Developmental origins of disease: Intra-uterine programming of offspring metabolic function and long-term markers of health

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Abstract

It is widely acknowledged that maternal obesity and metabolic disorder during pregnancy may trigger long-lasting cardiovascular dysfunction in the mother. In obese pregnancy, there is progressive insulin resistance and a systemic inflammatory end-organ structure and function. Emerging evidence suggests that the offspring may also be subject to these metabolic changes and endure these long-lasting effects. The 'Developmental Origins of Health and Disease' (DOHaD) model suggests that exposure to an abnormal environment in utero, such as in obese or diabetic pregnancy, may set a trajectory of greater offspring adiposity throughout the life-course. This is demonstrated by the fact that exposure to maternal adiposity during pregnancy is associated with higher offspring birthweight and greater adiposity through childhood and into adulthood. Offspring of mothers with diabetes or hypertensive disorders of pregnancy (HDP), such as pre-eclampsia (PE), may also adopt a greater risk of cardiovascular disease in later life. This cycle is then perpetuated when the affected offspring become pregnant and extend this exponential risk profile onto the next generation. Epigenetic modification has been proposed in the pathogenesis of disease but shared familial lifestyle and behaviours may also be causal. With the increasing prevalence of childhood obesity and metabolic disorders, such as non-alcoholic fatty liver disease (NAFLD), there is a greater need to examine these causal relationships and determinants of the fetal origins of–disease.

Adipokines have both endocrine and paracrine function and are secreted by adipose tissue, as well as the placenta. Leptin is required for the regulation of maternal appetite and despite high levels during pregnancy, pregnancy induces a state of leptin resistance. Leptin is also a valid biomarker of neonatal fat mass and may be used as a proxy for neonatal anthropometry. Adiponectin has an opposing function to leptin during pregnancy and correlates with fasting insulin levels. Low adiponectin amongst obese adults is associated with metabolic dysfunction and systemic insulin resistance.

Using data from the large prospective cohort, The Avon Longitudinal Study of Parents

and Children (ALSPAC), analysis of 5011 cord blood samples was performed for the purpose of this thesis. Offspring biomarkers and birthweight were examined in relation to obese, diabetic and hypertensive disordered pregnancy. Since biomarkers have shown evidence of tracking in later life, repeat measures at serial time points from birth (referred to as 'like for like' measures), using data available from the offspring at later time points, were also examined. Lastly, cord blood adipokines and birthweight were examined in relation to later obesity or development of NAFLD amongst the offspring to determine the impact of fetal over-nutrition, as demonstrated by higher cord blood leptin for example, on the long-term metabolic function of the offspring.

This study has demonstrated that maternal body mass index (BMI), rather than appropriate GWG, was more consistently associated with a wider range of offspring cardio-metabolic markers at birth. Whilst there was limited evidence of GDM influencing cord blood at birth, offspring of mothers with pre-eclampsia displayed higher cord blood leptin and lipids.

There was limited evidence of tracking of cord blood measures from birth into adolescence, however. Specifically, gamma-glutamyl transferase (GGT) and adiponectin correlated with repeat measures taken at birth and age 9. Whilst the association with adiponectin was lost in later childhood, this study has shown that GGT may track from birth to age 17. Lastly, this study has demonstrated that cord blood leptin was weakly, and adiponectin strongly related to adiposity in childhood and adolescence respectively. As cord blood leptin may be used as a reflection of fetal fat mass, the positive association with adiposity in later life demonstrates that the offspring may be 'programmed' at birth and set on a trajectory of enhanced adiposity in later life. Neither birthweight, nor adiposity at birth were related to offspring markers of liver structure or function in adolescence, however. This suggested that behavioural and environmental factors may have contributed to the pathogenesis of NAFLD, and similar disorders, rather than exposure to an adverse perinatal environment itself.

In conclusion, this study has demonstrated a limited association between disordered metabolism in pregnancy and offspring metabolic markers at birth. Whilst cord blood

biomarkers being used as clinical predictors of disease may be the focus of future research, they are already established biomarkers of neonatal anthropometry. Given the direction of the association between cord blood leptin and birthweight with later offspring adiposity, exposure to over-nutrition in pregnancy may therefore have a long-lasting impact on the offspring's phenotype rather than the metabolic function itself.

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Finally, thank you to my family. I am immensely grateful for their constant support. This Thesis is dedicated to the very special loved ones I have lost along the way.

Declaration

The work described in this thesis has not been previously accepted for or is currently being submitted in candidature for another degree. For each chapter in this thesis, I acknowledge the assistance that has been granted. All the laboratory cord blood analyses were performed independently after initial in-house training.

Chapter 3: Gestational weight gain in pregnancy: association with cord blood lipids, adipokines, and birthweight

I acknowledge ALSPAC for the collection of maternal and offspring co-variables used in the analyses. Included in this, is the creation of a linear spline multilevel model relating maternal weight to gestational age so that variables 'pre-pregnancy weight' and gestational weight gain over 3 gestational periods could be created. Clinically this was applied by ALSPAC so that gestational weight could be analysed per 1kg increase at conception and per 40g increase per week of pregnancy for the assessment of gestational weight gain. The cord blood samples collected by ALSPAC were analysed by myself for this thesis. I acknowledge Professor Nelson and Dr. Abigail Fraser for their guidance on the conceptualisation of this Chapter and in the development of the statistical analyses.

Chapter 4: Diabetic disorders in pregnancy: association with cord blood lipids, adipokines, and birthweight

I acknowledge ALSPAC for the collection of maternal and offspring co-variables and for creating the variable gestational weight gain (GWG), as detailed in the Chapter. I also acknowledge ALSPAC for collecting urine samples and performing glucose tolerance tests so that participants could be categorised as no diabetes (healthy), pre-gestational diabetes, gestational diabetes, and those with glycosuria. The cord blood samples collected by ALSPAC were analysed by myself for this thesis. I acknowledge Professor Nelson and Dr. Abigail Fraser for their guidance on the conceptualisation of this Chapter and in the development of the statistical analyses.

Chapter 5: Blood pressure in pregnancy: association with cord blood lipids, adipokines, and birthweight

I acknowledge ALSPAC for obtaining maternal data: blood pressure and urine dipsticks for protein (to create the variables pre-existing hypertension, gestational hypertension, and pre-eclampsia) and maternal weight (to create the variable absolute weight gain and BMI). I also acknowledge ALSPAC for obtaining maternal and offspring cohort characteristics: age, social class, education, smoking, parity, height (BMI), birthweight, gestational age, and mode of delivery. The cord blood samples collected by ALSPAC were analysed by myself for this thesis. Using data created by ALSPAC, it was possible to examine blood pressure across different gestational periods as detailed in the Chapter.

Chapter 6: Programming of offspring cardio-metabolic risk: tracking analytes from birth to adolescence

I acknowledge ALSPAC in the collection of all offspring and maternal co-variables used in the analyses. I acknowledge ALSPAC for the collection and analysis of the offspring serum samples at ages 9 and 17. The cord blood samples collected by ALSPAC were analysed by myself for this thesis. The statistical analyses for this Chapter were performed in part by statistician Dr. Andrew Smith, University of Bristol, on behalf of ALSPAC, and tables are labelled accordingly. The results section was written in conjunction with Dr. Andrew Smith. I acknowledge Dr. Andrew Smith, Dr. Abigail Fraser, and Professor Nelson for their guidance in the interpretation of the results displayed in the Discussion section.

Chapter 7: Programming of adiposity in late childhood and adolescence: association with birth weight and cord blood adipokines

I acknowledge ALSPAC for the collection of maternal co-variables and for obtaining the offspring adiposity measurements (including weight, height, and fat mass), as detailed in the Chapter. The cord blood samples collected by ALSPAC were analysed by myself for this thesis. I acknowledge Dr. Andrew Smith for his contribution to the statistical analysis and contributing

to the data interpretation. I acknowledge Dr. Abigail Fraser, Dr. Professor Naveed Sattar, Dr. Robert Lindsay, Dr. Susan Ring, Professor George Davey Smith, and Professor Debbie Lawlor in their contribution to data interpretation. I acknowledge Professor Scott Nelson for the conceptualisation of the study and his contribution to the statistical analysis and data interpretation and guided revision of the manuscript. All the above authors are acknowledged for their contributions to the final published manuscript.

Chapter 8: Programming of adolescent liver outcomes: association with birth weight and cord blood adipokines

I acknowledge ALSPAC for the collection of maternal co-variables and for obtaining the offspring adiposity measurements, as detailed in the Chapter. The cord blood samples collected by ALSPAC were analysed by myself for this thesis. I acknowledge the assistance of Dr. Andrew Smith for his contribution to the statistical analysis and contributing to the data interpretation. I acknowledge Dr. Abigail Fraser, Dr. Professor Naveed Sattar, Dr. Robert Lindsay, Dr. Susan Ring, Professor George Davey Smith, and Professor Debbie Lawlor in their contribution to data interpretation. I acknowledge Professor Scott Nelson for the conceptualisation of the study and his contribution to the statistical analysis, data interpretation, and guided revision of the manuscript. All the above authors were acknowledged for their contributions to the final published manuscript.

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Chapter 7

Simpson, J., Smith, A. D. A. C., Fraser, A., Sattar, N. ed, Lindsay, R. S., Ring, S. M., Tilling, K., Davey Smith, G., Lawlor, D. A. and Nelson, S. M. (2016) Programming of adiposity in childhood and adolescence: Associations with birth weight and cord blood adipokines. Journal of Clinical Endocrinology and Metabolism, 102 (2). pp. 499506. ISSN 0021-972X Available from: http://eprints.uwe.ac.uk/30422

Chapter 8

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Presentations relating to this thesis

Fetal programming and offspring adiposity, Scottish Annual Consultants Conference, Dunblane Hydro, 2018

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(Winner: Best oral presentation)

Cord blood adipokines and lipids and adolescent non-alcoholic fatty liver disease, Endo 2016, Boston, Massachusetts, US, 2016

Programming of adiposity in childhood and adolescence: Associations with birth weight and cord blood adipokines, Endo 2016, Boston, Massachusetts, US, 2016

Annual presentation of progress, University of Glasgow, 2013-2016

Cord blood adipokines and offspring adiposity, Blair Bell annual academic meeting, Royal College of Obstetricians and Gynaecologists, London, 2013

Abbreviations

ACEI	Angiotensin-converting enzyme inhibitor
ALSPAC	Avon Longitudinal Study of Parents and Children
AST	Aspartate transaminase
ALT	Alanine transaminase
β	Beta, correlation
BP	Blood pressure
BMI	Body mass index
MBRRACE-UK	Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries across the UK
CI	Confidence interval
CMAR	Completely missing at random
CRP	C-reactive protein
CT	Computerised tomography
DAG	Directed acyclic graph
DBP	Diastolic blood pressure
DEXA	Dual-energy X-ray absorptiometry
DNA	Deoxyribonucleic acid
DOHaD	Developmental origins of health and disease
ELISA	Enzyme-linked immunosorbent assay
EPOCH	Exploring Perinatal Outcomes among Children
FM	Fat mass
FLD	Fatty liver disease
GH	Gestational hypertension
GDM	Gestational diabetes mellitus
GGT	Gamma-glutamyl transferase
GnRH	Gonadotrophin releasing hormone
GUS	Growing Up Study
GWG	Gestational weight gain
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome
HCG	Human chorionic gonadotrophin
HDL	High-density lipoprotein

HDLc	High-density lipoprotein cholesterol
HDP	Hypertensive disorders of pregnancy
HMW	High molecular weight
HPL	Human placental lactogen
ICD	International Classification of Disease
IOM	Institute of Medicine
IQR	Interquartile range
IOM	Institute of Medicine
LGA	Large for gestational age
LDLc	Low-density lipoprotein cholesterol
LFTs	Liver function tests
LLD	Lower limit of detection
LMW	Low molecular weight
LPL	Lipoprotein lipase
MAR	Missing at random
MI	Multiple imputation
MNAR	Missing not at random
MRI	Magnetic resonance imaging
MRNA	Messenger ribonucleic acid
MSD	Mesoscale Diagnostics
NAFLD	Non-alcoholic fatty liver disease
NHS	National Health Service
Non-HDLc	Non-high-density lipoprotein cholesterol
NMR	Nuclear Magnetic Resonance
PE	Pre-eclampsia
PEH	Pre-existing hypertension
PGDM	Pre-gestational diabetes mellitus
POMC	Pro-opiomelanocortin
PPW	Pre-pregnancy weight
R	Correlation
SEM	Structural equation modelling
SBP	Systolic blood pressure
SD	Standard deviation

SVD	Spontaneous vaginal delivery
TAG	Triacylglycerol
T2DM	Type 2 diabetes mellitus
TNF-α	Tumour necrosis factor-alpha
USS	Ultrasound scan
VLDLc	Very low-density lipoprotein cholesterol
Vs	Versus
WC	Waist circumference
WHO	World Health Organisation

Chapter1: Introduction

This thesis will examine the relationship between the intrauterine environment, as measured by cord blood analytes (reflecting maternal metabolic health and the environment to which the offspring was exposed during pregnancy) on offspring cardio-metabolic function at birth and in later life. By examining the relationship between these cord blood indices and long-term offspring outcomes, including adiposity and disease relating to metabolic dysfunction (such as non-alcoholic fatty liver disease), their relationship in the causal pathway is examined. Disordered metabolism in pregnancy, namely excessive gestational weight gain (GWG), diabetic and hypertensive disorders, may also influence the intrauterine environment and provide abnormal exposure to the offspring. The extent to which these maternal conditions influence cord blood analytes, reflecting potential programming of offspring metabolism at birth will also be determined. Finally, this thesis will examine the extent to which this biochemical imprint at birth tracks throughout the life course by examining metabolic analytes at serial time-points and comparing repeated measures of analytes ('like for like' measures) at serial time points from birth to adolescence.

Several reviews and publications have highlighted the need for large long-term follow-up studies examining the true effect of the intrauterine environment over environmental and familial factors influencing adiposity later in life (Debbie A. Lawlor *et al.*, 2012; Lawlor, 2013). Using data obtained by the large prospective cohort - the Avon Longitudinal Study of Parents and Children (ALSPAC) - in addition to novel data created for this thesis from analyses of 5011 cord blood samples, it provides an overview of the biochemical influences of the maternal environment, offspring cardio-metabolic function at birth and the impact this may have on the offspring in later life.

1.1 Maternal metabolism in pregnancy

Maternal metabolism alters significantly throughout each stage of pregnancy as energy requirements change accordingly. Accrual of lipid is characteristic of the first trimester, while insulin sensitivity remains under normal pre-gravid regulation (Franz, 1978; Catalano *et al.*, 1999). This accretion of fat is to provide reserve energy stores required for late pregnancy where the demands are greater as the fetus grows and the mother prepares for birth and subsequent breastfeeding. With advancing gestation, the mother becomes progressively insulin resistant (Catalano *et al.*, 1991) and there is compensatory hyperinsulinemia (Spellacy and Goetz, 1963). This process triggers the release of free fatty acids and gluconeogenesis so that the increasing energy requirements of the developing fetus may be met, particularly during the third trimester. This is thought to be secondary to hormones produced by the placenta at this late stage, namely human placental lactogen and leptin. The degree of insulin sensitivity is inversely proportional to placental and birthweight and is,, therefore, a significant adaption in the growth of a healthy fetus (Catalano, Drago, and Amini, 1995). The HAPO study highlights the importance of good glycaemic control in pregnancy and demonstrated that maternal glucose levels are strongly associated with pregnancy outcomes. Birthweight, for example, correlated positively with maternal glucose levels, even where glucose levels did not meet the threshold for gestational diabetes (GDM) (Metzger *et al.*, 2008). As such, it is becoming increasingly apparent that the maternal health and intrauterine environment that the fetus is exposed to in utero may have a significant impact on the offspring.

1.1.1 Obesity

Defined by the World Health Organisation (WHO) in 2000, obesity is characterised by an 'abnormal or excessive fat accumulation that may impair health'. This is further classified by the International Classification of Disease (ICD-10), recognising extreme obesity (E66.8) as a subgroup of its own.

In keeping with the global health pandemic, the incidence of obesity in the UK continues to increase. In 2015, 58% of females and 68% of males were classified as overweight or obese, with a BMI of greater than30kg/m^2 (World Health Organization, 2018). Whilst there is some evidence to suggest that the incidence of obesity in the US may have plateaued (Ogden *et al.*, 2014), the prevalence of obesity in the UK has risen dramatically from 15% in 1993 to 27% in 2015, with morbid obesity increasing three-fold during this time (World Health Organization, 2018). Despite considerable public health campaigns, particularly in pre-school children (Department of Health, Physical Activity, 2011), this phenotype appears to be trans-generational as currently 1 in 3 primary school children in England are classed as overweight or obese (World Health Organization, 2018). Being overweight at age 7 or 13 is positively associated with overweight in adulthood as well as the risk of type 2 diabetes (Bjerregaard *et al.*, 2018). Childhood adiposity is also associated with metabolic dysfunction with elevated cholesterol, triglyceride, glycosylated haemoglobin, systolic blood pressure, and low-density lipoprotein cholesterol (LDLc) (Skinner *et al.*, 2008, 2015).

The rise in obesity, particularly in adolescents and younger age groups (Flegal *et al.*, 2010; Ogden *et al.*, 2016), has particular concern during childbearing years as almost one-third of women aged 20 or over in the US are obese (Flegal *et al.*, 2016). In keeping with national trends in the UK, maternal BMI at first midwife appointment (booking) is rising. In a large retrospective analysis of births between 1989 and 2007, 40% of women were reported as being overweight or obese at their booking

appointment (Barber, Rankin, and Heslehurst, 2017). Cross-sectional data from the UK in 2015 however demonstrates that 45% of women fulfil these criteria (Health & Social Care Information Centre (HSCIC), 2015) and there is a disturbing trend to morbid obesity (BMI ≥40kg/m^2) (Heslehurst *et al.*, 2010). Whilst highlighting the current obesogenic climate, this also emphasises the need to limit escalating trends in obesity amongst women of childbearing age and address the impact on both mother and child (Deierlein *et al.*, 2012) in the short and long-term.

1.1.2 Adipose tissue, obesity, and insulin resistance

Over-expansion of adipose tissue in obesity causes local and systemic dysfunction. Locally, there is dysregulation of adipose function and abnormal secretion of adipokines and pro-inflammatory cytokines as their pathways are upregulated. Leptin and adiponectin for example are expressed differently and the systemic metabolic response to them significantly altered. There are also widespread multisystem effects of obesity. Targeting the liver, skeletal muscle, pancreas, and brain specifically, this will clinically manifest as systemic insulin resistance and the pathophysiological features associated with obesity vascular dysfunction. Collectively this may lead to diabetes, hypertension, development of metabolic syndrome, and ultimately greater cardiovascular risk.

An excess supply of free fatty acid in the liver (due to reduced storage capacity of adipose resulting from insulin resistance) contributes to greater intracellular lipid levels. Excess free fatty acid in conjunction with lipogenesis also contributes to greater production of very-low-density lipoprotein cholesterol (VLDLc) (Lewis *et al.*, 2002). Collectively they compound the greater risk of cardiovascular disease such as atherosclerosis. Locally, greater lipid levels lead to greater oxidative stress which may lead to hepatotoxicity and stimulation of hepatic inflammation (Sanyal *et al.*, 2001). Whilst obesity is a recognised risk factor for fatty liver disease, genetic variants may also pose a greater risk to greater accumulation of hepatic fat (Romeo

et al., 2008). Within the lean population, there is a recognised group that has metabolic dysfunction but normal weight. It is thought that the greater proportion of mat mass may contribute to this predisposition. In this subset of the population, low physical activity was the main aggravator for the development of this phenotype (Karelis *et al.*, 2004). Conversely, among the obese population, not all subjects have metabolic dysfunction and insulin resistance although many display early atherosclerotic changes and vascular abnormalities even in the absence of insulin resistance (Bonora *et al.*, 1998; Karelis *et al.*, 2004).

Accrual of ectopic intracellular fat is also associated with insulin resistance, independent of BMI or total body fat content (Korenblat *et al.*, 2008). Whilst bariatric surgery and weight loss may reduce liver fat and lessen insulin resistance (Petersen *et al.*, 2005; Vitola *et al.*, 2009; Lim *et al.*, 2011; Rossi *et al.*, 2012), exercise alone has even been shown to improve hepatic fat content irrespective of the outcome on body weight (Hallsworth *et al.*, 2011). Hepatic fat content, therefore, plays a key role in hepatic glucose production and insulin sensitivity.

Obesity induces dysregulation of metabolic pathways and stimulates inflammatory processes which have a local and systemic effect. Clinically these may manifest in disease associated with disturbed end-organ structure and function. This cycle is then perpetuated by an obese-state and consequent dysfunction of normal metabolism. Pregnancy, with its progressive insulin resistance and fat deposition with increasing gestation, therefore carries a greater risk of worsening metabolism not only for the mother but also for the developing fetus.

1.2 Adipose tissue

1.2.1 Anatomical function of adipose tissue

In humans', there are three types of fat depots – brown, white, and beige adipose tissue. Adults have mostly white adipose tissue, and it is this tissue that increases in obesity. In contrast, brown adipose tissue is found mostly in the fetus and neonate with its role mainly in thermogenesis. It has been shown to generate significantly more heat than any other organ in the body (Symonds, Pope, and Budge, 2015) via its unique uncoupling protein (UCP1) that allows free passage of protons across the mitochondrial membrane (Cannon and Nedergaard, 2012). Brown adipose tissue is activated at birth as a result of cold exposure to the ex-utero environment, endocrine stimulation, and increase in UCP1 at birth that occurs during this time. It has been shown that although it may have a role in energy homeostasis in adult humans, it represents less than 1% of the total adipose tissue (Cypess *et al.*, 2009). Although it, therefore, has a relatively minor role in adults, it remains responsive to sold stimuli (Virtanen *et al.*, 2009) and the amount of brown adipose tissue is inversely proportional to BMI. This may support less of a requirement for non-shivering thermogenesis in obese humans or reflect the inability in obese subjects to dispose of excess calories and therefore gain even more adipose tissue (Cypess *et al.*, 2009).

Beige adipose tissue is a more recent discovery and although it contains less UCP1 than brown adipose tissue it resides in white adipose tissue, which has greater mass than any other adipose tissue and therefore has potential for more functional significance (Sepa-Kishi and Ceddia, 2018)

Adipose tissue is a highly active organ of the endocrine system and has a specific function according to its anatomical site. As an organ of mesodermal origin, it is formerly thought to perform a sole function as energy storage as fat in the form of

triglyceride. Its role however has emerged to be more complex and multifaceted as it is emerging that it is a highly active endocrine organ rather than an inert lipid storage organ. While fetal adipose tissue is composed of both brown and white adipose tissue, adults tend to have mostly white adipose tissue. Lymph nodes are also found within adipose tissue and the stromal cells found within demonstrate a localised immune cell response. In combination with the adipocytes found within adipose tissue, macrophages, fibroblasts, and endothelial cells activated during the immune cell response form the majority of the secretory products of adipose tissue.

In contrast, white adipose tissue is widespread and found mainly subcutaneously and viscerally. Muscle and bone marrow are also thought to contain some white adipose as well. Composed of mainly adipocytes mainly derived from mesenchymal cells, white adipose tissue begins to form in the second trimester of pregnancy although most of the accrual and deposition of fetal adipose tissue occurs in the third trimester (Symonds *et al.*, 2003). White adipose tissues' function is mainly in supporting stromal cells and the vascular network.

There may be regional differences in the characteristics and function of adipose depots as the site and size of the adipose depot confers specific metabolic risk. The more metabolically active visceral depots, which include intra-abdominal adipose tissue, are associated with greater cardiovascular and metabolic risk. Visceral adipocytes tend to be smaller in size compared to subcutaneous depots and there is greater stromal cell infiltration into visceral depots, especially in obesity (Harman-Boehm *et al.*, 2007) thus contributing to the greater metabolic risk associated with visceral adipose tissue. Subcutaneous depots in contrast, despite comprising 80% of total body adipose, tend to confer less cardiovascular and metabolic risk. Its role however is more of lipid storage.

1.2.2 Biochemical function of adipose tissue

Under endocrine regulation, one of the main roles of adipocytes within adipose tissue is energy storage. Depending on whether the subject is in an energy-requiring or energy-replete state, determines the relative rate of lipid synthesis and lipolysis. Chylomicrons or VLDLc circulate bound to protein and are hydrolysed by lipoprotein lipase and are bound to the endothelial surfaces of adipose tissue capillaries. The non-esterified fatty acid is then bound to protein and converted to fatty acid coenzyme A. It is then re-esterified with glycerol – 3 – phosphate to form triacylglyceride (TAG) in the endoplasmic reticulum. Hence lipid is stored in the form of TAG as a single anhydrous lipid droplet in the cytoplasm of the adipocyte. During fasting conditions, hormone-sensitive lipase is phosphorylated and relocates from the cytoplasm to the surface of the lipid droplet (Brasaemle *et al.*, 2000). Triglyceride is then hydrolysed, and one molecule of glycerol and three molecules of fatty acid are released into the circulation. The free fatty acid is released to the plasma membrane from the lipid droplet bound to the fatty acid-binding protein (Baar *et al.*, 2005). The free fatty acid is then bound to albumin and transported to the liver where they undergo beta-oxidation whereas glycerol is exported and used as a substrate for gluconeogenesis in the liver.

Insulin is the principal hormone involved in enhanced lipid storage as it promotes lipid esterification and inhibition of lipolytic enzymes. It also promotes translocation of the Glucose Transporter 4 to the cell surface which in turn increases glucose uptake. Glucocorticoids (cortisol) also promote lipid storage by limiting lipolysis. Glucose is then phosphorylated and enters the lipid synthesis pathway. In contrast, catecholamines (adrenaline and noradrenaline) are mainly responsible for the release of lipids via lipolysis. Acting via adrenoceptors beta 1 and beta 2, they promote lipolysis of the adipocytes.

1.2.3 Endocrine function of adipose tissue

Adipose tissue is a highly active endocrine organ and its role in lipid storage is only one element of its function. Adipokines are hormones with endocrine and paracrine functions. They are secretory products of adipose tissue. They form a group of hormones that include adipokines leptin and adiponectin, as well as inflammatory cytokines interleukin-6 and tumour necrosis factor-alpha. In obesity, the synthesis and secretion of inflammatory products become dysregulated although their role in the physiological control of adipose tissue is unclear. Whilst leptin has been shown to correlate with insulin sensitivity in some studies, leptins' counterpart, adiponectin, may be more closely associated with this (Kirwan *et al.*, 2002; McLachlan *et al.*, 2006).

Leptin has an important role in pregnancy for both the mother and the fetus. High levels of leptin in pregnancy are thought to be for the regulation of maternal appetite. Leptin is a 16kDa hormone that is mainly produced by white adipose tissue (Zhang *et al.*, 1994) but it is also produced by the ovary (Cioffi, 1997), pituitary (Jin *et al.*, 2000), and by the placenta in pregnancy (Masuzaki *et al.*, 1997; Jin *et al.*, 2000). Subcutaneous adipose specifically is thought to be the main contributor to circulating leptin concentrations and leptin mRNA is greater in subcutaneous compared to omental adipose tissue (Montague *et al.*, 1998). Leptin has a diverse role as it has pleiotropic effects on multiple organs. It is involved in the regulation of appetite (suppression), satiety, reproductive function, body mass, and immune regulation (Friedman and Halaas, 1998; Chan *et al.*, 2006; Kelesidis *et al.*, 2010). Circulating levels of leptin are proportional to fat mass (Maffei *et al.*, 1995; Considine *et al.*, 1996; Rönnemaa *et al.*, 1997) and correlate inversely with insulin sensitivity independently of fat mass (Silha *et al.*, 2003).

In obesity, there are high levels of leptin (Maffei *et al.*, 1995; Considine *et al.*, 1996). Although leptin enhances insulin-sensitive pathways, in severe obesity there is often severe insulin resistance despite elevated levels of leptin (Rossetti *et al.*, 1997).

This may reflect end-organ insensitivity to leptin in obese subjects causing reduced hypothalamic signalling in response to leptin. In human pregnancy, the placenta is a major source of leptin (Masuzaki *et al.*, 1997). In contrast, mouse studies show that leptin is mainly produced by adipose tissue in pregnancy and not by the placenta at all (Malik *et al.*, 2005). In humans, circulating leptin levels correlate with hormones human chorionic gonadotrophin (hCG) and oestradiol and with insulin sensitivity (McLachlan *et al.*, 2006). As such, serum leptin levels are consistently higher in pregnant women compared to non-pregnant women (Highman *et al.*, 1998; Hendler, Blackwell, *et al.*, 2005; McLachlan *et al.*, 2006). Leptin levels continue to rise with advancing gestation, in keeping with fetal and placental development (Sattar *et al.*, 1998; Melczer *et al.*, 2003), until around the third trimester of pregnancy. It is around this stage that leptin levels may begin to plateau or even fall, as placental leptin transcript levels are significantly lower at term compared to the first trimester (Sattar *et al.*, 1998; Henson and Castracane, 2006). This reflects the trend in fat mass accumulation although leptin levels are still higher in the third trimester than in the non-pregnant state (Eriksson *et al.*, 2010).

The rise in serum leptin in pregnancy is lesser in obese women compared to lean women (Misra and Trudeau, 2011) which may also be in keeping with obese women gaining less fat mass, but also supporting the hypothesis that other tissues, supplementary to adipose tissue, may also account for the upregulation of leptin production with advanced gestation. These studies are however limited due to the inaccuracies of measuring fat mass and leptin in pregnancy. Fat mass, for example, is difficult to accurately quantify in a pregnant woman because of the distribution of fat and the gravid uterus. Measurement of serum leptin in obese versus lean women in pregnancy may also lead to inaccuracy as obese women have a larger circulating volume. The same mass of leptin will equate to a greater volume of distribution and therefore a lower concentration of leptin in obese women.

Pregnancy, whilst a state of hyperleptinemia, is also a state of relative leptin resistance. Women may develop relative leptin resistance during pregnancy and

therefore eat excessively, stimulating more fat stores. Infusion of leptin in rats failed to suppress maternal appetite (Ladyman and Grattan, 2004). In ob/ob mice, withdrawal of leptin at different points throughout gestation appeared to influence maternal appetite and weight gain (Mounzih *et al.*, 1998). In pregnant rats, there is also increased leptin binding protein (Seeber, Smith and Waddell, 2002) and downregulation of leptin receptors in the hypothalamus (Ladyman and Grattan, 2004) thereby limiting the supply and uptake of leptin centrally. Pregnancy is therefore a state of relative maternal leptin (and insulin) resistance despite the development of hyperleptinemia due to increasing fat stores and placental secretion with advancing gestation. Greater maternal adiposity may alter leptin levels in pregnancy as obesity (along with greater maternal fat mass) is associated with maternal hyperleptinemia as well as reduced amino acid transport and uptake by the placenta (Farley *et al.*, 2009). Intracerebroventricular infusion of leptin in pregnant rats, for example, does not suppress appetite unlike in non-pregnant rats (Ladyman and Grattan, 2004). There is also increased levels of leptin binding protein in pregnant rats (Seeber, Smith and Waddell, 2002), and downregulation of leptin receptors in the hypothalamus (Ladyman and Grattan, 2004) contributing to reduced availability of leptin to the brain. Once released into the maternal circulation, placental leptin (Linnemann *et al.*, 2000) may regulate the energy supply to the fetus via altered amino acid uptake pathways (despite very little placental leptin being released to the fetal side of the placenta) (von Versen-Höynck *et al.*, 2009). Collectively, increased fat stores, as well as placental secretion of leptin, may lead to a state of hyperleptinemia in pregnancy. Relative leptin resistance may ensue during pregnancy as shown in animal models which may perpetuate the process of laying down fat stores in pregnancy.

Adiponectin is a 30kDa hormone protein that is secreted by adipocytes (Scherer *et al.*, 1995) as well as salivary gland epithelial cells (Katsiougiannis *et al.*, 2006), leucocytes (Crawford *et al.*, 2010), skeletal muscle (Liu *et al.*, 2009) and also by the placenta (Caminos *et al.*, 2005; Lappas *et al.*, 2005; Chen *et al.*, 2006). Circulating in the plasma in a multimeric (high molecular weight) form, adiponectin takes on an opposing role to leptin. Despite both being synthesised in adipose tissue, adiponectin

is inversely associated with fat mass, especially in men (Zhang *et al.*, 2005). In obese subjects, there is reduced expression and secretion of adiponectin from adipose tissue (Arita *et al.*, 1999).

In contrast to placental leptin, adiponectin correlates more closely with fasting insulin levels, reflecting insulin sensitivity (Hotta *et al.*, 2000; Weyer *et al.*, 2001; Catalano *et al.*, 2006). Since adiponectin itself is insulin-sensitising, reduced levels of the hormone in obesity may in fact compound the insulin resistance seen in obese subjects. Out-with pregnancy, adiponectin has insulin-sensitising effects but as adiponectin levels fall in pregnancy, women develop relative insulin-resistance (Catalano *et al.*, 2006; Nien *et al.*, 2007). In those that meet the criteria for GDM, adiponectin levels are further suppressed compared to weight-matched non-diabetic pregnant individuals (Retnakaran *et al.*, 2004; Williams *et al.*, 2004). Overweight women, who already have lower baseline adiponectin levels, demonstrate maintenance of circulating adiponectin and there is less of an impact of the pregnant state than that seen in normal-weight pregnant individuals (Nien *et al.*, 2007). As such, treatment with adiponectin can reverse the insulin resistance phenotype of obesity in mice and reduces hepatocyte gluconeogenesis (Yamauchi *et al.*, 2001, 2002). Treatment with adiponectin has also been proposed in adult human subjects (Achari and Jain, 2017).

Targeting a variety of tissues, adiponectin's function is also site-dependent. In skeletal muscle, adiponectin upregulates the expression of fatty acid transporter proteins and stimulates metabolic oxidation (Myeong *et al.*, 2006). In addition, it stimulates glucose uptake by increasing GLUT4 translocation (Ceddia *et al.*, 2005) and enhances mitochondrial biogenesis (Qiao, Kinney, *et al.*, 2012). In the liver, adiponectin is insulin-sensitising (Qiao, Kinney, *et al.*, 2012) and adiponectin levels are, as aforementioned, negatively associated with glucose production (Stefan *et al.*, 2003; Howe *et al.*, 2012).

In pregnancy, circulating adiponectin levels remain, like leptin, fairly constant in the first trimester, but decline with advancing gestation and are lowest in the third trimester. As with adiponectin in the non-pregnant state, circulating adiponectin levels in pregnancy correlate with insulin sensitivity. With reduced expression and secretion of adiponectin, it may assume a specific role in contributing to pregnancy-induced insulin resistance (rather than insulin resistance developing due to dysregulation of maternal metabolism and greater fat mass that occurs during pregnancy). Adiponectin may also alter placental function in pregnancy by negatively regulating placental amino acid transport, collectively altering the placental response to insulin and placental transport of the hormone (Jones, Jansson and Powell, 2010), thereby potentially limiting fetal growth in obese pregnant women.

Adiponectin, therefore, plays a direct role in the development of insulin resistance during pregnancy although this will be multifactorial and will be influenced by the dysregulation of other metabolic processes that also occur during pregnancy.

Whilst other maternal hormones, namely pro-inflammatory cytokines, sex steroids (oestrogens and progesterone), and the maternal pituitary hormone prolactin have all been implicated, placental adiponectin (and resistin) appear to be key in the development of the insulin resistance that occurs during pregnancy. In contrast, placental leptin is more strongly associated with fetal growth and placental function. Collectively, the expression of these adipokines by the placenta demonstrates their complex role in fetal development and physiological adaptions required for a normal pregnancy.

1.3 Obesity in pregnancy

1.3.1 Maternal Effects of obesity in pregnancy

Obesity in pregnancy has implications for weight gain during pregnancy, metabolic and vascular dysfunction, as well as a greater risk of adverse outcomes of labour and delivery. Obese pregnancy has implication s for both mother and baby both in the short and long term (Poston *et al.*, 2016). Whilst the long-term impact obesity may have on offspring's cardiovascular and metabolic health throughout the life-course is starting to emerge, there is also a greater appreciation of the impact it may have on the mother herself. In Chapter 3,the impact of maternal GWG in pregnancy on cord blood biomarkers and birthweight, as well as maternal characteristics and GWG during each gestational period.

Whilst maternal obesity is a recognised risk factor for developing GDM (Q. Wang *et al.*, 2013), with progressive insulin resistance exacerbated in obese pregnancy, it also poses a greater risk of developing multi-system disorders such as pre-eclampsia (PE) (Z. Wang *et al.*, 2013). These disorders in pregnancy will be examined in Chapters 4 and 5, where the prevalence of diabetic and hypertensive disorders in pregnancy, the maternal offspring characteristics, as well as the impact of such metabolic disorders on offspring birthweight and offspring metabolism at birth, will be examined. Endothelial dysfunction, inflammation, dyslipidaemia, and oxidative stress are all characteristic of obesity-related hypertensive disorders in pregnancy. Chronic or essential hypertension may not have been recognised prior to pregnancy and may simply be detected at the onset of pregnancy; pregnancy-induced hypertension may develop during the pregnancy itself (Duckitt and Harrington, 2005). Obese pregnant women are at much greater risk of developing any hypertensive disorder of pregnancy (HDP) and there is around a 2-fold increased risk in developing PE in obese pregnant women compared to lean subjects (Duckitt and Harrington, 2005).

Whilst weight gain in pregnancy may be less in obese compared to lean women (Soltani and Fraser, 2000; Ehrenberg, Huston-Presley and Catalano, 2003), accrual may be more central than subcutaneous and it is this central adiposity that confers greater risk in pregnancy, such as the greater risk of GDM, hypertension and PE and preterm delivery (Ebrahimi-Mameghani *et al.*, 2013). The distribution of fatness appears to convey greater maternal risk as even non-obese women with a central distribution of fat ('apple-shaped' women) have a greater pro-inflammatory response at pregnancy booking compared to non-obese women with a lower body fat distribution ('pear-shaped' women) (E. Jarvie *et al.*, 2010). Combining this central distribution at the beginning of pregnancy with its accrual actually during pregnancy in an already obese woman may be the trigger of greater metabolic dysfunction, greater insulin resistance, and consequently GDM in obese pregnant women (Chu *et al.*, 2007).

Obesity amongst women of child-bearing age impacts fertility from conception through to the postnatal period. It adversely affects ovulation and thus conception, such as in polycystic ovarian syndrome, commonly known as PCOS. In those that do manage to conceive, the risk of miscarriage and pregnancy loss is greater (Poston *et al.*, 2016; Koh *et al.*, 2017). During pregnancy itself, women may develop co-morbidities which range from minor nuances in pregnancy to maternal death. Symphysis pubis disorder (SPD), oesophageal reflux disease, carpal tunnel syndrome, and chest infections all feature more frequently in those with obesity in pregnancy (Denison *et al.*, 2008). As discussed, in normal pregnancy, there is also progressive insulin resistance with compensatory hyperinsulinemia (Spellacy and Goetz, 1963). In obese pregnant women, there is an impaired beta-cell function and impaired insulin response to glucose. In a prospective multi-centre study, The UK Pregnancies Better Eating and Activity Trial (UPBEAT), 1555 obese women were recruited between 2009 and 2004. From this 64% of obese women developed complications during pregnancy of which GDM was the most common complication occurring antenatally (Vieira *et al.*, 2017).

Obesity in pregnancy may also have a negative effect on parturition and the delivery may be more complex. Individuals are more likely to require induction of labour and instrumental or operative delivery (Sebire *et al.*, 2001; Arrowsmith, Wray, and Quenby, 2011). More complex deliveries in obese pregnant women were also recognised by UPBEAT, as an emergency caesarean section was the most common delivery-related complication amongst obese women included in the study (Vieira *et al.*, 2017). Greater caesarean section rates may be due to a greater risk of induction of labour as obese women are less likely to labour spontaneously and require post-date induction of labour. Induction is also more likely to fail amongst obese women due to inefficient myometrial contractility but for those who did establish labour, they were less likely to progress in labour, thus requiring emergency caesarean section (Sherrard *et al.*, 2007; Denison *et al.*, 2008, 2014; Maged *et al.*, 2018).

