecture surrounding them, such as nerve fibres and macromolecules in the brain. As such, water molecules diffuse more easily when hindrance caused by cellular walls and organelles is less, whether intracellularly or extracellularly if cells are tightly packed. Anisotropy refers to the “orientationally dependent water diffusion” (Jones, 2012) characteristic of what is observed when water is constricted and runs parallel in the direction of a fibrous tissue such as the white matter. This is illustrated in a prolate or oblate ellipsoid.

At the voxel level, diffusion *tensor* imaging aimed to quantify the extend of an/isotropy and directionality to model white-matter fibre bundle to produce metrics such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). However, this model is highly limited by its ability to only provide one fiber orientation. It carries the following assumptions: the tissue within a voxel is homogenous (e.g. only white matter), the voxel contains fibers belonging to the same bundle only in one direction and the diffusion is represented by a Gaussian function with one directional maximum (Tuch et al., 2003). In the case of partial-voluming (e.g. CSF and WM within the same voxel) or crossing fibres, the tensor model provides only the *average* fibre orientation in the voxel offers poor specificity to the complex axonal arrangement in the brain.

A substantial marked advancement to this issue was proposed by integrating a distribution function which could integrate more than one peak into the spherical function. This is incorporated by the diffusion orientation distribution function (ODF). The ODF can describe multimodal diffusion, the probability of of a water molecule to diffuse in any direction in a diffusion time. With the modern high angular resolution diffusion imaging (HARDI) protocol (Tuch et al., 2003; Tournier et al., 2011) more complex angular frequencies can be measured. HARDI was then combined with Constrained Spherical Deconvolution (CSD, Tournier et al. (2004)). Further combination within multi-shell schemes (with more than one constant diffusion weighting applied, or b-values) markably improved obtaining sensitivity in the voxel-wise fibre orientation distribution function in the WM (Jeurissen et al., 2014; Dell'Acqua and Tournier, 2019).

While validating diffusion models often requires optical imaging in fixed tissue, the challenges remains in modeling diffusivities within tissues containing different compartments, such as intra-axonal and extra-axonal diffusivities in WM. Adapting models in disease states and development which may present individual diffusivity signatures should also be considered since a universal model may not be applicable and/or limit the sensitivity and specificity of quantitative dMRI measures (Jelescu and Budde, 2017).

One of the challenges in interpreting DWI, which is accentuated in a neonatal brain of higher water content, is that increased anisotropy as measured by DTI models is still often attributed to increased myelin. However, the neonatal brain tissue itself is still undergoing growth and is at a pre-myelinating stage, which implies a heterogenous water fraction and density of e.g. glial cells and other intra-axonal organelles across possibly short time intervals and regions while myelination and axonal growth is ongoing. As seen on Figure 3.3) the contrast between GM and WM is very little in the neonates at the early stages of myelination, however DTI here provides a visualisation of major tracts from their principal orientation.

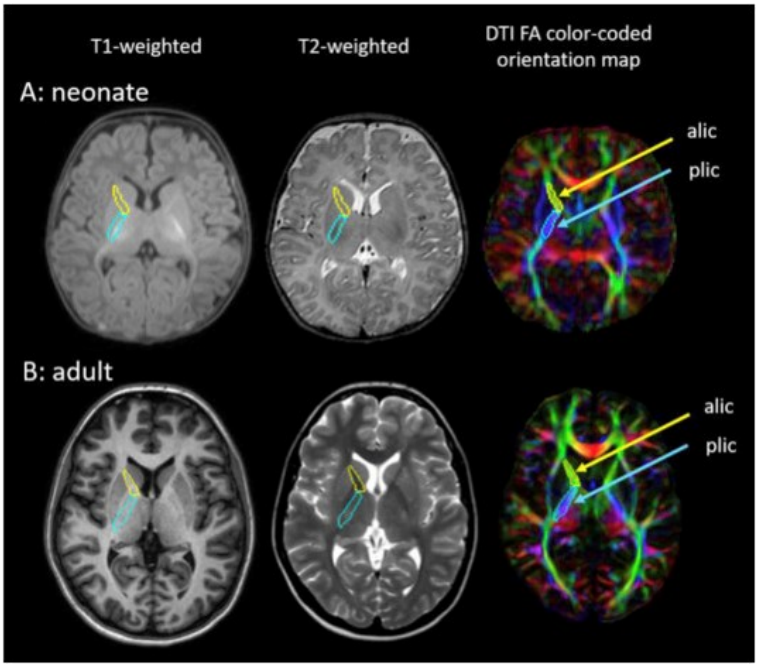

Figure 3.3: *T1 and T2 structural MRI images are illustrated in the neonate and adult brains to exemplify how lack of maturation of the white matter in the neonate, in addition to a higher water content, reduces the visualiation of clear boundaries with grey matter. Diffusion tensor imaging can be used to depict major white-matter fibers in both adult and neonates, maps are color coded by orientation of the fractional anisotropy within the brain. alic: anterior limb of the internal capsule; plic: posterior limb of the internal capsule. Reproduced from Oishi et al. (2019).*

Nevertheless, in the dHCP multi-shell HARDI acquisitions are incorporated. Together with bespoke neonatal headcoils and gradient coil systems, this permits highly sensitive microstructural assessment of neurodevelopment. The state-of-the-art dMRI acquisition (Hutter et al., 2018), pre and post-processing pipelines within the dHCP (Christiaens et al., 2021) support the application of CSD methods and tractography for delineating WM tracts of interest in the developing brain with the highest accuracy. An example of ODF maps in a subject neonate is in Figure 3.4. For instance, this data has also been utilised to exemplify how isotropy and therefore the perinatal DWI signature, can be modelled more specifically to show variability across areas of the brain and the spatial pattern in which this occurs over the period of 33 to 42 weeks (Pietsch et al., 2019).

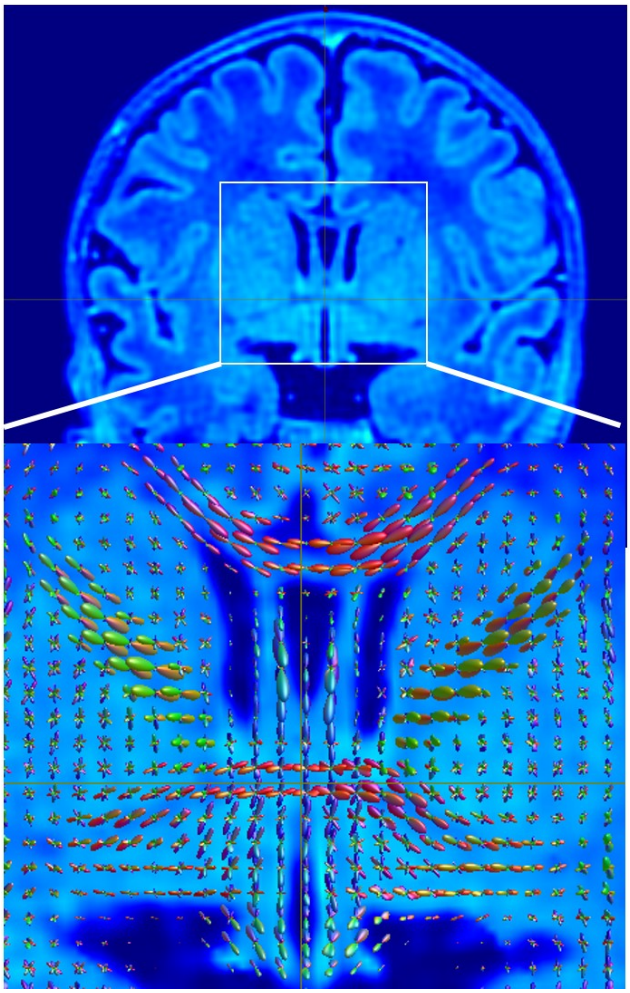

Figure 3.4: *Example of ODF maps overlaid on a subject T1 image (14 days-old term born female) at the level of the anterior hypothalamus demonstrate the several diffusion peaks and their amplitutes within each voxel color-coded by conventional direction i.e. red: left-right, blue: dorsal-ventral, green: posterio-anterior. Here the commissural fibres of the corpus callosum and anterior commissure which have started to myelinate are clear (red) and the anterior limb of the internal capsule (green).*

3.10 Tractography

Capitalising on dMRI, tractography is a method which allows for the delineation and therefore visualisation of white matter tracts in the brain in 3D. It more specifically refers to algorithm-dependent, delineation of the WM is represented by “streamlines” or groups of parallel streamlines (“bundles”) as determined by the quality of the diffusion signal and the estimated orientation of the fibres within the bundles. With DTI, the propagation of a streamline trajectory is based on the primary direction of diffusion in a voxel as estimated by the eigenvector associated with the largest eigenvalue in each voxel and subsequently the propagation continues in all adjacent voxels until a specific termination threshold (e.g. anisotropy) is met. In CSD-based tractography termination relies on the amplitude to the fODF. Tractography can produce whole-brain tractograms (or structural connectomes) or tracts specific delineation using seed and target regions.

The exercise of delineating streamlines for tracts of interest (TOI) will always be subject to the user knowledge of the ground truth, i.e. neural anatomy, and is not void of erroneous outputs when the aim is to produce an outcome which would stand up to the scrutiny of experts in that field. While tractography in this thesis is probabilitistic, deterministic tractography is the common alternative approach. The distinction between probabilistic and deterministic methods has conventionally been that the latter is viewed as more conservative (higher specificity and lower sensitivity, Grisot et al. (2021)). Histological tracing studies demonstrate that probabilistic tractography can improve sensitivity at the same level of specificity (Girard et al., 2020).

While WM tracts can be configured in tightly packed parallel fibers, it is more often arranged in a more complex configuration as crossing, bending and kissing fibers and this can be observed in up to 90% of brain WM. It should be noted that such configuration is also difficult to observe using post-mortem dissection which most of the anatomical literature relies on to establish “ground truth”.

More recent tractography models which are able to account for these more complex configurations have enabled the exploration of the anatomy in a non-invasive manner with increased detail (Jeurissen et al., 2014). In the context of this thesis, their main benefit lies in the capacity of tractography to allow for the exploration of fibre tract development *in vivo* to complement the established, yet limited, collection of histological findings in the fetal and neonatal brain literature. One central aim of the thesis was to contribute to this by delineating streamlines underlying white-matter tracts which have been under-studied possibly both due to the absence of high enough resolution dMRI data in the neonates but also the software to produce them in *vivo*. The work described in Chapter 7 relies on tractography using *MRtrix3* and describes this investigation in more detail.

3.10.1 Fixel Based Analysis - FBA

Tensor models offer measures of FA, MD, RD and AD to quantify WM “integrity” and the associated caveats of this at the voxel level have been described. Fixel-Based Analysis (FBA) aims to characterise WM structural integrity at the microscopic and macroscopic levels (Raffelt et al., 2017a; Dhollander et al., 2021). It relies on the CSD-based estimation of the fiber orientation distribution (FOD) and the Apparent Fiber Density (AFD, Raffelt et al. (2012)) derived from it. AFD is conceptualised by the fact that intra-axonal diffusivity is hindered in the direction perpendicular to the fiber orientation so that it is inferred that the radial diffusion-weighted signal reflects the intra-axonal volume, which is captured by the FOD amplitude to provide a fiber density (FD). Further, because registration to a template or a target requires voxel-wise expansion and contraction (captured by the Jacobian maps in that process), the morphological volumetric changes applied perpendicular to the main fibre orientation are then utilised as a metric for the cross-sectional volume of the bundle, the fibre cross-section (FC). A combined Fiber Density x Fiber Cross-section is also computed (FDC) and thus provide an estimation of the macroscopical morphology of the bundle (depicted in Figure 3.6). Importantly, since the FOD maps in this model can target multiple fiber orientations within one voxel, this method separates fibres from distinct fiber bundles, each fiber unit is then called a *fixel*. All these features are applied in this thesis using *MRtrix3* (Tournier et al., 2019). An example of this advantage over the tensor model is found in Figure 3.5.

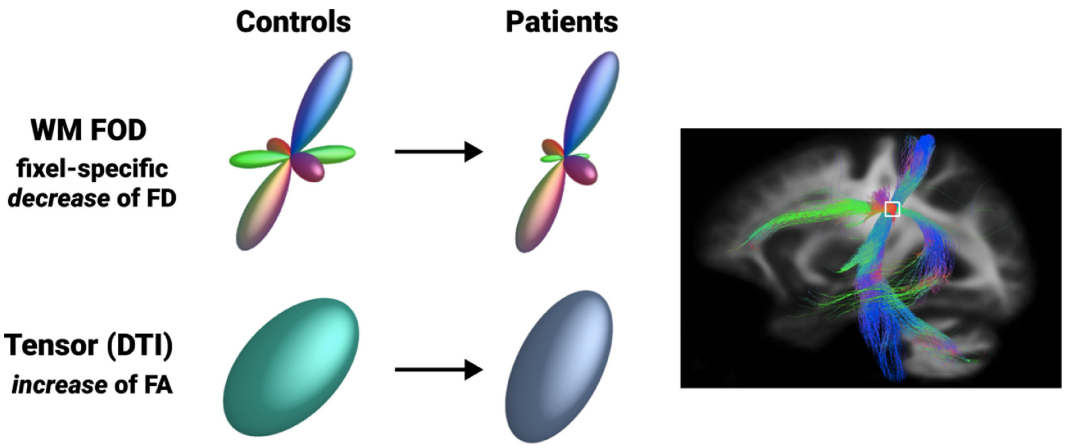

Figure 3.5: *Fixel-based analysis based on a constrained-spherical devonvolution model of the diffusion signal enables the disantangling of three different fibre bundles crossing at one junction. The tensor signal shows an increase in the average FA within this voxel whereas the FBA indicates that, among the patients, there is a decrease in the apparent fiber density of one fibre bundle (green) and not the other two. Reproduced from Dhollander et al. (2021)*

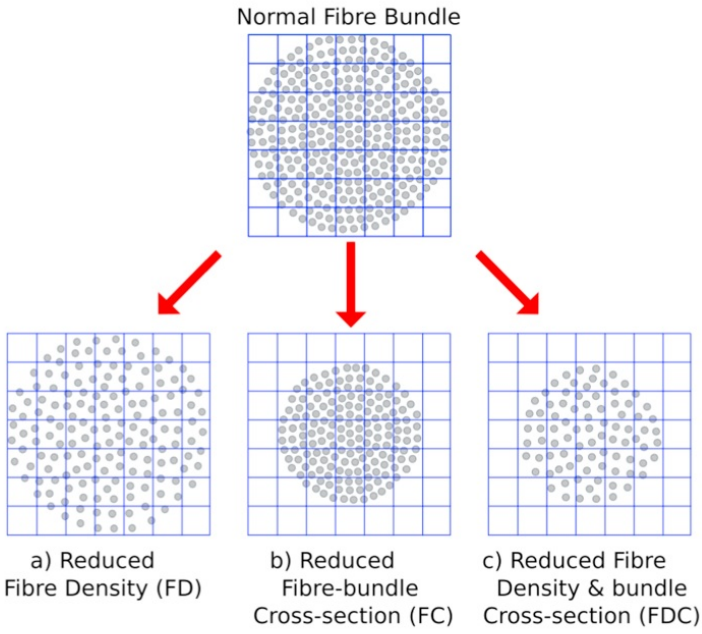

Figure 3.6: *Fixel-based Analysis provides metrics of Fibre Density (FD), Fibre Cross-section (FC) and their combination (FDC) to assess microscopic and morphological characteristics of white-matter tracts. Reproduced from Raffelt et al., 2017.*

Other analytical tools which utilise dMRI to provide measures of WM include tract-specific analysis (Yushkevich et al., 2008) and tract-based spatial statistics (Smith et al., 2006) which have been applied in the neonates including preterm-born infants (Pecheva et al., 2017). However, the utility of voxel-based-analysis (including tensor based methods) and TBSS to produce sensitive estimation of fibre microstructure in a brain

of reduced size and for tracts whose anatomy are not yet clarified in the neonate is viewed insufficient in light of the improvement offered by FBA. A comparison is presented in Table 3.1 (Dhollander et al., 2021).

Table 3.1: *Comparisons between voxel-bases analysis, tract-based spatial statistics and fixel-based statistics in uncovering properties of the brain and group comparisons using diffusion MRI.*

	Voxel-based analysis (VBA)	Tract-based spatial statistics	Fixel-based analysis (FBA)
Domain of analysis	Entire voxel grid within the brain.	Only voxels on a mean (template) FA "skeleton".	Entire fixel grid: mostly WM, some (sub)cortical GM.
Specificity	Voxel-level (spatial) specificity.	Voxel-level specificity; limited to the mean FA skeleton.	Fixel-level specificity for individual fixels in a voxel.
Alignment & correspondence	Image registration to a common template space and spatial interpolation.	Image registration to a common template space.	FOD-based image registration to a common FOD template.
		Thinning of FA template to obtain a mean FA skeleton.	Segmentation of template fixels and subject fixels.
		Project maximum subject FA value perpendicular to mean FA skeleton onto the skeleton voxels.	Bespoke fixel correspondence criteria to assign reoriented subject fixels to template fixels.
Statistics	Correction for a large number of comparisons.	Correction for a reduced number of comparisons (less voxels on the FA skeleton).	Correction for a very large number of comparisons (typically more fixels than voxels).
	Spatial smoothing and threshold-free cluster enhancement (TFCE).		Connectivity-based fixel-wise smoothing and connectivity-based fixel enhancement (CFE).

Note:
Reproduced from Dhollander et al., 2021

Chapter 4

Longitudinal phenotyping of maternal antenatal depression in obese pregnant women supports multiple-hit hypothesis for fetal brain development, a secondary analysis of the UPBEAT study

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4.1 Introduction

Depression during pregnancy occurs in approximately 17% of women in developed countries (Dadi et al., 2020) and has been repeatedly associated with increased risk of neurodevelopmental and affective disorders in the exposed offspring (Manzari et al., 2019). Causal mechanisms suggested include disturbances in the maternal and the fetal hypothalamic-pituitary-adrenal axes through the action of cortisol, mediated via the placenta (Glover, 2015; Shook et al., 2020). Other plausible pathways and confounders are often neglected such as exposures associated with maternal obesity, occurring in over 20% in UK pregnancies. Indeed, obesity in pregnancy has been repeatedly associated with both maternal depression and autism and attention-deficit hyperactivity disorder (ADHD) in the child (Godfrey et al., 2017; Kong et al., 2020). However, depression and obesity share abnormalities in glucose homeostasis and lipid metabolism and with inflammatory mediators and can interact with diet, all of which may influence the developing brain (Penninx et al., 2013; Catalano and Shankar, 2017). Putative mechanisms include the quality of dietary fat intake which can induce a pro-inflammatory state and deficiencies in omega-3 fatty acids found in depression and obesity (Lindsay et al., 2019).

Previous studies addressing these relationships have placed little importance on the interaction between obesity and depression nor with other exposures adverse to fetal neurodevelopment (e.g. infection, diet) and sample size has been a limiting factor (Burg et al., 2016; Catalano and Shankar, 2017; Lindsay et al., 2019). Importantly, the frequent use of single-point measurement or dichotomisation of diagnosis oversimplifies the burden of co-morbidities and assumes symptom stability in depression but also disorders such as gestational diabetes mellitus (GDM), the most common complication in obesity. Such approaches may conceal outcome heterogeneity in "control" pregnancies, possibly introducing bias and undermining optimal antenatal management. In the few studies reporting longitudinal depressive symptom trajectories across pregnancy, sample have been of heterogeneous BMI and include post-partum time points (Baron et al., 2017; Yu et al., 2020). Therefore there is a need for a more comprehensive description of the associations between maternal depression and obesity and fetal exposures in which depression is assessed longitudinally throughout pregnancy.

In anticipation of understanding offspring long-term psychological outcomes, we have addressed these gaps in one of the largest exclusively obese multi-ethnic cohorts of pregnant women from whom longitudinal measures of depressive symptoms, maternal blood biomarkers and a range of clinical and lifestyle factors relevant to fetal neurodevelopment are available. Using Latent Class Growth Analysis, a data driven approach, we modelled the most likely longitudinal depressive symptom profiles, comparing trajectories at 17, 27 and 34 weeks of pregnancy. We sought demographic profiles and mechanistic pathways that could affect neurodevelopment through depression, and possible novel confounders. This study directly answers a recent call for the integration of cumulative maternal states which have inflammation in common, either acutely (infection) or chronically (obesity, diabetes, preeclampsia, low socioeconomic status, depression) and are associated with neurodevelopmental disorders in the child (Han et al., 2021).

4.2 METHODS

4.2.1 Participants

We studied pregnant women with obesity (BMI $\geq$ 30kg/m^2) who participated in the UK Pregnancies Better Eating and Activity Trial (UPBEAT; Poston et al. (2015)) of a lifestyle intervention which primary outcome was the prevention of GDM and large-for-gestational age neonates. 1554 women, age >16 years, carrying singleton pregnancies, void of hypertension, renal disease, pre-gestational diabetes, sick cell disease, thalassemia, coeliac disease, renal disease, systemic lupus erythematous, antiphospholipid syndrome, thyroid disease, current psychosis or under current metformin medication were consented to participate from 8 UK NHS trusts (Bradford, Glasgow, 3 London Centres, Manchester, Newcastle and Sunderland) (Poston et al., 2015). The trial was designed to randomize women between 15^{+0}-18^{+6} weeks gestation (intervention start), invite them for the oral glucose tolerance test (OGTT) at 27^{+0}-28^{+6} weeks (intervention end) and a last visit between 34^{+0}-36^{+0} weeks of gestation. Randomization was minimized by ethnicity, BMI groups (obesity class I:30.0–34.9, II:35.0–39.9, III: $\geq$ 40 kg/m²), age (<24, 25–29, 30–34, $\geq$ 35 years) and centre.

UPBEAT complied with criteria set by the Declaration of Helsinki. Ethical approval was given by the National Health Service (NHS) Research Ethics Committee (09/H0802/5) and all participants provided informed consent.

From 1554 in the original UPBEAT trial, 24 experienced fetal loss or miscarriages, 4 terminated, 5 neonatal

deaths occurred (within 28 days of birth), 14 withdrew consent to use data or were lost to follow-up and 17 had unconfirmed fetal/neonatal outcomes. Thus 1490 pregnancies resulted in known live births. Of those, 1369 women had at least one completed Edinburgh Postnatal Depression Scale (EPDS)(Cox et al., 1987) questionnaire and were included for the purpose of studying depressive symptom trajectories in pregnancy and their outcomes. Questionnaires were completed and blood samples collected at three trial visits (median 17, 27 and 34 weeks gestation). Women who were missing all EPDS scores and were excluded did not differ on entry demographics from those included but rather on trial site (Table 4.1). 935/1369 (68.3%) participants provided all three EPDS questionnaires.

Table 4.1: *Comparison between excluded and included participants who answered to at least one EPDS questionnaire (total n=1490).*

	Excluded	Included	p
n	121	1,369	
BMI (median [IQR])	35.40 [32.70, 39.10]	35.00 [32.80, 38.50]	0.538
Age(years) (mean (SD))	30.50 (5.68)	30.48 (5.47)	0.968
Main ethnicity (%)			0.397
White	79 (65.3)	857 (62.6)	
Black	31 (25.6)	351 (25.6)	
Asian	3 (2.5)	85 (6.2)	
Other	8 (6.6)	76 (5.6)	
IMD (%)			0.461
Least Deprived	5 (4.2)	54 (4.0)	
2nd quintile	8 (6.7)	90 (6.6)	
3rd quintile	11 (9.2)	155 (11.4)	
4th quintile	50 (42.0)	462 (33.8)	
Most deprived	45 (37.8)	604 (44.2)	
Income (%)			0.275
< £12,688	27 (22.3)	247 (18.0)	
£12,688 - £17,628	7 (5.8)	159 (11.6)	
£17,629 - £23,452	14 (11.6)	112 (8.2)	
£23,453 - £32,500	13 (10.7)	169 (12.3)	
> £32,500	41 (33.9)	479 (35.0)	
Prefers not to answer	19 (15.7)	203 (14.8)	
Born in the UK (%)	81 (66.9)	922 (67.3)	1.000
Nulliparous (%)	52 (43.0)	598 (43.7)	0.956
Centre (%)			<0.001
St Thomas'	41 (33.9)	327 (23.9)	
King's College Hospital	29 (24.0)	240 (17.5)	
Newcastle	24 (19.8)	206 (15.0)	
Glasgow	25 (20.7)	234 (17.1)	
Manchester	1 (0.8)	134 (9.8)	
Bradford	0 (0.0)	50 (3.7)	
Sunderland	0 (0.0)	82 (6.0)	
St Georges'	1 (0.8)	96 (7.0)	

Note:
From 1554 women enrolled, 1490 pregnancies resulted in confirmed live births, 1369 provided at least one EPDS questionnaire and were included in this study. Normally and non-normally distributed variables are compared with a t-test and a Kruskal-Wallis test, respectively. Categorical variables were analysed with a Chi-squared test. IMD: Index of Multiple Deprivation; BMI: Body Mass Index.

4.3 Antenatal depression

Depressive symptoms were evaluated using the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987), a freely accessible 10-item self-reported screening tool of depression, validated in pregnancy in multiple languages. Ten items ask about experiences and feelings occurring seven days previously, rated on a four-point scale according to frequency (e.g. Item 9: "I have been so unhappy that I have been crying";

"Yes, most of the time"=3, "No, Never"=0). Items three and five to 10 are reverse coded and the total sum score ranges from 0-30 points. Major depression is suspected at the cutoff of 14/15 and minor at 12/13 points (Murray and Cox, 1990).

Responses were provided by 1300 women at median[IQR] 17[16 to 17] weeks gestation (baseline visit), 1156 women at 27[27 to 28] weeks (visit 2) and 1004 women at 34[34 to 35] weeks (visit 3). A frequency matrix is provided in Table 4.2. We report on Item 10 (self-harm) in Table 4.3 as women presenting as high-risk on the EPDS or those scoring high on the *self-harm* item were referred to NHS perinatal mental health services as according to local protocols.

We first interrogated whether (latent) groups of women could be identified based on the antenatal trajectories as measured by their total EPDS scores. Then, we explored whether heterogeneity in trajectories was also reflected in heterogeneity in maternal and neonatal factors known to influence neurodevelopment.

Table 4.2: *Frequency matrix of available EPDS.*

	Time point 1	Time point 2	Time point 3
Time point 1	1300		
Time point 2	1116	1158	
Time point 3	939	974	1005

Note:
The responses for the EPDS were provided by 1300 women at median[IQR] 17[16,17] weeks GA, 1156 women at 27[27,28] weeks and 1004 women at 34[34,35] weeks. The count(%) of women who responded above the 13 point threshold was 157/1300 (12.1%), 118/1158(10.2%) and 82/1005(8.2%) at the 1st,2nd and 3rd time points respectively.

Table 4.3: *Participant response on item 10 ('self-harm') of the EPDS at median 17, 27 and 34 weeks gestation*

	Time 1	Time 2	Time 3
Never	1256	1116	978
Hardly	25	31	20
Sometimes	18	8	7
Quite often	1	3	0
Missing	*69*	*211*	*364*

Note:
From 1369 women who responded to at least one questionnaire.

4.4 Variables and outcomes

Baseline demographic variables and anthropometric measurements were recorded at median 17 weeks. Other than providing demographic characterisation, demographic variables were chosen based on reported associations with obesity, mood disorders and/or fetal outcomes such as socio-economic status (SES),

asthma and smoking. Anthropometric measures were included as a more informative reflection of maternal adiposity compared to BMI alone and in order to derive a latent “Obesity” factor. This latent obesity factor helps minimise the confounding adiposity-induced effects (i.e.”meta-inflammation” and metabolic disturbance) on outcomes when the depressive status is the main factor of interest.

Sum of skinfold thickness measurements (mean triceps + mean biceps + mean subscapular + mean suprailiac taken in triplicate) were obtained with Harpenden skinfold callipers to the nearest millimeter. Waist, hip and thigh circumferences were measured to the nearest centimeter and the neck to the nearest millimeter with a plastic tape taken.

Table 4.4: *Anthropometric characteristics at first visit in the whole sample of 1369 obese pregnant women.*

Factor		N=1369
Anthropometrics	BMI	35.0 (32.8, 38.5)
	Neck (cm)	36.6(2.5)
	Waist (cm)	106(100,113)
	Hip (cm)	121(116,128)
	Thigh (cm)	68.5 (6.6)

Note:
Continuous variables presented as mean(standard deviation) or median (interquartile range) if non-normally distributed. Missing: neck/hip/waist/thigh measurement=10.

Dietary intake, was evaluated by food frequency questionnaire for the previous month at 17 weeks (intervention start) and 27 weeks (intervention end). Nutrient intake was calculated from food/beverage codes using the WISP 3.0 software (Tinuviel Software) as described (Poston et al., 2015; Flynn et al., 2016). 997 (72.8%) and 858 (62.7%) of 1369 women had data at 17 and 27 weeks respectively (Appendix Table A.3) for Total energy (kcal), Glycaemic load (per 100g; calculated for each food as the glycemic index x carbohydrates amount/100), Saturated fat (g) and dietary composition for Carbohydrates, Saturated fat, Protein, Sugar as a % of total energy.

The first and last blood samples were random samples and the second after overnight fast (as per OGTT protocol). All were kept at -80 degrees after processing (within 2 hours of collection). The count of blood samples at each time point is provided in table A.1 and the laboratory methods in table A.2 of the Appendix A. All the markers obtained by conventional biochemical assays were processed by blinded technicians at the University of Glasgow following manufacturer’s calibrators and quality controls apart from human placental lactogen which was obtained by DiabetOmics, Inc, Beaverton, Oregan, USA. Glucose levels at the OGTT was processed at each trial center to obtain GDM status by the IADPSG criteria (see below). Amino acids, fatty acids and glycoprotein acetyles were measure by targeted NMR metabolomics platform (Nightingale Health, Finland) with no batch effect as described previously (White et al., 2017).

Analysed as a binary event, infection was self-reported for the period prior to each visit, prespecified for respiratory infection/flu, lower urinary tract infection (UTI), pyelonephritis, gastroenteritis, vaginal candida

(VC), suspected VC or “other”, see details in Tables 4.5 and 4.6.

Other pregnancy outcomes included GDM and preeclampsia (defined according to international guidelines, only included if not comorbid with the other) and gestational weight gain at 34 weeks. The obstetric diagnoses were those which received clinical diagnoses during pregnancy (GDM) or after revision of the pregnancy outcomes from the electronic records and pregnancy notes after birth (preeclampsia or gestational hypertension[GHT]). Due to the low frequency in GHT and the established adverse effect of PE in the literature only PE and GDM are included in group comparisons of outcomes but all comorbidity patterns are reported below for clarity (Table 4.8).

Table 4.5: *Frequency of infections in 1369 pregnancies at three visits.*

n=1369	Time point 1	Time point 2	Time point 3	n(%)
All				
No	986(72.0%)	866(63.2%)	773(56.5%)	
Yes	358(26.2%)	313(22.9%)	234(17.1%)	
Total	1344	1179	1007	
Missing	25(1.8%)	190(13.9%)	362(26.4%)	
Missing data pattern				
				980(71.59%)
			X	181(13.22%)
		X		9(0.66%)
		X	X	174(12.71%)
	X			16(1.17%)
	X		X	2(0.15%)
	X	X		2(0.15%)
	X	X	X	5(0.37%)
Infection in complete cases (n=980)				
	Yes	Yes	Yes	51(5.20%)
	No	Yes	Yes	49(5.00%)
	Yes	No	Yes	31(3.16%)
	No	No	Yes	97(9.90%)
	Yes	Yes	No	54(5.51%)
	No	Yes	No	117(11.93%)
	Yes	No	No	116(11.83%)
	No	No	No	465(47.45%)

Note:
Women were asked about whether they suffered from at least one infection in the period prior to the visit, i.e. before 17 weeks, between 1 and 27 weeks, and between 27 and 34 weeks. The frequency of any infection for participants who responded at all 3 time points (n=980): never = 465(47.4%), one visit = 330(33.6%), two visits = 134(13.6%) and all three visits = 51(5.2%).’X’ refers to missing data.

Table 4.6: *Infections in 1369 pregnancies*

	Time point 1	Time point 2	Time point 3
Infections	n(%)	n(%)	n(%)
Any	361 (26.4%)	317 (23.2%)	234 (17.1%)
None	983 (71.8%)	862 (63.0%)	773 (56.5%)
Type			
Flu/Respiratory tract	81 (5.9%)	71 (5.2%)	64 (4.7%)
Lower UTI	147 (10.7%)	95 (6.9%)	55 (4.0%)
Pyelonephritis	1 (0.1%)	2 (0.1%)	2 (0.1%)
Gastroenteritis	12 (0.9%)	20 (1.5%)	6 (0.4%)
Vaginal candida (VC)	52 (3.8%)	63 (4.6%)	61 (4.5%)
Suspected VC	24 (1.8%)	21 (1.5%)	17 (1.2%)
Other infection	78 (5.7%)	78 (5.7%)	49 (3.6%)
Treatment			
None	103 (28.5%)	81 (25.6%)	74 (31.6%)
Yes	248 (68.7%)	232 (73.2%)	159 (67.9%)

Note:
Women were asked about whether they experienced any infection in the period prior to each visit. Women could answer positive for more than one type. There were 1344 responses at Time point 1, 1179 at time point 2 and 1007 at time point 3. Percentages in the table are of the total sample (1369).

GDM was diagnosed according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, from 27 to 28^{+6} weeks of gestation at OGTT visit, if fasting glucose $\geq$ 5.1 mmol/L and/or if 1-hour glucose $\geq$ 10mm/L and/or 2-hour glucose $\geq$ 8.5 mmol/L. Women who received GDM diagnosis were referred to standard antenatal care based on the NICE guidelines.

Preeclampsia was diagnosed following the International Society for the study of Hypertension in Pregnancy (ISSHP; Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (2000)) criteria as: The presence of proteinuria (spot urine protein/creatinine $\geq$ 30mg/mmol [0.3mg/mg] or $\geq$ 300 mg/day or minimum 1g/L ["2 +"] on dipstick testing) with two measures of systolic ($\geq$ 140mmHg) or diastolic blood pressure ($\geq$ 90 mmHg) taken four hours apart. Preeclampsia was recorded by the research team after revision of the recorded blood pressure and proteinuria values (Vieira et al., 2018).

Gestational hypertension was recorded as two measures of systolic ($\geq$ 140mmHg) or diastolic blood pressure ($\geq$ 90 mmHg) taken four hours apart in the absence of proteinuria.

Table 4.7: *Obstetric comorbidities in n=1369 participants.*

n=1369	GDM	PE	GHT
All			
No	882 (64.4%)	1271 (92.8%)	1292 (94.4%)
Yes	303 (22.1%)	82 (6.0%)	56 (4.1%)
missing	184 (13.4%)	16 (1.2%)	21 (1.5%)

Note:
GDM diagnosed according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, missing due to no-show at the Oral Glucose Tolerance Test. PE and GHT marked at revision of all recorded blood pressure measurement and test of proteinuria. GDM: Gestational Diabetes Mellitus, PE:preeclampsia, GHT: Gestational Hypertension.

Table 4.8: *Obstetric commorbidity patterns*

n=1369	GDM	PE	GHT	Met criteria	n(%)	Missing n(%)
	X	X		No	1143 (83.5%)	195 (14.2%)
	X	X		Yes	31 (2.3%)	
	X		X	No	1157 (84.6%)	198 (14.4%)
	X		X	Yes	14 (1.0%)	
	X			No	927 (67.7%)	188 (13.7%)
	X			Yes	254 (18.6%)	
		X		No	1130 (82.5%)	193 (14.1%)
		X		Yes	46 (3.4%)	
			X	No	1143 (83.5%)	194 (14.2%)
			X	Yes	32 (2.3%)	
All absent					789 (57.6%)	184 (13.4%)
One or more					396 (28.9%)	

Note:
Missing value counted if any obstetric diagnosis had missing for each pattern. PE and GHT diagnoses are mutually exclusive. GDM: Gestational Diabetes Mellitus, PE:preeclampsia, GHT: Gestational Hypertension.

Hospital admission: data was collected from summary notes or medical records for any admission to hospital during pregnancy. The indications were available but not used as outcomes in this study. 129 women (9.4%) were admitted to hospital during pregnancy

4.5 Birth and neonatal outcomes

The following variables were included as birth outcomes and frequencies found in Table 4.9 and were collected from routine clinical data or the national NICU database.

Gestational age at birth which was calculated in postmenstrual days and a new variable created where GA birth was dichotomised as $\leq$ 34 week, $\leq$ 37 weeks to estimate rate of early and late preterm birth respectively but only preterm <37 weeks is included as an outcome in our comparative analyses. Spontaneous/Premature Rupture of Membranes (PROM) are also included in analyses to differentiate against indicated premature birth given the physiological etiologies of spontaneous birth and clinical context of the event.

Birth weight centile by WHO: centile calculated according to WHO is adjusted for sex and gestational age and is included as an outcome for comparative value. Large-for-gestational age (LGA) was defined as $\geq$ 90th centile and small-for-gestational age (SGA) as < 10th centile.

C-section (vs no CS), *induction of labour* (IOL), and *blood loss*, were retrieved from medical records/pregnancy notes.
NICU admission was reviewed from discharge summaries or from UK NICU database and included admission from birth or from the postnatal ward and included in comparative analyses. The clinical indication recorded (may be more than 1) were SGA, respiratory distress, birth asphyxia, infection, congenital abnormality, feeding problem, phototherapy, hypoglycemia, cyanosis, drug withdrawal or other indications.

Other outcomes reported but not included in analyses: *Mode of delivery* , *Ceasarian-section indication*, *Birthweight* , *sex*, *NICU indication and length*, *Apgar score* and *Neonatal length of stay* were all collected from the discharge summaries. *Birth weight centile*: a customised centile using the GROW calculator, adjusted for maternal weight, height, ethnicity, parity, birthweight, sex and gestational age at delivery. Large-for-gestational age (LGA) was defined as $\geq$ 90th centile and small-for-gestational age (SGA) as < 10th centile.

Table 4.9: *Birth and infant outcomes in n=1369 pregnancies.*

Outcome		n=1369	Missing
Birth			
Induction of labour	No induction performed	892 (65.2%)	3 (0.2%)
	Induction performed	474 (34.6%)	
Mode of Delivery	LSCS in labour	227 (16.6%)	3 (0.2%)
	Operative vaginal	160 (11.7%)	
	Prelabour LSCS	263 (19.2%)	
	Unassisted vaginal	716 (52.3%)	
CS indication	CS not applicable	875 (63.9%)	5 (0.4%)
	Delivery timed to suit the woman and staff	169 (12.3%)	
	Immediate threat to life of the woman or fetus	96 (7.0%)	
	Maternal/fetal compromise which is not immediately life threatening	132 (9.6%)	
	No maternal or fetal compromise but needs early delivery	92 (6.7%)	

Table 4.9: *Birth and infant outcomes in n=1369 pregnancies. (continued)*

Outcome		n=1369	Missing
Blood loss	Blood loss <1000mls	1165 (85.1%)	22 (1.6%)
	Blood loss >=1000mls	182 (13.3%)	
Sex	Male	695 (50.8%)	3 (0.2%)
	Female	671 (49.0%)	
Gestation age at birth	Days	279 [271, 286]	3 (0.2%)
	Weeks	39.9[38.7,40.9]	
Infant			
Preterm birth	Delivery >34 weeks	1342 (98.0%)	3 (0.2%)
	Delivery <34 weeks	24 (1.8%)	
	Delivered >37 weeks	1285 (93.9%)	
	Delivered < 37 weeks	81 (5.9%)	
Birthweight (g)		3447[3120,3788]	3 (0.2%)
Low birthweight <1.5kg		12(0.88%)	3 (0.2%)
SGA 10% WHO		83(6.06%)	3 (0.2%)
SGA 10% customised		150(10.96%)	3 (0.2%)
LGA 90% WHO		158(11.54%)	3 (0.2%)
LGA 90% customised		111(8.11%)	3 (0.2%)
Apgar at 5 min	Score	10[9,10]	26(1.9%)
	>=7	1324(98.6%)	
	<7	19(1.4%)	
NICU	Not admitted	1264 (92.3%)	3 (0.2%)
	Admitted	102 (7.5%)	
NICU Indication	Preterm	32 (2.3%)	2/102(2.0%)
	SGA	3 (0.2%)	
	Respiratory distress	41 (3.0%)	
	Birth asphyxia	3 (0.2%)	
	Infection	22 (1.6%)	
	Congenital abnormality	3 (0.2%)	
	Feeding problem	2 (0.1%)	
	Phototherapy	9 (0.7%)	
	Hypoglycemia	24 (1.8%)	
	Cyanosis	2 (0.1%)	
	Other NICU indication	29 (2.1%)	
		3[1,14]	8/102(7.8%)

Table 4.9: *Birth and infant outcomes in n=1369 pregnancies. (continued)*

Outcome		n=1369	Missing
NICU Days admitted	1 day	29 (2.1%)	
	2-3 days	19 (1.4%)	
	4-13 days	22 (1.6%)	
	14+ days	24 (1.8%)	
Neonatal nights	0 nights	111 (8.1%)	22 (1.6%)
Neonatal nights	1-3nights	1033 (75.5%)	
	4-10 nights	174 (12.7%)	
	More than 10 nights	29 (2.1%)	

Note:
102 neonates were admitted to the NICU after revision of discharge summaries and search on UK database among whom two had missing NICU indication (one born at 28+6 weeks, weighing 1130gr with unknown apgar score and one born at 38+1 weeks weighing 3890g with a 5-min apgar score of 6). LGA: Large for gestational age; NICU: Neonatal Intensive Care Unit; SGA: Small for gestational age.

4.6 Missing data

Three responses were missing out of all the 3463 EPDS questionaires included in this study. At time point two, one participant had an answer missing for the item six ("overwhelmed") which was inputed to zero as her two other scores for this item were zero. Another participant had a missing answer for item seven ("poor sleep") also at time point two which was inputed to 2 based on the her answer scoring two at the first time point and one at third time point. At time point three one participant did not answer item six which was inputed to zero because she scored zero for this item at the two previous time points. A frequency matrix is provided in table 4.2 and the responses to the Self-harm item in table 4.3 for it is the only item which requires direct referral to mental health services regardless of the total score.

Demographic and lifestyle variables collected at 17 weeks gestation were available for all women, expect four (0.29%) had missing data on the index of multiple deprivation, 39 (2.85%) on accommodation and ten (0.73%) on waist/neck/hip and thigh circumferences.

Blood samples were not collected from two centres (Sunderland and King's College Hospital). Logistic regression of missing blood sample at the first visit was associated with center variable only ($p<0.05$). At time point 2 (OGTT) missing blood sample was associated with younger maternal age and centre. Missing blood at time point 3 was associated with centre, younger maternal age, black ethnicity and intervention arm. On obstetric complications, 184(13.4%) had missing values, primarily due to no-show at the OGTT so the GDM diagnosis was missing, see Table 4.8. There was no infant outcome for 3 pregnancies: One participant withdrew before the OGTT visit (i.e. second) and one participant prior to the third visit who consented for the team to obtain outcome data but these were only partially retrieved. One participant was lost to follow-up before the OGTT for whom there were no infant data (Table 4.9).

The LCGA and auxiliary group-wise comparisions in adjusted models (of continuous and categorical outcomes) in *Mplus* relied on the Full Information Maximum Likelihood (FIML) function, under the missing at

random (MAR) assumption i.e. missingness could be explained by the observed variables. FIML is asymptotically equivalent to multiple imputation and thus helps avoid listwise deletion. This allows computation from all available data and therefore increasing power. The MAR assumption was assumed in this LCGA so modeling the longitudinal trajectories from all time points meant previous EPDS scores could predict missingness on the next score, i.e. based on the reasoning that high levels of depression being associated with attrition or not attending the next visit. Equally, given the known predictors for missingness on other outcomes, values are presented without adjustment and adjusted by age, randomisation (visit 2 and 3 only), ethnicity (white/other) to meet the FIML assumption and latent obesity and latent SES to remove the effects of baseline differences and for theoretical reasons.

4.7 Statistical Analysis

4.7.1 Latent Class Growth Analysis - LCGA

Reporting of the LCGA followed the *Guidelines for Reporting on Latent Trajectory Studies (GRoLTS)* Checklist (Schoot et al., 2017b). LCGA can detect the presence of subgroups of individuals which share a pattern of scores and change over time and assigns them probabilistically to latent classes (Muthén and Muthén, 2000). Unlike repeated measure ANOVA, it provides an intercept and a rate of change (slope) (Fan, 2003) and retrieves classes in a data-driven fashion, here from the sum EPDS scores at from each time point. *Mplus v8.3* (Muthén and Muthén, 2017) was used to generate all the models described and the outputs integrated with the R package *MplusAutomation* (Hallquist and Wiley, 2018). To handle missing data, *Mplus* implements full information maximum likelihood (FIML) under the assumption of missing at random (MAR) and the robust maximum likelihood (MLR) estimator dealt with non-normal distributions of EPDS scores. Each run was checked it had reached global maximum i.e. the best log-likelihood value had successfully replicated.

We favoured LCGA over Growth Mixture Modelling because we did not expect that within group variation in EPDS scores to be easily interpretable when depressive symptoms are inferred form the total EPDS score which measures feelings over the previous short window of 7-days. LCGA can offer more parsimonious results once an overall pattern of score is established longitudinally, one can more confidently assume that effects of mental health may have been present on a continuum of the score and relative to the trajectory identified. Therefore, variance in the intercepts and slopes within-group was fixed to 0 rather than allowing for within group intercept and slope variance to be estimated (i.e., GMM) across pregnancy. Nevertheless GMM models were generated in accordance to the reporting guidelines, as well as the complete case LCGA (see below). The analysis commands for the LCGA in *Mplus* are included below.

```
DATA:
!listwise=ON # uncommented in full case analysis

ANALYSIS:
TYPE = MIXTURE;
starts=700 90;
stiterations=10;
estimator =mlr;
LRTSTARTS= 20 5 200 50;

MODEL:

%overall%
i s | EPDStotal_T1 @ 0 EPDStotal_T2@1 EPDStotal_T3 @ 1.6; i-s @ 0; # removed in LGMM modelling
```

The first timepoint was set at 0 as the intercept and the second and third time points set at 1 and 1.6 to reflect the time line at which the questionnaires were obtained. Only a linear model was tested given the 3 timepoints available. The final choice of 700 repeats and 90 final optimisations was chosen based on previous optimisation steps (so as to faciliate running the model in the loop script in the final stage).

4.8 Criteria

One to five-class solutions were generated as the range often identified in similar studies (Baron et al., 2017). We evaluated several indices in order to assess and select the best fitting model: for parsimonious fit we present the Aikaike Information Criterion (AIC, Akaike (1987)), Bayesian Information Criterion (BIC), sample-size Adjusted BIC (aBIC) where the smallest values is prefered. We also evaluated the entropy (measures the accuracy of group assignment and membership) where entropy =1 reflects perfect classification and 0 is poor and > 0.6 is considered good classification in large sample sizes (Asparouhov and Muthén, 2014). Additionally, to select which final model would be carried for further analyses we evaluated class sizes (minimum count and % of total), and a significant Lo-Mendell-Rubin Likekihood Ratio Test (LMR-LRT, Lo (2001)) was interpreted as an improvement in fit compared to the n-1 class model. A qualitative judgment was also made, opting to apply the principle of parsimony and placing an importance on ease of interpretability and theoretical justifications in the model selection (Wickrama et al., 2016).

4.8.1 Group comparisons

From the chosen model we first compared the classes against a reference class (see below) across baseline socio-demographic characteristics. Further comparisons involved unadjusted and adjusted multinomial/logistic regressions for categorical outcomes and pairwise comparison of means for continuous outcomes. To control for group-wise baseline differences and assess the specific influence of maternal mental health, maternal age, nulliparity, white ethnicity, latent obesity and latent SES served as covariates in adjusted (regression) analyses of other outcomes and the intervention effect was included at 27 weeks and 34 week only. .

Unadjusted analyses are provided by the AUXILIARY option in *Mplus* (Asparouhov and Muthén, 2014; Bakk and Vermunt, 2016) using the “automatic” method. The DCAT function for categorical outcomes and the BCH function for continuous outcomes allowed for group comparison while accounting for the classification error (i.e. fractional class membership) performed during the LCGA, employing a 3-step approach.

The three steps involve

1) building the unconditional Latent Class Growth model.

2) assigning individuals to classes based on their posterior class membership probabilities *W*.

3) compares classes on auxiliaries (i.e. class predictors or distal outcomes) accounting for the assignment error in 2).

In the second step of the 3-step approach using the BCH method, weights are computed per individual based on their inverse logits error rates and brought into the third step (Nylund-Gibson et al., 2019). These methods are thus unbiased by the lack of perfect class assignment (Nylund-Gibson and Masyn, 2016) (Table 4.12). Means and categorical probability estimates are robust in large samples with good class assignment (entropy >0.6, (Asparouhov and Muthén, 2014)). This 3-step approach is viewed as superior and indicated, regardless of entropy, over the “classify-analyse” approach which only compares the classes based on most probable participant class membership (Nylund-Gibson et al., 2019). The BCH function has shown to be

robust and unbiased even in the assumption of homoskedasticity is in the distal outcome is violated and the variable distribution is non-normal or bimodal rather than normally distributed (Nylund-Gibson and Masyn, 2016).

Adjusted analyses are done using the "manual" method(Asparouhov and Muthén, 2014) because the automatic methods described above cannot include covariates. All dietary variables, blood biomarkers, pregnancy and birth outcomes were defined as distal. When distal variables were binary (e.g. SGA) and adjustment included latent covariates (SES and Obesity) the model requires numeric integration and the BCH function is not available. In this case, the 3-step method relies on saving the modal (most likely) class assignment in the first step and their estimated conditional probabilities are computed in the second step to represent the classification error.

Standard deviation unit difference in group means or Odds ratio [95%CIs] against the reference class are presented. When variables are categorical/binary, we provide the probability (%) of response for each class. If CIs excluded 1 or 0 for the ORs and mean differences, respectively, the effect was viewed as significant.

Blood and dietary variables (continuous) were standardized and log transformed if non-normally distributed. We visually report the main effects of covariates on blood and dietary outcomes as these are underreported in the literature but may have clinical and epidemiological value. The interaction of covariates with classes was evaluated by within-class regressions. To improve estimation of the all covariate main effects, the covariance of latent SES and obesity were included in regression models since theoretically plausible.

Additionally, MLR provided robust estimates and standard errors. In a sensitivity analysis, we excluded blood markers from 120 participants taken at 27 weeks from one study site which deviated from the UPBEAT processing protocol (analytes were retrieved from a 1h OGTT sample rather than fasting blood).

4.8.2 Latent factor modelling

As a further approach taken to minimise bias, we implemented an additional theoretically directed application to the structural framework described in this study: *socio-economic status* is, by definition, a multifaceted social construct and we opted to represent this by creating a new latent variable ("SES") using the following variables as indicators: income (ordinal), index of multiple deprivation (ordinal) and highest education attained (ordinal). Similarly, we derived a latent *Obesity* variable using sum of skinfold, waist and hip circumferences and BMI as indicators as a more valid/closer representation of the implied physiological impact of adiposity. A strength in the present study is a large variability of BMI above 30 kg/m^2 which this latent variable can capture in further depth. We verified that the measurement models of each latent variable was valid (i.e. standardized factor loadings were all significant, see Figure 4.5).

Overall, this strategy allows for the reduction in the the measurement error, and therefore bias, introduced by otherwise using each indicator as independent proxies of SES or obesity in regression analyses, so their latent forms were used in all adjusted models. To improve model fit further and improve the estimation of the main effects of the covariates, in models including both SES and Obesity, we included their covariance as this is theoretically plausible relationship.

4.9 RESULTS

Baseline characteristics for the study sample (n=1369) at baseline are in Table 4.10. The median [IQR] EPDS scores at 17, 27 and 34 weeks were 6[3 to 10], 5[2 to 9] and 5[2 to 8] and frequencies for scores >= 13 points threshold were 157/1300 (12.1%), 118/1158(10.2%) and 82/1005(8.2%) respectively. One to five class models were generated and all reached convergence and the best log-likelihood was replicated in each solution. Model estimation, selection criteria and summaries are provided in Tables 4.11 and 4.12).

Table 4.10: *Entry characteristics at first visit in the whole sample of 1369 obese pregnant women.*

Factor		N=1369
Age(years)		30.5 (5.47)
Main ethnicity	White	857 (62.6%)
	Black	351 (25.6%)
	Asian	85 (6.2%)
	Other	76 (5.6%)
UK born	No	447(32.6%)
	Yes	922(67.3%)
Index Multiple Deprivation	Least Deprived	54 (3.9%)
	2nd quintile	90 (6.6%)
	3rd quintile	155 (11.3%)
	4th quintile	462 (33.7%)
	Most deprived	604 (44.1%)
Highest education attained	None	57 (4.2%)
	GCE (or equivalent)	224 (16.4%)
	Vocational qualification	333 (24.3%)
	A level (or equivalent)	214 (15.6%)
	First degree	365 (26.7%)
	Higher degree	176 (12.9%)
Job situation	School	60 (4.4%)
	In paid/self employement	894 (65.3%)
	Looking after home or family	240 (17.5%)
	Not doing paid work	147 (10.7%)
	Government scheme training	6 (0.4%)
	Permanently unable to work	9 (0.7%)
	Retired	1 (<0.1%)
	Doing something else	12 (0.9%)

Table 4.10: *Entry characteristics at first visit in the whole sample of 1369 obese pregnant women. (continued)*

Factor		N=1369
Parity	Multiparous	771 (56.3%)
	Nulliparous	598 (43.6%)
Child(ren) in household age < 2yrs	0	1,182 (86.3%)
	1+	187 (13.7%)
Living with partner	Yes	1,060 (77.4%)
	No	309(22.6%)
Type of accommodation	Own house/flat	443 (32.4%)
	Temporary	6 (0.4%)
	Family/Friends rent free	80 (5.8%)
	Private rental	413 (30.2%)
	Council rental	359 (26.2%)
	Other	29 (2.8%)
Income at entry (gross)	< £12,688	247 (18.0%)
	£12,688 - £17,628	159 (11.6%)
	£17,629 - £23,452	112 (8.2%)
	£23,453 - £32,500	169 (12.3%)
	> £32,500	479 (35.0%)
	Prefers not to answer	203 (14.8%)
Anthropometrics	BMI	35.0 (32.8, 38.5)
Folate supplement	Daily	800(58.4%)
	Less than daily	91(6.6%)
	None	478(34.9%)
Asthma	No	1113(81.3%)
	Yes	256(18.7%)
Smoking	No	1279(93.4%)
	Current	90(6.6%)
Randomisation	Controls	676(49.38%)
	Intervention	693 (50.62%)

Note:
Continuous variables presented as mean(standard deviation) or median (interquartile range) if non-normally distributed. Asthma was coded as 'yes' if it had been diagnosed by a medical practitioner. Missing: Index of Multiple Deprivation=4, accommodation=39.

Table 4.11: *Model fit estimates of LCGA 1-5 class solutions and counts per class (%)*

							Count(%) per class				
n-class Model	LL	BIC	AIC	aBIC	Entropy	LMR aLRT p-value	Class 1	Class 2	Class 3	Class 4	Class 5
1	-2282	4601	4575	4585			1369(100)				
2	-1774	3607	3565	3581	0.76	0.00	395(29.8)	975(71.2)			
3	-1589	3257	3199	3222	0.77	0.00	96(7.0)	524(38.3)	749(54.7)		
4	**-1531**	**3163**	**3090**	**3119**	**0.72**	**0.02**	**62(4.5)**	**219(16.0)**	**513(37.5)**	**575(42.0)**	
5	-1496	3114	3026	3060	0.74	0.30	32(2.3)	62(4.5)	217(15.9)	488(35.6)	570 (41.6)

Note:
One to five class models were generated by Latent Class Growth Analaysis using all available data and compared accross model fit indices, entropy and whether a n-class model significantly improves the data fit compared to a (n-1)-class model using the Lo-Mendell-Rubin adjusted Likekihood Ratio Test (LMR-aLRT). AIC:Aikaike Information Criterion; BIC: Bayesian Information Criterion; aBIC: sample-size Adjusted BIC; LL:log likelihood.

Table 4.12: *Class-wise average probabilities, slopes and intercepts for the 4-class model.*

Average latent class probabilities for most likely latent class membership (row) by latent class (column)					Intercept			Slope		
Class	1	2	3	4	Estimate	95% CI	p-value	Estimate	95% CI	p-value
1	0.79	0.00	0.16	0.05	11.3	[10.24,12.38]	p<0.001	-0.34	[-1.25,0.57]	0.468
2	0.00	0.88	0.11	0.00	3.4	[2.97,3.82]	p<0.001	-0.90	[-1.10,-0.70]	p<0.001
3	0.09	0.14	0.77	0.00	7.5	[6.46,8.48]	p<0.001	-0.74	[-1.13,-0.35]	p<0.001
4	0.12	0.00	0.00	0.87	16.1	[14.37,17.79]	p<0.001	0.77	[-0.34,1.87]	0.174

Note:
The diagonal in the average latent class probabilities reflect the classification accuracy. Entropy of 0.72 is mostly contributed by Class 1 ('Subclinical') which share observations with Class 3 ('Moderate') and class 3 sharing observation with class 2 'Not Depressed'.In the 4-class model selected, each class intercept represents the mean score at baseline visit and the mean growth estimate in symptom scores is represented by the slope. A significant intercept refers to a value different from 0. A positive and significant slope estimate is interpreted as a worsening in depressive symptoms (as evaluated from the total EPDS score) whereas a negative significant slope is interpreted as an improvement in symptoms across pregnancy.

4.9.1 Model selection and interpretation

The 1-class model has the worst fit (highest BIC/AIC/aBIC values) suggesting heterogeneity in depressive symptom trajectories from 17 to 34 weeks gestation.

Although the 5-class model had the lowest AIC/BIC/aBIC values, the smallest class size was n=32 (2.3%) in the 5-class model which was judged to be insufficient for meaningful and powered group comparisions whereas the smallest count was n=62 (4.5%) in the 4-class model. Additionally, the Lo-Mendell-Rubin adjusted LRT p-value was above 0.05 in the 5-class solution suggesting that it was not a significant improvement to the 4-class model whereas the p-value for the 4-class model was significant at 0.0239 over the 3-class model.

Despite the 4-class model having the lowest entropy (0.72) it is still considered of good standard i.e. > 0.6 for further analyses of group comparisions especially in large samples such as this one (Asparouhov and Muthén, 2014).

In the 4-class solution selected here, we viewed the mean intercepts and growth estimates to label the groups, see Table 4.12. The unstandardized mean intercepts and slopes obtained from the 4-class solution were the following:

Class 1 (n=219, 16%) had a mean intercept of 11.3 points (i.e. at baseline, standard error [SE]=0.55, p<0.001) and mean slope of -0.34 (SE=0.46, p=0.47) which was labeled as *"Moderate"* and stable.
Class 2 (n=575, 42%) had a mean intercept of 3.4 points (SE=0.22, p<0.001) and a slope of -0.9 points (SE=0.1, p<0.001) which was labelled as *"Not Depressed"* with symptoms improving over pregnancy.

Class 3 (n=513, 37.5%) had a mean intercept of 7.5 points (SE=0.5, p<0.001) and a mean slope of -0.74 (SE=0.20,p<0.001) which was labelled *"Mild"* and showed improvement over pregnancy.