Greater intervention in labour, resulting in operative delivery, is also associated with a greater risk of sepsis and post-partum complications including wound infections and thromboembolism. According to the recent report from Mothers and Babies: Reducing Risk through Audit and Confidential Enquiries across the UK (MBRRACE-UK), 34% of mothers who died between 2013 and 2015 were obese and 19% were overweight (Koh *et al.*, 2017). Venous thromboembolism and subsequent thromboembolic events remained the leading cause of direct maternal deaths during this triennium (Koh *et al.*, 2017). As previously discussed, obesity may also impair myometrial contractility and as a result, there may be a greater risk of atonic postpartum haemorrhage (Sebire *et al.*, 2001; Blomberg, 2011). Anaesthetic complications also feature more frequently. Difficulty inserting regional anaesthesia may result in women requiring a general anaesthetic and there is a greater risk of Mendelson syndrome. Collectively these complications prolong hospital stay and present a significant burden on the NHS (Denison *et al.*, 2014; Solmi and Morris, 2018). Whilst it is clear that greater maternal adiposity poses a significant risk to the mother during pregnancy, obesity also confers a greater risk to the fetus during pregnancy.

1.3.2 Fetal effects of obesity in pregnancy

Maternal obesity adversely affects fetal health throughout the entire gestational period and there is emerging evidence to support a long-lasting impact on the offspring. Offspring of obese mothers are at greater risk of obesity themselves (Whitaker, 2004; Oken *et al.*, 2007; Perng *et al.*, 2014) although the interpretation of the relationships drawn from these studies are complex and are limited by environmental and lifestyle factors which may play a significant role in the causal pathway. Shared dietary patterns and activity levels within families, for example, may all contribute to the development of obesity in the offspring. Metabolic dysfunction in these offspring,, has potential for transfer of this dysfunction across generations as highlighted in epigenetic studies (Drake and Walker, 2004; Aagaard-Tillery *et al.*, 2008; Davis *et al.*, 2008; Waterland *et al.*, 2008; Li *et al.*, 2012). Whilst it is recognised that interventions are required to prevent the propagation of obesity-related disease to subsequent generations, specific treatment options are limited due to the complex physiological processes that occur in pregnancy that are susceptible to environmental influences.

In early pregnancy, there is a greater risk of fetal demise amongst obese pregnant women (Lashen, Fear, and Sturdee, 2004). There is also a greater risk of congenital anomalies (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009; Stothard *et al.*, 2009; Mills *et al.*, 2010; Zhu *et al.*, 2018). This may be due to the poor quality of the oocyte at the peri-conceptual stage of pregnancy. Murine models suggest that there is an excess accumulation of lipid in oocytes of those fed a high-fat diet (Wu *et al.*, 2010) which is associated with maternal mitochondrial dysfunction and impaired ability to maintain a pregnancy (Igosheva *et al.*, 2010; Wu *et al.*, 2010). Whilst this may account for subfertility in obese women, it may also implicate this peri-conceptual stage in translating this adverse metabolic environment onto the embryo which may develop abnormally. Congenital anomalies in the offspring of obese mothers may result from teratogenesis as seen in diabetic pregnancies due to metabolic

dysfunction (Ramsay *et al.*, 2004), folate deficiency due to inadequate dietary intake and absorption of folate (Kaidar-Person *et al.*, 2008), as well as decreased sensitivity of antenatal screening tests such as ultrasound (Eastwood *et al.*, 2017).

With advancing obese pregnancy, there is a greater risk of large for gestational age (LGA) offspring and fetal macrosomia (Yu *et al.*, 2013). Maternal hyperglycaemia, as a result of maternal obesity, triggers diffusion of nutrients, such as glucose (but not insulin), along a concentration gradient from the maternal to the fetal circulation via the placenta. LGA and fetal macrosomia occur in offspring of these mothers, particularly if they have developed GDM, as there are relative hyperglycaemia and compensatory fetal hyperinsulinemia (Skyler, O'Sullivan, and Holsinger, 1980; Catalano *et al.*, 2009; Modi *et al.*, 2011) as well as placental fatty acid transport dysfunction (Dubé *et al.*, 2012).

The impact of maternal obesity on the fetus is not only restricted to the duration of pregnancy itself as labour and delivery are more likely to sway from the 'normal' delivery pathway. Obese women are less likely to labour spontaneously (Hendler, Goldenberg, *et al.*, 2005) as discussed previously. They are also more likely to deliver pre-term (McDonald *et al.*, 2010; Denison *et al.*, 2014). This may be iatrogenic as there is a greater risk of preterm induction of labour (Hendler, Goldenberg, *et al.*, 2005) as a result of medical complications arising in pregnancy such as PE and GDM. Deliveries are more complicated as a result of fetal macrosomia, and there are consequent adverse outcomes for the fetus such as shoulder dystocia. With this, there is a greater risk of hypoxic-ischaemic encephalopathy, brachial plexus, or bony injury and stillbirth. Fetal monitoring during labour may technically be more challenging, heightening the risk to the fetus. Collectively, offspring of obese mothers are more likely to require admission to the neonatal unit (Denison *et al.*, 2014).

There is also evidence demonstrating that maternal obesity adversely affects the long-term health of the offspring. An adverse maternal metabolic environment, such as in maternal obesity, has been associated with altered fetal metabolism as

reflected by dysregulation of adipokines and insulin (Catalano *et al.*, 2009). Neonatal offspring of obese mothers, even in the absence of GDM, are also predisposed to greater adiposity (Sewell *et al.*, 2006). This may extend though child into adulthood as greater birthweight is associated with offspring obesity throughout the life-course (Curhan *et al.*, 1996; Whitaker, 2004). This is examined further in Chapters 6 and 7 which explore how cord blood markers track through childhood into adolescence as well as how cord blood analytes relate to later offspring adiposity. Not all infants born to obese mothers are however of large birthweight. Adult central obesity, as previously discussed a risk factor for cardiovascular disease, can be associated with low birthweight (Kuh *et al.*, 2002), as are hypertension, diabetes, and cardiovascular disease (Curhan *et al.*, 1996) and there is evidence to show that placental dysfunction, in keeping with maternal obesity, may induce fetal growth restriction instead (McDonald *et al.*, 2010). Nevertheless, there is evidence to suggest the offspring of obese mothers, even in the case of low or high birthweight, are predisposed to metabolic dysfunction.

Animal models demonstrate the association between maternal nutritional status and adverse offspring phenotype. In primate models, greater offspring adiposity was seen in those of obese baboons (Farley *et al.*, 2009) and obese macaques (McCurdy *et al.*, 2009). Whilst the offspring of rats fed a high-fat diet in pregnancy were of low birthweight, the offspring became obese later in life (Howie *et al.*, 2009; Nivoit *et al.*, 2009). Dysregulation of rodent offspring insulin and leptin levels as well as fat mass and blood pressure have also been described (Khan *et al.*, 2004; Samuelsson *et al.*, 2008). Furthermore, the offspring of rats fed a high-fat diet in pregnancy demonstrated greater weight gain compared to offspring of lean dams, despite no difference in pup birth weight between the groups (Shankar *et al.*, 2008).

Animal models have also been used to show the association between an adverse maternal environment and adverse offspring metabolism. The offspring of diet-induced obese rats demonstrated adipocyte hypertrophy and dysregulation of adrenoceptors (Samuelsson *et al.*, 2008). Maternal over-nutrition in an ovine model

may also lead to overexpression of adipokines leptin and adiponectin, among other lipogenesis-stimulating hormones (Muhlhausler, Duffield and McMillen, 2007). Finally, alteration of thalamic neurotransmitters has also been implicated in the translation of this adverse maternal metabolic profile onto the offspring (Chang *et al.*, 2008; Kirk *et al.*, 2009; Sullivan *et al.*, 2010).

Collectively it is clear that maternal diet, distribution, and degree of adiposity, albeit peri-conceptually or during pregnancy, may adversely influence the offspring's metabolic profile and phenotype at birth and this has the potential for translation across subsequent generations.

1.4 Fetal programming

1.4.1 The intrauterine environment and long-term programming of offspring

Distinguishing between the concept of programming in utero and environment-induced developmental modelling is complex. Examining the outcomes of one mechanism without the other is a challenging process, with many co-variables to consider. Acting via distinct or converging pathways, both theories imply that the individual is at increased risk of adverse health conditions later in life via altered growth trajectories and metabolic disruption. By identifying biomarkers at birth and recognising periods of greatest developmental modelling and impact on the phenotype there is potential to put in place stratified intervention models that generate positive changes in health. In doing so, there may be significant economic benefit by treating disease early on or even avoid the need for treatment altogether by preventing the onset of disease (Gluckman *et al.*, 2008).

1.4.2 Developmental Origins of Health and Disease

The intrauterine environment is a critical time to ‘determine’ the offspring's risk pathway for later life. The Developmental Origins of Health and Disease (DOHaD) concepts encompass mechanisms and early life exposures that create a ‘memory’ and fetal adaption to an abnormal early life environment which is then retained in later life 4). Several processes have been proposed to account for this DOHaD hypothesis, including maternal over as well as undernutrition, epigenetic changes, and abnormal environmental exposures.

During this process, mal-adaption, and long-lasting changes in the morphological structure of the fetal tissues or organs, known as developmental organ plasticity,

may predispose to a greater risk of non-communicable disease in later life such as cardiovascular and metabolic dysfunction. Fetal exposure to maternal obesity in pregnancy, for example, may set an abnormal trajectory of offspring health and predispose that individual to ill health in later life. This then in turns continues throughout the life cycle and facilitates the propagation of the ill-health onto future generations.

Barkers' hypothesis (Barker and Osmond, 1986; Barker *et al.*, 1989, 1993; Barker, 2007), first proposed in 1986, demonstrated a direct link between prenatal nutrition and late-onset coronary artery disease. This hypothesis has not only demonstrated that fetal programming exists, but also that it significantly contributes to the origin of type 2 diabetes, metabolic syndrome, and cardiovascular disease. Historically, heart disease was thought to be solely accounted for by lifestyle and genetic factors. Whilst genetic variations have been directly associated with type 2 diabetes, it is thought that they only account for around 10% of primary constitutional type 2 diabetes hence there are major aetiological factors involved in the origin of type 2 diabetes that remain unexplained (Ahlqvist, Ahluwalia and Groop, 2011). There is therefore potential for early life exposures to act as determinants of type 2 diabetes and its sequelae.

Heart disease in adult life was also more prevalent amongst individuals with low birthweight. Replicated worldwide, the offspring are thought to have permanently adapted in utero to the abnormal environment and the physiology continues to respond in this abnormal way once born. While maternal undernutrition is more apparent in low-income countries, metabolic programming is thought to be more prevalent in any population with the greatest maternal exposure to underweight. The intrauterine environment, as determined by external exposures such as maternal obesity, acts as a 'taster' for what life may be like 'ex-utero' but this may be disproportionate to the world of birth. This world at birth may vary dramatically from the conditions the fetus was exposed to in utero. Nutritional resources for example may be scarce or in abundance and directly opposed to what the fetus

became accustomed to in utero. The adaptions made by the fetus may be long lasting and the physiological response of the fetus may not be appropriate or meet the new demands of the new environment outside the womb. GDM may reflect an early manifestation of type 2 diabetes and is associated with low (and high) birthweight offspring as a result of an abnormal intrauterine environment. Pregnancy is therefore a window of opportunity to prevent type 2 diabetes in both the mother and their offspring. This is a public health issue as the global diabetes epidemic continues to grow as a result of the propagation of maternal over-nutrition and physical inactivity and decreased birthweight in their offspring who are predisposed to metabolic dysfunction (NICE (National Institute for Health and Care Excellence), 2011; National Institute for Health and Care Excellence, 2015).

1.4.3 Developmental undernutrition

Developmental undernutrition, also known as the 'thrifty phenotype', describes a low nutrient supply in utero, occurring in malnourished and mothers with a low BMI. The fetus adapts in utero to a low nutritional supply during the gestational period and maybe small for gestational age or growth-restricted at birth. In childhood, however, and with the supply of processed foods containing low nutritional and high calorific content, the offspring sustains permanent disruption to the regulation of glucose and insulin pathways hence greater predisposition to metabolic disease such as type 2 diabetes. This thrifty phenotype, and its association with developmental organ plasticity, has been proposed as the most plausible hypothesis for the aetiological development as well as the molecular mechanisms in the origin of type 2 diabetes.

A low birth weight itself, although a crude marker of the intrauterine environment, has repeatedly been associated with type 2 diabetes in later life, as demonstrated by population-based studies (Ahlqvist, Ahluwalia, and Groop, 2011). This is of clinical significance as insulin resistance is a prominent feature of early metabolic

syndrome. Prematurity, which is independent of birthweight has also been independently linked to the development of type 2 diabetes, however, it is evident that growth velocity within the first and second trimesters have the greatest impact on the development of the metabolic syndrome in later life, rather than growth trajectory in a later gestational period (Vielwerth *et al.*, 2008).

Dutch Famine Birth Cohort Study demonstrated the permanent fetal adaption to a suboptimal intrauterine environment (Roseboom, de Rooij, and Painter, 2006). From 1944-1945, the blockade of the Netherlands meant that no food was reaching the Dutch population and a famine ensued. The population, which included pregnant women, became severely malnourished. This study found that offspring born to these mothers were at greater risk of diabetes, cardiovascular disease, obesity, and other non-communicable diseases (Roseboom, de Rooij, and Painter, 2006). Exposure to famine at any gestational period, in the first, second, or third trimester, or throughout the entire gestational period predisposed the offspring to glucose intolerance in later life. The fetus is most vulnerable during the first trimester however as maternal exposure to famine during this period was also associated with cardiovascular disease and a more atherogenic profile in the offspring.

Whilst organ dysfunction and cellular re-modelling (reflecting organ plasticity) involving cardiac, pancreatic, and hepatic tissues have been demonstrated in response to fetal under-nutrition studies, the structure of the kidney may be permanently altered by exposure to an abnormal intrauterine environment. Nephrons are developed in utero and a deficit created in this early gestational period has long-lasting effects. Low birthweight and prematurity, as influenced by maternal BMI, may lead to inadequate kidney development in utero and predispose the offspring to hypertension and risk of chronic kidney disease in later life (Luyckx *et al.*, 2013).

Twin studies also demonstrate the discordance in the development of diabetes according to birthweight. Studying twins eliminates inter-familial genetic variations,

as they are born of the same mother, but also removes variation in the gestational age at delivery and its association with offspring development of type 2 diabetes. Twins born with a lower birthweight were at greater risk of type 2 diabetes compared to their 'normal weight' and genetically identical co-twin, as found in monozygotic twins, but also dizygotic twins (Poulsen *et al.*, 1997).

1.4.4 Developmental overnutrition

The DOHaD hypothesis also supports developmental overnutrition as a mechanism for translating metabolic dysfunction onto future generations. This is of particular relevance with the maternal population getting heavier and the prevalence of maternal diabetes in pregnancy greater. Normal physiological adaptions that occur during 'healthy' pregnancy include the development of some insulin resistance and elevated circulating glucose levels. This occurs to meet the demands of the developing fetus. There is a linear relationship between maternal glucose levels and offspring birthweight, even out with the diagnosis of maternal diabetes (Metzger *et al.*, 2009). This risk however extends into childhood as maternal glucose concentration has also been shown to be related to childhood adiposity (Ehrlich *et al.*, 2013) and insulin resistance which is independent of maternal BMI (Hamilton *et al.*, 2010).

Maternal obesity, compounded by maternal diabetes in pregnancy, may lead to fetal growth restriction (via the development of placental insufficiency), or fetal over-growth (via greater in utero exposure to glucose). This is demonstrated by the fact that exposure to maternal adiposity during pregnancy is associated with higher offspring birth weight and greater adiposity through childhood and adult life (Nelson, Matthews, and Poston, 2009). The greater this degree of adiposity, the greater the transplacental passage of nutrients (glucose and fatty acids) are transferred to the fetus (Fraser *et al.*, 2010; Lawlor *et al.*, 2011). As a result, there is excessive pancreatic stimulation and the fetus secretes more insulin, thereby enabling the

development of larger babies with greater fat mass. The offspring born to obese mothers are not only at greater risk of adiposity but the metabolic disruption extends from childhood to adult life as offspring are at greater risk of cardiovascular-related death (Gaillard *et al.*, 2013; Godfrey *et al.*, 2017). Whilst accounting for environmental and shared lifestyle factors may be challenging, the DOHaD concept is further strengthened by the fact that offspring born to mothers before and after bariatric surgery had a greater risk of adiposity when the maternal BMI was greater (Smith *et al.*, 2009). In addition, higher maternal weight gain in pregnancy is also associated with greater childhood adiposity, highlighting the intrauterine period as a key period of offspring programming (Godfrey *et al.*, 2017). The fetus may be at risk from as early as the peri-conception stage as there is also mounting evidence that maternal obesity at the time of conception may adversely affect the metabolic health of the offspring (Lillycrop and Burdge, 2012; Fleming *et al.*, 2018).

It was first considered in 1980 that fetal exposure to obesity and 'fuel mediated teratogenesis' would cause long-lasting effects on the offspring (Freinkel, 1980). As demonstrated by Pima Indian population, offspring born to mothers with diabetes were at greater risk of obesity and early-onset type 2 diabetes in later life compared to non-diabetic mothers (Dabelea *et al.*, 2000). This Study also compared siblings within the family unit which also showed discordance in their risk when born before and after maternal development of diabetes in pregnancy (Dabelea *et al.*, 2000). A Study of Indian children born to mothers with GDM also showed that the offspring had a greater risk of obesity, as well as higher glucose and insulin concentrations compared to those born to non-diabetic mothers (Krishnaveni *et al.*, 2010).

The phenotype at birth is well known to be related to long-term outcomes and this concept is strengthened by observations that birth weight is positively associated with the mean infant, childhood, and adulthood BMI, and adiposity (Pietiläinen *et al.*, 2001). This is explored further in Chapter 6. It is also proposed that this increased birthweight may result in irreversible changes to the neuroendocrine systems and adipose and musculoskeletal tissue in the offspring. Consequently, there

is a greater degree of adiposity throughout life (Oken and Gillman, 2003; Taylor and Poston, 2007; Lawlor *et al.*, 2011). This pathway is perpetuated when female offspring who then become pregnant pass over the cycle of developmental over-nutrition onto subsequent generations (Ebbeling, Pawlak and Ludwig, 2002; Symonds *et al.*, 2003; Taylor and Poston, 2007).

The neuroendocrine feedback loop, the adipo-insular axis, encompasses the pancreas, adipose tissue, and the brain and regulates hunger and fat storage by controlling insulin and leptin. As insulin is adipogenic, it stimulates the production and secretion of leptin. Leptin, the satiety hormone, acts by suppressing energy intake and suppressing insulin secretion. Disruption to the neuroendocrine axis via fetal overnutrition (including maternal obesity and diabetes), causes altered energy and appetite regulation and disrupted adipocyte metabolism. From this, there is potential to induce greater fetal and neonatal adiposity as well as predispose the offspring to metabolic dysfunction in later life, such as type 2 diabetes and cardiovascular disease (Kieffer and Habener, 2000).

Evidence from within sibling studies, comparisons of maternal and paternal exposures, and the use of genetic variants as proxies for the maternal exposures support developmental overnutrition causing greater adiposity in offspring at birth (Debbie A. Lawlor *et al.*, 2012; Lawlor, 2013). However, studies to date have been unable to determine the extent to which greater fat mass at birth tracks into later life and explain the association or whether any longer-term impact is irrespective of fatness at birth. This is because the provision of excessive nutrients facilitates an increase in fetal fat mass, with the assessment of birth weight unable to distinguish its relative contribution from the lean mass (Sewell *et al.*, 2006; Jain *et al.*, 2016).

1.4.5 Epigenetics

There is increasing awareness of the impact of epigenetic changes in the developmental origins of disease. These changes occur as a result of intrauterine adaptions that have a long-lasting impact on the offspring's physiological responses. Epigenetics encompasses the study of heritable phenotypes that do not involve changes in the DNA sequence. The prefix ‘epi’ suggests that these changes are in addition to genetic changes and account for differentiation in gene expression and activity. Predetermined epigenetics describes a predictable developmental change and is in contrast to probabilistic epigenetics, which describes alterations to structure and function as a result of environmental exposures. Epigenetics encompasses novel ideas and mechanisms that may underlie the development of organ dysfunction relevant to insulin resistance and type 2 diabetes and include changes in DNA methylation, histone modification and regulation, and non-coding micro RNA’s.

DNA methylation, or differential methylation, occurs during key developmental periods, particularly in fetal life but also in early childhood and puberty. The ‘methylome’ is determined mainly in utero and follows a period between fertilisation and implantation in which DNA in the fertilised egg (other than imprinted genes) is ‘demethylated’. This accounts for differentiation in the cell types in the fetus with varying gene expression. This process may be influenced by maternal diet and the exposed environment as demonstrated by maternal famine during pregnancy, as previously discussed, as well as the season of conception. Consequently, varying offspring phenotypes ensue and may lead to offspring development of cardiovascular disease, obesity, hypertension, insulin resistance, and altered leptin and lipid profile.

Altered methylation of the pro-opiomelanocortin (POMC) gene, which contributes to the regulation of body weight, has been demonstrated in seasonal exposure to

famine in a study conducted in The Gambia (Kühnen *et al.*, 2016). This Study highlights the peri-conceptual phase as a critical period of epigenetic change and developmental plasticity. Offspring conceived during the rainy 'hungry' season had greater methylation of the POMC gene and overall greater offspring BMI and risk of obesity compared to those conceived during the dry season where nutrition was more plentiful (Kühnen *et al.*, 2016).

Epigenetic modifications and their association with low birthweight have been demonstrated in both animal and human models. Transcriptional regulation by promoter DNA methylation and histone modifications of the key pancreatic proliferation and transcription factor Pdx1 as a result of fetal undernutrition (Park *et al.*, 2008). Furthermore, there is an increased expression of MiR-483-3p in subcutaneous adipose tissues from humans with low birthweight and from rats that were protein undernourished in utero. Collectively they have demonstrated a greater risk of lipotoxicity and organ dysfunction, culminating in a greater risk of type 2 diabetes in the offspring (Ferland-Mccollough *et al.*, 2012).

1.4.6 Other causes

It is not only maternal and fetal over or undernutrition that may permanently influence the physiology of the offspring. Environmental exposures via shared lifestyle factors and familial dietary patterns may also account for the positive associations between maternal obesity in pregnancy for example, and offspring adiposity. Hormonal disruption may also contribute to this, as inadequate thyroid control and supply in pregnancy may lead to abnormal brain development, particularly during the first trimester (before the fetus starts to produce endogenous thyroid hormone). Chronic under-exposure to thyroid hormone in utero can lead to neurological conditions in later life. Furthermore, the maternal mental state may 'expose' the offspring to mental health disorders. Maternal anxiety and depression may influence the offspring's risk of developing this in later life. Similarly, maternal

stress, which may accompany these conditions, may lead to preterm labour and resulting low birthweight baby, which then inherently predisposes the offspring to metabolic dysfunction in later life.

Intrauterine exposure to toxins, particularly during critical periods of fetal development may also permanently alter the hypothalamic-pituitary axis. Transplacental passage of alcohol for example alters the neuroendocrine, metabolic, and physiological responses of the offspring as demonstrated in fetal alcohol spectrum disorder. In addition, smoking may contribute to a greater risk of preterm labour and small for gestational age offspring with resultant altered lung development and potential for greater childhood obesity in the offspring (Rayfield and Plugge, 2017). Differentiating confounding factors, such as mothers who smoke in pregnancy may vary in socioeconomic status compared to mothers who don't smoke, and the shared lifestyle in childhood and beyond may alone account for the greater predisposition in the offspring to obesity. Animal studies however have shown that pregnant rats given nicotine in pregnancy are associated with greater fat in the pups suggesting a causal relationship between the exposure and the offspring outcome (Behl *et al.*, 2013). Maternal consumption of medication, such as certain beta-blockers and angiotensin receptor enzyme inhibitors (ACEI), may also contribute to impaired fetal growth, especially in the context of maternal hypertensive disorders of pregnancy.

1.5 Programming of offspring metabolic dysfunction

1.5.1 Gestational weight gain (GWG) in pregnancy on offspring metabolic markers

Chapter 3 will focus on the impact of maternal obesity and GWG, reflecting maternal over (and under) nutrition in pregnancy and their role in the causal pathway on offspring birthweight and metabolic markers. The accumulation of adipose tissue leading to weight gain varies between individuals in pregnancy with average weight gain between 10 and 12kgs. Of which, around 3kg is due to an increase in adipose tissue. Approximately 3-4kg weight gain is due to the developing fetus, 0.5kg due to the placenta, 0.8kg due to amniotic fluid, 1.2kg from blood, 1kg due to the enlarged uterus, and 1.5kg due to extracellular extravascular fluid (Hytten and Leitch, 1971). There is evidence to suggest that obese pregnant women tend to gain less weight than lean women (Soltani and Fraser, 2000; Ehrenberg, Huston-Presley and Catalano, 2003), however, accrual tends to be more central than subcutaneous (Sohlstrom, Wahlund and Forsum, 1993; Sohlstrom and Forsum, 1995; Stevens-Simon *et al.*, 2001), which as previously discussed may carry greater metabolic risk. Guidelines for weight gain in pregnancy were developed by the Institute of Medicine (IOM) in 2009 as weight gain greater than the recommended thresholds have been associated with adverse outcome (Crane *et al.*, 2009; Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009).

Greater maternal adiposity, due to elevated pre-pregnancy BMI or excessive GWG, are well recognised ‘risk factors’ for adverse maternal and offspring outcomes in the perinatal period (Viswanathan *et al.*, 2008; Poston, Harthoorn and Van Der Beek, 2011). The ‘risk’ to the offspring appears to extend well beyond the neonatal period, however, as GWG has not only been strongly positively associated with birthweight (Fraser *et al.*, 2010; Ludwig and Currie, 2010), but also with body mass index (BMI) in childhood (Fraser *et al.*, 2010), adolescence (Oken *et al.*, 2008) and adulthood

(Mamun *et al.*, 2009; Hochner *et al.*, 2012). GWG has also been linked to elevated triglyceride and leptin and reduced high-density lipoprotein cholesterol (HDLc) levels in child and adulthood (A. Lawlor *et al.*, 2010; Fraser *et al.*, 2010; Hochner *et al.*, 2012), but, as yet there is limited evidence of the effect of inappropriate GWG on the offspring's cardio-metabolic profile at birth. Evidence does however support a greater association between GWG in already obese mothers and offspring adiposity (Oken *et al.*, 2008). Pregnancy is therefore a crucial window of opportunity for optimising weight gain as there is increasing potential for the translation of the phenotype and potential inherent metabolic dysfunction onto the offspring.

1.5.2 Institute of Medicine (IOM) Guidance on GWG

The 2009 IOM guidance was devised to advise pregnant women on appropriate GWG according to their pre-pregnancy BMI, so that offspring growth patterns and the trajectory of metabolic health may be optimised (Rasmussen, Catalano and Yaktine, 2009). By gaining weight appropriately, the guidance aimed to minimise the risk and potential health consequences for both mother and baby that are associated with low or excessive maternal weight gain during pregnancy. Appropriate GWG, for example, is associated with better obstetric outcomes (Olson, 2008; Langford *et al.*, 2011). More than half of pregnant Americans gained inadequately or excessively, which highlighted the need for a guideline and to standardise care and provide individualised advice before and during pregnancy (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). Their focus was to examine both the short and long-term outcomes for both the mother and fetus. Specifically, the guidance aimed to better advise on optimal weight gain to reduce the risk of a small or LGA fetus, reduce the need for unexpected caesarean section, and limit excessive maternal weight retention. The recommendations were to be used 'in concert with good clinical judgement as well as a discussion between the mother and her prenatal care provider about diet and exercise' (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009).

Recommendations on weight gain were first issued in 1990 (IOM, 1990) and vary dramatically from the current 2009 guidance available. Primary objectives were similar however and included reducing the risk of preterm birth, reducing the rate of non-scheduled caesarean section delivery, as well as reducing the risk of low offspring birthweight. Both have found that weight gain above the guidance was associated with higher caesarean section rates (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009), and higher weight gain overall was associated with greater maternal post-partum weight and greater birthweight. One of the main recommendations from both publications was however to encourage a normal weight at the time of conception as this then has implications for the duration of the pregnancy and remains a large determinant of pregnancy outcome (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009; Rasmussen, Catalano and Yaktine, 2009).

There was the need for these 1990 guidelines to be revised and updated for several reasons. Firstly, changing demographics incorporating a heavier, more obese maternal population with more chronic health conditions. Populations are more culturally diverse and in addition, maternal age at first index pregnancy has risen, as has the number of multiple pregnancies. Secondly, the BMI thresholds used in 1990 were not following World Health Organisation (WHO) cut-offs of BMI, as these had not yet been determined at this stage ('Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee.', 1995). For example, 'normal' weight was defined as 19.8-26.0 kg/m^2 and differs from current BMI thresholds, seen in Table 1.1 and Chapter 2, Table 2.1. Furthermore, the recommendation of weight gain for obese women was only a minimal weight gain but there was no upper limit specified. Lastly, its recommendations, based on observational data, drew conclusions that if maternal weight gain was within the recommendations issued in 1990, the pregnancy outcomes would be overall better. Pregnancy outcomes however are multi-factorial and consideration should also be given to a wider range of influential factors. A range of weight gain (kg) is provided

in the current IOM guidance to account for these factors which may influence GWG, thereby influencing the pregnancy outcome.

The current guidance on GWG however also has its limitations, which are more apparent in the contemporary population. Several publications have documented the association of GWG on gestational health conditions, such as GDM and HDP (Yee *et al.*, 2011; Siegel *et al.*, 2015; Egan and Dunne, 2018). Not all studies however account for the duration of pregnancy, likely to be shortened in the presence of PE for example, or the weight gain before the actual diagnosis of diabetes in pregnancy (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). The latter being of particular importance in GDM where the diagnosis of the condition, and implementation of dietary changes or the addition of an anti-glycaemic agent, may limit the GWG thereby introducing bias. Furthermore, the current 2009 guidance does not account for maternal short stature, young age (adolescents), or specific ethnic groups which may all influence GWG. Weight gain in Asian women for example may have varying impact on pregnancy or fetal outcome compared to Western populations, in which the 2009 recommendations were based. The recommendations were also limited to wide ranges of BMI, in particular for extreme obesity as it does not give guidance specifically on Class II (BMI: 35-39.9 kg/m^2) or III obesity (BMI: 40 kg/m^2), also shown in Table 1.1. Conversely, the guidance did not provide any recommendations on weight loss in pregnancy as the safety and implications of this have not yet been determined. As discussed, weight loss before pregnancy, to achieve optimal pre-conceptual weight, is however widely recommended but with most pregnancies being unplanned, may not be applied in daily practice. Collectively, these factors highlight the need to revise the current 2009 guidance, with specific recommendations for a more diverse and obese contemporary population.

There is a wide variety of literature available on the interventions adopted in pregnancy to encourage women to achieve optimal GWG (Polley, Wing, and Sims, 2002; Asbee *et al.*, 2009). For example, advice on diet, exercise, monitoring of

weight gain during pregnancy, and providing support and counselling. Many have limited statistical power however to draw assumptions on the pregnancy outcome. They conclude however that models of care should be available for all women of childbearing age, and not simply to those 'planning a pregnancy' (as discussed, as most pregnancies are unplanned), in addition to being available throughout pregnancy as well as in the postpartum period. By optimising the weight gain in pregnancy, according to the 2009 guidance, it lowers the obstetric risk, normalises offspring birthweight, improves the long-term health outcomes, reduces the postpartum weight retention risk of obesity in subsequent pregnancies as well as reducing the risk of childhood obesity (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009).

Table 1.1 IOM Recommended levels of GWG according to pre-pregnancy BMI categories (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009)

Pre-pregnancy BMI (kg/m^2)	**Recommended absolute weight gain (kg)**
<18.5 (Underweight)	12.5-18.0
18.5-24.9 (Health weight)	11.5-16.0
25.0-29.9 (Overweight)	7.0-11.5
≥30 (Obese)	5.0-9.0

1.5.3 Maternal diabetes in pregnancy

Whilst GWG and maternal obesity, and their role in the causal pathway, in relation to offspring birthweight and metabolic markers, are the focus in Chapter 3, Chapter 4 will examine the impact of maternal diabetes in pregnancy, and fetal overnutrition in pregnancy. GDM is a common complication of pregnancy and accounts for 90% of all maternal diabetes in pregnancy (National Institute for Health and Care Excellence, 2015). Evidence from human studies suggests there is a possible linear (causal) association between elevated maternal glucose levels in utero and greater offspring birthweight and metabolic dysfunction (Metzger *et al.*, 2008; Poston, Harthoorn and Van Der Beek, 2011; Thaware *et al.*, 2015). Both leptin and insulin are higher in babies of mothers with GDM, as a result of fetal overnutrition as well as a consequence of leptin resistance or altered leptin signalling, so the offspring become less able to control weight gain (Simmons, 2011). It has been proposed that these offspring outcomes may be worsened by excessive GWG as it enhances the insulin-resistant state present during pregnancy and diabetes (Egan *et al.*, 2014). As the 2009 IOM guidelines on GWG in pregnancy were devised for a general obstetric population, it is as yet unclear if they are relevant to higher risk groups, such as those with diabetes mellitus (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). It also remains uncertain as to whether regulating GWG limits the translation of this metabolic dysfunction onto the offspring at birth.

The large, multicentre study, Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) revolutionised clinical management of diabetes in pregnancy as it showed a clear association between maternal glycaemia, offspring birthweight, and cord blood c-peptide (Metzger *et al.*, 2008). A more recent report from this study however has shown that the relationship between mild, untreated hyperglycaemia in pregnancy (that does not meet the criteria for GDM) and offspring adiposity in childhood is limited (Thaware *et al.*, 2015). It has been proposed that the effect of exposure to diabetes in utero may only become apparent in the offspring in early

adolescence as they transition through puberty. The Exploring Perinatal Outcomes among Children (EPOCH) study demonstrated this by showing higher BMI at age 10 to 13 in the offspring of mothers who developed GDM during pregnancy (Crume *et al.*, 2011). Undoubtedly, maternal diabetes emphasises the fetal overnutrition hypothesis with the offspring, and mothers, at substantial long-term risk.

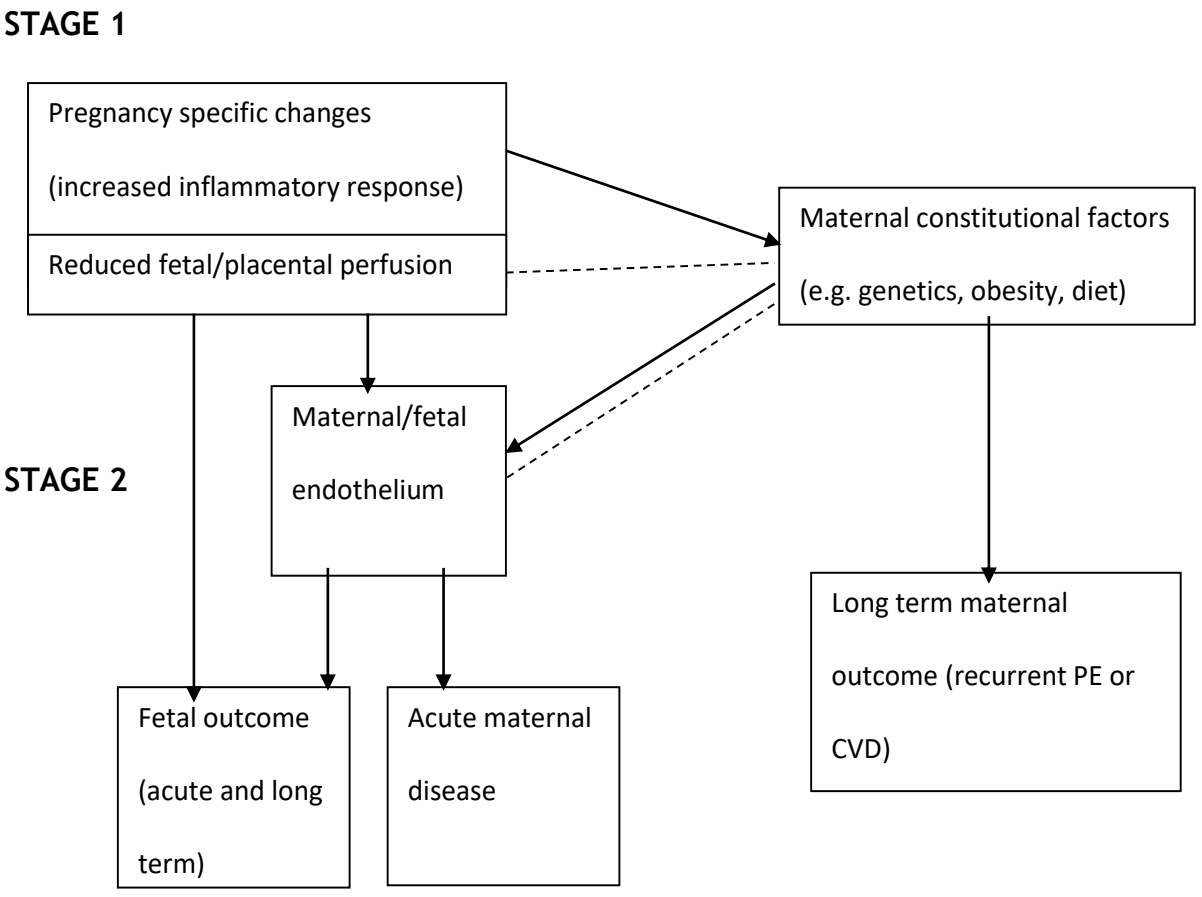
C-peptide is a short chain of amino acids that are released into the circulation as a byproduct of the formation of insulin from biologically inactive proinsulin in the pancreas. Both insulin and c-peptide are released in equimolar amounts but as insulin has a short half-life it is cleared quicker and therefore more prone to fluctuations, making it difficult to measure from peripheral venous samples (Nesbitt *et al.*, 2006; Metzger *et al.*, 2008). In addition, c-peptide does not appear to be altered by haemolysis, unlike insulin which shows signs of degradation (O'Rahilly *et al.*, 1987). As around 15% of all venous samples show signs of haemolysis, c-peptide is a therefore a more reliable indicator of insulin concentrations (Metzger *et al.*, 2008).

1.5.4 Hypertensive disorders in pregnancy (HDP)

HDP is increasingly prevalent amongst the current obstetric population and includes pre-eclampsia (PE) and gestational hypertension (GH). Chapter 5 will examine the association of HDP in pregnancy and offspring birthweight and metabolic markers. PE is of unclear origin but is thought to be a two-stage disorder, as shown in Figure 1.1 (Roberts and Hubel, 2009). Firstly, there is abnormal implantation and invasion by the trophoblast leading to insufficient spiral artery re-modelling and ultimately decreased placental perfusion, thereby limiting the passage of nutrients to the developing fetus (Pijnenborg, Vercruysse and Hanssens, 2006; Roberts and Hubel, 2009). Following this, there is an exaggerated maternal systemic inflammatory response and oxidative stress. Altered vascular endothelial function ensue which may result in PE and possibly multiorgan damage. It is thought that the metabolic

and biochemical disruption that is associated with greater maternal adiposity may be the 'maternal milieu' that leads to this second stage in PE or may indeed trigger the onset of the first stage. Overweight and obesity are associated with a chronic low-grade inflammatory state and elevated C-reactive protein (CRP), as well as some inflammatory cytokines, have been demonstrated in such a population. There is also systemic inflammation as shown by elevated neutrophils, that release toxic compounds, which may adversely affect the integrity of the endothelium and may also contribute to the development of PE.

Figure 1.1: Two-stage process of (PE) (Roberts and Hubel, 2009)



Current guidance for women at high risk of developing pre-eclampsia is to commence low-dose Aspirin from 12 weeks' gestation (NICE, 2010). As shown in Table 1.2, those

with one or more high-risk factors or women with two or more moderate risk factors should be advised to commence therapy during pregnancy. This treatment given to those at moderate to high risk of PE can reduce their risk of developing PE by 15% (Duley, 2009).

Table 1.2: Risk factors for PE according to NICE guidelines (NICE, 2010)

High risk	Moderate risk
History of hypertension in a previous pregnancy	Nulliparous
Chronic renal disease	Age ≥40
Autoimmune disease e.g. Antiphospholipid syndrome	Pregnancy interval more than 10 years
	BMI ≥35 at the first visit
Type 1 or 2 diabetes mellitus	Family history of PE
Chronic hypertension	Multiple pregnancy

PE in particular has well documented long-term sequelae for the mother, such as chronic hypertension, cardiac and cerebrovascular disease (Bellamy *et al.*, 2007; Fraser *et al.*, 2010; McDonald *et al.*, 2010). Dyslipidaemia in the maternal serum has also been reported (Khatua *et al.*, 1989; Vanessa A. Rodie *et al.*, 2004; Ophir *et al.*, 2006; Catarino *et al.*, 2008). Fewer extreme cases of PE are seen prior to 28 weeks' gestation, with only 0.5% of all nulliparous women delivering before 34 weeks as a result of developing the severe disease (NICE, 2010).