Class 4 (n=62,4.5%) had a mean intercept of 16.1 points (SE=0.87, p<0.001) and a mean slope of 0.77 (SE=0.56, p=0.174) which was labelled as *"Severe"* with a chronic/stable feature.

Hence we find that among 1369 obese women there are 4 latent groups which are distinguished by their average baseline EPDS score and growth trajectories. Two groups of women ("Not Depressed" and "Mild") form 79.47% of the total sample and show low and moderate symptoms which on average improve from baseline 15-18 weeks until 34-36 weeks.

4.9.2 Class formation and the effect of gestational age and randomisation

To ascertain the robustness of the classification we also assessed whether the variability in gestational age at each visit could have an influence so we also performed the analyses adjusting the EPDS scores for GA at each time point within the LCGA and found there was no significant influence of GA (p=0.481, p=0.253 and p=0.198 at visit 1,2 and 3 respectively) so that the group trajectories and proportions stayed equivalent (4.3% vs 4.5%, 16.5% vs 16%, 36.3% vs 37.4% and 43% vs 42%), with the 4-class solution also yielding the best solution by aforementioned criteria.

Furthermore, because the intervention may have influenced the EPDS score at visit 2 and 3, we repeated the 4-class LCGA analysis adjusting for the effect of randomization and we found no significant effect on the second (p=0.300) or the third EPDS score (p=0.860) and no change in the class proportions described in the model above.

4.9.3 Other growth models considered

Additionally for completedness and in line with the guidelines (Schoot et al., 2017b), Tables 4.13 and 4.15 with Figure 4.1 are provided for comparisons between 1-4 class solutions when using 935 cases with complete data (EPDS available at three time points) vs all available data LCGA as well as the the LCGA vs GMM model solutions using all data.

The robustness of FIML to deal with missing data is reflected in the model solutions generated from 935 participants who provided EPDS scores at all 3 timepoints. There the change in model fit indices is only of a degree of magnitude (4.1) while it also agrees to a 4-class model as the most appropriate according to the LMR-LRT (Table 4.13). Figure 4.2 and table 4.14 show that the overall distribution of means intercept and mean slopes for the 4 classes is almost identical in the "Not Depressed","Mild" and "Moderate" to the LCGA using all available data. The complete case sample (n=935) also yielded a 4-class model. The

difference between these two models, for the “Severe” class, the intercept is 1 point lower in the complete case analysis (15.06 vs 16.08) and the slope is positively significant (1.16, p=0.039, vs 0.77 p=0.174 in the full data analysis), indicating a worsening of symptoms among these women.

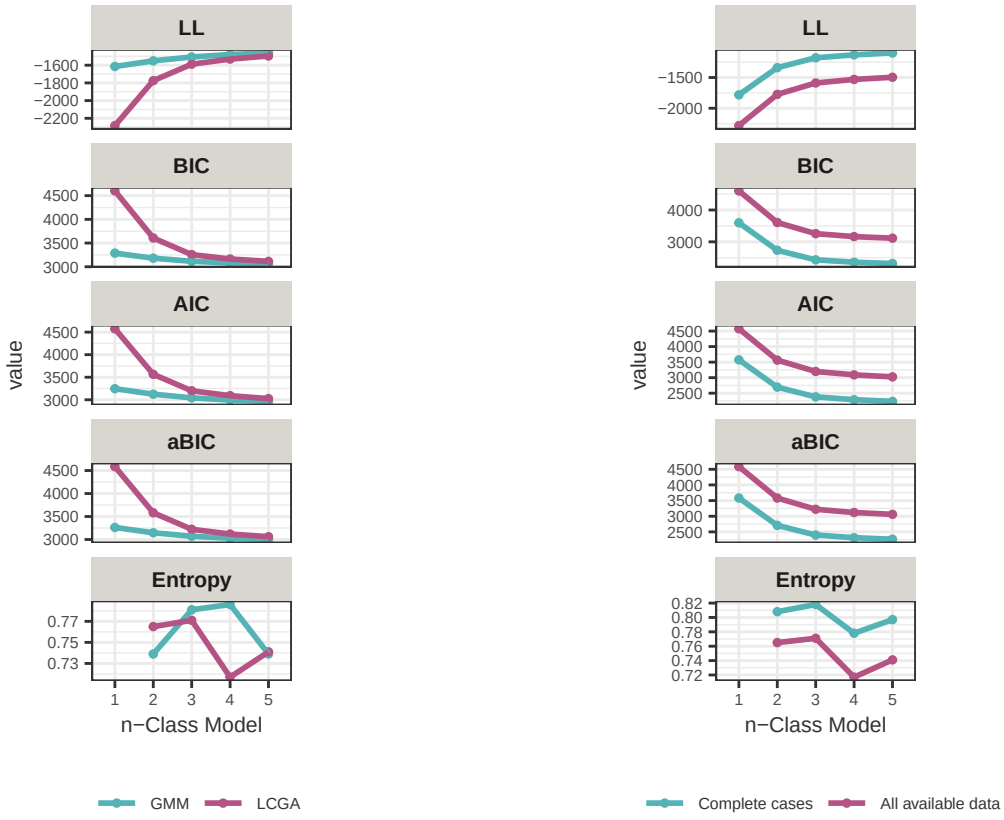

Figure 4.1: *Distribution of Model indices across 1-5 class model solutions using LCGA vs GMM (left panel) with all available data (n=1369) and the 1-5 class model solution by LCGA with all available data (n=1369) vs LCGA with complete cases only (n=935, right panel) . AIC:Aikaike Information Criterion; BIC: Bayesian Information Criterion; aBIC: sample-size Adjusted BIC; LMR-LRT :Lo-Mendell-Rubin Likekihood Ratio Test; LL:log likelihood.*

Table 4.13: *Model fit indices for the 1-5 class solutions from subgroup of women with 3 EPDS scores (n=935).*

n-class	LL	BIC	AIC	aBIC	Entropy	LMR-LRT p-value
1	-1782	3599	3574	3583		
2	-1339	2733	2695	2708	0.81	0.000
3	-1179	2434	2381	2399	0.82	0.004
4	**-1131**	**2359**	**2291**	**2314**	**0.78**	**0.002**
5	-1101	2319	2237	2265	0.80	0.146

Note:

The Latent Class Growth Analysis is generated for the complete cases analysis by listwise deletion. Models representing the best fit according to indices and LMR-LRT are in bold. AIC: Aikaike Information Criterion, BIC: Bayesian Information Criterion, aBIC: sample-size Adjusted BIC; LMR-LRT: Lo-Mendell-Rubin Likekihood Ratio Test; LL: log likelihood.

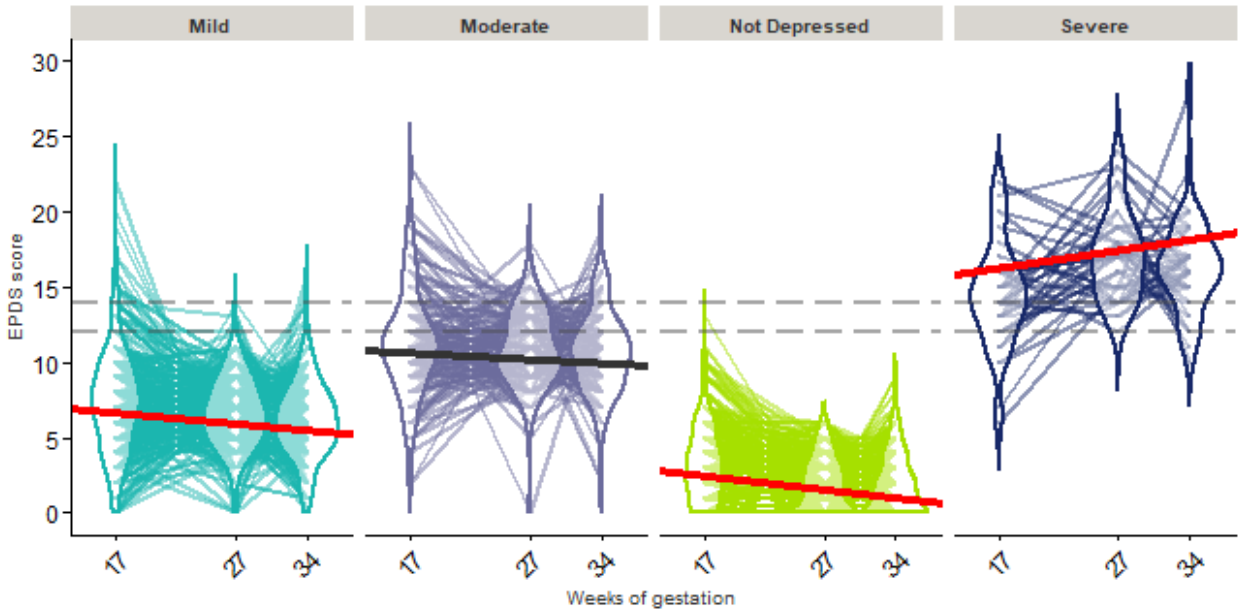

Figure 4.2: *Depressive symptom trajectories and distributions for women who had 3 EPDS scores available (n=935) based on the best fitting solution (4-class model). Each line refers to a subject except bold lines which represent the average trajectory for a class which is red if the slope is significantly different from zero (p<0.05). Violin plots represent the score distribution at each time point.*

Table 4.14: *Mean slopes and Intercepts for the 4-class model using cases with complete data.*

Class	Intercept	p-value	Slope	p-value
1	3.4	p<0.001	-0.92	p<0.001
2	15.1	p<0.001	1.16	0.039
3	7.3	p<0.001	-0.72	0.001
4	11.1	p<0.001	-0.42	0.347

Table 4.15: *Model fit indices using the GMM method.*

n-class	LL	BIC	AIC	aBIC	Entropy	LMR-LRT p-value
1	-1615	3287	3246	3262		
2	-1551	3181	3123	3146	0.74	0.00
3	**-1507**	**3116**	**3042**	**3071**	**0.78**	**0.00**
4	-1480	3082	2994	3028	0.79	0.21
5	-1462	3069	2964	3005	0.74	0.18

Note:
Models generated from all available data by Growth Mixture Modelling under the Full Information Maximum Likelihood for missing data handling. Model representing the best fit according to indices and LMR-LRT are in bold. AIC: Aikaike Information Criterion; BIC: Bayesian Information Criterion; aBIC: sample-size Adjusted BIC; LMR-LRT: Lo-Mendell-Rubin Likekihood Ratio Test; LL:log likelihood.

4.10 4-class Model

As mentioned, a 4-class model was accepted and item response probabilities and trajectories by class are shown in Figure 4.3. Classes were labelled based on their means symptom severity and slope as: “Not Depressed” (n=575,42%),“Mild”(n=513, 37.5%),“Moderate”(n=219, 16%) and “Severe”(n=62, 4.5%). The “Not Depressed” class provided the opportunity to obtain a reference “control” class against which the other three classes were compared.

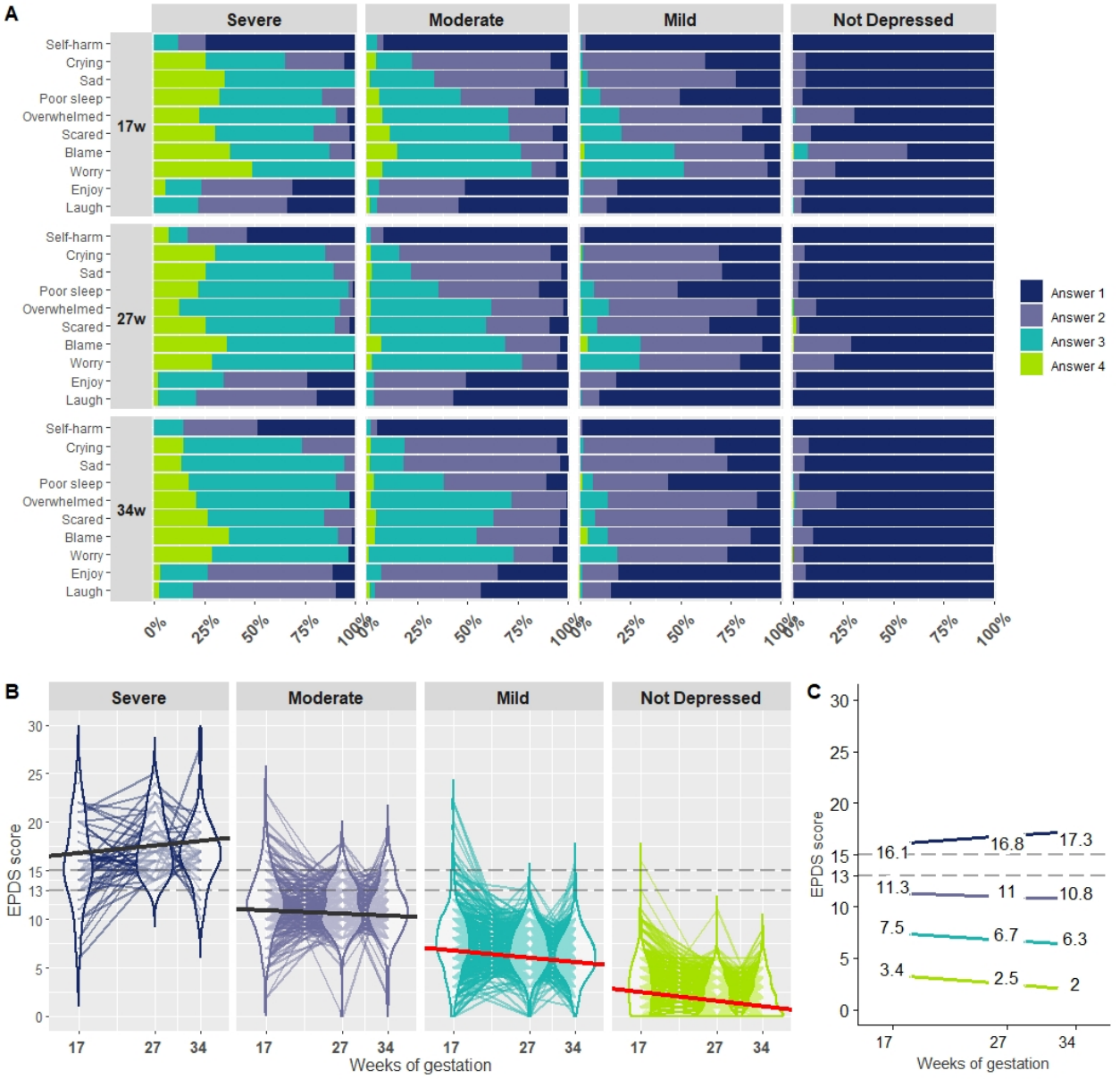

Figure 4.3: *EPDS item response probabilities and total score trajectories of the 4-class model. A. Item response probabilities were estimated for each class where Answer 4 is the highest scoring response (i.e. highest severity/frequency). B. Observed EPDS scores split according to most likely class membership following LCGA using all data available (n=1369). Each line refers to a subject and violin plots illustrate the score distributions. Solid black/red lines are within group average trajectories (slopes different from 0 at p<0.05 are in red). C. Estimated mean score at each time point in each class, colors same as in B. Dashed lines in B. and C. are references to the 12/13 and 14/15 cutoff scores for all and major depression respectively (Murray et al., 1990).*

4.10.1 Baseline characteristics by class

Comparisons on baseline factors against the Not Depressed class are represented in Tables 4.16, Figures 4.4 and 4.5 which also shows the latent factor model. Women in the Severe class were more socio-economically deprived (Mean Difference -0.77[-1.13 to -0.42]), more likely to be of Asian or Other ethnicity

(than White), to live in temporary/council rental or with family rather than their own property, not living with their partners, unemployed, and asthmatic. Women in the Moderate group were more socio-economically deprived (MD -0.41 [-0.64 to -0.17]), more likely Asian or Black than White, and living in council rentals than their own homes. Women in the Mild group were socio-economically comparable (MD 0.00 [-0.20 to 0.21]) to the Not Depressed women but more likely to be Asian, born outside the UK and less likely multiparous. There were no differences in latent obesity nor age between classes.

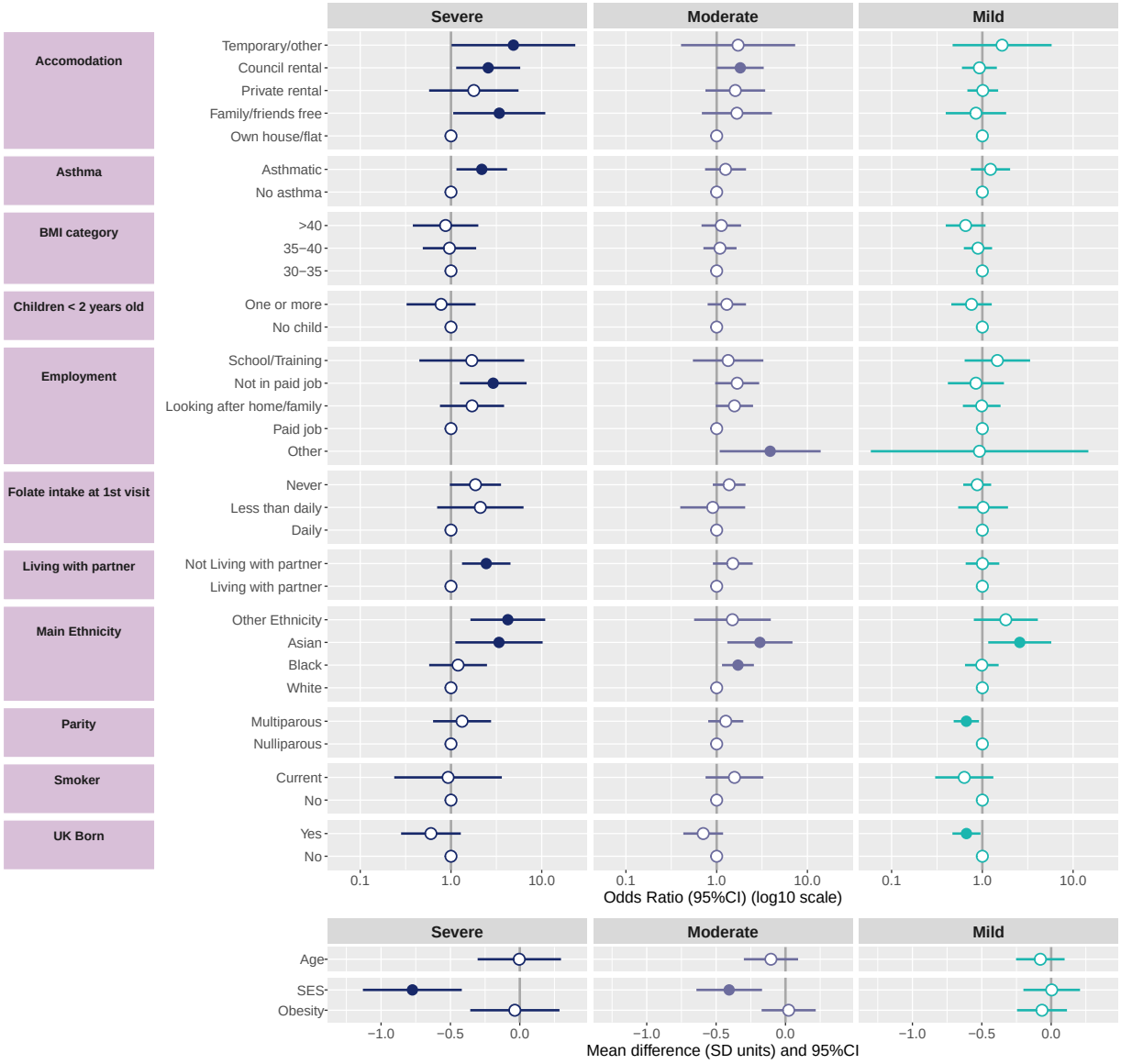

Figure 4.4: *Comparisons against the Not Depressed class on baseline characteristics. The participant counts based on the most likely class membership was n=62 (Severe), n=219 (Moderate), n=513 (Mild) and n=575 (Not Depressed, reference). Odds ratio below 1 indicate the latent group's odds for that category relative the reference category are lower against the same odds in the Not depressed class. Dots are filled if the OR CIs exclude 1 or the mean difference CIs exclude 0, which is interpreted as significant. Measurement error attributed to the LCGA was taken into account. SES and Obesity are latent factors (see appendix page 27). Missing: Index of Multiple Deprivation n=4, Accommodation n= 39.*

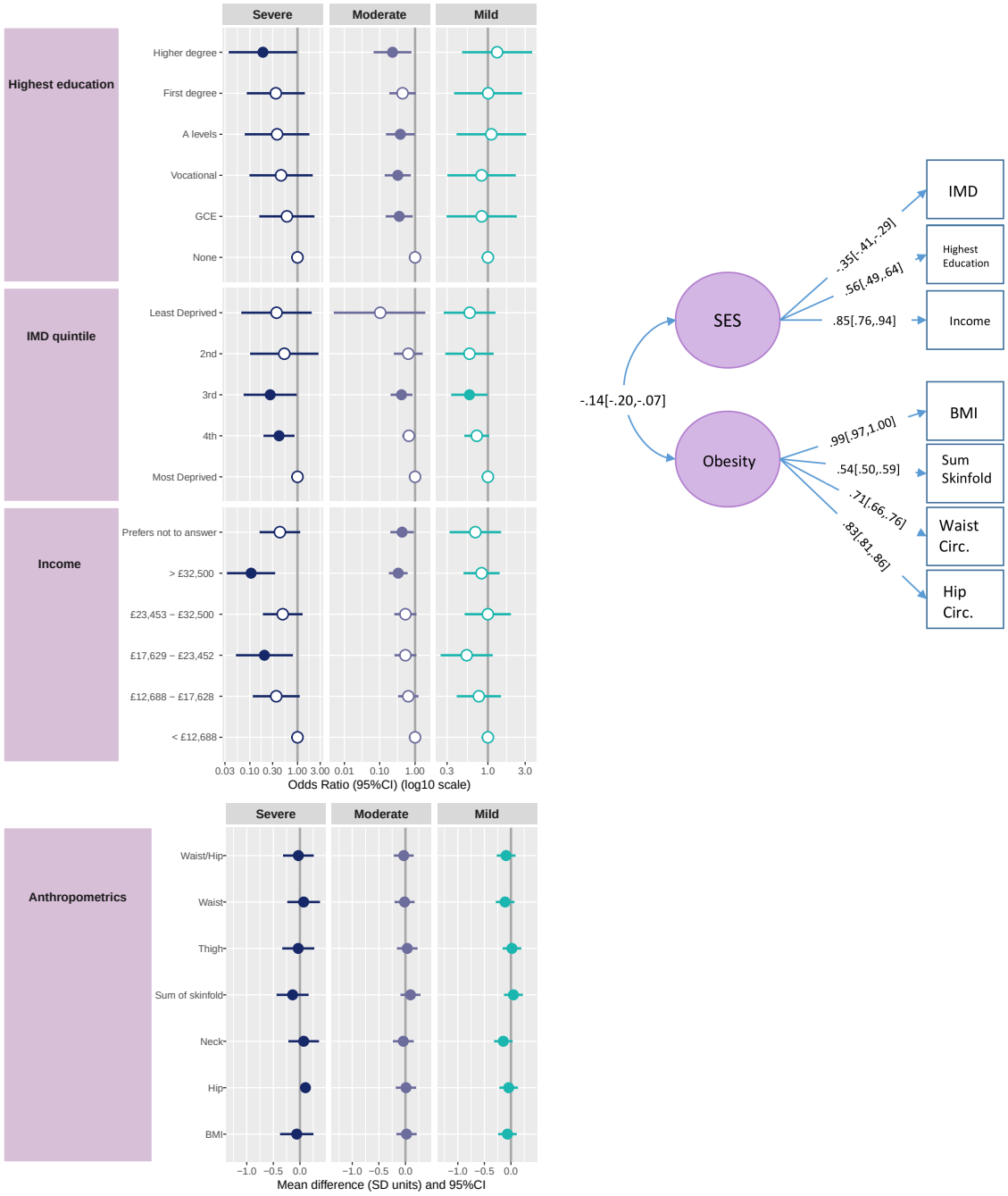

Figure 4.5: *Plot presenting unadjusted Odds Ratios (ORs) and standardized differences of the means and their 95% Confidence intervals (CIs) of the associations betweeen participant socio-economic status and anthropometrics against the reference 'Not Depressed' class. The variables were then used as indicators to model the latent constructs 'SES' and 'Obesity' used as covariates in adjusted models. On the right is the measurement model showing the standardized factor loadings[95%CI] and latent factors covariance (all significant at p<0.05).*

Table 4.16: *Baseline probabilities and Odds ratio against the Not Depressed class.*

		Not Depressed	Severe		Moderate		Mild	
		Probability %	Probability %	OR[95%CI]	Probability %	OR[95%CI]	Probability %	OR[95%CI]
Index Multiple Deprivation (quintile)	Most Deprived	37.10	60.30	ref	51.30	ref	47.10	ref
	4th	36.00	24.00	0.41 [0.19 to 0.87]	33.20	0.67 [0.43 to 1.03]	33.00	0.72 [0.50 to 1.04]
	3rd	14.10	6.10	0.27 [0.07 to 0.96]	8.10	0.41 [0.20 to 0.84]	10.40	0.58 [0.34 to 0.99]
	2nd	7.50	6.50	0.53 [0.10 to 2.77]	6.70	0.64 [0.25 to 1.65]	5.50	0.58 [0.28 to 1.19]
	Least Deprived	5.30	3.10	0.36 [0.07 to 1.99]	0.80	0.10 [0.00 to 1.96]	3.90	0.58 [0.27 to 1.25]
Smoker	No	93.10	93.60	ref	89.60	ref	95.50	ref
	Current	6.90	6.40	0.93 [0.24 to 3.63]	10.40	1.57 [0.75 to 3.27]	4.50	0.63 [0.30 to 1.32]
Main Ethnicity	White	67.70	53.80	ref	53.50	ref	62.50	ref
	Black	24.90	23.70	1.20 [0.58 to 2.49]	33.80	1.72 [1.15 to 2.57]	22.70	0.99 [0.64 to 1.51]
	Asian	3.40	9.10	3.38 [1.12 to 10.24]	8.00	3.00 [1.31 to 6.85]	8.10	2.58 [1.16 to 5.75]
	Other Ethnicity	4.00	13.40	4.23 [1.64 to 10.93]	4.70	1.49 [0.56 to 3.95]	6.70	1.81 [0.80 to 4.09]
Living with partner	Living with partner	79.50	61.40	ref	72.10	ref	79.50	ref
	Not Living with partner	20.50	38.60	2.45 [1.32 to 4.53]	27.90	1.51 [0.91 to 2.50]	20.50	1.00 [0.66 to 1.54]
Children < 2 years old	No child	85.70	88.50	ref	82.20	ref	88.70	ref
	One or more	14.30	11.50	0.78 [0.32 to 1.87]	17.80	1.29 [0.80 to 2.10]	11.30	0.76 [0.46 to 1.27]
Highest education attained	None	3.10	7.40	ref	8.30	ref	3.20	ref
	GCE	17.10	24.30	0.60 [0.16 to 2.27]	16.10	0.36 [0.15 to 0.86]	14.50	0.83 [0.30 to 2.35]
	Vocational	26.40	28.10	0.45 [0.10 to 2.09]	22.50	0.32 [0.14 to 0.76]	22.20	0.83 [0.30 to 2.27]
	A levels	15.00	13.20	0.37 [0.08 to 1.78]	15.20	0.38 [0.15 to 0.98]	16.90	1.11 [0.40 to 3.09]
	First degree	26.00	21.50	0.35 [0.09 to 1.43]	30.30	0.44 [0.19 to 1.04]	26.60	1.01 [0.37 to 2.75]
	Higher degree	12.40	5.50	0.19 [0.04 to 0.98]	7.50	0.23 [0.07 to 0.79]	16.60	1.31 [0.47 to 3.69]
BMI category	30-35	48.40	50.30	ref	46.00	ref	53.80	ref
	35-40	32.20	32.20	0.96 [0.49 to 1.90]	33.30	1.09 [0.71 to 1.66]	32.10	0.90 [0.62 to 1.28]
	>40	19.40	17.50	0.87 [0.38 to 2.00]	20.70	1.12 [0.68 to 1.86]	14.10	0.65 [0.40 to 1.08]
Folate intake at 1st visit	Daily	59.30	43.40	ref	52.90	ref	61.80	ref
	Less than daily	6.50	10.10	2.11 [0.70 to 6.30]	5.30	0.90 [0.40 to 2.06]	6.90	1.02 [0.54 to 1.92]
	Never	34.20	46.50	1.86 [0.97 to 3.56]	41.80	1.37 [0.91 to 2.08]	31.20	0.88 [0.62 to 1.25]
UK Born	No	27.70	38.90	ref	35.00	ref	36.50	ref
	Yes	72.30	61.10	0.60 [0.28 to 1.28]	65.00	0.71 [0.43 to 1.18]	63.50	0.67 [0.47 to 0.95]
Income	< £12,688	13.80	38.10	ref	26.60	ref	16.90	ref
	£12,688 - £17,628	11.40	11.30	0.36 [0.12 to 1.11]	14.20	0.64 [0.33 to 1.25]	10.70	0.77 [0.40 to 1.47]
	£17,629 - £23,452	9.50	5.30	0.20 [0.05 to 0.80]	9.80	0.53 [0.26 to 1.09]	6.30	0.54 [0.25 to 1.16]
	£23,453 - £32,500	11.20	15.20	0.49 [0.19 to 1.28]	11.50	0.53 [0.26 to 1.10]	13.70	1.00 [0.50 to 1.97]
	> £32,500	38.10	11.10	0.11 [0.03 to 0.34]	24.60	0.33 [0.18 to 0.62]	38.80	0.83 [0.49 to 1.41]
	Prefers not to answer	16.00	18.90	0.43 [0.16 to 1.14]	13.30	0.43 [0.20 to 0.92]	13.60	0.69 [0.32 to 1.48]
Parity	Nulliparous	41.20	34.60	ref	35.70	ref	51.30	ref
	Multiparous	58.80	65.40	1.32 [0.64 to 2.77]	64.30	1.26 [0.81 to 1.97]	48.70	0.67 [0.48 to 0.92]
Accomodation	Own house/flat	35.80	19.20	ref	24.70	ref	36.20	ref
	Temporary/other	1.90	5.10	4.88 [1.01 to 23.43]	2.30	1.72 [0.41 to 7.33]	3.20	1.65 [0.47 to 5.80]
	Family/friends free	5.90	10.80	3.40 [1.06 to 10.95]	6.80	1.67 [0.69 to 4.07]	5.10	0.85 [0.40 to 1.83]
	Private rental	30.30	29.00	1.78 [0.57 to 5.55]	33.50	1.60 [0.75 to 3.42]	31.00	1.01 [0.68 to 1.49]
	Council rental	26.00	35.80	2.57 [1.14 to 5.79]	32.60	1.82 [1.01 to 3.30]	24.40	0.93 [0.60 to 1.45]
Employment	Paid job	68.30	51.40	ref	56.40	ref	68.30	ref
	Looking after home/family	16.50	21.20	1.71 [0.76 to 3.85]	21.40	1.57 [0.98 to 2.52]	16.20	0.98 [0.61 to 1.59]
	Not in paid job	10.00	21.90	2.92 [1.25 to 6.81]	13.90	1.68 [0.96 to 2.94]	8.50	0.85 [0.42 to 1.73]
	School/Training	4.00	5.10	1.70 [0.45 to 6.42]	4.40	1.34 [0.55 to 3.27]	5.90	1.46 [0.64 to 3.36]
	Other	1.20	0.40	0.45 [0.00 to NA]	3.90	3.89 [1.08 to 13.99]	1.10	0.93 [0.06 to 14.73]
Asthma	No asthma	83.60	70.00	ref	80.30	ref	80.60	ref
	Asthmatic	16.40	30.00	2.18 [1.15 to 4.15]	19.70	1.25 [0.74 to 2.11]	19.40	1.23 [0.75 to 2.02]

4.11 Diet

Comparisons on dietary intake are shown in Figure 4.6A. Intake as composition (% Energy) and other details are found in Supplementary Table 4.20 and Figure 4.17 at the end of the chapter. There were notable associations between depression and obesogenic dietary factors. Women in the Severe depression class reported at 17 weeks higher glycaemic load (per 100g, aMD: 0.46[0.00 to 0.92]) and lower protein composition (% Energy, aMD: -0.50[-0.85 to -0.15]) after adjustment for baseline factors. At 27 weeks they showed higher energy intake (kcal) after adjustments. For the Moderate group, glycaemic load (per 100g) and energy intake (kcal) was higher in undadjusted comparisions only. However, at 27 weeks, these women reported higher glycemic load, energy intake(kcal, aMD: 0.39[0.14 to 0.65]) and saturated fat intake (grams, aMD 0.32[0.07 to 0.57]) than the Not Depressed group in adjusted comparisons. The only difference between the Mild and Not Depressed classes was at 27 weeks where diet composition in total fats and saturated fats was higher and lower in carbohydrates (all as % of total energy) for the Mild women, after adjustment.

Age, SES and white (vs non-white) ethnicity had overall the strongest effects on the diet at 17 weeks. The intervention, SES and white ethnicity had the largest effect on diet at 27 weeks (Figure 4.7). To explore interaction, within-group regressions suggest that the effect of the intervention was evident in the Mild and Not Depressed classes only while a heterogeneous effects of age, white ethnicity and SES across the classes also suggest interactions with class membership (Figure 4.8).

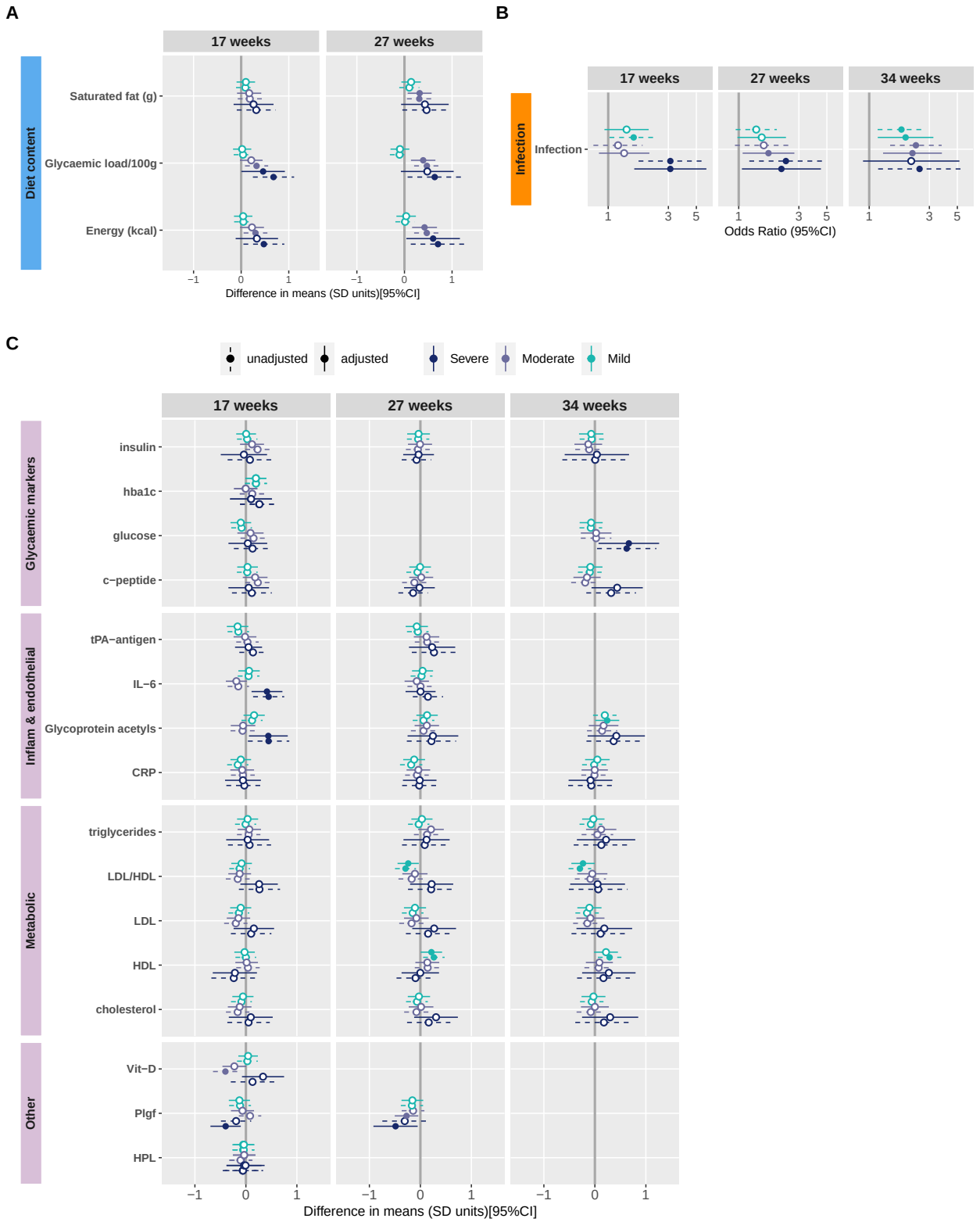

Figure 4.6: *Classes compared against the Not Depressed class on their macronutrient intake (A), self-reported infection prior to each visit (B) and blood biomarkers (C), each presented unadjusted and adjusted for maternal age, nulliparity, white ethnicity (vs non-white), latent SES and latent Obesity, and the intervention effect at 27 and 34 weeks only. Filled circles: CIs exclude 0 for the mean differences in A and C or 1 for the ORs in B, interpreted as significant.*

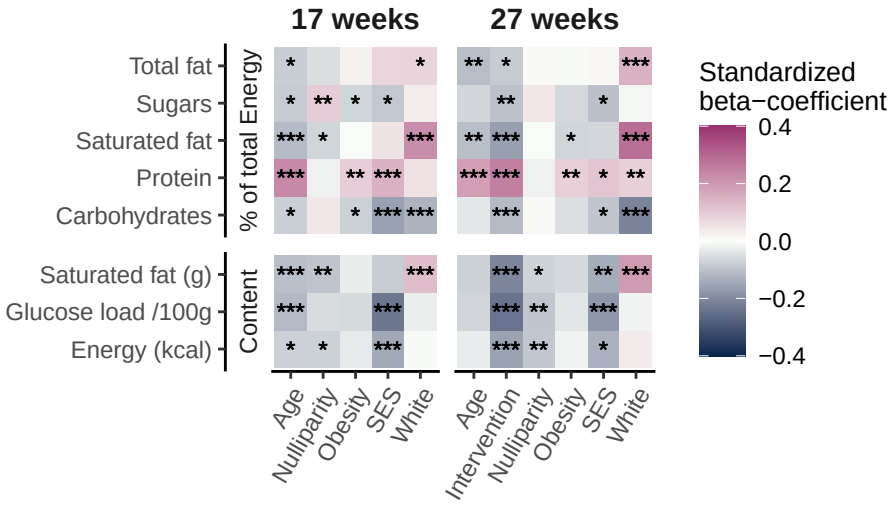

Figure 4.7: *Main effects of covariates on dietary intake at 17 and 27 weeks. Maternal age, nulliparity (vs Multiparity), White (vs non-White), latent SES, latent Obesity and Intervention (vs Controls, included at 27 weeks only) were entered into multiple linear regression models for each dietary factor. Negative SES indicates more adverse SES. Heatmap colors represent the sign and estimate of the standardized coefficients which can be used to evaluate the influence of a covariate when the others are held constant. Variables significantly associated with diet macronutrient are annotated '*':p<0.05,'**':p<0.01,'***'p<0.001.*

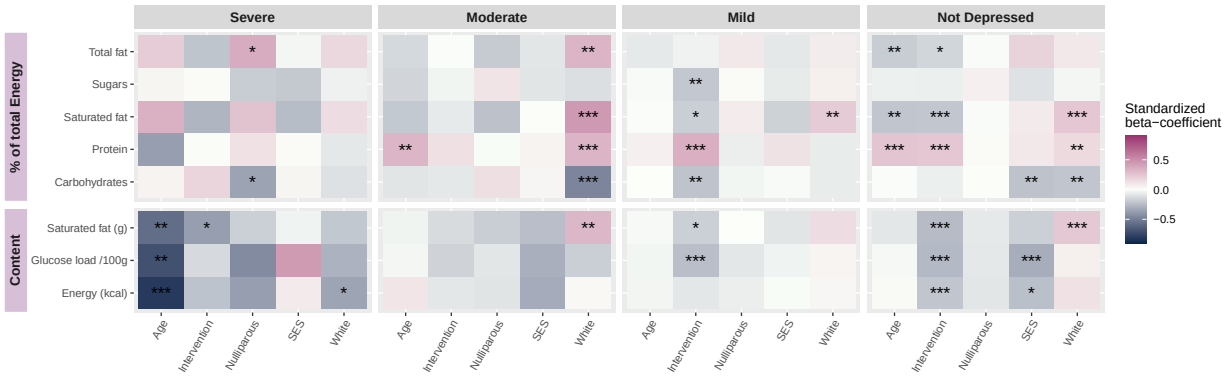

Figure 4.8: *Interaction between the depressive symptom classes and effect of the intervention, conducted bu within-class regressions with age, nulliparity, white (vs non-white) ethnicity and latent socioeconomic status (with income, highest education attained and index of multiple deprivation as indicators). Over- and under reporting was controlled by excluding participants if calorie intake was less than 20Mj (=4,780kcal) and higher than 4.5 Mj (=1,076 kcal).*

4.11.1 Blood markers

Figure 4.6C presents results on inflammatory, metabolic and placental markers (for results on adipokines, amino acids and fatty acids see Figure 4.9). There was no difference in CRP >10mg/L (clinical cut-off for active infection at sampling) between groups at any of the three visits (see Supplementary at the end of the

chapter for details).

At 17 weeks the Severe class had significantly higher adjusted IL-6 and Glycoprotein acetyls and phenylalanine levels and lower PlGF than the Not Depressed group. At 27 weeks, the Severe class phenylalanine remained higher and PlGF lower. At 34 weeks, these women had higher adjusted blood glucose and lower Omega-6/Omega-3 ratio. At 27 weeks Moderate women presented lower PlGF and higher isoleucine and higher alanine in adjusted comparisons. There were no differences between the Mild and Not Depressed groups on blood markers at 17 weeks. However in adjusted comparisons at 27 weeks, women in the Mild class showed lower LDL/HDL ratio (with accompanying higher blood HDL), lower Omega-6/ Omega-3 ratio and higher degree of blood unsaturation and proportion of Omega-3/Total FA. At 34 weeks, they showed higher glycoprotein acetyls, higher degree of unsaturation, lower Saturated FA/Total FA proportion and lower LDL/HDL ratio compared to the Not Depressed women. Sensitivity analyses at 27 weeks generated small changes i.e. adjusted triglycerides was higher in the Moderate group and PlGF was no longer lower in the Severe and Moderate group (Figure 4.12).

Overall, white ethnicity appeared to have the strongest effect on blood biomarkers, followed by latent obesity and SES (Figure 4.10). The effect of the UPBEAT intervention on blood markers at 27 and 34 weeks was not evident when including all other covariates.

At 17 weeks there were no difference between the Moderate women and the Not Depressed women except in the unadjusted vitamin-D level which was no significant after adjustments.

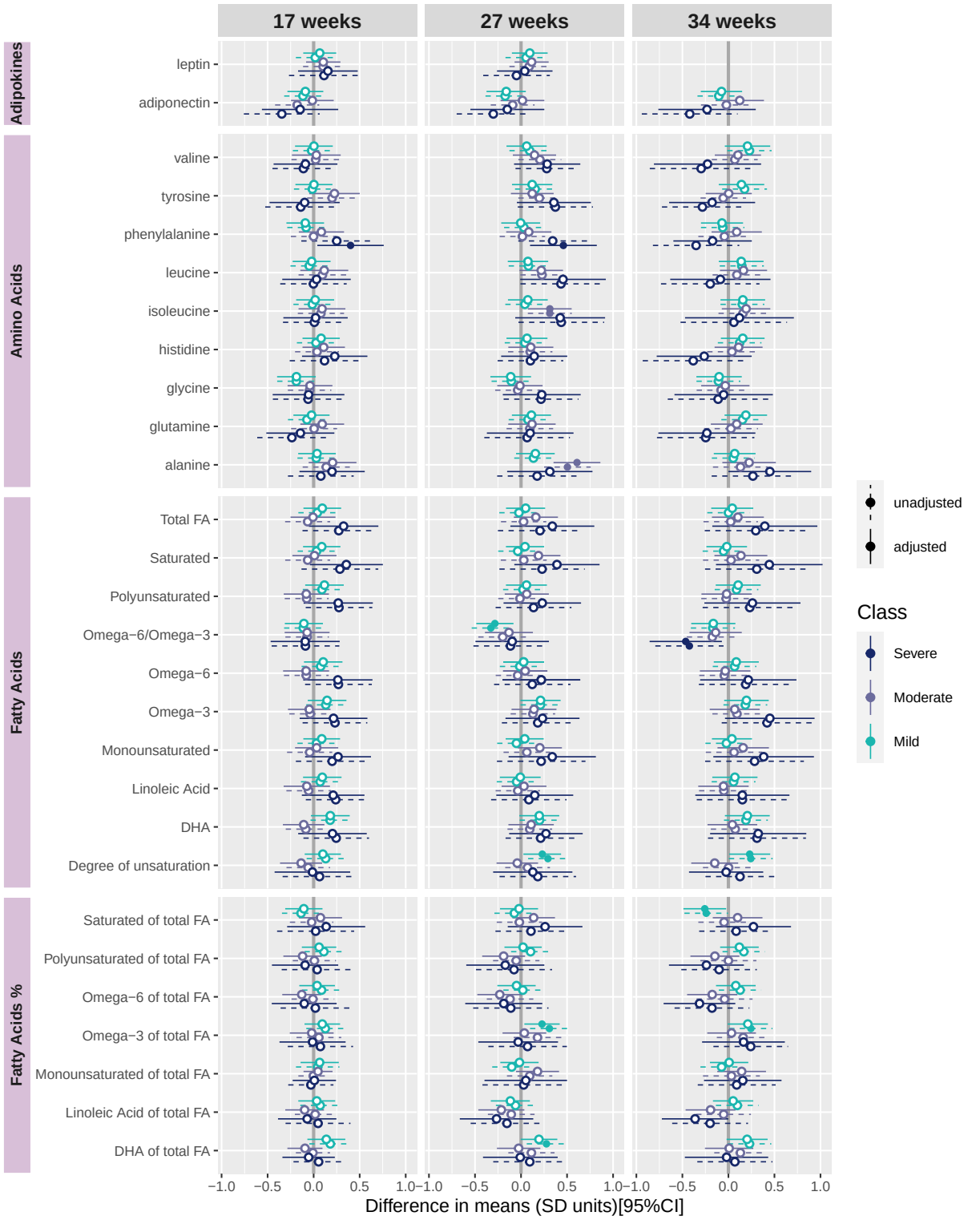

Figure 4.9: *Blood markers were compared between the Severe, Moderate and Mild against the Not depressed class and presented as mean difference [95% CIs] in standardized units unadjusted and adjusted for age, nulliparity and white ethnicity, latent socio-economic status and latent obesity. All estimates are calculated taking into account the class membership measurement error.*

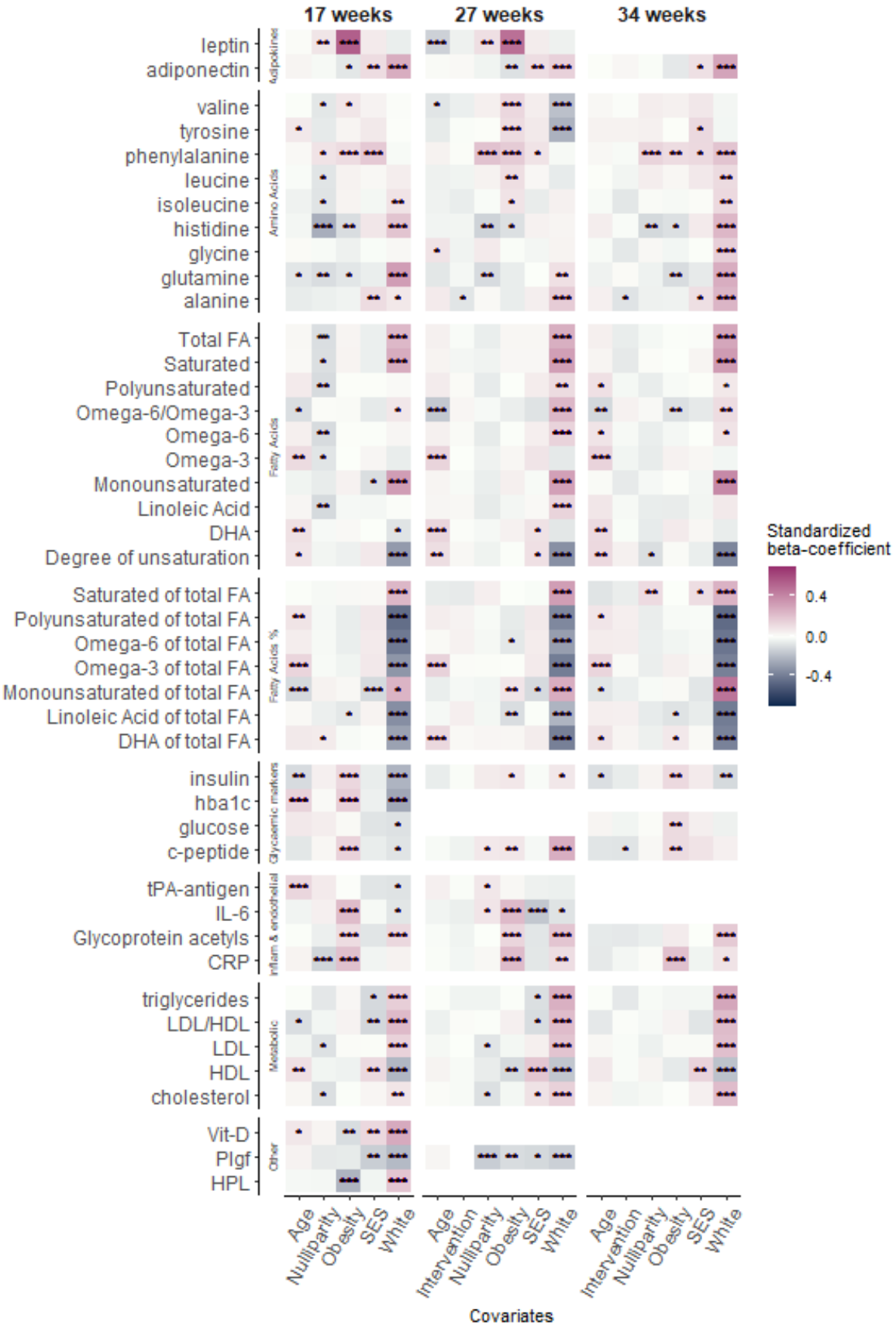

Figure 4.10: *Main effects of covariates included in multiple regressions with each blood marker as an outcome, at 17, 27 and 34 weeks gestation. The correlation of latent SES and latent Obesity was included in each model. Variables significantly associated with metabolites are annotated '*':p<0.05,'**':p<0.01,'***'p<0.001.*

Figure 4.11: *In order to explore an interaction between depressive symptom class and covariates, we performed a within-class regressions. Blood samples at the second visit were taken after overnight fast during the oral glucose tolerance test. Variables significantly associated with metabolites are annotated '*':p<0.05,'**':p<0.01,'***'p<0.001.*

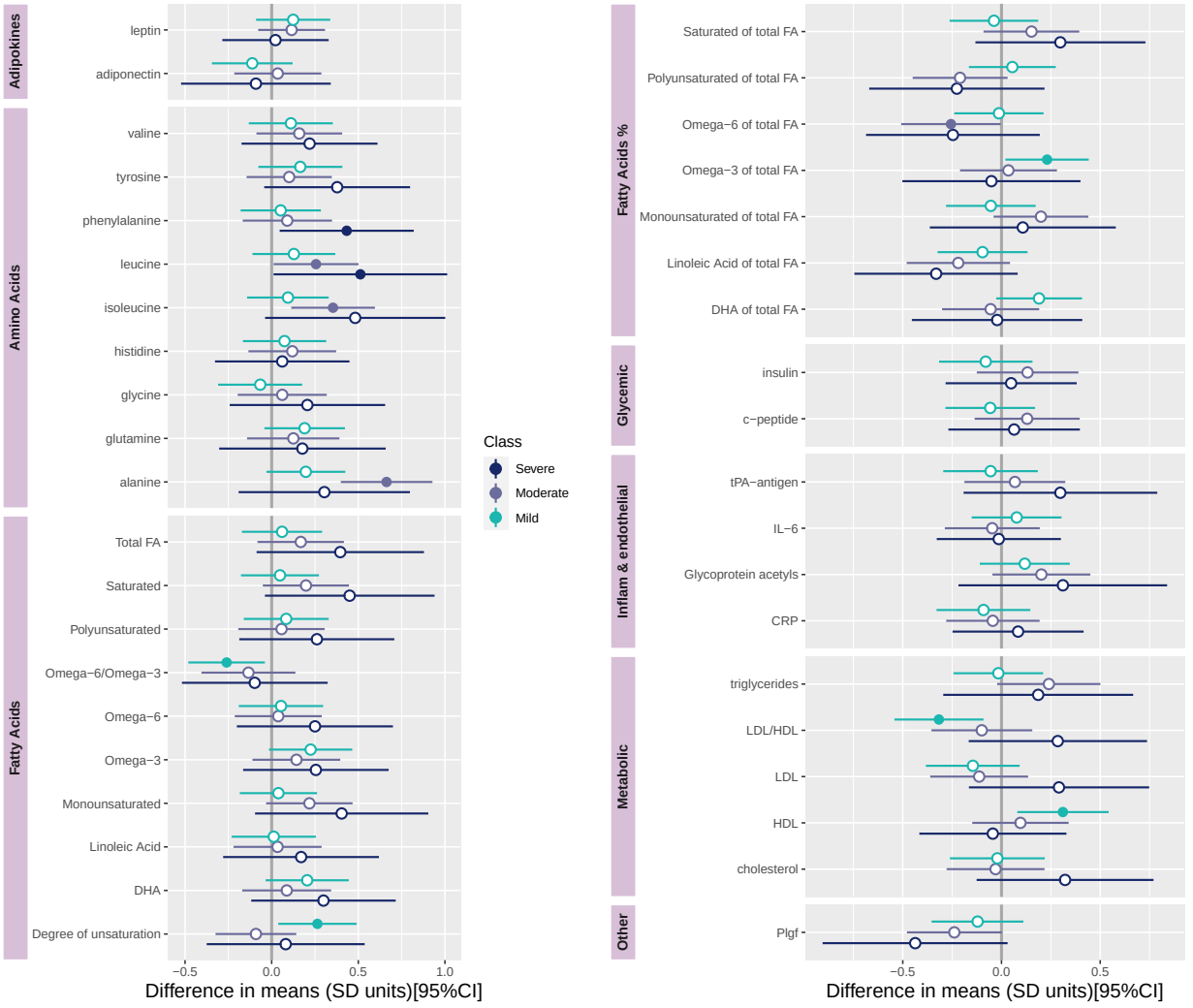

Figure 4.12: *Blood samples at 27 weeks were analysed excluding participants from one centre who had 1h post OGTT research samples rather than the fasting sample (0h). Mean standardized differences are presented after adjustement for maternal age, ethnicity(white vs non-white), nulliparity, latent obesity, latent SES and intervention.*

4.11.2 Pregnancy outcomes

4.11.2.1 Infection

The Severe group was 3.11 times more likely (aOR, a95%CI: 1.61 to 6.00) than the Not Depressed group to report an infection occurring prior to 17 weeks (46.5% probability vs 21.9%) and 2.18 times [1.06 to 4.49] at 27 weeks (40.6% vs 22.4%). The Moderate group was 1.72 times [1.07 to 2.76] and 2.20 times [1.29 to 3.77] more likely at 27 and 34 weeks respectively. The Mild group was 1.94 times [1.17 to 3.23] more likely at 34 weeks, see Figure 4.6B and Table 4.17.

Table 4.17: *Class comparison on reported infections at each visit.*

		17 weeks n= 1344			27 weeks n=1179			34 weeks n=1007		
		Prob (%)	OR[95%CI]	adjOR[95%CI]	Prob (%)	OR[95%CI]	adjOR[95%CI]	Prob (%)	OR[95%CI]	adjOR[95%CI]
Severe	No infection	53.50	ref	ref	59.40	ref	ref	67.00	ref	ref
	Infection	46.50	3.11 [1.74 to 5.56]	3.11 [1.61 to 6.00]	40.60	2.37 [1.21 to 4.62]	2.18 [1.06 to 4.49]	33.00	2.51 [1.18 to 5.34]	2.15 [0.89 to 5.19]
Moderate	No infection	74.90	ref	ref	68.70	ref	ref	68.40	ref	ref
	Infection	25.10	1.20 [0.76 to 1.87]	1.34 [0.84 to 2.12]	31.30	1.58 [0.93 to 2.67]	1.72 [1.07 to 2.76]	31.60	2.35 [1.47 to 3.76]	2.20 [1.29 to 3.77]
Mild	No infection	69.20	ref	ref	71.60	ref	ref	73.90	ref	ref
	Infection	30.80	1.59 [1.02 to 2.47]	1.40 [0.93 to 2.09]	28.40	1.38 [0.95 to 2.01]	1.52 [0.98 to 2.37]	26.10	1.80 [1.17 to 2.78]	1.94 [1.17 to 3.23]
Not Depressed	No infection	78.10	ref	ref	77.60	ref	ref	83.60	ref	ref
	Infection	21.90	ref	ref	22.40	ref	ref	16.40	ref	ref

Note:
Women were asked to report any infections prior to the first visit, between the first and the second visit and between the second and the third visit. Classes Odds ratios (ORs) and confidence intervals (CIs) are presented against the Not Depressed class as the reference class. Adjustment for maternal age, nulliparity, white (vs non-white) ethnicity, latent SES, latent obesity and the intervention (at 27 and 34 weeks only).

4.11.2.2 Obstetric complications

We did not find differences in odds of GDM and preeclampsia between classes (Figure 4.13). However, as GDM was diagnosed from a single OGTT, clinically indicated for all obese women, we inquired on the missing data and found that the OR of missing a diagnosis was 2.9 times more likely for the Severe group (a95%CI: 1.32 to 6.36; 31% probability vs a 13.2% probability), but odds across the other classes were not different. Moreover, the Severe class was 2.69 times more likely (a95%CI [1.18 to 6.13]) than the Not Depressed class to be admitted to hospital during pregnancy (probability 20.60% vs 7.60%, Figure 4.13).

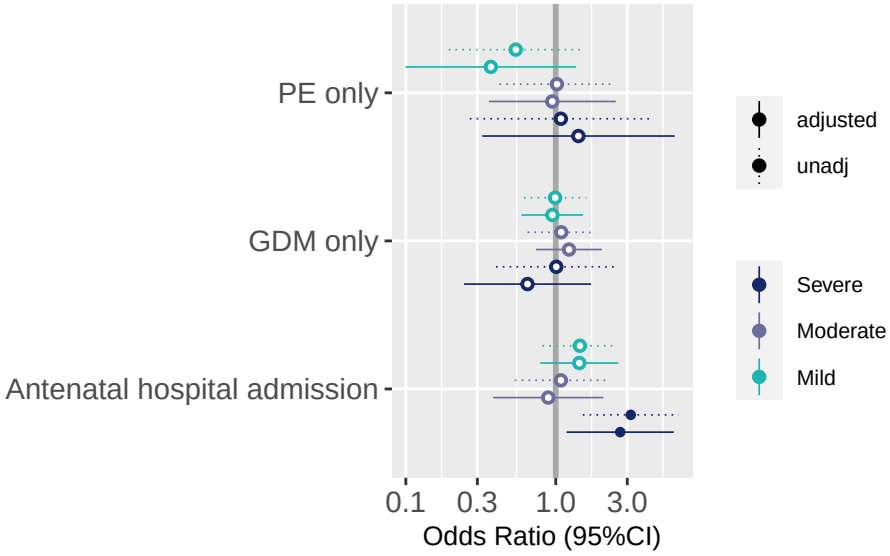

Figure 4.13: *ORs and 95%CIs are plotted for outcomes during pregnancy with the Not Depressed class are the reference. Participant with only either GDM or PE were included (i.e., excluded participant with superimposed GDM with PE/GHT). Covariates in adjusted models included maternal age, nulliparity, white ethnicity, latent SES, latent Obesity and intervention. GDM= gestational diabetes mellitus, PE=Preclampisa.*

4.11.2.3 Weight gain

After adjustment, the Severe group gained a mean 2.64kg more [0.63kg to 4.64kg] than the Not Depressed women at 34 weeks (Table 4.18). Odds of induction of labour, Caesarean section, blood loss at birth >1000ml and NICU admission and gestational age at birth (days) were similar between classes.

Table 4.18: *Adjusted mean gestational weight gain accross classes.*

	Unadjusted		Adjusted	
Class	Mean GWG (kg)[95%CI]	Difference(kg) [95%CI]	Mean GWG (kg)[95%CI]	Difference(kg) [95%CI]
Severe	9.00[6.99 to 11.01]	1.71[-0.37 to 3.78]	12.45[9.64 to 15.26]	2.64[0.63 to 4.64]
Moderate	7.77[6.77 to 8.77]	0.47[-0.62 to 1.56]	10.70[8.65 to 12.74]	0.88[-0.16 to 1.93]
Mild	7.46[6.87 to 8.05]	0.16[-0.72 to 1.04]	9.66[7.71 to 11.61]	-0.16[-1.02 to 0.70]
Not Depressed	7.30[6.80 to 7.79]		9.81[7.93 to 11.70]	

Note:
Mean Gestational weight gain (GWG) at 34 weeks and difference in GWG against the Not Depressed class, after adjustment for maternal age, parity, white ethnicity, SES, Obesity, Intervention.

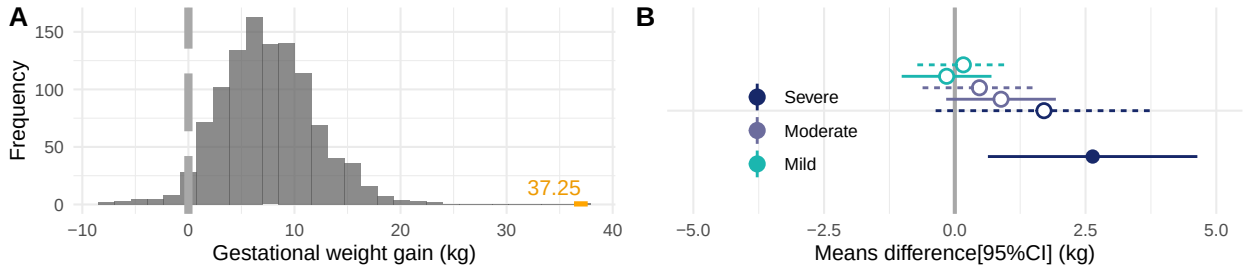

Figure 4.14: *Gestational weight gain at 34 weeks. A) Distribution of weight gains (kg) shows outlier value 37.25kg which is excluded in further analyses. C) Plot representing the mean difference in GWG against the Not Depressed class after adjustement for maternal age, parity, white ethnicity, latent SES, latent obesity and intervention, bars represent the 95%CI. Available data n=1004.*

4.11.2.4 Infant outcomes

However, preterm birth (<37 weeks) was three times more likely for the Severe class (aOR:3.05 [1.11 to 8.36]) with probability of 14.6% compared to 2.9% in the Not Depressed. The OR[95%CI] for the Mild group was 3.07[1.22 to 7.72] with a 8.4% probability but no longer significant after adjustment (aOR:1.84[0.81 to 4.18]). To differentiate from indicated prematurity, the OR of spontaneous preterm birth or premature rupture of membrane was 4.63 [1.36 to 15.73] for the Severe and 3.19[1.11 to 9.22] for the Mild group, this difference was not significant after adjustment. Large-for-gestational-age infants by WHO standards was less likely for the Mild class (OR:0.51[0.30 to 0.88]) but no longer significant after adjustment (aOR 0.54 [0.29 to 1.02]). Odds of small-for-gestational age infants were similar across groups, see Figure 4.15.

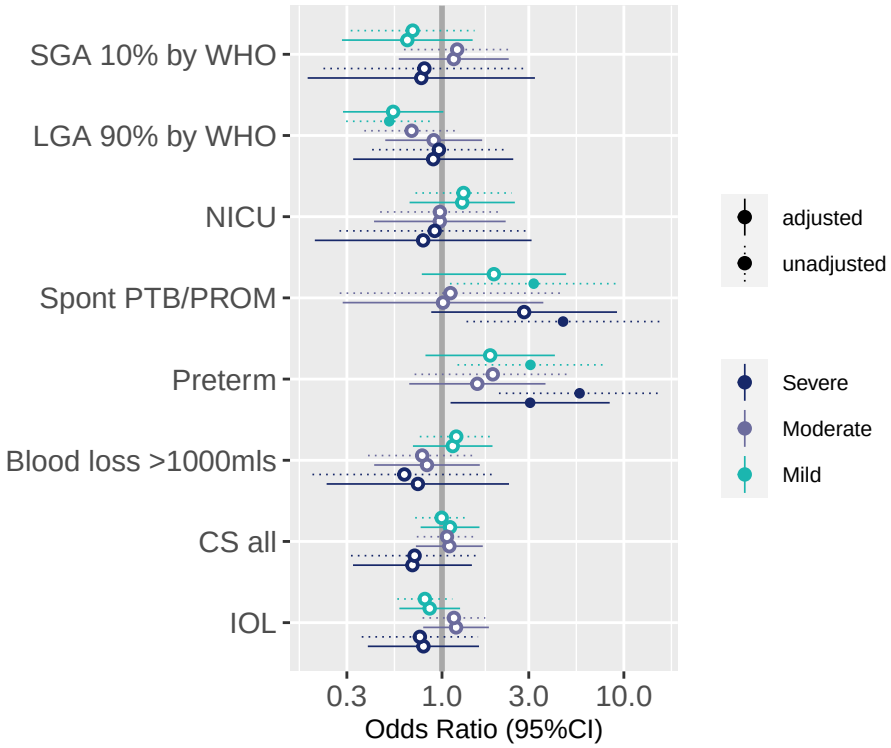

Figure 4.15: *Odds Ratios (95%CIs) obtained from logistic regressions on birth outcomes are plotted where the Not Depressed class is the reference and adjusted models included maternal age, white ethnicity,nulliparity, latent SES, latent Obesity and intervention were covariates. Filled circles indicate CIs excluded 1 and is interpreted as significant. IOL: Induction of Labour, NICU: Neonatal Intensive Care Unit. PROM: Premature Rupture of Membrane, PTB: Preterm birth, SGA/LGA: Small/large for gestational age.*

4.12 Discussion

This study is the first to report the multiple exposures related to maternal antenatal depression and obesity which may adversely influence fetal neurodevelopment. While we cannot infer causality here, the analysis identified several important lifestyle factors including diet and biomarkers which could contribute to the relationships previously observed between maternal obesity/depression and offspring neurodevelopment. In the context of psychopathological risks factors, it highlights the complexities of causal models within the framework of the Developmental Origins of Health and Disease.

The Severe and Moderate depression classes reported a higher obesogenic diet compared to Not Depressed women, independent of socio-economic factors. Overall, the model indicates a dose-dependent effect of maternal symptom severity and reported obesogenic dietary intake not previously described. The implication for the fetus lies in the known effects of maternal high-fat and high-sugar diets on neurodevelopment (Davis and Mire, 2021). Additionally, lower dietary protein composition in the Severe and Moderate classes could indicate a variability in serotonin precursor availability within the fetal brain, necessarily derived from dietary protein. High maternal phenylalanine has almost exclusively been studied in relation to phenylketonuria and demonstrated severe intellectual impairment in the exposed offspring (Levy and Waisbren, 1983). A notable

dietary source of phenylalanine is artificial sweeteners (aspartame).

The heightened concentration of the pro-inflammatory cytokine IL-6 in the Severe class is consistent with it being a biomarker of depression (Roohi et al., 2021). This was after removing the effect of obesity and SES. Glycoprotein acetyls (GP) was also higher and has recently been identified as a marker of chronic inflammation and shown to increase throughout pregnancy (Wang et al., 2016) and has been recently implicated in antenatal depression symptomatology (Lahti-Pulkkinen et al., 2020). The higher glucose at the 34 week visit could also reflect an inflammatory response. Importantly, antenatal maternal immune activation has been associated with increased risk of offspring psychiatric disorders (Ozaki et al., 2020). The observation that recurring maternal infections was common and probability of infection was least in the Not Depressed class. Maternal antenatal infection, including UTI, has frequently been associated with offspring outcomes of ASD and depression (Al-Haddad et al., 2019) and animal models have shown that maternal immune activation increases inflammatory responses in the fetal brain and subsequent offspring behavioural abnormalities. This association was mediated by placental IL-6 (Wu et al., 2017). The degree and extent to which such insult affects the fetal brain will vary by its timing during gestation given the known critical windows in neurodevelopment and since placental permeability is highest early in pregnancy (Dahlgren et al., 2006). Whereas others have frequently implicated cortisol in the mechanisms linking maternal depression and offspring psychological outcome, our results emphasize the need to include infection and inflammation as confounder or interacting factors (Murphy et al., 2017).

It was interesting to observe there was no differences in odds of GDM and PE between the classes as both have been implicated in adverse neurodevelopmental outcomes. However, the higher probability in the Severe class a missing their GDM diagnostic test suggests under-diagnosis and would explain the higher glucose at 34 weeks and may constitute an additional risk factor for adverse offspring outcomes.

The Severe class showed a threefold risk of preterm birth, a recognised population risk factor for neurodevelopmental disorders and our findings agree with the literature associating preterm-birth with depressed/stress pregnancies, and higher IL-6 concentrations (Osborne et al., 2018). The 2.7 fold risk of hospital admission has not previously been reported in relation to depression in obese pregnancy but have been implicated in risks of autism in the child if due to viral infection (Atladóttir et al., 2010). Finally, the lower PlGF in the Severe and Moderate classes implies poorer placental function which could influence nutrient availability to the to fetal brain.

This is to our knowledge the first study to show a cumulative increase in socio-economic disparity and ethnic diversity associated with a stepwise increase in longitudinal depressive symptoms in obese pregnant women. The observation that women with severe symptoms had the lowest SES, were less likely to be living with their partner and more likely unemployed, reflects other reports (Biaggi et al., 2016; Dadi et al., 2020) including a small UK study of women with Major Depressive Disorder (Osborne et al., 2018). These parallels further strengthen the validity of our longitudinal phenotyping of depression in an unselected sample. Socio-economic deprivation is strongly linked to adverse pregnancy outcomes such as preterm birth and our observation that Severely depressed women had less secure accommodation is consistent also with findings that extreme housing insecurity increase risks of preterm birth by 73% (Leifheit et al., 2020). More ethnic diversity in all classes other than the Not Depressed class could reflect recent reports that UK ethnic minorities are less likely to access mental health services, to be referred to primary/secondary care(Jankovic et al., 2020) or to be asked about their mental health during pregnancy (Sambrook Smith et al., 2019).

The primary strengths of our study lie in the advantages of the LCGA and our implementation of robust methods within the structural equation modelling framework, the comprehensive data available and the large sample size in an exclusively obese cohort. This is, to our knowledge, the most extensive characterisation of heterogeneity of outcomes across longitudinal antenatal depressive symptom profiles derived from a screening tool such as the EPDS, providing reference estimates against a clear low-risk group for three antenatal time points. It emphasises the importance of repeated measures in establishing stability of the fetal exposures in obese pregnancies.

The study was limited by the trial exclusion criteria which bias representation. We also cannot exclude any effect of medication e.g. anti-depressants or antibiotics on the biomarkers of interest. Whilst the analyses were based on comparisons of confidence intervals, the retrospective and explorative nature of LCGA may lead to underpowered analyses in the smallest group (Severe). Some biomarkers (e.g., IL-6, CRP) are functionally related but were treated as being independent in this exploratory analysis. Additionally, no epigenetic/genetic variables were considered, nor the contribution of paternal mental health. Analyses into causal mechanisms with outcomes were beyond the scope of this study but will follow for offspring psychological outcomes. We cannot rule out bias from a retrospective self-report of diet, or of infection although the former may be validated by the higher gestational weight gain in the Severe class and latter was predefined according to standard clinical guidelines.

Our findings have several implications. Our study explored trajectories of antenatal depression from a data-driven approach which showed improvement in symptoms in women with already low baseline risks which single-measure and dichotomised designs cannot detect.Further, fluctuations in symptoms at different antenatal periods may lead to an overestimation of cases of depressed women in these designs. The Moderate group scored on average under the threshold for suspected depression while the intervention effect in this class and the Severe classes was unlikely. This implies that the assumption of homogeneity in subthreshold individuals (i.e. in dichotomised studies) is not upheld and therefore bias statistical analyses. Moreover, a reappraisal of the influence of a maternal lifestyle intervention in this RCT, or in any future intervention trial among those experiencing low mood, is warranted in the context of prevention of suboptimal fetal neurodevelopment. Finally, the socio-demographic profiling provided could have clinical value in detecting at-risk pregnant women and promote policies on improving access to care, especially among ethnic minorities.

To conclude, this study reveals that obesity, now highly prevalent amongst women in antenatal care world wide, complicates causal modelling between maternal depression to offspring short and long-term outcomes. By providing cross-sectional estimates at three time points, our study invites the generation of new hypotheses into a multifactorial and multi-hit model of transgenerational transfer of psychopathological risks.

4.13 Supplementary

Table 4.19: *Properties of blood biochemical analyses.*

Group	Biomarker	Units	Sample type	Method	Platform	CV(%)
Conventional Biochemical Platforms						
Glycaemic Markers	Insulin	mU/l	plasma	Electrochemiluminescence immunoassay	Roche, Cobas e411	< 10.3
Glycaemic Markers	HbA1c	mmol/mol	whole blood	Turbidimetric inhibition immunoassay	Roche, Cobas c311	< 1.4
Glycaemic Markers	HbA1c	% (old units)	whole blood	Turbidimetric inhibition immunoassay	Roche, Cobas c312	< 1.5
Glycaemic Markers	C-peptide	ng/ml	serum	Electrochemiluminescence immunoassay	Roche, Cobas e411	< 6.2
Glycaemic Markers	glucose	mmol/l	plasma	Enzymatic hexokinase	Roche Cobas c311	< 2.4
Metabolic Markers	Cholesterol	mmol/l	plasma	Enzymatic, colorimetric	Roche Cobas c311	< 2.4
Metabolic Markers	Triglycerides	mmol/l	plasma	Enzymatic, colorimetric	Roche Cobas c311	< 3.6
Metabolic Markers	HDL	mmol/l	plasma	Homogeneous enzymatic, colorimetric	Roche Cobas c311	< 4.5
Metabolic Markers	LDL	mmol/l	plasma	Homogeneous enzymatic, colorimetric	Roche Cobas c311	< 3.3
Adipokines	Adiponectin	ug/ml	plasma	Enzyme-linked immunosorbent assay	R and D Systems	< 6.9
Adipokines	Leptin	pg/ml	plasma	Enzyme-linked immunosorbent assay	R and D Systems	< 2.0
Inflammation	hs-IL-6	pg/ml	plasma	Enzyme-linked immunosorbent assay	R and D Systems	< 9.8
Inflammation	hs-CRP	mg/L	plasma	Particle enhanced immunoturbidimetric	Roche, Cobas c311	< 7.1
Endothelial marker	t-PA antigen	ng/ml	plasma	Enzyme-linked immunosorbent assay	Asserchrom (Stago)	< 5.7
Placenta	Human placental lactogen	ng/ml	serum	Enzyme-linked immunoassay	R and D Systems	< 5.0
Placenta	Placental growth factor	pg/ml	Plasma	Fluorescence Immunoassay	Alere, Triage Meter Pro	
Vitamin	Vitamin D	ng/ml	serum	Electrochemiluminescence immunoassay	Roche, Cobas e411	< 11.2
NMR metabolomics platform						
Amino acids	Alanine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids	Glutamine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids	Glycine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids	Histadine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Branched-chain)	Isoleucine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Branched-chain)	Leucine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Branched-chain)	Valine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Aromatic)	Phenylalanine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Aromatic)	Tyrosine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Total fatty acids	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	estimated degree of unsaturation		serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Omega-3	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Omega-6	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Polyunsaturated fatty acids	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Monounsaturated fatty acids 16:1;18:1	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Saturated fatty acids	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Docosahexaenoic acid (DHA) 22:6	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Linoleic acid 18:2	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Inflammation	Glycoprotein acetyls (a1-acid glycoprotein)	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	

Note:

The Coefficient of Variation (CV) based on the highest value at time 1 or 2. Glucose at time point 2 issued by centre as per OGTT protocol.

Table 4.20: *Mean differences in dietary intake and composition compared against the Not Depressed class in standardised units at 17 and 27 weeks gestation.*

			Severe		Moderate		Mild	
Timepoint			MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]
17 weeks	% of total Energy	Carbohydrates	0.44[0.05 to 0.82]	0.28[-0.10 to 0.66]	0.23[0.00 to 0.47]	0.14[-0.09 to 0.37]	-0.06 [-0.26 to 0.14]	-0.10[-0.30 to 0.09]
		Protein	-0.64[-0.99 to -0.30]	-0.50[-0.85 to -0.15]	-0.19[-0.41 to 0.04]	-0.12[-0.34 to 0.10]	-0.06 [-0.26 to 0.14]	-0.01[-0.20 to 0.19]
		Saturated fat	-0.05[-0.39 to 0.28]	0.04[-0.29 to 0.37]	-0.05[-0.30 to 0.20]	0.03[-0.22 to 0.27]	0.14 [-0.06 to 0.34]	0.16[-0.04 to 0.36]
		Sugars	0.32[-0.08 to 0.72]	0.28[-0.11 to 0.67]	0.19[-0.05 to 0.42]	0.18[-0.06 to 0.42]	-0.01 [-0.21 to 0.19]	-0.04[-0.24 to 0.16]
		Total fat	-0.04[-0.42 to 0.33]	0.03[-0.35 to 0.41]	-0.15[-0.40 to 0.09]	-0.10[-0.35 to 0.14]	0.15 [-0.05 to 0.35]	0.16[-0.04 to 0.36]
	Content	Energy (kcal)	0.48[0.05 to 0.91]	0.33[-0.12 to 0.78]	0.30[0.05 to 0.55]	0.23[-0.02 to 0.48]	0.05 [-0.15 to 0.24]	0.04[-0.15 to 0.24]
		Glucose load /100g	0.68[0.25 to 1.11]	0.46[0.00 to 0.92]	0.32[0.08 to 0.57]	0.21[-0.03 to 0.45]	0.04 [-0.16 to 0.24]	0.02[-0.17 to 0.21]
		Saturated fat (g)	0.32[-0.09 to 0.73]	0.26[-0.16 to 0.68]	0.18[-0.07 to 0.43]	0.17[-0.09 to 0.42]	0.09 [-0.11 to 0.28]	0.10[-0.10 to 0.29]
27 weeks	% of total Energy	Carbohydrates	-0.03[-0.51 to 0.46]	-0.18[-0.66 to 0.30]	0.26[0.01 to 0.51]	0.17[-0.07 to 0.41]	-0.24 [-0.46 to -0.03]	-0.29[-0.50 to -0.08]
		Protein	-0.17[-0.60 to 0.26]	-0.07[-0.53 to 0.39]	-0.28[-0.52 to -0.04]	-0.23[-0.45 to -0.01]	0.10 [-0.12 to 0.33]	0.15[-0.06 to 0.36]
		Saturated fat	0.05[-0.34 to 0.44]	0.14[-0.26 to 0.53]	-0.04[-0.30 to 0.22]	0.04[-0.21 to 0.29]	0.17 [-0.04 to 0.39]	0.21[0.00 to 0.42]
		Sugars	-0.01[-0.49 to 0.47]	-0.07[-0.54 to 0.40]	0.12[-0.13 to 0.38]	0.10[-0.16 to 0.36]	-0.12 [-0.34 to 0.09]	-0.15[-0.36 to 0.07]
		Total fat	0.19[-0.25 to 0.63]	0.28[-0.15 to 0.71]	-0.09[-0.34 to 0.16]	-0.03[-0.28 to 0.22]	0.25 [0.03 to 0.46]	0.26[0.04 to 0.47]
	Content	Energy (kcal)	0.70[0.13 to 1.27]	0.60[0.04 to 1.17]	0.47[0.20 to 0.73]	0.42[0.16 to 0.68]	0.01 [-0.19 to 0.22]	0.04[-0.17 to 0.24]
		Glucose load /100g	0.64[0.07 to 1.20]	0.48[-0.07 to 1.03]	0.47[0.20 to 0.74]	0.39[0.14 to 0.65]	-0.10 [-0.31 to 0.10]	-0.10[-0.29 to 0.10]
		Saturated fat (g)	0.46[-0.04 to 0.97]	0.43[-0.07 to 0.93]	0.31[0.05 to 0.57]	0.32[0.07 to 0.57]	0.10 [-0.11 to 0.31]	0.14[-0.06 to 0.34]

Note:
AdjMD=adjusted mean difference, all presented in SD units, with maternal age, nulliparity (vs multiparity), white ethnicity (vs other), latent socio-economic status, latent obesity and intervention (at 27 weeks only) as covariates.

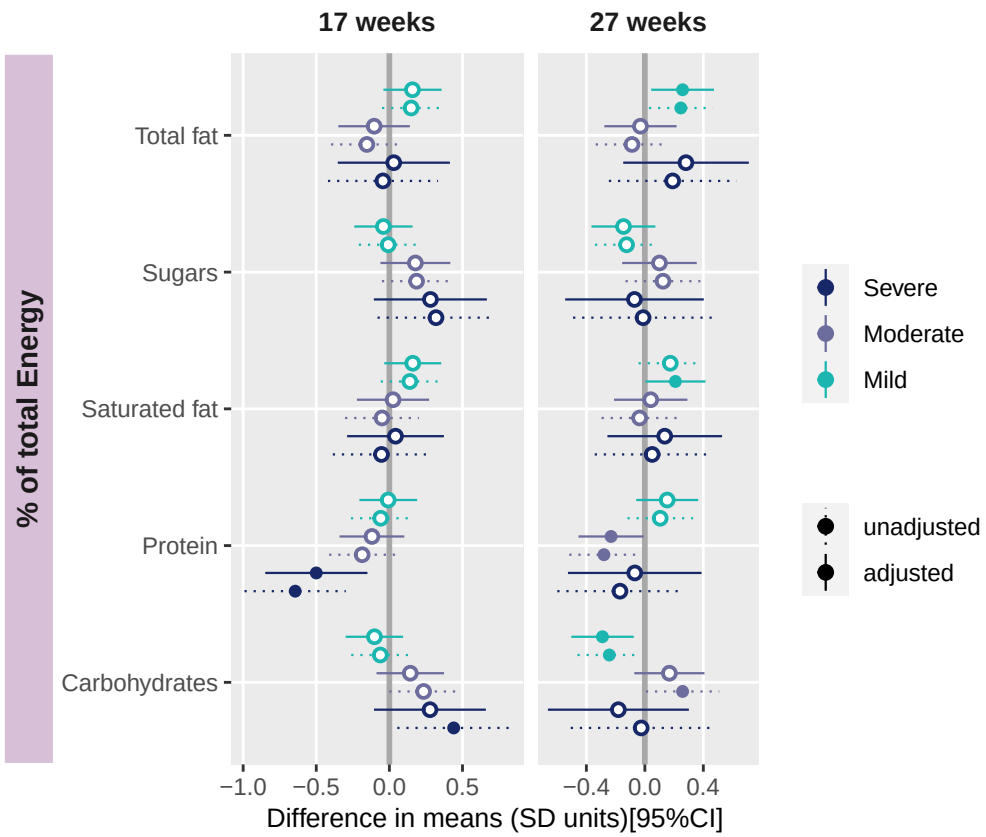

Figure 4.16: *Classes were compared on their diet composition (as % of total Energy) against the Not Depressed class based on self-reported diet obtained at the trial entry (17 weeks) and trial end (27 weeks) which is presented in standardized mean differences from unadjusted and adjusted models with maternal age, nulliparity, white ethnicity, latent SES and latent Obesity as covariates, and the intervention effect at 27 weeks only. Circles are filled if the Confidence Intervals (CIs) did not contain 0, which was interpreted as significant.*

Table 4.21: *Unadjusted means [Standard Error] dietary intake in raw units.*

	Severe	Moderate	Mild	Not Depressed
17 weeks, trial entry				
Energy (kcal)	2064.02 [124.35]	1961.93 [69.90]	1811.95 [40.12]	1784.01 [32.99]
Glycaemic load (/100g)	166.94 [11.02]	148.72 [5.96]	133.99 [3.62]	131.95 [2.82]
Saturated fat (g)	28.57 [2.21]	27.07 [1.31]	26.05 [0.74]	25.07 [0.63]
Total fat (% of Energy)	30.69 [0.97]	30.09 [0.61]	31.72 [0.36]	30.92 [0.31]
Carbohydrates (% of Energy)	52.13 [1.37]	50.63 [0.80]	48.44 [0.50]	48.90 [0.43]
Sugars (% of Energy)	26.17 [1.56]	25.12 [0.89]	23.57 [0.57]	23.63 [0.45]
Saturated fat (% of Energy)	12.33 [0.47]	12.34 [0.35]	12.90 [0.20]	12.49 [0.17]
Protein (% of Energy)	17.45 [0.74]	19.48 [0.47]	20.05 [0.32]	20.31 [0.25]
27 weeks, trial end				
Energy (kcal)	2022.85 [145.49]	1904.15 [64.79]	1670.46 [37.31]	1663.68 [28.96]
Glycaemic load (/100g)	148.40 [12.60]	141.27 [5.72]	115.67 [3.25]	120.27 [2.49]
Saturated fat (g)	27.92 [2.50]	26.42 [1.22]	24.32 [0.75]	23.32 [0.58]
Total fat (% of Energy)	31.62 [1.10]	30.18 [0.59]	31.92 [0.40]	30.63 [0.32]
Carbohydrates (% of Energy)	48.09 [1.66]	50.07 [0.82]	46.57 [0.56]	48.27 [0.40]
Sugars (% of Energy)	23.25 [1.76]	24.26 [0.90]	22.40 [0.58]	23.34 [0.44]
Saturated fat (% of Energy)	12.61 [0.56]	12.35 [0.36]	12.97 [0.22]	12.46 [0.18]
Protein (% of Energy)	20.42 [0.92]	19.93 [0.49]	21.63 [0.37]	21.16 [0.25]

Note:

Over- and under reporting in the dietary variables was controlled by excluding participant data if total calorie count >= 20Mj (=4,780kcal) or =< 4.5 Mj (=1,076 kcal).

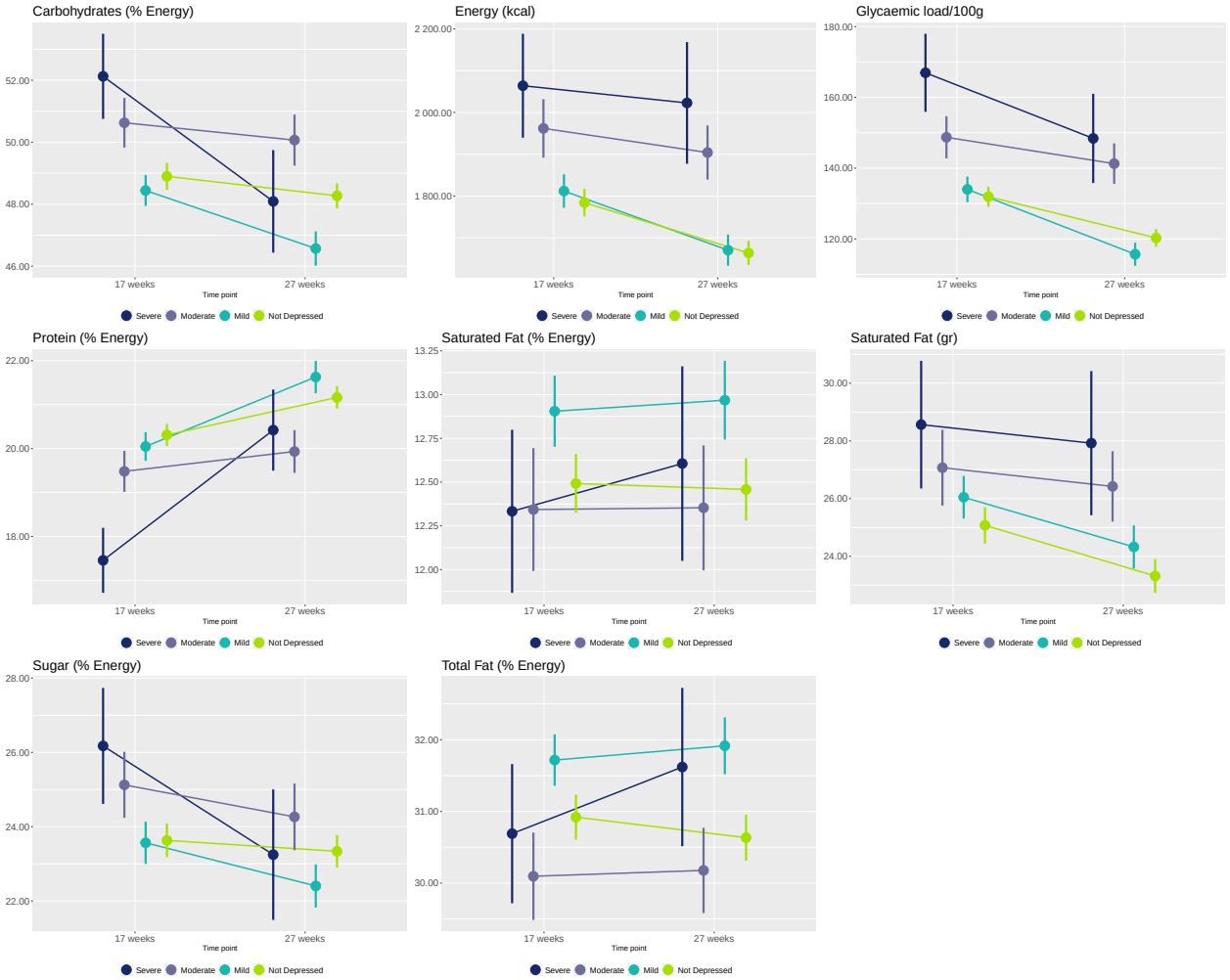

Figure 4.17: *Women self-reported their dietary intake over the previous month at the first and second visit(at median 17[16 to 17] and median 27[27 to 28] weeks gestation respectively). Diet content and composition (% of total energy) were calculated and plotted as means and standard-errors, estimated for each class taking into account measurement error. Values are presented in raw units.*

Table 4.22: *Mean differences in Adipokines Inflammation and endothelial function, Amino Acids, Placental,Glycaemic markers against the Not Depressed class in standardised units at 17 weeks gestation.*

		Depressed		Subclinical		Moderate	
Group	Biomarker	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]
Adipokines	adiponectin	-0.35[-0.76 to 0.06]	-0.15[-0.56 to 0.27]	-0.18[-0.42 to 0.06]	-0.01[-0.25 to 0.22]	-0.12 [-0.32 to 0.09]	-0.09[-0.28 to 0.10]
	leptin	0.11[-0.27 to 0.49]	0.16[-0.17 to 0.48]	0.10[-0.12 to 0.32]	0.10[-0.08 to 0.29]	0.02 [-0.19 to 0.23]	0.07[-0.11 to 0.24]
Amino Acids	alanine	0.08[-0.28 to 0.44]	0.20[-0.15 to 0.56]	0.14[-0.12 to 0.39]	0.21[-0.05 to 0.47]	0.03 [-0.18 to 0.23]	0.04[-0.17 to 0.24]
	glutamine	-0.24[-0.61 to 0.14]	-0.14[-0.51 to 0.22]	0.00[-0.24 to 0.26]	0.09[-0.14 to 0.33]	-0.07 [-0.28 to 0.13]	-0.02[-0.22 to 0.17]
	glycine	-0.06[-0.44 to 0.32]	-0.06[-0.44 to 0.33]	-0.04[-0.28 to 0.19]	-0.04[-0.28 to 0.20]	-0.19 [-0.40 to 0.02]	-0.19[-0.40 to 0.02]
	histidine	0.12[-0.26 to 0.50]	0.23[-0.13 to 0.58]	0.04[-0.20 to 0.28]	0.11[-0.12 to 0.34]	0.03 [-0.18 to 0.23]	0.08[-0.12 to 0.28]
	isoleucine	0.01[-0.33 to 0.35]	0.02[-0.33 to 0.37]	0.08[-0.17 to 0.33]	0.09[-0.16 to 0.34]	-0.01 [-0.22 to 0.19]	0.02[-0.19 to 0.22]
	leucine	0.00[-0.36 to 0.36]	0.03[-0.34 to 0.40]	0.10[-0.16 to 0.36]	0.12[-0.14 to 0.38]	-0.05 [-0.25 to 0.16]	-0.02[-0.23 to 0.18]
	phenylalanine	0.25[-0.13 to 0.64]	0.40[0.04 to 0.76]	0.00[-0.25 to 0.25]	0.08[-0.16 to 0.33]	-0.08 [-0.29 to 0.12]	-0.09[-0.30 to 0.11]
	tyrosine	-0.14[-0.53 to 0.25]	-0.10[-0.48 to 0.28]	0.20[-0.07 to 0.47]	0.23[-0.05 to 0.50]	-0.01 [-0.21 to 0.19]	0.00[-0.20 to 0.20]
	valine	-0.11[-0.45 to 0.23]	-0.09[-0.44 to 0.26]	0.02[-0.24 to 0.28]	0.03[-0.24 to 0.29]	-0.02 [-0.23 to 0.18]	0.00[-0.20 to 0.21]
Glycaemic markers	c-peptide	0.12[-0.27 to 0.51]	0.06[-0.34 to 0.45]	0.23[-0.01 to 0.48]	0.18[-0.06 to 0.42]	0.03 [-0.18 to 0.23]	0.03[-0.17 to 0.23]
	glucose	0.13[-0.24 to 0.50]	0.04[-0.34 to 0.42]	0.15[-0.11 to 0.41]	0.09[-0.17 to 0.35]	-0.08 [-0.29 to 0.12]	-0.10[-0.30 to 0.10]
	hba1c	0.26[-0.12 to 0.64]	0.10[-0.31 to 0.51]	0.12[-0.12 to 0.37]	0.00[-0.23 to 0.22]	0.20 [-0.02 to 0.41]	0.20[-0.01 to 0.40]
	insulin	0.08[-0.36 to 0.52]	-0.04[-0.49 to 0.42]	0.23[-0.01 to 0.47]	0.12[-0.12 to 0.36]	0.03 [-0.17 to 0.23]	0.00[-0.19 to 0.20]
Inflammation and endothelial function	CRP	-0.03[-0.38 to 0.32]	-0.06[-0.41 to 0.29]	-0.06[-0.29 to 0.17]	-0.07[-0.31 to 0.16]	-0.16 [-0.37 to 0.05]	-0.10[-0.30 to 0.10]
	IL-6	0.45[0.14 to 0.75]	0.41[0.11 to 0.72]	-0.15[-0.36 to 0.06]	-0.19[-0.39 to 0.02]	0.05 [-0.16 to 0.27]	0.06[-0.15 to 0.27]
	tPA-antigen	0.14[-0.13 to 0.41]	0.05[-0.21 to 0.32]	0.03[-0.19 to 0.25]	-0.02[-0.24 to 0.21]	-0.15 [-0.36 to 0.06]	-0.16[-0.37 to 0.05]
	Glycoprotein acetyls	0.44[0.04 to 0.85]	0.44[0.06 to 0.82]	-0.06[-0.30 to 0.17]	-0.06[-0.30 to 0.18]	0.12 [-0.09 to 0.33]	0.16[-0.05 to 0.37]
Other	HPL	-0.06[-0.46 to 0.34]	-0.01[-0.38 to 0.37]	-0.11[-0.33 to 0.12]	-0.03[-0.26 to 0.19]	-0.04 [-0.25 to 0.17]	-0.04[-0.25 to 0.17]
	Plgf	-0.19[-0.49 to 0.10]	-0.40[-0.70 to -0.10]	0.08[-0.14 to 0.30]	-0.07[-0.29 to 0.16]	-0.12 [-0.33 to 0.10]	-0.13[-0.34 to 0.08]
	Vit-D	0.13[-0.30 to 0.56]	0.34[-0.08 to 0.75]	-0.40[-0.64 to -0.16]	-0.23[-0.46 to 0.01]	0.03 [-0.17 to 0.23]	0.04[-0.15 to 0.23]

Note:

AdjMD=adjusted mean difference, all presented in SD units, with maternal age, nulliparity (vs multiparity), white ethnicity (vs other), latent socio-economic status, latent obesity as covariates.

Table 4.23: *Mean differences in Fatty Acids, Fatty Acids (of Total Fatty acids) and Metabolic markers against the Not Depressed class in standardised units at 17 weeks gestation.*

		Severe		Moderate		Mild	
Group	Biomarker	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]
Fatty Acids	DHA	0.24[-0.11 to 0.60]	0.20[-0.17 to 0.58]	-0.09[-0.30 to 0.13]	-0.11[-0.33 to 0.12]	0.18 [-0.03 to 0.39]	0.18[-0.03 to 0.39]
	Omega-3	0.23[-0.12 to 0.58]	0.22[-0.15 to 0.58]	-0.04[-0.26 to 0.19]	-0.05[-0.28 to 0.18]	0.13 [-0.08 to 0.34]	0.15[-0.06 to 0.36]
	Omega-6	0.27[-0.09 to 0.63]	0.26[-0.11 to 0.64]	-0.08[-0.32 to 0.16]	-0.08[-0.33 to 0.17]	0.08 [-0.13 to 0.28]	0.10[-0.10 to 0.31]
	Linoleic Acid	0.24[-0.09 to 0.57]	0.21[-0.13 to 0.55]	-0.05[-0.30 to 0.19]	-0.07[-0.33 to 0.18]	0.07 [-0.14 to 0.28]	0.09[-0.12 to 0.30]
	Monounsaturated	0.20[-0.19 to 0.60]	0.26[-0.09 to 0.62]	-0.04[-0.29 to 0.20]	0.03[-0.20 to 0.27]	0.03 [-0.18 to 0.24]	0.09[-0.11 to 0.29]
	Polyunsaturated	0.28[-0.09 to 0.64]	0.27[-0.11 to 0.64]	-0.08[-0.31 to 0.16]	-0.08[-0.32 to 0.17]	0.09 [-0.12 to 0.30]	0.12[-0.09 to 0.33]
	Saturated	0.28[-0.13 to 0.70]	0.36[-0.04 to 0.75]	-0.06[-0.31 to 0.18]	0.01[-0.23 to 0.25]	0.03 [-0.17 to 0.24]	0.09[-0.12 to 0.29]
	Total FA	0.27[-0.12 to 0.67]	0.32[-0.05 to 0.70]	-0.06[-0.31 to 0.18]	-0.01[-0.25 to 0.24]	0.04 [-0.17 to 0.25]	0.09[-0.11 to 0.30]
	Degree of unsaturation	0.06[-0.34 to 0.47]	-0.01[-0.42 to 0.40]	-0.06[-0.30 to 0.18]	-0.14[-0.36 to 0.09]	0.13 [-0.08 to 0.34]	0.10[-0.10 to 0.30]
	Omega-6/Omega-3	-0.09[-0.46 to 0.28]	-0.09[-0.47 to 0.28]	-0.07[-0.31 to 0.16]	-0.07[-0.32 to 0.17]	-0.11 [-0.32 to 0.10]	-0.11[-0.31 to 0.10]
Fatty Acids %	DHA of total FA	0.05[-0.22 to 0.33]	-0.05[-0.34 to 0.23]	-0.01[-0.19 to 0.18]	-0.09[-0.28 to 0.10]	0.18 [-0.05 to 0.41]	0.14[-0.07 to 0.34]
	Omega-3 of total FA	0.07[-0.28 to 0.43]	-0.01[-0.37 to 0.35]	0.06[-0.19 to 0.30]	-0.02[-0.26 to 0.22]	0.13 [-0.08 to 0.33]	0.09[-0.10 to 0.29]
	Omega-6 of total FA	0.02[-0.36 to 0.40]	-0.10[-0.46 to 0.25]	-0.01[-0.24 to 0.22]	-0.13[-0.34 to 0.08]	0.09 [-0.12 to 0.30]	0.04[-0.16 to 0.23]
	Linoleic Acid of total FA	0.05[-0.30 to 0.40]	-0.07[-0.39 to 0.25]	0.02[-0.20 to 0.24]	-0.10[-0.31 to 0.11]	0.07 [-0.14 to 0.29]	0.03[-0.17 to 0.23]
	Monounsaturated of total FA	-0.03[-0.28 to 0.22]	0.01[-0.23 to 0.24]	0.00[-0.17 to 0.16]	0.05[-0.11 to 0.20]	0.04 [-0.19 to 0.28]	0.06[-0.14 to 0.27]
	Polyunsaturated of total FA	0.04[-0.35 to 0.42]	-0.09[-0.45 to 0.27]	0.01[-0.23 to 0.24]	-0.12[-0.33 to 0.09]	0.11 [-0.10 to 0.32]	0.06[-0.13 to 0.25]
	Saturated of total FA	0.02[-0.40 to 0.44]	0.14[-0.29 to 0.56]	-0.02[-0.26 to 0.21]	0.07[-0.16 to 0.31]	-0.14 [-0.34 to 0.07]	-0.11[-0.31 to 0.10]
Metabolic	cholesterol	0.05[-0.36 to 0.47]	0.10[-0.34 to 0.53]	-0.16[-0.39 to 0.07]	-0.12[-0.35 to 0.11]	-0.09 [-0.29 to 0.12]	-0.06[-0.27 to 0.15]
	HDL	-0.24[-0.68 to 0.20]	-0.22[-0.65 to 0.22]	0.04[-0.19 to 0.27]	0.01[-0.21 to 0.24]	0.00 [-0.20 to 0.20]	-0.03[-0.23 to 0.17]
	LDL	0.10[-0.29 to 0.50]	0.16[-0.24 to 0.55]	-0.20[-0.43 to 0.03]	-0.15[-0.38 to 0.08]	-0.14 [-0.34 to 0.07]	-0.10[-0.31 to 0.10]
	LDL/HDL	0.27[-0.14 to 0.67]	0.26[-0.11 to 0.63]	-0.16[-0.39 to 0.07]	-0.12[-0.34 to 0.10]	-0.12 [-0.33 to 0.08]	-0.08[-0.29 to 0.12]
	triglycerides	0.07[-0.35 to 0.49]	0.03[-0.39 to 0.46]	0.05[-0.17 to 0.28]	0.07[-0.16 to 0.30]	-0.01 [-0.21 to 0.20]	0.03[-0.17 to 0.24]

Note:

AdjMD= adjusted mean difference, all presented in SD units, with maternal age, nulliparity (vs multiparity), white ethnicity (vs other), latent socio-economic status, latent obesity as covariates.

4.13.1 Tables at 27 weeks

Table 4.24: *Mean differences in Adipokines Inflammation and endothelial function, Amino Acids, Placental, Glycaemic markers against the Not Depressed class in standardised units at 27 weeks gestation.*

		Severe		Moderate		Mild	
Group	Biomarker	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]
Adipokines	adiponectin	-0.30[-0.70 to 0.10]	-0.15[-0.55 to 0.25]	-0.09[-0.33 to 0.15]	0.02[-0.22 to 0.25]	-0.17 [-0.39 to 0.04]	-0.16[-0.38 to 0.05]
	leptin	-0.05[-0.41 to 0.31]	0.04[-0.26 to 0.34]	0.09[-0.13 to 0.30]	0.12[-0.07 to 0.30]	0.06 [-0.17 to 0.28]	0.10[-0.10 to 0.29]
Amino Acids	alanine	0.17[-0.26 to 0.61]	0.31[-0.15 to 0.78]	0.50[0.25 to 0.76]	0.61[0.35 to 0.86]	0.14 [-0.07 to 0.34]	0.16[-0.05 to 0.37]
	glutamine	0.07[-0.40 to 0.53]	0.10[-0.37 to 0.57]	0.10[-0.16 to 0.35]	0.12[-0.14 to 0.38]	0.07 [-0.13 to 0.28]	0.11[-0.10 to 0.33]
	glycine	0.22[-0.19 to 0.62]	0.22[-0.20 to 0.65]	-0.04[-0.28 to 0.21]	-0.01[-0.26 to 0.23]	-0.10 [-0.32 to 0.11]	-0.11[-0.33 to 0.11]
	histidine	0.10[-0.25 to 0.46]	0.14[-0.22 to 0.50]	0.10[-0.14 to 0.34]	0.11[-0.14 to 0.35]	0.04 [-0.18 to 0.26]	0.06[-0.16 to 0.29]
	isoleucine	0.44[-0.03 to 0.90]	0.42[-0.06 to 0.91]	0.31[0.07 to 0.55]	0.31[0.07 to 0.55]	0.04 [-0.17 to 0.25]	0.07[-0.14 to 0.29]
	leucine	0.43[-0.01 to 0.87]	0.46[-0.01 to 0.92]	0.23[-0.01 to 0.46]	0.22[-0.02 to 0.46]	0.08 [-0.13 to 0.30]	0.07[-0.14 to 0.30]
	phenylalanine	0.34[-0.03 to 0.72]	0.46[0.10 to 0.82]	0.01[-0.23 to 0.26]	0.08[-0.16 to 0.33]	0.03 [-0.18 to 0.25]	0.00[-0.22 to 0.21]
	tyrosine	0.37[-0.04 to 0.78]	0.36[-0.04 to 0.76]	0.20[-0.03 to 0.43]	0.12[-0.11 to 0.35]	0.15 [-0.06 to 0.37]	0.12[-0.10 to 0.34]
	valine	0.28[-0.06 to 0.62]	0.28[-0.08 to 0.64]	0.20[-0.03 to 0.44]	0.15[-0.09 to 0.38]	0.09 [-0.13 to 0.31]	0.06[-0.16 to 0.28]
Glycaemic markers	c-peptide	-0.14[-0.43 to 0.15]	-0.02[-0.32 to 0.28]	-0.12[-0.36 to 0.13]	0.02[-0.22 to 0.25]	-0.06 [-0.28 to 0.16]	-0.01[-0.22 to 0.21]
	insulin	-0.07[-0.37 to 0.22]	-0.04[-0.34 to 0.27]	-0.05[-0.28 to 0.18]	-0.01[-0.24 to 0.23]	-0.04 [-0.26 to 0.17]	-0.04[-0.26 to 0.19]
Inflammation and endothelial function	CRP	-0.02[-0.36 to 0.31]	-0.01[-0.34 to 0.32]	-0.07[-0.30 to 0.16]	-0.04[-0.27 to 0.19]	-0.18 [-0.40 to 0.04]	-0.12[-0.34 to 0.09]
	IL-6	0.15[-0.15 to 0.44]	0.00[-0.29 to 0.30]	0.00[-0.25 to 0.26]	-0.07[-0.31 to 0.17]	0.02 [-0.19 to 0.24]	0.04[-0.16 to 0.25]
	tPA-antigen	0.27[-0.17 to 0.70]	0.23[-0.22 to 0.68]	0.13[-0.11 to 0.38]	0.12[-0.13 to 0.37]	-0.05 [-0.26 to 0.16]	-0.07[-0.29 to 0.15]
	Glycoprotein acetyls	0.22[-0.27 to 0.70]	0.25[-0.25 to 0.74]	0.06[-0.19 to 0.31]	0.13[-0.11 to 0.37]	0.06 [-0.15 to 0.27]	0.13[-0.08 to 0.34]
Other	Plgf	-0.31[-0.75 to 0.13]	-0.49[-0.92 to -0.05]	-0.14[-0.37 to 0.08]	-0.27[-0.50 to -0.04]	-0.17 [-0.38 to 0.05]	-0.16[-0.37 to 0.06]

Note:
AdjMD=adjusted mean difference, all presented in SD units, with maternal age, nulliparity (vs multiparity), white ethnicity (vs other), latent socio-economic status, latent obesity and intervention as covariates.

Table 4.25: *Mean differences in Fatty Acids, Fatty Acids (of Total Fatty acids) and Metabolic markers against the Not Depressed class in standardised units at 27 weeks gestation.*

		Severe		Moderate		Mild	
Group	Biomarker	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]	MD[95%CI]	adjMD[95%CI]
Fatty Acids	DHA	0.21[-0.17 to 0.60]	0.27[-0.13 to 0.67]	0.10[-0.15 to 0.34]	0.11[-0.14 to 0.36]	0.20 [-0.01 to 0.42]	0.20[-0.02 to 0.42]
	Omega-3	0.18[-0.21 to 0.57]	0.23[-0.17 to 0.64]	0.13[-0.11 to 0.37]	0.14[-0.10 to 0.38]	0.21 [0.00 to 0.43]	0.21[-0.01 to 0.43]
	Omega-6	0.12[-0.29 to 0.54]	0.22[-0.20 to 0.64]	-0.04[-0.28 to 0.20]	0.04[-0.20 to 0.29]	-0.01 [-0.23 to 0.20]	0.03[-0.19 to 0.25]
	Linoleic Acid	0.09[-0.32 to 0.50]	0.15[-0.27 to 0.57]	-0.04[-0.28 to 0.21]	0.03[-0.21 to 0.28]	-0.05 [-0.26 to 0.17]	-0.01[-0.23 to 0.21]
	Monounsaturated	0.22[-0.26 to 0.70]	0.34[-0.14 to 0.81]	0.06[-0.19 to 0.32]	0.20[-0.04 to 0.44]	-0.05 [-0.26 to 0.16]	0.04[-0.17 to 0.25]
	Polyunsaturated	0.14[-0.28 to 0.55]	0.23[-0.19 to 0.65]	-0.01[-0.25 to 0.22]	0.06[-0.18 to 0.30]	0.02 [-0.20 to 0.24]	0.06[-0.16 to 0.28]
	Saturated	0.23[-0.23 to 0.69]	0.39[-0.07 to 0.85]	0.03[-0.22 to 0.28]	0.19[-0.05 to 0.43]	-0.04 [-0.25 to 0.17]	0.04[-0.16 to 0.25]
	Total FA	0.21[-0.25 to 0.66]	0.34[-0.12 to 0.80]	0.03[-0.22 to 0.27]	0.16[-0.08 to 0.40]	-0.02 [-0.24 to 0.19]	0.05[-0.16 to 0.26]
	Degree of unsaturation	0.18[-0.23 to 0.60]	0.13[-0.30 to 0.56]	0.07[-0.17 to 0.31]	-0.04[-0.27 to 0.18]	0.29 [0.08 to 0.51]	0.23[0.03 to 0.44]
	Omega-6/Omega-3	-0.12[-0.52 to 0.29]	-0.10[-0.50 to 0.30]	-0.20[-0.46 to 0.06]	-0.13[-0.39 to 0.13]	-0.33 [-0.54 to -0.12]	-0.28[-0.49 to -0.08]
Fatty Acids %	DHA of total FA	0.10[-0.30 to 0.49]	-0.01[-0.41 to 0.40]	0.11[-0.14 to 0.36]	-0.03[-0.26 to 0.21]	0.28 [0.06 to 0.49]	0.20[0.00 to 0.39]
	Omega-3 of total FA	0.07[-0.36 to 0.50]	-0.03[-0.46 to 0.40]	0.18[-0.08 to 0.44]	0.04[-0.20 to 0.27]	0.31 [0.10 to 0.52]	0.23[0.04 to 0.42]
	Omega-6 of total FA	-0.11[-0.52 to 0.30]	-0.19[-0.61 to 0.23]	-0.12[-0.37 to 0.13]	-0.23[-0.48 to 0.01]	0.02 [-0.19 to 0.23]	-0.05[-0.26 to 0.16]
	Linoleic Acid of total FA	-0.15[-0.55 to 0.24]	-0.27[-0.66 to 0.13]	-0.11[-0.36 to 0.15]	-0.21[-0.46 to 0.04]	-0.06 [-0.27 to 0.15]	-0.12[-0.33 to 0.10]
	Monounsaturated of total FA	0.03[-0.42 to 0.48]	0.05[-0.40 to 0.50]	0.09[-0.15 to 0.34]	0.18[-0.05 to 0.41]	-0.10 [-0.31 to 0.11]	-0.02[-0.22 to 0.19]
	Polyunsaturated of total FA	-0.08[-0.49 to 0.34]	-0.17[-0.60 to 0.25]	-0.05[-0.30 to 0.20]	-0.19[-0.42 to 0.04]	0.10 [-0.11 to 0.32]	0.02[-0.18 to 0.22]
	Saturated of total FA	0.11[-0.28 to 0.49]	0.26[-0.14 to 0.67]	-0.02[-0.26 to 0.23]	0.14[-0.09 to 0.37]	-0.07 [-0.29 to 0.14]	-0.02[-0.23 to 0.18]
Metabolic	cholesterol	0.16[-0.26 to 0.59]	0.31[-0.12 to 0.74]	-0.08[-0.32 to 0.16]	0.01[-0.23 to 0.26]	-0.07 [-0.28 to 0.15]	-0.03[-0.25 to 0.19]
	HDL	-0.10[-0.47 to 0.28]	0.00[-0.37 to 0.36]	0.14[-0.11 to 0.40]	0.14[-0.10 to 0.37]	0.26 [0.05 to 0.48]	0.22[0.00 to 0.43]
	LDL	0.15[-0.28 to 0.59]	0.27[-0.16 to 0.70]	-0.17[-0.42 to 0.07]	-0.08[-0.32 to 0.16]	-0.15 [-0.36 to 0.06]	-0.11[-0.32 to 0.11]
	LDL/HDL	0.21[-0.24 to 0.67]	0.22[-0.20 to 0.64]	-0.17[-0.43 to 0.09]	-0.11[-0.35 to 0.14]	-0.29 [-0.50 to -0.09]	-0.24[-0.45 to -0.03]
	triglycerides	0.09[-0.36 to 0.53]	0.12[-0.33 to 0.57]	0.13[-0.13 to 0.39]	0.21[-0.04 to 0.46]	-0.03 [-0.24 to 0.18]	0.03[-0.18 to 0.24]

Note:
AdjMD= adjusted mean difference, all presented in SD units, with maternal age, nulliparity (vs multiparity), white ethnicity (vs other), latent socio-economic status, latent obesity and intervention as covariates.

Chapter 5

Relationships between psychological outcomes and adiposity in 3 year old children of women with obesity, and the role of maternal antenatal depression, glycaemic status and inflammation.

5.1 Introduction

Currently, 20-30% of women of child-bearing age in England are obese, defined as a body mass index (BMI) equal or over 30kg/m^2 (National Health Service Digital, 2021). The higher prevalence of obstetric complications such as gestational diabetes mellitus (GDM) and preeclampsia and large for gestational age delivery associated with maternal obesity results in higher health care costs in the perinatal period (Morgan et al., 2014). However, the potential burden of poorer long-term health outcomes and quality of life for both women and their children is not confined to health economics.

Large cohort studies and meta-analyses have provided evidence that exposure to maternal obesity *in utero* increases odds of autism and attention-deficit hyperactivity disorder (ADHD) and poorer cognitive outcomes in the offspring, and that maternal hyperglycaemia appears to compound these effects (Adane et al., 2016; Li et al., 2016a). While maternal or paternal genetic susceptibility to neurodevelopmental disorders is rarely accounted for in these analyses, animals models of obesity and/or hyperglycaemia induced by high-fat or high-sugar diets have demonstrated changes in fetal brains potentially via epigenomic mechanisms (Money et al., 2018). Also, in the human, GDM can alter postprandial fetal brain response (Linder et al., 2015). Additionally, substantial literature describes relationships between maternal obesity and risk of offspring obesity, type-2 diabetes and cardiovascular disease (Vogt et al., 2014; Park et al., 2020b).

Several studies have addressed the potential overlap between psychological and metabolic outcomes and

reported a higher rate of obesity and overweight in children diagnosed with autism and/or ADHD (Broder-Fingert et al., 2014; Cortese et al., 2016; Güngör et al., 2016; Ptacek et al., 2016; Racicka et al., 2018). Such findings may be explained by a common neurological system which links health outcomes in the offspring born of obese mothers. This includes the CNS pathways of the reward system and a role for the hypothalamus. The hypothalamus relays neuronal transmission within the reward/dopamine system and the limbic network and is the master regulator of energy homeostasis and the autonomic nervous system (Wang et al., 2005; Gali Ramamoorthy et al., 2015; Volkow et al., 2017). These brain pathways have been associated with traits of the autistic spectrum and the psychobehavioural phenotype of ADHD, as well as food seeking behaviour and food response (Wang et al., 2005; Kenny, 2011; Volkow et al., 2017).

However, in the characterisation of these long-term offspring outcomes, the causal pathways to neurodevelopmental disorders are complex to define. Studying disruption of the uterine processes which support normal offspring neurodevelopment is complicated in the human population where the socioeconomic context in which women experience their pregnancies can influence both antenatal and postnatal exposures to the offspring brain, which grows rapidly in the first years of life. Antenatal stressors which may act as potential contributors to adverse offspring neurodevelopment include infection (Boulanger-Bertolus et al., 2018; Al-Haddad et al., 2019), poorer diet and inadequate resources for mental health care. These antenatal stressors can both co-occur in pregnancy and be recurrent postpartum, having direct and indirect effects on the child (Barker et al., 2013; Ornoy et al., 2016). Furthermore, C-section, which is more likely to occur in obese pregnancies, is associated partly to changes in brain development (Castillo-Ruiz et al., 2018) through various mechanisms, including injury, exposure to pathogens at birth and changes to the infant gut microbiome, which in turn is associated with overweight and obesity when compared to vaginally delivered infants (Li et al., 2013). Additionally, the recognised higher incidence of neurodevelopmental disorders among males has potential biological underpinning through for example, a sexual dimorphic fetal brain immune response to maternal immune activation (Oertelt-Prigione, 2012; Keever et al., 2020) or to maternal stress in pregnancy (Mueller and Bale, 2008) .

Maternal depression, which is associated with obesity in the general population, is also a risk factor for preterm birth (Ding et al., 2014), autism, ADHD and psychopathology in the offspring (Manzari et al., 2019). Nutritional and socioeconomic disparity linked with maternal depression are associated with lower cognitive outcomes in the exposed child (Barker et al., 2013). Importantly, prematurity and C-sections are also higher in obese and depressed pregnancies (Hoffman et al., 2016). However it was also shown that maternal stress is additionally associated with changes in the offspring gut microbiome and in a sex-dependent manner (Jašarević et al., 2017) and in the paraventricular nucleus of the hypothalamus (Jašarević et al., 2018).