HDP may also contribute to an altered metabolic risk profile in the offspring. This may stem from the lower associated birthweight (Levine *et al.*, 2004; Catarino *et*

al., 2008) in the offspring of those with PE, or from the pronounced endothelial dysfunction (Tenhola *et al.*, 2003) and dyslipidaemia (Ophir *et al.*, 2006; Catarino *et al.*, 2008; Akcakus *et al.*, 2010) that may ultimately lead to higher blood pressure (BP) and greater risk of cerebrovascular disease (Kajantie *et al.*, 2009) in the offspring. As greater maternal BMI and excessive GWG are risk factors for developing HDP, it is unclear as to whether appropriate GWG may mitigate any additional metabolic risk in the offspring of mothers with HDP. There is also limited evidence on how these disorders during pregnancy influence the biochemical imprint at birth, as measured by cord blood adipokines and lipids.

Women who develop PE are counselled about their recurrence risk and the risk of future cardiovascular disease. Whilst PE may recur in future pregnancies, the phenotype tends to be less severe. From those that had developed PE, there is a 13% increased risk of GH in a future pregnancy and a 16% risk of re-developing PE (NICE, 2010). This may increase to 55% however if the original PE led to delivery before 28 weeks' gestation (NICE, 2010). In terms of lifetime risk, a 3.7-fold increased risk of hypertension, as well as a 2.2-fold increased risk of coronary heart disease and a 1.8-fold increased risk of stroke in this cohort have been reported (Bellamy *et al.*, 2007).

1.5.5 Tracking of cord blood analytes

Whilst cord blood measures may be used to describe the intrauterine environment at birth, it remains uncertain whether these markers track throughout the life course. It has also yet to be established whether these cord blood measures may be used at birth for predictive purposes, due to the lack of longitudinal studies examining these associations. Chapter 6 will examine biomarkers in the cord blood at birth with subsequent repeat 'like for like' analyte measures obtained in childhood and adolescence. A 12-year follow-up study has shown that serum lipoproteins do show evidence of tracking (Porkka *et al.*, 1994; Raitakari *et al.*,

2003), even though there is evidence from another that lipids positively correlate with cardiovascular disease in later life (Klag *et al.*, 1993; Raitakari *et al.*, 2003). Furthermore, liver function tests (LFT's) have shown evidence of tracking in adulthood (Patel *et al.*, 2007), as has leptin measured in childhood and 3 years later (Nishimura *et al.*, 2009; Volberg, Heggeseth, *et al.*, 2013). Cord blood leptin is also positively associated with leptin at age 3, unlike cord blood adiponectin which wasn't associated with adiponectin at age 3 (Mantzoros *et al.*, 2009). Studies from birth using cord blood indices are limited however and it is,, therefore, difficult to determine whether they may be used as predictors of long-term metabolic health. Should this be possible, it has significant therapeutic and health implications as 'higher risk' individuals may be identified even before the onset of the disease. Certainly, the associations between lipids and later life cardiovascular events give promise to the concept that early detection of analytes may herald the early onset of metabolic disease.

1.5.6 Adipokines and fetal programming

Whether or not it is the maternal health and degree of adiposity in pregnancy that triggers a disruption in cord blood measures and resultant higher offspring birth weight or fact cord blood analytes act independently of maternal health is unclear. It has been proposed that it is the maternal health status that has the most significant impact on levels of fetal adipokines such as leptin and adiponectin in utero. From this, a 'pathway' may be created passing the maternal susceptibility to obesity and insulin resistance onto the fetus (Luo *et al.*, 2013). Another mechanism may be that obese mothers may produce large for gestational age offspring which may then lead to increased adiposity later in life. These may however be all secondary mechanisms to the dominant cause which is shared lifestyle and genes that create an obesogenic environment for the offspring throughout late life (Debbie A. Lawlor *et al.*, 2012). In Chapter 7, the associations between cord blood adipokines and birthweight on offspring anthropometry in childhood and adolescence are

examined and account for variables such as maternal BMI which, as previously discussed, are also associated with greater offspring adiposity.

The key role that adipokine leptin plays in regulating energy homeostasis is well documented. Circulating cord blood leptin will likely be influenced by maternal adiposity (Geary *et al.*, 1999; Karakosta *et al.*, 2011). This in turn is positively associated with an increase in cord blood leptin (Helland *et al.*, 1998). Leptin is present at significant levels in the cord blood making it a reliable biomarker to measure (Tamura *et al.*, 1998; Schubring *et al.*, 1999). The role leptin plays in fetal programming however remains relatively unknown (Karakosta *et al.*, 2013). There is a vast amount of research demonstrating the link between leptin and the development of the fetus in utero through to the neonatal period. It is thought to act as a 'growth-promoting signal for fetal development. It correlates positively with anthropometric markers of fetal growth in utero, increasing gestational age and birth weight (Tamura *et al.*, 1998; Geary *et al.*, 1999; Nakano, Itabashi and Maruyama, 2009; Tung *et al.*, 2009; Karakosta *et al.*, 2011, 2013). In particular, umbilical cord blood leptin is strongly related to birthweight (Laml, Hartmann, *et al.*, 2001; Laml, Preyer, *et al.*, 2001; Domali and Messinis, 2002) and is a true reflection of neonatal fat mass (Schubring *et al.*, 1999; Schulz, Häckel and Weise, 2000; Pighetti *et al.*, 2003; Hauguel-de Mouzon, Lepercq and Catalano, 2006). Leptin is also related to the ponderal index in the neonatal period (Bellone *et al.*, 2004). From this, it may be deduced that leptin may be used as a biomarker of fetal and neonatal adiposity which has the potential to programme the degree of adiposity in later life (Varvarigou, Mantzoros, and Beratis, 1999; Hauguel-de Mouzon, Lepercq and Catalano, 2006).

Evidence of the long-term effects of fetal programming, as measured by tracking fat mass from the neonatal period into adulthood for example, and its relationship to cord blood leptin is limited as studies are mainly confined to the neonatal period, rather than later in the life-course (Mantzoros *et al.*, 2009; Nakano, Itabashi and Maruyama, 2009; Lindsay *et al.*, 2010; Boeke *et al.*, 2013; Kaar *et al.*, 2014). This

primarily reflects the availability of prospective birth cohorts with cord blood samples (N=56-580) and detailed measures of offspring adiposity as well as potential maternal and offspring confounders. Despite the robust positive association of cord blood leptin with birth weight, skin-folds and neonatal fat mass (Mantzoros *et al.*, 2009; Kaar *et al.*, 2014), lower cord blood leptin has been associated with faster growth and infant weight gain in the first year (Mantzoros *et al.*, 2009; Kaar *et al.*, 2014) and a higher BMI at 3 and 7 years (Mantzoros *et al.*, 2009; Boeke *et al.*, 2013). In contrast, from age 7 years through to adult life, higher circulating leptin predicts greater subsequent weight gain, BMI, and fat mass (Savoye *et al.*, 2002; Fleisch *et al.*, 2007). These contrasting directions of association with subsequent adiposity for cord blood leptin and childhood and adult leptin have been principally interpreted as reflecting distinct periods of leptin sensitivity (Blüher and Mantzoros, 2009; Mantzoros *et al.*, 2009). In animal models, neonatal exposure to exogenous leptin matures the hypothalamic neurons responsible for appetite balance, thereby moderating subsequent energy balance and the trajectory of weight gain (Vickers *et al.*, 2005). In adult mice exposure to exogenous leptin has little impact on subsequent weight gain, suggesting a loss of central sensitivity (Halaas *et al.*, 1997). In humans, equivalent age-dependent periods of leptin sensitivity have been proposed (Mantzoros *et al.*, 2009). However, the accuracy of circulating leptin even in cord blood as a measure of central leptin sensitivity as compared to its established role as an indicator of neonatal fat mass is unknown. In the offspring of mothers with type 1 diabetes, a classical model of developmental overnutrition (Pettitt *et al.*, 1983), a higher cord blood leptin was associated with greater neonatal fat mass and higher BMI at age 7 years (Lindsay *et al.*, 2010). Fundamentally if there is a positive correlation between cord blood leptin and growth and development later in life, cord blood leptin may be used as a surrogate marker for adiposity in future health intervention studies.

The adipocyte-derived hormone adiponectin has also been shown to be related to birth weight and neonatal fat mass (Inoue *et al.*, 2008). Its role in fetal physiology and programming is less clear, as maternal adiponectin does not cross the placenta (D'Ippolito *et al.*, 2012), it has been suggested that adiponectin may drive fetal

growth as well as having insulin-sensitizing effects in adults. Adiponectin is also present in high levels in the cord blood which aids accurate measurement of the analyte (Tsai *et al.*, 2004). Neonatal levels are approximately 4 to 7 times higher than maternal levels, and in contrast to maternal circulating concentrations, where adiponectin is inversely associated with BMI, higher levels of cord blood adiponectin are associated with anthropometric parameters of adiposity, including increased birth weight and fat mass in some (Inoue *et al.*, 2008; Mantzoros *et al.*, 2009; Teague *et al.*, 2015) but not all studies (Nelson *et al.*, 2008; Lindsay *et al.*, 2010). This shift in the association is evident in childhood where low serum levels of adiponectin have been associated with childhood obesity (Araki *et al.*, 2006). The large longitudinal study of women and children, Project Viva, however, demonstrated high maternal and cord blood leptin and low cord blood adiponectin to be associated with lower offspring adiposity in childhood from age 3 to 13 years (Li *et al.*, 2018). Adiponectin, therefore, exhibits different directions of association with adiposity in the neonate as compared to later on in life. That these higher cord blood adiponectin concentrations might reflect increased fat mass is suggested by mouse studies where transgenic over-expression of fetal adiponectin increased the size of fat depots in early life, while adiponectin knockout fetuses, with adiponectin deficiency, display lower body weight and lower fat content (Qiao, Yoo, *et al.*, 2012). This effect however was lost on the 15th postnatal day suggesting that adiponectin may not have a significant role in programming the degree of long-term offspring adiposity. These differing directions of associations highlight the complex role that adiponectin plays in early fetal life but suggest that the strength of their effect may diminish with time (Li *et al.*, 2018). Collectively, from this established and emerging evidence examining the role of adipokines in pregnancy, it is clear that the hormonal status of the intrauterine environment may induce significant changes in growth patterns in infancy which may trigger the onset of obesity in later life.

1.5.7 Lipids and fetal programming

During early pregnancy, there is deposition and hypertrophy of maternal adipocytes, with increased expression of insulin receptors, enabling maternal glucose to meet the increased metabolic demand of the fetus (Herrera, 2002). With rising insulin and progesterone, there is subsequent lipogenesis and lipolysis, thereby increasing circulating lipids and enhancing transplacental passage of lipids to the fetus. With advancing gestation, there is further dysregulation of metabolic pathways, in particular the lipid pathway. Normal pregnancy is characterised by hypertriglyceridemia and hypercholesterolemia (Eslamian *et al.*, 2013) as a result of disruption to lipid transport and metabolism. By the third trimester, triglyceride levels may be up to four times higher than that of pre-pregnancy levels (Hadden and McLaughlin, 2009). The disrupted lipid profile is also characterised by smaller, denser LDLc particles, which are thought to be more atherogenic (Belo *et al.*, 2004). Elevated HDLc and lipoprotein-A, which peak in the second trimester, also feature in normal pregnancy. HDL offers vasodilatory, anti-inflammatory, anti-oxidant, and anti-thrombotic effects and thus protection against endothelial damage (Wan Sulaiman *et al.*, 2016).

Co-morbid conditions during pregnancy, such as PE and GDM, may also exaggerate the disruption to lipid profile during pregnancy and present additional risk to the mother and baby. In mild PE, high triglyceride and cholesterol levels (especially during the second trimester) feature, whereas lower LDLc may be seen in more severe disease (Baker *et al.*, 2009). Reduced levels of HDL have also been reported in PE and may account for the vascular dysfunction that ensues (Wan Sulaiman *et al.*, 2016).

Maternal diabetes exaggerates this disruption of lipid profile and is also associated with altered lipoprotein levels, hence greater cardiovascular risk. This was evident in a small, contemporary cohort where normal-weight women with GDM displayed

elevated triglyceride and decreased HDL levels compared to controls (Dubé, Ethier-Chiasson, and Lafond, 2013). In contrast, overweight or obese women with GDM had lower total cholesterol and LDLc levels compared to controls (Dubé, Ethier-Chiasson and Lafond, 2013). It is difficult to ascertain the metabolic risk adopted by the offspring, however. While offspring of diabetic mothers displayed elevated cord blood triglyceride and LDLc and lower HDLc levels, their associations were not significant compared to offspring of non-diabetic mothers (Dubé, Ethier-Chiasson and Lafond, 2013; Eslamian *et al.*, 2013). Another study showed elevated triglyceride in mothers with GDM, higher fat mass in their offspring but lower triglyceride levels in the cord blood (Ortega-Senovilla *et al.*, 2013). A further study showed that whilst maternal diabetes has been consistently associated with higher offspring glucose and insulin levels in adolescence, it was not associated with offspring lipids at all, nor was it associated with other metabolic markers including offspring blood pressure or CRP (Patel *et al.*, 2012).

Maternal obesity in pregnancy may also feature more atherogenic lipid profiles, which are associated with adverse pregnancy outcomes (Meyer *et al.*, 2013; Barrett *et al.*, 2014). In obese pregnancy triglyceride levels increase, and the anti-inflammatory properties of HDL may be diminished. Combined with a rise in LDLc, these changes may account for decreasing vascular function and adverse pregnancy outcomes that are associated with obesity in pregnancy (Meyer *et al.*, 2013). Dyslipidaemia is also a feature of pregnancies delivered pre-term, as well as the growth-restricted pregnancy, whose offspring assume greater cardiovascular risk in later life (Gluckman *et al.*, 2008; Pecks *et al.*, 2012).

Despite evidence of lipids tracking through life from childhood (Raitakari *et al.*, 2003), their role, if any, in contributing to long-term offspring adiposity is unknown. HDL has been inversely related to fetal growth and BMI at age 9 (Nayak, Agarwal and Nayak, 2013), whereas triglycerides have been positively associated with neonatal anthropometry and BMI at age 9 (Nayak, Agarwal and Nayak, 2013; Volberg, Harley, *et al.*, 2013). In contrast, higher concentrations of fatty acids in cord blood have

been associated with lower adiposity in children at age 3 years (Donahue *et al.*, 2011). Recent mendelian randomization studies however did not support a causal association between maternal triglyceride, HDL, or adiponectin levels and offspring birth weight or ponderal index (Tyrrell *et al.*, 2016).

Whilst normal pregnancy features disruption to the maternal lipid profile, the extent of offspring dyslipidaemia may be subject to the maternal health status of the mother during pregnancy, and as yet there is conflicting evidence to support abnormal lipid profiles in pregnancy causing a long-lasting adverse clinical impact on the offspring.

1.5.8 Non-Alcoholic Fatty Liver Disease in the offspring

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children and adults in the developed world (Younossi *et al.*, 2011). The increasing prevalence of the disease in childhood and adolescence has led to further investigation into potential fetal origins of the disease and developmental overnutrition has been proposed in its pathogenesis (Brumbaugh *et al.*, 2013). Associations to date however have been mixed. Increasing maternal adiposity and greater GWG may both contribute to the adverse environmental exposure in utero and thus 'programme' the offspring to a greater predisposition to the disease. Maternal BMI has also been positively associated with increased intrahepatocellular lipid content in neonatal offspring (Modi *et al.*, 2011). Animal models of a maternal high-fat diet during pregnancy equally display greater adiposity and elevated hepatic lipid accumulation in their offspring (McCurdy *et al.*, 2009; Oben *et al.*, 2010). Conversely, lower birth weight has also been associated with elevated liver transaminases and NAFLD in adolescence and adulthood (Nobili *et al.*, 2006; Fraser *et al.*, 2008; Faienza *et al.*, 2013; Anderson *et al.*, 2014), which is thought to be as a result of impeded organ development (Andersen and Osler, 2004; Nobili *et al.*, 2006; Barker, 2007; Faienza *et al.*, 2013). Whilst cord blood lipids and adipokines at

birth have been linked to neonatal fat mass and anthropometric measurements in early life, their association with childhood NAFLD remains unclear. In Chapter 8, the associations between cord blood adipokines, lipids, and birthweight, and structural and functional evidence of NAFLD in adolescence are examined.

1.6 Summary

This thesis aims to explore the impact of the intrauterine environment, as a reflection of maternal health in pregnancy, on the offspring's health in the short and long-term. Emerging and established evidence suggests that the intrauterine environment plays a considerable role in offspring development and by utilising cord blood indices from the large prospective cohort, ALSPAC, this may be examined further. As few epidemiological studies address the long-term impact of adverse metabolism in utero, this Thesis will aim to provide an overview of the relationship between altered maternal metabolism, offspring biomarkers at birth, and their association with later life anthropometry.

Maternal 'ill health' may be determined by a wide range of physiological processes. Maternal obesity and inappropriate GWG may lead to dysregulation of adipose tissue, disruption to circulating adipokines, and may stimulate systemic pro-inflammatory responses which collectively may result in multi-system effects on the mother and baby. Metabolic complications in pregnancy, such as GDM, may arise as a result of an inadequate response to increased circulating glucose with resultant systemic insulin resistance. In addition, HDP may develop as a result of widespread vascular dysfunction. Collectively, these may influence fetal growth and development in utero but may also leave long-lasting metabolic disruption to the health of the offspring.

Although birthweight is a recognised anthropometric marker that is positively associated with adolescent adiposity, it has yet to be established whether biomarkers at birth, such as cord blood leptin and adiponectin, track throughout the life course. Whilst positive associations have been established between repeat measures of adipokines, lipids, and liver function in later life, this Thesis aims to examine the direction of associations and degree of tracking between cord blood indices and repeat measures in later life. Similarly, as leptin is an established

surrogate of measured fat mass, by identifying evidence of tracking throughout the life course, there is potential to use leptin measurements, from cord blood at birth for example, as an indicator of the fat mass in the offspring in later life.

Lastly, given the associations established in later life, the aim is to establish whether a higher cord blood leptin, as a surrogate for greater neonatal fat mass, would be associated with greater adiposity in late childhood and adolescence. Conversely, acknowledging the direct effect of adiponectin on body composition and specifically enhancing fat deposition, and the different direction of associations in infancy compared to adulthood, this study aims to determine whether adiponectin would be positively associated with offspring adiposity from mid-childhood into adolescence.

1.7 Hypothesis

This study was designed with the aims of addressing:

a) How a dysregulated intrauterine environment, such as in maternal diabetes, hypertension, or in abnormal GWG, may influence cord blood lipids, adipokines, and birthweight. Specifically, it was hypothesised that greater maternal BMI or GWG would be positively associated with cord blood leptin and lipids and negatively associated with cord blood adiponectin, given the direction of association that existed amongst obese adult subjects. In addition, it was proposed that maternal diabetes in pregnancy was not associated with elevated cord blood lipids levels, as associations in childhood previously reported by ALSPAC were null. It was also proposed that cord blood adiponectin specifically would be lower in the offspring of diabetic mothers, given the inverse relationship that has existed between adiponectin and maternal diabetes. As maternal PE has previously been associated with disturbed lipid profile in the offspring, this study also hypothesised that the presence of PE would be associated with higher cord blood triglyceride and LDLc, as well as lower HDL levels.

b) How the baseline measures of cord blood lipids, adipokines, and liver function tests, reflecting the offspring's cardio-metabolic profile at birth, may track from birth to adolescence. As positive associations have previously been demonstrated between LFTs, lipids, and adipokines in later life, it was hypothesised that cord blood measures would also be positively associated with repeat measures of the analyte in childhood and adolescence.

c) How birthweight and cord blood adipokines, reflecting the intrauterine environment at birth, may be associated with long-term metabolic function of the child, as measured by adiposity in late childhood and adolescence and

by the presence of NAFLD in late adolescence. Given the positive associations that have existed between cord blood leptin and fat mass at birth and in early childhood, as well as the direct effect adiponectin has on body composition and fat deposition, it was hypothesised that higher cord blood leptin and lower cord blood adiponectin, would be associated with greater adiposity in later childhood and adolescence. In addition, it was hypothesised that cord blood leptin and lipids would be positively, and adiponectin negatively associated with NAFLD in adolescence, given the already established associations between these measures and presence of NAFLD activity in later life (Chapter 8).

Specific Hypothesis for each Chapter

Chapter 3: Greater maternal adiposity (as measured by higher maternal BMI and greater or excessive GWG) would be positively associated with offspring birthweight, cord blood leptin, and lipids (higher cord blood cholesterol and triglycerides and lower cord blood HDLc) and lower cord blood adiponectin.

Chapter 4: Maternal diabetes in pregnancy would be positively associated with offspring birthweight, negatively associated with cord blood adiponectin, and limited or no impact on cord blood lipids.

Chapter 5: Elevated blood pressure in pregnancy would be negatively associated with offspring birthweight and cord blood adiponectin and positively associated with cord blood leptin and lipids (elevated cord blood cholesterol, triglyceride and lower HDLc).

Chapter 6: Cord blood LFTs (AST, ALT, and GGT), lipids (cholesterol, triglyceride and HDLc), CRP, and leptin would track positively, and cord blood adiponectin track negatively when repeat measures of each analyte were obtained at ages 9 (LFTs, lipids, leptin, and adiponectin), 15 (lipids and CRP) and 17 (LFTs, lipids and CRP).

Chapter 7: Cord blood leptin would be positively, and cord blood adiponectin negatively associated with offspring birthweight and BMI, waist circumference, and fat mass at ages 9 and 17.

Chapter 8: Cord blood leptin, lipids, and birthweight would be positively, and cord blood adiponectin negatively associated with an ultrasound (measured by liver stiffness, volume, and presence of a fatty liver) and biochemical (measured by ALT, AST, and GGT) diagnosis of NAFLD.

Chapter 2: General Methods

2.1 The Avon Longitudinal Study of Parents and Children (ALPSAC)

2.1.1 Study population and ethical approval

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective birth cohort study investigating the health and development of children (Boyd *et al.*, 2013; Fraser, Macdonald-wallis, *et al.*, 2013). The study website contains details of all the data that is available through a fully searchable data dictionary; http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/. ALSPAC had sought prior ethical approval from the ALSPAC Law and Ethics Committee (Institutional Review Board 00003312) and the National Health Service local ethics committee. Participants provided written consent to participate in the study, including information from medical records to be abstracted. Written informed consent was obtained from parents/guardians providing consent for offspring up to age 16 years, with offspring providing consent thereafter.

A total of 14,541 women were initially enrolled, with expected dates of delivery between 1st April 1991 and 31st December 1992. Of those recruited, there were 5011 mother-offspring pairs with a cord blood sample.

2.1.2 Obstetric and perinatal data

Six trained research midwives from ALSPAC extracted data from obstetric medical records. There was no between midwife variation in the mean values of data abstracted from the case notes as repeat data entry checks showed error rates

consistently less than 1%, as previously reported by ALSPAC (Fraser, Macdonald-wallis, *et al.*, 2013). This data included maternal age, GWG, complications during pregnancy (hypertensive or diabetic disorders), gestational age at birth, mode of delivery, offspring's sex, and birthweight. Maternal pre-pregnancy height and weight, smoking status (defined as never smoked, smoked before but not during pregnancy and smoked during pregnancy), parity, occupational social class, and highest educational attainment were obtained from questionnaires completed by the mothers in early and advanced stages of pregnancy. Self-reporting of pre-pregnancy weight correlated highly with weight at the first antenatal visit (Geelhoed *et al.*, 2010). The occupation was used to allocate social class groups using the 1991 British Office of Population and Census Statistics classification. To quantify GWG during pregnancy, maternal weight was recorded by research midwives at regular intervals during the pregnancy (median 10, IQR 8, 10) along with the corresponding gestational age and date. Although the exact models of confounder adjusted analysis are discussed later and according to each Chapter, maternal confounders obtained from data extraction by ALSPAC included age, smoking, parity, occupational social class, education, and pre-pregnancy BMI. Further analysis included adjusting for pregnancy confounders obtained from ALSPAC and accounted for gestational age at birth, mode of delivery, gestational weight gain, hypertensive disorders, and diabetic disorders of pregnancy.

Gestational age was included as a confounder in the models of analysis as it was proposed that a longer duration of pregnancy may influence the circulating metabolites within the intrauterine environment. Literature supports increasing circulating leptin levels with advanced gestational age, in particular in those who are already overweight or obese (Misra, Straughen and Trudeau, 2013). Cord blood adiponectin also rises with advanced gestational age, particularly from 24 weeks' gestation (Kajantie *et al.*, 2004). Greater gestational age would also likely be associated with greater maternal GWG, which has also been positively associated with higher maternal and offspring leptin levels (Logan *et al.*, 2017). Also in keeping with advanced gestational age is larger offspring birthweight and evidence supports higher leptin and a lower rate of change of HDL levels amongst those with larger

offspring birthweight (Farias *et al.*, 2017). As leptin is additionally secreted by the placenta during pregnancy, advanced gestation and therefore a larger placenta is likely to yield higher circulating leptin levels (Masuzaki *et al.*, 1997). There is also the risk of placental dysfunction and consequent metabolic dysfunction with very advanced gestational age. Collectively this may have influenced the circulating levels of adipokines and lipids and justifies including gestational age as a cofounder, and mediator, in the analysis. However, for most individuals included in the Study, delivery was around 'term' (40 weeks) and was unlikely to exceed 42 weeks, which reflects obstetric practice throughout the UK. Conversely, deliveries before 37 weeks (pre-term pregnancies) were not well represented in this Study for several reasons. The pre-term umbilical cord would be less likely to yield sufficient blood volume to be included in the study, partly because the cord would have been narrower and technically more difficult to access the umbilical vein, but also because the cord would have contained less blood volume. In addition, pre-term deliveries were also encouraged to allow passive flow of cord blood into the baby following delivery (and before clamping of the cord) to increase delivery of red blood cells to the preterm offspring, therefore limiting the abundance of available cord blood. As a result of the impact gestational age may have on the offspring and the circulating analyte concentrations, it was therefore adjusted for in the analysis.

Mode of delivery was also adjusted for in the analysis, owing to its influence on cord blood adipokines at birth. Vaginal birth has been associated with higher cord blood leptin levels compared to caesarean section deliveries (Yoshimitsu *et al.*, 2000). This finding however has not been demonstrated by all, with one study concluding that leptin was associated with duration of labour rather than the mode of delivery alone (Logan *et al.*, 2016) and another study demonstrated that adiponectin was not associated with mode of delivery (Kajantie *et al.*, 2004).

Mediator adjusted analysis has also been also described within the analysis in Chapters 3 and 4, as maternal BMI or GWG may have influenced the cord blood outcomes. In Chapter 4, birthweight and gestational age were also included as

potential mediating factors when examining maternal diabetes and its offspring cord blood analytes. In this case, maternal diabetes and its association with GWG may have positively influenced offspring birthweight which may then have also influenced the cord blood adipokine levels, justifying their inclusion as potential mediators. These variables may also have been examined as potential confounders however as there was progressive glucose dysregulation with advancing gestational age. Furthermore, in Chapter 7, offspring height and fat mass in the preceding age group feature in the causal pathway and were accounted for in the analysis. In Chapter 8, where offspring liver outcomes were examined in relation to cord blood measures, the offspring's height and weight were also accounted for given the associations with offspring anthropometry in adolescence, which may in turn have influenced the risk of metabolic dysfunction, as measured by offspring NAFLD.

2.1.3 Offspring data

Offspring were followed up at specified clinics and anthropometric measurements were obtained by ALSPAC, as previously reported (Boyd *et al.*, 2013). Specific details on offspring variables have been listed according to each Chapter. Identical protocols were used at all ALSPAC follow-up clinics. At each clinic assessment participants' age in months was recorded and their weight and height were measured in light clothing and without shoes. Weight was measured by ALSPAC to the nearest 0.1kg using Tanita scales. Height was measured by ALSPAC to the nearest 0.1cm using a Harpenden stadiometer. Dual-energy X-ray absorptiometry (DEXA) scans were used by ALSPAC to measure total fat mass. Waist circumference was measured by ALSPAC to the nearest 1mm at the midpoint between the lower ribs and the pelvic bone with a flexible tape and with the child breathing normally. BMI was represented by z-scores, adjusted for age and sex according to the 1990 British Growth Charts (Cole *et al.*, 2000). Offspring obesity was classified using BMI and criteria defined by the International Obesity Task Force (Cole *et al.*, 2000).

For the purpose of this Thesis, offspring birthweight was examined as an exposure variable. In Chapter 7, relations between birthweight (the exposure) and offspring adiposity outcomes have been addressed and in Chapter 8, birthweight was further analysed as an exposure in relation to offspring liver outcomes. Birthweight, however, has also been acknowledged as an outcome of the 'exposed' intrauterine environment. As discussed in Chapter 1, umbilical cord blood leptin has been widely recognized as an accurate biomarker for neonatal fat mass (Hauguel-de Mouzon, Lepercq and Catalano, 2006) with its concentration at birth proportional to birthweight. In animal studies, overexpression of adiponectin has been associated with greater fat depots in early life, and in contrast, adiponectin knockout fetuses have lower fat content and body weight (Qiao, Yoo, *et al.*, 2012). Birthweight has also been related to maternal BMI (Stamnes Koepp *et al.*, 2012), uncontrolled GDM (Gillman *et al.*, 2003), and GWG (Stamnes Koepp *et al.*, 2012), highlighting birthweight as a product of the intrauterine environment rather than simply the primary exposure for that individual.

2.1.4 Body Mass Index (BMI)

Body Mass Index (BMI) is the most commonly used method to quantify adiposity. It is easily reproducible, simple to calculate, and is used universally so that data can be calculated and compared between studies globally. By assessing weight relative to height, BMI is expressed in kg/m^2 and is calculated as weight (kg) / (height (m) ^{2}. Table 2.1 displays BMI categories as defined by the World Health Organisation (WHO).

Table 2.1: BMI as defined by the World Health Organisation (WHO)

Category	BMI Range (kg/m^2)
Underweight	<19.9
Normal	20.0 - 24.9
Overweight	25.0 – 29.9
Obese Class I	30.0 – 34.9
Obese Class II	35.0 – 39.9
Obese Class III	>40.0

Whilst the use of BMI is adequate in general population studies, it may have its limitations. As it is a simplistic measurement of total body fatness, it assumes that relative proportions of muscles, water, and fat mass remain constant. BMI or adiposity may be overestimated in athletes for example, with a greater proportion of lean body mass. The distribution of the fat may also determine the risk of cardiovascular disease and as discussed in Chapter 1, it has been proposed that central (or abdominal) adiposity poses a greater risk (Eleanor Jarvie *et al.*, 2010). The categories of BMI may also vary according to ethnicity as the relative cardiovascular risk may be greater at lower thresholds of BMI in South East Asian and Japanese populations (Razak *et al.*, 2007). In these populations, a BMI of >23.0 kg/m^2 may be considered overweight and at greater cardiovascular risk compared to their European counterparts, where a BMI of >25.0 kg/m^2 would meet this criterion.

For the purpose of this study, childhood BMI was represented by z-scores, adjusted for age and sex according to the 1990 British Growth Charts (Cole, Freeman and Preece, 1998).

In order to assess body composition and distribution of fatness, direct and indirect methods were employed. BMI is an example of an indirect method, in addition to anthropometric measurements such as waist-hip ratio (WHR), waist circumference (WC) and skinfold thickness. Direct methods include DEXA, computerised tomography (CT) and magnetic resonance imaging (MRI).

2.1.5 Indirect Methods: Anthropometric measurements

The WHR is the simplest measurement of body distribution and assesses the ratio of waist circumference to hip circumference. A WHR >0.85 has been shown to demonstrate greater central adiposity and therefore cardiovascular disease and type II diabetes mellitus (Kannel *et al.*, 1991). For the purpose of this thesis WC in the offspring was measured by ALSPAC.

Skinfold thickness is a more detailed measurement of fat distribution. It is a means of quantifying total body fat as a percentage of total body weight. With the use of callipers to gauge fatness at specific anatomical sites, namely subscapular, biceps, triceps, and supra-iliac locations. At birth, skinfold thickness is considered the best method of determining body fat composition (Strydom, Van Niekerk and Dhansay, 2019).

Implementation of the WHR or skinfold thickness has major advantages in larger-sized studies in that they are low cost, easily accessible, and acceptable by patients involved in the study. As the method of measurement is not exactly entirely reproducible, however, identifying the correct site for measurement as well as employing the use of the calipers will vary according to each operator which will lead to a lot of variation. Whilst repetition and training may lower this variation, inaccuracy in the method may also stem from the population being studied, should

they be of extremes of weight in the Gaussian curve. It is however a good method of assessing body composition in longitudinal studies over a long period.

2.1.6 Direct methods: Imaging

DEXA, CT and MRI compose the main imaging techniques used to measure body fat content as well as providing an assessment of fat distribution. For the purpose of this thesis, we have also used data obtained by ALSPAC on offspring fat mass using DEXA. DEXA makes use of the differential attenuation of X-ray radiation by bone, adipose, and non-adipose soft tissue. It was relatively operator-independent and it was a quick method to perform. Other advantages were that as well as providing information on total adipose tissue, it also reported specific sites of adipose deposits. Body composition, using the 4-compartment model, was also measured more accurately with the measurement of bone mass. These 4 compartments included fat mass, and fat-free mass including bone mass, body water, and protein or muscle mass. Disadvantages however included the expense of the assessment and exposure to ionising radiation. Although radiation exposure with X-ray is low, it did however limit its usefulness as it was not suitable in pregnant women or children.

Whole-body CT is an alternative to DEXA but it was a more expensive option and would have exposed the individual to significantly more X-ray radiation. MRI has the advantage that it avoids radiation exposure. It provides a detailed assessment of adipose tissue and organ lipid content due to its increased resolution of soft-tissue and therefore greater delineation of adipose tissue deposits.

2.1.7 Cord blood samples

Cord blood samples were collected and prepared by ALSPAC at the time of delivery. Specimens were separated into 1ml aliquots of cord blood plasma along with 500µl of heparin and stored at -70°C. The samples were transferred frozen from ALSPAC to the University of Glasgow (British Heart Foundation building), where all of the cord blood sample analyses were performed for the purpose of this thesis. Cord blood leptin and adiponectin were measured using commercially available ELISA (Enzyme-Linked ImmunoSorbent Assay) kits (Quantikine human leptin immunoassay, R&D systems, Quantikine human adiponectin immunoassay, R&D systems). Cord blood total lipids (cholesterol, triglyceride, HDLc and non-high-density lipoprotein cholesterol (non-HDLc), LFT's and CRP were measured using Roche Cobas C311 auto-analyser using enzymatic reagents for lipid determination. Non-HDLc was calculated as total cholesterol minus HDLc. Cord blood c-peptide was measured using Roche Cobas E411 auto-analyser. This is summarised in Table 2.2. Analysis of the cord blood was completed within a maximum of three freeze-thaw cycles, over twelve months, and remained at -80°C in between thaws.

2.2 Materials

Table 2.2: Materials used for laboratory analysis

Cord blood analyte	Manufacturer		
Leptin	Quantikine human leptin ELISA	R&D Systems, UK	DLP00
Adiponectin	Quantikine human adiponectin ELISA	R&D Systems, UK	DRP300
Cholesterol	Cobas C311 auto-analyser	Roche	CHOL2
Triglyceride	Cobas C311 auto-analyser	Roche	TRIGL
High-density lipoprotein cholesterol (HDLc)	Cobas C311 auto-analyser	Roche	HDLC3
C-reactive protein (CRP)	Cobas C311 auto-analyser	Roche	CRP
Alananine transaminase (ALT)	Cobas C311 auto-analyser	Roche	ALT/GPT
Aspartate transaminase (AST)	Cobas C311 auto-analyser	Roche	AST/GOT
Gamma-glutamyl transferase (GGT)	Cobas C311 auto-analyser	Roche	GGT
C-peptide	Cobas E411 auto-analyser	Roche	CPEP

2.2.1 Analyte Assays

Types of ELISA and procedure protocol

ELISA is an immunoassay used to detect the presence of proteins or other antibodies, known as 'antigens'. Commercially available ELISA kits were used for leptin and adiponectin quantification (R&D Systems, UK) for the purpose of this thesis. This method has previously been used for the quantification of both cord blood leptin and adiponectin (Teague *et al.*, 2015; Logan *et al.*, 2017), and this study will also later directly compare the precision of two separate ELISA kits. In ELISA, the capture antibody or protein, specific to the target analyte, was bound to each well so that many samples could be analysed and processed simultaneously. The ELISA assay typically entailed a 96-well microplate that enabled efficient analysis of the samples of interest. A conjugated detection antibody was then used to bind to the epitope on the target analyte. This was followed by the addition of a substrate solution which reflected the amount of analyte bound by releasing a signal in proportion to it. The presence of a signal meant that the antigen of interest was present.

ELISA is the widely preferred method of examining and quantifying specific protein levels in biological samples. Whilst there are other methods available, including Western Blots and other immunoassay platforms, they are not as specific or as sensitive tests and do not produce entirely quantitative results. The binding characteristics of the antibodies, the amplification process involved in some, as well as the display system for the signal produced all contributed to the highly sensitive nature of the ELISA process. Furthermore, ELISA is a robust method that can withstand various buffers or pre-treatments already used. As the signal produced was specific for the amount of analyte of interest present within the sample, detection or amplification of other proteins not being examined was unlikely. When the protein of interest was very low, the lower limit of detection could be adjusted

and there was also the option to add in an additional amplification step to aid the sensitivity of the analyte.

There are four different types of ELISA: direct, indirect, sandwich, and competitive ELISA. The advantages and disadvantages are described in Table 2.3

Table 2.3: Advantages and Disadvantages of Four Types of ELISA

Type of ELISA	Advantages	Disadvantages
Direct	Rapid process Protocol easy to follow (only one antibody used)	Less specific and less sensitive Risk of interference
Indirect	A secondary antibody is used allowing greater amplification of the analyte	Risk of cross-reactivity
Sandwich	Most specific and sensitive ELISA as two antibodies required to bind to the protein of interest	ELISA takes longer More complex protocol
Competitive	Quantification of smaller molecules (using one antibody)	Less specific Conjugated antigen required

Direct ELISA

Direct ELISAs are a preferred method of analysis where rapid processing is required as it only uses one antibody. As a result of this, however, they are less specific than other types of ELISA, such as sandwich ELISA. Typically, this method is used when

examining antibody affinity and specificity or when assessing blocking or inhibitory interactions. During this process, the antigen or sample is immobilized directly on the plate and a conjugated primary detection antibody binds to the target protein (antigen). With the addition of the substrate solution, the amount of analyte in the sample is represented by the proportion of the signal that is produced.

Indirect ELISA

Indirect ELISAs are typically used when examining endogenous antibodies. They differ from the Direct ELISA due to the addition of an extra amplification step to detect the immobilized antigen. As a result, Indirect ELISAs take longer. Similar to the direct ELISA, the antigen is immobilized on the plate using an unconjugated primary detection antibody. In contrast to the direct ELISA, a conjugated secondary antibody is then added and is directed against the host species of the primary antibody. Finally, with the addition of the substrate solution, a signal proportional to the amount of antigen bound in the well is then released. As the desired antigen may be present in very low quantities compared to the proportion of other proteins present in the sample, Indirect ELISA may not be able to detect the presence of the antigen in the sample. Whilst Indirect ELISA may be more sensitive than Direct ELISA, there is also a risk of cross-reactivity with the antigen.

Sandwich ELISA

Sandwich ELISAs were used in the current study and examined the concentration of an analyte in a biological sample. They were therefore the most common type of ELISA. The process took longer and the protocol more complex to follow as it used two specific antibodies to ‘sandwich’ the antigen. As a result of this, they are highly specific assays. With the ‘capture’ antibody coated on the microplate, the addition of the sample enabled the protein of interest to bind and immobilize. This was

followed by the addition of a conjugated-detection antibody which bound to an additional epitope on the target protein. As displayed by the other types of ELISA, a signal was produced that reflected the amount of the analyte within the sample when the substrate solution was added.

Competitive ELISA

Competitive ELISAs use only one antibody and are used for the detection of small molecules and hormones. In this case, the protein of interest cannot be 'sandwiched' effectively as it is too small, so a conjugated antigen is used to bind to the antigen in the sample. With greater quantities of antigen within the sample, the less conjugated antigen will bind to the capture antibody. Similar to the other types of ELISAs, a substrate solution is then added but in contrast, the signal produced in Competitive ELISA is inversely proportional to the amount of protein within the sample.

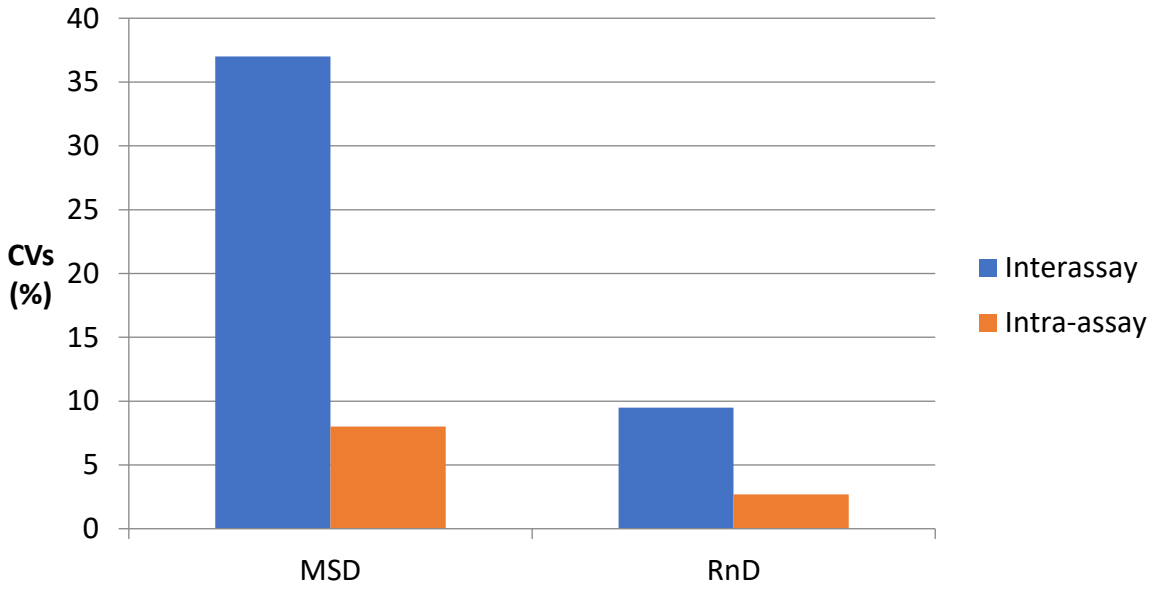
2.2.2 A comparison of leptin ELISA kits

A comparison was performed between leptin ELISA kits from 2 different manufacturers: Meso Scale Diagnostics (MSD) and R&D systems. This was required to determine which method would provide sufficient reliability results. Using the MSD platform, 10 plates were analysed, and using the R&D systems platform, 43 plates were analysed. Inconsistency in the results was demonstrated early on in the study, justifying why only 10 plates from the MSD manufacturer were used. The inter-and intra-assay coefficients of variability (CVs) were consistently better using the R&D systems kits which led to their use for the entirety of the Study (Table 2.4 And Figure 2.1).

Table 2.4: Inter and intra-assay CVs between MSD and R&D leptin ELISA kits

		MSD (10 plates)	R+D (43 plates)
Inter-assay	**Mean (ng/ml)**	9.72	11.53
	St dev (ng/ml)	3.61	1.10
	CV	0.37	0.096
Intra-assay	**CV**	0.08	0.03

Figure 2.1: Inter and intra-assay CVs between MSD and R&D leptin ELISA kits

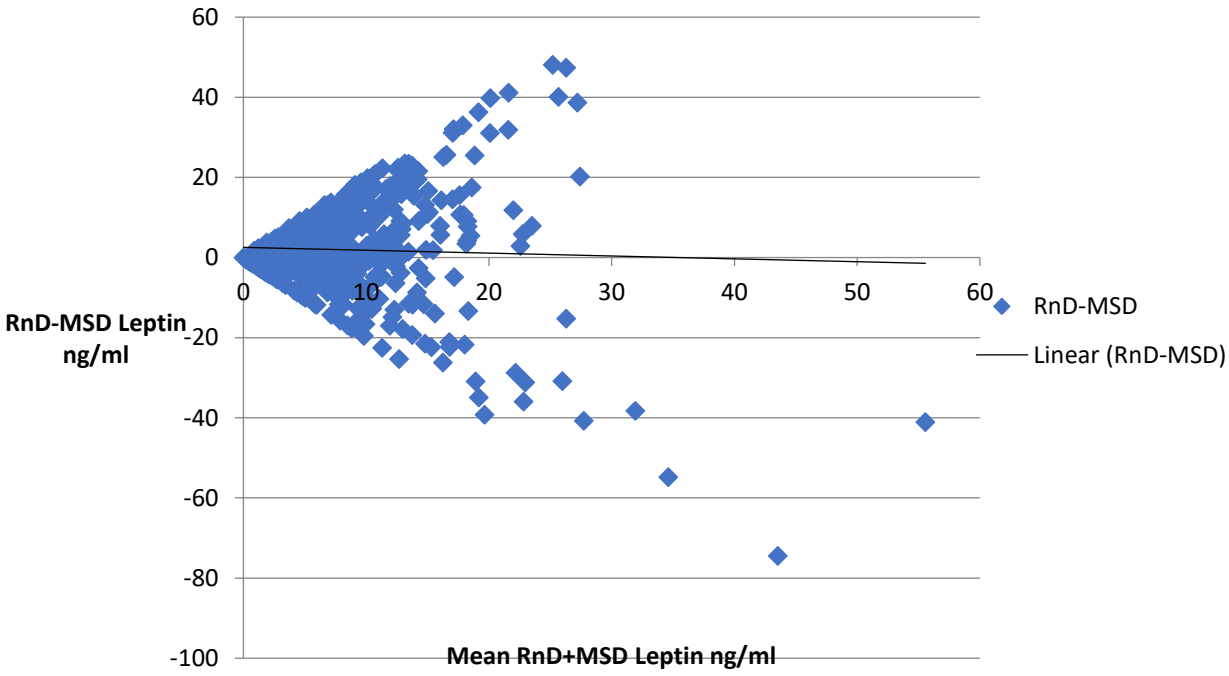


In order to identify the presence of systematic differences and possible outliers between measurements in the R&D and the MSD groups, a Bland Altman plot was developed. Figure 2.2 shows poor agreement between the two methods of leptin ELISA. The x-axis represents the mean of the two measurements, whereas the y-axis represents the difference between the two values. The Plot was used as a

means of evaluating bias between the mean differences. This method of examining the data was explicitly different from examining relationships between values. In correlation studies, for example, one value is compared to another rather than examining the actual difference between the two groups. Based on this assessment of two different commercially available kits, R&D ELISA kits were used for the analysis of the cord blood samples for the purpose of this thesis.

Figure 2.2: Bland Altman plot showing poor agreement between leptin assays

Mean (bias): 2.01 ng/ml
SD: 10.9 ng//ml
Upper limit of agreement: 23.4 ng/ml
Lower limit of agreement: 19.4 ng/ml



2.2.3 Leptin procedure protocol:

Reagents and samples were brought to room temperature before use and the assay was performed according to manufacturers' instructions. Samples were diluted 100-fold prior to the assay. The reagents were prepared to produce a substrate solution, the wash buffer was diluted, and a stock solution was used to prepare a leptin standard.

100 µL of Assay Diluent was added to each well on the 96-well plate. Then, 100 µL of sample or control was also added to each well of the pre-coated plate. The plate was covered and left to incubate for 2 hours at room temperature. The incubation process enabled the antigen to be adsorbed entirely to the well surface.

The plate was then aspirated, and each well was washed in an automated plate washer 4 times. For this, a phosphate-buffered saline with Tween detergent (PBS-T) was used. This process removes unbound or poorly bound protein and leaves the protein being assayed bound to the plate. 200 µL of Conjugate was then added to each well. The plate was then again covered and left to incubate at room temperature for 1 hour. A secondary detection antibody conjugate, which had been biotinylated and was specific to the protein being assayed, was then added. This ensured that the primary antibody bound non-specifically to the well.

The samples were then aspirated and each well was washed in an automated plate washer 4 times, again removing any unbound detection antibody. The protein being assayed was then anchored to the primary and secondary antibodies. 200 µL of Substrate Solution was then added to each well in the plate following which, the plate was covered and incubated at room temperature for 30 minutes, ensuring its protection from light.

The Substrate Solution was composed of horseradish peroxidase-conjugated to streptavidin. It detected the antibody being quantified as streptavidin bound biotin (in the Conjugate) specifically. It released a blue coloured dye in response to peroxidase activity (3,3',5,5'-tetramethylbenzidine becomes 3,3',5,5'-tetramethylbenzidine diamine), reflecting the amount of protein of interest detected. 50 µL of Stop Solution was then added to each well in the plate. Stop solution (sulphuric acid) was then added and caused a colour change in the wells from blue to yellow.

The plate was then placed in the plate reader within 30 minutes of adding the Stop Solution. The plate reader used spectrophotometry to measure light absorption at 450nm. The amount of absorption reflected the concentration of the yellow solution produced by adding the Stop Solution. This was directly proportional to the concentration of the adipokine protein being assayed.

2.2.4 High and low molecular weight adiponectin

Adiponectin circulates in the plasma in three distinct forms: 70 kDa low molecular weight (LMW) noncovalently-linked trimers, disulphide-linked medium molecular weight (MMW) hexamers, and high (>300 kDa) molecular weight (HMW) multimers (Ebinuma *et al.*, 2007). Each has a specific function and action on individual target tissues. Circulating in peripheral tissues (Oh, Ciaraldi and Henry, 2007; Heidemann *et al.*, 2008), the primary bioactive fraction of adiponectin resides in the form of HMW adiponectin (Hara *et al.*, 2006). It is widely favoured for its best approach to measuring adiponectin concentrations. It is recognised for its insulin sensitising effects whilst being negatively associated with obesity, insulin resistance, and coronary artery disease. Repeatedly, HMW (and total) adiponectin have proven to be the more useful indicators of metabolic conditions as there are greater fold differences between HMW and downregulation of insulin-resistant states and in the extremes of BMI (Daimon *et al.*, 2003; Nakashima *et al.*, 2006; Zhu *et al.*, 2010; Goto *et al.*, 2014). HMW adiponectin however may be implicated more specifically in vascular dysfunction associated with obesity (Kobayashi *et al.*, 2004). Furthermore, in studies examining high-risk populations, such as those with diabetes or demonstrable coronary artery disease, HMW adiponectin may be a more reliable marker of cardiometabolic risk than total adiponectin (Aso *et al.*, 2006; Hara *et al.*, 2006). In these studies, HMW adiponectin displayed its association with (rather than its predictive value of) already established individuals with insulin resistance and cardio-metabolic dysfunction. In a healthy population, however, both total and HMW adiponectin have comparable predictive values of impending insulin resistance and metabolic dysfunction (Almeda-Valdes *et al.*, 2010).

Although the role of LMW adiponectin is less defined, there is growing evidence to also implicate LMW adiponectin in the control of glucose metabolism (Graessler *et al.*, 2011; Goto *et al.*, 2014). Found predominantly in cerebrospinal fluid (Ebinuma *et al.*, 2007; Kusminski *et al.*, 2007), LMW adiponectin is not as widely associated with insulin resistance or metabolic dysfunction. In a case-control study comparing

a Japanese population of diabetic patients with matched controls however LMW adiponectin, in addition to validated biomarkers total and HMW adiponectin, demonstrated an inverse association with diabetes (Goto *et al.*, 2014).

For the purpose of this thesis, total adiponectin rather than HMW adiponectin was measured as it would have required pre-treatment of the plasma sample with a specific protease enzyme so that the differential forms of the protein (trimers, hexamers) would be removed. Measurement of total adiponectin was therefore a standardised, cost-effective, and accurate assessment of the circulating adiponectin concentration.

2.2.5 Adiponectin procedure protocol

Samples and controls were removed from the freezer and transferred to a rack to defrost to reach room temperature. The control used was pooled plasma EDTA from healthy individuals. 2 mls of calibrator diluent RD6-39 was added to the adiponectin standard. This produced a stock solution of 250ng/ml. The sample was mixed to ensure complete reconstitution and allowed to sit for a minimum of 15 minutes prior to making dilutions. Within the assay plate, there were 78 specimen samples (labelled 1 to 78), 4 control samples (labelled C), and 7 calibrator samples (standards, labelled 1 (low) to 6 (high) and 7 (blank)).

2ml of the calibrator diluent RD6-39 was pipetted into the tubes labelled 1-78 and then pipetted into each of the 4 controls. Once completely defrosted, 10µl of the sample was added to tubes 1-78, and each of the controls and mixed.

Using a multichannel pipette, 100µl of the assay diluent RD1W was added to each well then 50µl of the standards, control, and sample were also added using a specified plate plan.

The plate was then covered with an adhesive strip and incubated for 2 hours at room temperature on a plate shaker.

The wash buffer was prepared and mixed, to ensure there were no crystals in the sample. The wash buffer was then diluted in deionised water. It was used to aspirate and wash each plate 4 times on the automated washer. Excess wash buffer was then removed and 200µl of adiponectin conjugate was added to each well. The plate was then re-covered and incubated for 2 hours at room temperature on a plate shaker.

The plate was then aspirated and washed again. The substrate solution was then prepared within 15 minutes of use and 200 µl was added to each well, ensuring that the plate was protected from light. This process turned the samples blue. The plate was then re-incubated for a further 30 minutes at room temperature.

Finally, 50 µl of stop solution was added to each well. The plate was read at 450 nanometres on a Tecan Magellan plate reader within 30 minutes of adding the stop solution.

2.2.6 Auto-analyser

The Roche Cobas auto-analysers were employed for analysis of cholesterol, triglyceride, HDLc, CRP, ALT, AST, and GGT (C311 model) and c-peptide (C411 model). They were selected for use within the study largely as they were already readily available within the lab, however, there were also demonstrable advantages to using them. They are stand-alone molecular platforms that allowed convenient reagent and sample handling. With up to 45 tests onboard capacity, they allowed rapid processing of samples. The main benefit of these analysers was that they could process very small sample sizes, which was of particular importance when processing finite cord blood samples. Over 70% of all the reagents required sample volumes of less than 5µl so this minimised the volume of sample wasted or required for the sample to be processed. In addition, the analysers were able to check the integrity of the sample via highly sensitive pressure sensors and a clot detection system. This reduced the level of repeated errors and highlighted which samples possessed a significant amount of clot and could not be processed as a result. There were also advantages in terms of the use of the reagent provided. These reagents were already conveniently prepared, thereby minimising the handling of the reagent so overall reduced time and increased the throughput of the specimens. The reagent was stable for 3-month periods, so the long inter-calibration intervals reduced the time spent processing this.

2.2.7 Quality control (CV)

CV is the standardised measure of the frequency of distribution. It demonstrates the precision of the assay as well as signifies its repeatability. The CV was calculated as the standard deviation divided by the mean. The standard deviation must always be understood in the context of the mean and it quantified the amount of variability ie. if the standard deviation was low, the value was close to the mean. The inter-assay CV represented the CV between assays whereas the

intra-assay CV demonstrated the CV within assays. The desired CV for biochemical analysis such as in this study was less than 10%. Both the inter-and intra-assay CVs met this criterion, as shown in Tables 2.5 and 2.6. The quality control data for all of the studies performed are displayed in the Appendix, Tables 1-3. Using the Roche C311 equipment to process the cord blood lipids and LFT's, a standard control was used to detect lower concentration (ensuring that this remained within the range 1.5-2.46 ng/ml), as well as a higher concentration of analytes (ensuring that this remained in the range 7.35-12.00 ng/ml). An adult control sample was used as well on each plate in the analyser to ensure these remained within desired reference ranges, as shown in Table 3 in the Appendix.

Table 2.5: Inter-assay CV

Analyte	Mean	Standard deviation (SD)	Coefficient of variability (CV, %)
Leptin (pg/ml)	11.38	1.08	0.09 (9.0)
Adiponectin (µg/ml)	14.41	0.47	0.03 (3.0)
HDLc (mmol/l)	1.11	0.04	0.04 (4.0)
Cholesterol (mmol/l)	2.52	0.08	0.03 (3.0)
Triglyceride (mmol/l)	1.25	0.03	0.03 (3.0)
ALT (u/l)	42.82	1.76	0.04 (4.0)
AST (u/l)	50.03	1.77	0.04 (4.0)
GGT (u/l)	51.56	1.30	0.03 (3.0)
CRP (mg/l)	3.95	0.05	0.01 (1.0)

Table 2.6: Intra-assay CV

	Intra-assay CV (%)
Leptin ELISA	0.03 (3.0)
Adiponectin ELISA	0.06 (6.0)
Roche C311	
Lower concentration control	0.02 (1.7)
Higher concentration control	0.00 (0.3)

2.3 Cord blood analyte reference ranges and stability with long-term storage

The cord blood samples used within the study were originally obtained between 1991 and 1992 and have been in freezer storage since. It was vital to examine the stability of the analytes included in the study over time in order to aid the interpretation of the results and ensure their validity in such prospective studies. Whilst preliminary studies encouraged the laboratory separation of the blood sample immediately after venesection to ensure greater accuracy of the assay (Diver *et al.*, 1994), more contemporary studies have highlighted many additional factors that may contribute to analyte variability.

The concentration of an analyte may be affected by the method of specimen collection, the conditions the sample was stored, or the number of freeze-thaw cycles prior to sample processing. Direct comparison of clinical studies needs this process to be standardised so that valid between sample analysis can be made. Variations may be present from the onset – from the fasting status of the patient to the time of day the sample was collected. In addition, the time that was taken for the sample to coagulate, the quantity or type of additive within the areceptacle, and the presence of sample haemolyisis may also influence the assay results. The time left to stand before processing and separating the sample, the method of centrifugation, and the type of storage vials used may also alter the outcome. The storage temperature of the sample could also range from the ambient temperature at the point of collection to the storage temperature in the biobank and there may be wide variations within these. Repeated freeze-thaw cycles may also contribute to sample degradation and so it is generally recommended that this is kept to a minimum.

Whilst studies using cord blood samples were limited, there was more detailed availability of child and adult blood samples that have been used to examine the effect of short and long-term storage. Overall, cord blood c-peptide has been shown

to degrade with long-term storage but in contrast, there did not appear to be the same degree of degradation with cord blood adipokines, lipids, or liver function tests included in this study, as demonstrated in Table 4 in the Appendix.

Flower et al. examined specific variations in specimen collection by assaying leptin, and pro-inflammatory cytokines in 22 healthy subjects (Flower *et al.*, 2000). Samples were allowed to stand at hourly intervals before or after sample separation and freezing at -70°C. The concentration of leptin was unchanged by allowing the specimen to stand for up to 6 hours (Flower *et al.*, 2000). The study also examined the effect of using 3 different anticoagulants (lithium heparin, sodium citrate, and EDTA) or no anticoagulant in the specimen collection tubes. EDTA specimens demonstrated stability for both leptin and cytokines however there was wide variation in the analytes when alternative anticoagulants or no anticoagulant was used (Flower *et al.*, 2000). The impact of freeze-thaw cycles was also addressed in this study by performing a series of freeze-thaw cycles prior to the analysis. This showed that leptin (along with IL-6) could withstand up to 6 freeze-thaw cycles (Flower *et al.*, 2000). Another study demonstrated high reproducibility of leptin sampled 1 year apart (Stattin *et al.*, 2004) and a further study has demonstrated its stability when taken 4 years apart (Chu *et al.*, 2001).

Leptin does not appear to vary according to fasting or non-fasting status in normal or obese subjects and was, therefore, a reliable biomarker to measure irrespective of recent dietary intake (Korbonits *et al.*, 1997). Although there was demonstrable stability of leptin with storage, it did display a significant circadian rhythm (Sinha *et al.*, 1996). For studies to be reproducible, leptin ideally would be sampled at the same time of day as the concentration doubles overnight compared to during the day (Sinha *et al.*, 1996). It was unlikely, however, that the ALSPAC specimens were obtained outwith daytime hours. This further validated leptin as a robust analyte as it did not appear to degrade with storage, nor did it vary depending on recent dietary intake and it could withstand repeated freeze-thaw cycles without adversely affecting the assay outcome.

In contrast to leptin, levels of pro-inflammatory cytokines, Interleukin-6 (IL-18) and TNF- α, as demonstrated by Flower et al. varied significantly if left unseparated for over 4 hours (Flower *et al.*, 2000). These cytokines are strongly related to obesity and metabolic disorders. They are both secreted by adipocytes and are responsible for the induction of acute-phase proteins producing proteins including CRP, culminating in inflammatory states (Popko *et al.*, 2010). Consequently, cord blood CRP was measured during this thesis and has previously demonstrated stability 4°C, -20°C, and -80°C over 3 months (Q. Li *et al.*, 2013)(24). That the findings were null (0), excluding CRP from the analysis, may reflect the very low levels of CRP expected in healthy pregnant women. Elevated levels may typically be found in those who have developed sepsis or an acute inflammatory state for example and therefore compose very small numbers within the study. These women may also have been excluded from the study due to ill-health at the initial point of cord blood sample collection.

The stability of adiponectin specifically has also been assessed in a small study by examining the effect of leaving the primary sample up to 36 hours before processing and repeating the sample 1 year later to assess reproducibility (Pischon, Hotamisligil and Rimm, 2003). Using both EDTA and sodium heparin products within the specimen tube, samples were processed at sequential intervals until 36 hours from the initial point of collection. There was no change in the concentration of adiponectin if left to stand for 36 hours before processing, compared to immediate analysis however the concentration increased if left for more than 24 hours in the EDTA sample. It has been proposed that this is either due to chance or that this observation may be a result of adiponectin's dissociation from polymeric and multimeric forms leading to a higher result (Pischon, Hotamisligil and Rimm, 2003). Overall the concentration of adiponectin decreased when stored at -150°C and was assayed again one year later. It has been proposed however that the individuals' change in BMI over time may account for this. There was no significant difference in the concentration of adiponectin one year later following adjustment for BMI indicating that it is a highly reproducible analyte (Pischon, Hotamisligil and Rimm, 2003).

There have been varying outcomes on the effect of storage on hepatic enzymes. LFT's appeared stable when stored over 10 hours at 21°C (Henriksen *et al.*, 2014) and again when LFT's were examined over 56 hours and stored at 25°C in either unseparated or separated (into plasma and serum) form (Boyanton and Blick, 2002). Furthermore, analysis of rat serum has shown that transaminases were stable when stored in the fridge for up to 7 days and when they examined the samples over a longer period (Cray *et al.*, 2009). They demonstrated that LFTs were stable for up to 90 days at -20°C in a frost-free freezer and up to one year at -70°C (Cray *et al.*, 2009). Other studies have supported this, reporting that both GGT and aminotransferases to be stable when stored at -20°C for 3 months and could withstand up to 10 freeze-thaw cycles (Cuhadar *et al.*, 2013). In addition, Jung et al. found relative stability of hepatic enzymes, including ALT, AST, GGT, and alkaline phosphatase, over 10 months when stored at -196°C (liquid nitrogen) (Jung, Bader and Grutzmann, 1984).

In contrast, others have shown that storage at ambient room temperatures or in the fridge prior to transport to the lab may adversely affect LFT concentration by up to 46% on day 6 of storage at -20°C (Williams, Kline and Dodd, 1987). ALT determination in particular may be influenced by storage conditions. Analysis of rat serum showed 50% demonstrable loss of the analyte when stored in a frost-free freezer at -20°C for up to 90 days (Cray *et al.*, 2009) and they recommend that this degradation should be accounted for when interpreting results of future studies.

Due to the conflicting evidence around the stability of AST, Niblock et al. analysed AST activity at room, fridge, and freezer conditions over 28 days. They demonstrated that AST was stable in ambient conditions for the first 24 hours, and at 4°C, -20°C, and -80°C for up to 28 days, and is in contrast to previous reports (Niblock, Leung and Henderson, 1986). An additional study concluded however that degradation of aminotransferases begins from initial venepuncture and encourages sample assay immediately after the specimen is obtained (Cuccherini *et al.*, 1983). This is further

supported by others who have concluded that time from sample collection to analysis was more influential than the temperature it was stored at but that the rate of analyte decay was limited during lower storage temperatures (An and Park, 2014).

There are limited studies observing the stability of hepatic enzymes over longer periods, whose conclusions could be transferable when examining analytes used in longitudinal studies. The Norwegian Mother and Baby biobank concluded that AST was stable over 2 years of storage at -80°C and could withstand up to thirty freeze-thaw cycles (Paltiel *et al.*, 2008). Overall it appeared that optimising conditions (such as at -80°C), along with improved sample collection, could minimise analyte decay during long-term storage periods (Williams, Kline and Dodd, 1987).

The stability of lipids has also been examined over the short and long term. When stored in the very short term, over 10 hours at 21°C, lipid levels did not appear to deteriorate (Henriksen *et al.*, 2014). Again, when lipids were examined over 56 hours and stored at 25°C in either unseparated or separated (into plasma and serum) form, there was no adverse effect on the variability of the samples (Boyanton and Blick, 2002). Shih et al. examined the stability of lipids (Cholesterol, HDLc, and triglyceride) when stored at -70°C and demonstrated the reliability of the assay after one year. There was however some (<3%) deterioration in triglyceride and cholesterol levels over 5 and 7 years respectively (Shih *et al.*, 2000). The Canadian Laboratory Initiative in Pediatric Reference Interval (CALIPER) study demonstrated the stability of all but one of the 57 routine biochemical markers assayed over a 10 to 13 month period stored at -80°C, with up to one freeze-thaw cycle (Brinc *et al.*, 2012). In addition, cholesterol and HDLc have been examined following storage at -20°C over 3 months and also for up to 10 freeze-thaw cycles and stability of both analytes has been observed (Cuhadar *et al.*, 2013). Further studies have demonstrated that cholesterol and triglycerides may withstand up to thirty freeze-thaw cycles over 2 years of storage at -80°C as there was no demonstrable difference from the analyte observed at baseline (39). Collectively there did appear to be sufficient evidence to support the stability of lipids included in the current study.

C-peptide is a 3kDA hormone and is formed when insulin and propeptide are cleaved to form the active hormone. As it has a longer half-life than insulin it is a useful measure of insulin secretion. Insulin is not a reliable biomarker with longer-term storage as demonstrated over 4 years (Chu *et al.*, 2001). Being a small polypeptide, however, c-peptide is also susceptible to attack from proteases and endopeptidase degradation of c-peptide has previously been described (Melles *et al.*, 2004).

The Janus Serum Bank was established in 1972 and is a population-based biobank for prospective systematized serum collection in Norway. Its primary aim was for the identification of specific biomarkers relevant to certain cancers however due to the long-term nature of the samples being stored (at -25°C), it has also been used to demonstrate analyte stability or degradation over varying timeframes. Samples were largely collected from men attending for routine cardiovascular health assessments and from male blood donors. The study included only males as this limited biological hormonal influence on the specimens collected and was made up of a mainly Caucasian population. A detailed description of the Janus Serum Bank Cohort, its data, and its establishment, has already been published (Langseth *et al.*, 2017). From this study, examining the stability of serum components, comparing analytes from fresh (obtained within 1 month) and samples that had been in long-term storage, they found marked differences in the concentration of c-peptide over time (Gislefoss, Grimsrud and Mørkrid, 2009). It displayed significant decay when assayed one year later (Stattin *et al.*, 2004), and there was a complete loss of the analyte after 25 years of storage (Gislefoss, Grimsrud and Mørkrid, 2009). This would suggest that c-peptide may degrade significantly with time and as a result introduce bias within future studies.

Although cord blood c-peptide was measured as the preferred index of fetal glucose exposure, the concentration of the analyte was much lower (median 0.1 ng/ml) than fresh cord blood c-peptide levels obtained during other studies (Metzger *et al.*, 2008; Regnault *et al.*, 2011; Hou *et al.*, 2014; Rifas-Shiman *et al.*, 2017), as shown in Table 4 in the Appendix. Variability in these findings may also be a result of inconsistent

methods of cord blood sample collection and variation in quantities of freeze-thaw cycles, methods of analysis, and duration and conditions of storage.

Both insulin and c-peptide are highly susceptible to glucose metabolism and will vary significantly according to fasting status. Although the fasting status of individuals from the cohort was not recorded, women in labour for example are likely to be in a near fasted state indicating that high levels of c-peptide would be significant for these individuals. That this study demonstrated very low or no c-peptide results indicated that the sample had degraded significantly and perhaps may reflect the near-fasted state of the ALSPAC cohort.

It is clear that specimen collection and the storage conditions and duration can vary dramatically between and even within studies. For epidemiological and clinical studies to be truly comparable, standardised operating procedures should be reproduced and adhered to. Comparing study outcomes and analyte concentration according to certain diseases for example may be misleading and not reliable if there was such variation in practice. There was limited literature or documentation from the process of the cord blood collection in ALSPAC which was also acknowledged when analysing the study outcomes. Many of the studies discussed were small in sample size and the duration of the storage period may be limited. In order to validate the results from ALSPAC, immediate analysis of the cord blood followed by the assessment of the specimen at timely intervals would have yielded significant information on the stability of analytes measured within a large cohort, over a long period. This would have been limited or not possible however due to the very small cord blood sample size obtained initially and also due to changing method of sample analysis and advancement in technology during this time frame.

Collectively, there does appear to be sufficient evidence to exclude any of the analytes assayed, except cord blood c-peptide and CRP, from this study but further longitudinal studies are required over more longer duration of time to provide

additional evidence for this. Most of the studies reported some assay variability which may be attributed to changes in the analyte concentration over time rather than significant degradation of the analyte.

2.4 Statistical analysis

All statistical analyses were performed using Stata (version 13.0) software (Stata Inc., College Station, TX.). A detailed description of analyses undertaken has been displayed according to each Chapter.

2.4.1 Regression Analysis

Regression analysis was used in each of the Chapters to examine the relationship and effect of the association between a dependent variable, the 'outcome' and an independent variable, the 'exposure'. Linear regression analysis has relied on the following assumptions: linearity, homoscedasticity, the absence of collinearity, and finally a normal distribution of the residuals. Consequently, the data were examined to ensure there was a linear relationship between the dependent and independent variables, that the data values for each had equal variance, that there was no correlation between two or more independent variables, and that the data were normally distributed. In certain cases, such as offspring WC and fat mass, the data were log-transformed to produce approximately normal distributions of residuals. BMI was assessed via BMI z-scores in children and therefore BMI z scores were created using the UK 1990 British growth reference (Cole, Freeman and Preece, 1998). To further validate the study, sex exposure interactions were tested for and included confounding variables in the analysis.

Chapters 3 to 5 used complete case data sets, including only those with a complete set of data variables. In Chapter 3, GWG was examined as a continuous variable (per 1kg increase) and according to IOM criteria (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). Chapter 4 further explored the association of GWG and pre-pregnancy weight (PPW) according to maternal diabetic status on cord blood analytes and the regression analysis additionally adjusted for potential confounders and mediators. In addition, this Chapter examined the association between maternal diabetic status and cord blood biomarkers, and offspring birthweight. Chapter 5 examined HDP in pregnancy using continuous measures during pregnancy in relation to cord blood biomarkers and birthweight.

In Chapter 7, multiple imputation methods were used to perform linear (offspring BMI, WC, and fat mass) and logistic (offspring obesity) regression models in order to examine the associations between birth weight and cord blood measures and offspring BMI, WC, fat mass, and obesity at age 9 and 17 years. A series of regression models were created and accounted for maternal and offspring confounders, as detailed in Chapter 7.

In Chapter 8, multiple imputation methods were employed again. Multivariable linear and logistic regression models were used to examine the associations between cord-blood measures, birthweight and fatty liver, liver volume, fibrosis, and liver function tests at age 17 years.

2.4.2 Missing Data and Multiple Imputation Methods

Missing data, particularly among large data sets occurs frequently. First described by Rubin in 1972, multiple imputation (MI) is a specific statistical technique used for handling data missing at random (Rubin, 1972). This key method was developed in

as a result of limited responses in surveys, thereby limiting the data that could be analysed. Values may be missing if there was a deletion of the case or data or if it was omitted or indeed was never available. Missing data may introduce bias (and therefore influence the outcome or representation of the result) and may make the data difficult to handle and examine.

The main objective in using MI was to enable handling of the missing data to allow valid statistical inferences, rather than to simply re-create the absent values as close as possible to the true ones (Rubin, 1978, 1996). The imputation process replaced the missing data with estimated values based on all the other values available and the imputed value is not a random number or simulated value.

In complete case analysis, values, and observations are excluded with any missing variable. In smaller data sets, for example, missing data removed from the analysis may have had a substantial impact on the overall outcomes. In contrast, missing data in much larger datasets may have had less impact. By maximising the number of useable data, however, the more robust the assumptions may be drawn from the study.

Missing data can be missing at random (MAR), completely missing at random (CMAR), and missing not at random (MNAR). MAR values describe data that were missing but the probability of the data being missing did not depend on unobserved data but may have depended on observed data. MCAR data accounts for data that was missing but the reason for it being missing was unrelated to the study itself. In this case, the probability of the data missing did not depend on observed or unobserved data. For example, the patient agreed to take part in the Study and had routine maternal and neonatal observations were taken but she moved away and she or her child did not attend for follow-up of the study. The missing data were therefore simply missing at random so that there was consistent disregard of any missing values in subsequent analysis. MNAR data describes deliberate missing values and their reason for being

missing should be accounted for. For example, the participant was very unwell at the time of delivery so obtaining a blood sample for Study purposes was not appropriate at that time and therefore no result was available.

MI uses a pre-defined number of imputations, known as M. There has been conflicting evidence on the number of M required to achieve optimal inferences. Historically, founders of the technique felt that M should equal five to achieve valid imputed data (Rubin, 1972; Van Buuren, Boshuizen and Knook, 1999). It was commonly accepted however that the actual number of imputations required depended on the amount of missing data as well as the analysis model used. As many variables as possible were recommended to be included particularly when there was a wide range of observations. The impact of a mis-specified imputation model depended on the quantity of observed versus imputed data. For example, in a case where there was a large number of observations with complete data, few incorrectly imputed values were unlikely to influence outcomes significantly. Overall, twenty imputations were acceptable in order to minimise the sampling error due to MI although other studies suggested that more imputations may have been required to obtain plausible results (Horton and Lipsitz, 2001). Some literature was available however on the sampling error of the MI estimates and the accuracy of the outcomes according to the number of imputations performed (Royston, Carlin and White, 2009; White, Royston and Wood, 2011).

In the current study, there were small amounts of missing data on some covariables included in the multivariable models, as detailed in Chapters 7 and 8. In Chapter 7, twenty imputation datasets were generated by chained equations (White, Royston and Wood, 2011), with all cord exposures, birth weight, covariates, and the measurements from the 11-year clinic and 15-year clinic informing imputation of missing values in the 9-year clinic and 17-year clinic, respectively (hereafter referred to as 9 and 17 years). Again, to deal with the missing data in Chapter 8, twenty imputation data sets were generated by chained equations (White, Royston and Wood, 2011), with measurements from the 15- year clinic informing the

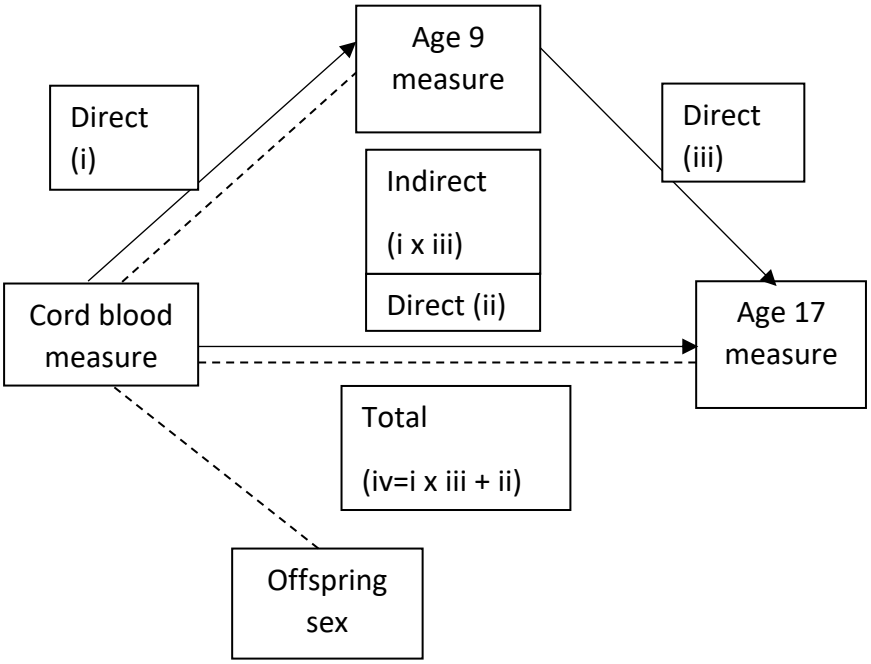
imputation of missing values in the 17-year clinic respectively. This imputation process generated up to 541 data sets for all participants with a liver scan and paired cord sample and up to 1037 for all participants with LFTs and paired cord samples. The distributions of observed (complete confounders) and imputed variables were also examined to ensure comparable results. This added strength to the outcomes of the current study and validated the use of MI analysis compared to using the observed data alone.

2.4.3 Structural Equation Modelling (SEM)

In Chapter 6: Programming of offspring cardio-metabolic risk: tracking analytes from birth to adolescence, Dr. Smith used multivariate statistical analysis to analyse structural relationships in form of structural equation modelling (SEM). It examined the structural relationship between measured or observed variables and unobserved constructs, known as latent variables. As blood analytes were obtained at varying time points (at birth (cord blood), aged 9 and aged 17), and their concentration may have depended on the participants' age for example as well as external factors, SEM was useful for describing the underlying mechanism and to why a relationship existed. Multiple factors may be accounted for in a single analysis and it incorporated independent variables, such as the cord blood analyte measure at birth, and the dependent variable such as the variable measure at age 17.

As SEM assumes normally distributed data, skewed values, including leptin and CRP, were log-transformed to ensure normally distributed data. In Figure 2.3, the direct association is demonstrated simplistically between cord blood and age 9 (i), cord blood and age 17 (ii), and between analytes measured at age 9 and age 17 (iii). In each case, the analyte is measured at the specified time points and the association is described between repeated measurements of the same variable. The direct effect bypasses the effect of any mediator and simply displays the effect of the exposure on the outcome via processes that do not involve the mediator. The indirect association between cord blood and the same analyte at age 17 additionally accounts for the direct association between cord blood and the analyte at age 9 and then the association between age 9 and age 17 (i x iii), thereby accounting for the mediator in the causal pathway. The total (cumulative, iv) effect of association is more complex as it encompasses the direct as well as the indirect effect (i x iii + ii). Mediation analysis is used to separate the total effect of the exposure on the outcome into these direct and indirect effects of associations.

Figure 2.3: Direct, indirect and total effect of associations



2.4.4 Mediation Analysis

Mediation analysis was also used and described in Chapters 3 to 5. This method examined the effect of an independent 'predictor' variable on a mediator variable, which then, in turn, may have influenced the dependent or 'outcome' variable. First described in 1986 (Baron and Kenny, 1986), mediation analysis provided an account of the mechanism or process that underlay the observed relationship between the independent and dependent variables. The mediator, or the 'intervening' variable, essentially mediated the relationship between the predictor and the outcome. This was of value, particularly when these main variables may not have any automatic connection and determined whether the mediator carried an effect between the two.

In contrast to confounding variables, which may affect both the independent and the dependent variables, mediators were part of the causal pathway. Regression analysis provided an estimate of the correlation between the independent and the dependent variable, but this process did not test for causality. In mediation analysis, the aim was to determine the extent to which an association between an exposure and an outcome operated through the mediator of interest.

2.4.5 Linear Spline Multilevel Modelling

Linear spline multilevel models were used to describe the shape of change of a variable over time and are readily interpretable. In handling longitudinal data, there were many challenges encountered including the time points where the measurement was taken and the total number of observations per participant obtained. Multilevel modelling overcame these challenges by adding flexibility to the data structure, as values did not need to be obtained at the same time points. It also allowed for varying numbers of observations per participant in the study

(Steele, 2008). Anyone with one or more observations could be included in the model under the assumption that data were missing at random ie. the probability of the missing observations were related to other observed variables for that participant and did not depend on the actual number of the missing value (Little and Rubin, 2002). This added power to the data, particularly if there was attrition during participant follow-up.

Linear spline modelling involved a series of 'knot points' that were used to model growth trajectories at specific time points. This methodology has previously been applied to ALSPAC data to study childhood growth (Howe et al., 2016). Traditional methods for the analysis of child growth, such as z-scores for childhood BMI (Cole, 2004), may have been employed however they didn't demonstrate the true shape of the observations or growth patterns observed. In addition, measurement error could have affected the results and may have differed with age. In contrast, linear spline multilevel modelling estimated average and person-specific linear growth rates across periods of time defined by 'knot points.' Specific 'knot points' were identified using fractional polynomial curves to enable a smooth function of the curve and this determined the position of the knots (Ben-Shlomo *et al.*, 2008; Howe *et al.*, 2012).