Studies addressing the hypothesis that obesity and/or GDM are key antenatal exposures to offspring neurodevelopmental and psychological abnormalities rarely account for maternal psychopathology (Daraki et al., 2017), nor differentiate between the birth events which result from the maternal condition directly and the clinical intervention implemented when the diagnosis of GDM is known. This is related to, for example, induction of labour or planned C-section. Conversely, studies on maternal antenatal depression have almost exclusively focused on pathways involving the stress hormone cortisol and in this literature the effects of maternal BMI/adiposity, inflammatory processes and hyperglycaemia are yet to be clarified.

It follows therefore, that the characterisation of potential causal pathways to offspring psychological and metabolic outcomes necessitate analytical models encompassing a large array of exposures inherent to

obesity and mediating factors including maternal physiological biomarkers, socio-economic and lifestyle factors and mental health. Depression, obesity, and hyperglycaemia such as GDM are also associated with inflammatory processes (Hryhorczuk et al., 2013) and a proinflammmatory uterine milieu can impact the fetal brain directly (Burg et al., 2016) potentially by disrupting placental function (Bronson and Bale, 2014; Bronson and Bale, 2016; Hirschmugl et al., 2017).

Overall, the above suggests how multifaceted the pregnancy with obesity is. Conventionally, studies relaying antenatal environment to postnatal outcome utilise conventional regression models. There, model fit is not established and the theorised interactions and relationships between the factors described are not characterised. Although never previously attempted in this context, methods for causal inference such as structural equation modeling (SEM) have the potential to inform and model the complex relationships just described and improve our insight into the maternal to child relationship. Moreover, previous conceptualisation of the constructs described above (depression, GDM, obesity, inflammation) rely on discreet classification of subjects into groups often along cut-off criteria and similarly of the children psychological and anthropometry status. Such classification and characterisation may underestimate the likely heterogeneity in the population. A more nuanced and granular description of phenotypes may increase our understanding of the maternal health profiles, of their children and the relationship between the two. Data-driven person-oriented mixture modelling such as Latent Class Analysis (LCA) (Nylund-Gibson and Choi, 2018) may be of benefit, and theory-based causal inference toward child health outcomes could provide meaningful and sensitive evidence with which to develop effective interventions.

The aims of this study are described below:

The first two steps aimed to characterise the relationships between psychological and anthropometric outcomes in 3-year old children born to mothers who were obese when they enrolled in the UK Pregnancies Better Eating and Activity Trial (UPBEAT) (Poston et al., 2015). This aimed to verify previously reported relationship between psychological phenotype and obesity.

1) The Strength and Difficulty Questionnaire SDQ total score and its subscales were used to define risk of psychopathology. Associations between risk of psychopathology, using published cutoff thresholds, and anthropometric measures were explored and subsequently relationship with antenatal and birth outcomes addressed..

2) Latent-Class Analysis (LCA) was performed as an alternative method to explore psychological phenotypes within the sample. The relationships between LCA-derived psychobehavioural profiles extracted from the SDQ were compared with the outcomes described in 1).

The third step aimed to offer an epidemiological framework of risks factors for the psychological outcomes in the child:

3) Building from the above child phenotyping, path models to adverse offspring psychological outcomes were built using SEMs iteratively where antenatal GDM, depression, inflammation and placental function are the predictors/mediators of interest. This aimed primarily at disentangling the associations between GDM and depression which have overlapping etiology in inflammatory processes and with the placenta as the necessary mediator in the transfer from the maternal milieu to the fetal brain. Such

characterisation was theoretically driven and underpinned by the principles of the Developmental Origins of Health and Disease (DOHaD) framework.

5.2 METHODS

5.2.1 Participants

The UPBEAT study, a randomised control trial, recruited 1555 obese pregnant women (BMI > 30kg/m^2) with the primary outcomes of GDM for the mother and of large for gestational age for the infant. The trial involved randomising pregnant women at 15^{+0}-18^{+6} weeks gestation into a 8-week lifestyle intervention delivered by health trainers or to standard antenatal care in order to increase maternal physical activity and reduce saturated fat intake and glycaemic load. Further visits occurred between 24-28 weeks (for an oral glucose tolerance test) and 34-36 weeks. All participants in the trial provided informed written consent. Ethical approval was granted by UK integrated research application system (IRAS: 09/H0802/5). Consent for follow up of the children at 3 years of age was provided by additional ethics approval (IRAS: 13/LO/1108).

At the 3-year follow-up 514 mother-child dyads participated and from these children the following exclusion criteria were applied in order to minimise the effects of important risk factors and confounders to psychological outcomes: birth prior to 34 weeks gestation and chromosomal or congenital abnormality (e.g. Down's syndrome, spina bifida, cardiac anomaly). Because the principal interest was to explore the contribution of antenatal GDM and depression on child outcomes, pregnancies complicated by preeclampsia were excluded (pre-eclampsia is also implicated in childhood long-term cardiometabolic outcomes). Those children missing the primary measure of psychological outcome (Strength and Difficulty Questionnaire, SDQ) (Goodman, 1997) were also excluded. A total of 462 eligible children were included.

The mothers of the 462 children included who attended the follow-up were compared to those excluded and gave birth to live born babies (n=1024). In their index pregnancies, the attending women were of lower BMI, older, a higher proportion were of white ethnicity and earned from the higher income brackets. Additionally, a lower proportion were in the Most Deprived IMD Quintile and they were more likely nulliparous, never smoked and had higher education (Supplemenatary Table 5.8 at the end of this chapter). There was no difference in proportion of those randomised to the intervention nor in GDM diagnosis or EPDS scores in the antenatal period. There were no differences in birth outcomes between children who attended and those who did not attend apart from a smaller proportion of preterm born children and they were born at higher gestational age days explained by the exclusion criteria of >34 weeks applied here. The offspring included were of higher WHO birthweight centile (Supplementary Table 5.9 at the end of this chapter).

In developing the SEM models described below, and to utilise all the available data from UPBEAT and increase power, antenatal variables from all women who gave birth to live born neonates were used even if they did not attend the follow-up visit, unless birth preceded 34 weeks and were complicated with pre-eclampsia. Therefore the number of observations increased for each variable.

5.2.2 Outcome variables

Primary outcome: *The Strength and Difficulty Questionnaire (SDQ)*

In UPBEAT parents/carers reported on the behaviour of children using the questionnaire designed for the

2-4 years age-range (www.sdqinfo.org). The SDQ (Goodman, 1997) comprises 25 items which are divided equally into 5 subscales: Emotional symptoms (*emotion*), Conduct problems (*conduct*), Hyperactivity/inattention (*hyperactive*), Peer relationship problems (*peer*) and Pro-social behaviour (*prosocial*). There are three possible responses per item: “Certainly true”, “Somewhat true”, “Not true”, scored 0, 1 and 2 depending on whether an item is worded negatively or positively. The total SDQ score provides an Overall Difficulty score derived from summing the first four scales and ranges from 0 to 40 points. Conduct and Hyperactivity have been used in conjunction to assess Externalising problems and the Emotion and Peer problem scales have been associated with Internalising problems (Goodman et al., 2010) so we only include the emotion, conduct, hyperactivity and peer subscales for further analyses.

The SDQ reported by carers or teachers has shown satisfactory psychometric properties (Stone et al., 2010; Kersten et al., 2016) and has been administered in British (Croft et al., 2015), German (Petermann et al., 2010) and Swedish pre-schoolers (Gustafsson et al., 2016). In a British sample of 16 659 families the Conduct and Hyperactivity scales demonstrated that assessment at 3 years of age had predictive validity for developmental and clinical diagnoses (ASD and ADHD) at 5 years of age (Croft et al., 2015).

Outcomes on the SDQ are described as total sum score and total subscales score as continuous variables. Additionally, total SDQ and subscale scores were dichotomised as high/low risks of problems. There are reported cut-offs for 2-4 year old children (www.sdqinfo.org) which categorises children within score bands as “normal”,“low-risk”,“high-risk” and “very high-risk”. Here, the lower bound cut-off scores of “high” for the total SDQ and total subscale scores were used to dichotomise the children as normal/low vs high/very-high risks into a “low risk” or “high-risk” category as follows:

- Total SDQ >= 16points
- Emotional Problems >=4
- Conduct Problems >=5
- Hyperactivity >=7
- Peer Problems >=4

Secondary outcome: *Anthropometry*

Outcomes for child anthropometric measurements included circumferences of waist and arm, sum of skinfold and z-scores of height-for-age, weight-for-age and BMI according to the WHO Child Growth Standards for each sex, based on optimal environmental conditions. The WHO standards apply irrespective of ethnicity and socio-economic status (Onis, 2007). Waist was measured at the halfway point between the inferior margin of the lowest rib and the iliac crest. Mid arm circumference was measured from the tip of the elbow to the edge of the acromion. Skinfold thicknesses were measured using Harpenden skinfold callipers. Sum of skinfolds thicknesses was calculated as the sum of abdominal, subscapular, suprailiac, triceps and biceps skinfolds. All thicknesses and circumference were each measured in triplicate by trained research staff. All variables were normally distributed except for sum of skinfold thicknesses. There was minimal missing data on demography in this sample (Supplementary Table 5.10 at the end of this chapter). The proportion of missing data on anthropometry was between 4.5% and 8.4%, except for sum of skinfold which was 24.7%.

5.2.3 Statistical Analysis

5.2.3.1 Group comparisons by SDQ dichotomisation

First, we performed univariate group-wise comparisons between males and females on psychological and anthropometric outcomes at the 3-year follow-up. Pregnancy birth events and antenatal maternal factors were also compared in order to assess the likely contribution of discordant exposures by sex in these periods as likely predictors.
T-tests were used for normally distributed continuous variables and Kruskal-Wallis test for non-parametric variables. Dichotomous/categorical variables were compared by Chi^2 tests. In order to study the association between psychological outcomes and anthropometry and provide comparative references to the literature, high and low-risk groups on the SDQ total score were compared on anthropometry, stratified again by sex.

5.2.3.2 Latent Class Analysis

SDQ subscales can help distinguish externalising and internalising problems (Goodman et al., 2010). Here, the presence of subgroups based on their probabilities to score high/very-high on any of the subscales was probed on the assumption that some children may present the phenotypes above, i.e. they would be mostly characterised as primarily at risk of internalising or externalising problems or could possibly show widescale psychological and behavioural issues.

Latent Class Analysis (LCA), a person-centered data-driven approach allows for these assumptions to be tested and clarify the psychological characteristics of the sample and thus potential heterogeneity by identifying the presence of these latent groups. This assumes that the subscales emotion, hyperactivity, conduct and peer may not completely tap into distinct (uncorrelated) constructs. Therefore the reported clustering of the subscales on Externalising (*hyperactivity* & *conduct*) and Internalising symptoms (*emotion* & *peer*) in the literature was tested in this sample. If distinct classes were observed these were then compared on anthropometric measurements. LCA was performed using the subscales dichotomised along the reported cutoff scores (normal/low vs high/very-high risk, coded 0/1).

One to five- Class LCA models were generated for the whole sample first and then for male and females separately due to the known sex differences in the incidence of internalising and externalising disorders in the population but also in fetal brain response to maternal exposures.

Model fit indices and other diagnostic criteria were assessed to decide on which model to accept as follows: Bayesian information criterion (BIC), the Akaike information criterion (AIC,Akaike (1987)), sample-size adjusted Bayesian information criterion (aBIC), where lower values infer better model fit (Nylund et al., 2007). The Vuong-Lo-Mendell-Rubin adjusted likelihood ratio test (LMR) indicates that if $p<0.05$ that an n-class model provides better fit than a n-1 class model (Lo, 2001). Entropy indicates the degree of separation of individuals into classes (Celeux and Soromenho, 1996) with a value over 0.60 deemed acceptable (Asparouhov and Muthén, 2014). Model selection is also at the discretion of the researcher to interpret, basing their judgement on class count(%) and interpretability in light of theory (Wickrama et al., 2016). The *Mplus* outputs were integrated into R with the R package *MplusAutomation* (Hallquist and Wiley, 2018) and the figures obtained with the *ggplot2* package.

Following the LCA, classes were compared on anthropometric measures using the BCH 3-step approach (Asparouhov and Muthén, 2021a) which takes into account the measurement error (fractional membership) of the LCGA and can also include covariates. Here only sex was considered (see below).

5.2.4 Pathway modelling to adverse offspring psychological outcomes after antenatal exposure to maternal obesity

Next, SEMs were built in order to understand the potential predictors of high risks for psychopathology in the children born to obese women. Since the class counts for the internalising and externalising phenotypes derived from the LCA were quite small (see below) when the both sexes were taken into account, the High/Low risks dichotomisation based on the total SDQ was the outcome variable. The number of covariates included in the SEMs was limited to the number of adverse outcome cases as to avoid overfitting in the presence of many exposures. Causal modelling of psychological outcomes from observational data is full of constrains and implication and discussed in Rohrer (2018). However, the building of structural equation models followed recommendations by Bollen and Noble (2011) and E. Kevin (2015) and was performed also in *Mplus 8.3* (Muthén and Muthén, 2017). The antenatal variables were associated by regression paths as those identified in the literature. For example, higher obesity is associated with higher risks of GDM diagnosis/hyperglycaemia. GDM diagnosis influences gestational age at birth as the clinical guideline recommend induction if labour has not commenced by 40 weeks, or by hyperglycaemia being on the causal pathway to early spontaneous labour and delivery.

5.2.5 Antenatal Exposures

The multiple exposures involved in the pregnancies complicated by obesity, specifically in relation to maternal depression were discussed previously (Chapter 3, Sigurdardottir et al. [2022], *under review*).

A strength of SEM is the incorporation of latent factors which can account for the measurement error otherwise introduced by proxy measures of a construct. It can also be used to model a physiological state using repeated measures as indicators when longitudinal trajectories are not of interest but rather to minimise (thus accounting for) the nuisance of time on the accuracy in measuring a construct. This latter concept is superior to averaging or summing i.e. scores or values across repeated assessments of the same variable into one measure. This is because one measure relies on one conditional distribution for parameter estimation whereas multiple repeated measures indicating a latent variable can utilise the joint and more informative distributions of all the measures available (Kim et al., 2020). Importantly, utilising repeated measures improves regression weights, reduces bias in small sample sizes (n=200) and/or when effects sizes are small. Additionally, repeated measures in regression show these benefits when the measures are unstable over time (Kim et al., 2020), which is assumed to be relevant in the course of pregnancy and for the factors included here.

Since GDM is diagnosed from a single glucose tolerance test at 24-28 weeks, there is a risk of underdiagnosis and the number of diagnoses may not reflect the potential burden of maternal hyperglycaemia throughout pregnancy. Therefore, a latent “glycaemia” factor indicated by glucose measures available at 15-18 weeks and at 36 weeks was also used in comparative models. In this scenario latent variable modelling of glycaemia replaces the dichotomous GDM diagnosis. Similarly *placental function* was indicated by two measures of placental growth factor (PlGF) as a biomarker of placental trophoblast function i.e. a surrogate for maternal to fetal nutrient transfer. Latent “inflammation” is similarly weighted by two measures of IL-6.

In summary, factors and biomarkers implicated in the causal pathways to suboptimal brain development *in utero* (see Burg et al. (2016)) were as follows:

- *GDM*, diagnosed at 24-28 weeks gestation according to the IADPSG guidelines: fasting glucose $\geq$ 5.1 mmol/L and/or 1-hour glucose $\geq$ 10mm/L and/or 2-hour glucose $\geq$ 8.5 mmol/L.
- *Maternal latent glycaemia*, indicated by 2h glucose measure available at two time points.
- *Maternal Latent obesity* was indicated by: maternal baseline BMI, sum of skinfold thicknesses, waist and hip circumferences (all continuous).
- *Latent maternal antenatal depression* was indicated by: EPDS sum score at three time points.
- *Latent inflammation (IL-6)*, indicated by log(IL-6) at two time points.
- *Latent placental function*, indicated by log placental growth factor (PlGF) at two time points.

Maternal depressive symptomology at the follow-up was the only postnatal factor considered. The 3 year EPDS score was modelled here for two reasons: to control for the fact that, when answering the SDQ, high levels of maternal depressive symptoms may affect/bias maternal perception of difficulties in the child. Second, postnatal depression can impact attachment with the child and is associated with child's neurodevelopment and behaviour and thus could be on the causal pathway to high risk of psychopathology as measured from the SDQ. Additionally, maternal depression in pregnancy is associated with postnatal depression so this regression was also included.

5.2.6 SEM specification and estimation

Several models aiming at understanding the causal pathways to child risks of psychopathology were specified. The models relied on theoretical assumptions in the first place but were constructed iteratively from simple to more complex. Several considerations had to be observed in choosing of the estimation method. The primary outcome variable was categorical (High/low SDQ), the inclusion of several latent factors, the overall complexity of the model, the data available and sample size, and the uncertainty of the distribution of the parameters included.

Here the Bayes estimator is used throughout for easier interpretation across the models due to issue of numerical integration (with the frequentist approach) using Maximum Likelihood as SEM complexity increases. However, to make it comparable to ML output, the Bayes estimation here included no priors (i.e. used non-informative prior distributions). In this exploratory analysis, Bayes provides several benefits over ML estimation: model convergence is easier in complex models since it does not rely on numerical integration, 2) Bayes does not require large samples, 3) nor that the parameter estimates be normally distributed, 4) the credibility intervals (akin confidence intervals in frequentist statistics) can be asymetrical (Muthén, 2010).

Relying on guidelines for reporting Bayes methodology (Schoot et al., 2014; Depaoli and Schoot, 2017), the following *Mplus* settings and evaluation criteria were used:

- 8 Markov chain Monte Carlo (MCMC) chains
- GIBBS algorithm
- FBIterations = 100000 to 200000
- processors=8

Latent factor indicators were free and the variance of the latent factors was set to 1.

Model convergence was assessed from the Proportional Scale Reduction (PSR) factor (Muthén, 2010). Convergence in Bayes models represent the variation between the MCMC chains, this should be small relative to the total variance (between and within-chain). The PSR should to be close to 1 (or <1.1 according to Fuente et al. (2014)) for the iterations after the burn-in and is provided by *Mplus*. The residual variance is visualised on a trace plot where the pattern of variation after the "burn-in" phase should have no distinct downward or upward trend and the chains have a good overlap (caterpillar shape). Note that instead of the PSR criterion we used the limited FBiteration option rather than the bconvergence= 0.05 option to reduce computational time and so the PSR has to be checked manually. No thinning was performed as per recommendation (Link and Eaton, 2012).

Model fit was assessed by the Posterior predictive checking (PPC) and that the Chi^2 test 95%CI do not contain 0 although it is recommended the Bayesian posterior predictive p-value is approximately 0.5 (Schoot et al., 2014).

The output provides the median of the posterior distribution as the point estimate and the 5% Bayesian *credibility* interval (CI) represents the 2.5 and 97.5 percentiles in the posterior distribution. The credibility interval is defined as "the 95% probability that in the population the parameter lies between the two values" (Schoot et al., 2014). Additionally, the posterior distribution histograms should also have smooth shape with no gaps and one peak and that the autocorrelation between lags (i.e. between iterations for a parameter) is not large. We report the standardized regression/covariance coefficient for direct (blue on diagrams) and indirect paths (green) and interpret the CIs excluding zero as significance for the coefficient.

The frequentist approach in *Mplus* relies on the full-information maximum likelihood estimation which also deals with missing data under the missing-at-random (MAR) criteria. The Bayes estimation also handles missing data under the same MAR missing data theory (Muthén, 2010; Asparouhov and Muthén, 2021b).

The models specified as of interest in this study are presented in Figure 5.1.

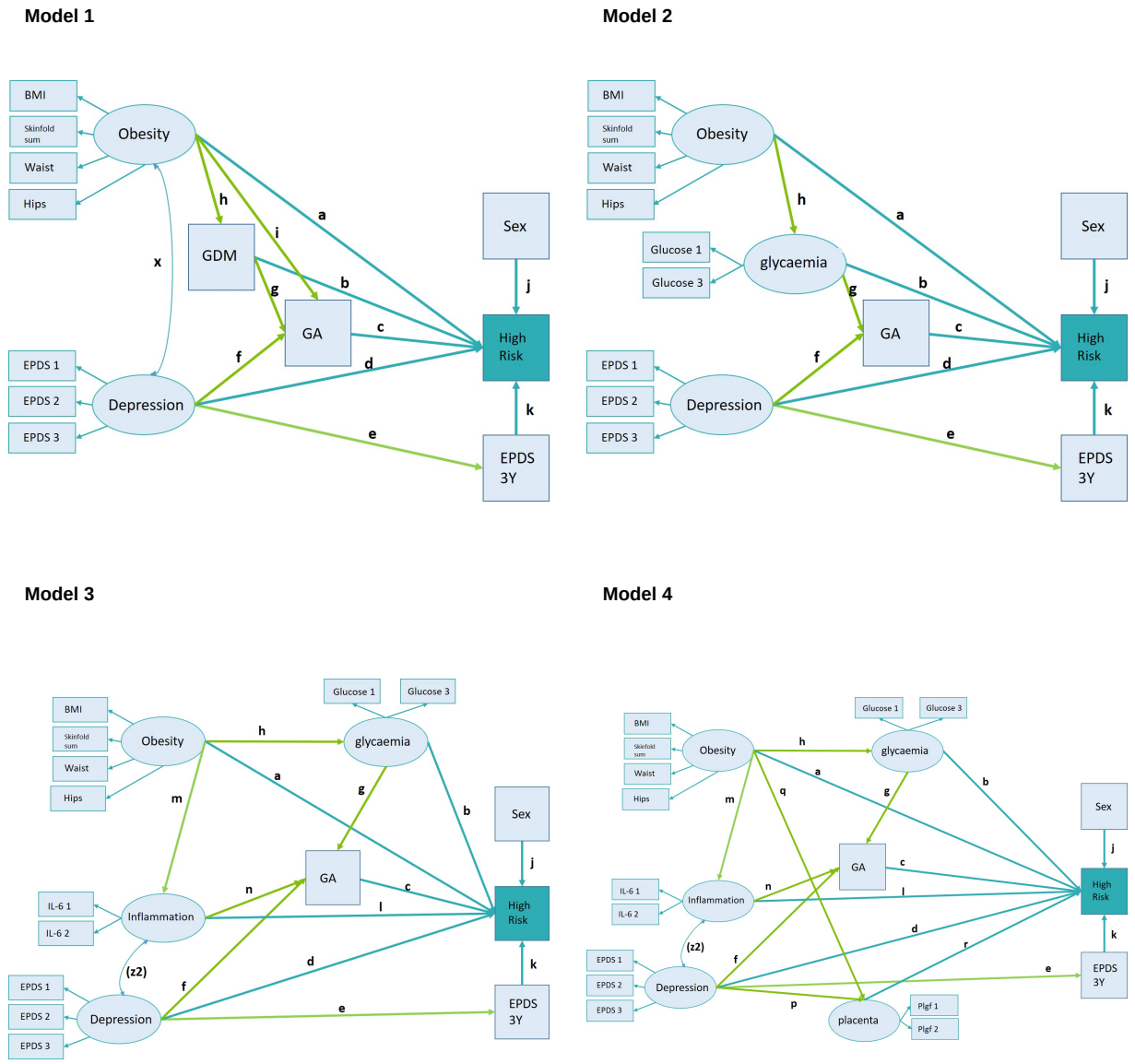

Figure 5.1: *Structural equation models were specified and run from Model 1 and in increasing complexity to Model 4. Main direct effect paths in blue and indirect in green. Latent variables are depicted in ovals and observed variables in squares. Curved bidirectional paths refers to a correlation. Variances of the latent variables were fixed at 1. Residuals are not shown. Available data: Body Mass Index (BMI)=1357, Edinburgh Postnatal Depression Scale 1 (EPDS 1)=1181, EPDS 2=1056, EPDS 3=932, gestational age (GA)=1357, gestational diabetes (GDM)=270 diagnosed and 887 not diagnosed, Glucose 1= 948, Glucose 3=735, High SDQ= 52 yes and 410 no, Hip circumference=1346, Interleukin-6 1 (IL-6 1)=905, IL-6 2=825, sex=1357, sum of skinfold(SSF)=1338, Waist=1346,*

5.3 RESULTS

5.4 Outcomes

5.4.1 SDQ

SDQ scores were non-normally distributed (Figure 5.2C) and the subscale scores were moderately correlated according to the Spearman coefficient, with *conduct* and *hyperactivity* showing a correlation of 0.47. However, child age at the assessment was not associated with scores (Figure 5.2B). .

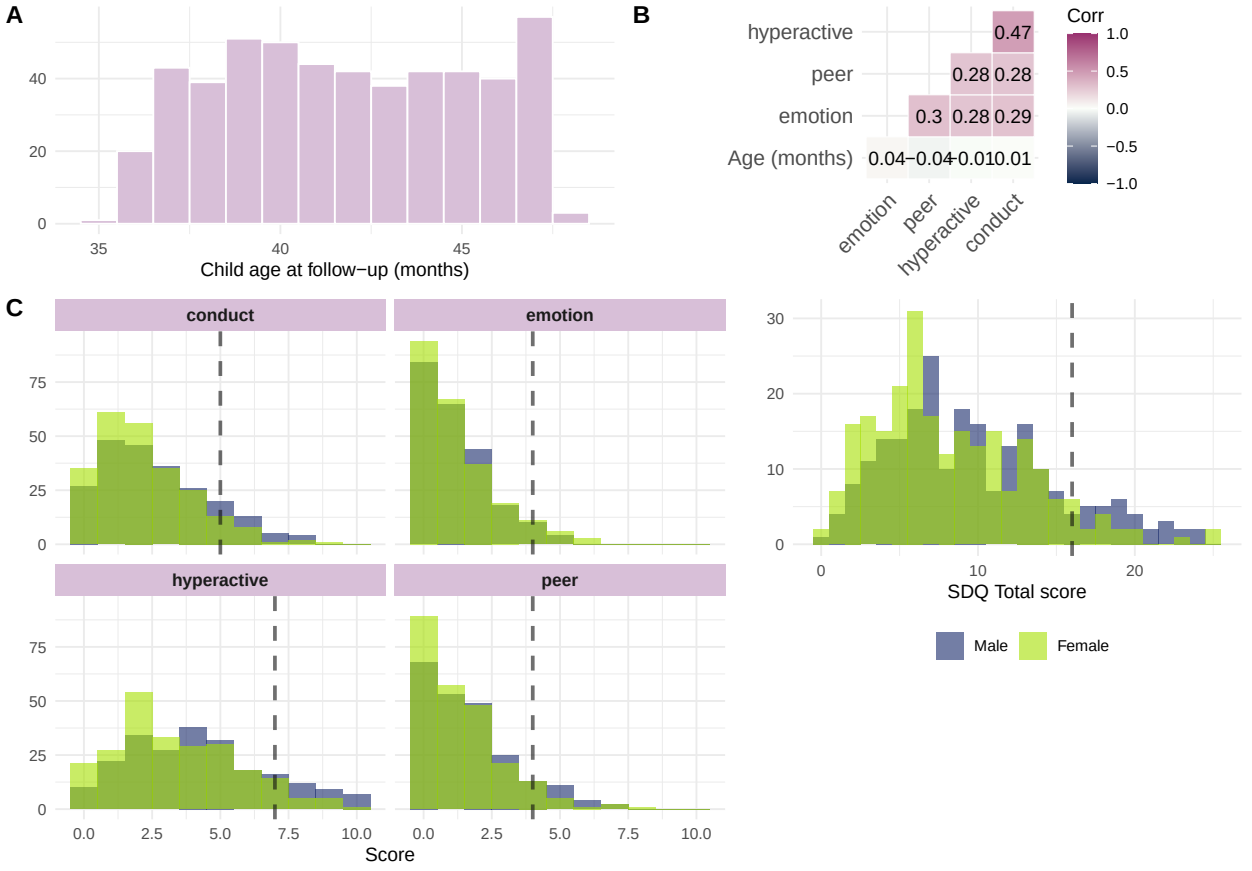

Figure 5.2: *Children were assessed between 3-4 years of age using the Strength and Difficulty Questionnaire (SDQ). A. Distribution of children's ages at 3 year follow-up. B. Correlation matrix of the SDQ total subscales with age, coefficients are based on spearman non-parametric correlation. C. The distribution of the SDQ total scores and subscales. Vertical lines represent the cut-offs for normal/low vs high/very high risk.*

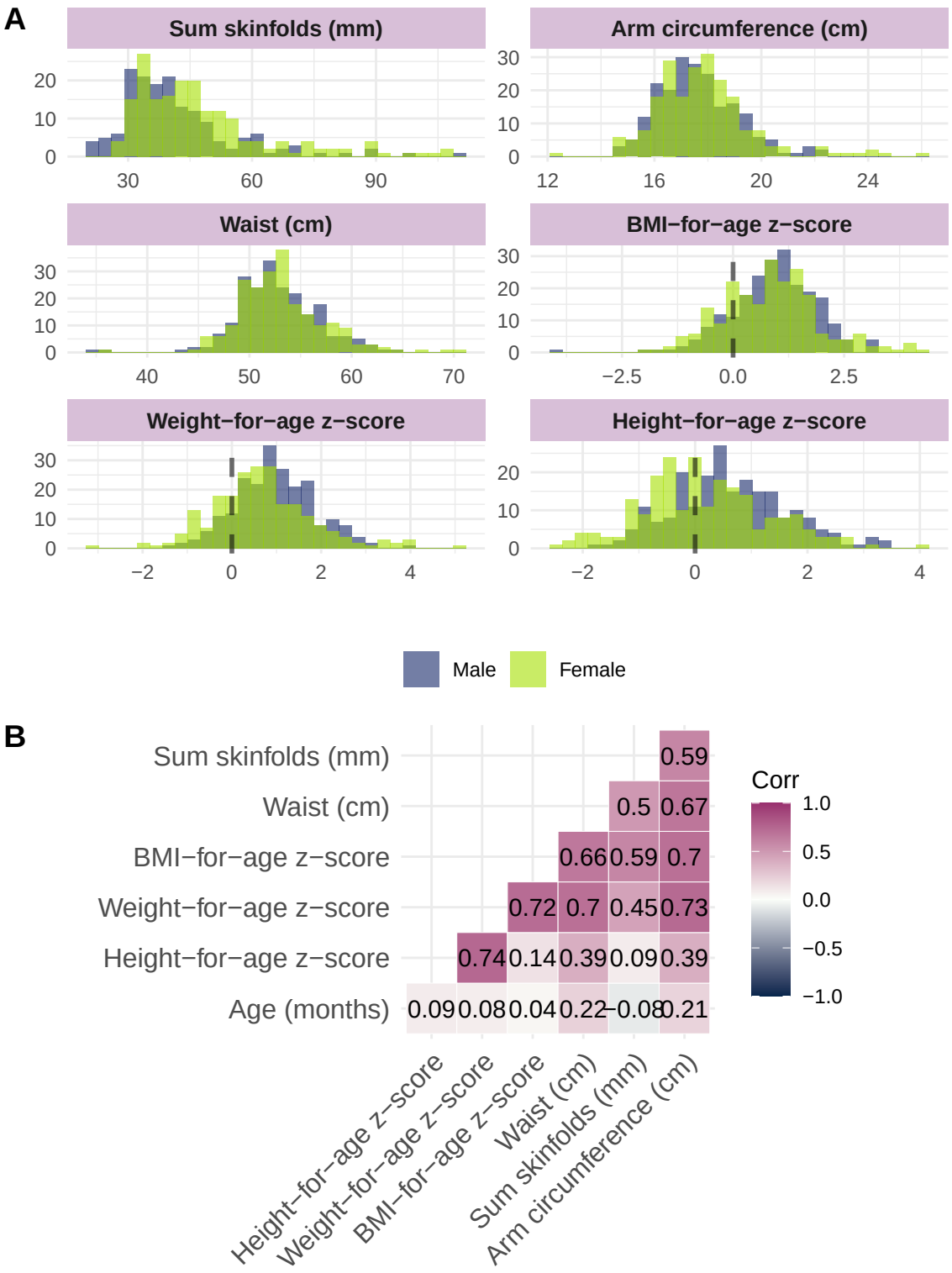

Figure 5.3: *Child anthropometric outcome at 3-year follow-up. A) Distributions of anthropometric outcomes for boys and girls and B) Spearman correlation matrix for non-normal variables indicate high correlation between anthropometric measures (r>0.7). All z-scores are according to WHO standards for each sex adjusted for age. Onis (2007).*

Child outcomes for the whole sample are presented in Table 5.1. The mean(SD) age of the 462 children at the time of the SDQ assessment was 41.8(3.4) months or 3.5(0.3) years. In this sample 52 of 462 children (11.3%) were considered at high or very high risks of psychopathology according to the SDQ total score. 14.5% were considered at high risk of Conduct Problems and 14.9% on the Hyperactivity subscales, 7.4% were at high risk on the Emotion and 11.3% on the Peer subscales. For their age the children had mean (SD) z-scores of 0.87 (1.04) for BMI, 0.39 (1.10) for height and 0.83 (1.06) for weight according to WHO standards, indicating this sample had a larger morphology on average than expected for their age.

5.4.2 Comparisons by sex

Comparisons between males and female outcomes as in Table 5.1. First, there was no difference in age at follow-up visit. However, a larger proportion of boys were considered high-risk based on the total SDQ (14.7% vs 8%, p=0.035), as well as at risk of Conduct Problem (18.7% vs 10.5%, p=0.019) and Hyperactivity (19.6% vs 10.5%, p=0.010) but proportion on the Emotional Problems (6.2% vs 8.4%) and Peer Problems (13.3% vs 9.3 %) were not significantly different.

Males height-for age z-score was higher at the follow-up (p<0.001) but there were no differences in BMI-for-age, arm circumference or waist circumference (cm). Females had higher mean sum of skinfold than males (p<0.001). Figure 5.3A presents the distributions on body measurements by sex Figure 5.3B shows that the some anthropometric measures are highly correlated (>0.70).

At birth the birthweight(g) was expectedly higher in males than females although no other outcome differed except for a higher proportion of boys being born of C-section (42.2% vs 32.5%, p=0.039) although the difference in Emergency C-section was not significant (21.8% vs 16%, p=0.144), see Table 5.12. In the index pregnancies, there were no difference in the maternal demography or pregnancy variables between women carrying the boys and those carrying girls (Table 5.11).

Table 5.1: *Child outcomes at 3-years follow-up by sex*

	Overall	Male	Female	p
n	462	225	237	
Age (months)	41.75 (3.40)	41.89 (3.36)	41.61 (3.44)	0.367
Strength and Difficulty Questionnaire				
SDQ Total Score	8.00 [5.00, 12.00]	9.00 [6.00, 13.00]	7.00 [5.00, 11.00]	0.002
SDQ>=16 (%)	52 (11.3)	33 (14.7)	19 (8.0)	0.035
Emotion total	1.00 [0.00, 2.00]	1.00 [0.00, 2.00]	1.00 [0.00, 2.00]	0.796
Emotion >=4 (%)	34 (7.4)	14 (6.2)	20 (8.4)	0.463
Conduct total	2.00 [1.00, 4.00]	2.00 [1.00, 4.00]	2.00 [1.00, 3.00]	0.016
Conduct >=5 (%)	67 (14.5)	42 (18.7)	25 (10.5)	0.019
Hyperactive total	4.00 [2.00, 5.00]	4.00 [2.00, 6.00]	3.00 [2.00, 5.00]	<0.001
Hyperactive >=7 (%)	69 (14.9)	44 (19.6)	25 (10.5)	0.010
Peer total	1.00 [0.00, 2.00]	1.00 [0.00, 2.00]	1.00 [0.00, 2.00]	0.040
Peer >=4 (%)	52 (11.3)	30 (13.3)	22 (9.3)	0.219
Anthropometric				
BMI for age z-score	0.87 (1.04)	0.90 (0.97)	0.85 (1.11)	0.617
Height for age z-score	0.39 (1.10)	0.62 (1.02)	0.18 (1.13)	<0.001
Weight for age z-score	0.83 (1.06)	0.99 (0.90)	0.68 (1.18)	0.002
Waist (cm)	52.96 (4.23)	52.74 (4.13)	53.17 (4.33)	0.294
Sum of skinfold (mm)	41.30 [33.98, 50.32]	38.45 [32.50, 46.30]	44.00 [35.32, 53.40]	<0.001
Arm circumference	17.72 (1.69)	17.63 (1.49)	17.81 (1.86)	0.289

Note:
Normally and non-normally distributed variables are presented as means(SD) and median[IQR] and compared with a t-test and a Kruskal-Wallis test, respectively. Categorical variables are shown as counts(%) and analysed with a Chi-squared test. BMI: Body Mass Index.

5.5 Relationships between psychopathological risk and anthropometric measures

The anthropometric outcomes are compared between those with overall high SDQ risk score i.e. 16 or above against those under 16, stratified by sex, see Table 5.2. Boys who are at high-risk vs low-risk of psychopathology at the 3-year follow-up were of higher BMI for age (z-score:1.27(0.80) vs 0.84 (0.98), p=0.024), higher weight for age (z-score 1.34(0.98) vs 0.93(0.87),p=0.017), larger waist circumference (54.81cm(4.05) vs 52.43cm (4.06), p=0.004) and arm circumference (17.54cm(1.45) vs 18.19cm (1.61), p=0.031). Birth outcomes did not differ significantly. However, there were no differences in anthropometry between high-risk and low-risk girls or in birth outcomes. This infers that, for a similar height, boys who present high-risk for psychopathology at 3 years have higher adiposity and larger morphology.

Table 5.2: *Anthropometry and birth outcomes by High/Low SDQ total risk*

	Boys			Girls		
	TotalSDQ_less16	TotalSDQ_16orMore	p	TotalSDQ_less16	TotalSDQ_16orMore	p
n	192	33		218	19	
Age (months) (mean (SD))	41.87 (3.35)	42.03 (3.52)	0.801	41.74 (3.45)	40.11 (2.98)	0.047
SDQ						
SDQ Total Score (median [IQR])	8.00 [5.00, 11.00]	19.00 [17.00, 21.00]	<0.001	6.50 [4.00, 10.00]	18.00 [16.00, 19.50]	<0.001
SDQ>=16 (%)	0 (0.0)	33 (100.0)	<0.001	0 (0.0)	19 (100.0)	<0.001
Emotion total (median [IQR])	1.00 [0.00, 2.00]	3.00 [2.00, 4.00]	<0.001	1.00 [0.00, 2.00]	3.00 [1.50, 5.00]	<0.001
Emotion >=4 (%)	4 (2.1)	10 (30.3)	<0.001	11 (5.0)	9 (47.4)	<0.001
Conduct total (median [IQR])	2.00 [1.00, 3.00]	6.00 [4.00, 6.00]	<0.001	2.00 [1.00, 3.00]	5.00 [4.50, 6.00]	<0.001
Conduct >=5 (%)	20 (10.4)	22 (66.7)	<0.001	11 (5.0)	14 (73.7)	<0.001
Hyperactive total (median [IQR])	4.00 [2.00, 5.00]	8.00 [7.00, 9.00]	<0.001	3.00 [2.00, 5.00]	6.00 [4.50, 7.50]	<0.001
Hyperactive >=7 (%)	19 (9.9)	25 (75.8)	<0.001	18 (8.3)	7 (36.8)	<0.001
Peer total (median [IQR])	1.00 [0.00, 2.00]	4.00 [3.00, 5.00]	<0.001	1.00 [0.00, 2.00]	4.00 [2.00, 5.00]	<0.001
Peer >=4 (%)	10 (5.2)	20 (60.6)	<0.001	12 (5.5)	10 (52.6)	<0.001
Anthropometry						
BMI for age z-score (mean (SD))	0.84 (0.98)	1.27 (0.80)	0.024	0.85 (1.13)	0.87 (0.95)	0.941
Height for age z-score (mean (SD))	0.58 (0.98)	0.85 (1.26)	0.172	0.17 (1.13)	0.21 (1.19)	0.894
Weight for age z-score (mean (SD))	0.93 (0.87)	1.34 (0.98)	0.017	0.68 (1.18)	0.71 (1.12)	0.894
Waist (cm) (mean (SD))	52.43 (4.06)	54.81 (4.05)	0.004	53.29 (4.27)	51.91 (4.89)	0.195
Sum of skinfold (mm) (median [IQR])	38.15 [32.18, 46.23]	41.40 [37.12, 49.20]	0.196	44.20 [35.40, 53.30]	39.70 [33.80, 51.70]	0.512
Arm circumference (mean (SD))	17.54 (1.45)	18.19 (1.61)	0.031	17.84 (1.89)	17.42 (1.43)	0.364
Birth outcomes						
Gestational age at birth (days) (median [IQR])	280.50 [272.00, 288.00]	278.00 [274.00, 284.00]	0.454	280.00 [273.00, 287.00]	274.00 [269.50, 286.00]	0.218
Preterm birth <37 weeks (%)	9 (4.7)	2 (6.1)	1.000	4 (1.8)	0 (0.0)	1.000
Apgar (median [IQR])	10.00 [9.00, 10.00]	10.00 [9.00, 10.00]	0.460	10.00 [9.00, 10.00]	10.00 [9.00, 10.00]	0.488
Birthweight (g) (mean (SD))	3,558.97 (499.56)	3,571.27 (553.75)	0.898	3,473.08 (467.58)	3,360.95 (509.00)	0.321
SGA WHO 10% (%)	11 (5.7)	1 (3.0)	0.827	14 (6.4)	3 (15.8)	0.292
LGA WHO 90% (%)	22 (11.5)	5 (15.2)	0.754	29 (13.3)	2 (10.5)	1.000
All C-section (%)	78 (40.6)	17 (51.5)	0.327	69 (31.7)	8 (42.1)	0.498
Emergency C-section (%)	39 (20.3)	10 (30.3)	0.291	35 (16.1)	3 (15.8)	1.000

Note:
LGA: Large for gestational age; SGA:Small for gestational age.

5.5.1 LCA

Table 5.3 provides the model fit indices, diagnostic criteria and class count(%) for the five models generated from the whole sample of 462 children. The AIC was the lowest for the 3-class model and entropy was >0.8 for this model which was preferred over the entropy of 0.66 and lower a/BIC in the 2-class model. The LMR also indicated that a 3-class model was an significant improvement in fit over the 2-class model. Furthermore, the smallest class count was deemed acceptable for this 3-class model. The number of children based on their likely class membership was n=37(8%) for class 1, 406(87.9%) for class 2 and 19(4.1%) for class 3.

After assessment of the the class average probabilities across the subscales (Figure 5.4) it was noticed that in the 3-class model there was a clearer identification of a externalising symptom class, with high probabilities of being in high-risk on both the conduct and hyperactivity subscales, and another class which indicated some individuals presented an internalising phenotype as represented in higher probabilities in the emotion and peer subscales but low risk in the hyperactivity and conduct subscales. This classification was viewed as of interest in relation to secondary anthropometric outcomes. The first class was labeled “Externalising”, the third class as “Internalising” and the second class was “Well-Adjusted”.

Table 5.3: *Latent Class Analysis in for 1 to 5 class models for total sample (n=462)*

	Model fit and diagnostic criteria								Count(%) per class				
N-class Model	LL	Parameters	AIC	BIC	aBIC	Entropy	LMR	LMR p-value	Class 1	Class 2	Class 3	Class 4	Class 5
1	-670	4	1348	1365	1352				462(100%)				
2	-630	9	1277	1315	1286	0.66	78.12	0.00	80(17.3%)	382(82.7%)			
3	**-623**	**14**	**1273**	**1331**	**1287**	**0.84**	**13.55**	**0.01**	**37(8%)**	**406(87.9%)**	**19(4.1%)**		
4	-622	19	1282	1361	1300	0.93	1.13	0.44	2(0.4%)	34(7.4%)	411(89%)	15(3.2%)	
5	-622	24	1292	1391	1315	0.93	0.09	0.79	9(1.9%)	30(6.5%)	3(0.6%)	407(88.1%)	13(2.8%)

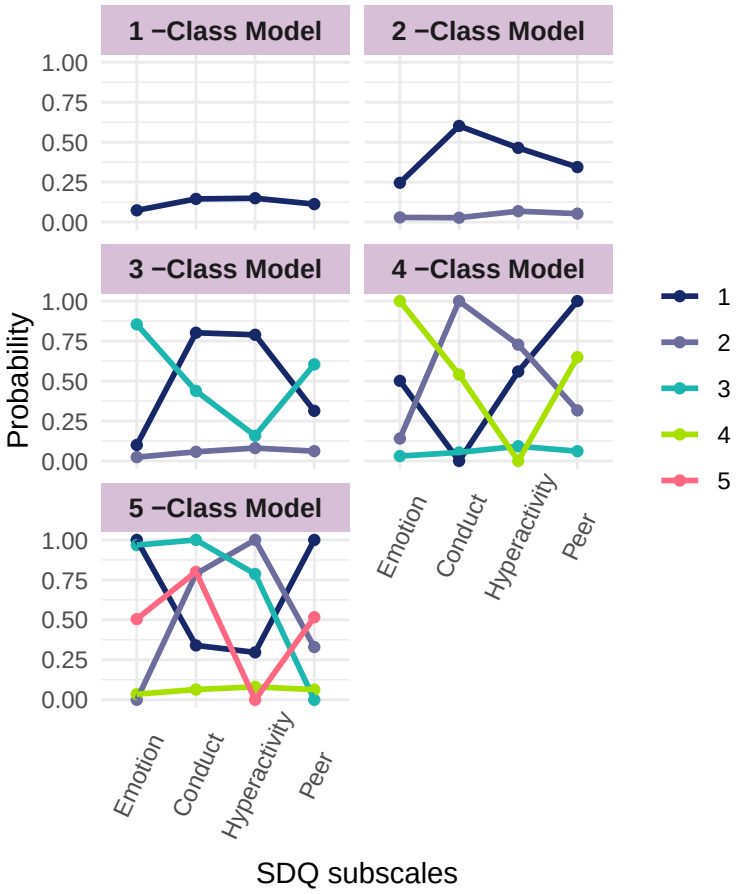

Figure 5.4: *Latent Class Analysis was performed for 462 children based on the subscales of the Strength and Difficulty Questionnaire. Average class probability of being at high-risk (vs low-risk) on each subscale for each n-class model are illustrated. Based on the assessing the Model fit and diagnostic criteria, a 3-class model was accepted.*

The probabilities of being male or female in each class was calculated using the *DCAT* auxiliary command in *Mplus* which takes into account fractional class membership error (Asparouhov and Muthén, 2014, 2021a). Probabilities of males in the Externalising class was 81.3%, 38.9% in the Internalising class and 45.7% in the well-Adjusted class. To assess the role of sex in predicting class membership (i.e. antecedent) the automated 3-step auxiliary model which also takes class fractional membership (using *R3STEP* auxiliary

command in *Mplus*) was used with sex as a predictor of class membership. Against the reference Well-Adjusted class, being a girl was negatively associated with the Externalising class (beta coefficient(standard error)=-1.74(0.64), p=0.007) but not associated with the Internalising class (beta coefficient (SE)=0.08(0.59), p=0.894).

5.5.2 LCA females vs males

In light of the above, 1-5 class LCA was performed for each sex separately in order to assess whether psychobehavioural phenotypes were similar among girls and boys. Results of the LCA for girls and boys are presented in Tables 5.4 and 5.5 and Figure 5.5.

Table 5.4: *Latent Class Analysis in for 1 to 5 class models for girl (n=237)*

	Model fit and diagnostic criteria								Count(%) per class				
N-class Model	LL	Parameters	AIC	BIC	aBIC	Entropy	LMR	LMR p-value	Class 1	Class 2	Class 3	Class 4	Class 5
1	-302	4	611	625	612				237(100%)				
2	**-288**	**9**	**595**	**626**	**598**	**0.73**	**25.18**	**0.08**	**29(12.2%)**	**208(87.8%)**			
3	-285	14	599	647	603	0.89	6.06	0.09	219(92.4%)	11(4.6%)	7(3%)		
4	-284	19	606	672	612	0.62	2.18	0.13	24(10.1%)	7(3%)	197(83.1%)	9(3.8%)	
5	-284	24	616	699	623	0.94	0.71	0.61	5(2.1%)	8(3.4%)	201(84.8%)	6(2.5%)	17(7.2%)

Table 5.5: *Latent Class Analysis in for 1 to 5 class models in boys (n=225)*

	Model fit and diagnostic criteria								Count(%) per class				
N-class Model	LL	Parameters	AIC	BIC	aBIC	Entropy	LMR	LMR p-value	Class 1	Class 2	Class 3	Class 4	Class 5
1	-360	4	729	742	730				225(100%)				
2	-331	9	680	711	682	0.64	56.27	0.00	159(70.7%)	66(29.3%)			
3	**-326**	**14**	**680**	**728**	**683**	**0.89**	**10.05**	**0.04**	**14(6.2%)**	**23(10.2%)**	**188(83.6%)**		
4	-325	19	688	753	693	0.90	1.43	0.41	6(2.7%)	23(10.2%)	185(82.2%)	11(4.9%)	
5	-325	24	698	780	704	0.75	0.22	0.65	3(1.3%)	8(3.6%)	6(2.7%)	185(82.2%)	23(10.2%)

Based on model fit indices and criteria a 3-class model is best fitting among the 225 boys. Group likely count and proportion were class 1= 14(6.2%), class 2= 23(10.2%) and class 3=188(83.6%). Visualising the average probability plots in the 3-class model (Figure 5.5B), the class 1 appears to have high peer problems with relative higher emotional problems and can be labeled as having an internalising phenotype. Class 2 shows a clear high probability of being at risk in both the conduct and hyperactivity domains and are labelled as having an externalising phenotype. The third class represent the children who are most likely persenting very low risk on all domains.

However, the 1-5 class models generated for the 237 girls did not yield as clear conclusions. The model fit indices present the lowest BIC for a 1-class model but lowest AIC and aBIC for a 2-class model. The LMR p-value is not significant at p=0.08 for the 2-class model nor the 3-class model (p=0.09) however the likely class counts in the smallest class is 29(12.2%) in the 2-class model and 7(3%) in the 3-class and 4-class models and 5(2.1%) in the 5-class model. All models provide the largest class of similar count which can be identified as low-risk on the profile plots (Figure 5.5A). However, the small class in the 2-class model could be considered medium-risk on most domains while in the 3-class model a clear “externalising” class (class 3), hence similar to the boys, and the second class could be considered of medium internalising/conduct

phenotype. However, given the small class count size in these two phenotypes it is arguable whether these could be valid and generalisable elsewhere. Taking the 3-class would imply that a very small proportion of girls are likely to have high externalising phenotypes.

Given the above observations, all further analyses of outcomes were performed on the whole sample LCA classes, adopting sex as a covariate.

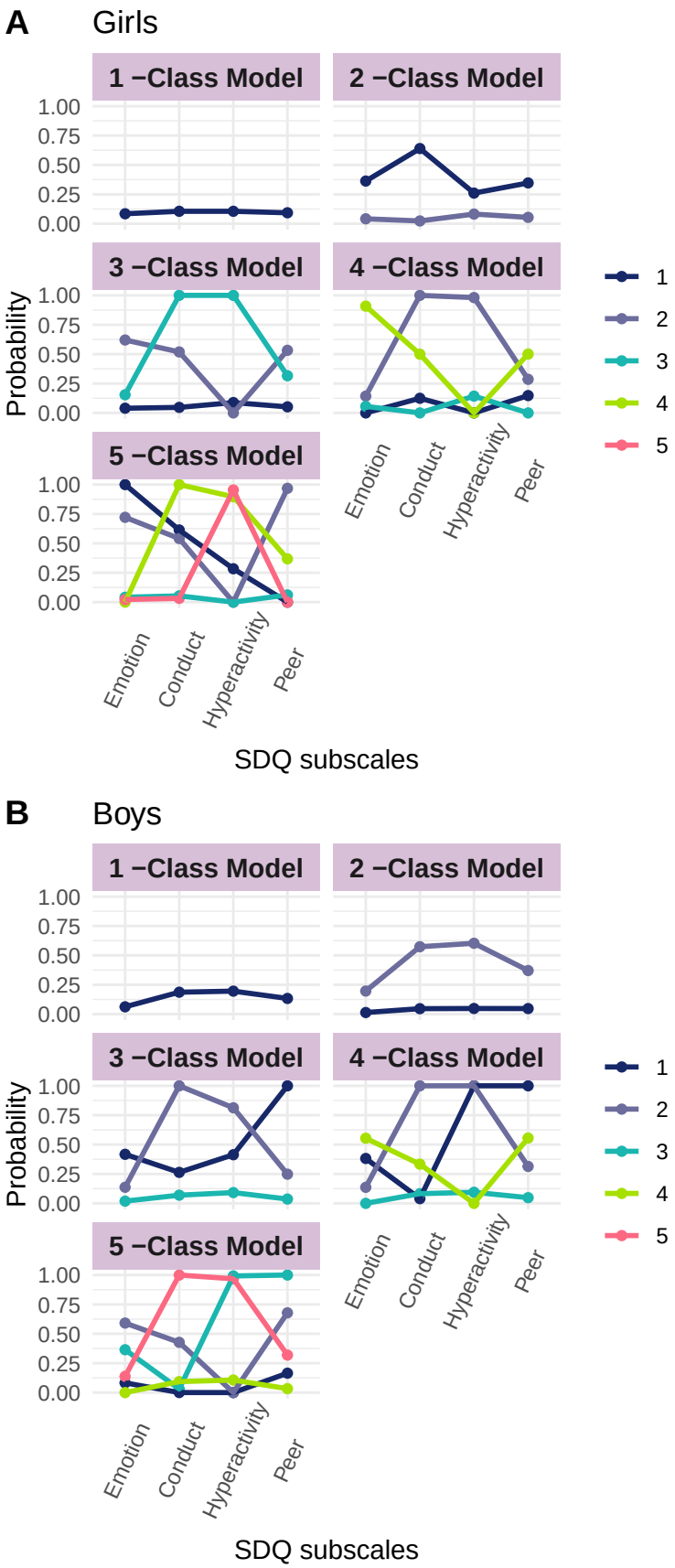

Figure 5.5: *LCA was performed for girls and boys separately and class subscale probabilities plotted. A 3-class model was accepted for boys and a 2-class model of girls.*

5.6 Class specific anthropometry

Next the latent classes were compared on anthropometric measurements, after adjustment for sex as a covariate. To align with the other scaled metrics, waist and sum of skinfold were standardised. Figure 5.6 shows that comparing to the children in the well-adjusted class, those presenting the externalising profile were heavier for their age, which reflected in a higher BMI for age, along with a larger waist circumference. In contrast, the children in the internalising class did not differ on these outcomes compared to the well-adjusted children. Further adjusting the child anthropometry for maternal antenatal (baseline) BMI did not change the results, expect for weight-for-age z-score was no longer different between the externalising and the well-adjusted children.

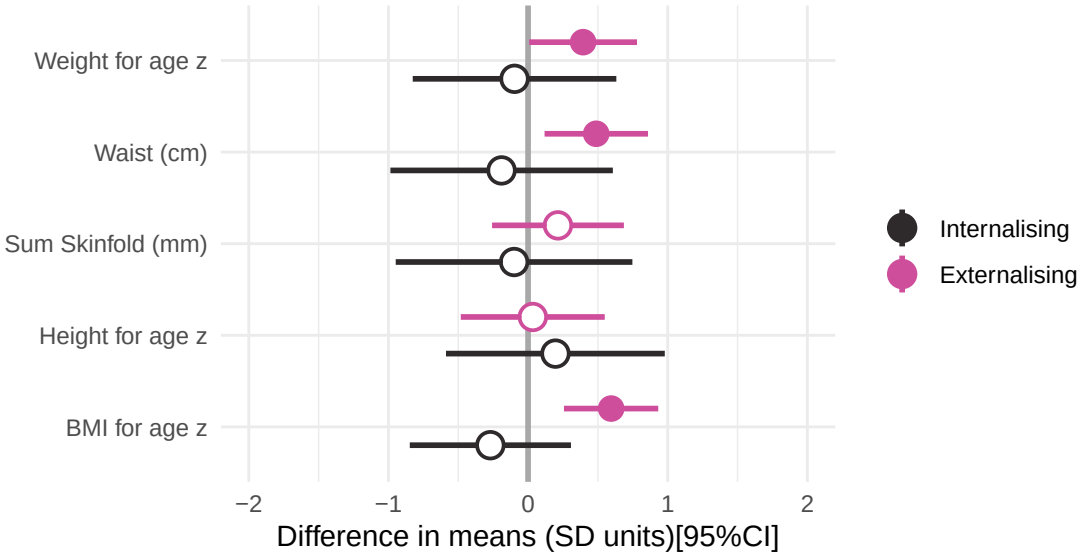

Figure 5.6: *The LCA-derived classes were compared on anthropometric outcomes, i.e. the externalising and internalising were compared against the children in the well-adjusted class. In the 3-step approach with BCH, the outcomes were adjusted for sex.*

5.7 Pathway models to risks of psychopathology.

5.7.1 Model fit and convergence

Four SEMs were specified (Figure 5.1). The model convergence and fit are found in Table 5.6 and the standardized coefficients in Table 5.7. The model 1, 2 and 3 reached convergence and were close to or below a PSR of 1.1. The Posterior predicted p-value was above >0.05 although only marginally, inferring the models were not a close fit. The trace plots were not all visualised for practicality reasons but we refer to Figure 5.7 for examples from Model 3. We note that those related to glycaemia or glucose were the least satisfactory. Model 4 did not converge and the glycaemia factor was replaced by GDM to evaluate whether convergence was due to the suboptimal factorisation, however, at 300k iteration, convergence was not achieved and no results are included for this model.

The measurement model for depression was good with standardized factor loadings of the indicator >0.7

Table 5.6: *Model convergence and fit.*

Model	Changes	Maximum PSR after burn-in	Chi2 95% CI	PP p-value	R2 (95%CI) for high-risk
1		1.129	-8.58 to 64.4	0.06	0.16(0.07 to 0.28)
2	remove x and i paths, add Latent glycemia for GDM, FB iteration 200k	<1.1	-16.67 to 59.48	0.13	0.26(0.1 to 0.49)
3	add Latent inflammation, FB iteration 200k	<1.1	-12.53 to 76.77	0.08	0.29(0.12 to 0.6)

Note:
PP p-value: posterior predicted p-value, PSR: Potential Scale Reduction. The Chi-squared test represent the 95% CI for difference between observed and replicated chi-square values

(p<0.001) and so for the Obesity factor (indicators >0.7, p<0.001). However, for the glycaemia factor made of two indicators, the measurement model was not as good (glucose 1 loading =0.37, glucose 3 loading =0.5, n.s.). The latent Inflammation factor had satisfactory factor loading (IL-6 1 at 0.64, IL-6 2 at 0.57, both p<0.001).

Model 1 explained 16% of the variance in the high-risk child psychological outcome and in Model 2, changing glycaemia for GDM, R^2 reached 26%. Adding latent inflammation (Model 3) increased R^2 to 29%.