In Chapter 8, average shape trajectories already devised by ALSPAC encompassed fractional polynomial curves and described trajectories of blood pressure measurements during pregnancy. During the gestational period, specific time points were identified and trajectories of the changes in blood pressure during pregnancy were summarised. From this, three-knot points were identified with changes in blood pressure identified over 4 gestational periods (MacDonald-Wallis *et al.*, 2011; Macdonald-Wallis *et al.*, 2014). The baseline BP was set by ALSPAC at 8 weeks' gestation as few participants had BP recorded prior to this. The best-fitting linear spline models had three knots at 18, 30, and 36 weeks and from 36 weeks until delivery (MacDonald-Wallis *et al.*, 2011). There were therefore 4 gestational periods where the rate of change in BP was assumed: from 8-18 weeks, 18-30 weeks,

30-36 weeks, and >36 weeks. These already established models from ALSPAC then allowed for a series of multivariable regression models to be formulated and confounders and mediators to be adjusted for using complete case analysis. It also allowed for the rate of change in maternal SBP or DBP according to each gestational period to be examined as well as the cord blood measures according to the presence of a hypertensive disorder of pregnancy.

2.4.6 Directed Acyclic Graph (DAG)

Directed Acyclic Graphs (DAGs) were used as directed graphs with no directional cycles and displayed the relationships amongst variables. In diagram format, they highlighted which variables were being examined and which variables were confounders or mediators. They incorporated a priori knowledge and clearly stated the assumptions. Typically, all common causes of exposure and disease of interest were included in the causal diagram. A DAG has been created for each Chapter to display the variables of interest and the potential confounding factors and mediators being accounted for in the analysis.

Chapter 3: Gestational weight gain (GWG) in pregnancy: association with cord blood lipids, adipokines and birth weight

3.1 Introduction

Maternal obesity in pregnancy is of public health concern. It poses a significant risk of medical complications during pregnancy, such as thromboembolism and pre-eclampsia, and there is a greater risk of complex caesarean section deliveries (Poston, Harthoorn and Van Der Beek, 2011; Poston *et al.*, 2016). There is also three times greater risk of developing GDM during obese pregnancy, but the risk may be even higher than this (Chu *et al.*, 2007). Fetal complications and poorer neonatal outcomes are also seen more frequently in the presence of maternal obesity, including pre-term birth, macrosomia, and stillbirth (Gaillard *et al.*, 2017). There is mounting evidence that maternal obesity in pregnancy can have a long-lasting impact on the offspring as well (Godfrey *et al.*, 2017), with systematic review demonstrating up to 3 times greater risk of offspring overweight when the mother was obese pre-pregnancy (Yu *et al.*, 2013).

Emerging evidence suggests that gestational weight gain (GWG) in pregnancy, independently or in addition to higher pre-pregnancy BMI, may also contribute significantly to this risk of greater offspring adiposity. A significant positive association has been identified between GWG and offspring birthweight, as observed in one large-scale retrospective study (Ludwig and Currie, 2010) and by ALPSAC (Fraser *et al.*, 2010). GWG has also been positively associated with BMI in childhood (Oken *et al.*, 2007; Fraser *et al.*, 2010; Ludwig and Currie, 2010; Ludwig, Rouse and Currie, 2013), adolescence (Oken *et al.*, 2008; Schack-Nielsen *et al.*, 2010) and adulthood (Mamun *et al.*, 2009; Schack-Nielsen *et al.*, 2010; Hochner *et al.*, 2012), culminating in a greater risk of adult obesity (Schack-Nielsen *et al.*, 2010). Associations between GWG and offspring birthweight, as previously reported by ALPSAC, are however weak and suggest that maternal BMI may be more consistently

associated with a wider range of offspring cardio-metabolic markers (Fraser *et al.*, 2010). Some studies suggest the risk of GWG on offspring overweight or obesity appears to increase with advancing age (Voerman *et al.*, 2019), even accounting for repeated measurements throughout childhood (Oostvogels *et al.*, 2017; Zalbahar *et al.*, 2017). It has therefore been proposed that in order to address the worldwide trend to increasing prevalence of obesity over past decades, including extreme obesity, (Brandkvist *et al.*, 2019), there is a need to examine the causal relationship between maternal BMI and GWG and offspring adiposity and the impact on their cardio-metabolic health both in the short and long-term.

As discussed in Chapter 1, the fetal overnutrition hypothesis suggests that increased offspring exposure to nutrients in utero may lead to persistent adaptations in the structure and function of adipose tissue, appetite regulation, and energy metabolism, leading to an increased susceptibility to later obesity. It is unclear however whether epigenetic processes may account for these associations. Changes in DNA methylation have been identified as a plausible mediating mechanism (Sharp *et al.*, 2015). Shared fetal-maternal genes, shared socioeconomic and lifestyle factors have also been implicated in the transgenerational passage of obesity (Silventoinen *et al.*, 2016; Tyrrell *et al.*, 2016).

The impact of GWG may not be limited to the degree of offspring adiposity, however. Since GWG has also been positively associated with blood pressure in the child (Oken *et al.*, 2007) and adulthood (Mamun *et al.*, 2009), there is growing evidence to support the adverse effect of inappropriate GWG on the offspring cardiovascular risk profile at birth. GWG throughout pregnancy has been positively associated with cord blood leptin (Karakosta *et al.*, 2013; Karachaliou *et al.*, 2015; Lemas *et al.*, 2015). This may be because higher leptin levels have been demonstrated in mothers with greater maternal BMI and GWG and maternal and cord blood leptin levels have been positively correlated (Li *et al.*, 2018), greater GWG may influence cord blood leptin levels. Evidence suggests that greater GWG does not appear to impact adiponectin levels at birth, however (Logan *et al.*, 2017), which is in contrast to the recognised

inverse relationship between adiponectin and maternal obesity during pregnancy (Haghiac *et al.*, 2014). In addition, lower adiponectin levels are also evident with obesity and progressive insulin resistance (Arita *et al.*, 1999; Hotta *et al.*, 2000). As umbilical cord blood leptin is widely recognised as an accurate biomarker for neonatal fat mass (Hauguel-de Mouzon, Lepercq and Catalano, 2006), the lack of association between GWG and cord blood adiponectin suggests that abnormal GWG has no bearing on offspring adiposity at birth. Greater GWG has however been associated with elevated maternal triglycerides throughout pregnancy (Shen *et al.*, 2016), and with dyslipidaemia in the offspring at age 9, 20, and 32 years, as demonstrated by elevated triglyceride and leptin and reduced HDLc levels (Fraser *et al.*, 2010; Hochner *et al.*, 2012). This may reflect the more atherogenic lipid profile overweight and obese mothers have at the start of pregnancy (Scifres, Catov and Simhan, 2014), who develop greater small dense LDLc, elevated leptin levels, and hypoadiponectinemia with advancing gestation and accumulating GWG (Farias *et al.*, 2016; Li *et al.*, 2018).

The 2009 IOM guidance recommends that GWG is modified according to pre-pregnancy BMI to optimise offspring growth patterns and the trajectory of metabolic health (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009), as discussed in Chapter 1. Whilst GWG for normal-weight women may vary between 6 and 18kg, the degree of weight gain varies between individuals and is often dependent on the pre-pregnancy weight (PPW) (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). Although there is limited evidence of the impact of inappropriate GWG on the cardio-metabolic profile of the offspring at birth, there is concern that excessive GWG poses a significant risk of offspring overweight and obesity (Gaillard *et al.*, 2017). Up to 30-40% risk of overweight has also been reported in the offspring of mothers who gained weight excessively during their pregnancy (Nehring, Lehmann and Von Kries, 2013; Mamun, Mannan and Doi, 2014; Tie *et al.*, 2014), although the risk may be even higher than this (Voerman *et al.*, 2019). As one study has shown, only part of the association with adult BMI is mediated by childhood BMI, suggesting

that excessive GWG may induce a persisting susceptibility to an obesogenic environment, (Schack-Nielsen *et al.*, 2010). excessive GWG has also demonstrated a positive association with cord blood leptin levels (Karakosta *et al.*, 2013; Karachaliou *et al.*, 2015).

The adverse impact of excessive GWG on the offspring may only be short-lived, however. Whilst increased overweight in 3 to6-year-old offspring of mothers with excessive GWG has been reported, beyond this only half of the offspring who remained overweight were born to obese mothers who also gained weight excessively (Guo *et al.*, 2015). This is supported by the fact that weight management intervention studies in the second or third trimesters had little or no impact on birth outcome or offspring body fat composition (Thangaratinam *et al.*, 2012; Poston *et al.*, 2015; Dodd *et al.*, 2016). Furthermore, a lifestyle intervention study in pregnancy showed no impact on offspring BMI at age 5 (Ronnberg, Hanson, and Nilsson, 2017). Providing advice to mothers on appropriate GWG, or tracking and recording weight during pregnancy may also be of limited value to the mother (Ferrari and Siega-Riz, 2013; Power *et al.*, 2018). This is in keeping with the fact that patterns of GWG in mothers over ten years since the IOM guidance was launched have not changed, despite a general acceptance of the recommendations. The same proportion of women with excessive GWG have been observed, even accounting for a rising background BMI during this time (Power and Schulkin, 2017).

Assessment of the timing of GWG and accrual of adiposity may also be crucial for the mother and the offspring. Pre- or early pregnancy obesity may reflect poor nutritional status, fat accumulation, and low-grade inflammation, whereas GWG in late pregnancy may additionally reflect fluid expansion, growth of the fetus, placenta, and uterus as well as the accumulation of amniotic fluid. The degree of GWG within the first trimester is important however as it may signify the final GWG category, according to the IOM recommendations (Logan *et al.*, 2017), and high GWG during this time has also been positively associated with the later development of

GDM, especially amongst normal-weight women (Chu *et al.*, 2007). As early as the first and second trimesters of pregnancy, maternal GWG may also positively influence fetal growth, as measured by ultrasound scan and fetal length at birth (Neufeld *et al.*, 2004). In addition, mid-pregnancy excessive GWG has been associated with decreased HDLc levels in 9-year old offspring from the ALSPAC cohort (Fraser *et al.*, 2010). It has therefore been proposed that early or mid-pregnancy GWG may have a significant impact on the translation of adverse cardio-metabolic health onto the offspring.

Collectively, the extent to which GWG may contribute to the degree of offspring adiposity remains unclear and the underlying mechanisms involved are not fully understood (Voerman *et al.*, 2019). The fact that maternal pre-gravid BMI has been shown to attenuate the adverse association between GWG and offspring BMI and HDLc at age 10 years (Kaar *et al.*, 2014) suggests that GWG has a limited role in the causal pathway. It has therefore been proposed that GWG may be a modifiable risk factor for offspring adiposity and future cardiometabolic health, but that optimising GWG may not minimise the risk entirely for the offspring of women who were already overweight or obese mothers (Gaillard *et al.*, 2013; Robinson *et al.*, 2014).

In this Chapter, the impact of fetal overnutrition, as demonstrated by greater maternal BMI and GWG, on offspring birthweight and cardiometabolic outcome at birth was explored. With greater maternal adiposity, higher cord blood leptin and lower cord blood adiponectin, reflecting disruption to adipokines in the presence of maternal obesity, was anticipated. Offspring dyslipidaemia, characterised by elevated levels of cord blood cholesterol, triglyceride, and lower levels of HDLc, reflecting the maternal lipid profile in obese pregnancy, was also expected. By examining GWG during specific gestational periods, as well as the impact of excessive GWG, according to the IOM guidelines, the aim was to establish whether early or mid-pregnancy GWG (as previously demonstrated by ALSPAC on later offspring outcomes) or excessive GWG may have contributed to greater offspring birthweight or an adverse cardiometabolic profile at birth.

3.2 Methods

3.2.1 Study Population

There were 5011 mother-offspring pairs from the ALSPAC cohort with a cord blood sample. Multiple pregnancies and those that were delivered before 37 weeks' gestation were excluded as this would likely influence birthweight and concentration of cord blood analytes. In total, there were 3479 mother-offspring pairs with a recorded maternal pre-pregnancy weight and height so that the BMI and GWG variables could be created.

Details of cord blood sample collection and analysis are presented in Chapter 2: General Methods. Details of obstetric and perinatal abstraction of data from patient case-notes and questionnaires completed are presented in Chapter 2: General Methods.

3.2.2 GWG

As discussed in Chapter 1, the 2009 Institute of Medicine guidelines on (GWG) were used to categorise women into inadequate, appropriate and excessive GWG (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). In order to allocate women according to these categories, maternal weight was obtained by ALSPAC from obstetric notes, who subtracted the first (<18 weeks) from the last (≥37 weeks) weight measurement in pregnancy to derive the variable absolute weight gain, as previously reported. This was further classified using the IOM criteria so that weight gain was either less, equal to or more than the recommendations, irrespective of BMI. As also discussed in Chapter 1, BMI was calculated as weight (kg)/height(m^2) and categorised according to World Health Organisation categories (underweight <18.5 kg/ m^2, normal 18.5 24.9 kg/ m^2, overweight 25.0-29.9 kg/ m^2, obese 30 kg/ m^2). All pregnancy weight measurements (median number of repeat measurements per woman, 10; range, 1, 17) have previously been used by ALSPAC to develop linear spline multilevel models

(with 2 levels: woman and measurement occasion) relating maternal weight (the outcome) to gestational age (the exposure) (Fraser *et al.*, 2011). There were high levels of agreement between estimated and observed weights and full details of statistical modelling have previously been published. In this multilevel model, specific knots were identified at 18 and 28 weeks. This model was then used by ALSPAC to predict maternal weight at 0 weeks (hereafter referred to as 'pre-pregnancy weight') and GWG per week from 0-18 weeks ('early pregnancy GWG'), 19-28 weeks ('mid pregnancy GWG'), and 29 weeks to delivery ('late pregnancy GWG'). PPW and gestational weight change were modified by ALSPAC to be clinically meaningful so that the variation in offspring outcomes could be examined per additional 1 kg of maternal weight at conception and per 400-g gain per week of gestation for GWG (the recommended rate of weight gain in pregnancy for women with a normal BMI) (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). By examining GWG in early, mid, and late pregnancy, this enabled specific gestational periods of GWG to be examined in relation to the cord blood or birthweight outcome.

3.2.3 Statistical Analysis

Cord blood cholesterol, triglyceride, HDLc and non-HDLc, leptin, adiponectin, and birthweight were log-transformed to produce approximately normal distributions of residuals. Linear regression models were used to examine the associations between GWG and cord blood lipids (cholesterol, triglyceride, HDLc, and non-HDLc), adipokines (leptin and adiponectin), and birthweight using complete case analysis. GWG was examined as a continuous variable (per 1kg increase) and as categorised according to IOM criteria. Using these recommendations, adequate GWG was classified as: 12.5-18kg for underweight women, 11.5-16kg for normal-weight women, 7-11.5kg for overweight women, and 5-9kg for obese women. Those gaining less than these ranges within each BMI category were defined as having inadequate GWG (<) and those gaining more as having excessive GWG (>). Maternal BMI was also examined as a continuous variable (per 1kg/m^2 unit increase) in relation to cord

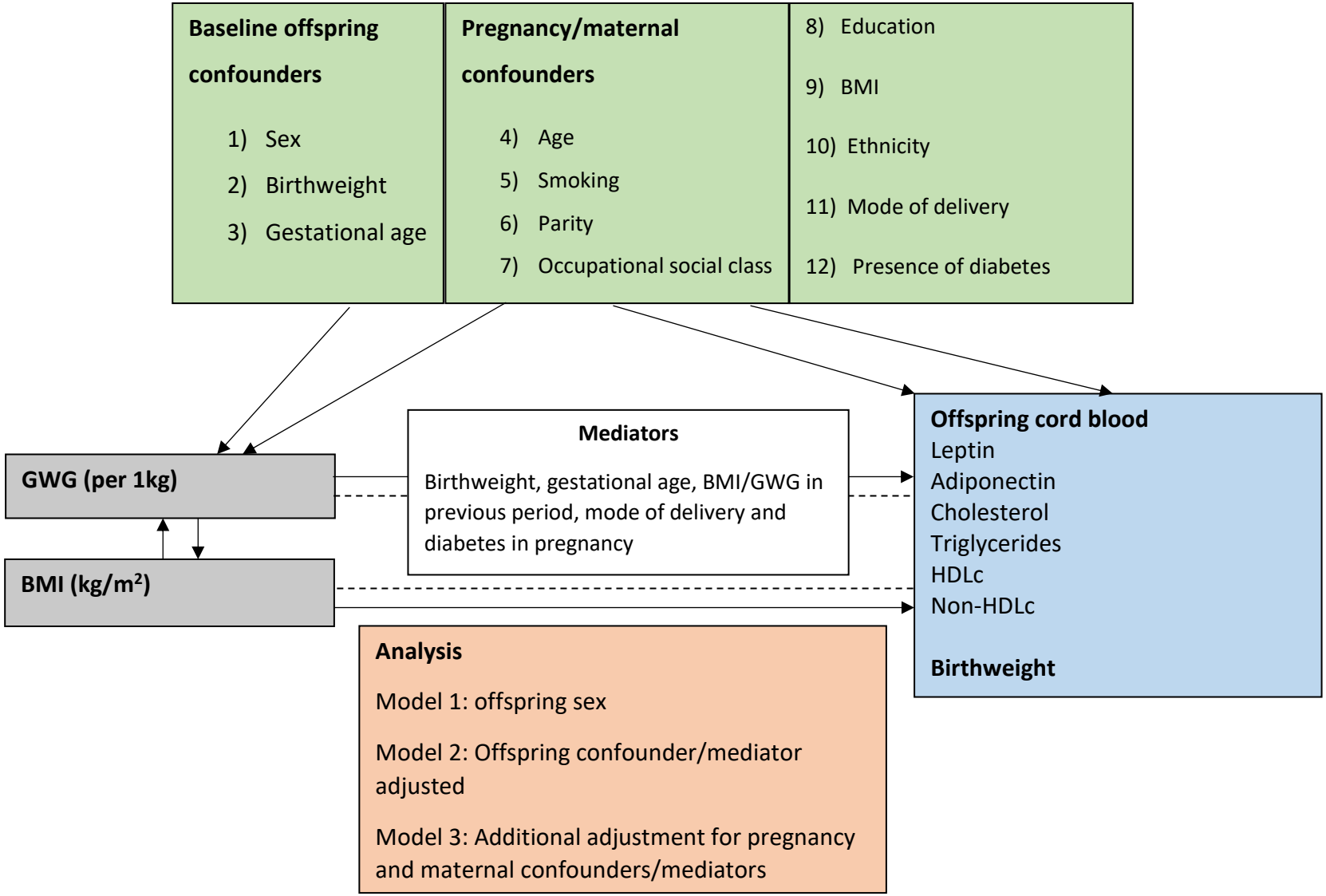
blood analytes and birthweight. Sex-exposure interactions were tested for and as there was no evidence to support these, results are reported for male and female offspring combined.

A series of incremental analyses was performed in order to adjust for potential confounders and mediators, as discussed in section 4. The basic model (model 1) adjusted for offspring sex. In model 2 birthweight was additionally adjusted for (birthweight was not included where the outcome was birthweight), as was gestational age. In the fully adjusted model (model 3) maternal and pregnancy confounders (age, smoking, parity, occupational social class, education, BMI, ethnicity, mode of delivery, and diabetes in pregnancy) were also adjusted for. Where the rate of GWG was examined for each gestational period (0-18, 19-28, ≥29 weeks) GWG in the previous period was also adjusted for.

3.2.4 Assessment of confounders and mediators

As shown in Figure 3.1: DAG, potential confounder and mediator variables were identified and considered as follows:

Figure 3.1: DAG



Offspring sex

Lower GWG in pregnancy has been shown to occur with female offspring more than male offspring (Navara, 2014). At delivery, male offspring may be on average 100g heavier than female offspring, thus contributing to the overall maternal weight gain (Tamimi *et al.*, 2003). Indeed, the rate of growth may also differ between sexes, with male offspring growing at a faster rate in the first and second trimesters before there is a comparable rate of growth between the sexes in the third trimester (De Zegher, Devlieger and Eeckels, 1999), and this may influence the GWG observed during each trimester. Offspring sex was also introduced as a confounder as higher leptin levels have been demonstrated in the cord blood of female compared to male offspring (Helland *et al.*, 1998; Kayemba-Kay's *et al.*, 2008) although some studies have not identified any gender difference for leptin (Iñiguez *et al.*, 2004). Lower adiponectin levels have also been identified in females compared to male offspring at age 2 years and adiponectin has also been inversely associated with offspring growth in childhood (Iñiguez *et al.*, 2004).

Birthweight

GWG is positively correlated with offspring birthweight (Fraser *et al.*, 2010; Ludwig and Currie, 2010), as is BMI across all ranges (Stamnes Koepp *et al.*, 2012). Birthweight may directly influence GWG or cord blood adipokines or lipids, indeed GWG may also be moderated by birthweight. Even though there is a strong correlation between maternal leptin and cord blood leptin, it appears that only cord leptin is directly associated with offspring birthweight and so maternal leptin was not adjusted for in the analysis (Schubring *et al.*, 1998; Logan *et al.*, 2016, 2017)

Gestational age

Gestational age was accounted for as a potential confounder as shorter duration of pregnancy limits the time frame for potential GWG. Deliveries of less than 37 weeks were also excluded and therefore adjusting for gestational age would have only considered those beyond 37 weeks' gestation. Gestational age may also mediate offspring birthweight by limiting the time for accrual of fat mass leading to a higher birthweight. In addition, the length of gestation positively influences cord blood leptin (Kayemba-Kay's *et al.*, 2008) and is associated with progressive dysregulation of lipids (Sattar *et al.*, 1997), with elevated triglyceride levels from as early as the first trimester in particular associated with adverse pregnancy outcome (Vrijkotte *et al.*, 2012). Gestational age is also a mediator as greater or lesser GWG may adversely affect the length of gestation since extremes of GWG or maternal BMI may influence the timing of delivery. Women with low BMI, for example, may labour earlier, compared to women with high GWG who may fail to labour spontaneously (Han *et al.*, 2011; N. Li *et al.*, 2013).

Maternal Age

Younger maternal age has been associated with excessive GWG (Restall *et al.*, 2014) as well as earlier deliveries, thereby limiting the opportunity for GWG (Aliyu *et al.*, 2010). More recently, however, younger age at conception has been associated with GWG less than recommended, although this association was weak (Power *et al.*, 2018) and not demonstrated universally.

Smoking

Smoking, during pregnancy, has been associated with excessive GWG in one study (Gaillard *et al.*, 2013), even when smoking has stopped by 14-16 weeks (Restall *et al.*, 2014). Maternal smoking is also consistently associated with lower birthweight in the offspring (Kayemba-Kay's *et al.*, 2008). Smoking is also associated with adipokine and cardio-metabolic disruption. Smoking cessation, for example, may lead to elevated leptin levels, possibly reflecting the changes in fat distribution after stopping smoking (Kryfti *et al.*, 2015). Smoking also induces dyslipidaemia and elevated HDLc levels have been observed after stopping smoking (Gepner *et al.*, 2011).

Parity

Higher parity is associated with maternal obesity as well as excessive GWG (Gaillard *et al.*, 2013). This finding is not universal, however, and a large retrospective study demonstrated that GWG above the recommendations was associated with lower parity and GWG below the recommendations associated with higher parity (Power *et al.*, 2018). Higher parity is also associated with progressively higher infant birthweight and risk of macrosomia (Ørskou *et al.*, 2003).

Occupational social class and education

Lower education levels are associated with maternal obesity (Gaillard *et al.*, 2013) but it has also been associated with GWG out with the recommended guidance during pregnancy (O'Brien, Alberdi and McAuliffe, 2018). There are inconsistent results when exploring social class as well as education as a risk factor for overall GWG. In one study it appears that excessive GWG was more prevalent amongst lower

educated normal-weight women specifically, rather than lesser educated obese women (Bjermo, Lind and Rasmussen, 2015). Overall higher education appears to protect against excessive GWG (C.K. *et al.*, 2013) whereas there does not appear to be the same or any association between social class and GWG (Fraser *et al.*, 2011; Restall *et al.*, 2014).

Pre-pregnancy BMI

One study showed that women that gain weight excessively in pregnancy are more likely to be already overweight or obese (Restall *et al.*, 2014). In a study across nine obstetric units in Canada, more than half of women with a BMI greater than 25 kg/m^2 (ie. overweight or obese women) gained weight excessively and the risk of excessive GWG increased exponentially according to BMI category (Morisset *et al.*, 2017). In addition, a large retrospective study of 18,217 women between 2006 and 2015, showed that more than half of women who were overweight or obese gained more than the recommended weight - in contrast to underweight women - who were more likely to gain less than recommended (Power *et al.*, 2018). Despite the threshold for exceeding the recommendations, GWG itself however declined with advancing BMI, suggesting that obese women gain less weight than lean women even though they may exceed the recommended GWG limit. They also highlighted the third trimester as the stage of pregnancy for most GWG (Gluckman *et al.*, 2008) and so GWG in the previous trimester (as well as pre-pregnancy BMI) were also accounted for in the analysis.

Overweight and obese mothers have more atherogenic lipid profiles during pregnancy, in addition to higher leptin and lower adiponectin levels (Scifres, Catov and Simhan, 2014; Farias *et al.*, 2016; Li *et al.*, 2018). As higher maternal leptin, reflecting higher maternal BMI, correlates with higher cord blood leptin (Li *et al.*, 2018) it was therefore appropriate to adjust for BMI in the mediation analysis of GWG and cord blood adipokines.

Ethnicity

European ethnicity has been associated with excessive GWG (Gaillard *et al.*, 2013) and more recently this has been demonstrated amongst the Caucasian population when compared to other ethnicities (Guo *et al.*, 2019). Observing GWG amongst diverse ethnicities across three continents, a recent systemic review indicated that women in the United States and Europe have a higher prevalence of GWG above IOM recommendations and lower rates of GWG below the recommendations than women in Asia (Goldstein *et al.*, 2018). Despite this, during pregnancy (and out with), Asian women also have a greater cardio-metabolic and diabetes risk profile at a lower BMI compared to Caucasian women (Deurenberg, Deurenberg-Yap and Guricci, 2002; Li *et al.*, 2002). Regulation of adipokines may therefore vary according to ethnicity, with lower adiponectin and higher leptin levels demonstrated amongst a South Asian population compared to Europeans with the same degree of adiposity (Mente *et al.*, 2010).

Mode of delivery

In one small study, cord blood leptin was higher amongst those who delivered vaginally compared to those who were delivered by caesarean section (Yoshimitsu *et al.*, 2000), suggesting that placental leptin release is augmented by advanced labour. Another study did not find any difference between the mode of delivery and that cord blood leptin was associated with the duration of labour alone (Logan *et al.*, 2016). Vaginal delivery has also been associated with fetal lipid dysregulation as demonstrated by elevated cord blood fatty acid levels compared to those undergoing caesarean section (Yoshimitsu *et al.*, 1999). Weight loss amongst obese women in pregnancy may reduce the risk of caesarean section delivery, compared to those who gained weight according to the IOM recommendations (Blomberg, 2011), although this is not recommended.

Presence of diabetes

GDM is associated with higher maternal BMI and GWG (National Institute for Health and Care Excellence, 2015). Higher maternal GWG may exaggerate the progressive insulin resistance during pregnancy, overall contributing to the risk of GDM. One recent retrospective study showed that women who were diagnosed with GDM were less likely to exceed the recommendations on GWG which may reflect the greater intervention such as counselling and dietician input in this patient group (Davis *et al.*, 2015; Power *et al.*, 2018). In contrast, excessive GWG prior to the diagnosis of GDM increased the odds of developing GDM, with similar strength of effect for normal-weight compared to overweight or obese women (Brunner *et al.*, 2015). GDM is also associated with higher maternal and cord blood leptin, perhaps as a result of higher leptin mRNA expression, as well as lower adiponectin (Meller *et al.*, 2006; Desoye and Hauguel-De Mouzon, 2007), and elevated triglyceride levels which have been reported from as early as the first trimester (Shen *et al.*, 2016).

3.3 Results

Figure 3.2 demonstrates the ALSPAC participant flow chart, the inclusion and exclusion criteria as well as introducing the demographics of the cohort.

Figure 3.2: ALSPAC participant flow chart

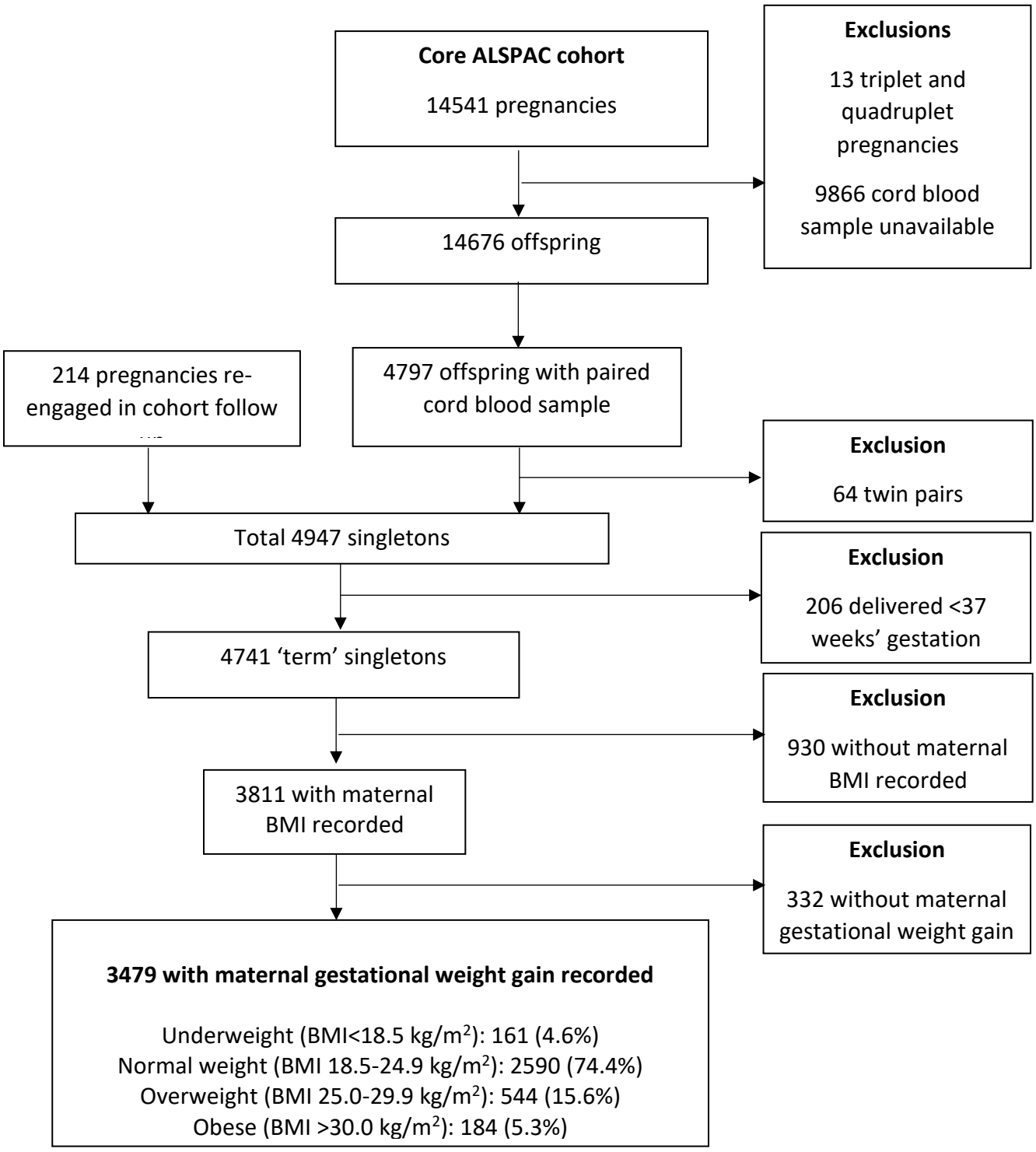


Table 3.1 summarises the characteristics of the entire cohort with GWG recorded and according to IOM criteria.

The median age was 28 years (25, 31 years). Women were mostly nulliparous (44.8%) and had never smoked (69.8%). Most mothers left school at 16 years old (63.8%) and few had gone on to further education. Less than 20% were socially disadvantaged, categorised as Class IIIb or below. The cohort consisted of a mainly Caucasian population (97.4%) with minimal ethnic diversity. Most women in the study had a normal BMI (74.4%), with only 5.3% of women classified as obese. The median GWG was 12 kg (9.6, 15.5kg). Most women achieved a vaginal birth (77.7% had a spontaneous vaginal delivery, SVD), with only 5.8% undergoing caesarean section. There were a similar number of male (50.6%) and female (49.4%) babies delivered and the median birthweight was 3.5 kg (3.2, 3.8kg) and 40 weeks' gestation. Table 3.1 also displays the median (and IQR) cord blood levels including leptin (6.6 (3.8, 12.2) pg/ml), adiponectin (77.2 (54.9, 100.0) µg/ml), cholesterol (1.7 (1.4, 2.0) mmol/ml), triglyceride (0.5 (0.4, 0.6) mmol/l), HDLc (0.5 (0.4, 0.7) mmol/ml) and non-HDLc (1.1 (0.9, 1.4) mmol/ml).

Table 3.1: Summary of characteristics of the entire cohort with GWG recorded and according to IOM criteria

Maternal Characteristics	Entire cohort N (%)	Entire cohort Median (IQR)	< N (%)	< Median (IQR)	= N (%)	= Median (IQR)	> N (%)	> Median (IQR)
Age	3479	28 (25, 31)	1191	29 (25, 32)	1305	28 (25, 31)	983	27 (25, 30)
Smoking								
Never	2385 (69.8)		807 (68.8)		938 (73.0)		640 (66.7)	
Before, not during	255 (7.5)		62 (5.3)		98 (7.6)		95 (9.9)	
During pregnancy	777 (22.7)		304 (25.9)		249 (19.4)		224 (23.4)	
BMI (kg/m^2)	3479	22.2 (20.5, 24.4)	1191	21.5 (20.0, 23.1)	1305	21.8 (20.5, 23.7)	951	24.0 (21.7, 26.7)
<18.5	161 (4.6)							
18.5-24.9	2590 (74.4)							
25.0-29.9	544 (15.6)							
>30.0	184 (5.3)							
Parity								
0	1499 (44.8)		450 (39.3)		577 (45.5)		472 (50.6)	
1	1226 (36.6)		430 (37.5)		485 (38.3)		311 (33.4)	
2	450 (13.5)		186 (16.2)		150 (11.8)		114 (12.2)	
3	134 (4.0)		60 (5.2)		45 (3.6)		29 (3.1)	
4+	37 (1.1)		20 (1.7)		11 (0.9)		6 (0.7)	
Education								
Left school at 16	2128 (63.6)		731 (64.4)		756 (60.1)		641 (67.4)	
A level	782 (23.4)		251 (22.1)		317 (25.2)		214 (22.5)	
Degree	434 (13.0)		154 (16.6)		184 (14.5)		96 (10.9)	
Social Class								
I (least)	149 (5.3)		59 (6.3)		56 (5.2)		34 (4.2)	
II	916 (32.5)		303 (32.3)		363 (33.9)		250 (30.7)	

Maternal Characteristics	Entire cohort N (%)	Entire cohort Median (IQR)	< N (%)	< Median (IQR)	= N (%)	= Median (IQR)	> N (%)	> Median (IQR)
IIIa	1229 (43.5)		400 (42.6)		468 (43.7)		361 (44.4)	
IIIb	214 (7.6)		70 (7.5)		75 (7.0)		69 (8.5)	
IV	263 (9.3)		87 (9.3)		88 (8.2)		88 (10.8)	
V (most disadvantaged)	52 (1.8)		19 (2.0)		21 (2.0)		6 (1.5)	
Ethnicity								
White Caucasian	3274 (97.4)		1112(97.7)		1234 (98.6)		928 (98.3)	
Black Caribbean	12 (0.4)		4 (0.4)		2 (0.2)		6 (0.6)	
Black African	3 (0.1)		1 (0.1)		1 (0.1)		1 (0.1)	
Black other	10 (0.3)		2 (0.2)		5 (0.4)		3 (0.3)	
Indian	10 (0.3)		6 (0.5)		3 (0.2)		1 (0.1)	
Pakistani	5 (0.2)		4 (0.4)		0 (0.0)		1 (0.1)	
Chinese	7 (0.2)		3 (0.3)		3 (0.2)		1 (0.1)	
Pregnancy Characteristics								
GWG(kg)	3479	12.0 (9.6, 15.5)	1191	9.0 (6.8, 10.2)	1305	13.2 (12.0, 14.5)	983	17.3 (15.5, 19.4)
With BMI <18.5		12.3*	86 (53.4)		56 (34.8)		19 (11.8)	
18.5-24.9		12.9*	987 (38.1)		1060 (40.9)		543 (21.0)	
25.0-29.9		12.6*	76 (14.0)		152 (27.9)		316 (58.1)	
>30.0		9.6*	42 (22.8)		37 (20.1)		105 (57.1)	
Diabetes								
Nil	3341 (96.0)		1151(96.6)		1252 (95.9)		3341(96.0)	
Existing	8 (0.2)		4 (0.3)		2 (0.2)		8 (0.2)	
GDM	16 (0.5)		7 (0.6)		5 (0.4)		16 (0.5)	
Glycosuria	114 (3.3)		29 (2.4)		46 (3.5)		114 (3.3)	
Mode of delivery								
SVD	2695 (77.7)		974 (82.0)		1013 (77.9)		708 (72.4)	
Breech	26 (0.8)		11 (0.9)		10 (0.8)		5 (0.5)	
Caesarean	313 (9.0)		102 (8.6)		111 (8.5)		100 (10.2)	

Maternal Characteristics	Entire cohort N (%)	Entire cohort Median (IQR)	< N (%)	< Median (IQR)	= N (%)	= Median (IQR)	> N (%)	> Median (IQR)
Forceps	202 (5.8)		49 (4.1)		70 (5.4)		83 (8.5)	
Vacuum	192 (5.5)		42 (3.5)		81 (6.2)		69 (7.1)	
Other	39 (1.1)		10 (0.8)		16 (1.2)		13 (1.3)	
Gestational age at birth (weeks)	3479	40 (39, 41)	1191	40 (39, 40)	1305	40 (39, 41)	983	40 (39, 41)
Offspring Characteristics								
Sex								
Male	1759 (50.6)		608 (51.1)		646 (49.5)		505 (51.4)	
Female	1720 (49.4)		583 (49.0)		659 (50.5)		478 (48.6)	
Birthweight (kg)	3448	3.5 (3.2, 3.8)	1177	3.3 (3.0, 3.6)	1292	3.5 (3.2, 3.8)	979	3.6 (3.3, 4.0)
Cord blood leptin (pg/ml)	3477	6.6 (3.8, 12.2)	1190	5.7 (3.1, 9.7)	1305	7.0 (3.9, 12.9)	982	8.0 (4.4, 14.2)
Cord blood adiponectin (µg/ml)	3447	77.2 (54.9, 100.0)	1182	76.4 (53.6, 96.8)	1295	77.5 (55.4,101.7)	968	78.2 (55.9, 101.6)
Cord blood cholesterol (mmol/l)	3434	1.7 (1.4, 2.0)	1175	1.7 (1.4, 2.0)	1292	1.6 (1.4, 2.0)	967	1.7 (1.4, 2.0)
Cord blood triglyceride (mmol/l)	3421	0.5 (0.4, 0.6)	1171	0.5 (0.4, 0.6)	1286	0.5 (0.4, 0.6)	964	0.5 (0.4, 0.7)
Cord blood HDLc (mmol/l)	3360	0.5 (0.4, 0.7)	1146	0.5 (0.4, 0.7)	1262	0.5 (0.4, 0.6)	952	0.5 (0.4, 0.6)
Cord blood non-HDLc (mmol/l)	3360	1.1 (0.9, 1.4)	1146	1.1 (0.9, 1.4)	1262	1.1 (0.9, 1.4)	952	1.1 (0.9, 1.4)

< represents GWG less than recommended, = represents GWG within recommendations, > represents GWG more than recommended. Median (Interquartile range). Figures are numbers (%) unless stated otherwise. *mean GWG (kg)

Table 3.1 also describes the cohort according to those who gained weight less than, within or more than the IOM recommendations on GWG. Of these women, 4.6% were underweight, 74.4% were normal weight, 15.6% were overweight and 5.3% were obese. More than half of overweight (58.1%) or obese (57.1%) women exceeded the recommendations on GWG, even though obese women gained overall less weight (mean GWG was 9.6kg in obese women and 12.9kg in normal-weight women), compared to only 21% of normal-weight women who also exceeded the recommendations on GWG.

In contrast to those with GWG within the recommendations, mothers with GWG less than recommended were older (median 29 years old compared to 28 years old), more likely to smoke during pregnancy (25.9% compared to 19.4%) although maternal BMI was comparable between these groups (median 21.5 kg/m^2 and 21.8 kg/m^2 respectively). They had the highest rate of SVD (82%) compared to 77.9% of healthy women although the rate of caesarean section was similar between groups. Offspring birthweight was lowest among women with inadequate GWG (3.3kg compared to 3.5kg in those that gained within the recommendations). Although cord blood cholesterol, triglyceride, HDLc, and non-HDLc were similar between each of the groups, those that gained less than the recommended weight during pregnancy had offspring with lower cord blood leptin (5.7 pg/ml compared to 7.0 pg/ml), and similar adiponectin levels (76.4 µg/ml compared to 77.5 µg/ml).

Characteristics of those that exceeded, in contrast to those that gained within, the GWG recommendations are also presented in Table 3.1. Overall, they were slightly younger (median 27 years old), more likely to smoke either before (9.9%) or during pregnancy (23.4%), and have a higher pre-pregnancy BMI (24 kg/m^2). Exceeding the GWG recommendations also included more nulliparous women (50.6% compared to 45.5 % of nulliparous who gained within the recommendations) who were least educated (67.4% left school at 16 years old compared to 60.1% of those who gained within the GWG recommendations). This group also had the lowest rate of SVD (72.4%) and the highest rate of caesarean section (10.2% compared to 8.5%). They

also had higher offspring birthweight (3.6kg compared to 3.5kg), cord blood leptin (8.0 pg/ml compared to 7.0 pg/ml), and cord blood adiponectin (78.2 µg/ml compared to 77.5 µg/ml) levels were also higher in those with excess GWG compared those who gained within the recommendations. Again, cord blood cholesterol, triglyceride, HDLc, and non-HDLc were comparable between groups.

Characteristics of the ALSPAC cohort are displayed in Figures 3.3 to 3.5. Figure 3.3 presents the GWG IOM category according to BMI; Figure 3.4 presents the mode of delivery according to the IOM category and Figure 3.5 shows the concentration of cord blood leptin and offspring birthweight according to the IOM recommendations on GWG.

Figure 3.3: GWG IOM category according to BMI

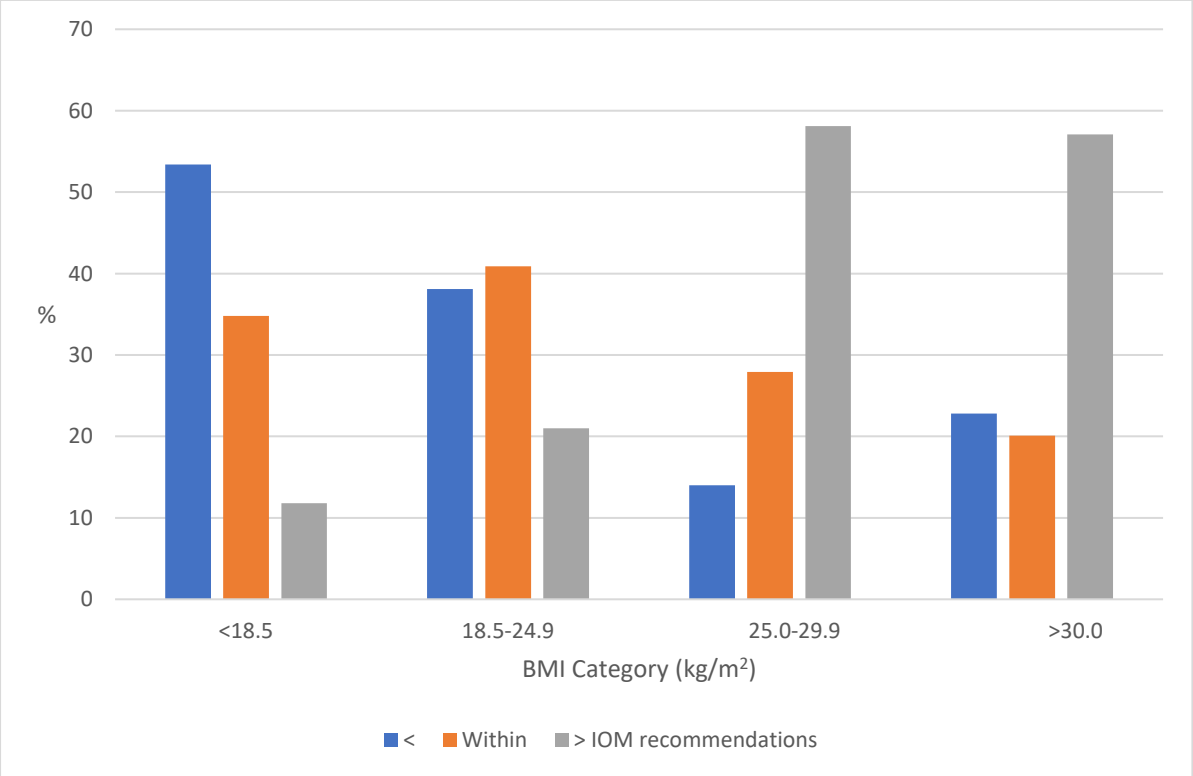


Figure 3.4: Mode of delivery according to IOM category

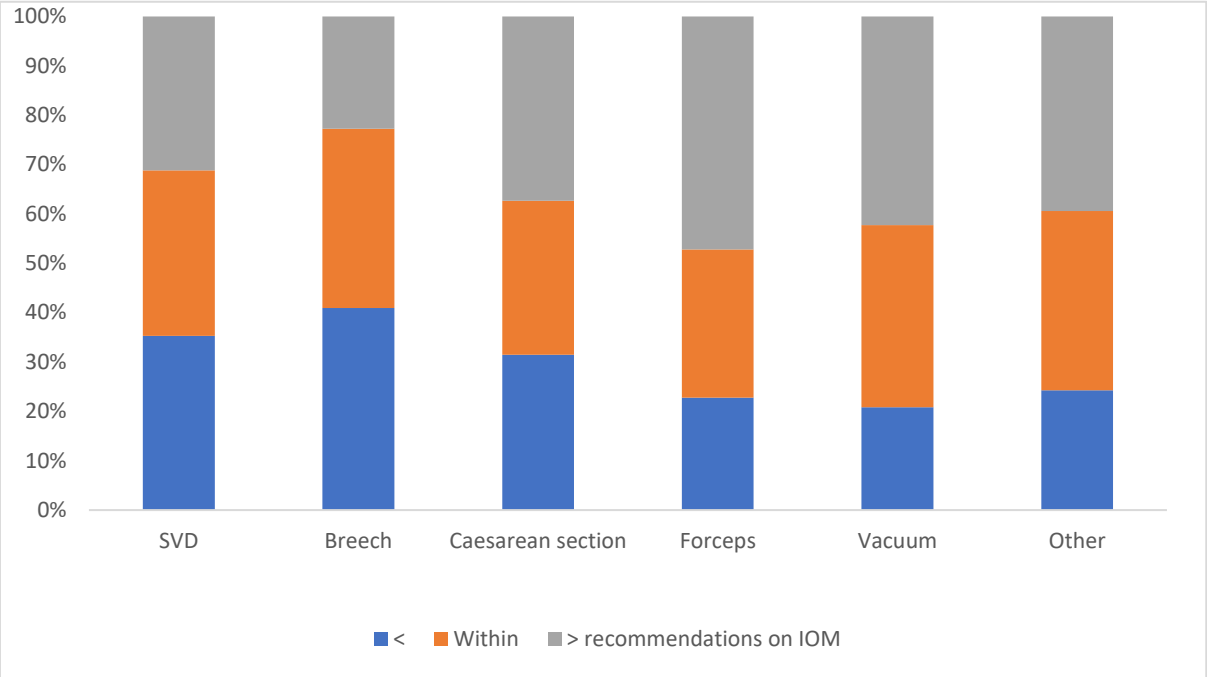
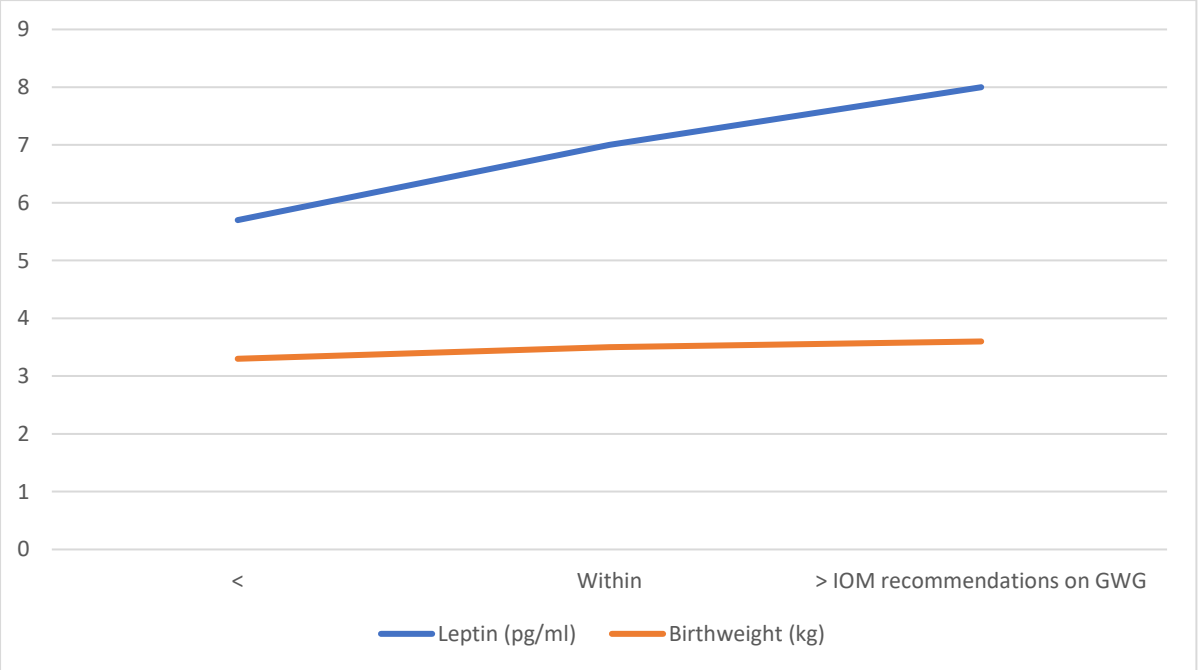


Figure 3.5: Concentration of cord blood leptin and offspring birthweight according to IOM recommendations on GWG



The association between the entire range of GWG (per 1kg) and BMI (per kg/m^2) and cord blood leptin, adiponectin, lipids, and birthweight is shown in Table 3.2 Both GWG and BMI were weakly positively associated with cord blood leptin (coefficient 0.01 (0.00, 0.02), p=0.002 and 0.02 (0.02, 0.03), p=0.000 respectively) in the fully adjusted analysis. GWG and BMI were also weakly positively associated with birthweight (coefficient 0.01 (0.01, 0.01), p=0.000 and 0.01 (0.01, 0.01), p=0.000). There was no significant association between GWG and cord blood adiponectin, cholesterol, HDLc, or non-HDLc. BMI however displayed weak positive association with cord blood triglycerides (coefficient 0.01 (0.01, 0.02), p=0.000) and a weak negative association with cord blood HDLc (coefficient -0.01 (-0.01, 0.00), p=0.011).

Table 3.2: Association between GWG (per 1kg) and BMI (per kg/m^2) and cord blood measures and birthweight

Outcome		Model 1 Coefficient (95% CI)	p	Model 2 Coefficient (95% CI)	p	Model 3 Coefficient (95% CI)	p
Cord blood leptin	GWG	0.03 (0.02, 0.03)	0.000	0.01 (0.00, 0.02)	0.006	0.01 (0.00, 0.02)	0.002
(pg/ml)	BMI	0.04 (0.03, 0.04)	0.000	0.02 (0.02, 0.03)	0.000	0.02 (0.02, 0.03)	0.000
N=2695							
Cord blood adiponectin	GWG	0.00 (0.00, 0.01)	0.572	0.00 (-0.01, 0.00)	0.684	0.00 (-0.01, 0.00)	0.369
(µg/ml)	BMI	0.00 (-0.01, 0.00)	0.339	-0.01 (-0.02, 0.00)	0.103	0.00 (-0.01, 0.00)	0.142
N=2669							
Cord blood cholesterol	GWG	0.00 (0.00, 0.01)	0.236	0.00 (0.00, 0.01)	0.171	0.00 (0.00, 0.01)	0.316
(mmol/l)	BMI	0.00 (0.00, 0.00)	0.869	0.00 (0.00, 0.01)	0.732	0.00 (0.00, 0.01)	0.515
N=2657							
Cord blood triglyceride	GWG	0.00 (0.00, 0.01)	0.097	0.01 (0.00, 0.01)	0.004	0.00 (0.00, 0.01)	0.060
(mmol/l)	BMI	0.01 (0.00, 0.01)	0.014	0.01 (0.01, 0.01)	0.000	0.01 (0.01, 0.02)	0.000
N=2645							
Cord blood HDLc	GWG	0.00 (0.00, 0.01)	0.593	0.00 (0.01, 0.00)	0.854	0.00 (0.00, 0.01)	0.805
(mmol/l)	BMI	0.01 (-0.01, 0.00)	0.040	-0.01 (-0.01, 0.00)	0.004	0.01 (0.01, 0.00)	0.011
N=2595							
Cord blood	GWG	0.00 (0.00, 0.01)	0.276	0.00 (0.00, 0.01)	0.104	0.00 (0.00, 0.01)	0.355
non-HDLc (mmol/l)	BMI	0.00 (0.00, 0.01)	0.430	0.00 (0.00, 0.01)	0.182	0.00 (0.00, 0.01)	0.119
n=2595							
Birthweight † (g)	GWG	0.01 (0.01, 0.01)	0.000	0.01 (0.01, 0.01)	0.000	0.01 (0.01, 0.01)	0.000
N=2696	BMI	0.01 (0.01, 0.01)	0.000	0.01 (0.01, 0.01)	0.000	0.01 (0.01, 0.01)	0.000

Models adjusted for 1: offspring sex, 2: offspring sex, birthweight, and gestational age, 3: offspring sex, birthweight, gestational age, maternal age, smoking, parity, social class, education, BMI (for the GWG analysis)/GWG (for the BMI analysis), ethnicity, mode of delivery and presence/absence of diabetes in pregnancy. †Birthweight not included as a confounder in models 2 and 3 where birthweight is the outcome

Displaying the association between GWG in those that gained weight excessively (according to the IOM criteria) and cord blood leptin and birthweight, Table 3.3 shows that the association was strongest for obese women who also gained weight excessively during pregnancy, although the association with birthweight was attenuated in the analysis (coefficient 0.06 (0.01, 0.12), p=0.034 for cord blood leptin and 0.01 (0.00, 0.16), p=0.052 for birthweight).

Table 3.3: Association between GWG in those that gained weight excessively according to the IOM criteria and cord blood leptin and birthweight

Outcome	Maternal BMI category	GWG (kg)		
		N	Coefficient (95% CI)	p
Cord blood leptin pg/ml	Normal	434	0.04 (0.00, 0.07)	0.033
	Overweight	244	0.00 (-0.03, 0.03)	0.835
	Obese	77	0.06 (0.01, 0.12)	0.034
Birthweight (g)	Normal	434	0.01 (0.00, 0.01)	0.022
	Overweight	245	0.00 (0.00, 0.01)	0.037
	Obese	77	0.01 (0.00, 0.16)	0.052

Adjusted for offspring sex, birthweight, gestational age, and maternal age, smoking, parity, social class, education, BMI, ethnicity, mode of delivery, and presence/absence of diabetes in pregnancy. †Birthweight not included as a confounder where birthweight is the outcome.

Table 3.4 shows the categorical association between GWG (classified as less or more than recommended) and cord blood leptin and birthweight. Gaining less weight than recommended was negatively associated with lower cord blood leptin and birthweight (-0.10 (95% CI -0.18, -0.02), p=0.012 and coefficient 0.054 (95% CI -0.07, -0.04), p=0.000 respectively) in the fully adjusted analysis. Excessive GWG was associated with higher offspring birthweight (0.03,(95% CI 0.02), 0.04, p<0.001). Excessive GWG was also associated with higher cord blood leptin but the association attenuated to null after adjusting for confounders. GWG categorised according to the IOM criteria was not associated with cord blood lipids.

Table 3.4: Association between GWG (per 1kg) (according to IOM categories) and cord blood leptin and birthweight

Outcome	IOM Category	Model 1 Coefficient (95% CI)	p	Model 2 Coefficient (95% CI)	p	Model 3 Coefficient (95% CI)	p
Cord blood leptin (pg/ml) N=2695	< Recommended	-0.23 (-0.31, -0.15)	0.000	-0.11 (-0.19, -0.04)	0.003	-0.10 (-0.18, -0.02)	0.012
	= Recommended	Reference (null, 0)		Reference (null, 0)		Reference (null, 0)	
	> Recommended	0.13 (0.05, 0.21)	0.002	0.07 (-0.01, 0.14)	0.099	0.02 (-0.06, 0.10)	0.674
Birthweight † (g) N=2696	< Recommended	-0.06 (-0.07, -0.05)	0.000	-0.05 (-0.07, -0.04)	0.000	-0.05 (-0.07, -0.04)	0.000
	= Recommended	Reference (null, 0)		Reference (null, 0)		Reference (null, 0)	
	> Recommended	0.03 (0.02, 0.05)	0.000	0.03 (0.02, 0.04)	0.000	0.03 (0.02, 0.04)	0.000

Models 1: adjusted for offspring sex, 2: additionally adjusted for birthweight and gestational age, 3: additionally adjusted for maternal age, smoking, parity, social class, education, BMI, ethnicity, mode of delivery, and presence of diabetes in pregnancy.
†Birthweight not included as a confounder in models 2 & 3 where birthweight is the outcome.

The association between GWG during specific gestational periods (0-18 weeks ‘early’, 19-28 weeks ‘mid’ and >28 weeks ‘late’ pregnancy) and cord blood leptin, adiponectin, lipids, and birthweight are shown in Table 3.5. Early GWG was associated with cord blood leptin, but this attenuated to the null when adjusted for in the analysis. Mid pregnancy GWG was weakly positively associated with cord blood leptin (coefficient 0.12 (95% CI 0.03, 0.20), p=0.010) and the association strengthened in late pregnancy (coefficient 0.14 (95% CI 0.05, 0.23), p=0.002). Mid pregnancy GWG was also weakly positively associated with triglyceride (coefficient 0.05 (95% CI 0.01, 0.10), p=0.025) but this association was lost during late pregnancy. There was no other significant association between early, mid, or late pregnancy GWG and cord blood cholesterol, HDLc or non-HDLc. GWG throughout each gestational period was positively associated with birthweight, with mid-pregnancy GWG demonstrating the strongest association (coefficient 0.06 (95% CI 0.05, 0.07), p=0.000).

Table 3.5: Association between early, mid-, and late pregnancy GWG* and cord blood measures and birthweight

		GWG (per 400g/wk change) *					
		0-18 wk 'early'		19-28 wk 'mid'		≥29 wk 'late'	
Outcome	Model	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p
Cord blood leptin	1	0.09 (0.02, 0.17)	0.017	0.21 (0.13, 0.28)	0.000	0.23 (0.16, 0.30)	0.000
(pg/ml)	2	0.01 (-0.07, 0.08)	0.873	0.08 (0.01, 0.16)	0.033	0.15 (0.08, 0.21)	0.000
	3	0.03 (-0.04, 0.11)	0.369	0.12 (0.03, 0.20)	0.010	0.14 (0.05, 0.23)	0.002
Cord blood adiponectin	1	-0.03 (-0.08, 0.01)	0.169	0.01 (-0.05, 0.04)	0.833	0.03 (-0.01, 0.08)	0.104
(µg/ml)	2	-0.05 (-0.09, 0.02)	0.058	-0.02 (-0.07, 0.03)	0.343	0.04 (-0.02, 0.07)	0.268
	3	-0.05 (-0.10, 0.00)	0.058	-0.03 (-0.08, 0.03)	0.351	0.05 (-0.01, 0.11)	0.073
Cord blood cholesterol	1	0.03 (0.01, 0.06)	0.116	0.02 (-0.01, 0.06)	0.176	0.00 (-0.03, 0.03)	0.777
(mmol/l)	2	0.03 (0.00, 0.07)	0.074	0.03 (-0.01, 0.07)	0.111	0.00 (-0.03, 0.03)	0.988
	3	0.03 (0.00, 0.07)	0.068	0.02 (-0.02, 0.06)	0.382	-0.03 (-0.07, 0.01)	0.133
Cord blood triglyceride	1	0.00 (-0.04, 0.04)	0.917	0.02 (-0.02, 0.07)	0.271	0.00 (-0.04, 0.03)	0.925
(mmol/l)	2	0.03 (-0.01, 0.07)	0.123	0.06 (0.02, 0.10)	0.006	0.04 (0.00, 0.07)	0.050
	3	0.04 (0.00, 0.08)	0.077	0.05 (0.01, 0.10)	0.025	-0.03 (-0.08, 0.02)	0.246
Cord blood HDLc	1	0.05 (0.00, 0.09)	0.039	0.03 (-0.02, 0.07)	0.234	0.03 (-0.03, 0.04)	0.878
(mmol/l)	2	0.03 (-0.01, 0.07)	0.194	0.01 (-0.04, 0.05)	0.785	-0.02 (-0.06, 0.02)	0.318
	3	0.02 (-0.02, 0.07)	0.273	0.00 (-0.05, 0.05)	0.903	-0.01 (-0.07, 0.04)	0.611
Cord blood	1	0.02 (-0.02, 0.06)	0.397	0.01 (-0.03, 0.05)	0.489	-0.01 (-0.04, 0.03)	0.778
non-HDLc (mmol/l)	2	0.03 (-0.01, 0.07)	0.150	0.03 (-0.01, 0.07)	0.166	0.01 (-0.03, 0.04)	0.580
	3	0.03 (-0.01, 0.07)	0.122	0.02 (-0.03, 0.06)	0.500	-0.03, (-0.07, 0.02)	0.275
Birthweight † (g)	1	0.04 (0.03, 0.05)	0.000	0.06 (0.05, 0.07)	0.000	0.04 (0.03, 0.05)	0.000
	2	0.05 (0.03, 0.06)	0.000	0.06 (0.05, 0.07)	0.000	0.05 (0.04, 0.06)	0.000
	3	0.05 (0.04, 0.06)	0.000	0.06 (0.05, 0.07)	0.000	0.03 (0.02, 0.04)	0.000

Models 1: adjusted for offspring sex, 2: additionally adjusted for birthweight, 3: additionally adjusted for maternal age, smoking, parity, social class, education, BMI, GWG in the previous period, ethnicity, mode of delivery, and presence of diabetes. †Birthweight not included as a confounder in where birthweight is the outcome. * A 400g per week change is the recommended rate of GWG in women with a normal BMI (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009)

3.4 Discussion

In this Chapter, GWG was examined in relation to cord blood leptin, adiponectin, lipids, and birthweight as well as the characteristics of women with inadequate and excessive GWG. Amongst this relatively lean Caucasian population, more than half of overweight or obese women gained weight excessively during pregnancy, compared to one-fifth of normal-weight women, even though obese women gained less weight overall. Excessive GWG occurred in younger, less educated, nulliparous women with a higher BMI, compared to women within the GWG recommendations. They also had offspring with higher birthweight, which may reflect the lower rate of vaginal birth and higher cord blood leptin and adiponectin levels. This study has shown that both GWG and BMI were positively associated with cord blood leptin and offspring birthweight, with the strongest association for cord blood leptin seen in obese women who gained excessive weight during pregnancy. BMI was also positively associated with cord blood triglyceride and negatively associated with cord blood HDLc, unlike GWG which showed a positive association with cord blood triglyceride during mid-pregnancy only. Whilst GWG throughout pregnancy was positively associated with both birthweight cord blood leptin, mid-pregnancy GWG demonstrated the strongest association with birthweight whereas late pregnancy GWG showed a stronger association with cord blood leptin.

Maternal pre-pregnancy weight and BMI have been more consistently and historically associated with offspring adiposity and cardiovascular risk factors than GWG alone (Fraser *et al.*, 2010; Poston, Harthoorn and Van Der Beek, 2011; Voerman *et al.*, 2019). This is supported by the current study whereby maternal BMI was associated with a wider range of cord blood biomarkers, and this has also been demonstrated by previous ALSPAC reports (Fraser *et al.*, 2010). GWG however has also been considered significant in contributing to offspring overweight and obesity (Lau *et al.*, 2014; Goldstein *et al.*, 2017). It remains unclear if these effects are causal and the underlying mechanistic influence of both BMI and GWG is not yet fully understood (Voerman *et al.*, 2019). In this study, BMI was positively correlated with

triglyceride and birthweight and negatively associated with cord blood HDLc. This is in contrast to GWG which was positively associated with cord blood leptin and birthweight alone (other than a weak positive association with cord blood triglyceride during mid-pregnancy GWG). GWG has also demonstrated a positive correlation with leptin at birth in other reports (Lemas *et al.*, 2015), and the association between GWG and leptin appears to track across the life-course as evident in child and early adulthood (Fraser *et al.*, 2010; Hrolfsdottir *et al.*, 2015).

Both elevated cord blood leptin and birthweight represent markers of fetal overnutrition as a result of higher maternal BMI or greater GWG. Umbilical cord blood leptin increased with advancing gestation, due to increased production by the placenta, and is an established biomarker of neonatal fat mass (Hauguel-de Mouzon, Lepercq and Catalano, 2006), with elevated leptin levels reflecting greater neonatal fat mass. Birthweight is also a crude marker of offspring anthropometry at birth that correlates positively with child and adult adiposity (Evensen *et al.*, 2017). As well as reflecting neonatal fat mass, leptin plays a central role in early life fetal programming, thereby altering adipocyte function (Thakali *et al.*, 2014) and as such, influences the propensity to greater adiposity throughout the life course. It has therefore been proposed that fetally-programmed adipokine levels may play a role as a potential biological mechanism for the link between fetal growth and long-term outcomes.

Emerging evidence highlights the importance of maternal BMI in pregnancy in relation to leptin gene expression and its effect on offspring body composition. Decreased methylation near the transcription start site of the leptin gene has been strongly associated with greater maternal BMI during pregnancy (Kadakia *et al.*, 2017). Consequently, enhanced expression of the leptin gene and overall greater leptin levels in infancy may have a long-lasting effect on the offspring. It is suggested that such offspring may adopt a leptin-resistant state from early on with subsequent dysregulation of metabolism and satiety leading to greater adiposity throughout childhood and beyond (Kadakia *et al.*, 2017). With the progressively increasing risk

of offspring overweight or obesity, it is becoming more evident that each incremental rise in maternal BMI has a greater effect on offspring adiposity compared to GWG (Voerman *et al.*, 2019). The current study has demonstrated this by displaying similar increased strength of effect when maternal BMI, rather than GWG, was considered. Examining excessive GWG, according to the IOM recommendations, it was felt that the additional risk of this is minimal and maternal BMI is the overriding factor. Accounting for greater offspring birthweight and leptin at birth, and the long-term implications of these findings, maternal BMI may account for the translation of the obesity epidemic across generations. Chapters 6 and 7 explored how these cord blood measures at birth tracked throughout childhood and into adolescence and how the phenotype at birth reflected the degree of adiposity in later life as well. Recent analysis has demonstrated that greater maternal pre-pregnancy BMI and GWG displayed the strongest effects on offspring adiposity in late childhood, rather than at birth (Voerman *et al.*, 2019), which suggested that maternal obesity had potential for long term programming of the offspring and the effects were not fully evident at birth or simply short-lived.

There was no association identified between GWG or BMI and cord blood adiponectin in the current study. This may be due to the fact the cohort included a relatively lean population and higher adiponectin levels are seen out-with pregnancy in obesity and Type 2 diabetes. Whilst one study found no difference between adiponectin in pregnant and non-pregnant controls, they did however identify lower adiponectin levels amongst those with GDM and PE (Cortelazzi *et al.*, 2007). They also reported no correlation between fetal and maternal adiponectin levels, even though fetal adiponectin is lower in the offspring of GDM mothers (Cortelazzi *et al.*, 2007). In addition, whilst adiponectin has been positively associated with offspring birthweight, no association was identified between cord blood adiponectin and maternal BMI (Sivan *et al.*, 2003). Since fetal adiponectin is significantly higher than maternal adiponectin, collectively this highlights that maternal and fetal adiponectin are distinct and that fetal adiponectin has an independent regulatory action on tissue differentiation and growth (Sivan *et al.*, 2003; Cortelazzi *et al.*, 2007).

The 2009 IOM guidance on GWG, as discussed in Chapter 1, was devised in order to guide pregnant women on appropriate weight gain in pregnancy and minimise their obstetric risks (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). Offspring of obese women with excessive GWG were considered to be at greatest risk, as demonstrated by the strength of association with birthweight and cord blood leptin, and this highlights the importance of optimising maternal BMI before pregnancy as well as optimising GWG during pregnancy. Despite this, obese women in the current study gained less weight overall than normal-weight women, which has been demonstrated in previous reports suggesting that early pregnancy BMI was associated with significant differences in GWG (Bergmann *et al.*, 1997; Cedergren, 2006), and may be due to the utilisation of fat stores during the third trimester when fetal energy requirements are greater. In the current study, over half of obese or overweight women exceeded the recommendations however and whilst GWG has been inversely related to pre-pregnancy BMI (Viswanathan *et al.*, 2008), it is clear that most women exceeding the recommendations on GWG are already overweight or obese (Kaar *et al.*, 2014).

ALSPAC has previously reported on the metabolic impact of excessive GWG on 9-year-old offspring (Fraser *et al.*, 2010). Offspring were at greater risk of obesity (Fraser *et al.*, 2010) and overweight in childhood (Nehring, Lehmann and Von Kries, 2013) due to excessive GWG. Greater fat mass, leptin, and HDLc have also been reported in the offspring of mothers with excessive GWG (Fraser *et al.*, 2010; Logan *et al.*, 2017), as has higher maternal leptin immediately following delivery (Logan *et al.*, 2017). The Rhea group further demonstrated a positive association between excessive weight gain in pregnancy and cord blood leptin levels (Karakosta *et al.*, 2013; Karachaliou *et al.*, 2015). Whilst excessive GWG alone has been shown to result in heavier and longer babies who grow more slowly in the first year of life (N. Li *et al.*, 2013; Diesel *et al.*, 2015), a more adipose maternal population (who also gained weight in pregnancy excessively) not only had babies that were heavier and longer, they also had greater growth trajectories in infancy (Deierlein *et al.*, 2012). Furthermore, it has been reported that whilst GWG has repeatedly been positively

associated with offspring BMI, the risk of offspring overweight doubled when the mother was overweight or obese and gained weight excessively (Guo *et al.*, 2015). Studies have concluded therefore that greater maternal PPW is associated with a wider range of cardiovascular risk factors and posed a greater risk of offspring adiposity overall (Fraser *et al.*, 2010). This was supported by the lack of association seen with GWG on offspring outcomes in the current study, although the association with birthweight was weakly positive throughout all BMI categories. Given the positive association between GWG in mothers that were already obese in pregnancy and offspring birthweight and cord blood leptin, this current study suggests that it was the offspring of mothers who not only exceed the GWG recommendations but also have a greater pre-gravid BMI that were at greatest risk of life-long adiposity and these findings add to previous reports on this (Robinson *et al.*, 2014).

GWG within the recommendations for BMI has however been shown to attenuate the adverse effect of maternal adiposity (Kaar *et al.*, 2014) and this may also be the focus of future research. Whilst GWG less than the recommendations has been associated with lower birthweight (Viswanathan *et al.*, 2008) and levels of adiposity in 9-year-old offspring, it has been reported that childhood cardio-metabolic profile did not differ from offspring of mothers who gained weight appropriately during pregnancy (Fraser *et al.*, 2010). This current study has demonstrated a similar lack of association with cord blood adiponectin or lipids is in keeping with this and emphasises the role of developmental overnutrition rather than undernutrition in programming offspring adiposity and cardio-metabolic dysfunction.

Although obese women may gain less weight during pregnancy overall compared to lean women, lean mothers may fail to lose the extra weight accrued during pregnancy for example before they become pregnant again. One study found that one-third of women with a normal pre-pregnancy BMI became overweight or obese by one year post-partum (Endres *et al.*, 2015). Greater rate of change in rising cholesterol and LDLc in normal-weight women has also been reported during pregnancy compared to overweight or obese women, even though they had a less

atherogenic lipid profile at the start of pregnancy and this may reflect the greater overall weight gain amongst normal-weight women (Scifres, Catov and Simhan, 2014). It has therefore been proposed that interventions to support appropriate GWG (rather than excessive GWG) should be encouraged for all ranges of BMI (Schack-Nielsen *et al.*, 2010). Recent intervention studies, to limit GWG to an appropriate level, have had varying outcomes. Involving obese women in pregnancy, group-based weight management sessions with changes in diet and physical activity observed lower GWG during pregnancy compared to a control group (Vesco *et al.*, 2014). Despite also observing lower mean GWG in those that received dietary counselling and exercise classes, another study concluded that most women still exceeded the recommendations despite the intervention (Sagedal *et al.*, 2017). They also suggested that the proportion of women that exceeded the guidance on GWG did not vary according to BMI and proposed that a more intense, individualized approach to weight management during pregnancy is recommended (Sagedal *et al.*, 2017). Whilst brief exposure to an intervention study based on an energy balance model, aimed at promoting better eating habits, behaviour and exercise, did not show any change in overall GWG although they did demonstrate positive changes in healthy eating and exercise during pregnancy. This study highlighted that more intense programmes may be of benefit and the limited impact on total GWG was perhaps restricted by the short duration of the intervention (6 weeks) (Pauley *et al.*, 2018).

The period in which GWG occurred may also be significant. Whilst first trimester weight gain reflects fat deposition and expansion of maternal tissues, late pregnancy GWG is closely related to fat-free mass and is attributable to increased circulating volume, growth of the fetus and placenta, and accumulation of amniotic fluid (Van Raaij *et al.*, 1988; Fraser *et al.*, 2010). Early GWG is important as it has been positively associated with later development of GDM, particularly amongst normal-weight women (Cho, Hur and Lee, 2015), as well as positively associated with fetal growth and infant length at birth (Neufeld *et al.*, 2004), and cord blood leptin levels (Logan *et al.*, 2017). Expansion of maternal fat stores due to GWG during this time results in increased circulating glucose, amino acid, and free fatty acids along with enhanced delivery to the fetus (Fraser *et al.*, 2010). This highlights that abnormal

GWG in early pregnancy may be a critical time for transmission of cardio-metabolic disease to the offspring. Second or third-trimester GWG has been associated with cardio-metabolic dysfunction in childhood (Fraser *et al.*, 2010; Karachaliou *et al.*, 2015), as well as higher maternal leptin levels (Logan *et al.*, 2017). Greatest strength of association with birthweight was demonstrated during mid-pregnancy GWG, positive association with cord blood triglycerides during mid-pregnancy GWG only, and greatest strength of association with cord blood leptin during late pregnancy GWG emphases these critical periods of fetal cardio-metabolic modelling during pregnancy. Although birthweight was positively associated with GWG throughout the entire gestational period, in keeping with previous reports (Tobi *et al.*, 2009; Fraser *et al.*, 2010), it is this peak during mid-pregnancy that may be of greatest significance to the offspring. Mid pregnancy GWG has also been associated with higher childhood BMI and fat mass, with loss of association with third-trimester GWG (Hivert *et al.*, 2016). By contrast, ALSPAC has previously reported that mid-pregnancy GWG was only positively associated with offspring adiposity aged 9 when the mother had excessive GWG during this time, although there was a linear positive association evident between first-trimester GWG and offspring adiposity (Fraser *et al.*, 2010).

In contrast to studies examining GWG and fetal over nutritional effect, the Dutch famine study demonstrated metabolic changes in the offspring of those exposed to famine. Maternal undernutrition in mid or late gestation, as a result of famine, displayed different cord blood metabolic profiles compared to those exposed in early gestation (Roseboom *et al.*, 2001). Changes in DNA methylation, as aforementioned, may occur due to changes in maternal BMI, may also occur as a result of weight gain during specific periods in pregnancy, and may also account for resultant offspring metabolic dysfunction (Tobi *et al.*, 2009). Given that the associations were seen with GWG during specific periods during pregnancy, targeted intervention during key time points may be the focus of future research.

In keeping with a smaller contemporary cohort (n=753), GWG was not consistently associated with cord blood lipids at birth (Lemas *et al.*, 2015). Associations with offspring lipids in later life have also been inconsistent. Whilst GWG between 14 and 36 weeks was positively and linearly associated with adverse lipid profile in the offspring at age 9, associations were mediated by offspring adiposity (Fraser *et al.*, 2010). Similarly, another study reported that associations with LDLc, HDLc, and triglycerides were attenuated to the null when adjusting for offspring BMI at age 32 (Hochner *et al.*, 2012). Although there were no consistent associations demonstrated between GWG and cord blood lipids, maternal pre-gravid BMI was however associated with offspring higher cord blood triglyceride and lower cord blood HDLc, indicating that maternal adiposity (rather than GWG alone) may alter the mechanisms involved in cholesterol transport and metabolism. This may be attributable to the more atherogenic profiles obese women have at the start of pregnancy. Higher maternal BMI is associated with higher cholesterol and triglycerides and lower HDLc levels at the start of pregnancy compared to normal-weight women, even though the rate of change of maternal lipids, as previously discussed, is lower during obese pregnancy than normal-weight women (Scifres, Catov and Simhan, 2014). The resulting disruption to the offspring's lipid profile that occurs in obese pregnancy may account for the vascular dysfunction and consequent predisposition to cardiovascular risk later in life (Cole, Freeman and Preece, 1998; Meyer *et al.*, 2013). This emphasises the value of maintaining a healthy PPW and supports weight management initiatives aimed at women of reproductive age. A positive association between mid-pregnancy GWG and cord blood triglycerides however was demonstrated, highlighting this short-lived period for potential fetal programming.

When examining GWG according to categories of appropriate weight gain, associations with lipids have also been inconsistent. Whilst one study showed that appropriate GWG may attenuate the inverse relationship between maternal BMI and offspring HDLc at age 10 (Kaar *et al.*, 2014), another showed that the negative association between maternal pre-gravid BMI and HDLc at age 9 remained in those that exceeded the GWG criteria (Fraser *et al.*, 2010). Programming of neonatal

cardio-metabolic profile, as measured by cord blood lipids, is therefore foremost determined by maternal pre-gravid BMI. This may be moderated by GWG, particularly during pregnancy, rather than determined by GWG alone.

This study has a number of strengths including the size of the cohort and the availability of data on a range of maternal, pregnancy, and social characteristics as well as being one of few studies with detailed recordings of maternal GWG and cord blood. Multiple pregnancies and deliveries <37 weeks were excluded as these would have influenced birthweight and cord blood measures. Potential confounders and mediators including maternal parity, ethnicity, smoking, and age were adjusted for in the analyses, enabling a more robust evaluation of the associations, as previously described. Pre-pregnancy BMI was also included as a confounder as it was inversely associated with GWG (Viswanathan *et al.*, 2008).

Limitations of the study should also be acknowledged. A mainly Caucasian population was included, so it is not possible to translate the findings across other ethnicities with a greater, or lesser predisposition to weight gain, which, as previously discussed is also a limitation of the IOM guidance. The maternal obesity rate in the current study is 5.3%, much lower than the contemporary population. Whilst it has been highly correlated with weight at the first antenatal visit (Geelhoed *et al.*, 2010), self-reporting maternal weight may have led to under-representation of BMI within the cohort. Maternal weight gain (last minus first weight measurement in pregnancy, kg) may be a more accurate reflection of the exposure as it is calculated using the first measured weight (around 10 weeks) and maternal weight (within 12 hours of delivery) as previously described (Fraser *et al.*, 2010; Lawlor *et al.*, 2011). This avoids contributions from the fetus, placenta and amniotic fluid (as in GWG) and reflects fat accrual instead. However, GWG is a more widely published exposure and was easier to classify according to the IOM guidelines (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). It was also useful to include GWG in the previous gestational period as a confounder and mediator in the analysis in order to account for weight

gain up until the reference point of examining GWG. This GWG may be due to increasing fat depots during pregnancy, mainly during the second trimester, as on average women gain between 2 and 5kg fat mass during pregnancy (Ehrenberg, Huston-Presley and Catalano, 2003). Whilst the gold standard assessment of body fat composition uses DEXA, this was not suitable during pregnancy as it uses ionising radiation. Alternative measures to assess fat mass include using water displacement, anthropometric measurement and bioelectrical impedance techniques. It is difficult to validate these as an assessment of fat mass in pregnancy however as they cannot be compared to DEXA. As such, maternal weight, and BMI instead of fat mass were examined for the purpose of this study.