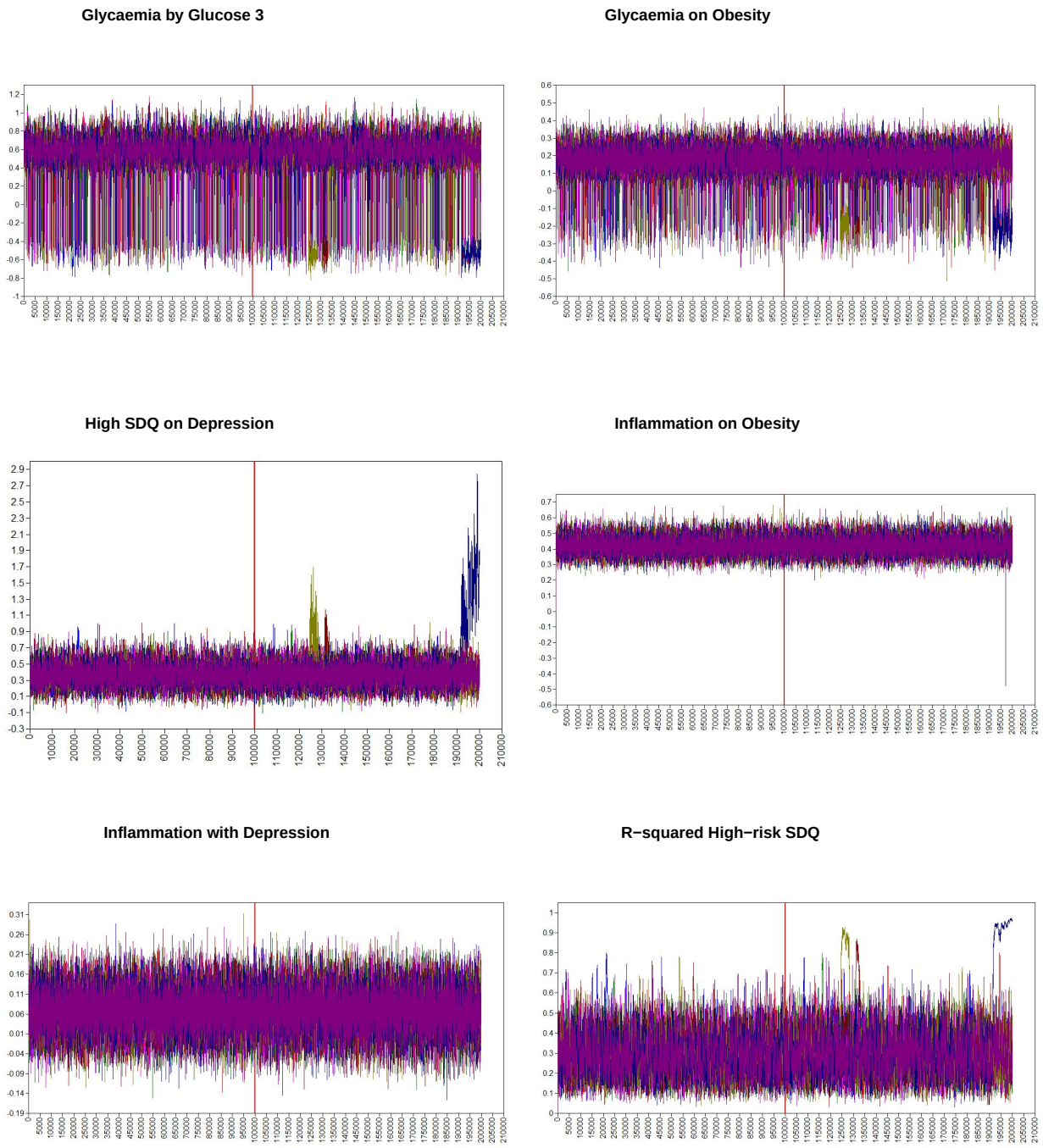

Figure 5.7: *Example of trace plots derived from Model 3.*

5.7.2 SEM results

Model 1 reveals that the main direct effects on child high-risks of psychopathology of latent maternal obesity, maternal GDM, gestational age at birth, female sex, and maternal 3-year EPDS score were all non-significant. The effect of female sex was not significant although the credibility interval suggests a trend (-0.14[-0.28 to 0.005]). In contrast, the main effect of latent depression on high-risk of psychopathology was significant and positive (standardized beta 95% Credibility Interval: 0.29[0.10 to 0.47]). This SEM also informs that antenatal depression predicted lower GA at birth (-0.09[-0.15 to -0.03]) and higher 3-year EPDS score (0.59[0.51 to 0.65]). However, GDM predicted earlier GA at birth (-0.37[-0.44 to -0.30]) and was in turn predicted by latent obesity(0.14[0.06 to 0.21]). Other paths were not significant.

Model 2 suggest latent glycaemia did not affect child high risks of psychopathology and the same significant effects of antenatal depression on child high-risk of psychopathology remained. In this model female sex was negatively associated with child high risks of psychopathology (-0.15[-0.29 to -0.004]).

Model 3 shows the same significant main effects of high antenatal depression on high-risk of psychopathology and the lower effect of female sex. Inflammation had no direct effect on the child outcomes, however the effect of latent obesity on latent inflammation was positive (0.39[0.30 to 0.47]).

Table 5.7: *Standardized coefficients for model paths*

			Model 1 GDM			Model 2 Glycaemia			Model 3 Inflammation		
Paths	Direct	Indirect	Estimate(SD)	95% PPI	Significant	Estimate(SD)	95% PPI	Significant	Estimate(SD)	95% PPI	Significant
a	Obesity		-0.034(0.083)	-0.2 to 0.125		0.024(0.095)	-0.159 to 0.216		-0.004(0.107)	-0.213 to 0.208	
b	GDM/Glycaemia		-0.107(0.116)	-0.328 to 0.123		-0.318(0.219)	-0.563 to 0.502		-0.331(0.267)	-0.564 to 0.667	
c	GA		-0.056(0.057)	-0.161 to 0.059		-0.067(0.052)	-0.169 to 0.035		-0.064(0.051)	-0.165 to 0.036	
d	Antenatal Depression		0.29(0.095)	0.098 to 0.468	*	0.309(0.094)	0.118 to 0.486	*	0.307(0.094)	0.114 to 0.484	*
e		3-y EPDS on Antenatal Depression	0.586(0.036)	0.512 to 0.652	*	0.586(0.036)	0.51 to 0.651	*	0.586(0.036)	0.51 to 0.651	*
f		GA on Depression	-0.087(0.03)	-0.145 to -0.028	*	-0.086(0.031)	-0.146 to -0.026	*	-0.084(0.031)	-0.144 to -0.023	*
g		GA on GDM/Glycaemia	-0.37(0.035)	-0.436 to -0.3	*	-0.226(0.094)	-0.348 to 0.125		-0.211(0.107)	-0.337 to 0.191	
h		GDM/Glycaemia on Obesity	0.136(0.039)	0.059 to 0.211	*	0.188(0.087)	-0.114 to 0.303		0.178(0.101)	-0.197 to 0.297	
i		GA on Obesity	0.001(0.028)	-0.054 to 0.057							
j	Female		-0.142(0.074)	-0.284 to 0.005		-0.148(0.073)	-0.288 to -0.004	*	-0.146(0.072)	-0.284 to -0.001	*
k	Postnatal Depression		0.033(0.089)	-0.144 to 0.206		0.02(0.089)	-0.156 to 0.193		0.019(0.088)	-0.155 to 0.191	
(x)		Obesity with Depression	0.024(0.032)	-0.038 to 0.085							
l	Inflammation								0.081(0.135)	-0.185 to 0.344	
m		Inflammation on Obesity							0.388(0.043)	0.302 to 0.471	*
n		GA on Inflammation							-0.039(0.043)	-0.123 to 0.047	
(z2)		Inflammation with Depression							0.069(0.054)	-0.037 to 0.174	

Note:
The letters in the first column refer to the paths in the models (Figure 5.1). Obesity, inflammation, depression and glycaemia are latent factors. EPDS: Edinburgh Postnatal Depression Scale, GA: Gestational age at birth, GDM: Gestational diabetes.

5.8 Discussion

In this chapter I combined the data from a longitudinal study involving over 1500 pregnant women and 462 of their children to investigate the association between exposure to maternal obesity *in utero* and offspring psychological outcomes at 3-years of age. With the rise of obesity among pregnant women, and associated increases in child obesity and psychological disorders, this study highlights how careful consideration should be given to the causal pathways involved. Additionally, potential associations between the two child outcomes were of interest given the reports of higher incidence of obesity with autism and ADHD. The proponents of DOHaD have characterised the effects to be of intrauterine origin, however further research is required to disentangle the individual roles of the multiple facets likely to be involved.

5.8.1 Child psychological and anthropometric outcomes

First, to provide comparable reference to the literature, we found that 11.3% of all 3-year old children of the obese mothers were high or very high-risk of psychopathology as per the total SDQ cut off score. The expected proportion based on the British norms for 3-year olds is 5.3 % (www.sdqinfo.org). The proportion of overall high-risk was higher among boys (14.7%, n=225) than girls (8%, n=237) which is higher than expected (boys:6.5 %, girls: 4 %). On the subscales of *hyperactivity* the higher incidence of problems among boys than girls (19.6% vs 10.5%) and of *conduct* problems (18.7% vs 10.5%) is in line with the norms but also higher than expected (hyperactivity for boys:5.2 % vs girls:2.9% and conduct problems for boys: 5.4 % and girls 3.5 %). Thus, 1 in 5 boys and 1 in 10 girls born of obese women in this sample had high risks of hyperactivity or conduct problems. Elsewhere, studies which have used the SDQ have reported a 4.8% incidence of total difficulties in Danish 5-7 year olds (Elberling et al., 2010), and 20% in very-preterm and 9% in term born 3-year old children (Delobel-Ayoub et al., 2006, cut off used 18.5 points).

It was interesting that using the WHO sex and age adjusted z-scores, boys were taller (0.62 vs 0.18) and heavier (0.99 vs 0.68) than girls even if the BMI was not different. The observation that boys in this cohort were on average 1 standard deviation above the expected weight for the age of 3 is of note. The fact that girls had a higher sum of skinfold for comparable waist and arm circumference to the boys reflects the known sex differences in adipose tissue distribution (Fitzgerald et al., 2018) and may reflect difference in lean mass between the sexes.

Further definition of psychological phenotypes in this sample of children was supported by the data-driven LCA. It revealed 37(8%) children were likely of externalising phenotype, 19(4.1%) as internalising phenotype and 406 (87.9%) were deemed well-adjusted. Therefore, based on these findings, the proportion of children likely to have any psychological problems is 12.1 %. which is close to the proportion based on the total SDQ. The LCA performed in each sex indicated a clear externalising class in boys but the sample size may have been too small in retrieving any pattern in the girls, given that in the population the prevalence of externalising disorders in girls is lower but prevalence of affective disorders is higher. Alternatively, girls may present symptoms later.

Furthermore, although children in this sample were on average larger than expected for their age, we found there were further morphological differences based on their psychological profile. The externalising class showed larger morphology than the well-adjusted group. This is in line with the findings that hyperactivity associates with increased adiposity in older children and adults. However, some consideration should be

given to the interpretation of these findings.

First, the directionality of the association in the two child outcomes cannot be clarified and other confounders may be at play such as shared genetics traits and unmeasured familial confounding factors (Chen et al., 2014). Second, although eating behaviour was not studied here, high food responsiveness precedes an increase in BMI, which in turn leads to decreased satiety response (Steinsbekk and Wichstrøm, 2015) and there may be a potential sex difference in these associations (De Decker et al., 2017). Previous studies in this sample showed that healthy and African/Caribbean dietary pattern was associated with lower child anthropometry and so was lower enjoyment of food, lower food responsiveness and greater satiety (Dalrymple et al., 2020). Therefore, the suggestion for further study is to investigate whether the association between the externalising phenotype in children and higher anthropometry is mediated by eating behaviour. This does not exclude child eating behaviour being an antecedent to their psychological outcomes. Should relationships be found between eating behaviour and psychological outcomes, this could point towards the hypothesised neurological overlap proposed between these outcomes.

Third, levels of physical activity and sedentary lifestyle (e.g. screen watching) and parental influences thereupon could also confound some of our findings. Fourth, although unlikely at this age, an effect of medication with a side effect of weight-gain cannot be excluded. Fifth, no maternal socio-economic differences were found between boys and girls in this sample but the maternal cohort from UPBEAT is overall categorised as deprived by the Index of Multiple Deprivation, which could in part explain the higher incidence of psychological risks compared to British population norms. Finally, although the SDQ hyperactivity and conduct subscales have predictive validity to autism and ADHD diagnosis (Croft et al., 2015), for this age group, parental perception of internalising (emotion/peer) problems may be difficult to ascertain. Some of the children may have different access and level of interaction to their peer depending, for example, whether they attended a nursery. Additionally, we cannot infer that the LCA profiles extracted here, i.e. externalising and internalising classes had significant long-term psychological outcomes and a follow-up of these children is indicated to answer this.

5.8.2 On pathways to high risks of psychopathology

If effects between maternal obesity and child outcomes are of intrauterine origin, defining the windows for early intervention requires a strong epidemiological framework. Predictive modelling of antenatal maternal effects is required in order to identify the strongest risk factors in the association between maternal obesity and child risks for further psychopathology. Previously, a large body of evidence has linked maternal obesity to child neurodevelopment through inflammation (Burg et al., 2016), however maternal psychopathology is largely underinvestigated as a potential strong confounding factor in these associations and the role of GDM is not clarified. In this study I asked the pertinent question of which of the facets of maternal obesity best explains child psychological outcomes.

Through the several SEMs presented, the assumptions of maternal latent obesity, GDM or inflammation influencing directly child high risks of psychopathology was not supported. Female sex and maternal antenatal depression had significant direct effect even after adjusting for maternal postnatal depressive symptoms. The biological mechanisms related to depression involve the effect of stimulation of the HPA pathway, and cortisol in particular which impacts on placental and fetal brain growth, as introduced in Chapter 1 and 2. It should be noted that the models described here were complex enough to answer the study aims but could

be further expanded.

The lack of further maternal variables may make the interpretations of the findings difficult because we found previously (Chapter 4) that maternal depression associates with various other exposures and these factors could influence the fetal growth. For reasons of parsimony and interpretation in this initial exploration, our models did not include these potential mediators, namely infection and diet, or maternal age and SES and C-section. C-section was mentioned to also influence both neurodevelopment and long-term obesity risks. However with such sample size and data availability and the necessary computational power these important variables could be implemented in further modelling and specifications in the future. Interestingly, the antenatal latent depression as a *continuous* factor also predicted shorter gestational age at birth. This reflect findings from Chapter 4 where we showed that Severely Depressed women had 3 times the risk of preterm birth. GA, however, had no direct effect on child outcome here but I acknowledge that many indirect effects were not tested in our models. A strength of this study is that I excluded early preterm born infant (<34 weeks) and pre-eclamptic pregnancies as etiologies of these outcomes may directly link to fetal growth.

Comparing to the previous literature is not straightforward. Firstly, this is because I used SEM and not conventional regression analyses and second due to the fact that effects of obesity elsewhere are studied in samples including normal-weight women. Rodriguez (2010) studied specifically if the relationship between maternal obesity in pregnancy and ADHD outcomes in the 5-year old children may be due to maternal distress. Using conventional regression analyses they adjusted for maternal depressive symptoms both in the antenatal and postnatal period as well as other covariates. They found that maternal obesity associated with twofold risks of difficulties in emotion regulation and predicted high inattention. However they found no relationship with hyperactivity symptoms in the child. Furthermore, when adjusting for child weight as a possible confounder, these association remained. It should be noted that in this study no maternal biomarkers were included nor any report on the incidence of obstetric complications across the BMI groups. In fact only 10% of the n=1,714 sample was obese during pregnancy and the normal-weight women comprised 54%.

One limitation of our study is that, due to low numbers, our SEMs included the overall (SDQ) high-risk dichotomy as an outcome and not the LCA-based classification. Causal models between the maternal milieu and the externalising and internalising phenotypes as outcomes differ especially in light of sexual dimorphism in effect of intrauterine insults on brain development and sex differences in externalising and internalising disorders in the population. Another limitation in regard to all women in our study being obese in pregnancy, the effect of latent obesity on child outcome along this obesity spectrum is diminished and as a result maternal mental health becomes the risk factor for child psychopathology. Furthermore, we did not include the effect of the UPBEAT intervention. It was recently reported that the intervention in pregnancy had no effect on anthropometry in the child (Dalrymple et al., 2021) but we cannot exclude that the intervention had effect on the psychological outcomes or interacted with the sex differences found in these outcomes.

The main strength of this study lies in the implementation of various SEM tools to categorise children by latent profiles and then to generate complex SEMs-regression models to understand the likely mechanisms. The benefit of SEM lies mostly in the characterisation of pathways between direct and indirect effects but also the inclusion of latent constructs which can improve on the statistical enumeration. The modelling of latent factors by repeated measures was chosen here to minimise measurement error and nuisance of time which has been shown to improve regression parameter estimation in simulation and applied study on sleep efficiency (Kim et al., 2020). Obtaining an average latent measure of a construct offers a parsimonious relation with

other predictors and outcomes in the models involved and eases interpretation. Alternative growth mixture modelling could be utilised to inform on whether trajectories in e.g. glycaemic status in pregnancy informs on outcomes. However this poses a different research question which was beyond the scope of this study. We note that we could not model glycaemia in lieu of GDM because of measurement model issue so the findings on the effect of latent glycaemia may not be robust and a new latent factorisation of glycaemic status should be explored. Nevertheless, other point estimates across models were stable. Moreover, using Bayesian statistics we were able to report 95% credibility intervals which directly translate in obtaining a range of point estimates, possibly intuitively more attractive than 95% confidence intervals in the frequentist framework. Importantly, our results could be utilised in future studies as priors. It is also probable that using noninformative priors (ie. the default priors in Mplus) was a limiting factor and caused non-convergence in Model 4. The Bayesian framework explicitly aims to facilitate computation and theoretical testing through the use of informative priors. Again, future models incorporating prior distributions could be specified such as the inclusion of small cross-loadings between the biological markers used as indicators.

In summary, this study used several methods within the SEM toolbox to uncover aspects of health in 3-year old offspring born of obese mothers which are both data-driven and can test theoretical assumptions. The implementation of such person-centered profiling (LCA) and SEM modelling method offers insight into the study of a common shared biological underpinning between mental health and adiposity. This applies to mothers and their children. In identifying risks factors in the obese pregnant population, researchers will eventually be well positioned to develop targeted interventions which serve long term projections. However, in light of the role of maternal mental health observed in this chapter and the extensive description of pregnancy along depressive profiles found in Chapter 4, particular attention should be paid to the impact of mental health in pregnancy on the developing fetal brain, and the dynamic between socio-economics, lifestyle and access to care in order to support mental wellbeing of parents in all sections of society.

5.8.3 Supplementary

Table 5.8: *Maternal factors between children who were included and excluded.*

	Excluded	Included	p
n	1,024	462	
Baseline			
BMI (median [IQR])	35.40 [32.90, 38.92]	34.70 [32.60, 37.80]	0.004
Age(years) (mean (SD))	31.11 (5.51)	32.29 (5.29)	<0.001
Main ethnicity (%)			0.013
White	618 (60.4)	318 (68.8)	
Black	277 (27.1)	103 (22.3)	
Asian	68 (6.6)	19 (4.1)	
Other	61 (6.0)	22 (4.8)	
IMD (%)			0.077
Least Deprived	32 (3.1)	27 (5.9)	
2nd quintile	66 (6.5)	32 (7.0)	
3rd quintile	115 (11.3)	51 (11.1)	
4th quintile	344 (33.7)	165 (35.9)	
Most deprived	463 (45.4)	185 (40.2)	
Income (%)			<0.001
< £12,688	213 (20.8)	61 (13.2)	
£12,688 - £17,628	118 (11.5)	47 (10.2)	
£17,629 - £23,452	93 (9.1)	32 (6.9)	
£23,453 - £32,500	120 (11.7)	62 (13.4)	
> £32,500	310 (30.3)	208 (45.0)	
Prefers not to answer	170 (16.6)	52 (11.3)	
Highest Education (%)			0.011
None	49 (4.8)	10 (2.2)	
GCE (or equivalent)	178 (17.4)	66 (14.3)	
Vocational qualification	245 (23.9)	113 (24.5)	
A level (or equivalent)	173 (16.9)	66 (14.3)	
First degree	261 (25.5)	134 (29.0)	
Higher degree	118 (11.5)	73 (15.8)	
Born in the UK (%)	674 (65.8)	328 (71.0)	0.056
Nulliparous (%)	427 (41.7)	223 (48.3)	0.021
Smoking (%)			0.004
Never	653 (63.8)	335 (72.5)	
Current	82 (8.0)	19 (4.1)	
Ex - gave up before pregnancy	185 (18.1)	69 (14.9)	
Ex - gave up in pregnancy	104 (10.2)	39 (8.4)	
Intervention (%)	521 (50.9)	227 (49.1)	0.571
Pregnancy outcomes			
All GDM (%)	221 (26.4)	105 (24.1)	0.405
GDM only (%)	187 (22.2)	105 (24.1)	0.493
All PE (%)	89 (8.8)	0 (0.0)	<0.001
PE only (%)	50 (5.0)	0 (0.0)	<0.001
EPDS 1st visit (median [IQR])	6.00 [3.00, 10.00]	6.00 [3.00, 9.00]	0.464
EPDS 2nd visit (median [IQR])	5.00 [2.00, 9.00]	5.00 [3.00, 9.00]	0.481
EPDS 3rd visit (median [IQR])	5.00 [2.00, 8.00]	5.00 [2.00, 8.00]	0.813

Note:

Comparison between the women whose children were included and those who were not. Normally and non-normally distributed variables are compared with a t-test and a Kruskal-Wallis test, respectively. Categorical variables were analysed with a Chi-squared test. IMD: Index of Multiple Deprivation; BMI: Body Mass Index. EPDS: Edinburgh Postnatal Depression Scale, GDM: Gestational Diabetes Mellitus, PE:Preeclampsia.

Table 5.9: *Birth factors between children who were included and excluded.*

	Excluded	Included	p
n	1,024	462	
Gestational age at birth (days) (median [IQR])	278.00 [270.00, 286.00]	280.00 [272.00, 287.00]	0.013
Born <37 weeks (%)	73 (7.1)	15 (3.2)	0.005
Female (%)	495 (48.5)	237 (51.3)	0.343
Apgar (median [IQR])	10.00 [9.00, 10.00]	10.00 [9.00, 10.00]	0.599
WHO centile (median [IQR])	52.39 [29.46, 76.73]	60.06 [33.18, 79.39]	0.027
SGA WHO 10% (%)	57 (5.6)	29 (6.3)	0.682
LGA WHO 90% (%)	119 (11.7)	58 (12.6)	0.683
All C-section (%)	360 (35.3)	172 (37.2)	0.500
Emergency C-section (%)	160 (15.7)	87 (18.8)	0.155

Note:
Normally and non-normally distributed variables are compared with a t-test and a Kruskal-Wallis test, respectively. Categorical variables were analysed with a Chi-squared test.

Table 5.10: *Data availability.*

Variable	Valid	Missing
Child Sex	462(100.0%)	0(0.0%)
SDQ		
SDQ Total Score	462(100.0%)	0(0.0%)
Emotion total	462(100.0%)	0(0.0%)
Peer total	462(100.0%)	0(0.0%)
Hyperactive total	462(100.0%)	0(0.0%)
Conduct total	462(100.0%)	0(0.0%)
Anthropometry		
Height for age z-score	438(94.8%)	24(5.2%)
Weight for age z-score	440(95.2%)	22(4.8%)
Weight for height z-score	435(94.2%)	27(5.8%)
BMI for age z-score	435(94.2%)	27(5.8%)
Weight (kg)	441(95.5%)	21(4.5%)
Height (cm)	440(95.2%)	22(4.8%)
Waist (cm)	428(92.6%)	34(7.4%)
Sum of skinfold (mm)	348(75.3%)	114(24.7%)
Arm circumference	423(91.6%)	39(8.4%)
Birth		
Gestational age at birth (days)	462(100.0%)	0(0.0%)
Preterm birth <37 weeks	462(100.0%)	0(0.0%)
Apgar	455(98.5%)	7(1.5%)
Birthweight	462(100.0%)	0(0.0%)
SGA WHO 10%	462(100.0%)	0(0.0%)
LGA WHO 90%	462(100.0%)	0(0.0%)
All C-section	462(100.0%)	0(0.0%)
Emergency C-section	462(100.0%)	0(0.0%)
Maternal factors		
BMI	462(100.0%)	0(0.0%)
Age(years)	462(100.0%)	0(0.0%)
Main ethnicity	462(100.0%)	0(0.0%)
IMD	460(99.6%)	2(0.4%)
Income	462(100.0%)	0(0.0%)
Highest Education	462(100.0%)	0(0.0%)
Born in the UK	462(100.0%)	0(0.0%)
Smoking	462(100.0%)	0(0.0%)
Nulliparous	462(100.0%)	0(0.0%)
GDM	436(94.4%)	26(5.6%)
Intervention	462(100.0%)	0(0.0%)
EPDS 1st visit	383(82.9%)	79(17.1%)
EPDS 2nd visit	381(82.5%)	81(17.5%)
EPDS 3rd visit	371(80.3%)	91(19.7%)

Table 5.11: *Maternal baseline and pregnancy outcomes by child sex.*

	Overall	Male	Female	p
n	462	225	237	
Baseline				
BMI (median [IQR])	34.70 [32.60, 37.80]	34.70 [32.60, 37.70]	34.70 [32.50, 37.90]	0.748
Age(years) (mean (SD))	32.29 (5.29)	32.38 (5.47)	32.20 (5.13)	0.715
Main ethnicity (%)				0.848
White	318 (68.8)	153 (68.0)	165 (69.6)	
Black	103 (22.3)	52 (23.1)	51 (21.5)	
Asian	19 (4.1)	8 (3.6)	11 (4.6)	
Other	22 (4.8)	12 (5.3)	10 (4.2)	
IMD (%)				0.187
Least Deprived	27 (5.9)	16 (7.1)	11 (4.7)	
2nd quintile	32 (7.0)	10 (4.5)	22 (9.3)	
3rd quintile	51 (11.1)	23 (10.3)	28 (11.9)	
4th quintile	165 (35.9)	86 (38.4)	79 (33.5)	
Most deprived	185 (40.2)	89 (39.7)	96 (40.7)	
Income (%)				0.636
< £12,688	61 (13.2)	28 (12.4)	33 (13.9)	
£12,688 - £17,628	47 (10.2)	21 (9.3)	26 (11.0)	
£17,629 - £23,452	32 (6.9)	14 (6.2)	18 (7.6)	
£23,453 - £32,500	62 (13.4)	27 (12.0)	35 (14.8)	
> £32,500	208 (45.0)	111 (49.3)	97 (40.9)	
Prefers not to answer	52 (11.3)	24 (10.7)	28 (11.8)	
Highest Education (%)				0.518
None	10 (2.2)	7 (3.1)	3 (1.3)	
GCE (or equivalent)	66 (14.3)	28 (12.4)	38 (16.0)	
Vocational qualification	113 (24.5)	51 (22.7)	62 (26.2)	
A level (or equivalent)	66 (14.3)	32 (14.2)	34 (14.3)	
First degree	134 (29.0)	70 (31.1)	64 (27.0)	
Higher degree	73 (15.8)	37 (16.4)	36 (15.2)	
Born in the UK (%)	328 (71.0)	159 (70.7)	169 (71.3)	0.961
Nulliparous (%)	223 (48.3)	116 (51.6)	107 (45.1)	0.199
Smoking (%)				0.202
Never	335 (72.5)	165 (73.3)	170 (71.7)	
Current	19 (4.1)	5 (2.2)	14 (5.9)	
Ex - gave up before pregnancy	69 (14.9)	37 (16.4)	32 (13.5)	
Ex - gave up in pregnancy	39 (8.4)	18 (8.0)	21 (8.9)	
Intervention (%)	227 (49.1)	111 (49.3)	116 (48.9)	1.000
Pregnancy outcomes				
GDM (%)	105 (24.1)	50 (23.7)	55 (24.4)	0.944
EPDS 1st visit (median [IQR])	6.00 [3.00, 9.00]	6.00 [4.00, 10.00]	6.00 [3.00, 9.00]	0.181
EPDS 2nd visit (median [IQR])	5.00 [3.00, 9.00]	6.00 [3.00, 9.00]	5.00 [2.00, 9.00]	0.200
EPDS 3rd visit (median [IQR])	5.00 [2.00, 8.00]	5.00 [2.00, 8.00]	5.00 [2.00, 8.00]	0.949

Note:

462 dyads were included. Normally and non-normally distributed variables are compared with a t-test and a Kruskal-Wallis test, respectively. Categorical variables were analysed with a Chi-squared test. BMI: Body Mass Index; EPDS: Edinburgh Postnatal Depression Scale; IMD: Index of Multiple Deprivation;GDM:Gestational Diabetes Mellitus.

Table 5.12: *Birth outcomes by sex.*

	Overall	Male	Female	p
n	462	225	237	
Gestational age at birth (days) (median [IQR])	280.00 [272.00, 287.00]	280.00 [272.00, 288.00]	280.00 [272.00, 287.00]	0.530
Preterm birth <37 weeks (%)	15 (3.2)	11 (4.9)	4 (1.7)	0.093
Apgar (median [IQR])	10.00 [9.00, 10.00]	10.00 [9.00, 10.00]	10.00 [9.00, 10.00]	0.280
Birthweight (mean (SD))	3,511.18 (490.45)	3,560.77 (506.58)	3,464.09 (470.87)	0.034
SGA WHO 10% (%)	29 (6.3)	12 (5.3)	17 (7.2)	0.533
LGA WHO 90% (%)	58 (12.6)	27 (12.0)	31 (13.1)	0.834
All C-section (%)	172 (37.2)	95 (42.2)	77 (32.5)	0.039
Emergency C-section (%)	87 (18.8)	49 (21.8)	38 (16.0)	0.144

Note:
462 dyads were included. Normally and non-normally distributed variables are compared with a t-test and a Kruskal-Wallis test, respectively. Categorical variables were analysed with a Chi-squared test. SGA: Small for gestational age, LGA: Large for gestational age. WHO: World Health Organisation. WHO centiles are adjusted for sex and gestational age at delivery.

Chapter 6

MRI segmentation of the neonatal hypothalamus, nucleus accumbens and ventral tegmental area.

6.1 Introduction

Advances in Magnetic resonance imaging (MRI) acquired during pregnancy and the early postnatal period provide essential opportunities to study brain development *in vivo*. It has the potential to increase our understanding of the chronology of critical periods and the place of insults in the timeline of emerging cognitive processes and behaviour (Keunen et al., 2017). Modeling physiological and functional dynamics in neurodevelopment could clarify etiological pathways for various adverse health outcomes which have a neurological underpinning.

The developing Human Connectome Project (dHCP, http://www.developingconnectome.org/) presents the opportunity to study brain structures and neuronal circuitry in the neonate, from high-resolution data acquired on a 3T scanner using state-of-the-art acquisition sequences and pre-processing methods bespoke to the neonatal brain. Several avenues of expanding interest which could rely on this data relate to the increased prevalence of obesity and incidence of neurodevelopmental disorders such as autism and attention-deficit hyperactivity disorder (ADHD). Metabolic and neurodevelopmental conditions, which can be co-occurring, are hypothesized to be in part mechanistically related to the limbic and reward systems. These conditions are also associated to antenatal maternal obesity exposures and fetal programming effects (Ramamoorthy et al., 2015), as proposed by the framework of the Developmental Origins of Health and Disease(Lee and Blackshaw, 2014; O'Donnell and Meaney, 2017).

The nucleus accumbens (NAcc) in the ventral striatum and the ventral tegmental area (VTA) of the midbrain (mesencephalon) are the primary areas involved in the reward pathway and have been implicated in anhedonia of depression (Russo and Nestler, 2013; Friedman et al., 2014) and addiction (e.g. food, drugs and sex) such as in stress-induced relapse to drugs (Wang et al., 2005), appetite for high-fat palatable food intake (Wang et al., 2015) and motivated feeding behaviour (Ferrario et al., 2016). The dopaminergic VTA neurons

project to the Nacc through the lateral hypothalamus via the white-matter medial forebrain bundle which further extends into the prefrontal cortex. This primary dopamine (mesolimbic) pathway is also implicated in reward processing related to associative/conditioned learning and social reinforcement learning (Solié et al., 2021) and is activated by palatable food cues (Kenny, 2011). In addition, these structures support an individual's assessment of the rewarding properties of novel interactions. Most recently, smaller NAcc volumes have also been identified in toddlers who subsequently received an autism diagnosis (Shiohama et al., 2021). It was shown that dysfunction in dopamine neurons in the VTA promotes the avoidance of exploration of unfamiliar social stimuli which relates strongly to traits found in autism spectrum conditions (Bariselli et al., 2018).

As part of the ventral diencephalon, the hypothalamus is also part of the reward system although it is widely recognised to play a crucial role in the neuroendocrine and homeostatic systems. As the master regulator of the autonomous nervous system (ANS) it serves, via afferents and efferents to visceral organs, the vital functions of, for instance, hunger and satiety, energy expenditure, thermoregulation, heart rate, blood pressure, renal output, sexual behaviour and the stress response (Timper and Brüning, 2017). Multiple animal models of obesity have focused on specific nuclei and hormones (e.g., leptin, ghrelin) in the hypothalamus in order to understand its etiology and of the related metabolic comorbidities (Bouret, 2004; Kirk et al., 2009; Bouret, 2012; Park et al., 2020a).

The hypothalamus is also essential to regulating affective cues, behavioural adaptation and cognitive processing which could explain food addiction but also wider psychological deficits of conduct, emotion regulation and social interaction. It regulates endocrine output through the pituitary (e.g. hypothalamic-pituitary-adrenal axis, HPA) and the sleep/wakefulness cycle both implicated in ADHD and autism (Baird et al., 2012; Fairchild, 2012; Logan and McClung, 2018). The neuropeptide oxytocin, released from the paraventricular nucleus of the hypothalamus has been a candidate therapeutic agent for autism (Watanabe et al., 2015; Walsh et al., 2018) and acts on the amygdala and NAcc where it triggers dopamine release (Uvnäs-Moberg et al., 2014). The hypothalamus is a central structure of the limbic system, essential in attention and emotion along with the orbital frontal lobe, cingulate cortex, amygdala and hippocampus. It takes part in emotion regulation and the top-down integration and cognitive processing of both intrinsic and extrinsic hedonistic value and the motivational/incentive salience of a stimuli.

Overall, the hypothalamus, NAcc and VTA together have attributes which have implication in the emergence of obesity and neurodevelopmental disorders (Posner et al., 2013; Nguyen et al., 2015). Although the hypothalamus, Nacc and VTA have been segmented manually and automatically as part of adult atlases at 1.5T, 3T and 7T (Baroncini et al., 2012; Makris et al., 2013; Schindler et al., 2013; Pauli et al., 2018; Neudorfer et al., 2020), they have largely been ignored in *in vivo* MRI studies of the neonate to date. Detailed research on the *human* subcortical anatomy during antenatal-to-early postnatal development is scarce and relies on post-mortem histological investigation (Bayer and Altman, 2003), e.g., Koutcherov et al. (2002) is one of the rare studies which have investigated human hypothalamic antenatal development. Nevertheless, advances in fetal and preterm and term neonatal brain MRI has increased the study of brain development and contributions to *in vivo* atlases has grown [Prayer et al. (2006); Habas et al. (2010);Kuklisova-Murgasova et al. (2011);Gousias et al. (2012);Serag et al. (2012);Gousias et al. (2013); Gholipour2017;Makropoulos2016a]

As part of dHCP processing pipeline, T1 and T2 weighted images were reconstructed to a $0.5mm^3$ resolution. Brain segmentation was performed based on the ALBERT brain atlas using the Draw-EM algorithm,

producing 87 labelled cortical/subcortical regions (Makropoulos et al., 2018) which improved earlier available 50-label atlases (Gousias et al., 2012; Serag et al., 2012). Subcortical labels include the hippocampus (label 1 and 2), amygdala (3/4), cerebellum (17/18), brainstem (19), caudate (40/41), thalami (42/43), subthalamic nuclei (44/45), lentiform nuclei (putamen & globus pallidus combined, label 46/47). Visual inspection reveals that in this atlas the nucleus accumbens is incorporated into the lentiform region and the hypothalamus as "inside//background" (label 85). Midbrain, pontine and medullary structures are all included under the brainstem region (label 19), see Figure 6.1. A subsequent segmentation using the anatomical automatic labelling (AAL; Tzourio-Mazoyer et al. (2002)) with Draw-EM adapted to dHCP neonates was also performed by Schuh et al. (2018) which contains 93 labels but excludes the regions of interest to this study.

Another neonatal atlas available is the Melbourne Children's Regional Infant Brain (M-CRIB) atlas (Alexander et al., 2017) which is based on the adult Desikan-Killiany adult atlas (Desikan, 2006) and provide manual parcellation scheme in 10 neonates scanned on a 3T Siemens scanner T2 acquired images reconstructed to a 0.63mm isotropic resolution. This effort aimed to expand cortical parcellation and provide some adjustment to subcortical segmentations, effectively separating the parts of the basal ganglia (caudate, putamen, pallidum and nucleus accumbens) and lobes of the cerebellum. de Macedo Rodrigues et al. (2015) provides some guidance for the Nacc in the neonate at $1mm^3$. However, neither in de Macedo Rodrigues et al. (2015) nor Alexander et al. (2017) are structures of the diencephalon and midbrain areas included.

The primary aim of the following chapter was to provide a manual segmentation of deep brain regions of the neonatal NAcc, VTA and hypothalamus, which are of interest in subsequently delineating white matter tracts within the limbic and reward pathways. The segmentations follow published studies and guidelines and are presented on the 40-post-menstrual week group structural template which as part of the dHCP data release. Such contribution could facilitate future studies relying on the dHCP data and others neonatal datasets.

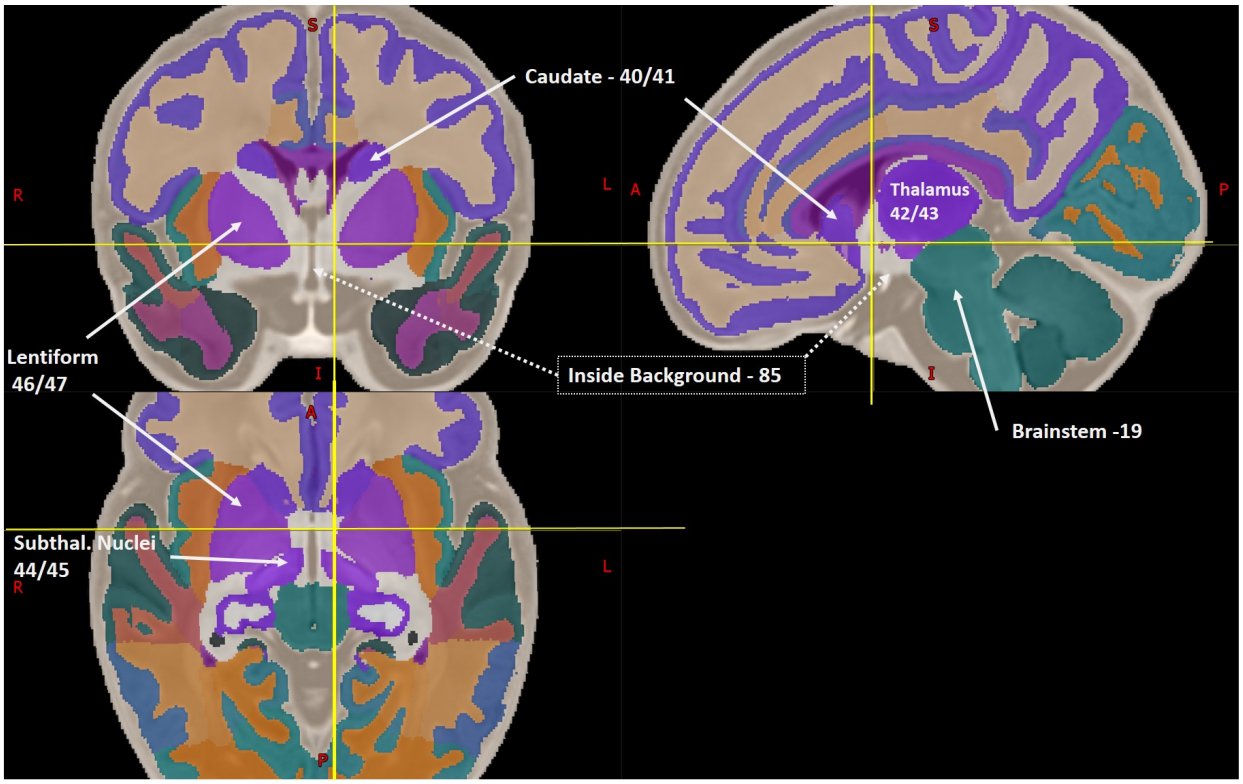

Figure 6.1: *The neonatal brain 40-week template T1 image is overlaid with the brain segmentation based on the ALBERT atlas using Draw-EM (Makropoulos et al. (2018)). It currently excludes primary regions of interest to the study of the limbic and reward networks i.e. hypothalamus is confined to the 'inside background', nucleus accumbens within the 'lentiform' regions and the ventral tegmental area within the 'brainstem' segmented region.*

6.2 Methods

6.2.0.1 Structural MRI acquisition and processing.

The acquisition protocol and processing pipelines of T1 and T2 neonatal images have been previously published (Makropoulos et al., 2018). For T2-weighted images, it used a Turbo Spin Echo (TSE) sequence: TR:12s, TE:156ms with a SENSE factor 2.11 (axial) and 2.58 (sagittal) with overlapping slices. T1-weighted images were acquired using a Inverse Recovery TSE: TR=4.8s, TE=8.7ms, SENSE factor 2.26 (axial) and 2.66 (sagittal. Images were acquired at $0.8mm^3$ resolution and 1.6 mm slice thickness. Both T1 and T2 data was reconstructed at a isotropic $0.5mm^3$ resolution (Kuklisova-Murgasova et al., 2012) following motion correction (Cordero-Grande et al., 2018), bias field correction (Tustison et al., 2010) and brain extraction (Smith, 2002).

Co-registered group T1 and T2 templates are averages from the subject images scanned at 40-weeks post-menstrual age, registered using nonlinear diffeomorphic symmetric image normalization and grey matter/white matter tissue probability maps (ANTs SyN, Avants et al. (2008)) into template space. Although the automated segmentation protocol with Draw-EM used the T2 dataset, the manual segmentation here used the contrasts provided from both T2 and T1 templates for visualisation and delineation.

6.2.1 Neonatal segmentations

The segmentations protocols described below and the resulting manual segmentation provided were performed by one operator and relied on publications from the adult brain segmentation from MRI imaging and/or postmortem dissections and histological studies. Many subcortical regions are also available to visualise in the three-planar mode from the NeuroVault.org anatomical repository. The photographic post-mortem 3rd-trimester fetal atlas by Bayer and Altman (2003) was also consulted for reference.

All manual segmentations were performed with visualisation in triplanar views. At a voxel size of 0.5mm isotropic the strategy was conservative (i.e., excluding voxels) in order to avoid partial voluming with cerebro spinal fluid (CSF) or grey matter e.g., an issue particularly important with the segmentation of the hypothalamus as it flanks the third ventricle and forms the ventral diencephalon. The segmentation did not have for aim to demarcate specific subregions of the structures (i.e. shell/core of the Nacc, nuclei of the hypothalamus) nor was it intended to provide accurate volumetric data.

Although neonatal white/grey matter contrast is low in the neonate and in early myelinating stage, relying on histological evidence and studies or repository available in the adult MRI literature was essential in establishing anatomical validity of the VTA, NAcc and hypothalamus and their adjacent brain structures. Further, extensive informal discussions with expert neuroanatomists and researchers on hypothalamic function provided additional support to the methodological strategies.

6.2.2 Hypothalamus

The hypothalamus is divided from the midline into medial and lateral regions and caudal to rostral into the Preoptic, Anterior(supraoptic), Middle (Tuberal) and Posterior (Mammilary) areas. Schindler et al. (2013) presented detailed guidelines in the form of a landmark matrix of preoptic/ anterior/ tuberal /posterior sections X inferior/ superior/ medial /lateral boundaries from their segmentation on 7T-acquired T1-weighted images. These were primarily relied on, see Figure 6.4. Reference was made to Baroncini et al. (2012) who provided stained histological slices and *in vivo* MRI references from 1.5 images (Figures 6.2 and 6.3). Makris et al. (2013) which also provided ex-vivo staining along with the corresponding tissues imaged by MRI at 7T.

In this current segmentation the hypothalamus included the mammillary bodies and the columns of the fornix. It should be pointed out that with low myelin content in the neonate there was limited visual information for landmarking provided by white-matter in the delineation, apart from the internal capsule and the anterior commissure.

The preoptic/anterior landmarks were easily visualised on the T1 and T2 templates i.e. the lamina terminalis and the anterior commissure which could be established in the sagittal plane on both T1 and T2. In the same plane the posterior boundary was established as a vertical line from the posterior mammillary body to the hypothalamic sulcus.

The lateral borders of the hypothalamus are knowingly difficult to establish (Schindler et al., 2013). In the coronal plane and anteroposterior direction, the anterior inferior lateral boundary was marked by the optic tract encircling it. However it is not obviously clear in the preoptic/anterior area where more superior limits should be established against the adjacent mixed white/grey matter of the substantia innominata. Still in coronal view, the tuberal to the posterior lateral boundaries were the globus pallidus and then the subthalamic nuclei which were clearly hyperintense on the T1. The medial landmark was the third ventricle and the

infundibular stalk was the ventral limit.

In addition we invited the segmentation to be reviewed by expert anatomists, including Dr Dick Swaab who published one of the earliest anatomical description of the human hypothalamus including in development and whose later contributions also relied on *in-vivo* and *ex-vivo* MRI and histological techniques (Swaab et al., 1993; Makris et al., 2013).

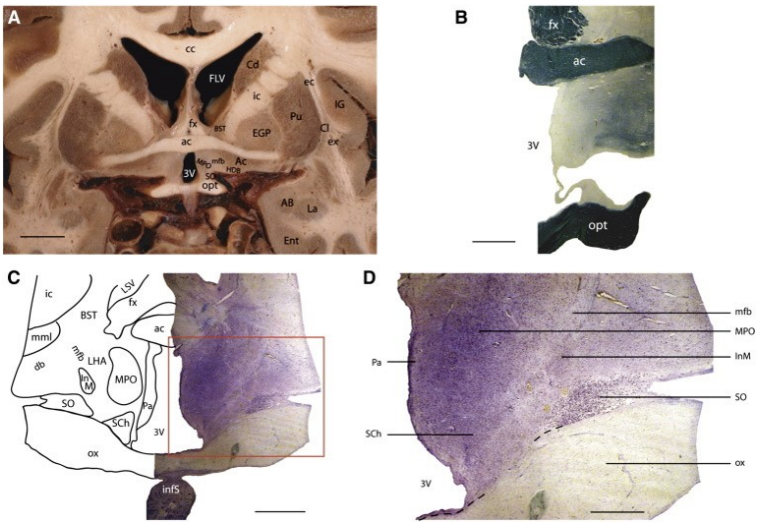

Figure 6.2: *Landmarks provided in the aim of producing an atlas of the adult the hypothalamus, from Baroncini et al. (2012). Here the anterior and preoptic regions are presented anatomically (A) histological with Sudan black (B) and Nissl staining (C, magnified in D).*

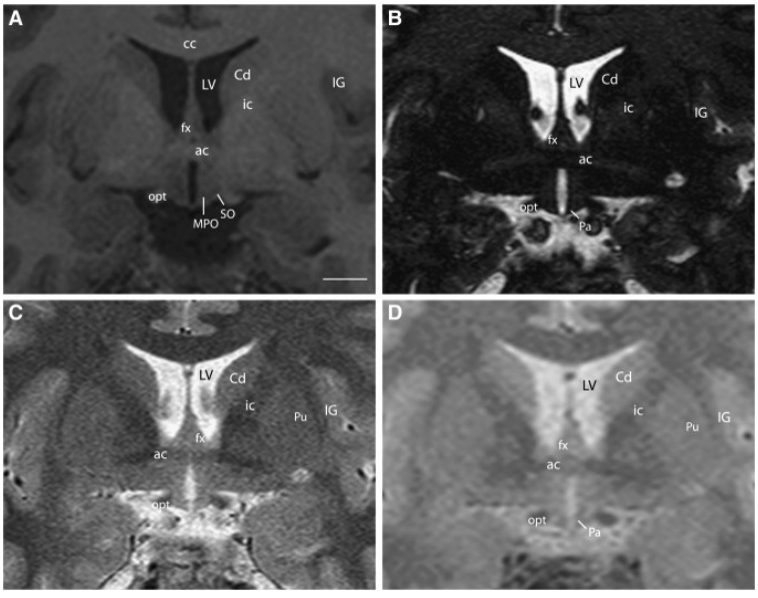

Figure 6.3: *From Baroncini et al. (2012), MRI sections obtained at 1.5T from an healthy volunteer and landmarks identified based based anatomical landmarks previously identified on post-mortem tissue. MRI sequences included representative sections from T1-weighted (A), T1-weighted imaging Inversion-Recovery (B), T2-weighted (C is Fast-Field Echno and D is Turbo Spin Echo).*

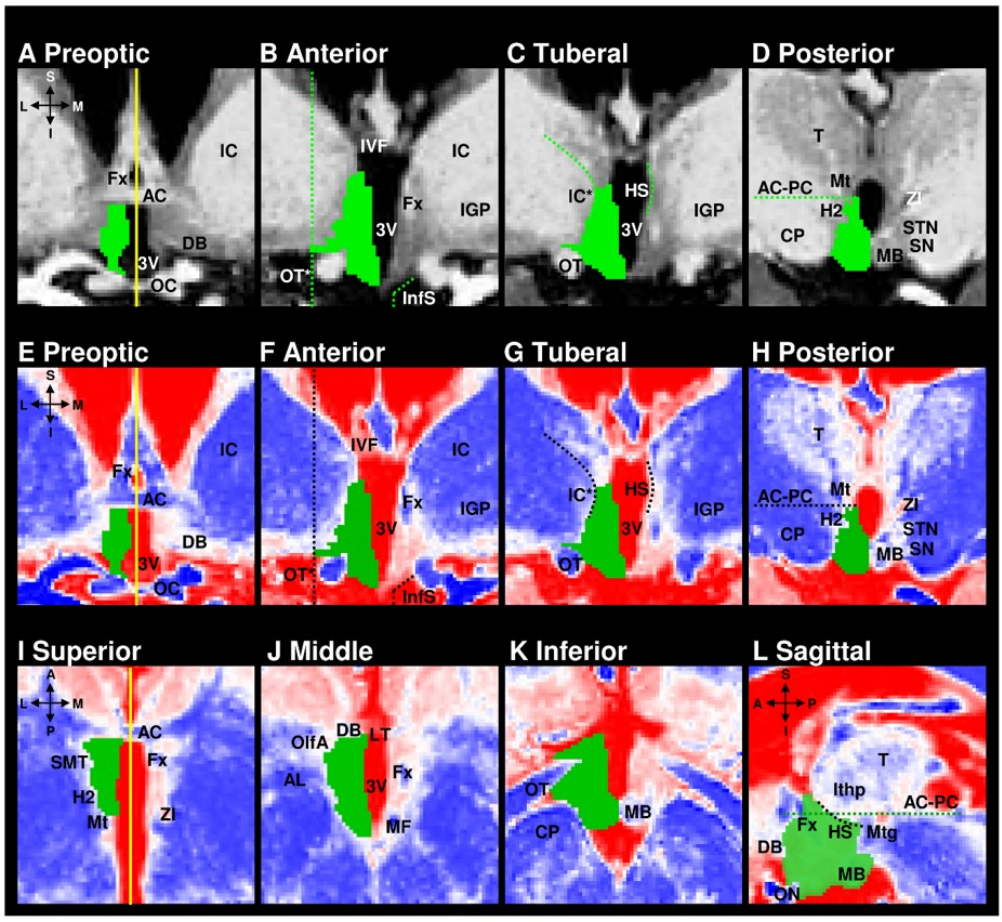

Figure 6.4: *From Schindler et al. (2013), adult human hypothalamus imaged at 7T was delineated in preoptic, anterior, tuberal and posterior regions in three planes. <U+064D>Schindler et al. provided the following annotation= 3V: third ventricle, A: anterior, AC: anterior commissure, AC-PC (dotted line): imaginary line between anterior and posterior commissures, AL: ansa lenticularis, CP: cerebral peduncle, DB: diagonal band of Broca, Fx: column of the fornix, H2: lenticular fasciculus (field H2), HS (dotted line): hypothalamic sulcus, I: inferior, IC: internal capsule; IC* (dotted line): medial pole of internal capsule, IGP: internal globus pallidus, InfS (dotted line): junction with infundibular stalk, Ithp: inferior thalamic peduncle, IVF: interventricular foramen, L: lateral, LT: lamina terminalis, M: medial, MB: mamillary body, MF: mamillary fasciculus, Mt: mamillothalamic tract, Mtg: mamillo-tegmental tract, OC: optic chiasma, OlfA: olfactory area, ON: optic nerve, OT: optic tract, OT* (dotted line): vertical line through lateral edge of optic tract, P: posterior, S: sagittal, SMT: stria medullaris of thalamus, SN: substantia nigra, STN: subthalamic nucleus, T: Thalamus, ZI: zona incerta.*

6.2.3 Ventral Tegmental Area

The literature is not unanimous in regards to anatomical boundaries of the VTA due to the large number of surrounding nuclei such as the parabrachial pigmented nucleus which is sometimes included (Halliday and Törk, 1986) but was separate in Pauli et al. (2018). The position of the first slice was horizontal to the superior limit of the mammillary bodies (which are hypointense on T1). In the axial plane, the segmentation was performed on two slices only and were identical and across 6 voxels in sagittal plane, in the lateral-medial direction. In the axial plane, the first posterior landmark to define the VTA was the clearly visible circular red

nuclei which are hypointense on the T1 template. The anterior limit was the CSF and the lateral limits were the bands of the assumed parabrachial pigmented nucleus and substantia nigra (slightly hyperintense on T1 and hypointense on T2).

6.2.4 Nucleus Accumbens (NAcc)

No distinction was made between the medioventral shell and a latero-dorsal core compartments of the NAcc (Bassareo and Di Chiara, 1999). de Macedo Rodrigues et al. (2015) offers some guidance in the neonate and similar to them and Langen et al. (2014), the delineation was performed primarily in the coronal view in an anterior-posterior direction. The supero-lateral limits for the Nacc were the base of the anterior limb of the internal capsule and the medial aspects of the caudate and putamen. The medial and inferior boundary of the NAcc was the white matter which was the clearly hypointense stripe on the T1. Still in coronal view, the posterior limit was the emerging of the globus pallidus. Overall, only three coronal slices bilaterally were included.

In addition, we invited the segmentation to be reviewed by expert anatomists. Prof Dick Swaab published one of the earliest anatomical description of the human hypothalamus including in development and whose later contributions also relied on in-vivo and ex-vivo MRI and histological techniques (Swaab et al. 1993; Makris et al. 2013). Prof Kevin O'Byrne is a specialist in neuroendocrinology and hypothalamic function and together they provided their views on the presented segmentation which they inspected visually. They approved that the landmarks used to delineate manually the hypothalamus and NAcc were appropriate and did not suggest any further changes.

6.3 Results

Figures 6.5 and 6.6 present the manual segmentation of the neonatal hypothalamus at the anterior and the tuberal/mammilary regions respectively. Figures 6.7 presents the NAcc, and Figures 6.8 and 6.9 the VTA.