We have used birthweight and umbilical cord blood leptin (reflecting neonatal fat) as surrogate markers of intrauterine growth and development and as direct measures of offspring anthropometry at birth. Recent studies however have used whole-body air-displacement plethysmography (PEA POD, Life Measurement) (Lemas *et al.*, 2015) and MRI (Kaar *et al.*, 2014) to quantify fat mass in infancy and childhood respectively. Whilst these were not readily available or universally suitable for infants they may be the focus of future research, providing a more accurate assessment of neonatal fat mass. In addition, glucose or insulin levels were not measured, unlike other studies (Lemas *et al.*, 2015). Significant cord blood sample degradation of c-peptide (the preferred index of fetal glucose exposure) was evident with long-term storage and this phenomenon has also been reported by others (Gislefoss, Grimsrud and Mørkrid, 2009), as previously discussed in Chapter 2.

In this current Chapter, BMI, but not GWG, was associated with a wider range of cardiometabolic risk factors at birth. Both BMI and GWG were however positively associated with offspring birthweight and cord blood leptin, reflecting greater offspring anthropometry at birth. With over half of overweight or obese mothers exceeding the recommendations, this work supports healthy weight gain initiatives aimed at obese mothers, whose offspring are at the greatest risk of adopting this phenotype. These findings also emphasise the value of women of reproductive age

maintaining a healthy pre-pregnancy BMI, as this was a more significant predictor of offspring cardio-metabolic profile at birth. By optimising weight gain during pregnancy, offspring may therefore adopt a lower risk of adiposity or cardio-metabolic dysfunction at birth. In addition, changes in dietary and lifestyle patterns during pregnancy may benefit the family unit longer term so pregnancy may be viewed as a window of opportunity to make long-term positive changes.

Chapter 4: Diabetic disorders in pregnancy: association with cord bloods lipids, adipokines and birthweight

4.1 Introduction

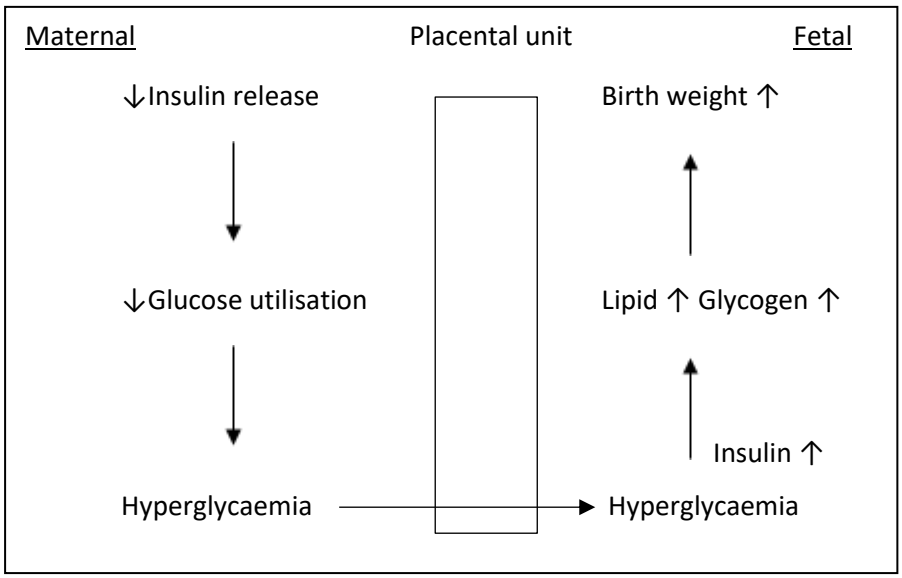
As discussed in Chapter 1, and examined in the previous Chapter (Chapter 3), the DOHaD paradigm suggests that exposure to an adverse intrauterine environment may lead to irreversible re-programming of fetal physiological systems (Barker, 2007). This abnormal intrauterine environment may be evident in maternal diabetes and obesity as insulin signalling pathways are disrupted due to chronic inflammation (Thakali *et al.*, 2014). As this inflammatory environment adversely affects the maternal-fetal interface, maternal insulin-resistance (exacerbated by GWG), exhibits a causal programming effect in utero (Poston, 2010), leading to offspring with a greater propensity to cardio-metabolic dysfunction. In addition, fetal exposure to maternal diabetes increases the offspring's risk of obesity also via intrauterine mechanisms, which is not solely accounted for by maternal pre-gravid BMI (Lawlor, Lichtenstein and Långström, 2011; Phillips *et al.*, 2014). This association is not universal however as studies have shown that mild hyperglycaemia (falling short of the threshold for diagnosis of GDM), as well as those diagnosed with GDM, are not directly at increased risk of obesity in childhood (Pettitt *et al.*, 2010; Thaware *et al.*, 2015). The association between maternal glycaemia and offspring cardiovascular risk in early and later life however remains unclear. There was therefore the need for a detailed assessment of maternal diabetes, reflecting over nutritional exposure to the fetus, in relation to cord blood metabolic profiles at birth.

GDM is one of the most common complications of pregnancy, defined as a carbohydrate intolerance identified in pregnancy (National Institute for Health and Care Excellence, 2015). Of the 5% of all pregnancies complicated by diabetes, 88% are recognised as GDM, 7.5% as Type 1 and 5%, and Type 2 (National Institute for Health and Care Excellence, 2015). Such pregnancies are deemed 'high risk' due to the maternal and fetal complications that may arise. Maternal risks include a greater

risk of miscarriage, GH, pre-term labour, caesarean section and worsening diabetic retinopathy throughout the gestational period (National Institute for Health and Care Excellence, 2015). The maternal risk extends beyond the pregnancy, however, as mothers face greater co-morbidity in later life such as obesity, type 2 diabetes, and cardiovascular disease (Catalano *et al.*, 2003; Egan *et al.*, 2014; Gante *et al.*, 2015).

Fetal risks during pregnancy include a greater risk of stillbirth, congenital malformations, large for gestational age, birth injury, and perinatal mortality. Evidence supports a direct causal role for exposure to maternal diabetes in utero in determining offspring's long-term greater adiposity and adverse cardio-metabolic health. GDM, for example, is associated with over a four-fold greater risk of offspring macrosomia, with birthweight over 4kg (A. Lawlor *et al.*, 2010). The HAPO study of over 23,000 pregnant women, as discussed in Chapter 1, found that the odds ratio for offspring birthweight greater than the 90th centile was 2.19 for non-obese women with GDM, 1.73 for obese women without GDM, and 3.62 for women with both obesity and GDM (Catalano *et al.*, 2012). This suggested that whilst maternal diabetes and maternal obesity may impact independently on fetal growth, the risk of the conditions combined had an additive and compounding effect on the offspring. The pathophysiology of macrosomia may be explained by the modified Pedersen's hypothesis in which exposure to maternal hyperglycaemia leads to fetal hyperinsulinemia and greater glucose utilisation resulting in greater fetal adipose tissue, as demonstrated in Figure 4.1 (Pedersen, 1952). Circulating glucose crosses the placenta but insulin does not and as a result, the fetal pancreas will secrete insulin in response to the hyperglycaemic environment. A combination of both fetal hyperinsulinemia and hyperglycaemia leads to anabolic accrual of greater fat and protein stores, resulting in offspring macrosomia.

Figure 4.1: Modified Pedersen's hypothesis



The presence of maternal obesity in maternal diabetes is of concern. As discussed in Chapter 3, the risk of developing GDM is 3 times greater in maternal obesity (Tamimi *et al.*, 2003). A retrospective study of 2773 women showed that 46% of those with pre-gestational diabetes (PGDM) and 18.3% of those with GDM demonstrated GWG above the 2009 IOM recommendations (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009; Egan and Dunne, 2018), as discussed in Chapter 3. Amongst those with excessive GWG, most women were either already overweight or obese (Egan and Dunne, 2018). The risk of macrosomia, especially for those with PGDM, was also higher amongst diabetic mothers with excessive GWG (Yee *et al.*, 2011; Harper *et al.*, 2013; Parellada *et al.*, 2014; Scifres *et al.*, 2014; Siegel *et al.*, 2015; Egan and Dunne, 2018). Excessive GWG, even amongst women with a normal BMI may still, however, impose a greater risk of LGA infants of mothers with Type 1 diabetes (Scifres *et al.*, 2014), suggesting that fetal overnutrition and hyperglycaemia may act independently of maternal BMI.

Greater risk of caesarean section and poorer neonatal condition at birth have also been reported amongst those with excessive GWG in some (Yee *et al.*, 2011; Harper *et al.*, 2013) but not all studies (Siegel *et al.*, 2015). Maternal outcomes at delivery however do not appear to be consistently adversely affected amongst mothers with excessive GWG and GDM (Harper *et al.*, 2013).

It is therefore becoming increasingly apparent that the insulin-resistant state in diabetes may be exaggerated by pregnancy weight gain, thereby worsening the obstetric and perinatal outcomes (Egan *et al.*, 2014; Gante *et al.*, 2015). Intervention studies suggest these outcomes or offspring metabolic risk may be modified as dietary and lifestyle changes in diabetic pregnancies, and therefore limiting GWG may moderate the translation of obesity and cardio-metabolic programming onto the offspring (Retnakaran *et al.*, 2013). A recent multicentre study of obese women with GDM reported that only a third of women achieved GWG within the recommendations (Gante *et al.*, 2015), although this has been reported as low as 10.9% in women with PGDM (Siegel *et al.*, 2015). Achieving appropriate GWG amongst this high-risk population will therefore be challenging. Gaining less than recommended amongst women with GDM is however not recommended as the benefits have yet to be established, as discussed previously, and the neonatal condition or the risk of caesarean section did not differ from those with GDM gaining within the recommendations on GWG (Harper *et al.*, 2013).

This risk of maternal hyperglycaemia and offspring adiposity may also extend into early childhood and there is a positive, albeit weak, correlation between maternal glucose levels at 28 weeks' gestation and offspring obesity at aged 2 years, (Pettitt *et al.*, 2010) although the association with offspring BMI or obesity was not independent of maternal BMI in the 5 to 7-year-old offspring (Thaware *et al.*, 2015). This association has been described in recent systematic review and meta-analysis, where GDM is a risk factor for childhood offspring overweight or obesity that is not independent of maternal BMI, even though maternal BMI itself is a risk factor for offspring obesity, independent of GDM (Kim *et al.*, 2011; Patro Golab *et al.*, 2018;

Voerman *et al.*, 2019). There is also the reported risk of greater offspring adiposity in later life. Maternal GDM has been associated with overweight and obesity in 9-11-year-old offspring, as previously reported by ALPSAC, and in adolescence, although the effect of GDM on offspring overweight or obesity may only partially be explained by its effect on birthweight as there was evidence of maternal BMI attenuating this association (Gillman *et al.*, 2003; A. Lawlor *et al.*, 2010). The association between maternal glycosuria, demonstrated by at least 2 episodes of 2+ glucose in urine during pregnancy in the absence of PGDM or GDM, did however show greater odds of overweight or obesity in the adjusted analysis of 9-11-year-old offspring (A. Lawlor *et al.*, 2010). In the 'Growing Up Scotland Study' of 4 and 6-year-old offspring, GDM was positively associated with childhood obesity, although the association attenuated to the null in the PGDM group (Abraham *et al.*, 2015). This indicates that disruption to maternal and fetal glucose metabolism in pregnancy may therefore play a modest role in the development of offspring obesity and that its role in the causal pathway is not simply accounted for by maternal pre-pregnancy BMI alone.

This metabolic risk not only applies to a greater risk of obesity in later-life, however. Diabetes in pregnancy poses a further risk of metabolic dysfunction in the offspring as demonstrated by human studies suggesting that maternal glycaemia exhibits a causal programming effect in utero and so may be used as a predictor of metabolic disorders as well in the offspring (Poston, 2010). The inflammatory and hyperglycaemic environment present in GDM may adversely affect the fetal-placental unit and disrupt the adipo-insular axis in early and late gestation, thereby establishing an adverse trajectory of metabolic health in the offspring (Dubé, Ethier-Chiasson and Lafond, 2013; Li, Chen and Li, 2013; Thakali *et al.*, 2014), although the exact mechanisms involved are not clear. As previously discussed, epigenetic modification of fetal genes in utero is of concern and have been suggested as a mediator on the pathways from maternal pregnancy diabetes to long-term offspring outcomes, including the transgenerational transmission of diabetic disorders in the offspring such as Type 2 diabetes (Dabelea, 2007; Fraser and Lawlor, 2014).

The effect of GDM on offspring insulin resistance was demonstrated by a small study of Chinese pregnant women in which cord blood insulin, proinsulin, and homeostasis model assessment of insulin resistance (HOMA-IR) levels were significantly higher in the offspring of GDM mothers compared to a control group (Q. Wang *et al.*, 2013). ALSPAC has also previously reported that both PGDM and GDM were positively associated with 15-year-old fasting glucose levels and that maternal glycosuria, not maternal diabetes, was positively associated with fasting insulin in the 15-year-old offspring (Patel *et al.*, 2012).

There have been inconsistent reports of disruption to the offspring's lipid, however. As discussed in Chapter 3, obese pregnant women display a more atherogenic lipid profile compared to normal-weight women. Higher cholesterol, LDLc, and triglycerides, as well as lower HDLc, have been reported in obese pregnant women compared to normal-weight pregnant women who develop GDM (Bozkurt *et al.*, 2016). Overweight or obese women however display significantly different trajectories (slower) of lipids with advancing pregnancy compared to normal-weight mothers (Bozkurt *et al.*, 2016). Amongst diabetic mothers, higher cord blood triglyceride and LDLc and lower HDLc have been reported (Dubé, Ethier-Chiasson and Lafond, 2013). In the UK Pregnancies Better Eating and Activity Trial (UPBEAT), a multicentre randomised controlled trial of a complex lifestyle intervention in obese pregnant women, there was marked dyslipidaemia (elevated lipids and lipoprotein constituents in VLDL subclasses) in mothers at least 10 weeks prior to the diagnosis of GDM (White *et al.*, 2017). This is thought to be due to greater insulin resistance in adipose tissue and reduced suppression of lipolysis with advancing pregnancy (Catalano *et al.*, 2002). There is less consistent evidence of GDM impacting the lipid profiles of the offspring, however. Whilst LDLc hypercholesterolaemia in the cord blood of mothers with GDM has been reported (Eslamian *et al.*, 2013), ALSPAC has previously demonstrated that neither PGDM, GDM, or maternal glycosuria were associated with offspring lipids, or other metabolic markers such as CRP or blood pressure in 15-year-old offspring (Patel *et al.*, 2012).

In addition, there is also limited evidence of the effect of maternal diabetes on offspring adipokines. As previously discussed, adiponectin is an adipocyte-derived hormone that is inversely associated with circulating insulin and insulin resistance, BMI, lipids, and type 2 diabetes in non-pregnant women (Cnop *et al.*, 2003; Yadav *et al.*, 2013). Women who are more insulin resistant prior to pregnancy may also have a concomitantly low adiponectin level, due to hyperinsulinemia, and as they become progressively more insulin resistant in pregnancy have a greater tendency to developing GDM (Iliodromiti *et al.*, 2016). Low levels of adiponectin prior to pregnancy may be used to predict the development in GDM in subsequent pregnancy and indeed there is growing evidence to support adiponectin testing in early pregnancy to detect those at high or low risk (Hedderson *et al.*, 2013; Iliodromiti *et al.*, 2016). In a healthy pregnancy, cord blood adiponectin levels correlate with maternal adiponectin levels (Ballesteros *et al.*, 2011; Retnakaran *et al.*, 2013). In GDM pregnancy, maternal adiponectin levels are lower than control groups, especially amongst obese women with GDM (Retnakaran *et al.*, 2013; Iliodromiti *et al.*, 2016). Maternal adiponectin in GDM pregnancy does not however correlate with offspring adiponectin levels at birth and there was no difference in adiponectin levels in 1-year-old offspring of mothers with GDM compared to offspring of healthy mothers (Ballesteros *et al.*, 2011; Retnakaran *et al.*, 2013). Collectively, the lack of consistent associations between maternal diabetes and a range of cardio-metabolic markers suggests that maternal diabetes may have a limited impact on the cardio-metabolic health of the offspring.

In contrast to maternal adiponectin, maternal leptin has been inconsistently associated with GDM. Whilst elevated leptin levels have been identified in those with GDM amongst (Kautzky-Willer *et al.*, 2001; Chen *et al.*, 2010; Fasshauer, Blüher and Stumvoll, 2014), this was not reproduced in the UPBEAT study, where leptin was not associated with GDM in the obese women and may reflect chronically elevated leptin levels in association with obesity (White *et al.*, 2017).

By examining associations between maternal diabetes (PGDM, GDM, and glycosuria) and offspring cord blood adipokines, lipids, and birthweight, the aim of this study was to identify whether fetal exposure to intrauterine hyperglycaemia, reflecting fetal overnutrition, poses a significant metabolic risk to the offspring at birth. Specifically, and as previously discussed, lower cord blood adiponectin in the offspring of diabetic mothers was anticipated, with limited or no effect on cord blood lipids of these offspring. By examining maternal weight in each of the diabetic groups, this study also aimed to examine whether PPW and GWG may modify this risk.

4.2 Methods

4.2.1 Study Population

There were 5011 participants with a paired cord blood sample. Multiple pregnancies and those without GWG or BMI recorded were excluded. Therefore, there were 3629 mother-offspring pairs included in the study. Women were categorised into one of four mutually exclusive categories of no diabetes or glycosuria (hereafter referred to as 'healthy' women), PGDM, GDM, and glycosuria. Details of cord blood sample collection and analysis are presented in Chapter 2.

4.2.2 Obstetric and Perinatal Data

Data were abstracted from obstetric medical records and questionnaires by ALSPAC. Full details of the study have been previously published (Fraser, Macdonald wallis, *et al.*, 2013) and are discussed in Chapter 2. Information on pre-pregnancy diabetic status was obtained from the patient questionnaire at the time of recruitment. A standard protocol was used by research midwives to obtain information on GDM and glycosuria (recorded as none, +, ++ and +++) for the index pregnancy from the participant's antenatal and postnatal records. Glycosuria is a valid marker of maternal glycemia as it positively associates with birth size and macrosomia (A. Lawlor *et al.*, 2010). All women were offered urine tests for glycosuria at each antenatal visit. Glycosuria in the current study was defined as a record of at least ++ (equal to 13.9mmol/l or 250mg/100ml) on at least two occasions at any time during the pregnancy in the absence of PGDM and GDM. Glycosuria below 250mg/100ml may occur in pregnancy as a result of normal physiological processes that lead to a greater glomerular filtration rate (Buhling *et al.*, 2004) and therefore testing for GDM below this level was not warranted. Women were either offered fasting glucose or oral glucose tolerance

and those with glucose greater or equal to 7.0 mmol/l or greater or equal to 9.0 mmol/l respectively were diagnosed with GDM.

At the time that this study was undertaken, screening for GDM was not universal and was selective to only those with established risk factors. These factors included established glycosuria in the index pregnancy, maternal obesity, family history of diabetes, personal history of GDM or macrosomic baby, and south Asian ethnicity. PGDM was defined as diabetes occurring prior to pregnancy and encompassed both 'type 1' and 'type2' diabetes mellitus. Offspring of PGDM mothers are universally at risk of impaired glucose tolerance so defining the type of maternal diabetes was not necessary (Poston, 2010).

4.2.3 GWG

GWG was recorded, as detailed in Chapters 2 and 3. This enabled the variable absolute weight gain to be created (Fraser *et al.*, 2010), which was then classified according to the IOM criteria (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). ALSPAC has previously created a multilevel model (Fraser *et al.*, 2010, 2011; Fraser, Macdonald wallis, *et al.*, 2013) relating gestational age to maternal weight so that pre-pregnancy weight (0 weeks), early (0-18 weeks), mid (19-28 weeks), and late (>29 weeks until delivery) pregnancy GWG could be examined. PPW was examined per 1 kg additional maternal weight at conception and rate of GWG was examined per400g GWG per week (the recommended rate of weight gain in pregnancy for women with a normal BMI) (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009).

4.2.4 Statistical Analysis

Cord blood cholesterol, triglyceride, HDLc and non-HDLc, leptin, adiponectin and birthweight were log-transformed to produce approximately normal distributions of residuals. Differences in the distribution of participant characteristics (maternal, obstetric and offspring co-variables) in relation to maternal diabetes or glycosuria status were also examined. These relations were tested using chi^2 tests for categorical variables and one-way anova or ttests for continuous variables, as previously reported by ALSPAC (Fraser *et al.*, 2010, 2011). These analyses tested for the null hypothesis of no difference between the four exposure groups so that there was no assumption of a linear trend across the exposure categories of healthy, PGDM, GDM, and glycosuria. The examination of PPW in the PGDM group was limited due to the small sample size. The power calculation, to determine the minimal detectable effect size suggested that the numbers were too small although it was possible to perform the analysis for GWG as there were fewer missing variables in this analysis.

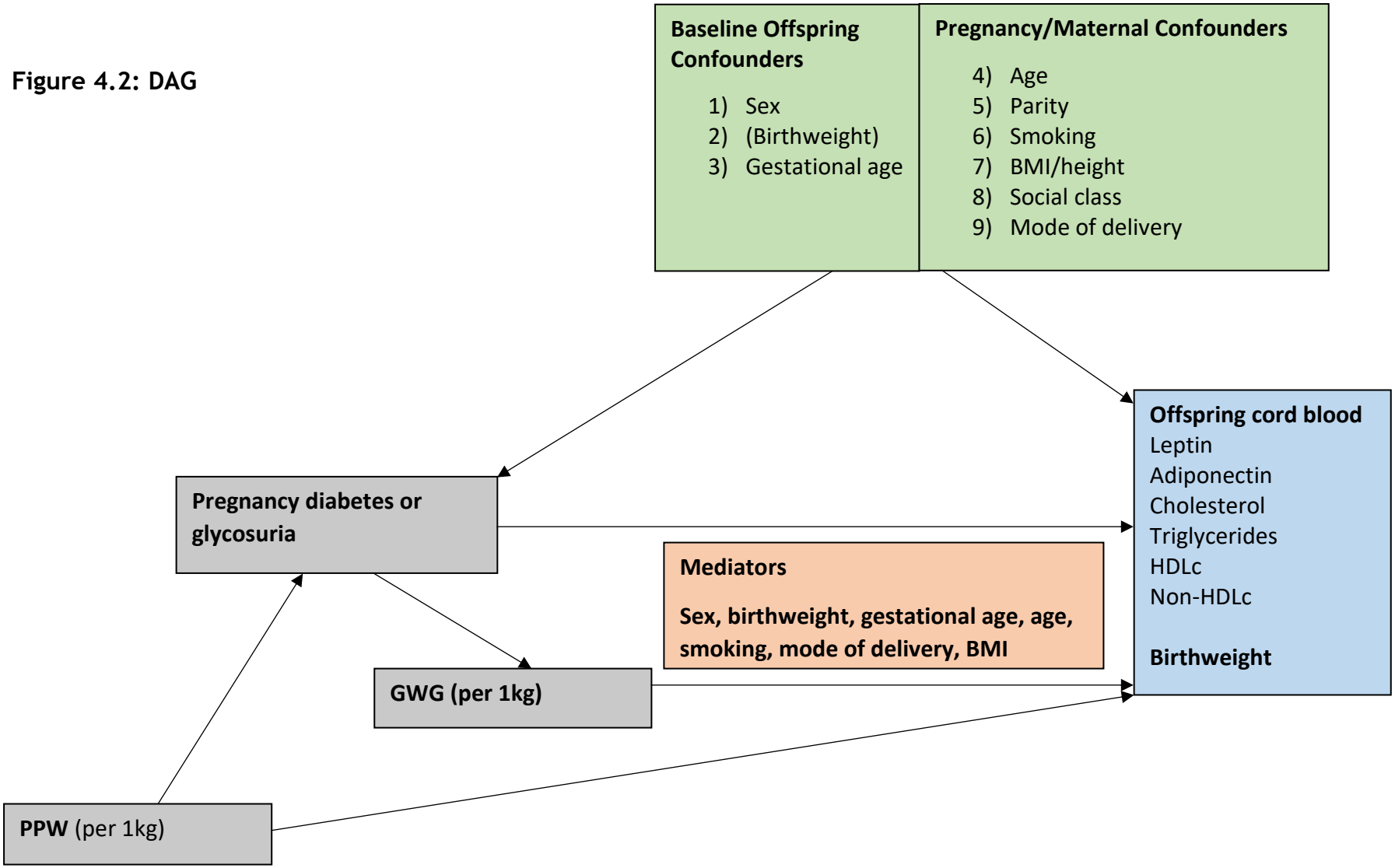
A series of multivariable regression models were formulated to examine the association of maternal diabetes or glycosuria with offspring outcomes and to examine the impact of adjusting for potential confounding and mediating characteristics on these associations. For each outcome, only participants with the complete data (complete case analysis) were included in each of the multivariable analysis models, and therefore the number of participants (N) varied between models. Associations did not differ for male and female offspring, and there was no statistical evidence of interaction with sex. Therefore, all results were presented with males and females combined. Maternal diabetic status was examined as a categorical variable in relation to cord blood measures and in the confounder adjusted model maternal age, social class, smoking, parity, and BMI were additionally adjusted for whereas potential mediation by birthweight, mode of delivery, and gestational age were also adjusted for.

Linear regression analysis was performed to examine pre-pregnancy weight (PPW, per 1kg) and GWG (per 1 kg) as a continuous variable in relation to cord blood adipokines, lipids, and birthweight. Again, potential offspring confounding or mediation by birthweight and gestational age were adjusted for. Where PPW was the exposure, maternal height replaced maternal BMI. Power calculation, to determine the minimal detectable effect size, and to minimise any errors in the effect size seen, suggested that the number of women with PGDM with PPW recorded, as well as all the co-variables included in the study, were too small. It was however possible to perform the analysis for GWG as there were fewer missing variables in this group, although the reproducibility of the effects demonstrated this would have been improved with a larger sample size.

4.2.5 Assessment of confounders and mediators

As shown in Figure 4.2: DAG, baseline offspring confounders (sex, birthweight, and gestational age) were identified and considered.

Figure 4.2: DAG



Similar confounders and mediators were included as those discussed in Chapter 3 since maternal diabetes, as well as maternal BMI and GWG, may reflect fetal overnutrition and be therefore considered in relation to offspring birthweight and cord blood adipokines and lipids. As an extension of the assessment of confounders and mediators considered in Chapter 3, the following variables were additionally considered:

Offspring sex

Male offspring of GDM mothers have demonstrated lower birthweight and lower neonatal fat mass indicating greater responsiveness to treatment of diabetes in pregnancy (Bahado-Singh *et al.*, 2012). Offspring sex was therefore additionally considered as a confounder as well as a mediator in the analysis as it may potentially influence not only the birthweight, but also cord blood leptin as a reflection of neonatal fat mass.

Birthweight

There is at least double the risk of offspring macrosomia in the presence of GDM, although this is even higher particularly in the presence of maternal obesity (A. Lawlor *et al.*, 2010; Catalano *et al.*, 2012) so birthweight was accounted for.

Gestational age

Gestational age was adjusted for as women become more insulin resistant with advancing gestation as well as the fact that women with diabetes tend to be delivered earlier than 40 weeks. Women with type 1 diabetes are at greater risk of

preterm delivery (Lepercq *et al.*, 1998), and even in the presence of good glycaemic control and appropriate fetal growth, mothers with GDM will be advised to deliver by 40 weeks and not beyond this.

Maternal Age

Greater maternal age is associated with a greater risk of developing GDM (Kuo *et al.*, 2017) which may display low adiponectin levels as a result, partly due to the progressive insulin resistance that occurs with advancing age but also due to physical inactivity displayed in older adults. In keeping with this, advancing age may also be associated with a more atherogenic baseline lipid profile which may influence the cord blood measures at birth.

Smoking

Whilst smoking does not appear to influence the risk of GDM, it is associated with lower GWG and lower offspring birthweight and it may also influence cord blood lipids at birth. The diagnosis of GDM in pregnancy, along with the lifestyle and dietary modifications offered, may however encourage women to stop smoking and therefore this may be considered also as a mediator in the analysis.

Parity

The recurrence risk of GDM is greater with higher parity, partly because of greater baseline BMI but also because of advancing age, and therefore maternal parity was considered as a confounder in the analysis.

Occupational social class and education

Lower socioeconomic class is a risk factor for developing Type 2 diabetes and may confer the same risk to women in pregnancy (NICE (National Institute for Health and Care Excellence), 2011), justifying its inclusion in the analysis.

Maternal BMI

High maternal BMI carries a greater risk of developing GDM. However, severity of GDM may also be moderated by optimising BMI during pregnancy (National Institute for Health and Care Excellence, 2015) and so it was important to account for this in the analysis.

Ethnicity

Women from a minority ethnic background may be more predisposed to developing GDM than Caucasian women (National Institute for Health and Care Excellence, 2015). This may be due to the greater visceral fat, particularly amongst the South East Asian community, and a greater propensity to cardiovascular disease at a lower BMI than the Caucasian population, as discussed in Chapter 2. Asian women may also have smaller birthweight babies, partly due to placental insufficiency. Ethnicity was therefore included as a confounder for the risk of developing GDM but also considered for the impact on birthweight and cord blood adipokines and lipids.

Mode of delivery

Women with GDM are more likely to require a caesarean section and so mode of delivery was accounted for. This may be due to fetal macrosomia or due to poor

glucose control in pregnancy warranting an emergency delivery (National Institute for Health and Care Excellence, 2015).

4.3 Results

Figure 4.3 shows the inclusion and exclusion criteria of the ALSPAC participants included in the study.

Figure 4.3: ALSPAC participant flow chart

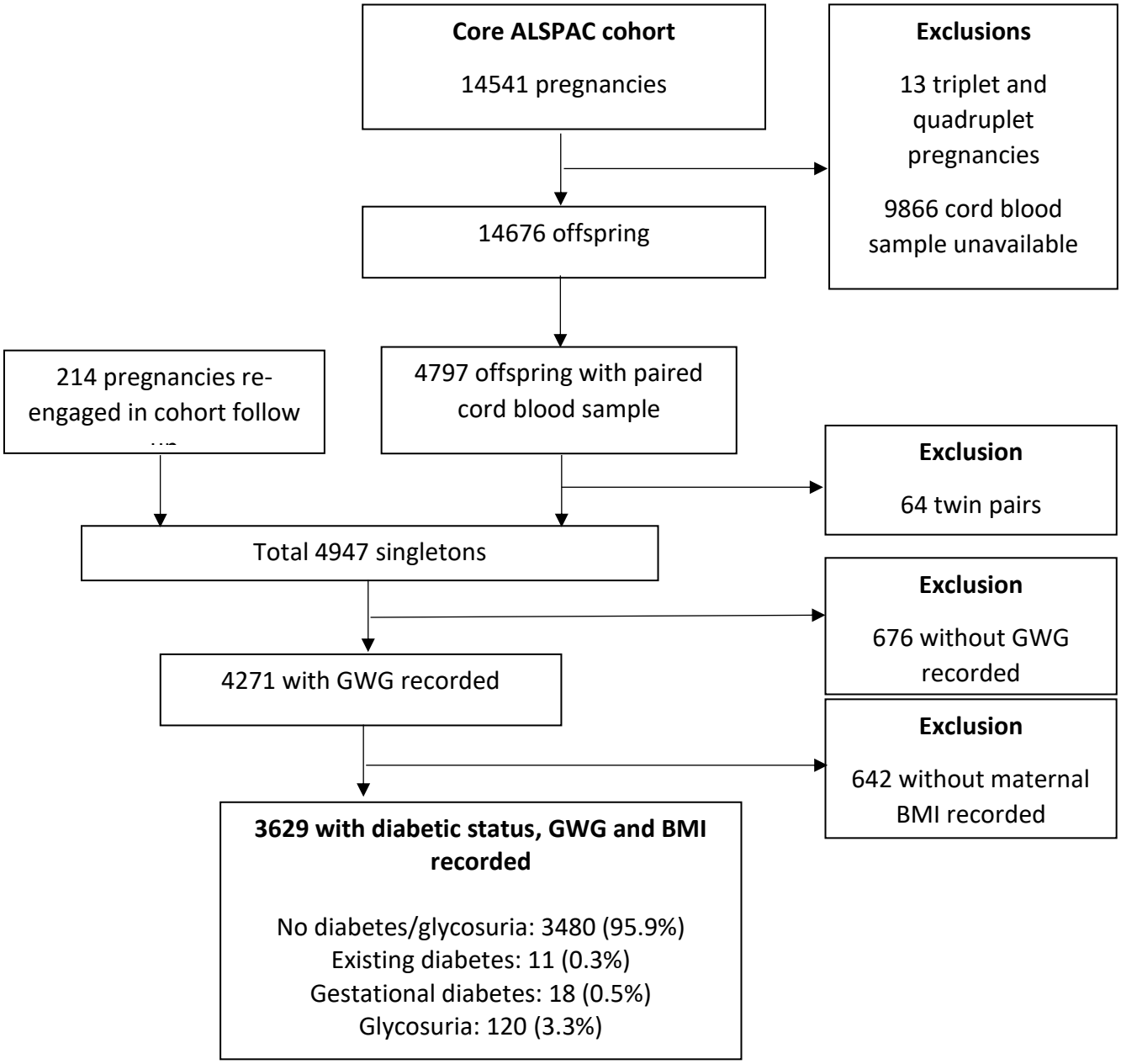


Table 4.1 summarises the cohort characteristics, according to their diabetic status in pregnancy. Age, parity (most nulliparous), ethnicity, education, and social class were comparable across all groups. Amongst the healthy women, 22.9% smoked during pregnancy, in contrast to 9.1% of those with PGDM or 5.6% of those with GDM. More women who developed GDM however smoked before, but not during, pregnancy (22.2%), which was higher than any other group. Maternal BMI was highest among mothers with PGDM (25.8kg/m^2) and lowest in the healthy (22.1kg/m^2) and GDM groups (23.9kg/m^2). GWG varied between groups with healthy mothers and those with glycosuria gaining the most (12.4kg) and mothers with GDM gaining the least (9.8kg). Most women gained either within or less than the recommendations on GWG although 33.3% of those with glycosuria exceeded these recommendations compared to only 22.2% of the GDM group.

Table 4.1 also demonstrates that the offspring birthweight was lowest among those with PGDM (3.2kg), perhaps due to the earlier gestation at delivery (37 weeks), and highest among those with glycosuria (3.6kg), who were delivered at 40 weeks' gestation. The offspring of mothers with GDM were of similar birthweight to offspring of the healthy mothers (3.5kg) and were delivered at 39 and 40 weeks, respectively. There were more male offspring amongst those with GDM (66.7%), and offspring sex was similar for males and females among all other groups. The caesarean section rate was much higher amongst women with diabetes with 18.2% of those with PGDM and 11.1% of those with GDM requiring a caesarean delivery, in contrast to 8.8% of the healthy women and only 1.8% of those who developed glycosuria.