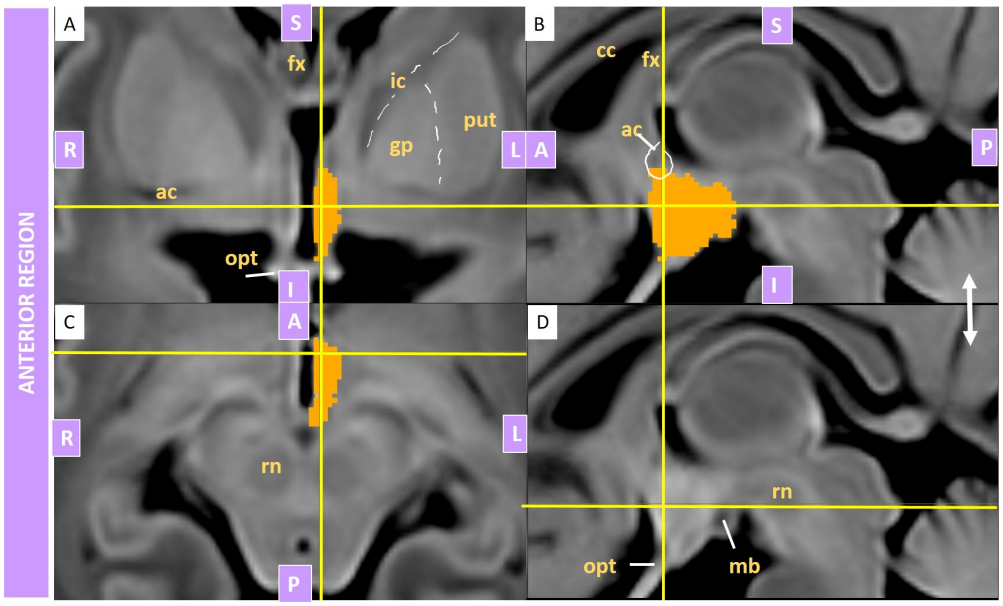

Figure 6.5: *Segmentation of the neonatal hypothalamus (orange) at the anterior region on the T1 40 week template provided in three-planar views :Coronal (A), Sagittal (B and D) and Axial (C). ac: anterior commissure, cc: corpus callosum, fx: fornix, ic: internal capsule, gp: globus pallidus, mb: mammilary bodies, opt: optic tract/chiasm, put: putamen, rn:red nucleus. Yellow lines are the cross-hair provided for easier planar orientation.*

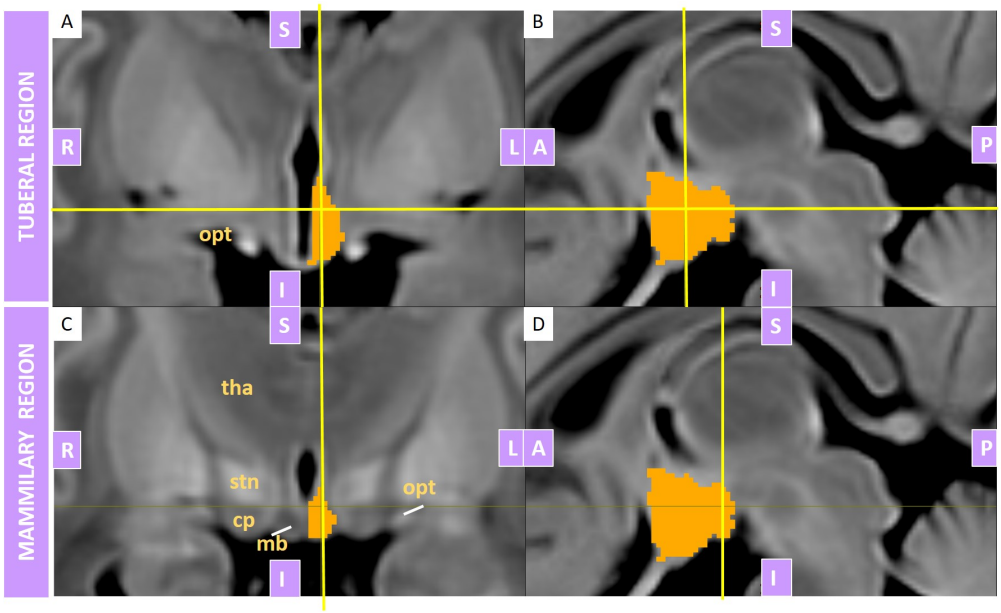

Figure 6.6: *Segmentation of the neonatal hypothalamus (orange) at the tuberal (middle) and mammilary (posterior) regions on the T1 40-week template provided in three-planar views :Coronal (A), Sagittal (B and D) and Axial (C). cp:cerebral peduncle, mb: mammilary bodies, opt: optic tract/chiasm, rn:red nucleus, stn: subthalamic nucleus, tha: thalamus.*

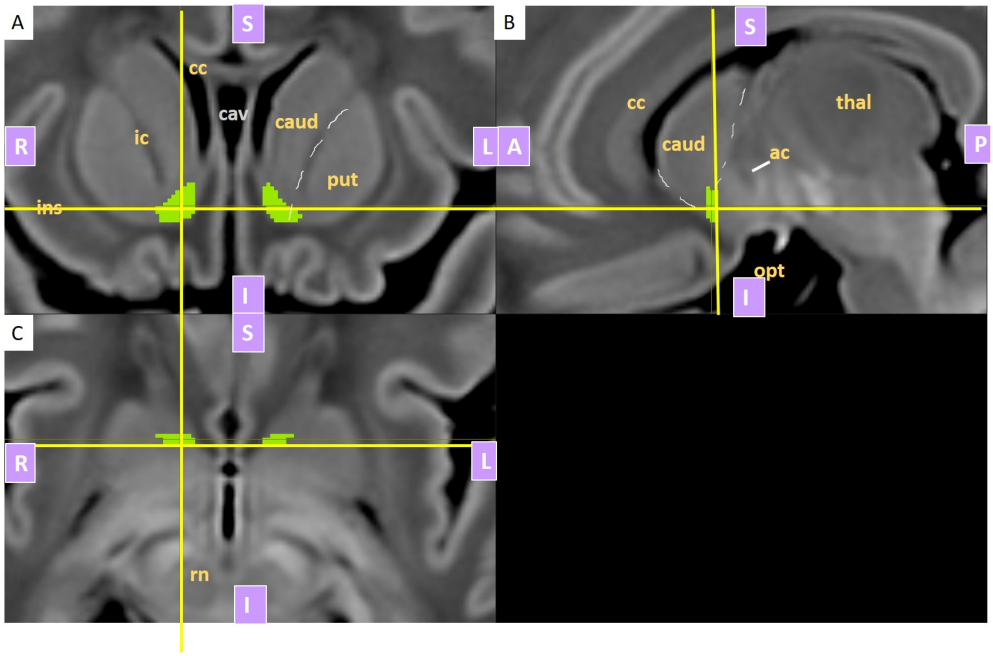

Figure 6.7: *Segmentation of the neonatal Nucleus Accumbens (green) on the T1 40-week template provided in three-planar views :Coronal (A), Sagittal (B) and Axial (C). Note the cavum is still present at this age. ac: anterior commissure, caud: caudate, cav: cavum pellucidum, cc: corpus callosum, ic: internal capsule (anterior), ins: insula, opt: optic tract/chiasm, put: putamen, rn:red nucleus, stn: subthalamic nucleus, tha: thalamus.*

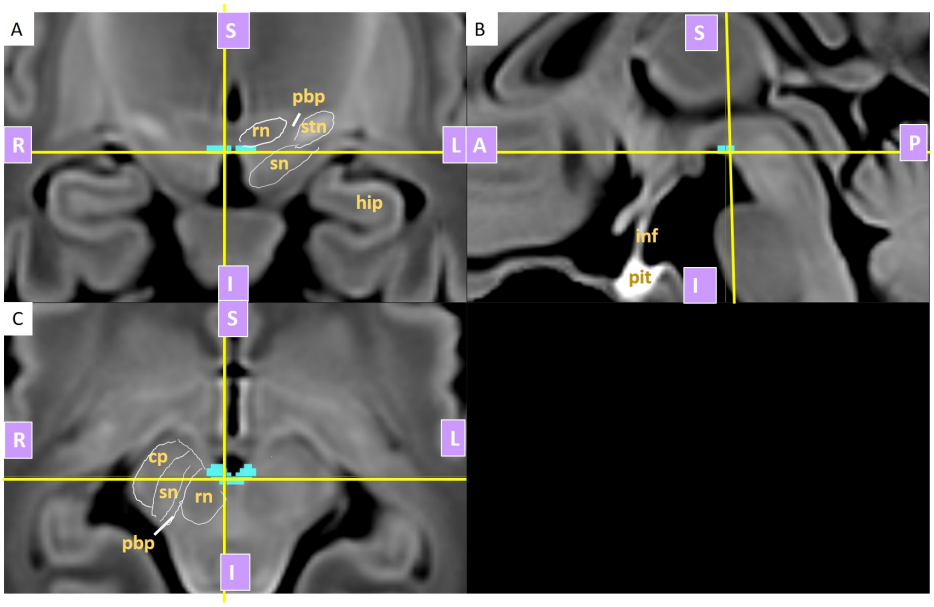

Figure 6.8: *Manual segmentation of the neonatal ventral tegmental area (cian) on the T1 40-week template provided in three-planar views :Coronal (A), Sagittal (B) and Axial (C). cp: cerebral peduncle, hip: hippocampus, inf: infundibulum (pituitary stalk), pbp: parabrachial pigmented nucleus, pit: pituitary, rn:red nucleus, sn: subtantia nigra, stn: subthalamic nucleus.*

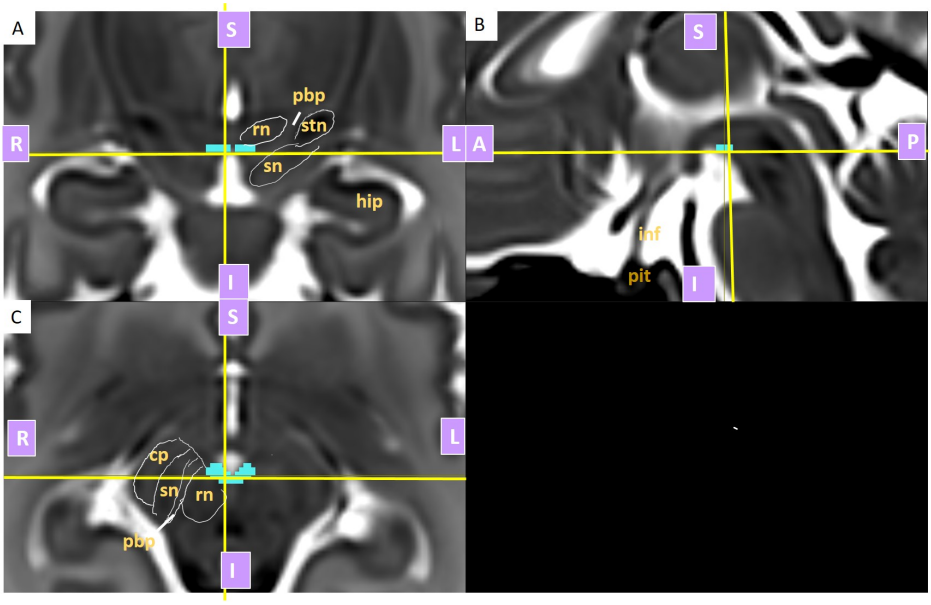

Figure 6.9: *Manual segmentation of the neonatal ventral tegmental area (cian) on the T2 40-week template provided in three-planar views :Coronal (A), Sagittal (B) and Axial (C). cp: cerebral peduncle, hip: hippocampus, inf: infidibulum (pituitary stalk), pbp: parabrachial pigmented nucleus, pit: pituitary, rn:red nucleus, sn: subtantia nigra, stn: subthalamic nucleus.*

6.4 Summary

Here the hypothalamus, nucleus accumbens and ventral tegmental area were segmented manually on a neonatal 40-week structural template. The above showcases the feasibility, at 0.5mm resolution, of delineating for the first time in the neonate these small subcortical areas. Using group templates rather than manual segmentation in subject MRI has the benefit of providing clearer regional boundaries which has facilitated this work.

Several future avenues could be contemplated using these novel segmentations. In the adult, further studies have produced detailed segmentations for the adult hypothalamus from T1, T2 and histological staining and further combining semi-automated algorithms (Wolff et al., 2018). Others have used the diffusion weighted signal to create hypothalamic brain masks which show potential to delineate subcompartments or clusters from the principal diffusion directions (Schönknecht et al., 2013) or diffusion orientation distribution functions (Spindler et al., 2020). Although the diffusion data is reconstructed at 1.5mm resolution in the dHCP there is a possibility that other segmentation techniques could be explored in the neonate using this modality in the hypothalamus but would be difficult in the smaller Nacc and VTA at this resolution.

The manual segmentation here relied on published adult high-quality segmentations protocols and atlases such as Pauli et al. (2018) are also recent contributions. Pauli et al. (2018) produced T1 and T2 templates at 700µm isotropic resolution from images available in 168 adults subjects in the HCP dataset and three observers used the Allen Institute Adult atlas (http://atlas.brain-map.org) as a reference. Although the neonatal regions here were delineated with the purpose of employing tractography of WM tracts of interest, the next steps in the neonate would be to conduct a template delineation and further validation of the regions included in this study and of other subcortical regions. This necessitates including multiple operators, val-

idation through intra-observer and inter-observer reliability with intra-class correlation and Dice coefficients (Zou et al., 2004).

However, it should be noted that, for the NAcc, inter-rater reliability on manual segmentations in *native space* of 0-2 year old infant 3T-acquired T1 is low (<50%) (de Macedo Rodrigues et al., 2015). Despite this, Zöllei et al. (2020) published the automated Infant (0-2) Freesurfer pipeline from their earlier labels at $1mm^3$ resolution (de Macedo Rodrigues et al., 2015). In this age range they also found that neonatal Dice indices were the lowest and importantly to our aims here, the midbrain and the hypothalamus are included in this pipeline. Despite being time consuming, it would be interesting to assess if using clear protocols, in weekly *templates* of $0.5mm^3$ resolution available in the dHCP could improve previously published results.

Since the transformation warps across weeks are also available as part of the dHCP data repository and pipelines, a validated segmentation in the the 40 week template could be applied easily in the younger or older templates and then into native space. Eventually, the production of multiple probabilistic atlases across gestation/early postnatal weeks could serve the scientific community studying neurodevelopment.

Chapter 7

Using tractography and fixel-based analysis to characterise the development of the reward and limbic pathways in human neonates, and following antenatal exposure to maternal obesity.

7.1 Introduction

Obesity in the female population of child-bearing age has been increasing globally with rates reaching 35%, including in the UK (National Health Service Digital, 2017; Chen et al., 2018). Maternal obesity in pregnancy has been associated with an exaggerated inflammatory state, a higher incidence of gestational diabetes mellitus (GDM) diagnosis, immune activation, depression and a suboptimal diet (Wolf et al., 2003; Brett et al., 2014; Pantham et al., 2015; Monk et al., 2016; Petursdottir Maack et al., 2019). Maternal obesity also confers higher risks of preterm birth and complications at delivery including C-section, infant shoulder dystocia due to larger birth size and intensive care admission (Ovesen et al., 2011; Mitanchez, 2015; Burg et al., 2016; Catalano and Shankar, 2017). In addition, pre-clinical research using models of high-fat-diet induced obesity as well as human clinical evidence suggest infants born of obese women have increased risk of obesity, metabolic and cardiovascular disease (Steculorum and Bouret, 2011b; Yu et al., 2013; Vogt et al., 2014; Park et al., 2020a) but also of neurodevelopmental disorders (Adane et al., 2016; Li et al., 2016a).

Notwithstanding the issues of confounding in the many human studies carried out to date (Ornoy et al., 2016), a proinflammatory maternal milieu with associated, and potentially adverse fetal exposure could independently or in synergy affect neurodevelopmental trajectories at any stage during neurogenesis, neuron migration and myelination (Grote et al., 2010; Bouret, 2012; Burg et al., 2016; Vohr et al., 2017; Boulanger-Bertolus et al., 2018; Moog et al., 2018; Reynolds et al., 2019). For example, mouse models of maternal

hyperglycemia with maternal immune activation have shown to alter both the inflammatory and neurodevelopmental transcriptome profile of the fetus but also produced disruption in the patterning genes involved in dopamine neuron differentiation and innate fetal immune response (Money et al., 2018).

Several parallels can be drawn between the metabolic and neuropsychological outcomes reported above with studies that have simultaneously addressed both childhood adiposity and psychological outcomes. Studies in children with and without autism (or autistic spectrum disorder [ASD]) or attention-deficit hyperactivity disorder (ADHD) diagnosis demonstrated that overweight or obesity is more likely among diagnosed children (Broder-Fingert et al., 2014; Cortese et al., 2016; Güngör et al., 2016; Ptacek et al., 2016; Racicka et al., 2018), with risks of obesity increased by 41% in children diagnosed with ASD (Kahathuduwa et al., 2019). Overall prevalence of obesity and overweight in ASD has been estimated to be 21.8% and 19.8%, and in ADHD 14.7% and 20.9% respectively (Li et al., 2020b). This is not a universal observation however, as some studies have reported a lower prevalence of obesity among diagnosed children (Dubnov-Raz et al., 2011). Others report sex differences, with lower prevalence in female compared to male autistic children (Broder-Fingert et al., 2014), or lower BMI z-score in autistic girls but overall similar incidence of overweight and obesity compared to neurotypical children (Barnhill et al., 2017), which may suggest some sexual dimorphism.

While shared genetic predisposition in mother and child has been considered (Locke et al., 2015), the relationship between maternal and childhood obesity, or surplus adiposity, as a dysfunction of energy homeostasis in the offspring rather than excess energy intake has been made (Schwartz et al., 2017). This has been extensively corroborated in research into the energy balance circuitry of the hypothalamus and its nuclei in animal models (Bouret, 2004; Kirk et al., 2009; Bouret, 2012; Park et al., 2020a), including during development (Vogt et al., 2014).

However, In relation to reported associations between neurodevelopmental outcomes and adiposity as summarised above, it should be noted that these important central pathways sustaining metabolic outcomes through feeding behaviour and energy expenditure are also directly or indirectly implicated in executive function, theory of mind (Hughes and Ensor, 2007) and social reinforcement and may thus participate in the phenotypes of autism and ADHD. In this study, potential shared biological pathways underpinning the neurodevelopmental disorders and metabolic disturbances and thus a predisposition to behavioural and psychological phenotypes are explored in the neonate. These include the neural systems implicated in the reward and the limbic system of the brain, specifically those relaying functions of the hypothalamus, nucleus accumbens (NAcc), ventral tegmental area, the amygdala, and cingulate.

7.2 The reward and limbic pathways and their relation with food intake, social reinforcement and impulsivity.

The (mesolimbic/dopamine) reward network, under the influence of the medial prefrontal cortex is to a large extent implicated in reward-seeking behaviour which integrate social reinforcement learning (Solié et al., 2021) and can explain addictions (food, drugs, sex) (Wang et al., 2005; Volkow et al., 2017), food seeking behaviour (Kenny, 2011) and also anhedonia following stress (Russo and Nestler, 2013; Friedman et al., 2014). This network is implicated in the social aspects of autistic traits (Bariselli et al., 2018) and is disrupted in adults with ADHD (Volkow et al., 2011) and has been shown to explain the association between the genetic

liability for BMI and higher odds for ADHD (Martins-Silva et al., 2021). Additionally the orbitofrontal area has been implicated in reward processing in the adults with ADHD who show lower activation in decision-making tasks (Yang et al., 2019).

Reward processing in autism is suggested to be different for social and non-social stimuli and neuroimaging studies suggest autistic children have higher functional response in the anterior cingular cortex and insula in response to food cues (Cascio et al., 2012). ADHD is associated with eating disorders (Quesada et al., 2018), mostly binge eating and bulimia nervosa (Ptacek et al., 2016), explained by the impulsivity and inattention part of ADHD, the likely leading factor explaining the increased unhealthy eating habit (Graziano et al., 2012). It has also been hypothesized that any observed eating behaviour disorder could be explained via the higher sleep disorder breathing symptoms (apnea) these patients experience (Cortese et al., 2008). Also implicated in reward and reward-seeking behaviour are the basolateral amygdala-to-NAcc projections (Am-NAcc) via a section of the ventral-amygdalofugal pathway (vAMFP) and another through the stria terminalis (ST). These glutamatergic neurons and these regions are involved in the valance of both rewarding and aversive stimuli (Namburi et al., 2015; Beyeler et al., 2016) and have been associated with a bias towards larger and more risky choices (i.e., when reward is more uncertain but larger) as demonstrated in animal models (Ghods-Sharifi et al., 2009; Averbeck and Costa, 2017; Bercovici et al., 2018).

Located in the ventral diencephalon, neuronal projections of the hypothalamus spread extensively throughout the CNS. As the master regulator of the autonomous nervous system (ANS), the hypothalamus regulates parasympathetic and sympathetic function but also sleep/wakefuless, neuroendocrine secretion, growth and the stress response via the pituitary gland and the hypothalamic-pituitary-adrenal axis. ANS function relies on neuronal fibres between the hypothalamus and the viscera via the dorsal longitudinal fasciculus (DLF), which indirectly also carries transmission to the vagus nerve, and the medial forebrain bundle (MFB). The vagal gut-brain pathway is implicated in both food addiction (Gupta et al., 2020) and neurodevelopment including in the paradigm of antenatal susceptibility to psychopathology (Cryan and Dinan, 2012). As an integral part of the limbic system, the hypothalamus links directly to the amygdala (Stria Terminalis and ventral amygdofugal pathway) and the hippocampus (fornix) and therefore integrates cognitive and emotion regulatory processing. Additionally, the lateral hypothalamus is traversed by the MFB which relays the ventral tegmental area to the NAcc, a primary circuit of the reward system noted above.

7.3 Neuroimaging following antenatal exposure to maternal obesity

Neuroimaging in the neonate offers opportunities to study the influence of genetic and environmental factors on brain development (Gao et al., 2019), including the comparison across maternal adiposity/BMI status in pregnancy (Dufford et al., 2021). Studying the neonate has the advantage of avoiding the potential early childhood exposure effects of treatment, illness, lifestyle influences including an obesogenic environment (e.g. parental feeding, low child exercise, picky eating) (Matheson et al., 2015), parenting strategies and parent mental health which could be implicated in the causal pathway of the childhood metabolic outcomes, neurodevelopmental diagnosis and their comorbidity (Dufford et al., 2021).

In neonates, Li et al. (2016b) used functional MRI and showed lower dorsal anterior cingular cortex to frontal connectivity with increasing maternal fat mass percentage. Ou et al. (2015) found global differences in neonatal fractional anisotropy (FA) between the normal-weight and obesity exposed groups, which included limbic pathways. In the same study, changes in gene methylation status in umbilical cord tissue

were associated with maternal adiposity, including differential methylation of 57 genes involved in nervous system development (e.g. white matter [WM] and neurogenesis). Furthermore, maternal cytokine IL-6, a pro-inflammatory marker of meta/inflammation associated with higher adiposity, has been negatively associated with FA in the neonatal fronto-limbic uncinate fasciculus (Rasmussen et al., 2019) and functional nodes of working-memory, language and negative emotionality (Rudolph et al., 2018). Maternal IL-6 has also been associated with larger neonatal amygdala volume and amygdala connectivity which predicted lower impulse control at 2 years (Graham et al., 2018).

7.4 Neuroimaging of the neonatal reward and limbic system

In order to evaluate the likely predisposition to the outcomes outlined above, and to further clarify neurological predisposition following exposures to maternal obesity *in utero*, we investigate the reward and limbic pathways in the human neonatal brain. Diffusion MRI allows for the *in vivo* study of WM to uncover both potential unexplored topographical development and define microstructural characteristics and morphology. However, several gaps in the *in vivo* segmentation of WM bundles of interest in the neonatal population have been identified. To date the most extensive neonatal WM atlas is the 54 WM tract delineation from the Melbourne Children's Regional Infant Brain atlas: M-CRIB-WM study (Alexander et al., 2020). This atlas is based on a manually segmentation of WM regions was inspired by the 52 WM regions in the Johns Hopkins University neonatal atlas ('JHU-neonate-SS') [Oishi et al. (2011);https://cmrm.med.jhmi.edu/]. The M-CRIB-WM used the T2-weighted structural images resampled at a 0.63 mm isotropic resolution and the DTI-derived direction-encoded colour (DEC) maps for direction orientation of the fibres. Their atlas contains the stria terminalis, the fornix (albeit without the precommissural columns) and UF but these were incomplete due to low resolution. The atlas does not include the MFB, DLF, ventral amygdalofugal pathway nor Amygdalo-accumbens fasciculus.

With the aim of assessing feasibility and to make contribution to *in vivo* neonatal atlases, I used tractography, rather than WM tract delineation by manual segmentation in the following study. I was also intent on characterising the topography of diencephalic tracts, many of which are associated with i.e. traverse or emanate, from the hypothalamus. To date the hypothalamus, NAcc and VTA are structures largely understudied in the neonatal human population and I offered their manual segmentation previously (Chapter 7).

The anatomical configuration of several of these pathways is complex due to their adjacent topographical localisation through the hypothalamus, into one or more hypothalamic nuclei and the issue of crossing fibre bundles. Conventional tensor-based acquisition and metrics used to assess axonal organisation and macrostructure are limited due to the absence of sensitivity in differentiating the diffusion signals from separate bundles crossing or kissing within the same voxel. A novel method able to first dissociate diffusion signal from separate bundles in one voxel is therefore necessary. Capitalising on constrained spherical deconvolution [CSD] to derive fibre orientation distributions (FODs) (Tournier et al. (2007);Jeurissen et al. (2014)), a technique to characterise micro and morphometric properties of WM tracts known as fixel-based analysis (FBA) is here preferred to evaluate fibre density (FD), fiber cross-section (FC) and combined fiber density/cross section (FDC) (Raffelt et al., 2017a).

Therefore the novel *in vivo* delineation of several white-matter pathways in the neonatal brain (medial forebrain bundle, ventral amygdalofugal pathway, dorsal longitudinal fasciculus) and others associated with psychological and metabolic outcomes could provide important anatomical references. Further, such work pro-

vides opportunities to study the development of these WM tracts in the child at risk, e.g., following compromising antenatal exposures such as maternal obesity, familial risk of autism and congenital heart disease. The cingulum and the UF are likewise important limbic pathways to the frontal brain associated in psychopathology and reward and are included in this investigation. Although already studied in the neonate, the UF has been implicated in many psychopathologies including autism, conduct disorders (of impulsivity and cognitive control) (Olson et al., 2015), and neonatal structural changes have been identified following maternal stress exposure *in utero* (Lautarescu et al., 2020). UF fractional anisotropy has been correlated with functional connectivity of the NAcc and reward sensitivity (Camara et al., 2010; Bjornebekk et al., 2012). Hence the UF and cingulum were also included.

7.5 Aims

The primary aim of this study was to assess the feasibility to delineate in the neonatal brain WM tracts of the reward and limbic pathways, using state-of-the-art neonatal DWI acquisition and processing methods. In this study I characterise white matter pathways which transmit between the regions of interest mentioned above. Furthermore, tractography in the subcortical areas demands refinement and anatomical knowledge often derived from histological studies.

Second, I correlated FD, FC and FDC in neonates born of uncomplicated normal-weight women and scanned between 37 to 44 week postmenstrual age to study the effects of sex, PMA, postnatal age, brain volume and birthweight centile on development in this time window.

Finally, in an exploratory study, I compared the FBA-derived metrics between these “control” neonates to those born to obese mothers who also had uncomplicated pregnancies. Since this was the first such investigation, performed as a pilot, I could not speculate on the direction of the effect but we hypothesized that infants born of obese women would differ in these pathways.

7.5.1 Tracts of interest

Uncinate Fasciculus (UF)
This association tract takes the shape of a hook from the anterior temporal pole, through the temporal stem, entering the extreme and external capsule and terminates in the orbitofrontal lobe.

The Dorsal longitudinal fasciculus (DLF)

The DLF originates from the medial and periventricular hypothalamic zones, passes through the periaqueductal grey matter (PAG) and caudal medulla oblongata.

Medial Forebrain Bundle (MFB)

The Medial Forebrain Bundle associates the VTA to the NAcc through the lateral hypothalamus, the inferomedial branch of the MFB. Identified in the human is the superolateral branch from the VTA which accesses the NAcc through the inferior portion of the anterior limb of the internal capsule and superior of the AC, briefly mingling with the anterior thalamic radiation (Coenen et al., 2012, 2018; Bracht et al., 2015; Fenoy et al., 2016). Some descending MFB fibres mix with the DLF in the brain stem but here only the segment VTA-NAcc is generated.

Ventral Amygdalofugal Pathway (vAMFP)

Fibres in the basolateral nuclear group and central nuclei of the amygdala run medially through the substance innominata and substantia perforata anterior, fanning out into the thalamic peduncle and joining the diagonal band of Broca. In the hypothalamus it reaches the lateral preoptic area (Willis and Haines, 2018) and more anteriorly the septal nuclei (Li et al., 2020a) and the NAcc (Zhao et al., 2018; Folloni et al., 2019).

Amygdala-NAcc pathway (Am-NAcc)

The Am-NAcc is also known as the amygdaloaccumbens fasciculus (Rigoard et al., 2011). It courses through the temporal section of the vAMFP from the anterolateral amygdala, it separates from the fibres reaching the hypothalamus as it travels to the NAcc anteriorly, preferentially to its shell, the ventromedial portion of the NAcc. This path is otherwise immediately adjacent to the UF.

Stria Terminalis (ST)

The ST originates from the corticomedial amygdala (Mori et al., 2017) and terminates in the bed nucleus of ST (BNST), the hypothalamus (anterior and medial preoptic area) and septal nuclei. These fibres loop around the medial caudate nucleus, associating with it within the grove with the thalamus (i.e. caudothalamic groove) and laterally parallel with the fornix. The BNST lies dorsal to the AC, lateral to the columns of the fornix and medial to the thalamostriate vein (Theiss et al., 2017). Corticomedial fibers of the amygdala pass through this pathway to reach the NAcc (Felten et al., 2016; Willis and Haines, 2018)

Cingulum

The cingulum is an association tract which is contained within the cingulate gyrus dorsally and the parahippocampal gyrus as it curves around the corpus callosum into the temporal pole. In this study the delineation was focused on the long curving fibres and not the short radiation fibres joining the cortex. It runs ventral to the subiculum of the hippocampus and does not contact the fornix, which must be differentiated accurately in the *in vivo* tractography.

7.6 Methods

7.6.1 Participants

Parents of neonates provided their written informed consent for the child to participate in the the developing Human Connectome Project (dHCP, ethics approval: 14/LO/1169). Demographic data was collected at enrollment and pregnancy and infant outcomes were obtained from the medical records, discharge notes and questionnaires. A birthweight centile was calculated using INTERGROWTH-21st, v.1.3.5, (Villar et al., 2014).

All scan IDs available at the start of the study were considered and then excluded based on *a priori* criteria of pregnancy characteristics and obstetric outcomes first followed by neonatal outcomes, then by MRI data quality (dMRI and anatomical) and radiological assessment. As the ultimate aim was to compare neonatal imaging parameters by maternal BMI group (normal/obese) as the main independent variable, it was important to exclude other antenatal exposures and birth outcomes which were potential confounders. All neonates included were therefore considered healthy and from uncomplicated pregnancies.

From 962 participant in the dHCP database at the time of enquiry, we excluded non-singleton pregnancies

(110), participants with missing obstetric outcomes (208) and missing BMI (39) and the following antenatal diagnoses : Obstetric cholestasis (6) ,preeclampsia (21), **H**amolysis, **E**levated **L**iver enzymes and **L**ow Platelets (HELLP) (1), pregnancy induced hypertension (25) and gestational diabetes mellitus (7). From the 545 remaining, 142 had no dMRI data available. 111 of 403 born were born outside the range 37-42 weeks gestational age. 174 and 29 pregnancies of normal-weight and obese women, respectively, remained. We excluded infants with apgar score below 7 at 5min (or missing) and who were admitted to the NICU. We reviewed the dMRI quality blinded to groups and the presence of anatomical imaging (T1w or T2w) which excluded 11 more for poor quality.

The radiological reports were reviewed blinded to maternal BMI and participants were excluded if any of the following was noted: germinal matrix hemorrhage (n=1), intraventricular hemorrhage (n=1) and bilateral temporal horn cyst (n=1). Many infants had WM punctate lesions (WMPL), so each scan was reviewed individually and the number of lesions and their location was noted. We chose to exclude scans with more than 4 lesions on the assumption of a potential wide-spread effect on our analysis and inference. In the remaining infants with WMPL we made sure none were within our regions of interest (ROIs) or within tracts of interests. We also excluded infants with significant congenital abnormalities (e.g. cardiac defect, n=3). The final sample count was 137 neonates born of normal-weight mothers (<25kg/m^2) and 28 of obese (BMI >=30kg/m^2) mothers.

Table 7.1: *Maternal and neonatal characteristics*

	Normal-weight (Controls)	Obese	p
n	137	28	
Mother			
Mother's age (mean (SD))	33.19 (5.03)	32.79 (4.04)	0.690
Mother's ethnicity (%)			<0.001
White	91 (67.9)	10 (35.7)	
Black	10 (7.5)	12 (42.9)	
Asian	16 (11.9)	1 (3.6)	
Chinese	7 (5.2)	0 (0.0)	
Mixed	3 (2.2)	2 (7.1)	
Other	7 (5.2)	3 (10.7)	
Mother's first language is English (%)	74 (55.2)	12 (42.9)	0.325
Mother's age when last in FT education (median [IQR])	23.00 [21.00, 25.00]	23.00 [21.50, 24.00]	0.930
Mother's BMI (median [IQR])	21.79 [20.20, 23.37]	32.20 [30.86, 35.18]	<0.001
Primiparous (%)	83 (60.6)	8 (28.6)	0.004
Delivery			
Meconium stained liquor (%)	32 (23.7)	7 (25.0)	1.000
Delivery Mode (%)			0.574
Elective caesarian section	14 (10.2)	6 (21.4)	
Emergency caesarian section - in labour	29 (21.2)	5 (17.9)	
Emergency caesarian section - not in labour	6 (4.4)	2 (7.1)	
Instrumental delivery - Forceps	26 (19.0)	4 (14.3)	
Instrumental delivery - Ventous	11 (8.0)	1 (3.6)	
Spontaneous vaginal delivery	51 (37.2)	10 (35.7)	
Neonate			
Baby sex Female (%)	60 (43.8)	10 (35.7)	0.563
GA at birth weeks (mean (SD))	40.25 (1.05)	39.87 (1.15)	0.085
Apgar 5 min (median [IQR])	10.00 [9.00, 10.00]	10.00 [9.00, 10.00]	0.550
Baby head circumference (mean (SD))	34.55 (1.54)	34.19 (1.67)	0.266
Baby birthweight (kg) (mean (SD))	3.40 (0.47)	3.46 (0.52)	0.522
Birthweight centile (mean (SD))	52.21 (30.07)	58.81 (29.43)	0.290
PMA at scan (mean (SD))	40.95 (1.31)	40.22 (1.09)	0.006

Note:
Mother's BMI (Body mass index) was booking BMI, GA: gestational age, PMA: Postmenstrual age (e.g. GA at birth + chronological postnatal age). Missing data: Maternal ethnicity=3, Maternal first language=3, Maternal education=16. Neonatal meconium=2. Birthweight centile was calculated from INTERGROWTH-21st standards.

7.6.2 Scanning of the newborn

All imaging data was acquired on a 3T Philips Achieva scanner within the Evelina Newborn Imaging Centre at St Thomas' Hospital, London, and included structural (T1 and T2-weighted), diffusion and functional imaging. The set up includes a bespoke neonatal imaging system (Hughes et al., 2017) aimed to maximise signal-to-noise ratio by minimizing movement and incorporating a close fitting 32-channel receiver coil. Infants were scanned during natural sleep usually after a feed. To minimise the noise from the scanner, moulded silicone earplugs ((President putty, Coltene Whaledent, Mahwah, NJ, USA)) are put in the infant ears in addition to neonatal earmuffs (MiniMuffs, Natus Medical Inc., San Carlos, CA, USA) and an acoustic foam hood which

is placed over the baby. A pediatrician was present throughout the scan and monitoring of the infant included electrocardiography, temperature, pulse oximetry and breathing rate. If infants woke up during the session the scan could be stopped and restarted once settled. The dHCP success rate for scan completion was 91%.

7.6.3 dMRI acquisition

Diffusion data of 300 volumes as acquired during a 19min scan through Multi-shell High Angular Resolution Diffusion Imaging (HARDI) single-shot spin-echo echo-planar sequence (Hutter et al., 2018; Tournier et al., 2020) with the following parameters:

- spin echo echo-planar sequencing
- multiband factor of 4, slice interleave factor 3
- TE/TR:90/3800msec
- Field of view 150 × 150 × 102 mm^3
- 1.5x1.5x3mm voxel resolution with 50% slice overlap.
- SENSE 1.2
- Half-Fourier 0.85
- 300 directions at b-value shells 0mm^2 (x20), 400mm^2(x64), 1000mm^2(x88) and 2600smm^2(x128) mm^2

Diffusion images were denoised and reconstructed to a 1.5mm^3 isotropic resolution (Bastiani et al., 2019; Cordero-Grande et al., 2019), Gibbs ringing suppressed (Kellner et al., 2016) and motion and distortion corrected (Christiaens et al., 2021). In *MRtrix3* (Tournier et al., 2019), the response functions for WM and CSF were estimated (Dhollander et al., 2019). For each subject, a Fibre Orientation Distributions (FODs) map was obtained with multi-shell multi-tissue constrained spherical deconvolution (Jeurissen et al., 2014). The FODs were intensity normalised (Raffelt et al., 2017b) and a brain mask was also produced from this process.

7.6.4 Fixel-based Analysis (FBA)

FBA was implemented on the sample using the documented pipeline (Dhollander et al., 2021) and with minimal adaptation to the neonatal data. It consists of a template FOD generation, anatomical registration of ROI to the FOD template, fixel masks generation and thresholding and the extraction of mean FD,logFC and FDC. The entire pipeline was performed in MRtrix3 (version 3.0). Given that the FBA pipeline produces FBA metric maps (FD/FC/FDC) within which all fixels have correspondence across subject (using *fixelcorrespondence*), the track-derived fixel mask also has correspondence at the subject level and therefore all FBA metrics can be extracted consistently within the subject maps for the given fixel-mask.

7.6.4.1 Template generation and fixel masks

In order to generate tracts of interest, a FOD template was produced from 20 randomly selected obesity-exposed and 20 controls (*population_template*). Next, all the other subjects FOD maps in native space were registered (*mrregister*) to the template FOD space which produced subjects-to-template and template-to-subject warps. The registration output was not inspected for each individual. The subject FOD masks in native space were then warped to FOD template space using these warps and then their intersection

(*mrmath -min*) was used to create the FOD template mask.

Subsequently a fixel mask was generated (*fod2fixel*) using the FOD template and the FOD mask with a threshold of 0.06 on the peak amplidtude of positive FOD lobes, from which quantitative analyses of fibre density and fibre crossing could be performed. Next, all subject FOD maps were warped to FOD template without reorientation applied (*mrtransform*). Thereafter, a whole brain fixel mask was generated for each subject FOD in FOD template space (*fod2fixel*), whereby the number and orientation of fixels per voxel was obtained but also the corresponding per-fixel apparent fibre density (AFD, now *fd*). Next, a reorientation of fixels of all subjects in template space relying on the warps was performed (*fixelreorient*). After, with all subject FODs spatially aligned to the FOD template, the *fixelcorrespondence* command allowed for matching every fixel across subjects. Lastly the fibre cross-section (fc) metric was computed to estimate an index of morphological differences between subjects using the warps employed in the earlier registration(*warp2metric*). This fc was log-transformed as per recommendations. Finally, the density and cross-section metric (fdc) was computed as the product of fd and fc.

Following tractography (see below) fixel masks were generated from each tract generated in the population template (*tck2fixel*). After visual inspection of the masks, streamline-per-voxel thresholds were applied to maintain only the core of each bundle (see Figure 7.4) and the mean FD,logFC and FDC were extracted for each subject for each tract.

7.6.5 Anatomical template

The subject T2 anatomical images from the dHCP dataset are reconstructed to a 0.5mm isotropic resolution. In order to produce a T2w anatomical template aligned in the FOD template space we obtained the T2w images in native space (co-registered with diffusion data) and these T2 anatomical images were warped to FOD template space using the warps generated in the subject FOD-to-template FOD registration and using the FOD template mask previously upsampled to 0.5mm resolution as the registration template. No issues with the output of these transformation was noted.

7.6.6 Region of inclusion and exclusion

In order to reconstruct the tracts of interest, mask regions of seeding, exclusion and inclusion were both manually drawn and derived from atlas-based masks. First, a T2 group template in the population FOD template space was produced. The FOD template mask was upsampled to 0.5mm resolution. Then the subject T2-weighted images (already in the same space as the diffusion images i.e. b0 at 0.5mm isotropic resolution) were warped into the FOD mask space using the transformation warps produced in the previous steps of the FOD population template generation. Then the mean of all subject T2 images was taken. After that, the available dHCP-specific atlas-based regional masks (Makropoulos et al., 2014) (i.e. bilateral temporal poles WM , mid-orbital frontal lobes WM, thalami, amygdala and hippocampus) were registered to the T2 template in the same way, taking the median of the subject ROI masks from 10 randomly selected participants per group used to produce the FOD population template. Other inclusion and exclusion regions were manually drawn on the FOD template using the T2 template to localise.

The subject ROIs derived from the atlas was in subject space at 0.5mm isotropic resolution and the FOD template at 1.5mm so some visual checks and manual adjustment were necessary. This primarily implicated a conservative approach to avoid partial-voluming of the hypothalamus with adjacent third ventricle.

Anatomical structures manually drawn were the hypothalamus (with mamillary bodies), NAcc and VTA, see Figure 7.3. Those derived from the anatomical automatic labelling (AAL) atlas adapted to the neonatal dHCP data (Schuh et al., 2018) were the amygdala, hippocampus, the anterior temporal lobe (medial and lateral) and the mid and orbitofrontal WM. There were visually inspected and did not require manual editing.

Additionally a FOD-based directionally encoded color (DEC) map (Dhollander et al., 2015) was produced (*fod2dec*) from the template FOD. Compared to the tensor derived DEC, it is more sensitive to the various intravoxel orientations especially at crossing and kissing fiber junctions, and provided additional visual information to place inclusion and exclusion regions for tractography.

The FOD-derived DEC map (Dhollander et al., 2015) across the hypothalamic regions (anterior, tuberal and mammilary) are provided in three-planar view in Figure 7.1. Figure 7.2 exemplifies the processed ODF estimation of the diffusion-derived signal by multi-tissue CSD from which tractography was initiated.

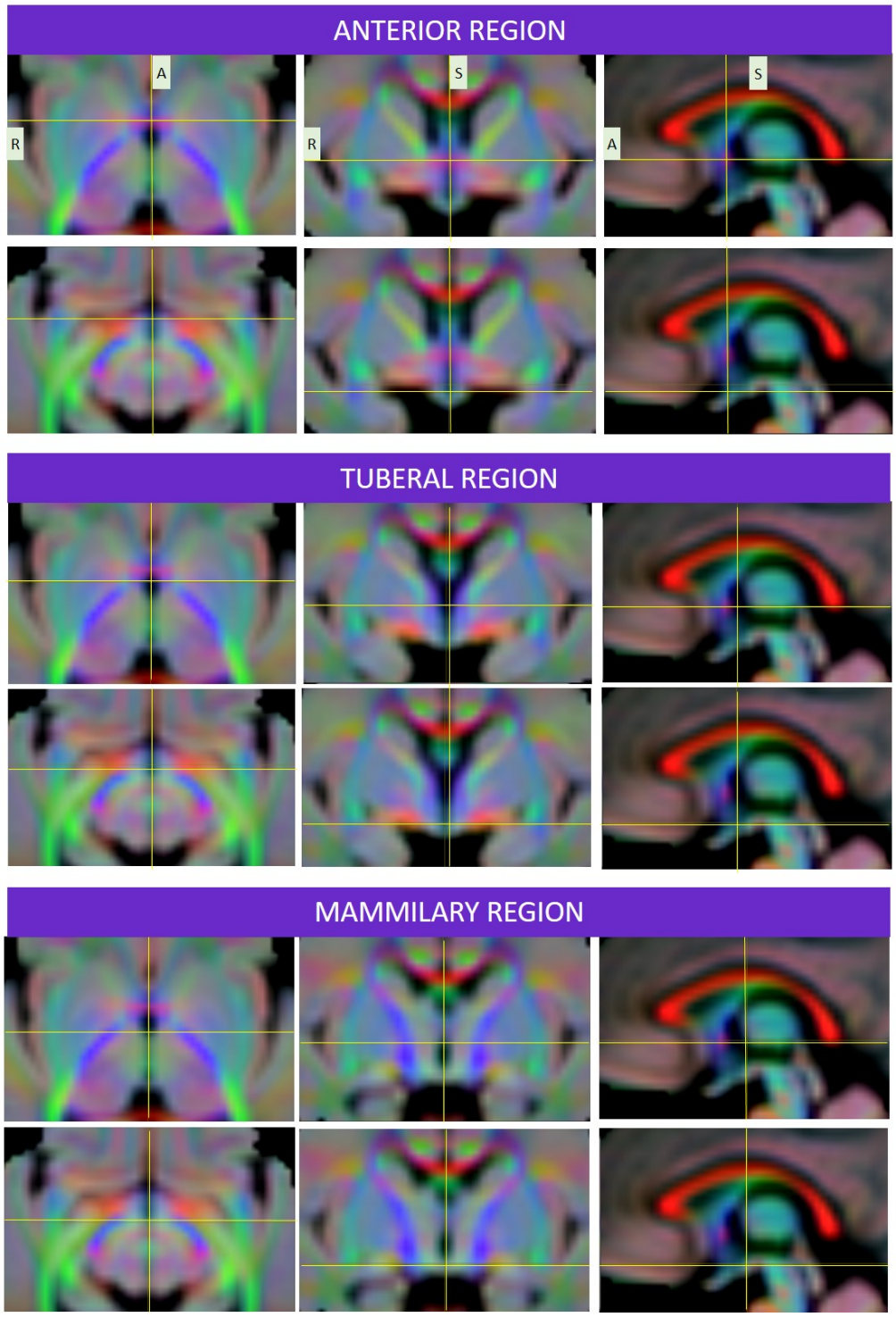

Figure 7.1: *The FOD-derived directionally encoded color (DEC) map, weighted by the integral of the FOD was generated. This offers further visual information to place exclusion and inclusion gates for the tractography of the bundles of interest. The maps are presented in axial (first column), coronal (middle) and sagittal (right) views across the Anterior (Anterior commissure), Tuberal and Mammilary regions of the hypothalamus, with the top rows showing a superior aspect and the bottom row in each region the inferior aspect, as can be seen with the yellow cross-hairs. Red: left-right, blue:superior-inferior and green: anterior-posterior orientations.*

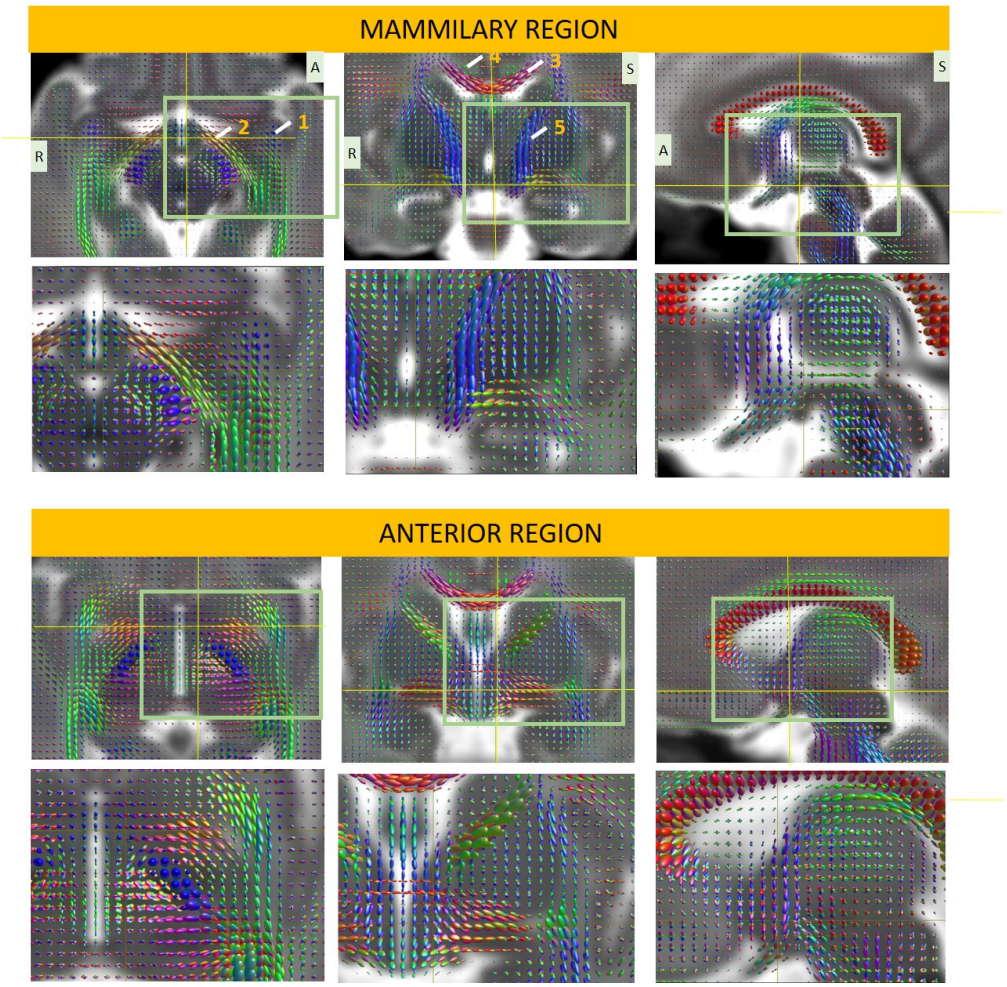

Figure 7.2: *The population FOD template is overlaid on the T2 template at the anterior (anterior commissural) region and mammilary region of the hypothalamus. Bottom rows are zoomed in boxes from the top rows. The maps are presented in axial (first column), coronal (middle) and sagittal (right) views. Yellow lines are cross-hairs provided for orientation. FOD lobes are sized by peak amplitude and directions are color coded, red: left-right, blue:superior-inferior and green: anterior-posterior orientations. Note how some groups of s underly WM bundles easily distinguishable in the neonatal brain i.e. 1: ascending temporal stem of the uncinate fasciculus, 2:optic tract, 3: corpus callosum, 4:cingulate, 5: corticospinal tract. Note the T2 template is in 0.5mm isotropic resolution and the FOD template map at 1.5mm.*

7.6.7 Tractography protocols

All tracks were generated in template FOD space only, using probabilistic tractography (iFOD2 algorithm) in MRtrix3. Each tractography protocol included a seeding region, regions of inclusion (ROI) and exclusion (ROE), seed number, max/min streamline length, cutoff threshold and whether unidirectional (rather than default bidirectional) streamline generation was necessary.

Since a few tracts had not been delineated in the neonate previously and others have used WM atlases based on manual delineation rather than tractography, certain tracts of interest (DLF, MFB) were first generated in a sample processed adult brain from the Human Connectome Project. This helped determine the location

of regions of inclusion which have not yet been delineated in the neonate and also because of the resolution available in our cohort. We relied on structural anatomical atlases of the fetus and neonate (Bayer and Altman, 2003) when possible. Each tract was evaluated in term of validity to known anatomy.

Note that, although coursing through the vAMFP, the amygdala to NAcc tract was differentiated and referred to as Am-NAcc (also known as Amygdalo-accumbens fasciculus), whereas the amygdala to hypothalamus tract is referred to as the vAMFP.

The same protocol was applied for tracts of the same type on both sides of the brain. However, protocols were adapted for each tract type (e.g. streamline length, cut-off threshold) to minimise the number of spurious and false positive tracts, to account for the FOD derived signal being lower in regions of high WM/GM mix within voxels in the subcortical areas studied here. Unless otherwise mentioned, all default parameters in MRtrix were kept. Left and right tracts were generated separately.

Furthermore, anatomical correspondence of the “core” body of a streamline bundle within each track was prioritised over the terminations of some fibre bundles which are known to “fan” out as they approach cortical surface (i.e. uncinate fasciculus) as we deemed this could influence the statistical metrics using mean FBA-derived measures. Further steps to minimise this was to utilise conservative fixel thresholding (count of streamline per fixel) within the fixel masks for each tract.

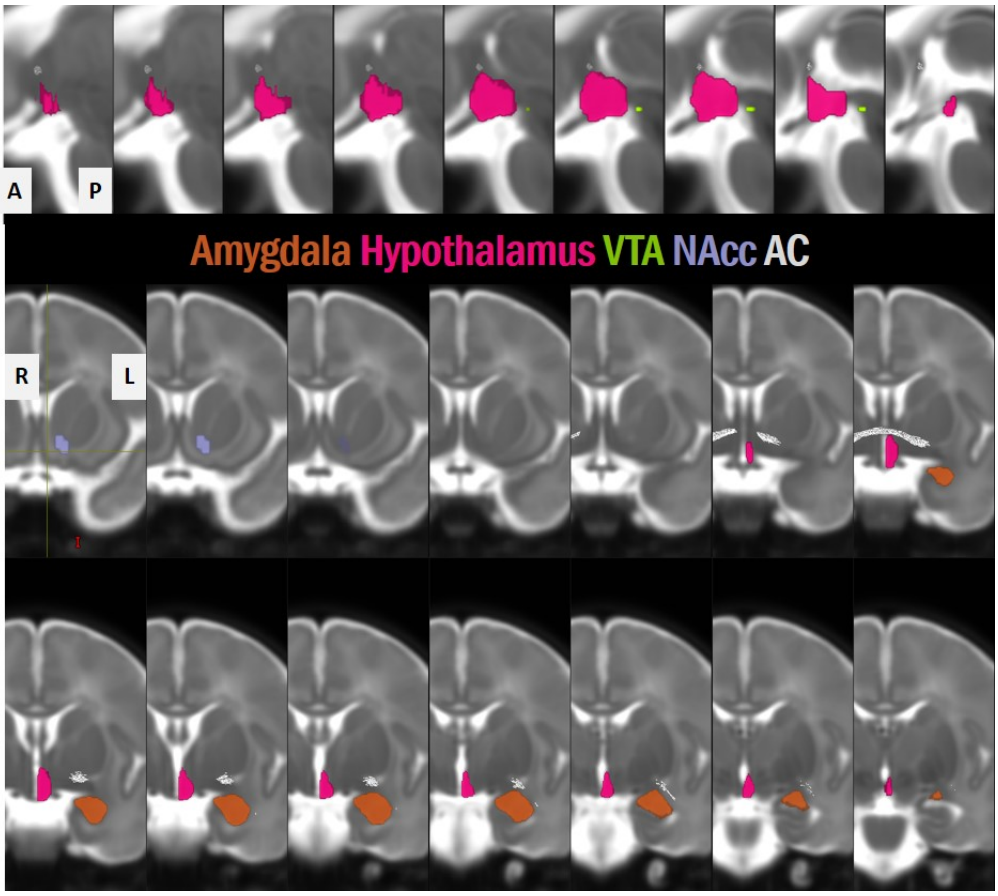

Figure 7.3: *Manually drawn regions for tractography: hypothalamus (pink), ventral tegmental area (green, VTA) and nucleus accumbens (lavender, NAcc) and the atlas-baed segmentation of the amygdala (orange). Top row are sagital planes through the hypothalamus in the lateral to medial direction. Bottom row are coronal slices arranged in the anterior to posterior directions. The anterior commissure (grey AC) was generated by tractography and here for visual reference.*

7.6.8 Statistical Analysis

The FBA metrics were considered as dependent variables for each tract in each hemisphere. The statistical plan included first studying development in the normal-weight exposed control neonates in order to evaluate the normal rate of change across the 37-44 weeks PMA window and of the effects of predictors: sex, neonatal birthweight centile (BWC, calculated adjusted for sex and age at birth by INTERGROWTH), total brain tissue volume (TBV cm^3) and postnatal age in days (PN age).

Thereafter the exposure variable (normal-weight/obese) was introduced and models rerun. Group-wise comparisons were initially performed to evaluate any sex-wise differences on maternal or delivery outcomes which could inform the inclusion of additional covariates. All FBA metrics were normalised for easier comparisons between tracts and variables were checked for normality. PN age was skewed as most infants were scanned in the first week of life.

The regression models implied here were constructed as path models. TBV is correlated with (or predicted by) PMA and it is known that males have larger brains than females so this scaling effect needs to be ac-

counted for in the analysis between sexes. Since conventional method of regression are saturated (just identified) models, fitness indices are not reported and it is not clear what is the contribution of these covariates in brain development. Hence, here there was also an interest in explaining these relationships based on theory, provide the main effects of predictors (sex, PMA, BWC, PNdays and exposure) on FBA-metrics along with model fit indices. All models were run in *Mplus* v8.3, using the robust maximum likelihood estimator (MLR) which can handle non-normality of predictors such as PN age. Since we show the main effects of the covariates for each FBA metric we also applied an FDR correction on each tract separately at <0.05 in the controls analysis only. There was no control for Type I error in the models including the exposure variable as these analyses were exploratory in such a convinience sample of obesity exposed neonates.

7.7 Results

7.7.1 Tractography

Figure 7.4 shows the generated streamline bundles for the tracts of interest in 3D, as well as the fixel masks from which the FBA metrics were obtained. I demonstrate to my knowledge for the first time the delineation of the neonatal the stria terminalis, medial forebrain bundle, dorsal longitudinal fasciculus, amygdalo-accumbens fasciculus and ventral amygdalofugal pathway by tractography.

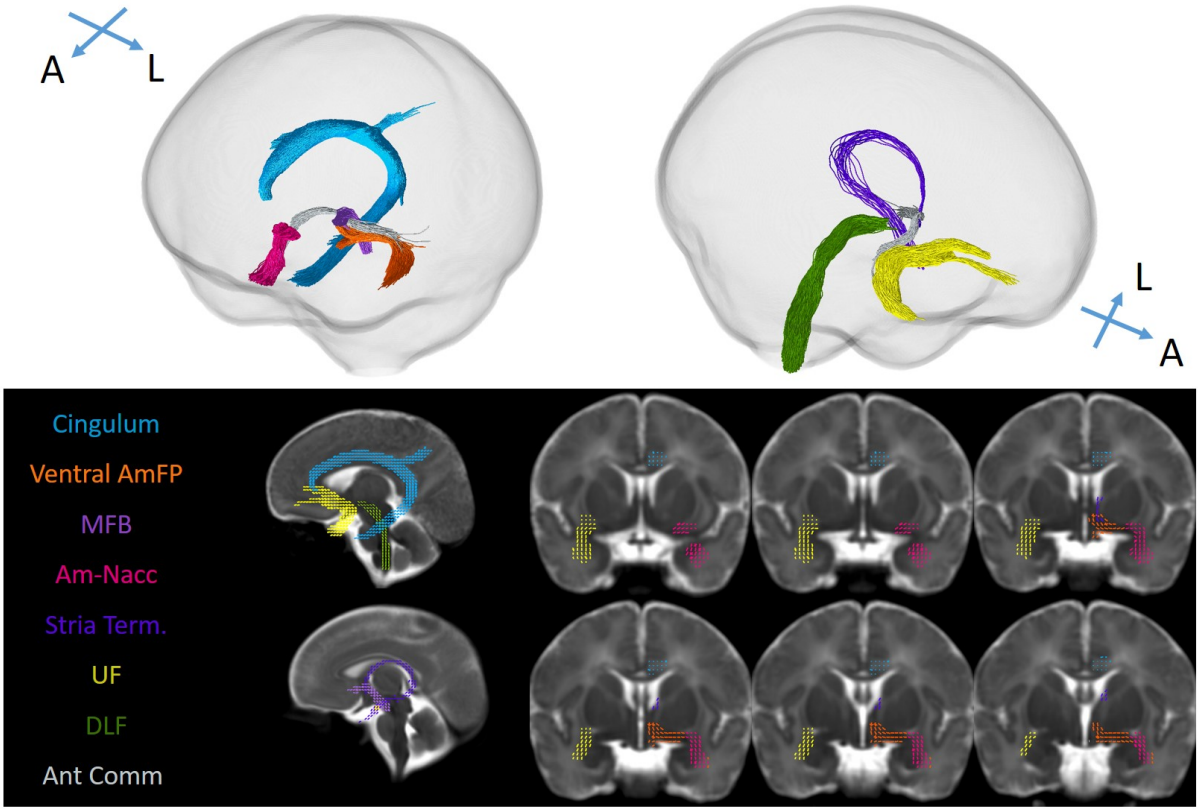

Figure 7.4: *Top: Probabilistic tractography was used to generate neonatal streamline bundles for the Amygdalo-accumbense tract (Am-NAcc), cingulum, dorsal longitudinal fasciculus (DLF), medial forebrain bundle (MFB), stria terminalis (Stria Term.), ventral amygdalofugal pathway (AMFP) and uncinate fasciculus (UF). The anterior commissure (Ant Comm) was also generated in order to offer a reference tract for visualisation. Bottom: Fixel masks were generated from the tractography from which the fixel based metrics are extracted. The coronal sections start top left at anterior to the anterior commissure and run posteriorly until the bottom right slice.*

7.8 Tract-wise topographical sensitivity in the neonatal brain

7.8.1 Projections in the amygdala and temporal pole

I demonstrate for the first time that the topography of the neonatal ST, vAMP and Am-NAcc. These generated streamline bundles follow the topography reported in the adult brain, presented in Figure 7.5 but this work also contributes further to the describe nuclear demarcation within the amygdala of the neonate.

An interesting observation is that the protocols included the whole amygdala as an inclusion area but the tract themselves are discreetly organised in the basolateral/central amygdala areas.

I found that the ST projections terminate preferentially inferior to those of the UF and vAMFP, as seen on the coronal slices. Overall we find the organisation of this bundles in the medial to lateral direction as ST, vAMFP, Am-NAcc and UF with little mixing between those families of projections. The UF brushes the amygdala lateral to the other fibre bundles as it enervates the cortical areas of the temporal pole. THe streamlines of the ST running caudal to the amygdala correspond to the centromedial and basolateral and lateral regions

mirrors the descriptions of this tract by Mori et al. (2017) in their DTI-based tractography in the postmortem tissue and anatomical resolution of 250um (refer to their figure 3)

On the axial and sagittal slices (Figure 7.5B and C) we find the topographical organisation anterior to posterior as such: UF, Am-NAcc, vAMFP and ST.

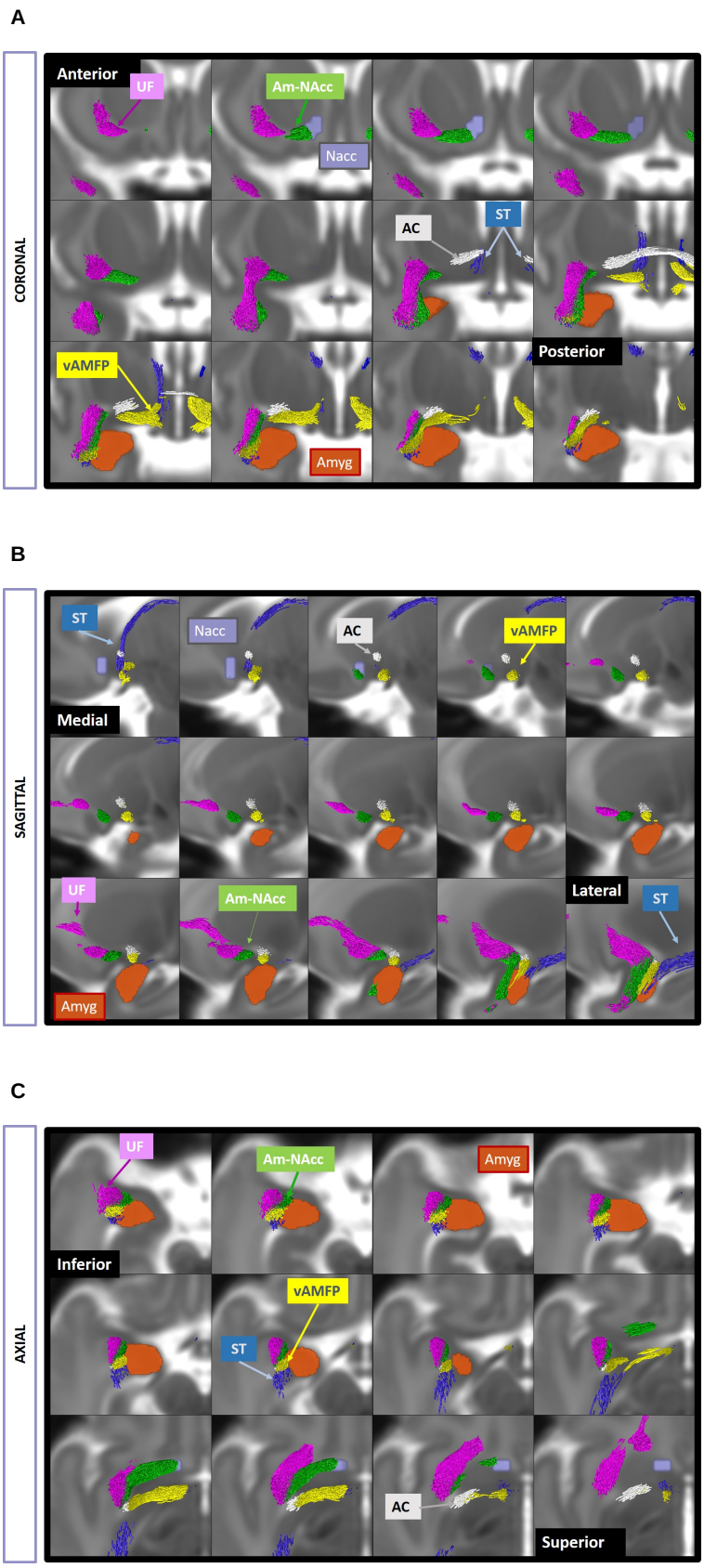

Figure 7.5: *Coronal, axial and sagittal sections through the temporal lobe and diencephalon demonstrate the topographical organisation of the Stria Terminalis (ST), Uncinate Fasciculus (UF), ventral amygdofugal pathway (vAMFP) and the Amygala-accumbens fasciculus (Am-NAcc).*

7.8.2 Projections through the hypothalamus

On their posterior approach, the DLF streamlines ascending toward the hypothalamus are contrived specifically to the border of the third ventricle, superior to the bundles of the MFB at the level of the subthalamic nuclei and mammilary bodies. The MFB becomes lateral to the DLF streamlines as the MFB bundle enters the lateral hypothalamus.

We point out that the whole hypothalamus was included in the tractography protocol but make the observation that the DLF streamlines terminate specifically in the medial and dorsal area of the hypothalamus, possibly the nucleus posterior hypothalami. We note in the left DLF that a few streamlines penetrate further ventrally (Figure 7.6A), which we can only speculate are the cross-nuclei projections from the posterior nucleus hypothalami into the ventromedialis nucleus.There are also some streamlines from the DFL which join into the MFB (Figure 7.6A, yellow box) but these could be the MFB fibres joining the DLF, which has been described before.

The neonatal vAMFP generated here shows an approach towards the hypothalamus which is posterior to the UF and Am-NAcc, inferior and parallel to the AC as it courses under the lentiform nuclei (internal globus pallidus). This is appreciated on all the plane in Figure 7.5. Then at its most medial termination point, the streamlines arrive at the anterior hypothalamic area. This follows the description of Kamali et al. (2016) in the adult but where as we find a split within the hypothalamus, of the fiber ascending towards the thalamic peduncle as described by Willis and Haines (2018). Unlike in the description by Kamali et al. (2016), we make the distinction between the tract towards the NAcc (Am-NAcc) which on the axial plane route towards through the substantia innominata.

7.8.3 Projections to the NAcc

The MFB joining the VTA to the NAcc showed differences bilaterally. The reported branches (superior and inferior of the AC) were only seen on the right bundles whereas the left MFB streams were confined to the superior aspect i.e. through the inferior limit of the anterior limb of the internal capsule (Figure 7.6). On the right tractogram we observe both the superior and inferior approach relative to the AC. In both MFBs we observe the preferred termination to what corresponds to the shell of the NAcc. This is in line with histology (Haber et al., 2000).

The Am-Nacc streamline bundle as mentioned leave the basolateral amygdala and show a course through what could appear the Ansa lenticularis-pathway which was described recently by Rusche et al. (2021) in their adult tractography. The termination points in the neonatal NAcc are preferential to ventral NAcc although Rusche et al. (2021) alluded to the central NAcc.

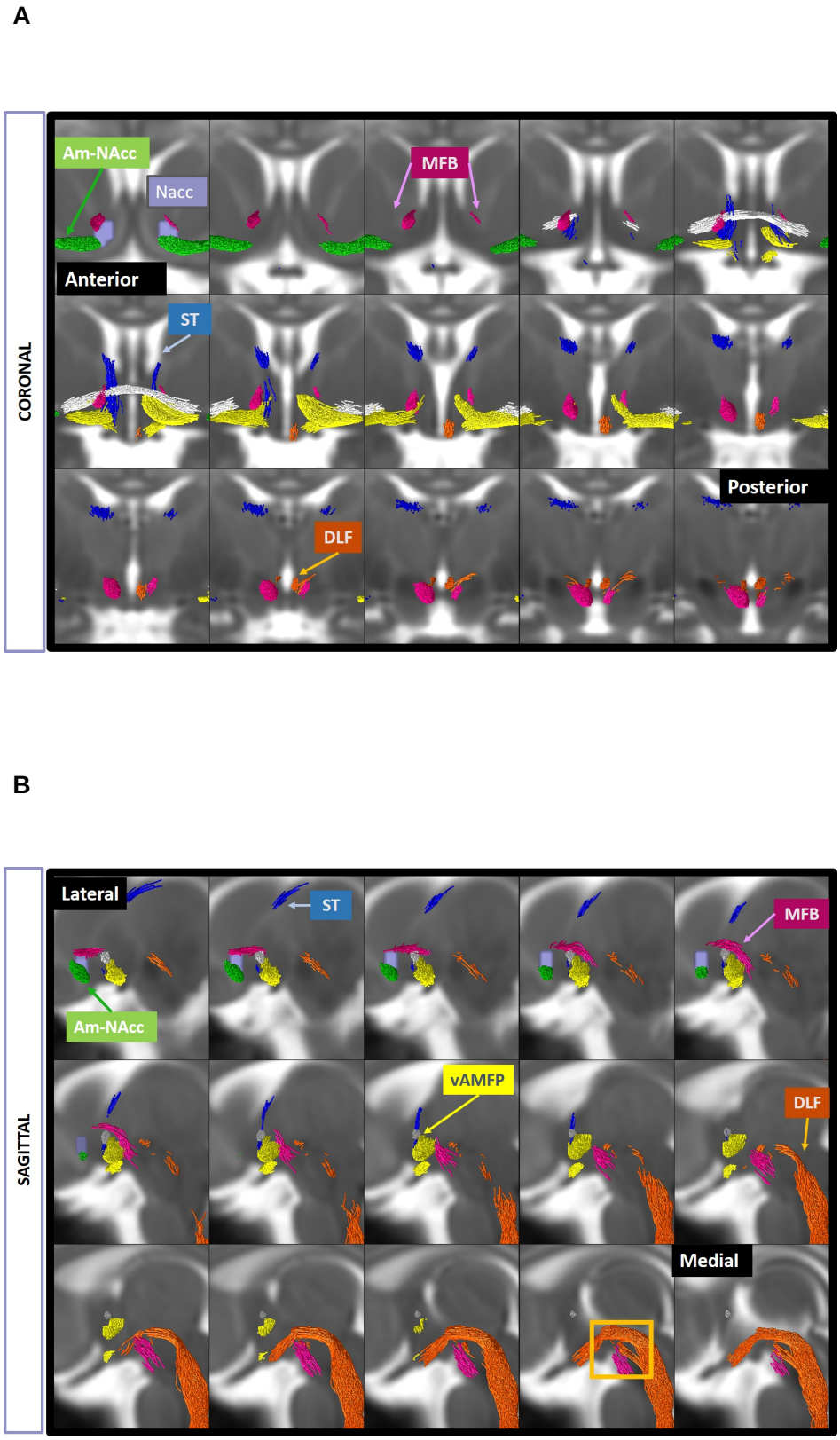

Figure 7.6: *Coronal and sagittal sections through the hypothalamus demonstrate the topographical organisation of the Stria Terminalis (ST), Dorsal longitudinal fasciculus (DLF), ventral amygdofugal pathway (vAMFP) and the Amygala-accumbens fasciculus (Am-NAcc) and Median forebrain bundle (MFB). In the sagital plane, the yellow box may be the joining of the MFB and DLF fibers.*

7.8.4 FBA metrics

7.8.4.1 Neonatal brain development in neonates born to mothers of normal-weight with an uncomplicated pregnancy

When comparing control male and female infants first, there were not differences on any maternal characteristics or delivery mode (Table 7.2 and the distributions of subjects across PMA, PN days, birthweight, total brain volume and head circumference is shown in Figure 7.7. The only difference was, as expected, a larger birth head circumference in boys (means(SD):34.81cm(1.43) vs girls 34.2cm(1.63), p=0.022). The girls were born a few days later than the boys (mean weeks(SD)= boys: 40.09 (1.01) vs girls: 40.46 (1.07),p=0.040). All control infants were scanned between 37.5 and 43.6 PMA. Figure 7.9 shows the correlation between FBA metrics and PMA.

Table 7.2: *Maternal and neonatal characteristics by sex in normal-weight pregnancies.*

	Male	Female	p
n	77	60	
Mother			
Mother's age (mean (SD))	33.78 (4.51)	32.43 (5.57)	0.120
Mother's ethnicity (%)			0.269
White	54 (72.0)	37 (62.7)	
Black	5 (6.7)	5 (8.5)	
Asian	6 (8.0)	10 (16.9)	
Chinese	6 (8.0)	1 (1.7)	
Mixed	1 (1.3)	2 (3.4)	
Other	3 (4.0)	4 (6.8)	
Mother's first language is English (%)	43 (56.6)	31 (53.4)	0.853
Mother's age when last in FT education (median [IQR])	22.00 [21.00, 24.00]	23.00 [21.00, 26.00]	0.802
Mother's BMI (median [IQR])	21.55 [20.23, 23.23]	21.88 [20.17, 23.77]	0.560
Primiparous (%)	47 (61.0)	36 (60.0)	1.000
Delivery			
Meconium stained liquor (%)	16 (21.3)	16 (26.7)	0.603
Delivery Mode (%)			0.988
Elective caesarian section	8 (10.4)	6 (10.0)	
Emergency caesarian section - in labour	18 (23.4)	11 (18.3)	
Emergency caesarian section - not in labour	3 (3.9)	3 (5.0)	
Instrumental delivery - Forceps	14 (18.2)	12 (20.0)	
Instrumental delivery - Ventous	6 (7.8)	5 (8.3)	
Spontaneous vaginal delivery	28 (36.4)	23 (38.3)	
Neonate			
GA at birth weeks (mean (SD))	40.09 (1.01)	40.46 (1.07)	0.040
Apgar 5 min (median [IQR])	10.00 [9.00, 10.00]	10.00 [9.00, 10.00]	0.283
Baby head circumference (mean (SD))	34.81 (1.43)	34.21 (1.63)	0.022
Baby birthweight (kg) (mean (SD))	3.43 (0.46)	3.35 (0.49)	0.374
Birthweight centile (mean (SD))	51.76 (30.38)	52.78 (29.92)	0.844
PMA at scan (mean (SD))	40.82 (1.33)	41.12 (1.29)	0.190

Note:

Mother's BMI (Body mass index) was booking BMI, GA: gestational age, PMA: Postmenstrual age (e.g. GA at birth + chronological postnatal age). Birthweight centile was calculated from INTERGROWTH-21st standards.

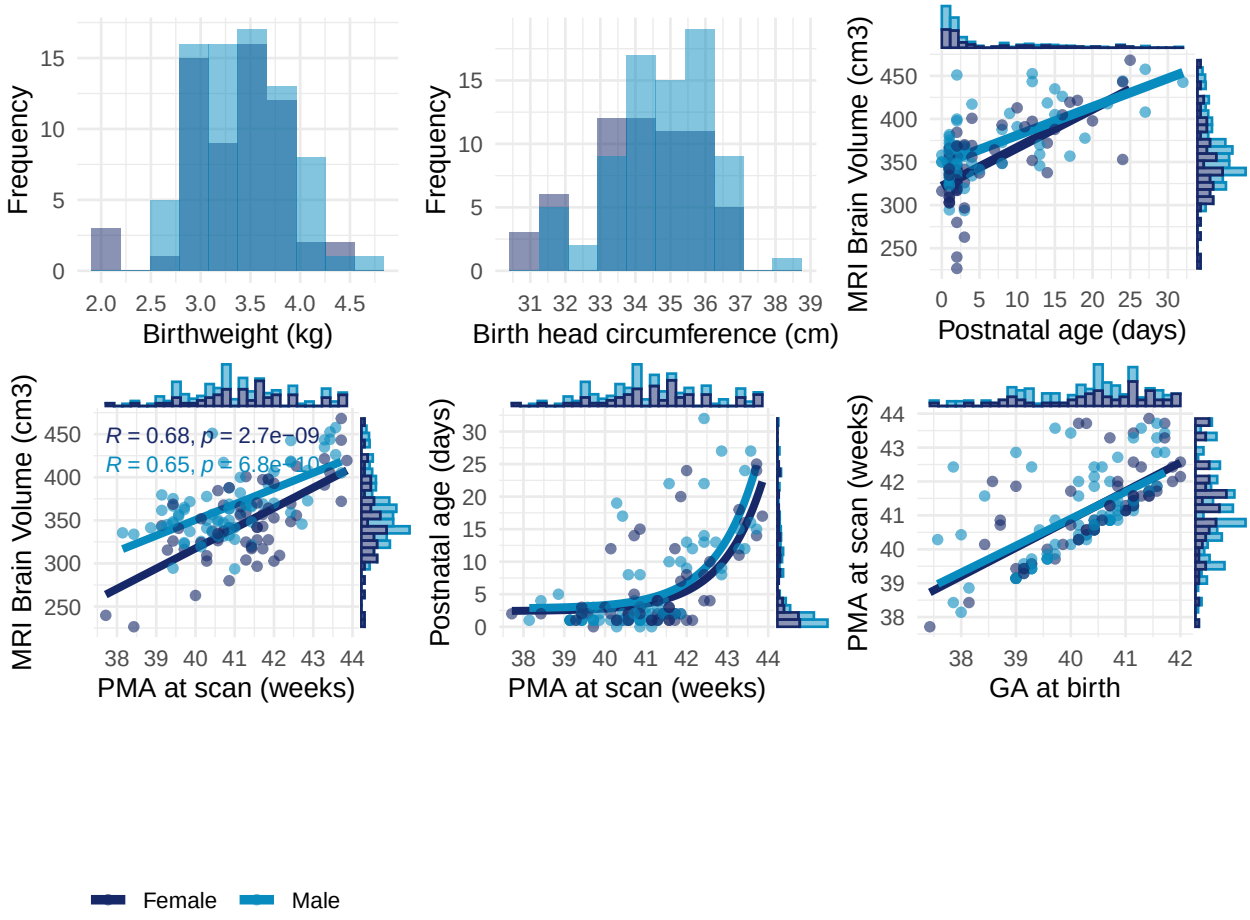

Figure 7.7: *Neonatal characteristics in males (n=77) and females (n=60) born to normal-weight uncomplicated pregnancies. PMA: Postmenstrual age. Note: Total brain volume was missing for 1 female and 4 males due to cropped image.*

I generated a path model for the prediction of the FBA metrics, illustrated in Figure 7.8. Based on a cutoff of <0.08 for SRMR and <0.05 for RMSEA, CFI and TLI >0.95 as good fit, all models showed good fit (CFI >= 9.89; TLI >= 0.972; RMSEA >= 0.058; SRMR >= 0.046).

All regression coefficients are standardised and provided with 95%CI. First, this model relays important relationships between the main effects of sex, PMA, TBV, birthweight centile and postnatal age on FBA metrics but also of the covariate effect on TBV and PMA as endogenous variables. TBV was predicted by sex (0.29[.20/.38]), as expected, PMA at scan [-0.13[-.26/-0.004] but also birthweight centile (0.37[.27/.46]) . Importantly, postnatal chronological age also associated positively with TBV event after accounting for the positive effect of PMA (0.43[0.30/.563]) suggesting longer time outside the womb is also influences infant brain growth. Birthweight centile was also positively associated with TBV in this model.

Figure 7.10 present the standardized coefficients of the main effects derived from the path models with the

tracts FBA metrics as dependent variables. Here, we show the development of WM tracts micro (FD) and macrostructure (FC,FDC) across 37 to 44 week PMA in children born of uncomplicated pregnancy.

In relation to log(FC), overall, TBV expectedly has the largest positive effect of all the covariates across all the tracts, although the largest effect seen in the cingulum. Infant scanned at later PMA had larger FC in the vAMP,DLF and Am-NAcc bilaterally but lower FC in the left UF. There was no effect of PMA on the FC of the bilateral cingulum, MFB and ST in this age period. Infants of higher BWC had larger FC bilateral ST, bilateral DLF and left MFB and right Cingulum. Postnatal age outside the womb had a negative effect on the left cingulum, and right AM-Nacc and right vAMFP. Males had larger FC in the bilateral UF and bilateral AM-NAcc but only the UF results survived FDR adjustement for multiple comparisons.

Regarding FD, it increases with increasing age in all tracts. TBV was negatively associated with the FD in the bilateral cingulum, bilateral MFB and right ST. BWC had no effect on FD and postnatal age in days was positively associated with the FD of the DLF. Males had lower FD in the left UF.

Only PMA at scan and total brain volume had effects on FDC although infant of larger BWC had large FDC in the DLF.

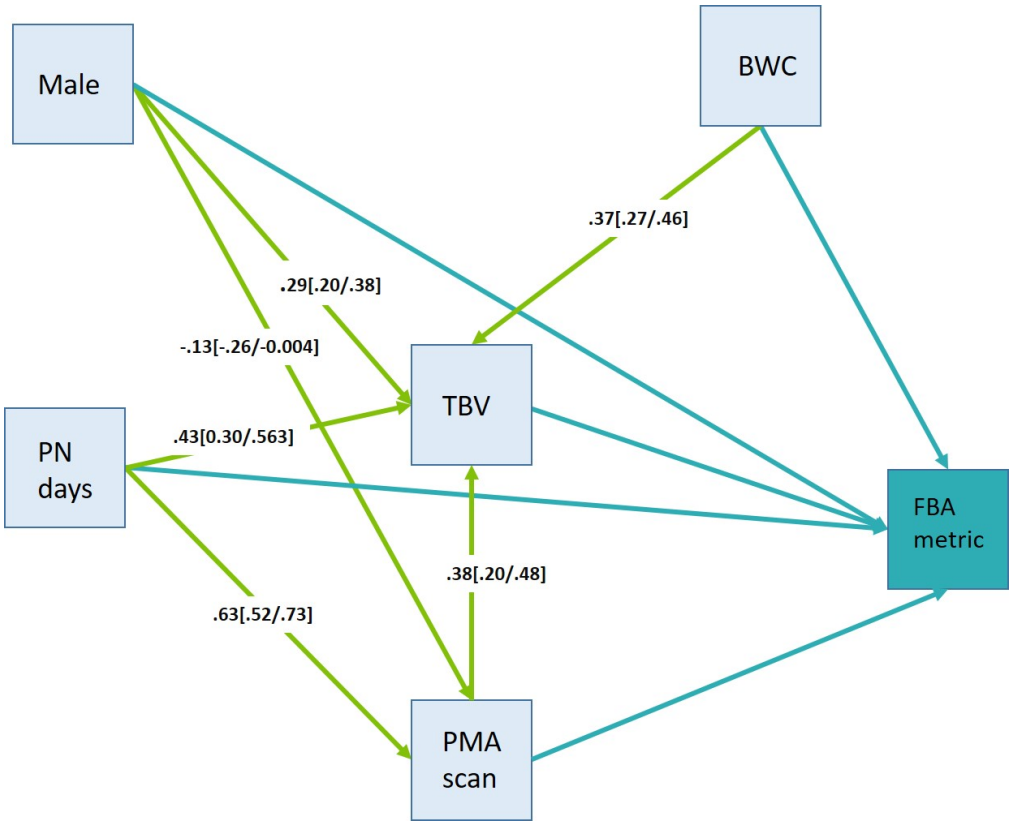

Figure 7.8: *Path model specified for the effect of covariates on FBA metrics. Blue lines represent the main covariate effects which are referred to in the main. The green lines are the regression paths for TBV and PMA as endogenous variables. All covariate effects (green lines) were significant.*

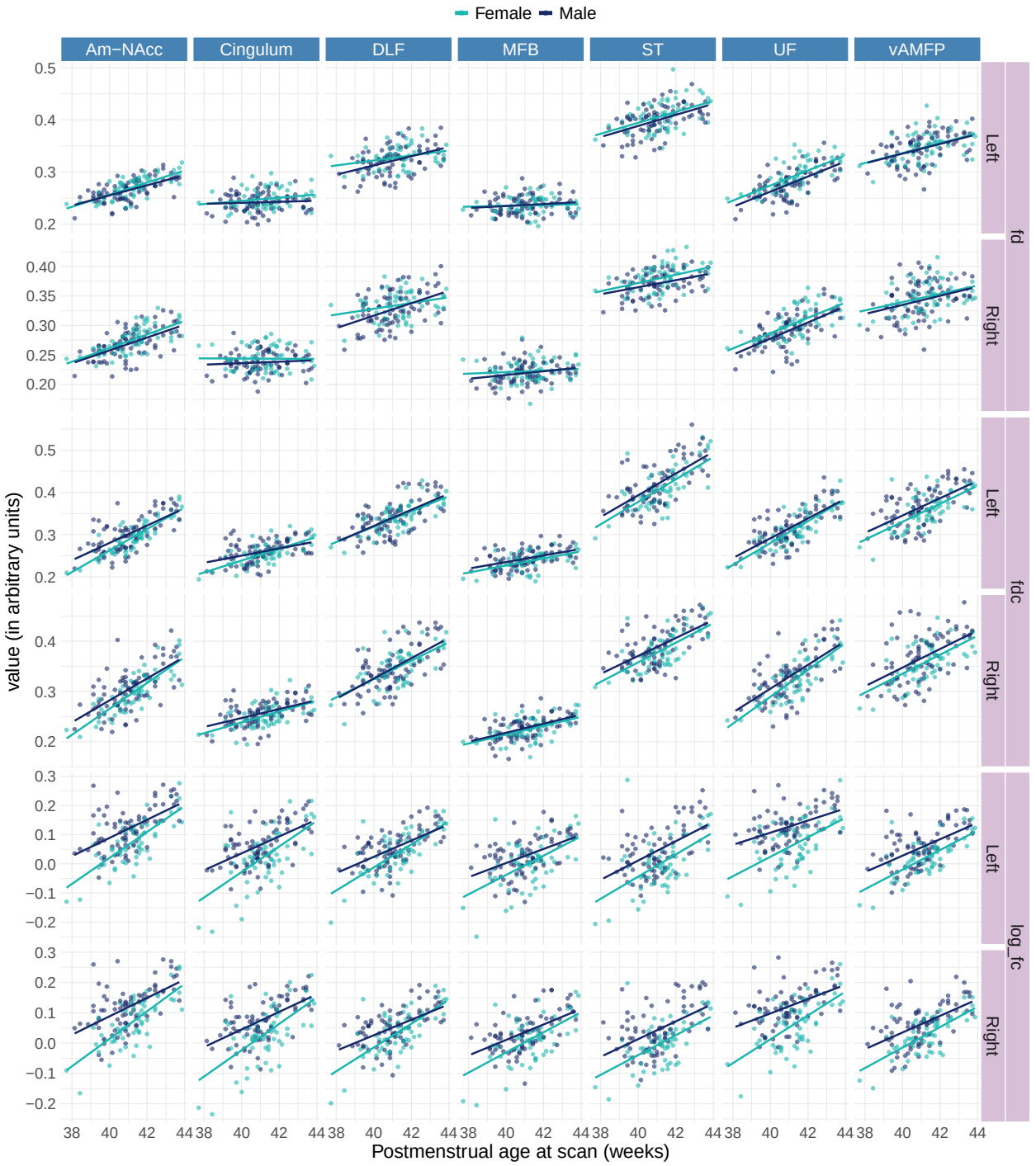

Figure 7.9: *Correlations between postmenstrual age at scan and fibre density (FD), fibre cross-section (FC) and combined fibre density and cross-section (FDC). Am-NAcc: Amygdala-Nucleus Accumens tracts, DLF: Dorsal longitudinal fasciculus, MFB: Medial forebrain bundle, Stria Term.: Stria terminalis, UF: Unicinate fasciculus, VAMPF: Ventral amygdofugal pathway.*

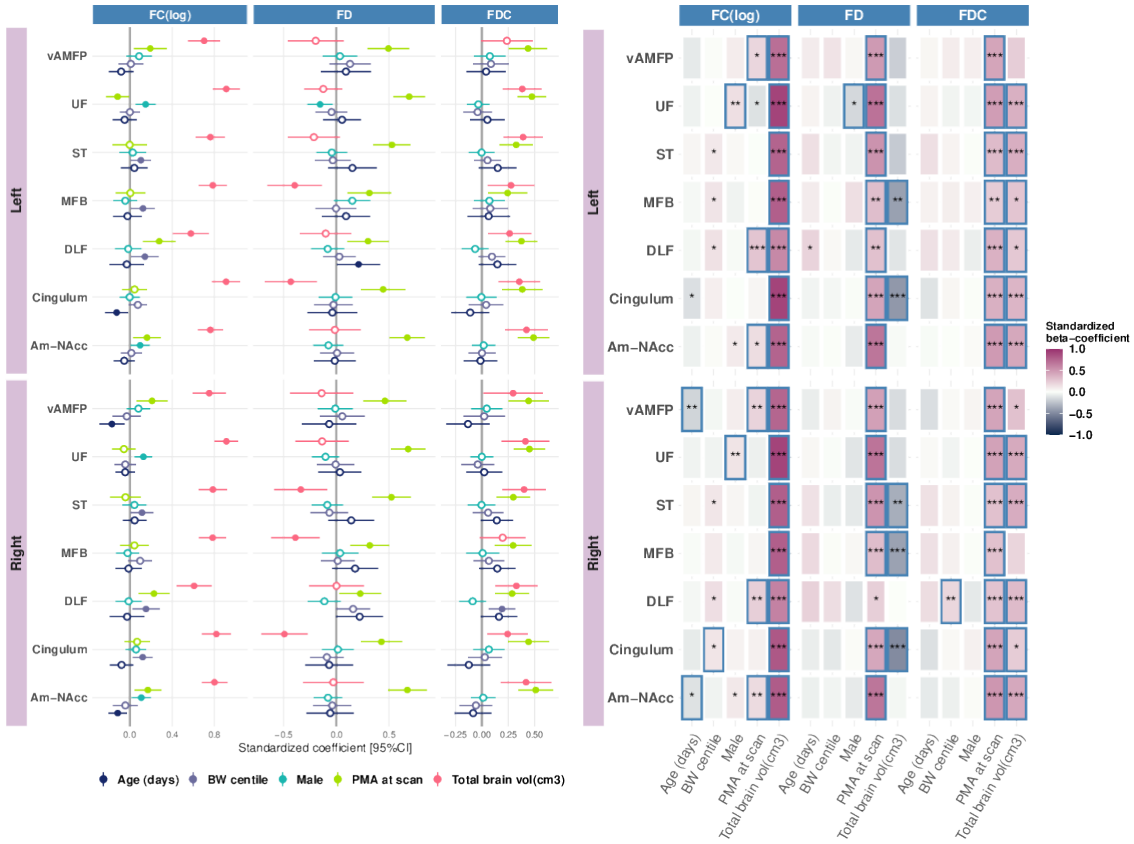

Figure 7.10: *By path analysis, fixel-based metrics were predicted from postmentrual age (PMA) at scan, total brain volume (TBV), sex, birthweight centile (BWC) and postnatal age (PN days) for 77 male and 60 female term-born neonates born of normal-weight pregnancies. Note the models included regression paths of TBV on covariates (BWC, PMA at scan, sex) and PMA on covariates (sex, PNage). Left: standardized effects sizes and 95 percent confidence intervals. Right: Beta coefficient and significance is denoted by * as p<0.05, ** p<0.01 and *** p<0.001. Boxed cells denote p-values surviving FDR adjustment at p<0.05. FD:fibre density, FC:fibre cross-section, FDC:fibre density x fibre cross-section. DLF: Dorsal longitudinal fasciculus, MFB: Medial forebrain bundle, ST: Stria terminalis, UF: Unscinate fasciculus, vAMPF: Ventral amygdalofugal pathway.*

7.8.5 Comparisons by exposure

Here I compare the 137 controls to the 28 neonates exposed to obesity *in utero*, both sexes combined. The correlations between the FBA metrics and postmenstrual age at scan with exposure as the grouping variables is shown in Figure 7.11.

Path analyses on FBA metrics were run with the main effects of the independent variables in the previous analyses, with the addition of the exposure variable and an interaction term Obese*PMA. The regression paths to PMA were removed. Model fit was good across all tracts (CFI 0.96 to 0.98, TLI: 0.93 to 0.97, RMSEA:0.062, SRMR 0.056 to 0.060).

As before the effects of sex, PMA, PN days, and birthweight centile on TBV remained. The effects of TBV and PMA on FBA metrics remained mostly as in the analysis in the controls only. In this model males had also lower FD in the bilateral UF and higher FC in both the bilateral UF and bilateral Am-NAcc.

There was no main effect of maternal exposure group (normal-weight/obesity) on TBV (std coefficient (SE):-0.075(0.057), p=0.189). There was no main effect of the exposure on any of the tracts of interest, see Figure 7.12. However, there was a significant negative interaction of exposure*PMA on the FD in the bilateral Am-NAcc tract and the right UF. This suggests that children exposed to maternal obesity do not see the same increase in FD as infants exposed to normal-weight women *in utero* during the postnatal period of 6 weeks, both in the bilateral Am-NAcc and the right UF.

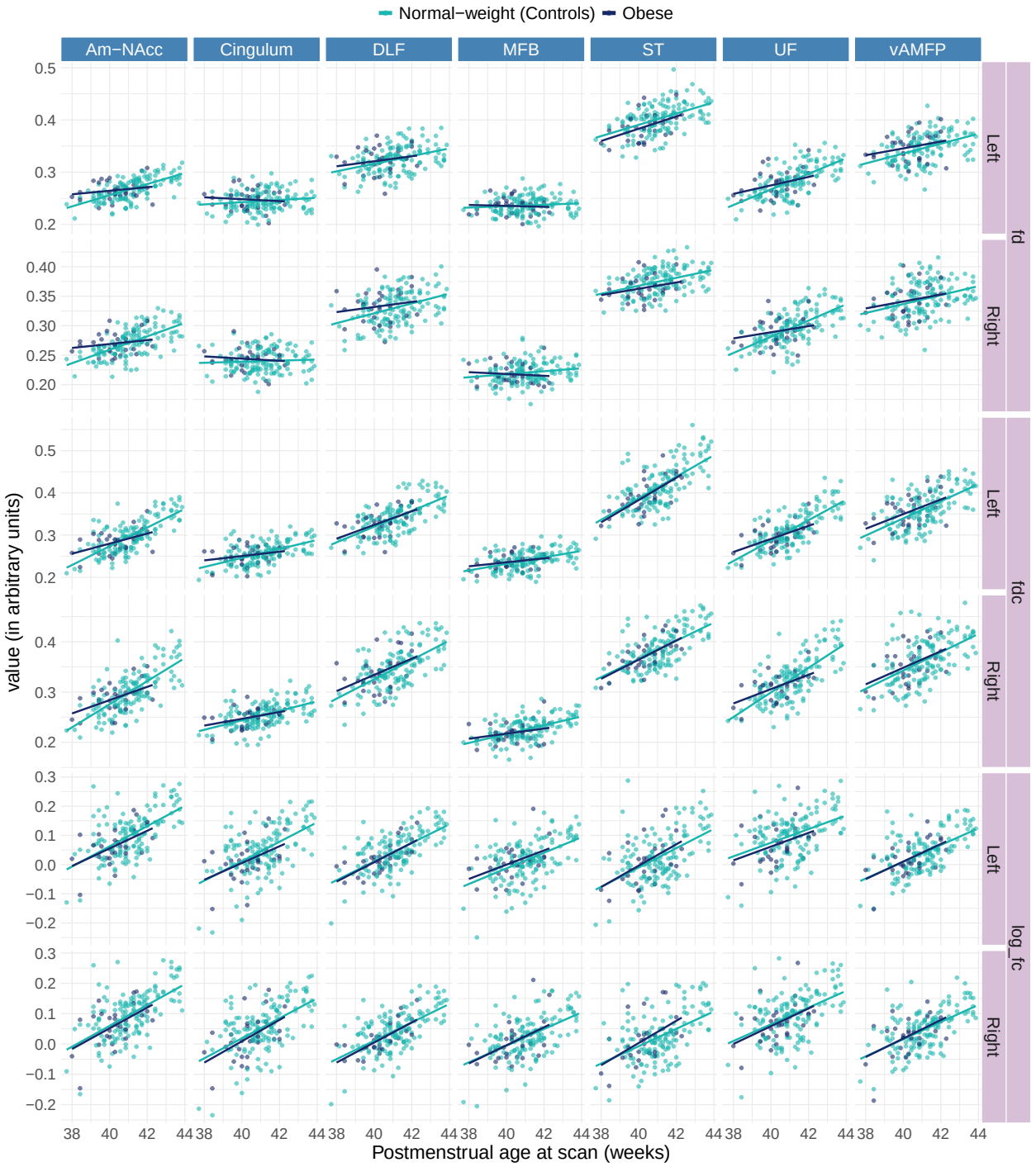

Figure 7.11: *Correlations between fixel-based metrics and postmenstrual age at scan by exposure (normal-weight/obesity), sexes combined. Normal-weight:n=137 and obesity : n=28.*

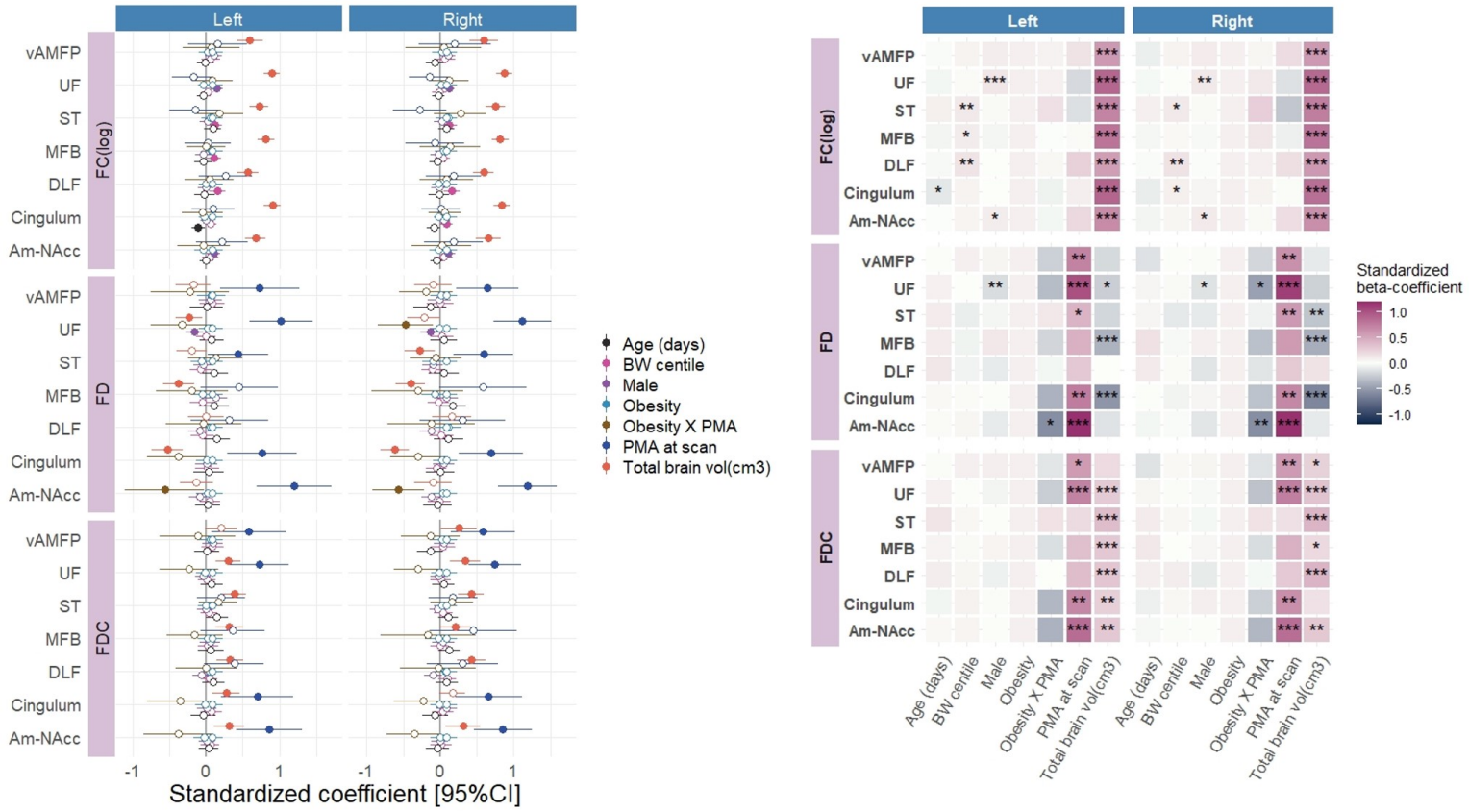

Figure 7.12: *Standardized beta coefficients obtained through path regression analyses are represented visually. The fixel based metrics were predicted from exposure (normal-weight/obese), postmenstrual age (PMA) at scan, total brain volume (TBV), sex, birthweight centile (BWC) and postnatal age (PN days) and the main effects only are represented. On the left, circles are filled if the confidence interval excludes 0. On the right, the size and direction standardized coefficients are represented by the color spectrum and stars denote significance levels *p<0.05,**p<0.01 and *** p<0.001. FD:fibre density, FC:fibre cross-section, fdc:fibre density x fibre cross-section. DLF: Dorsal longitudinal fasciculus, MFB: Medial forebrain bundle, Stria Term.: Stria terminalis, UF: Unicinate fasciculus, VAMPF: Ventral amygdalofugal pathway.*

7.9 Discussion

This study contributes to the field of neuroimaging and epidemiology in several ways. First, WM bundles associated with the reward, limbic and homeostasis systems in the human neonates were successfully delineated *in vivo*. Here, the vAMFP, Am-NAcc, DLF, MFB were distinguished in the first time in the neonate using tractography, in addition to the ST which had only been partially included in a segmentation-based neonatal WM atlas (Alexander et al., 2020).

Second, robust tractography protocols enabled the visualisation of the topographical organisation of these fibres which reflect overall ground truth from histological and post-mortem studies of adults and other *in vivo* studies using tractography. I demonstrated this topographical fibre organisation within the hypothalamic subregions not seen before in the neonate, either *ex vivo* or *in vivo* using this method.

Third, I employed FBA, a method sensitive to voxel-wise tissue microstructure of density and cross-section of *distinct* bundles which allowed for the assessment of WM rate of change across a relatively short window of growth of approximately 6 weeks and provide, through path analysis, a robust representation of the effects of time over TBV, BWC, sex and days outside the womb. Lastly, I interrogated whether there were differences in WM structure in tracts involved in the reward and limbic system which could explain the higher risks of neurodevelopmental and metabolic outcomes in the offspring born of obese women. This tested the assumption that maternal obesity has an impact on the fetal brain and this could be detected with dMRI in the neonatal brain. I found an interaction in the bilateral Am-NAcc bundle and the right UF.

7.9.1 Anatomical findings from tractography in the neonatal brain

First this chapter contributes to the field of *in vivo* tractography and the study of anatomy overall. I have devised tractography protocols which show topographical correspondence to what is known in the human and animal brain but has not been shown in the neonate before nor to this precision *in vivo*.

I show that organisation of the vAMFP and Am-NAcc in relation with the UF shown here in the neonate agrees with previous reports (Folloni et al., 2019). More recently Rusche et al. (2021) provided Nacc derived probabilistic tractography and our tractography agrees with their delineation of the VTA-NAcc path (i.e. MFB) and Am-NAcc path. However our study further demarcates the lateralisation of these tracts in relation to each other within the amygdala and the hypothalamus. We show the distinction between the bundles of the Am-NAcc and those of the vAMFP to the hypothalamus, although they share the temporal portion emanating from the amygdala.

Surprisingly, although the inclusion area of the NAcc was the same in their respective protocols, the termination of the MFB streamlines and Am-NAcc streamlines inside the Nacc were distinct from one another, possibly relating to their respective targets of the NAcc core and shell related in the literature.

In the tractography of the MFB, there was a lateralisation so that in the right MFB the superior and inferior branches could be generated but only the superior in the left MFB. It is likely a false negative effect although it must be acknowledged that the area under study covers many crossing and kissing fibers. Elsewhere, the MFB superior branch mean FA has been negatively associated with hedonistic capacity (Bracht et al., 2015). MacNiven et al. (2020) used probabilistic tractography in the adult and also found two VTA-Nacc branches (i.e. MFB) and demonstrated lower FA in the inferior MFB associated with higher impulsivity.

The inclusion of the DLF in our study may have several future implication for research. The DLF carries the transmission between the vagus nerve to the posterior hypothalamus where the sympathetic activation originates. Vagal activity (and the "gut-brain" axis) has been an extensive and novel domain of research in neurodevelopment, for example among children born of c-section, more susceptible to have their gut invaded by pathogens at birth than vaginally delivered children. Some evidence also shows c-section could influence infant white-matter development (Deoni et al., 2019). The gut-brain axis is implicated in neurodevelopment. In food addiction the gut-brain axis is suggested to shift from a homeostatic to a hedonistic function which could explain food addiction (Gupta et al., 2020). Therefore the avenues to utilise the protocol devised in this study are vast. The development of the DLF, e.g. especially in children at higher risks of neurodevelopmental disorders such as those born preterm or with familial risks of autism could be further interrogated. Additionally, brainstem tractography has served great purpose in planning neurosurgical approaches (Meola et al., 2016).

Ultimately comparing tractography protocols with those use in the adult population is not simple. Tractography protocols differ and so do the algorithm used, whether they are performed in template or subject space, the dMRI acquisition (shell-number, HARDI), tensor model vs CSD-derived FODs, and level of adjustment to software default settings applied. Nevertheless, here I used MRtrix which is open-source and contains a large amount of documentation and online support although not specific to tractography in the young brain. New tools for automated probabilistic tractography in the newborn have been specifically developed (Zöllei et al., 2019).

The purpose of this study was not to explore the intersubject variability in tracts in vivo since any method is subjective to the data quality and from our attempts it is very difficult to construct tracts with the same protocol across all subjects. Therefore, employing template based tractography and fixel-correspondence there is some confidence that extracting quantitative measures were done in the same brain areas across the subjects. However, in contrast to Alexander et al. (2020), tractography in template space has the limitation to not uncover heterogeneity among neonates in topography of these WM tract which may be of interest in explaining future outcomes.

Tractography to this precision remains an iterative process whereby we apply a priori knowledge to characterise the tracts of interest rather than explore the extent to which different algorithms produce true positives. The balance has to be struck between the limit of the diffusion signal and the aims of the tractography. Here we primarily started by evaluating the *feasibility* to generate WM bundles not studied in the neonate and the extent to which state-of-the-art dMRI and processing pipelines can facilitate this exploration.

It could be argued that prioritising the core body of the fibre bundle of interest could limits the validity of the tractography in achieving its aim of topographical sensitivity. However, this choice can be explained in several ways: 1) it is known that tractography can produce false positive and false negative streamlines, including e.g. spurious streamlines which are therefore likely to also apply to the terminations points in areas of various FOD amplitude where the GM meets the WM, 2) many fibre bundles by definition (and is well evidence using *in vivo* tractography) have a "core" body where the density of the axonal bundle is assumed to be greater (e.g. temporal stem for the uncinate fasciculus) than the distal termination at the GM/WM interface. I assumed that the direct transmission capacity through the structural enervation between two cortical/subcortical areas to be relatively dependent and represented by the cross-sectional area and fibre density within this core. 3) given that the FBA metrics used here was the mean over a tract and given point 1),

voxels at the extremities will contain fewer streamlines than those within the “core” of the tracts and therefore quantification of a single metric over the tract the strategy aimed to reduce issues of partial voluming effect within voxels at the periphery.

Hence, while the protocols presented here are based on the adult anatomical evidence, we cannot exclude false-positive streamlines were generated and that thresholding and seeding/targeting/gating methods influenced the findings. Probabilistic tractography has shown to provide sensitivity and specificity, as validation studies using histological tracing have shown (Girard et al., 2020; Grisot et al., 2021). Establishing validity against ground truth of the WM bundles delineated here in the neonate presents specific challenges due to the scarcity of histological evidence in postmortem tissue. Importantly, the brain in the perinatal period is dynamically changing which presents significant challenges in incorporating and accounting for processes such as fasciculation, axonal growth and myelination which may not be complete or vary across regions of the brain. This means attempts to generate bundles, specifically those less known in the adult and understood from post-mortem studies to be very thin would be even more difficult to propagate in the neonate. The Stria Terminalis is a good example of this. The ST is poorly myelinated in the adult (Mori et al., 2017). Furthermore, any antenatal effects on development of discrete and thinner neuronal bundles (e.g. ST) and disruption of neuronal maturation (e.g., dopamine availability in the maturation of the projections from the VTA to Nacc) in this fasciculation (Ádám et al., 2020) may not be identified when tractography is performed in template rather than native space. This contrasts to WM bundles established earlier in development and clearly observed by birth on MRI i.e. the major commissural (corpus callossum), projection (corticothalamic tract), associative (UF) and limbic (cingulate and fornix) WM bundles (Dubois et al., 2014).

Tractography in the living adult brain has shown wide benefits in devising the stereotactic targets for neurosurgery including ablations necessary to overcome some behavioural and psychopathological disorders. I demonstrate that in the neonate there is a promising avenue to import knowledge from this kind of tractography into the clinical field of pediatrics. Already, structural perinatal MRI of the brain is used to prepare for neurosurgery. Inspired by the novel advancement in precision cardiac imaging for intervention on the heart in this infant population it is possible that more sensitive tractography protocols of the neonatal brain could see applications to neurosurgery.

7.9.2 Macrostructural and microstructural changes in the perinatal period

Overall, the work presented here shows that not all WM bundles expand with age such as the MFB, the cingulum and ST although FD increased with PMA in all tracts. The cingulum and MFB had lower density in larger brains however it is difficult to interpret what this could mean on function. FD cannot distinguish between a change in axonal count or axonal diameter and is not directly sensitive to myelin (Dhollander et al., 2021). As a voxel-wise measure of axonal matter it is also influenced by the other components in the extra-axonal space surrounding a fibre bundle. The findings that BWC had a positive effect on the FC of the DLF as well as the MFB is yet to be explained.

7.9.3 Sex differences

There were several findings in the comparisons between the sexes in the normal-weight exposed neonates. Differences in brain anatomy between the sexes have been identified in the brain (Kim et al., 2017) and extensively studied. For example in the hypothalamus in animals such that the volume of the anteroventral

periventricular nucleus is larger in female mice which contains ten times more kisspeptin neurons (Vries and Södersten, 2009). Vassoppresin neurons in the BNST and media amygdaloid nucleus which related to sexual and aggressive behaviour (Vries and Södersten, 2009). However, not all studies find sex differences in WM in the term born neonate such as measured by mean diffusivity (Akazawa et al., 2016).

Dean et al. (2017) studied WM development by diffusion tensor imaging and neurite orientation dispersion and density imaging and did not detect sex dimorphism in WM development of the short neonatal period they studied. However, growth rate of some WM regions showed sex dimorphism in later childhood development (3 months through 5 years of age) as measured by the myelin water fraction (MWF) (Deoni et al., 2012). However, these studies included large WM tracts or regions and different metrics of assessing WM development than FBA metrics used in this chapter.

In the adult human Liu et al. (2020) showed that in grey cortical matter (CGM), males have larger areas in the temporal pole, hypothalamus, amygdala and BNST but in prefrontal and parietal regions CGM was larger in females. The differences for larger temporal cortex (medial) was demonstrated in the neonate by Knickmeyer et al. (2014). These grey matter volume differences are conserved throughout adult 21-90 year lifespan (Lotze et al., 2019). Additionally, amygdala volume growth trajectories are highly dimorphic between 5 and 25 years of age which is particularly prominent in the centromedial nuclear group of the amygdala (Fish et al., 2020).

Therefore, the larger UF and Am-Nacc tract cross-section in male neonates showed in this present study could be in agreement with these previous findings since the two tracts serve the temporal/amygdalar connectivity to frontal areas. We note that only the UF survived FDR correction in this sample. Nevertheless, this also aligns with findings of sex differences in maturation of the frontal and temporal regions in an overlapping neonatal sample using morphometric similarity networks (Fenchel et al., 2020). At this stage no causation can be inferred on any association these findings may have with the higher incidence of neurodevelopmental disorders in the males (Polanczyk et al., 2007). Furthermore, males show higher within-hemispheric connectivity but females dominate on between -hemisphere connectivity (Ingalhalikar et al., 2014). Although not studied here, it would be interesting therefore to see whether commissural WM bundles show a higher female cross section or density to explain the reported interhemispheric connectivity dominance.

7.9.4 The effects of maternal obesity in pregnancy on brain development

In light of the feasibility to use tractography in the neonatal brain, many study questions on the effect of various antenatal exposures can be investigated with minimal confounding effect of postnatal experience on brain plasticity. This study included a small group of 28 neonates exposed to obesity *in utero*. Although there was no direct effect of exposure on the FBA metrics, the results show that there is an interaction of PMA by exposure so that FD in the bilateral Am-NAcc and right UF does not increase with age as in the controls after accounting for the other covariates of sex, total brain volume, postnatal age and birthweight centile.

An important epidemiological question this work aimed to answer was if children born of obese women have a predisposition to neurodevelopmental and metabolic disorders. Although our sample of obesity-exposed infant was small a strength of this study is the strict exclusion for preterm birth and obstetric complications. However, the findings reported here cannot imply causation, but in their novelty, may offers some insights. The amygdala is implicated in the stress response and in pleasure-inducing behaviour such as eating palatable food (Ulrich-Lai et al., 2010). Further interaction between the stress-response circuit and reward net-

works implicate an aberrant structural connectivity between the amygdala with other areas of the reward system (mPFC) in pleasure-inducing contexts as studied in models of chronic early life adversity (Bolton et al., 2018). To note, the results were presented unadjusted for multiple comparisons and these analyses should be considered exploratory from this convinience sample. Other methods such as multi-level modelling may be more appropriate and the findings should be replicated in a larger sample. Nevertheless, the finding of a slower growth rate of Am-NAcc density in the obesity-exposed neonate is an important new finding in the research question of a predisposition to metabolic and neurodevelopmental disorders but the impact on function warrants further discussion.

Comparing these results with Ou et al. (2015) who used the tensor FA value and whole-brain approach is complicated due to their acquisition protocol (diffusion tensor imaging, 15 directions, one b-value (700 s/mm^2) at a voxel size of 2×2×3mm resolution using a 1.5T scanner and 8-channel head coil) and the CSD-derived FBA method employed here, more sensitive to the neonatal macrostructure and crossing fibres. However, the authors were able to detect in their modest sample wide spread differences in FA but none of the tracts they uncovered were targeted in this study. We did not find differences between exposure group and our obesity-exposed sample was larger. However our study is void of the genetic complementation and maternal fat mass percentage included in Ou et al. (2015) but interestingly Ou et al. (2015) exclusion criteria (GDM, preeclampsia) were similar to ours and the neonates were born full term.

Nevertheless, it is possible that the sample size was too small to find the effect of exposure in other tracts but this could be that excluding obstetric complications, preterm births and brain abnormalities removes many of the mediating or additive risks on the causal path between maternal obesity and the adverse offspring neurodevelopmental reported in the literature. Prematurity is the leading risk factor for neurodevelopmental disorders and lower maturation in several regions of the brain has been reported in preterms scanned at term-equivalent age, using tractography and tensor based metrics (Akazawa et al., 2016) or by FBA (Pannek et al., 2018). In Pannek et al. (2018) they included the corpus callosum, anterior commissure, cortico-spinal tract, optic radiations, and cingulum. Alternatively, effects of obesity exposure on brain microstructure and morphology may be sex-dependent and including them together in the analysis may have masked an interaction by sex. It could also be that differences in WM structure between obesity and normal-weight exposed offspring and the sexes appear later in development (Akazawa et al., 2016). There are, however, future avenues of research for this sample. Recently Na et al. (2021) found lower cortical thickness in several frontal lobe regions in neonates exposed to maternal obesity in pregnancy (28 normal-weight, 16 obese), with mean cortical thickness associated negatively with maternal fat mass percentage. It would be interesting to replicate this in the current sample.

An important strength of this study is that the scaling effect of TBV was included. However, it is possible that some volumetric differences at the subcortical level exist which could impact the findings. Volumetric analysis of the neonatal hypothalamus and NAcc have yet to be reported and is a potential future direction. However, given that studies in larger adult samples have not found changes of volume with BMI in this region of 1.1 cm3 bilaterally (e.g. on 7T, Schindler et al., 2013) it is possible that the power to detect a difference would be too small in a relatively small case-control sample and may be affected by the voxel resolution available in the delineation in the neonate.

7.9.5 Using path analysis

Another important strength of this study is that the statistical analysis of the effects on FBA metrics are provided from path analyses rather than conventional saturated regression analysis. First, this allows for the model fit to be reported, in this case the theorised model show good fit and so the coefficients were also better estimated. Employing this method also promote the awareness of important relationships in the study of brain development, e.g. the effect of days outside the womb on TBV. However, as alluded above, the model here did not include important confounders and thus the effect of obesity on neonatal brain should be interpreted with caution. It is clear that a genetic predisposition cannot be excluded. Importantly, and in line with the findings from Chapter 4 and 5, there was no adjustment made to maternal depression or maternal variables. Although in the latter I showed that there were not differences between the groups, it is imperative the place of genetics and maternal mental health in these associations be not ignored (Wilson et al., 2018). The dHCP included some information on maternal psychiatric history and a EPDS questionnaire at time of scan. However, most of the neonates were scanned within 5 days of birth and the EPDS is designed to measure depression outside the 1 month postpartum period to exclude effect of the hormone-induced “blues” many women experience soon after delivery. Therefore, the decision was made to not include it. Similarly, only maternal education was available as a proxy of SES in our database.

7.9.6 Summary

This chapter has presented what state-of-the-art dMRI acquisition and processing can deliver in tractography of the neonate. Topographical preferences of the tracts of interest correspond to their known and documented areas which was especially pertinent to observe within the hypothalamic “subcompartment”, only previously derived from animal retrograde/anterograde histology and postmortem examination. Although it is difficult to infer directly about the specific nuclei involved in this *in vivo* study it can be argued that the termination point, lateralisation (medial/lateral termination/initiation) with respect to the third ventricle is highly accurate to bring confidence that these are not spurious findings. Further, important exploration as to the functional relevance the sex differences and effect of exposure found in WM development may have in the long-term is warranted. This is especially interesting considering the findings reported in Chapter 5, where child psychological phenotype associated with adiposity at 3 years of age.

Summary

The work presented in this thesis had as an overall aim to contribute to our understanding of the likely causal pathways which may explain the increased incidence of neurodevelopmental disorders in children born to obese women compared to those born to normal-weight women.

An important gap which the study described in Chapter 4 fulfilled was the lack of longitudinal profiling of maternal depressive symptomatology in obese pregnancy and its likely burden for this population. I found that four "latent" groups of women can be distinguished on their trajectories and that socio-economic disparity was, unsurprisingly, a clear theme in the profiles of the women with moderate to severe depression. Then, I also found that when using the latent modelling techniques and classification it is possible to unveil other heterogeneities in blood markers, rate of infection and dietary habits across these groups.

In Chapter 5, I found that child psychological outcomes were associated with anthropometry in the 3 year old children of the UPBEAT women and that maternal antenatal depression was a significant predictor for increased risks of psychopathology in the child. This study provided evidence that GDM or inflammation in this sample were unlikely precipitating factors while at the same time using SEM enabled some theoretical assumptions to be tested. It was noted that these models were not of perfect fit to the data and so further SEMs could be proposed and markers such, as glycaemia, better defined.

Then, on the hypothesis that both child psychological outcomes and anthropometry had the same or overlapping etiologies involving the brain, in Chapter 6 and 7 I utilised state-of-the-art neonatal MRI data to investigate the white matter (WM) pathways potentially implicated. I am the first to produce the segmentation of the neonatal hypothalamus, nucleus accumbens and ventral tegmental area. I also demonstrated the feasibility of using diffusion tractography for the *in vivo* reconstruction of the neonatal stria terminalis, medial forebrain bundle, ventral amygdalofugal pathway, amygdaloaccumbens fasciculus and dorsal longitudinal fasciculus. My results show interesting sex differences in macro and microstructural development and that in the amygdaloaccumbens tracts and right uncinate fasciculus there was a low fibre density growth across the perinatal period in the obesity exposed neonates. However, not having included maternal mental health measures in this smaller feasibility study or other risks factors was a limitation. This study does not establish causation between obesity/normal-weight and the observation on WM development but it adds to other neuroimaging studies, which have focused on the social, behavioural lifestyle and psychological factors detrimental to fetal neurodevelopment (reviewed in Dufford et al., 2021). Nevertheless, it is the first time that a study addressed the common neurological etiology between neurodevelopmental and metabolic disorders and a possible predisposition to long-term adverse health outcomes using the methods described.

7.10 Implications

The study in the UPBEAT women has demonstrated that more granular and comprehensive approach to assessing the role of potential etiological factors may lead to a better understanding of complex interactions over the course of pregnancy. I have shown that there are tools available to enable this exploration. When taking a holistic approach to defining the exposures that may have adverse effects on the fetus, it is shown that there may be multiple "hits" to the fetus across gestation. Any one such "insult" has the potential to disrupt fetal brain development to various extents, depending on the severity of the insult and the timing in gestation (Chapter 2). As the data showed in this unselected sample, severe and *constant* depression in pregnancy was associated with a *threefold* risk of preterm birth and this cannot be ignored. With this in mind, some of the factors related the maternal health in pregnancy can carry over into the postpartum period and continue to affect the offspring, such as through her caretaking and the diet provided to the child.

Overall, uncovering heterogeneity in depressive symptom trajectories demonstrated that studying maternal mental health using dichotomisation on the basis of cutoff scores or diagnostic criteria may be inadequate and may introduce bias. This also applies to the GDM diagnosis which is based on a one-off measure of glucose after an oral glucose load in pregnancy. Further, finding that antenatal depression was a significant predictor of high-risk of psychopathology at 3 years beyond GDM and inflammation should motivate researchers, clinicians and public health bodies to scrutinise the exposures which were presented in Chapter 4. However, what becomes clear from my findings is that devising preventive strategies to improve outcomes in children born of obese women in such a physiologically multidimensional and dynamic context is very complicated.

There are several implications to my study on the neonatal brain. Dissemination of the protocols I have produced for tractography will expand the opportunities for others to explore biomarkers for long-term psychological and non-psychological outcomes following various antenatal exposures, congenital anomalies or injury at birth.

My studies to date may have provided some small pieces of the puzzle into the transgenerational transfer of disease risk. It is obvious that the extensive data made available to me both through UPBEAT and the dHCP presented the ideal opportunity to devise interesting empirical studies which could offer true insights in to the DOHaD framework. I believe that my reliance on SEM-based methods showed my attempt at providing robust estimates throughout my work. It is also possible that others can use the findings to generate new hypotheses and future studies limited in sample size could rely on my estimates as priors in a Bayesian framework.

7.11 Some reflections

Although animals studies enable the characterisation of specific biological mechanisms involved in the transfer for disease risk, the translation into humans who live outside these controlled environments becomes difficult.

The thesis focuses on maternal depression in obesity as an important risk factor but it should still be noted that 79% of the UPBEAT women were at low or mild risk so this should be viewed positively. Nonetheless, detecting poor mental health in the antenatal care pathway in general is not a priority, unless a pre-pregnancy diagnosis is disclosed or registered. Maternal obesity, in the clinical setting, is seldom considered to be a

result of depression (e.g. through comfort eating). Additionally, the very limited time afforded to asking a woman about her feelings in a 10 min antenatal appointment is inadequate. This is beside the fact that some women with obesity experience stigmatisation obese women due to their weight as they engage with healthcare providers. Adverse health outcomes are further exacerbated by racial disparity in access to and experience of health care, at least in the UK. It is particularly alarming to read reports of maternal death in pregnancy and child birth being five times higher among Black women and twice among Asian women (https://www.npeu.ox.ac.uk/mbrrace-uk/reports), and suicide is a leading cause of maternal death postpartum (Mangla et al., 2019). When women associate negatively with the care service, their lack of motivation and engagement with that system may have dramatic implication on their outcomes and of their children. What makes one hopeful is that since I started my PhD, multiple initiatives have been put forward to tackle specifically the issue of maternal (and paternal) mental health in the UK. This include increasing nation-wide provisions of care in perinatal psychiatry, the building of in-patient mother-baby units across the UK, and the increase discussion on this topic in the media.

In relation to the neuroimaging findings and the underlying hypothesis, it is difficult to establish how a potential neurological predisposition at birth in the brain pathways described here could explain the “remission” of neurodevelopmental diagnosis. For example, while the worldwide childhood prevalence of ADHD was around 5% in 2007 (Weissenberger et al., 2017), 50% no longer meet criteria of ADHD in adulthood (Wender, 1998). However it should be noted that formerly diagnosed ADHD may receive a antisocial personality disorder diagnosis in adulthood (Wender, 1998) possibly because early studies of children’s diagnosis had not accounted for conduct disorder (Wender, 1998). Alternatively, a remission could be explained by treatment history, aging or diagnostic criteria/scales used between decades (Moreno-Alcázar et al., 2016) or changes in symptoms so that adults are less hyperactive and more inattentive (Larsson et al., 2011). Although overall robust, similar concerns arise over the inconsistency of the association between ADHD and overweight/obesity across anthropometric criteria (Racicka-Pawlukiewicz et al., 2021) and Kahathuduwa et al. (2019) noted that associations between childhood ASD and obesity are difficult to interpret in light of the symptom severity and subtypes of ASD varying across studies. The field of psychometrics will mostly likely continue to contribute to this debate.

7.12 Future Directions

7.12.1 From UPBEAT

UPBEAT offers an unprecedented access to a wide range of pregnancy outcomes from which several avenues of research could be explored.

- Including infection and diet and mode of delivery into SEMs to elucidate additional pathways through which depression influences outcomes in the 3 year old.
- Implementing latent modelling of glycaemia through longitudinal modelling such as LCGA to understand heterogeneity in the pregnant population and the impact of both GDM diagnosis, under diagnosis and treatment on maternal and infant outcomes.
- Performing longer-term follow-up of the children to understand if the psychological and morphological outcomes associate to health at an older age.

- Using the latent class with low-risk of depression found in Chapter 4 as a control group for further investigation into metabolism in pregnancy.

7.12.2 My Baby brain and Me

In light of the findings included in this thesis, I designed “myBBM” (www.mybbm.co.uk),a prospective study which aimed at using MRI to investigate the associations between maternal obesity and GDM on brain development in the offspring. The overall design focused particularly on collecting a wide range of variables from the mother and control for the effect of maternal psychopathology by applying strict exclusion criteria. I drafted all the documentation required, including for ethics, and received final approval to start the study in April 2020. Due to the effect of the pandemic on recruitment it was not possible to consider within the timeframe of the PhD. However, the protocol for implementing a prospective study on this research question, including the rationale, aims and measures are included in Appendix B.

7.12.3 From the dHCP

Further analysis including the cohort described in Chapter 7 is warranted:

- Investigate the correlates between the findings and tractography in the WM with functional activity in the cortical areas which are structurally connected via these tracts.

- Explore structural connectomes in the whole brain and implement methods such as network analysis to infer on wider scale differences between obesity-exposed and normal-weight exposed children.

- Assess the 18-month follow-up psychological outcome data and interrogate whether the FBA metrics of tracts which I generated in the neonate correlate with traits on the autism spectrum (Q-CHAT questionnaire) and other areas such as cognition, language and social-emotional development as measured by the Bayley Scales of Infant and Toddler Development.

7.13 Other data

Other research avenues include validating the findings in other study samples. This includes prospective studies in pregnancy and neuroimaging such as the ABCD initiative, which has collected one of the most comprehensive and large longitudinal datasets containing data on child MRI, socio-demographic factors and genetics.

The reported associations between maternal obesity and offspring psychological outcomes found in meta-analyses (e.g Sanchez et al., 2018) and in this thesis do not take into account the share genetic and/or family environment. Future sibling designs can help dissipate such limitation (Chen et al., 2014).

7.14 Conclusion

The field of life-course epidemiology and supporters of DOHaD now emphasize strongly that pre-conception health should be a primary target for intervention. Under the DOHaD framework, this would include nutrition

and lifestyle adaptation to readdressing the population-wide patterns of transgenerational transfer of disease risks (Poston et al., 2016; Fleming et al., 2018; Lindsay et al., 2019, 2020). This thesis provides a profile of overall vulnerability among a selected group of pregnant women and their children which will further promote the field and I hope, increase investment in this important area of research.

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Appendix A

Appendix A

Table A.1: *Biomarkers available from a total of 1369 participants.*

Group	Biomarker	Time point 1	Time point 2	Time point 3
Adipokines	adiponectin	967 (70.6%)	898 (65.6%)	733 (53.5%)
Adipokines	leptin	967 (70.6%)	897 (65.5%)	
Amino Acids	alanine	945 (69.0%)	887 (64.8%)	715 (52.2%)
Amino Acids	glutamine	943 (68.9%)	886 (64.7%)	714 (52.2%)
Amino Acids	glycine	945 (69.0%)	887 (64.8%)	715 (52.2%)
Amino Acids	histidine	944 (69.0%)	886 (64.7%)	715 (52.2%)
Amino Acids	isoleucine	945 (69.0%)	887 (64.8%)	715 (52.2%)
Amino Acids	leucine	945 (69.0%)	887 (64.8%)	715 (52.2%)
Amino Acids	phenylalanine	945 (69.0%)	887 (64.8%)	715 (52.2%)
Amino Acids	tyrosine	944 (69.0%)	886 (64.7%)	715 (52.2%)
Amino Acids	valine	945 (69.0%)	887 (64.8%)	715 (52.2%)
Fatty Acids	Degree of unsaturation	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids	DHA	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids	Linoleic Acid	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids	Monounsaturated	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids	Omega-3	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids	Omega-6	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids	Polyunsaturated	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids	Saturated	944 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids	Total FA	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids %	DHA of total FA	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids %	Linoleic Acid of total FA	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids %	Monounsaturated of total FA	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids %	Omega-3 of total FA	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids %	Omega-6 of total FA	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids %	Polyunsaturated of total FA	945 (69.0%)	886 (64.7%)	716 (52.3%)
Fatty Acids %	Saturated of total FA	944 (69.0%)	886 (64.7%)	716 (52.3%)
Glycaemic markers	c-peptide	959 (70.1%)	890 (65.0%)	728 (53.2%)
Glycaemic markers	glucose	959 (70.1%)		733 (53.5%)
Glycaemic markers	hba1c	905 (66.1%)		
Glycaemic markers	HOMA-2IR		878 (64.1%)	
Glycaemic markers	insulin	972 (71.0%)	898 (65.6%)	704 (51.4%)
Inflammation and endothelial function	CRP	969 (70.8%)	894 (65.3%)	733 (53.5%)
Inflammation and endothelial function	Glycoprotein acetyls	945 (69.0%)	887 (64.8%)	715 (52.2%)
Inflammation and endothelial function	IL-6	968 (70.7%)	895 (65.4%)	
Inflammation and endothelial function	tPA-antigen	968 (70.7%)	898 (65.6%)	
Metabolic	cholesterol	972 (71.0%)	896 (65.4%)	733 (53.5%)
Metabolic	HDL	969 (70.8%)	896 (65.4%)	733 (53.5%)
Metabolic	LDL	969 (70.8%)	896 (65.4%)	733 (53.5%)
Metabolic	triglycerides	969 (70.8%)	891 (65.1%)	733 (53.5%)
Other	HPL	944 (69.0%)		
Other	Plgf	970 (70.9%)	897 (65.5%)	
Other	Vit-D	953 (69.6%)		

Note:
FA: Fatty Acids, DHA: Docosahexaenoic acid, tPA-antigen: Tissue plasminogen activator antigen, IL-6: Interleukin-6, CRP: C-reactive protein, LDL: low-density lipoproteins, HDL: high-density lipoproteins, HPL: Human Placental Lactogen, Plgf: Placental growth factor.

Table A.2: *Properties of blood biochemical analyses.*

Group	Biomarker	Units	Sample type	Method	Platform	CV(%)
Conventional Biochemical Platforms						
Glycaemic Markers	Insulin	mU/l	plasma	Electrochemiluminescence immunoassay	Roche, Cobas e411	< 10.3
Glycaemic Markers	HbA1c	mmol/mol	whole blood	Turbidimetric inhibition immunoassay	Roche, Cobas c311	< 1.4
Glycaemic Markers	HbA1c	% (old units)	whole blood	Turbidimetric inhibition immunoassay	Roche, Cobas c312	< 1.5
Glycaemic Markers	C-peptide	ng/ml	serum	Electrochemiluminescence immunoassay	Roche, Cobas e411	< 6.2
Glycaemic Markers	glucose	mmol/l	plasma	Enzymatic hexokinase	Roche Cobas c311	< 2.4
Metabolic Markers	Cholesterol	mmol/l	plasma	Enzymatic, colorimetric	Roche Cobas c311	< 2.4
Metabolic Markers	Triglycerides	mmol/l	plasma	Enzymatic, colorimetric	Roche Cobas c311	< 3.6
Metabolic Markers	HDL	mmol/l	plasma	Homogeneous enzymatic, colorimetric	Roche Cobas c311	< 4.5
Metabolic Markers	LDL	mmol/l	plasma	Homogeneous enzymatic, colorimetric	Roche Cobas c311	< 3.3
Adipokines	Adiponectin	ug/ml	plasma	Enzyme-linked immunosorbent assay	R and D Systems	< 6.9
Adipokines	Leptin	pg/ml	plasma	Enzyme-linked immunosorbent assay	R and D Systems	< 2.0
Inflammation	hs-IL-6	pg/ml	plasma	Enzyme-linked immunosorbent assay	R and D Systems	< 9.8
Inflammation	hs-CRP	mg/L	plasma	Particle enhanced immunoturbidimetric	Roche, Cobas c311	< 7.1
Endothelial marker	t-PA antigen	ng/ml	plasma	Enzyme-linked immunosorbent assay	Asserchrom (Stago)	< 5.7
Placenta	Human placental lactogen	ng/ml	serum	Enzyme-linked immunoassay	R and D Systems	< 5.0
Placenta	Placental growth factor	pg/ml	Plasma	Fluorescence Immunoassay	Alere, Triage Meter Pro	
Vitamin	Vitamin D	ng/ml	serum	Electrochemiluminescence immunoassay	Roche, Cobas e411	< 11.2
NMR metabolomics platform						
Amino acids	Alanine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids	Glutamine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids	Glycine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids	Histadine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Branched-chain)	Isoleucine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Branched-chain)	Leucine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Branched-chain)	Valine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Aromatic)	Phenylalanine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Amino acids (Aromatic)	Tyrosine	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Total fatty acids	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	estimated degree of unsaturation		serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Omega-3	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Omega-6	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Polyunsaturated fatty acids	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Monounsaturated fatty acids 16:1;18:1	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Saturated fatty acids	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Docosahexaenoic acid (DHA) 22:6	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Fatty Acids	Linoleic acid 18:2	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	
Inflammation	Glycoprotein acetyls (a1-acid glycoprotein)	mmol/l	serum or EDTA plasma	NMR	Nightingal Health, Finland	

Note:

The Coefficient of Variation (CV) based on the highest value at time 1 or 2. Glucose at time point 2 issued by centre as per OGTT protocol.

Table A.3: *Dietary outcomes available from a total of 1369 participants.*

	17 weeks	27 weeks
Energy (kcal)	997 (72.8%)	858 (62.7%)
Glycaemic load/100g	997 (72.8%)	858 (62.7%)
Saturated fat (gr)	997 (72.8%)	858 (62.7%)
Total Fat (% Energy)	997 (72.8%)	858 (62.7%)
Carbohydrates (% Energy)	997 (72.8%)	858 (62.7%)
Saturated fat (% Energy)	997 (72.8%)	858 (62.7%)
Protein (% Energy)	997 (72.8%)	858 (62.7%)
Sugar (% Energy)	997 (72.8%)	858 (62.7%)

Note:
Participant dietary intake was included if total energy count was within 1076 to 4780 kcal range to control for over- and under-reporting.

Appendix B

Appendix B

B.1 My Baby's Brain and Me study (MyBBM)

As a consequence of the pandemic, myBBM was not conducted but given the previous chapters built up to its design it is described here as within the remit of the thesis. Future plans to implement such design should serve the interests of the field in exploring the associations between GDM and neurodevelopmental outcomes in exposed offspring.

The text below is the protocol presented and approved by the London - West London & GTAC Research Ethics Committee on the 8th of April 2020 (19/LO/1934), see www.mybbm.co.uk

B.2 Summary

"My Baby's Brain and Me" (myBBM) study was designed as a longitudinal prospective observational study which aimed to investigate the effect of maternal obesity and GDM on brain development in the offspring employing state-of-the-art imaging techniques. Equipped with ultra-precise magnetic resonance imaging (MRI) technologies, we hope to show if higher incidence of neurodevelopmental disorders in the offspring are related to antenatal factors or more likely explained by postpartum events such as birth related events. The opportunity to measure placental function in pregnancy would allow us to explore its role in offspring brain measurements. If there was evidence to show that the exposed offspring brain is already altered during pregnancy, this will help adapt current antenatal management and/or develop tailored and timed interventions to improve both maternal and infant outcomes.

Our research question was: Does maternal obesity and GDM in pregnancy confer adverse neurological and developmental outcomes in the exposed offspring in the short and long-term and can measures of in-utero placental function support this?

B.3 Objectives

Primary :

To characterize offspring brain development following exposure to maternal obesity alone and comorbid with GDM in order to detect alterations of offspring brain microstructure and function which could explain their higher long-term incidence of neurodevelopmental disorders.

Secondary :

1. To characterize offspring brain development following exposure to maternal obesity alone and comorbid with GDM in order to detect alterations of offspring brain microstructure and function which could explain their higher long-term incidence of neurodevelopmental disorders.
2. To explore the potential mechanisms through which the placenta may mediate any effect of maternal obesity and GDM on the offspring brain measures.
3. To understand the effects of maternal cortisol and other blood markers (e.g. inflammatory cytokines) in these associations.
4. To measure the child's development at 12-18 months and compare performances between those exposed to maternal GDM to the control non-GDM group and how this associates with their brain scans.

B.4 Hypothesis

We hypothesize that important brain regions which are implicated in both energy metabolism and cognitive/emotion processing will be different in the offspring exposed to GDM compared to obesity alone. We also hypothesize that placental function as measured by MRI will correlate with GDM diagnostic status and offspring brain development.

B.5 Background and Rationale

B.5.1 The Problem

Gestational diabetes mellitus (GDM) is a type of diabetes first manifesting or diagnosed in pregnancy and for which obesity is a dominant risk factor. 30% of women of reproductive age are now considered obese (BMI>30 kg/m2) and circa 15-30% will develop GDM in pregnancy (Torloni et al., 2009, White et al., 2016). Current trajectories indicate rates of obesity, and thus GDM, are increasing globally (Stephenson et al., 2018). It has become imperative to identify the causative pathways subjecting the offspring of these women to substantially higher risks of neurodevelopmental disorders, such as autism and attention-deficit hyperactivity disorder (Krakowiak et al., 2012, Li et al. 2016). However, uncertainty remains about the independent and additive contributions of antenatal exposure on one hand, and labour and postnatal events on the other hand, in these causal pathways. This is due to the fact that maternal obesity and diabetes are both risk factors for other pregnancy and labour complications and is also associated with maternal depression and immunological disturbances, which are themselves independently linked to these long-term outcomes and thus could confound the reported associations. Current reports most commonly employ cross-sectional designs which have shown inconsistencies but also fail to discuss or control for these pertinent confounders or adopt methods which could sensitively quantify brain development.

The proposed study will target the following gaps in the current literature on the subject of obese and GDM pregnancies and offspring health:

1. No prospective MRI study has been conducted in human obese and GDM pregnancies employing both fetal and neonatal brain imaging data and considering important factoring in important exclusion criteria (maternal depression).

2. Pre-gestational diabetes is often included in case groups.

3. Reporting of glycemic control and treatment type in pregnancy is often lacking.

4. Controlling or reporting of confounding comorbid conditions e.g. pre-eclampsia, antenatal infections, maternal psychopathology, medication is often lacking.

5. Developmental outcome measures are variable.

6. In GDM-focused studies only: absence of measures of adiposity/BMI or immunological status (e.g. markers of inflammation, infections) and inconsistent GDM diagnostic cut-offs.

7. No strict characterization of maternal mental health during pregnancy nor any biological measure of cortisol (a hormone implicated in mental health and placental/fetal development) has been used in previous studies to control of its effect against maternal glycaemic status.

Thus far, one study using perinatal MRI in obesity and GDM pregnancies has been reported. Denison et al. (2017) conducted a feasibility study comparing fetal brain volumes in pregnancies of healthy mothers or mothers with diabetes (GDM, Type 1 and Type 2) where 25/26 women were treated with oral metformin or insulin.

B.5.2 Our background work

The UPBEAT study (Poston et al., 2015) evaluated the impact of an intervention improving exercise and diet routines in obese women at risk of developing GDM. This trial was conducted in over 1500 obese women and was followed by the UPBEAT-TEMPO study in which > 500 offspring returned for various health assessments at and 3-4 years of age. To inform the design of myBBM study, given the inconsistencies found in the present literature, we conducted secondary analyses on the extensive database provided by UPBEAT and UPBEAT-TEMPO to understand the likely factors predisposing the offspring to adverse psychological outcomes. Our unpublished analysis so far indicates that maternal antenatal depression is a considerable predictor of psychobehavioural risks in the offspring at 3-4 years, in addition to mode of delivery, length of gestation and the presence/absence of GDM diagnosis. The literature has previously emphasized the role of antenatal diet on fetal brain development and we also showed how maternal depression was associated with antenatal dietary quality.

B.5.3 Rationale of design

Our preliminary work will now help define our participants' exclusion criteria in answering our research question. We thus argue that in order to investigate the extent to which GDM (i.e., the assumption of increased fetal exposure to glucose in pregnancy), can independently alter the brain of the offspring, a prospective

study must then at least exclude maternal antenatal depression as a confounder. A GDM prediction tool, based on results from the UPBEAT study, is currently being validated in a sample of 1102 obese pregnant women. This study is from now on referred to as “the GDM prediction study” and we plan to recruit into the myBBM study women whose participation in this study has ceased at 26-28 weeks’ gestation when they have their GTT which will facilitate data collection, screening.

B.5.4 Biomarkers

The hormone cortisol, implicated in mood disorders, is understood to have a pivotal negative effect on placental function and fetal brain development in pregnancies complicated by maternal depression (Caparros-Gonzalez et al., 2017). Cortisol however, also interacts with glucose metabolism (de Kloet & Herman, 2017). Hence, we will also take measures of cortisol (from hair samples) to further control for its effect on offspring and maternal outcomes. Hair cortisol concentration (HCC) will provide a longitudinal estimation of the trajectory of cortisol in pregnancy. One cm of hair from the base of the scalp provides an average concentration of systemic cortisol for the past month. Additionally, the approach to HCC reduces inter and intra-subject variability seen in the acute measures of cortisol in urine and saliva. At the same time, HCC shows significant correspondence with 30-day integrated daily salivary cortisol concentration (Short et al., 2016). Thus, for example, a sample of 6 cm from the scalp can provide a longitudinal index of maternal cortisol for the 6 previous months, which can then be segmented by month or semester of pregnancy.

B.5.5 Antenatal and Postnatal exposure

Because the health of the mother can have detrimental effects on the fetal development and health, we will record pregnancy exposures extensively. This will include (but not limited): history of infections, comorbidities other than GDM, maternal diet, hospital admission, prescriptions and infant feeding (breast milk or formula or mixed; see 6.6. for Assessments).

Incorporating antenatal and postnatal measures of the offspring brain, could then unveil a predisposition of the child to neurodevelopmental deficits following exposure to maternal obesity and GDM independent of other events occurring around birth and beyond. To some extent this will test our previous findings based on the UPBEAT data analysis, using a prospective approach but importantly have the advantage of implementing a much more rigorous design than any published study on the matter has been able to provide.

B.5.6 Perinatal MRI

Employing MRI in utero and within the first weeks after birth, lends itself particularly well to disentangling the pre- and postnatal effects. Another strength of perinatal MRI is that it is more sensitive and precise than methods used in previous studies associating maternal diabetes/obesity to offspring brain measures such as magneto- and electro-encephalography (Cai et al., 2016). Furthermore, these studies were conducted in older samples of children and given that the brain continues to develop well beyond the first year of life, these studies cannot remove the influence of postnatal environmental factors and experiences.

To our advantage, novel MRI techniques developed in our department can now also characterize placental function in this in utero milieu to further enhance our understanding of potentially important physiological mechanisms and identify critical periods. Importantly there is virtually no prospective study which has at-

tempted to longitudinally associate maternal obesity/GDM, antenatal blood markers (inflammatory cytokines and glucose), hair cortisol, ante/postnatal MRI measures (brain and placenta) and later offspring developmental outcomes. We deem this approach necessary to clarify and add evidence to the mechanisms involved in increasing neurodevelopmental adverse outcomes observed in the offspring.

B.5.7 Fetal Electrocardiogram (ECG)

Placental dysfunction results in fetal compromise both in overall somatic growth but also in alterations or even injury to the developing fetal nervous system. Fetal electrocardiographic (fECG) measures of heart rate variability provide a measure of the fetal autonomic nervous system. Heart rate variability has been linked to measures on subsequent neonatal Electroencephalogram (EEG) and to the neurodevelopmental outcome of the infant. In addition fetal heart rate variability has been linked to US Doppler parameters of reduced placental function. FECG is easy to record and represents a maternally acceptable, non-invasive, cheap, transportable and potentially widely available tool for the assessment of fetal wellbeing. It has recently been used in over 10,000 maternal recordings in the NIH funded Safe Passage study (Hofmeyr et al., 2014), data from which are currently being analysed. Assessment of the fetus throughout gestation is imperative to understanding placental function. We plan to collect recordings of fetal heart rate variability overnight to compare with our placental MR parameters.

B.5.8 Study benefits

Obese women who received GDM diagnosis are conventionally closely monitored by multidisciplinary medical teams to encourage adequate glucose control through dietary advice or metformin/insulin treatment. The main purpose of treatment is to maintain fetal size within normal range since larger fetuses confer higher incidence of birth difficulties but also potentially obesity and metabolic disorders in their own lifetime (Boney et al., 2005). We argue that if we can find evidence for an antenatal influence on offspring brain development, this could argue for the ongoing careful implementation of glucose control monitoring. Given the burden of adverse neurodevelopmental on the individual and society beyond that of obesity, our findings could potentially call for adaptation of current guidelines and policies surrounding reproductive and antenatal care provided to obese women. Moreover, such enquiry will add valuable insight into the literature on intergenerational transmission of disease risks as well as proposing mechanisms potentiating a predisposition to psychiatric disorders. Importantly, with such knowledge on hand, this could then guide the development of larger studies which could inform the optimization of timely interventions aiming to reduce adverse offspring outcomes.

B.6 STUDY DESIGN & FLOWCHART

B.6.1 Study Design

This is a longitudinal observational study involving scanning pregnant women after their OGTT (26-28weeks) weeks gestation, subsequently their newborn up to 44 weeks postmenstrual age using MRI at both time points. We will then relate the perinatal brain measurements to the children's 12-18 months developmental outcomes.

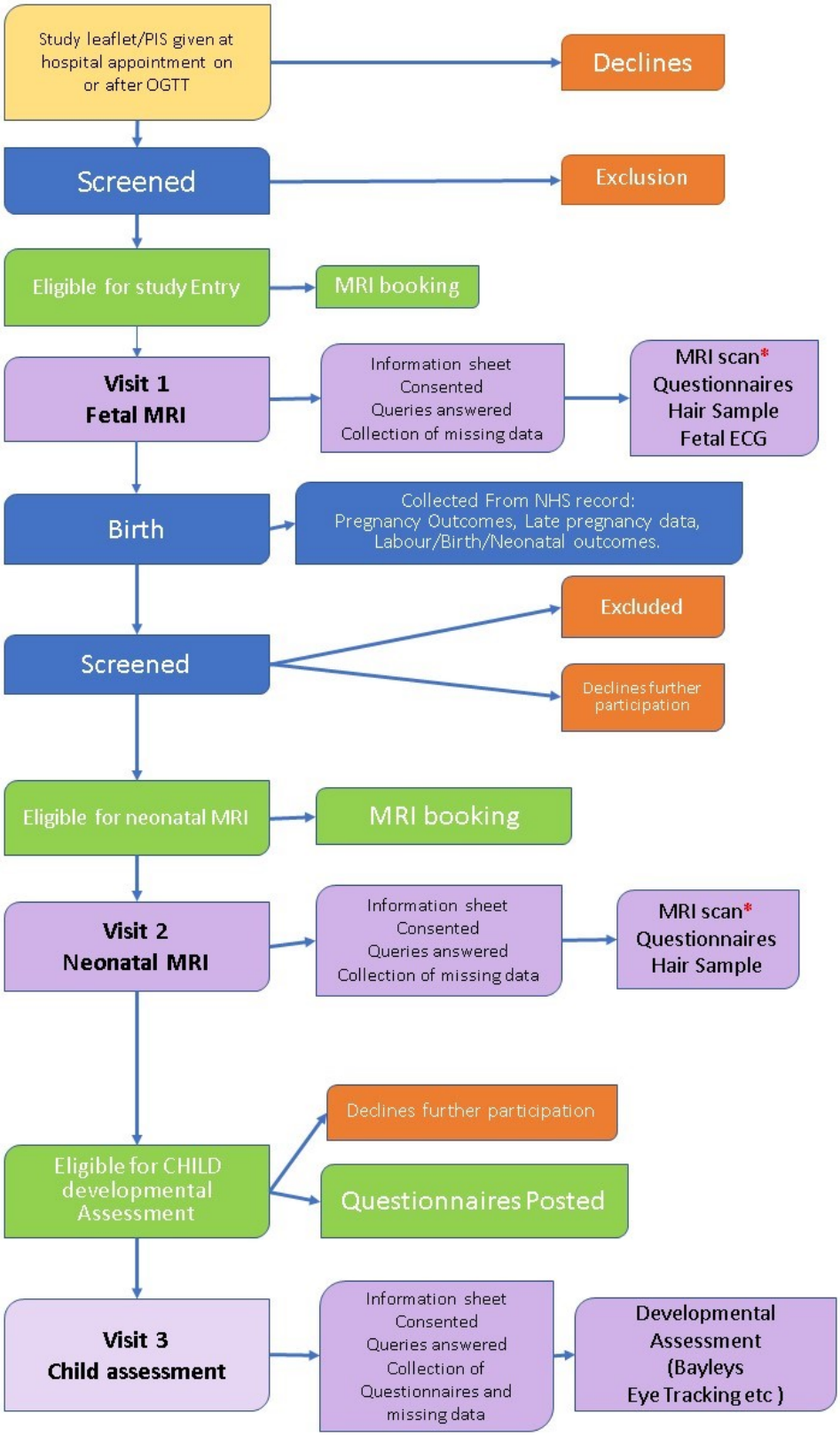
B.6.2 Primary Endpoint

Fetal and neonatal MRI measures of structure, function (fMRI) and diffusion (dMRI). Antenatal MRI measures of placental function. Maternal hair cortisol concentration collected at fetal or neonatal scan.

B.6.3 Secondary Endpoints

Children 12-18 months, developmental outcome in offspring (including but not restricted to Bayley's developmental scale, eye tracking).

B.6.4 Flowchart



B.6.5 Subject selection

As part of the GDM prediction study (IRAS 224961), >1000 obese pregnant women will be recruited between 11-18 weeks of pregnancy when anthropometric measurements, demographic data and blood samples will be collected. The second and last point of contact from the validation study occurs at the routine 26-28 week OGTT. The women's involvement in the validation study ends at this point.

These women are informed about myBBM study after they have provided the final endpoint measures (blood at the OGTT at 26-28 weeks' gestation) of the validation study. Therefore, only women previously enrolled in the validation study and have provided a blood sample at the OGTT will be approached by the direct care team on the day of their visit for recruitment into the MRI (myBBM) study.

We chose to only include these women for the MRI follow-up study so that we can access their demographic, clinical and blood results obtained already through this validation study to introduce the fewest additional measures. As part of the validation study participants will provide blood samples for research use at baseline and follow up visits and answer relevant questionnaires which myBBM study will utilize so as not to burden them with additional requirements. An additional advantage of recruiting from this group is that they would have already been screened against primary exclusion criteria and represent the same demographic population as the UPBEAT study from which our hypotheses and prior analyses are based. We will apply simple additional exclusion criteria based on the already available data about these women (see exclusion criteria). Moreover, the women would have been in contact with clinical and research staff for the purpose of research and we deem they will consider an additional MRI study also during pregnancy.

Following ethical approval, a member of the research team will obtain relevant information from the electronic medical records which will be accessed by the care team and which are obtained through their routine antenatal care. Women who meet inclusion criteria based on their medical record (e.g. show low risk of depression: no history of depression, no antipsychotic or antidepressant prescription or history thereof and normal score on Whooley questions) will be eligible to join the MRI study. Additional information obtained through the validation study and not part of routine antenatal data collection may also be accessed to assess eligibility. As part of the GDM prediction study, women have consented their sample to be used for that specific research and for future research. In their current version (v.2; 01/12/18) of the consent form for the prediction study point 8 states "I understand that my data and samples may be analysed in collaboration with other institutions, for future studies and/or in commercial collaborations with appropriate ethical approval" and section 10 states "I agree to samples and data that I have provided in the GDM Prediction study being shared with other investigators following appropriate approvals….."

B.7 SUBJECT INCLUSION/EXCLUSION CRITERIA

Below is a table explaining the screening against inclusion/exclusion criteria which these participants will have gone through and which additional ones will be applied as part of the MRI study.

B.8 STUDY PROCEDURES

Table B.1: *Inclusion and Exclusion criteria additional to the GDM validation study.*

Inclusion criteria for enrolment to validation study	Additional inclusion criteria applied for enrolment to the MRI study
Aged 18 years or more Singleton pregnancy Gestation 11+0-18+6 weeks' Willing and able to give informed consent	
Exclusion criteria for enrolment to validation study	**Additional exclusion criteria applied for enrolment to the MRI study for FETAL scan**

Exclusion criteria for enrolment to validation study	Additional exclusion criteria applied for enrolment to the MRI study for FETAL scan
Pre-existing diabetes (by HbA1c or early pregnancy Oral Glucose Tolerance Test)	Any alcohol and drug consumptions in pregnancy.
Hypertension requiring treatment pre-pregnancy/in pregnancy	Any smoking in pregnancy.
Chronic renal disease	Infection and treatment known to impact fetal brain development (e.g. Chicken pox)
Thyroid disease	Maternal developmental/intellectual/learning disability (Dyslexia, Autism, Tourette etc)
Systemic Lupus Erythematosus	Counter indication to MRI imaging (e.g. metal implants).
Antiphospholipid syndrome (APS)	Previous history of depression
Thalassaemia	Antidepressant use prior to or during pregnancy.
Coeliac Disease	Positive score on the Whooley questions at 11-14w.
Current psychosis	Above cut-off (12 points or more) on the Edinburgh Postnatal Depression Scale after 20 weeks of gestation.
Taking metformin	Maternal comorbid diagnosis other than GDM including but not limited to: pre-eclampsia, chronic hypertension, autoimmune disease.
Taking medications that affect insulin sensitivity (oral antihypoglycemic agents, antipsychotic drugs, steroids)	Fetal abnormalities e.g genetic, neurological or physical malformations detected at anomaly ultrasound scan (performed routinely as part of antenatal care)
Bariatric surgery	

Exclusion criteria for enrolment to validation study	Additional exclusion criteria applied for enrolment to the MRI study for NEONATAL scan
	Severe delivery complications (including and not limited to: NICU admission, resuscitation, hypoxia-ischemia, spontaneous preterm birth) although information will be kept and documented.

B.8.0.1 Subject recruitment

Women entering the “GDM prediction study” (n>1000; IRAS ID 224961) will be eligible for recruitment for myBBM study.

Recruitment will occur from three entry points/pathways: 1. As part of routine clinical care pathways for obese pregnant women the OGTT is performed at 26-28 weeks gestation and requires 3 hours to complete, it includes several waiting periods. We will take advantage of this long appointment to approach eligible women. 2. Additionally, women who received a GDM diagnosis following their OGTT are invited shortly after diagnosis to formulate a care plan with the multidisciplinary health care team. This visit will add an opportunity to approach eligible women if missed at the OGTT visit. 3. If we deem recruitment to be slow or that we are practically unable to meet and hand a PIS/leaflet to every woman face-to-face at any hospital visit, we will contact previous participants of the validation study by phone (under this study ethics) and present the same information to them, sending leaflets/PIS via email or post and/or answering on the phone. Only those women who have consented, as part of the validation study, to be contacted in the future (clause 11 in their consent form) will be contacted via this recruitment pathway.

In 1 and 2: From antenatal records or appointment lists, clinical/research staff directly involved in the women’s care (a member of the direct health care team) will flag up names of women present on-site who are part of the validation study and happy to be approached for research. Thereupon, a member of the research or antenatal care team (including but not limited to research midwives briefed on the aims of the study) will approach them and introduce the MRI study. Prior to leaving their appointment, eligible women will be handed a study leaflet and asked to verbally approve that their contact information can be passed to the myBBM team. With the potential recruits permission a referral/contact form will be completed to facilitate later contact to gauge interest in the MRI study

All obese women will receive a GDM diagnosis or a clear result within a week of the OGTT in accordance with the WHO criteria adopted by the Trust. Women will be classified as GDM or No-GDM. They will be contacted by phone first to confirm their interest in the MRI study and have their questions answered or allowed longer to reconsider joining the MRI study after the OGTT appointment.

Potential participants will be given as long as they require to read the provided written study information sheet, to discuss the details with relatives and / or their doctor, and to ask any questions to the research team. No research or data collection will be conducted on pregnant women from whom valid consent has not been received. An appointment for the MRI examination will be given.

B.8.1 Screening

The antenatal pregnancy electronic records of all women handed the leaflet for the MRI study and have approved to be contacted will be checked by a direct care or research team member against main exclusion criteria (e.g. BMI eligible for MRI scanning, low risk of depression, smoking). This process will also be applied before calling women as per point 3 in the recruitment pathway. For example, women who have low risks of antenatal depression as per their medical notes/records provided as part of their antenatal care and through the GDM prediction study will be eligible for recruitment to the MRI study at first instance. For example, any woman who poses risk for psychopathology will be excluded from further contact: if in pregnancy notes they report antidepressant medication or are positive on Whooley questions which assess low mood or

alcohol/smoking during pregnancy. All screened participants will be logged into the study specific screening. Only researchers on the study delegation log are authorized to complete this task.

Following this initial screening, eligible women will be contacted and if they are willing to take part they will be asked to review the PIS and complete an additional screening. This will entail filling, online or in paper form, a consent form prior to completing a questionnaire screening against depression. This consists of the Edinburgh Postnatal Depression Scale (EPDS;). and a question about history of psychiatric disorders and a question on learning disabilities and developmental disorders , substance for screening purposes. The EPDS is a validated questionnaire for detecting both antenatal and postnatal depressive symptoms. The 10-question (EPDS) is a valuable and efficient way of identifying patients at risk for “perinatal” depression and will provide data regarding any additional risks of maternal depression not previously detected via recruitment screening. The EPDS is quick and easy to administer. The EPDS will be sent to them by post or as a link via email to the online platform Qualtrics. Qualtrics data is stored securely on the Qualtrics server located in the EU, to meet EU data requirements. Participants will be asked to read the initial information sheet and sign consent form prior to completing the Qualtrics questionnaire . They will have the choice of sending the paper form to send back by post or with a choice of sending the electronic format or a photo of the paper format to the research team using an encrypted GSTT email address.

Any woman who scores above the EPDS cut-off of 13 points, or reports a history of psychiatric disorder or learning disability or developmental diagnosis will no longer be eligible for the MRI study. The mother’s routine care providers (GP, midwives, obstetrician etc) will be alerted to high scores obtained on the EPDS and results will be discussed with the patient. Women scoring below 12 remain eligible to participate in the MRI study.

Eligible women will then be contacted again by the investigator or another trained MRI recruiter by phone to ask standard questions related to MRI safety screening. Given they declare no contraindications to MRI (e.g. metal implants) they will be booked for a scan at a mutually agreeable time some time in pregnancy thereafter. We will contact women until we have 35 non-GDM and 35 GDM participants booked for an MRI. 6.3. Recruitment schedule From previous findings, in the sample of 1000 women aim to be joining the validation study, 15% will develop GDM (n=150). To reach 30 GDM and 30 non-GDM successfully imaged cases, we allow for 10 additional participants in the event some imaging data has to be rejected due to technical faults so that 70 women (35 GDM diagnosed and 35 without GDM) will be approached in total. We expect to be recruiting 1 woman per week so that recruitment should take around 18 months.

B.8.2 Participant Visiting Schedule

1. The first visit will be the fetal MRI appointment where the procedure and aims of the study will be re-explained and women will be asked to provide informed written consent and screened for MRI safety prior to start. The first outcome measures will be collected here: MRI of the maternal abdomen, including but not limited to fetal brain/body and placenta. She wil also fill questionnaires (see 6.6.). After the MRI examination, a portable fECG monitor (Monica AN24) will be used to collect fetal heart rate recordings. This device will be attached to the participant’s abdomen with several small adhesive pads and a short recording will be taken at the visit. If any abnormality is detected that may be of clinical significance such as a fetal bradycardia, we will repeat the trace or continue recording to ensure if recovers. If it does not recover, we will refer the mother to maternity services for immediate follow

up. The participant will then be shown how to connect and disconnect the device which they will then take home in order to collect an overnight recording of the fetus' heart rate. Full instructions on how to attach and detach the fetal ECG monitor will be given to the mother by a trained midwife or doctor. They will be scheduled to have the collected from the participant's home via a courier service. Maternal hair cortisol will be collected here if they consented to it.

Subsequent to the birth, participating mothers will be invited by telephone call and/or email to bring their newborns to the hospital for a follow-up neonatal scan before 44 weeks postmenstrual age.

2. A second visit will involve a MRI scan of the neonate using similar measures to those acquired in the fetus (e.g., dMRI and structural MRI) and will also include functional connectivity (fcMRI). The neonatal scan offers the opportunity to measure the offspring brain using state-of-the-art and ultra-precise MRI technology which helps detected very subtle brain differences between GDM and absence of GDM exposure. Women will fill questionnaires during this visit (see section 6.6). Maternal hair cortisol will be collected here if they consented to it.

This will conclude collection of primary outcome measures. We hope to maintain retention for the neonatal MRI by building a rapport with the women during our interactions in the antenatal period. The research team will maintain contact with participants by sending birthday cards on the child's first birthday.

Participating women will be contacted by telephone and/or email near 12 months post-birth to invite them for a developmental assessment of their child.

3. The third visit is anticipated approximately 12-18 months after birth. This will entail (but not exclusively) employing eye-tracking technology and the commonly employed Bayley's Infant Development Scale, as conventionally used in our department. Questionnaire data (see 6.6.) will be collected here. This will conclude the collection of secondary outcome measures and will correspond to the end of participation for women and their children. The MRI data acquired antenatally and in the newborn will be correlated longitudinally with cognitive and behavioural measure taken at 12-18 months.

B.8.3 Consenting

In all parts of the recruitment, it will be made clear that women can consent to part of the study (fetal or neonatal scans, with or without follow-up) or its entirety and that a request for a sample of their hair sample at the MRI appointment will also be optional.

Written consent will be received from the participant prior to any data collection. Consent will be taken by researchers who have valid GCP certification and study specific training. If there are any concerns about the mother or parents' ability to understand the information being given to them because of language barrier, we will endeavour where possible to use interpreters to confirm full understanding. In case of non-availability of those no consent will be sought and the participants not enrolled in the study.

Written consent will be obtained from the mother for fetal scans and the parent(s) with legal parental responsibility for neonatal scans using a study-specific consent form (CF) . Consent will only be given once the mother or parent has had the opportunity to read the provided information sheet, discuss the study face to

face with the research team or staff (radiographers, midwives, clinicians who are employed by our department and have been fully briefed on the aims of the study and on the delegation log), and had any questions answered to her satisfaction.

For the follow up visits, and more specifically for the 12-18 months assessment, it is not always possible for parents to attend the appointment with their child (e.g. due to work commitments). In such cases, the parent(s) may prefer that a carer (e.g. nanny, family member) bring their child to the appointment. As the carer would not have parental responsibility for the child, parental consent would have to be given before the appointment visit. If the parent agrees and has already received and read the PIS and ICF, verbal consent can be taken over the telephone whereby the consenter would write a telephone summary and post/email a copy (with a copy of the consent form) for them to review, sign and ask that a witness sign the form and send back. Alternatively, face to face consent can be taken place if the parent is situated locally to the research site (St Thomas' Hospital) e.g. working in London. A member of the research team would visit the parent and go through the informed consent process as they would if this process took place on site. Individuals lacking mental capacity to consent during recruitment or lose capacity after enrolment will be excluded from the study.

B.8.4 Collection of data on medical status and clinical observations

Following consent, data will be collected from mothers and parents by talking to them, by the administration of a short questionnaire and by review of the medical notes or hospital record during pregnancy or at discharge from their point of delivery (i.e. discharge summaries). This provides patient demographics, social history and subsequently to inform assessment of the impact of multiple factors including gender, maternal illness, medications and obstetric factors.

B.8.5 Schedule of assessment and questionnaires for each visit

The following questionnaires allow for the collection of information relating to other important exposures above and beyond maternal obesity and GDM which could confound our findings on fetal/neonatal brain and long-term outcomes. The timing of when these are administered is found after.

A. Edinburgh Postnatal Depression Scale (EPDS). We would like to request permission to administer this short questionnaire as it is possible that, although screened prior to enrolment, the women may develop depression after enrolment. The 10-question Edinburgh Postnatal Depression Scale (EPDS) is a valuable and efficient way of identifying patients at risk for "perinatal" depression and will provide data regarding maternal depression and infant brain development. The EPDS is quick and easy to administer. The mother's routine care providers will be alerted to any high scores obtained and results will be discussed with the patient.

B. The Prenatal Distress Questionnaire (PDQ, Yali & Lobel, 1999) The PDQ is a brief, 12-item self-report questionnaire with scores ranging from zero to 48 on a 5-point Likert-type scale ranging from zero (Not at all worried) to four (Extremely worried). It was designed to assess women's worries and anxiety regarding pregnancy and birth. The items ask about pregnancy-specific worries, including the baby's health, labour and delivery, medical problems, parenting, physical symptoms, pregnancy related bodily changes, and relationships. It is an easy-to-complete questionnaire that has good face validity, con-

current validity and internal consistency (Alderdice & Lynn, 2010). We would like to include it in our study as pregnancy specific anxiety has been shown to be a better predictor of poor developmental outcomes than general anxiety (Huizink et al., 2003).

C. Perceived Stress Scale (PSS, Cohen et al., 1994) PSS is one of the most widely used instruments to assess perceived stress. It is a 10 item self-reported questionnaire that was designed to measure "the degree to which individuals appraise situations in their lives as stressful". PSS is an easy-to-use questionnaire with established acceptable psychometric properties and has been widely used in pregnancy (Lee, 2012).

D. Food Frequency Questionnaire (FFQ). Diet will be assessed in all participants using a semi-quantitative food frequency questionnaire (FFQ) adapted from the UK arm of the European Prospective Investigation into Cancer Study (EPIC) (Flynn et al., 2016). The FFQ was a shortened version (50 items) of the EPIC questionnaire and focused primarily on assessing intake of food groups relevant to the UPBEAT intervention and modified for a multi-ethnic population, and which we employ here to keep consistent with our previous findings from the UPBEAT cohort and understand the role of antenatal diet in our associations of interest.

E. EQ-5D-3L is a self-reported standardized measure of health status which provide a simple, generic measure of health for clinical appraisal on aspects of 5 items: MOBILITY, SELF-CARE, USUAL ACTIVITIES, PAIN / DISCOMFORT and ANXIETY / DEPRESSION. Severity is label at 3 levels to form a numerical description of a health state. In addition the participants is asked to rate their overall health on the day on a scale of 0-100.

F. Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in the adult, including pregnant women. It differentiates "poor" from "good" sleep by measuring seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction over the last month. Sleep abnormalities such as Obstructive sleep apnea are more frequent in the obese population and can impact fetal growth and placental function (Telerant et al., 2018)

G. Study specific Health Questionnaire: We would like to obtain data on maternal health which may not be recorded as part of routine care but also to ensure the mother has not developed conditions which would impact our results. This includes asking her for any event of infections, comorbidities and medications being prescribed throughout pregnancy.

H. Hair sample questionnaire: Information about hair-related characteristics is collected via a questionnaire administered when the hair sample was collected. Participants will be asked whether they dyed or treated (perm or chemical treatment) their hair. They were also asked about the number of times per week they washed their hair with shampoo. Participants are also asked about their natural hair colour, if they had used hair dye. This is done so that hair treatment can be taken into account in the accuracy of cortisol extraction (Abell et al., 2016).

1st visit (after the Fetal MRI, paper, 15-20 min) the pregnant woman will complete: - EPDS questionnaire for depressive symptoms. - PDQ - EQ-5D-3L on general health status - PSS perceived stress scale - FFQ - PSQI - Study Specific Mother Questionnaire

2nd visit (after the neonatal MRI, in paper, 15 min) the mother will fill: - EPDS - EQ-5D-3L - PSS - FFQ - PSQI - Study Specific Health Outcome questionnaire

B.8.6 Follow up Procedures

Women who consent to take part in the MRI study will be invited to come back with their child for a 12-18 month follow-up assessment. They will be provided with a separate PIS and required to provide written informed consent for this aspect of the study. Parent(s) will be sent the questionnaire booklets by post prior to their appointment which takes 45min to fill. Mothers will be asked to complete the following questionnaires (See Appendices): A. Early Childhood Behaviour Questionnaire (ECBQ; Putnam et al., 2010: Achenbach et al., 2000,) or IBQ-R (Garstein & Rothbart, 2003) depending on age in months of the child. They both measure temperament. B. Quantitative Checklist for Autism in Toddlers (Q-CHAT) (Allison et al., 2008.)) C. Child Behavioural Checklist for Ages 1.5 to 5 (CBCA; Putnam et al., 2006,)) D. Child Eating Behaviour Questionnaire (CEBQ). E. EPDS ( ) The parents will be asked to bring the completed questionnaire booklets to the assessment session. The visit at 12-18 month follow-up child assessment will include: A. Bayley Scales of Infant and Toddler Development (3rd edition; Bayley-III) B. Eye tracking

The Bayley Scales of Infant and Toddler Development (3rd edition; Bayley-III; Bayley et al., 2006) will be used to assess the child's cognitive, language and motor abilities. In addition, gaze behaviour will be characterised with gaze tracking using a Tobii TX-300 eye tracking equipment using validated tasks that assess visual attention, cognitive control and social behaviour. Atypical gaze behaviour is an early sign of an emerging neuropsychiatric condition, which can be detected from around 6 months of age (Jones et al., 2013), well before a clinical diagnosis is feasible which is typically after 3 years of age. Gaze tracking allows characterising gaze behaviour through tasks that require no complex cognitive or motor skills. This is of particular interest in the preterm population, where existing parent questionnaire-based screening tools for neuropsychiatric conditions are confounded by the high prevalence of coexisting cognitive and motor impairments [31]. The eye tracking session will take approximately 40 minutes and will be video recorded (recordings will be stored on encrypted and password protected hard drives). The assessment will be carried out by trained researchers, paediatricians and psychologists. The duration of the whole assessment and follow up visit will be approximately 3 hours and includes rest breaks. Our department has many years of experience receiving young children for such assessment.

B.9 RADIOLOGY ASSESSMENTS

B.9.1 MRI scanning process

MR scans will be performed on either the neonatal Philips (Best, Netherlands) 3-Tesla Achieva MR scanner in the Evelina Newborn Imaging Centre at St Thomas' Hospital or the Philips 1.5-Tesla Ingenia MR scanner in the X-Ray Department of St Thomas' Hospital and follow the Standard Operating Procedures in place in the Centre.

B.9.1.1 Fetal scans

Fetal scans will be acquired by scanning mothers. Scans will take approximately 60 minutes per fetus and no longer than 90 minutes, and mothers will be able to interrupt imaging to leave the scanner if they wish.

Pregnancy pillows will be used to ensure maximum comfort for the duration of the scan. Mothers will be provided with earphones for music during the scan. Communication with the radiographers through the earphones will be frequent. The participant will be shown the MRI images after the scan and will be provided with an electronic copy of the images.

For incidental findings of the MRI images in participants of myBBM study, the results will be discussed with the participant and a referral made to the appropriate clinician. Participants will be given the option to withdraw from the study without this effecting their ongoing care. Any unexpected MR findings will be discussed with the parents. If any further action is deemed appropriate, this will be discussed with the mother and then organised by her obstetrician or relevant clinician. A copy of the MR report will also be sent to the mother's obstetrician and/or relevant clinician.*

B.9.1.2 Neonatal scans:

Neonatal scans will be performed during sleep without the use of anaesthesia. Imaging will be undertaken after feeding the infants and allowing the baby to fall asleep according to conventional protocol (Huges et al., 2017). A paediatrician or research nurse trained in neonatal and paediatric life support will be present at all times during the preparation and scanning of neonates.

MRI-compatible heart rate, oxygen saturation, and temperature monitoring, along with ear protection will be used to ensure safety during the scan. MR scanning will take approximately 60 minutes but may take as long as 90 minutes if the infant wakes and needs to be settled; image data will be anonymised and stored. Any unexpected MR findings will be discussed with the parents. If any further action is deemed an appropriate clinicial referral will be made

B.10 END OF STUDY DEFINITION

The MRI data acquired will be correlated longitudinally with offspring cognitive and behavioural measure taken at 12-18 months. This will be measured employing eye-tracking technology, the Bayley's Infant Development Scale and collecting other standardized questionnaires. Trial will end upon completion of this last assessment or at any other visit if subject decline further participation.

B.11 LABORATORIES

B.11.1 Biomarkers

As part of the GDM prediction study, women would have provide 2 blood samples and have consented to have their data and anonymized blood samples shared with other ethically approved studies. These anonymized blood samples are stored within KCL at -80oC in dedicated fridges in the deparmentdepartment of Women's and Child Health , St. Thomas' Hospital and will be analysed within KCL (same department) and thus a Material Transfer Agreement will not be required. myBBM study will retrieve these existing blood samples from storage to look at biomarkers of interest (e.g. inflammation). All blood samples will be taken by trained staff using universal precautions. Each blood study sample are given a unique study specific code by the GDM prediction study, which will be linked to a the myBBM password protected participant database (a spreadsheet created for this study) held electronically on encrypted hard drives and on NHS/KCL networks

behind the Trust or University firewall. The collection, arrival at the Women's Health Academic Centre, and any use of the sample will be recorded in the study database. Anonymised unused samples will be put back into storage facility in accordance with King's Health Partners procedures and policies or securely disposed at the Women's Health Academic Centre, St. Thomas' Hospital and in accordance with all applicable legal and regulatory requirements.

Women are asked to consent to provide hair samples as part of myBBM at the fetal scan and/or neonatal scan. At the fetal or neonatal scan visit (visit 1 and 2) women will be asked to voluntarily provide a hair sample. If they consent (as per clause on the fetal or neonatal CFs) , the researcher will cut up to app. 0.5 cm2 patch with the back of the neck, 4-7 cm above the bottom hairline. All samples of hair will be stored in an aluminium sheet and in a plastic zip-locked bag, marked with anonymous study identifier and stored in a fridge at the department of Women's and Child Health or at room temperature as per conventional protocol (Caparros-Gonzalez,et al.,. 2017) for later analysis. Extraction of maternal cortisol will be performed from the hair also at the department of Women's and Child Health. Hair without root does not fall under "relevant material" under the Human Tissue Act. However, following good practice we will provide a Materal Transfer Arrangment to transfer the hair from the 1.5T suite (GSTT) into the storage facility of KCL (Department of Women's and Child Health), both at St Thomas' Hospital. The 3T neonatal scaning suite is KCL infrastructure and as such will not require the MTA.

B.12 Sample Analysis Procedures

Analysis procedures will follow local protocol agreed with the chief investigator of this study.

B.13 DATA RECORDING/REPORTING

Data from questionnaires will be collected in paper form at enrolment and MRI visits. Paper forms will be stored in locked facilities at the Department of Perinatal Imaging and Health. Data extracted will be logged onto a spreadsheet which includes anonymized participants' ID's only.

The Chief Investigator will act as custodian for the study data. Patient data will be pseudo-anonymised. All pseudo-anonymised data will be stored on a password-protected computer with restricted access. Any data exported from the personal computer to outside the hospital or university firewall will be pseudo-anonymised and stored on Trust approved encrypted military grade memory sticks. Personal data will not be used freely for further research if this research is beyond the scope of the participants' original consent. Any personal identifiable information will be stored in databases in the NHS network and copy documents in access restricted locked cabinets inaccessible to the public and staff not involved directly with the work.

B.14 SAMPLE RECEIPT/CHAIN OF CUSTODY/ACCOUNTABILITY

Handling of the samples upon arrival at the laboratory will be documented by the investigator. Upon receipt of the samples, the laboratory should ensure that the physical integrity of these samples have not been compromised in transit. If it has, it is important that the study teams, as well as the sponsor, are informed of this. Upon receipt of samples laboratory staff should ensure that all samples are accounted as per the

labelling. All samples received should be logged in an accountability log.

(omitted from this chapter :Adverse events)

B.15 COMPLIANCE AND WITHDRAWAL

B.15.1 Withdrawal / dropout of subjects

The participant's involvement with the project will last approximately 24 months from 24 weeks of pregnancy. If during this time they lose the capacity to consent they will be withdrawn from any future parts of the study. Any data already collected will be retained; it will not be used to plan or influence clinical decisions. As such it will have no immediate benefit or risk burden for the withdrawn participant. Should the woman or her family request for the collected data to be discarded their wishes will be upheld. A participant may choose to withdraw herself and her child at any stage during the study. This option will be provided within the written information on the participant information sheet and discussed at the time of informed consent. She/parent would be told that withdrawal at any time during the study would not affect her/the child's clinical care and management. Any data or samples collected will be retained unless otherwise specifically requested by the woman/parent.

B.16 DATA / SAMPLE STORAGE AND CONFIDENTIALITY

All study data will be stored for a minimum of 25years in a dedicated study database established to contain both imaging and ancillary data. Patient identifiable data required for administrative purposes (e.g. name and contact details) will be stored in a separate database established within the NHS firewall at St Thomas' Hospital and each patient given a unique patient code. All study MRI data will be kept in a password protected anonymised linked database: patient identifiers will be removed from the images prior to data storage and linkage will only be possible by decision of the Principal Investigator. Each MRI scan will be assigned a sequential reference number prior to data storage. Image data will be exported directly from the MR scanner to the database and access to the database controlled by the chief investigator and accessible only from within the Perinatal Imaging and Health department of King's College London.

The reports of MRI scans and neurodevelopmental assessments will be made available to patients' medical attendants and put in the patient's clinical records, as per convention. Gaze tracking, performed as part of the follow-up visit, will involve video recording of the child during performing the tasks. Anonymization of video is not possible, therefore such records will be stored securely on encrypted hard disks at premises of King's College London in line with the General Data Protection Regulation (2018), Information Governance guidelines, and local data protection policies.

Hair samples will be held at St Thomas' Hospital, in a secure research laboratory and will be stored there for the duration of the study. Hair sample will be destroyed conventionally as not under HTA regulation criteria.

B.17 STATISTICAL CONSIDERATIONS

B.17.1 Sample size

This is an observational study. As this is the first study of this kind and design we cannot produce formal power estimation. Rudolph et al. (2018) detected differences in neonatal brains from a sample of 84 neonates. Aligning with recruitment procedures already established at our department for longitudinal studies using perinatal MRI, we estimate retention rate from neonatal MRI to the 12-18 month follow-up to be 80%, representative of what similar studies obtain in our department (e.g. Edwards et al., 2017). Given the effect size of neurodevelopmental risk in the offspring of obese mothers and previous sample sizes deemed adequate for perinatal imaging analyses we estimate 30 case and 30 control participant with successful MRI data to be adequately powered against a control sample of 234 cases (born of normal-weight no-GDM pregnancies) for which MRI imaging and follow-up outcomes are available already in our department.

B.17.2 Software

MR data analysis will be performed off-line using computer software specifically designed for processing MR data. The software packages used will include Philips ViewForum, FSL, ITK-SNAP, IRTK, and other software being developed for MRI data analysis of placenta and brain images. Data will be used in multiple analyses including group comparisons. Statistical analyses between groups will include softwares such as SPSS and Mplus.

(Omitted sections : Approvals, Finances, Insurance, Direct Access to Source of Data and Documents)

B.18 REPORTING AND DISSEMINATION:

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. Progress of the study and results will also be published on the study website (www.mybbm.co.uk) and twitter. No patient identifiable information will be published. Publications resulting from data shared with collaborators or academic institutions require the agreement of the immediate research team.

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