Table 4.1: Summary of cohort characteristics according to diabetic groups

	Healthy (n=3480)		PGDM (n=11)		GDM (n=18)		Glycosuria (n=120)		
	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	P
Maternal Characteristics									
Age	3480	28 (25, 31)	11	29 (24, 31)	18	28 (25, 35)	120	28 (25, 32)	0.345
Smoking									
Never	2381 (69.7)		10 (90.9)		13 (72.2)		82 (68.3)		0.117
Before, not during	254 (7.4)		0 (0.0)		4 (22.2)		10 (8.3)		
During pregnancy	781 (22.9)		1 (9.1)		1 (5.6)		28 (23.3)		
BMI (kg/m^2)	3480	22.1 (20.5, 24.4)	11	25.8 (22.7, 29.4)	18	23.9 (21.5, 25.7)	120	22.8 (21.2, 26.5)	0.000
Parity									
0	1502 (44.9)		7 (63.6)		8 (44.4)		55 (46.6)		0.786
1	1223 (36.5)		4 (36.4)		6 (33.3)		35 (29.7)		
2	455 (13.6)		0 (0.0)		1 (5.6)		20 (17.0)		
3	130 (3.9)		0 (0.0)		3 (16.7)		6 (5.1)		
4+	37 (1.1)		0 (0.0)		0 (0.0)		2 (1.7)		
Education									
Left school at 16	2128 (63.6)		4 (40.0)		8 (50.0)		75 (67.6)		0.538
A level	785 (23.5)		4 (40.0)		6 (37.5)		24 (21.6)		
Degree	433 (12.9)		2 (20.0)		2 (12.5)		12 (10.8)		
Social Class									
I (least disadvantaged)	143 (5.1)		1 (9.1)		1 (8.3)		9 (9.4)		0.272
II	932 (33.0)		2 (18.2)		3 (25.0)		23 (24.0)		
IIIa	1224 (43.4)		5 (45.5)		5 (41.7)		45 (46.9)		

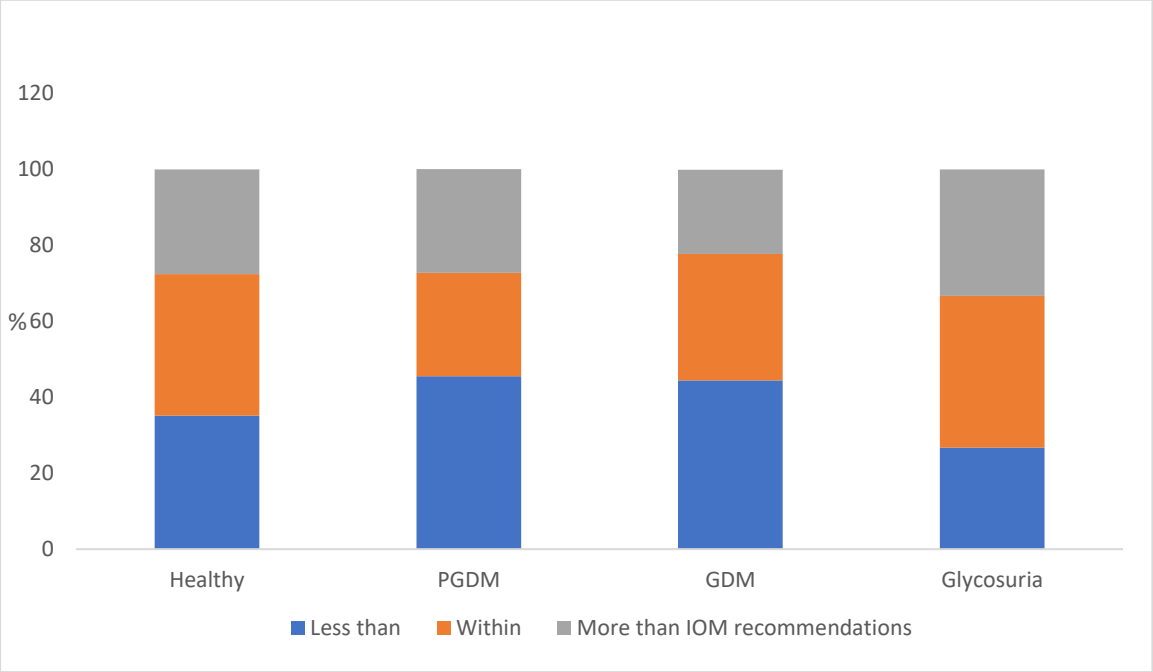
	Healthy (n=3480)		PGDM (n=11)		GDM (n=18)		Glycosuria (n=120)		
IIIb	217 (7.7)		0 (0.0)		1 (8.3)		4 (4.2)		
IV	254 (9.0)		2 (18.2)		2 (16.7)		11 (11.5)		
V (most disadvantaged)	52 (1.8)		1 (9.1)		0 (0.0)		4 (4.2)		
Ethnicity									0.994
White	3274 (98.2)		11 (100.0)		16 (88.9)		110 (97.4)		
Caucasian	12 (0.4)		0 (0.0)		0 (0.0)		0 (0.0)		
Black Caribbean	4 (0.1)		0 (0.0)		0 (0.0)		0 (0.0)		
Black African	9 (0.3)		0 (0.0)		0 (0.0)		1 (0.9)		
Black other	11 (0.3)		0 (0.0)		0 (0.0)		1 (0.9)		
Indian	4 (0.1)		0 (0.0)		0 (0.0)		0 (0.0)		
Pakistani	7 (0.2)		0 (0.0)		0 (0.0)		0 (0.0)		
Chinese	13 (0.4)		0 (0.0)		0 (0.0)		1 (0.9)		
Other									
Pregnancy characteristics									
GWG (kg)	3480	12.4 (9.5, 15.4)	11	10.6 (6.2, 11.7)	18	9.8 (5.5, 14.5)	120	12.4 (9.7, 15.5)	0.000
= Recommend	1294 (37.2)		3 (27.3)		6 (33.3)		48 (40.0)		
< Recommend	1224 (35.2)		5 (45.5)		8 (44.4)		32 (26.7)		
> Recommend	962 (27.6)		3 (27.3)		4 (22.2)		40 (33.3)		
Mode of delivery									0.000
Spontaneous	2705 (78.0)		4 (36.4)		8 (44.4)		91 (75.8)		
Breech	28 (0.8)		0 (0.0)		0 (0.0)		1 (0.8)		
Caesarean	305 (8.8)		5 (45.5)		8 (44.4)		15 (12.5)		
Forceps	204 (5.9)		2 (18.2)		2 (11.1)		2 (1.7)		
Vacuum	190 (5.5)		0 (0.0)		0 (0.0)		7 (5.8)		
Other	36 (1.0)		0 (0.0)		0 (0.0)		4 (3.3)		
Gestational age at birth (weeks)	3480	40 (39, 41)	11	37 (36, 38)	18	39 (38, 40)	120	40 (38, 40)	0.000

Offspring characteristics	Healthy (n=3480)		PGDM (n=11)		GDM (n=18)		Glycosuria (n=120)		
Sex									
Male	1767 (50.8)		6 (54.6)		12 (66.7)		62 (51.7)		0.594
Female	1713 (49.2)		5 (45.5)		6 (33.3)		58 (48.3)		
Birthweight (kg)	3448	3.5 (3.2, 3.8)	11	3.2 (3.1, 7.8)	18	3.5 (3.2, 4.1)	119	3.6 (3.2, 4.0)	0.000
Cord blood leptin (pg/ml)	3477	6.3 (3.6, 11.8)	11	8.3 (5.4, 13.9)	18	5.8 (2.3, 11.6)	120	7.9 (4.6, 15.3)	0.000
Cord blood adiponectin (µg/ml)	3477	76.6 (54.4, 99.8)	11	71.8 (36.1, 105.6)	18	63.8 (37.5, 84.3)	117	67.0 (52.2, 91.7)	0.029
Cord blood cholesterol (mmol/l)	3436	1.7 (1.4, 2.0)	10	2.2 (1.6, 2.6)	18	1.6 (1.2, 1.9)	118	1.6 (1.3, 1.9)	0.000
Cord blood triglyceride (mmol/l)	3422	0.5 (0.4, 0.6)	10	0.6 (0.5, 0.6)	18	0.4 (0.3, 0.6)	118	0.4 (0.4, 0.6)	0.000
Cord blood HDLc (mmol/l)	3365	0.5 (0.4, 0.7)	10	0.6 (0.4, 0.8)	18	0.6 (0.3, 0.7)	115	0.5 (0.4, 0.6)	0.142
Cord blood non-HDLc (mmol/l)	3365	1.1 (0.9, 1.4)	10	1.4 (1.1, 1.8)	18	1.1 (0.9, 1.2)	115	1.1 (0.9, 1.4)	0.010

Median (Interquartile range) Figures are numbers (%) unless stated otherwise

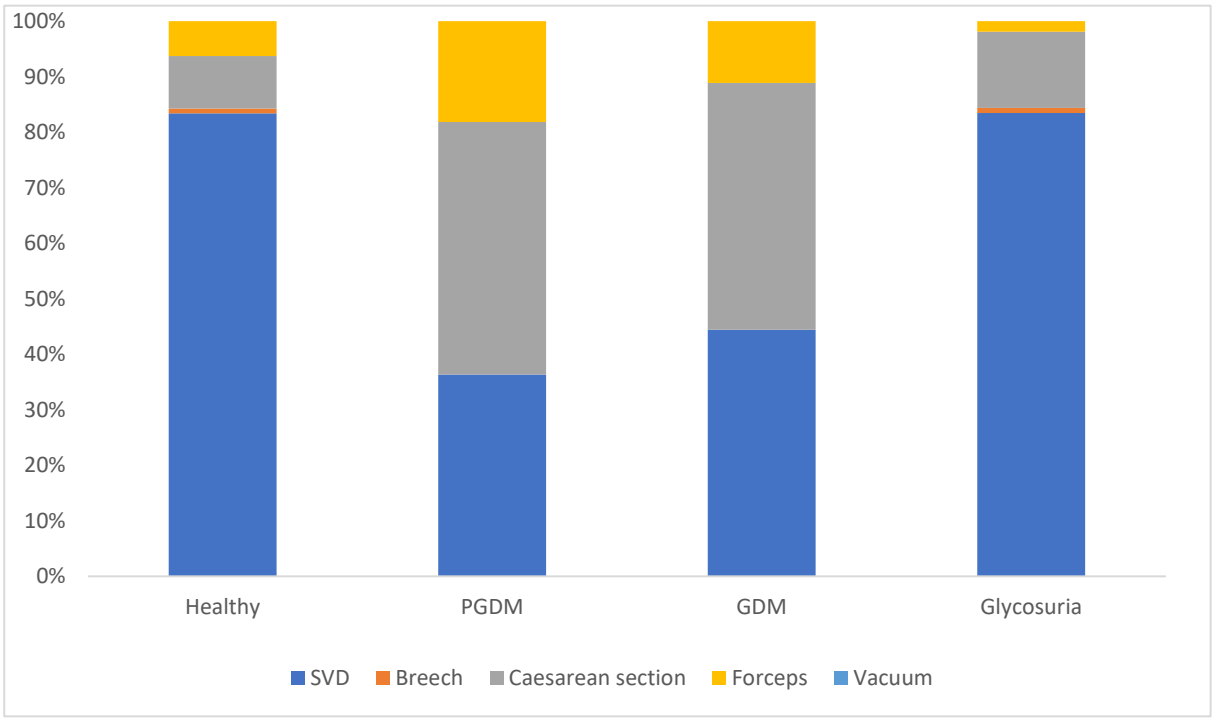
GWG according to the IOM recommendations for each diabetic group is displayed in Figure 4.4.

Figure 4.4: IOM category according to diabetic status



The mode of delivery according to diabetic status is displayed in Figure 4.5.

Figure 4.5: Mode of delivery according to diabetic status



The concentration of cord blood leptin was highest (8.3pg/ml) amongst women with PGDM and lowest among those with GDM (5.8pg/ml). In contrast, cord blood adiponectin from both PGDM and GDM groups had the lowest values (71.8µg/ml and 63.8µg/ml respectively). Cord blood cholesterol was highest among those with PGDM (2.2mmol/l), as was cord blood triglyceride (0.6mmol/l) and non-HDLc (1.4mmol/l). The concentration of cord blood analytes according to diabetic status is displayed in Figure 4.6.

Figure 4.6: Concentration of cord blood analytes according to diabetic status

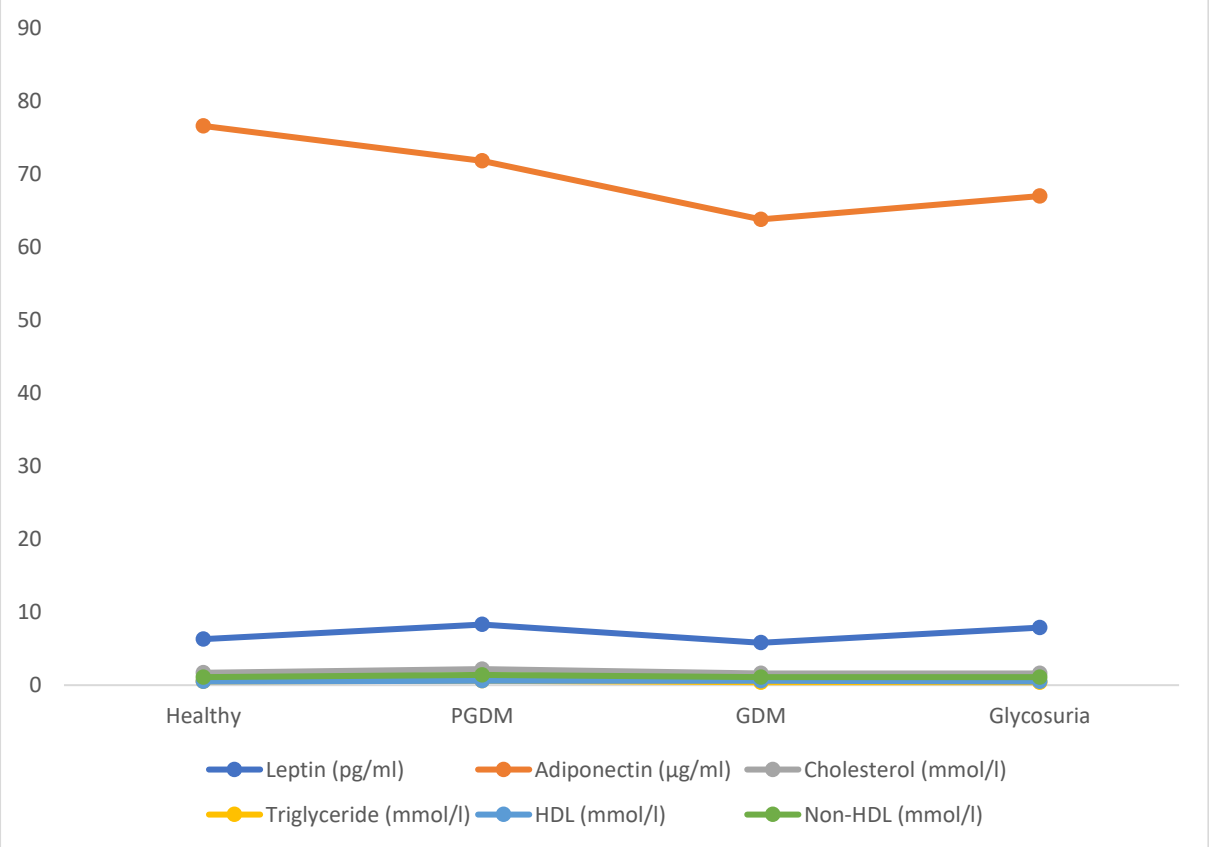


Table 4.2 shows the multivariable associations between maternal diabetic status (compared to the healthy reference group) and cord blood adipokines, lipids, and birthweight. PGDM was associated with cord blood cholesterol (0.31, 95% CI 0.07, 0.54, p=0.011), triglyceride (0.28, 95% CI 0.02, 0.54, p=0.036) and non-HDLc (0.34 95% CI 0.10, 0.61, p=0.009) after adjusting for potential confounding and mediation. PGDM was also associated with lower birthweight although the confidence interval included the null and was mediated by gestational age. GDM was associated with lower cord blood adiponectin in the simplest model but this was attenuated to the null when maternal co-variables were considered. GDM was associated with higher offspring birthweight (0.10, 95% CI 0.03, 0.16, p= 0.004) in the fully adjusted analysis, but not with any other variable. Glycosuria was associated with lower cord blood cholesterol (-0.09, 95% CI -0.17, -0.00, p=0.040) and triglyceride (-0.11, 95% CI -0.208, -0.02, p=0.021) and higher offspring birthweight (0.03, 95% CI 0.00, 0.05, p=0.031).

Table 4.2: Multivariable models for the association between maternal diabetes/glycosuria status and offspring cardio-metabolic risk factors at birth compared to the healthy reference group (0)

			PGDM		GDM		Glycosuria	
Outcome		N	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p
Cord blood	Model 1	4258	0.09 (-0.40, 0.58)	0.716	0.22 (-0.16, 0.60)	0.262	0.26 (0.11, 0.41)	0.001
leptin	Model 2	3097	-0.15 (-0.65, 0.35)	0.557	0.17 (-0.29, 0.64)	0.472	0.15 (-0.03, 0.33)	0.107
(pg/ml)	Model 3	3060	0.08 (-0.09, 0.25)	0.359	0.06 (-0.38, 0.50)	0.789	0.089 (-0.09, 0.25)	0.359
Cord blood	Model 1	4218	-0.19 (-0.48, 0.10)	0.194	-0.26 (-0.49, -0.03)	0.027	-0.05 (-0.14, 0.04)	0.292
adiponectin	Model 2	3069	-0.18 (-0.49, 0.12)	0.236	-0.18 (-0.47, 0.10)	0.206	-0.06 (-0.17, 0.05)	0.318
(µg/ml)	Model 3	3031	-0.08 (-0.39, 0.22)	0.589	-0.18 (-0.46, 0.10)	0.202	-0.05 (-0.16, 0.06)	0.414
Cord blood	Model 1	4203	0.25 (0.03, 0.05)	0.024	-0.02 (-0.19, 0.14)	0.789	-0.06 (-0.13, 0.00)	0.061
cholesterol	Model 2	3057	0.29 (0.05, 0.52)	0.016	-0.03 (-0.24, 0.18)	0.767	-0.07 (-0.15, 0.01)	0.079
(mmol/l)	Model 3	3019	0.31 (0.07, 0.54)	0.011	-0.02 (-0.22, 0.19)	0.886	-0.09 (-0.17, 0.00)	0.040
Cord blood	Model 1	4188	0.13 (-0.13, 0.39)	0.324	-0.11 (-0.30, 0.09)	0.294	-0.08 (-0.15, 0.00)	0.049
triglyceride	Model 2	3045	0.07 (-0.20, 0.35)	0.595	-0.01 (-0.25, 0.23)	0.945	-0.11 (-0.21, -0.02)	0.019
(mmol/l)	Model 3	3007	0.28 (0.02, 0.54)	0.036	0.09 (-0.14, 0.33)	0.428	-0.11 (-0.20, -0.02)	0.021
Cord blood	Model 1	4124	0.23 (-0.04, 0.50)	0.088	0.03 (-0.18, 0.23)	0.780	-0.06 (-0.14, 0.03)	0.175
HDLc	Model 2	2993	0.28 (0.00, 0.56)	0.053	-0.11 (-0.36, 0.14)	0.399	0.08 (-0.174 0.03)	0.142
(mmol/l)	Model 3	2955	0.20 (0.09, 0.48)	0.179	-0.16 (-0.41, 0.10)	0.224	-0.10 (-0.20, 0.00)	0.060
Cord blood	Model 1	4124	0.27 (0.02, 0.51)	0.033	-0.06 (-0.24, 0.13)	0.535	-0.06 (-0.13, 0.02)	0.135
non-HDLc	Model 2	2993	0.29 (0.03, 0.55)	0.029	-0.01 (-0.25, 0.22)	0.906	-0.070 (-0.16, 0.02)	0.143
(mmol/l)	Model 3	2955	0.34 (0.09, 0.61)	0.009	0.03 (-0.21, 0.26)	0.826	-0.08 (-0.17, 0.01)	0.096
Birthweight †	Model 1	4221	-0.10 (-0.19, -0.02)	0.015	0.06 (0.00, 0.13)	0.059	0.04 (0.01, 0.06)	0.008
(g)	Model 2	3072	-0.11 (-0.19, -0.03)	0.007	0.06 (-0.02, 0.14)	0.121	0.02 (-0.01, 0.05)	0.171
	Model 3	3061	0.01 (-0.06, 0.09)	0.695	0.10 (0.03, 0.16)	0.004	0.03 (0.00, 0.05)	0.031

Models 1: adjusted for offspring sex, 2: additionally adjusted for maternal age, social class, smoking, parity, and BMI, 3: additionally adjusted for birthweight, mode of delivery, and gestational age †Birthweight not included as a confounder in models 2+3 where birthweight is the outcome

Table 4.3 shows the multivariable association of PPW (per 1kg) and GWG (per 1kg) and cord blood adipokines, lipids, and birthweight. Assessment of PPW in the PGDM group and cord blood adipokines, lipids, and birthweight were limited due to the small sample size, where 7 to 8 out of the 11 women with PGDM had complete confounders available. For the remaining groups, there were complete confounder sets available for 2599 to 3167 out of 3480 in the healthy group, 15 out of 18 of those with GDM, and 88 to 110 out of 120 women with glycosuria. PPW was weakly positively associated with cord blood leptin (0.01, 95% CI 0.00, 0.01, p=0.000) in healthy women and weakly negatively associated with cord blood leptin in the glycosuria group (0.01, 95% CI 0.00, 0.02, p=0.016). PPW was also weakly negatively associated with cord blood HDLc (-0.01, 95% CI -0.02, 0.01, p=0.000) in the glycosuria group. PPW was not associated with cord blood adipokines, lipids, or birthweight across diabetic groups.

GWG was weakly positively associated with cord blood leptin and triglyceride in the healthy groups (0.01, 95% CI 0.01, 0.02, p=0.00) but there was not any association identified between GWG and cord blood leptin, adiponectin, cholesterol, HDLc, or non-HDLc in any of the diabetic groups. GWG was however weakly positively associated with birthweight in the offspring of healthy mothers (0.01, 95% CI 0.01, 0.01, p=0.000), and in those with GDM (0.01, 95% CI 0.00, 0.03, p=0.035) and glycosuria (0.01, 95% CI 0.00, 0.01, p=0.000).

Table 4.3: Association between pre-pregnancy weight (PPW) (per 1kg) and GWG (per 1kg) and cord blood measures and birthweight according to diabetic group

			Model 1			Model 2			Model 3		
Outcome			N	Coefficient (95% CI)	p	N	Coefficient (95% CI)	p	N	Coefficient (95% CI)	p
Cord blood	Healthy	PPW	3325	0.01 (0.01, 0.01)	0.000	3296	0.01 (0.00, 0.01)	0.000	3167	0.01 (0.00, 0.01)	0.000
leptin		GWG	347	0.03 (0.02, 0.03)	0.000	3446	0.01 (0.00, 0.02)	0.007	3314	0.01 (0.01, 0.02)	0.000
(pg/ml)	PGDM	PPW	8	0.01(-0.06, 0.08)	0.823	8	0.02 (-0.09, 0.12)	0.640			
		GWG	11	0.07 (-0.10, 0.24)	0.364	11	0.06 (-0.15, 0.27)	0.533	11	0.02 (-0.95, 1.00)	0.925
	GDM	PPW	15	0.02 (-0.06, 0.10)	0.551	15	0.01 (-0.06, 0.08)	0.726	15	0.01 (-0.19, 0.21)	0.902
		GWG	18	0.09 (-0.01, 0.19)	0.062	18	0.01 (-0.10, 0.11)	0.877	18	-0.03 (-0.20, 0.40)	0.684
	Glycosuria	PPW	113	0.02 (0.01, 0.03)	0.001	112	0.01 (0.00, 0.02)	0.026	110	0.01 (0.00, 0.02)	0.016
		GWG	120	0.02 (-0.01, 0.06)	0.177	119	-0.01 (-0.05, 0.02)	0.403	117	-0.00 (-0.04, 0.03)	0.916
Cord blood	Healthy	PPW	3295	0.00 (0.00, 0.00)	0.265	3265	0.00 (0.00, 0.00)	0.027	3137	0.00 (-0.01, 0.00)	0.072
adiponectin		GWG	3447	0.00 (0.00, 0.01)	0.045	3415	0.00 (0.00, 0.00)	0.831	3284	-0.01 (-0.01, 0.00)	0.765
(µg/ml)	PGDM	PPW	8	-0.02 (-0.09, 0.05)	0.570	8	-0.03 (-0.12, 0.06)	0.336			
		GWG	11	0.01 (-0.10, 0.13)	0.789	11	0.01 (-0.15, 0.17)	0.858	11	0.06 (-0.71, 0.82)	0.786
	GDM	PPW	15	-0.01 (-0.05, 0.03)	0.699	15	-0.02 (-0.06, 0.02)	0.373	15	0.03 (-0.05, 0.10)	0.374
		GWG	18	0.04 (-0.01, 0.09)	0.094	18	0.03 (-0.01, 0.02)	0.589	18	0.06 (-0.03, 0.15)	0.145
	Glycosuria	PPW	111	0.01 (-0.01, 0.00)	0.615	110	0.00 (-0.01, 0.01)	0.851	108	0.00 (-0.01, 0.01)	0.906
		GWG	117	0.00 (-0.02, 0.01)	0.684	116	0.01 (0.00, 0.02)	0.113	114	0.00 (0.00, 0.00)	0.377
Cord blood	Healthy	PPW	3284	0.00 (0.00, 0.00)	0.654	3254	0.00 (0.00, 0.00)	0.597	3125	0.00 (0.00, 0.00)	0.761
cholesterol		GWG	3436	0.00 (0.00, 0.00)	0.427	3404	0.00 (0.00, 0.00)	0.388	3272	0.00 (0.00, 0.01)	0.378
(mmol/l)	PGDM	PPW	7	0.00 (-0.13, 0.17)	0.925	7	0.06 (-0.59, 0.70)	0.743			
		GWG	10	-0.06 (-0.17, 0.04)	0.198	10	-0.07 (-0.21, 0.08)	0.275	10	-0.14 (-1.28, 1.00)	0.353
	GDM	PPW	15	0.01 (-0.02, 0.03)	0.640	15	0.01 (-0.02, 0.04)	0.618	15	-0.02 (-0.07, -0.03)	0.260
		GWG	18	-0.02 (-0.05, 0.02)	0.381	18	-0.01 (-0.06, 0.04)	0.712	18	-0.01 (-0.07, 0.06)	0.856
	Glycosuria	PPW	112	0.00 (-0.01, 0.00)	0.377	111	0.00 (-0.01, 0.00)	0.179	109	0.00 (-0.01, 0.00)	0.208
		GWG	118	0.01 (0.00, 0.03)	0.023	117	0.01 (0.00, 0.02)	0.113	91	0.01 (-0.01, 0.03)	0.353
Cord blood	Healthy	PPW	3271	0.00 (0.00, 0.00)	0.023	3241	0.00 (0.00, 0.00)	0.000	3112	0.00 (0.00, 0.01)	0.000
triglyceride		GWG	3422	0.01 (0.00, 0.01)	0.001	3390	0.01 (0.00, 0.01)	0.000	3258	0.01 (0.00, 0.01)	0.001
(mmol/l)	PGDM	PPW	7	-0.02 (-0.13, 0.08)	0.551	7	0.09 (-0.34, 0.52)	0.464			
		GWG	10	-0.04 (-0.15, 0.06)	0.353	10	-0.04 (-0.17, 0.09)	0.435	10	-0.07 (-1.43, 1.29)	0.627
	GDM	PPW	15	0.02 (-0.02, 0.05)	0.338	15	0.02 (-0.02, 0.06)	0.294	15	0.00 (-0.09, 0.09)	0.970

Outcome			Model 1 N	Model 1 Coefficient (95% CI)	Model 1 p	Model 2 N	Model 2 Coefficient (95% CI)	Model 2 p	Model 3 N	Model 3 Coefficient (95% CI)	Model 3 p
		GWG	18	0.00 (-0.05, 0.05)	0.946	18	0.02 (-0.05, 0.08)	0.617	18	0.02 (-0.05, 0.09)	0.589
	Glycosuria	PPW	112	0.01 (0.00, 0.01)	0.032	111	0.01 (0.00, 0.01)	0.002	109	0.01 (0.00, 0.01)	0.005
		GWG	118	-0.01 (-0.02, 0.01)	0.394	117	-0.01 (-0.03, 0.01)	0.194	115	-0.01 (-0.02, 0.01)	0.546
Cord blood	Healthy	PPW	3213	0.00 (0.00, 0.00)	0.361	3183	0.00 (0.00, 0.00)	0.025	3056	0.00 (0.00, 0.00)	0.005
HDLc		GWG	3365	0.00 (-0.00, 0.00)	0.908	3333	0.00 (-0.01, 0.00)	0.382	3203	0.00 (-0.01, 0.00)	0.471
(mmol/l)	PGDM	PPW	7	0.01 (-0.07, 0.08)	0.841	7	-0.02 (-0.39, 0.36)	0.872			
		GWG	10	-0.05 (-0.11, 0.02)	0.124	10	-0.06 (-0.13, 0.01)	0.082	10	-0.12 (-0.28, 0.05)	0.072
	GDM	PPW	15	-0.01 (-0.04, 0.02)	0.660	15	-0.01 (-0.04, 0.03)	0.715	15	-0.03 (-0.12, 0.05)	0.390
		GWG	18	-0.01 (-0.05, 0.03)	0.611	18	-0.01 (-0.06, 0.05)	0.834	18	-0.02 (-0.11, 0.07)	0.660
	Glycosuria	PPW	109	-0.01 (-0.02, -0.01)	0.000	108	-0.01 (-0.02, -0.01)	0.000	106	-0.01 (-0.02, 0.01)	0.000
		GWG	115	0.02 (-0.01, 0.04)	0.180	114	0.01 (-0.01, 0.04)	0.301	112	0.00 (-0.03, 0.02)	0.807
Cord blood	Healthy	PPW	3213	0.00 (0.00, 0.00)	0.342	3183	0.00 (0.00, 0.00)	0.095	3056	0.00 (0.00, 0.00)	0.119
non-HDLc		GWG	3365	0.00 (0.00, 0.01)	0.277	3333	0.00 (0.00, 0.01)	0.127	3164	0.00 (0.00, 0.01)	0.113
(mmol/l)	PGDM	PPW	7	-0.01 (-0.15, 0.13)	0.901	7	0.08 (-0.63, 0.79)	0.664			
		GWG	10	-0.07 (-0.19, 0.05)	0.229	10	-0.07 (-0.23, 0.10)	0.336	10	-0.15 (-1.57, 1.28)	0.414
	GDM	PPW	15	0.01 (-0.02, 0.04)	0.549	15	0.01 (-0.02, 0.04)	0.521	15	-0.02 (-0.09, -0.05)	0.490
		GWG	18	-0.01 (-0.05, 0.03)	0.490	18	-0.01 (-0.06, 0.05)	0.831	18	0.01 (-0.06, 0.07)	0.854
	Glycosuria	PPW	109	0.00 -0.00, 0.01)	0.862	108	0.00 (0.00, 0.00)	0.974	106	0.00 (-0.01, 0.01)	0.956
		GWG	115	0.01 (0.00, 0.02)	0.177	114	0.01 (-0.01, 0.02)	0.456	112	0.00 (-0.01, 0.02)	0.621
Birthweight †	Healthy	PPW	3297	0.00 (0.00, 0.00)	0.000	3297	0.00 (0.02, 0.00)	0.000	2599	0.00 (0.00, 0.00)	0.000
(g)		GWG	3448	0.01 (0.01, 0.01)	0.000	3448	0.01 (0.01, 0.01)	0.000	3316	0.01 (0.01, 0.01)	0.000
	PGDM	PPW	8	0.01 (-0.01, 0.02)	0.416	8	0.01 (-0.01, 0.02)	0.420			
		GWG	11	0.02 (-0.02, 0.06)	0.280	11	0.02 (-0.01, 0.04)	0.244	11	0.03 (-0.04, 0.09)	0.302
	GDM	PPW	15	0.00 (-0.01, 0.01)	0.299	15	0.00 (-0.01, 0.01)	0.493	15	0.00 (-0.01, 0.01)	0.284
		GWG	18	0.01 (0.00, 0.03)	0.040	18	0.01 (0.00, 0.02)	0.010	18	0.01 (0.00, 0.03)	0.035
	Glycosuria	PPW	112	0.00 (0.00, 0.00)	0.002	112	0.00 (0.00, 0.00)	0.002	88	0.00 (0.00, 0.01)	0.017
		GWG	119	0.01 (0.00, 0.01)	0.002	119	0.01 (0.00, 0.01)	0.001	117	0.01 (0.00, 0.01)	0.000

Models 1: adjusted for offspring sex, 2: additionally adjusted for offspring sex, birthweight and gestational age at birth, 3: additionally adjusted for maternal age, parity, smoking status and BMI (for GWG), and maternal height (for pre-pregnancy weight). †Birthweight not included as a confounder in models 2+3 where birthweight is the outcome

4.4 Discussion

This Chapter examined the impact of fuel-mediated teratogenesis, as displayed in diabetic pregnancy, on mothers and their offspring in a mainly Caucasian population. Maternal, pregnancy, and offspring characteristics of each diabetic group (mothers with PGDM, GDM, and glycosuria) have been described, and the association between maternal PPW and GWG, previously described indiscriminately in Chapter 3, on cord blood leptin, adiponectin, lipids and birthweight have also been examined.

Mothers with PGDM had higher pre-pregnancy BMI than any other group, were delivered earlier, and had the highest caesarean section rate. Offspring of mothers with PGDM had the lowest birthweight and displayed higher cord blood leptin, cholesterol, triglyceride, and non-HDLc and lower adiponectin than any other group, although the association was only significantly positive for cord blood cholesterol, triglyceride, and non-HDLc.

Mothers with GDM had a lower BMI at booking, and many were smokers prior to but not during pregnancy. Overall, they had lower GWG and lower caesarean section rates than those with PGDM. GDM and GWG within this group were positively associated with offspring birthweight, even though birthweight was a similar normal weight to the offspring of healthy mothers. Mothers with GDM displayed the lowest levels of cord blood leptin and adiponectin compared to any other group, but this was subject to attenuation and mediation by covariables, including offspring birthweight.

Amongst mothers with glycosuria during pregnancy, GWG and offspring birthweight were the highest, and yet the caesarean section rate was lowest. Glycosuria was associated with lower cord blood cholesterol, triglyceride, and higher birthweight.

PPW, in those that developed glycosuria, was weakly negatively associated with cord blood leptin and HDLc.

Offspring birthweight in the GDM group was unexpected and of similar 'normal' value to the healthy group, and the group with the highest offspring birthweight was among those with glycosuria. This may partly be explained by lower pre-pregnancy BMI (which itself is independently associated with lower offspring birthweight) amongst those with GDM and greater intervention following the diagnosis of GDM such as dietician input, lifestyle modification, or pharmaceutical intervention (consequently limiting GWG, as demonstrated previously (Retnakaran *et al.*, 2013)). Treatment of GDM has demonstrated a reduction in offspring birthweight and neonatal fat mass, even in the presence of mild hyperglycaemia (Landon *et al.*, 2009). GWG was lowest among women with GDM, which may have limited fetal growth, and fewer women with GDM gained weight excessively than any other group which is in contrast to previous reports (Gante *et al.*, 2015). The lower birthweight among offspring of GDM mothers may also be explained by the fact that these offspring were delivered 1 week earlier than the 'healthy' or the glycosuria offspring (further limiting maternal opportunity for GWG and limiting fetal accruing of fat). As more women smoked prior to, but not during pregnancy, this may have instead led to higher GWG amongst this group of GDM mothers due to compensatory eating habits however this was not the case but may explain the lower pre-pregnancy BMI in this group.

In contrast, those with glycosuria had a higher baseline BMI than those with GDM (already predisposing the offspring to higher birthweight), and as they were not 'diabetic' they were not offered any intervention or diabetes management (and therefore were unlikely to have adjusted their dietary or lifestyle habits). This group gained more weight overall during pregnancy partly because of the longer duration of pregnancy and lack of lifestyle changes (and one-third of women exceeded the recommendations on GWG, more than any other group), so there was more opportunity for the offspring to gain more weight and consequently, mothers with

glycosuria had offspring with higher birthweight. In addition, there were more male offspring amongst women with GDM than any other diabetic or non-diabetic category, and whilst this did not reach significance, may additionally explain the unexpectedly 'normal' birthweight in offspring of GDM mothers. Treatment of GDM in mothers carrying male offspring has been shown to yield lower offspring birthweight and lower neonatal fat mass that was only significant amongst male offspring (Bahado-Singh *et al.*, 2012). This may be because GDM is characterised by increased oxidative stress, which in turn is also associated with lipogenesis (Sekiya *et al.*, 2008), male offspring are more susceptible to the insults of oxidative stress and therefore may be more responsive to improved glycaemic control in pregnancy (Brandes and Mügge, 1997; Malorni *et al.*, 2008; Bahado-Singh *et al.*, 2012).

The pathophysiology of GDM involves many complex maternal, fetal, and placental factors that are interrelated and lead to fetal insulin resistance. Firstly, the fetal placental unit plays an important role in the development of GDM. With advancing gestation and the growing placenta, there is a greater release of the hormones oestrogen, progesterone, cortisol, and human placental lactogen (HPL) (which is ten times higher in pregnancy) into the maternal circulation (Ryan and Enns, 1988). HPL is an insulin antagonist in pregnancy and acts by increasing lipolysis and promoting free fatty acid transmission to the mother in order to preserve glucose and amino acid for the developing fetus (Buchanan and Xiang, 2005). With this, insulin resistance ensues from 20-24 weeks' gestation. The fact that once delivery has occurred, the placental hormone production stops and GDM resolves in most cases, suggests that these placental hormones are responsible for the progressive insulin resistance in GDM pregnancy (Buchanan and Xiang, 2005).

Secondly, adipose tissue also plays an additional important role in the development of GDM. As discussed in Chapter 1, adipose tissue produces adipokines, including leptin, adiponectin, and TNF-α, which have been implicated in developing insulin resistance in pregnancy (Briana and Malamitsi-Puchner, 2009). Maternal obesity or high GWG may enhance the production of adipokines as a result of the greater

abundance of adipose tissue, thus predisposing women to the condition. High first-trimester GWG, particularly amongst normal-weight women, has been strongly associated with the development of GDM in later pregnancy (Chu *et al.*, 2007). The resulting offspring of diabetic mothers display a unique pattern of fetal overgrowth with the accrual of fat centrally leading to wider shoulders and increased abdominal circumference, hence the greater risk of birth injury.

An early manifestation of offspring exposed to hyperglycaemia, as a result of maternal GDM, is macrosomia. Accelerated growth patterns may be detected in GDM pregnancy, and may even pre-date the diagnosis of the disease (Sovio, Murphy and Smith, 2016). There is at least double the risk of offspring macrosomia in the presence of GDM, although this is even higher particularly in the presence of maternal obesity (which itself is an independent risk factor for higher birthweight) (A. Lawlor *et al.*, 2010; Catalano *et al.*, 2012). Maternal obesity may itself be a stronger predictor of large for gestational age babies than hyperglycaemia, as demonstrated in Chapter 3, where BMI and GWG were positively associated with offspring birthweight. HAPO has previously reported that obesity alone doubles the risk of macrosomia in the presence or absence of GDM (Metzger *et al.*, 2008). The limited number of women included in the current study with GDM, who were relatively lean, may have only had a very mild manifestation of the disease and could also account for the lower birthweight amongst this group and lack of associations evident in this study. In addition, the prevalence of GDM may be inaccurate due to screening methods.

A later manifestation of offspring exposed to GDM is demonstrated in a Pima Indian population in Arizona. Mothers, who either had Type 2 diabetes or GDM in pregnancy, had offspring that were LGA and heavier at 5 years of age compared to offspring of non-diabetic mothers (Pettitt *et al.*, 2010) (Pettitt *et al.*, 1993). In addition, the EPOCH study showed that exposure to GDM was associated with higher offspring BMI (and greater BMI growth trajectory), waist circumference, visceral and subcutaneous fat amongst 6 to 13-year-olds in a multi-ethnic community (Crume *et al.*, 2011).

ALSPAC has previously reported higher offspring BMI and fat mass in the 9 to 11-year old (A. Lawlor *et al.*, 2010) and 15-year old (Patel *et al.*, 2012) offspring of mothers with diabetes or glycosuria (a marker of hyperglycaemia). Reporting similar strength of association at each age group suggested that intrauterine exposure to diabetes may induce long-lasting influence on adiposity on the offspring. The fact that an inverse relationship between GDM and birthweight has been identified suggests that GDM itself does not influence offspring anthropometry at birth and that perhaps the associations with adiposity become more apparent in later life. This is unexpected however as higher offspring birthweight is more prevalent in diabetic groups and may simply reflect the leaner cohort with limited GWG, as well as the impact of a diabetes treatment programme in pregnancy, thus lowering the offspring birthweight in this current cohort. The fact that there was a positive association between glycosuria and birthweight suggests that in untreated hyperglycaemia, which is under the threshold for diagnosis GDM, may still exhibit causal programming of the offspring in utero, particularly as birthweight was highest among this group. More recently, the Growing Up in Scotland (GUS) Birth Cohort has demonstrated greater risk of obesity at age 4 and 6 years amongst offspring of GDM mothers (Abraham *et al.*, 2015), emphasising the impact GDM has on childhood offspring anthropometry. The association between maternal glucose and offspring adiposity however is not linear. HAPO has previously demonstrated that whilst maternal glucose positively correlated with offspring birthweight and cord blood c-peptide, they did not report any association between maternal glucose and offspring BMI z-score at age 2 (Thaware *et al.*, 2015). In addition, they did not identify any association between mild, untreated maternal hyperglycemia (falling short of the threshold for GDM) and offspring adiposity at age 5 to 7 (Pettitt *et al.*, 2010).

Maternal diabetes is associated with higher caesarean section delivery and this has been demonstrated in the current study, particularly amongst mothers with PGDM, even though they had a lower birth weight and earlier gestational at delivery than any other group. Anticipated large for gestational age infants detected at antenatal scan may promote this decision for caesarean delivery. Antenatal scanning, particularly amongst obese women, however, is inaccurate so could lead to

unnecessary earlier or surgical intervention, particularly amongst diabetic women (Hall, 1996). This may be either electively, in order to minimise the birth-related injury to the mother and baby of vaginal birth, or due to the unstable or malpresentation that can occur in fetal macrosomia and polyhydramnios, as displayed in uncontrolled diabetes. Similarly, an emergency caesarean section in the diabetic group may be higher due to failed induction of labour, perhaps due to iatrogenic preterm intervention (and PGDM group was delivered earlier than other groups) or failure to progress or poor descent in labour as a result of greater offspring birthweight.

There is growing evidence demonstrating this transgenerational transmission of GDM and gene modification and epigenetic changes may account for this, although the exact mechanisms are unclear. The abnormal metabolic environment offspring are exposed to in utero impacts fetal development, by inducing changes in gene expression by epigenetic mechanisms of susceptible cells. This may lead to the development of diabetes in later life or GDM in a future pregnancy. This is a vicious cycle as the next generation of offspring are then exposed to this abnormal metabolic environment and adopt further epigenetic changes making them more prone to metabolic disease in later life. Decreased gene methylation may be responsible for these epigenetic modifications, and has previously been demonstrated in the placenta and cord blood of GDM pregnancy (El Hajj *et al.*, 2013). Epigenetic changes to adipokines have also been reported in GDM pregnancy. Specifically, lower placental adiponectin and leptin gene expression has been demonstrated in the presence of maternal insulin resistance (Bouchard *et al.*, 2010, 2012). The increased fetal need for insulin, in conjunction with exposure to hyperglycaemia in GDM pregnancy, maybe the trigger for these epigenetic changes in early pregnancy and lead to disruption in glucose and insulin regulation.

There is limited evidence of cord blood lipids being examined in relation to maternal diabetic status. In addition, the influence of GDM on offspring lipids is difficult to interpret as normal pregnancy is characterised by hypertriglyceridemia, as a result

of altered lipid transport and metabolism (Eslamian *et al.*, 2013). Early lipogenesis followed by lipolysis with advancing gestation occurs in healthy pregnancy but there is more marked dyslipidaemia in obese pregnancy. This is thought to result from enhanced insulin resistance in adipose tissue and reduced suppression of lipolysis in order to support fetal growth as well as meet the maternal energy demands for labour and the postpartum period (Catalano *et al.*, 2002). Recently, NMR has been employed to examine the maternal metabolome in pregnancy and from mid-pregnancy, before the diagnosis of GDM, obese pregnant women display a markedly different metabolic profile to non-obese pregnant women (White *et al.*, 2017). Placental lipoprotein lipase (LPL) DNA methylation dysregulation has been reported in GDM pregnancy and has been implicated in the translation of cardio-metabolic ill-health onto the fetus (Houde *et al.*, 2014). The impact of GDM on the lipid profile is not clear however and whilst aortic fatty streaks have been demonstrated in the fetal offspring of mothers with hypercholesterolaemia (Napoli *et al.*, 1997), intervention studies suggest that offspring exposed to GDM were not at any greater cardiovascular risk (Retnakaran *et al.*, 2013). Dube et al. examined maternal and cord blood lipids at birth in normal and overweight mothers with GDM (Dubé, Ethier-Chiasson and Lafond, 2013). They found that normal-weight mothers with GDM had higher levels of triglycerides and lower levels of HDLc compared to overweight mothers with GDM, who had lower levels of cholesterol and LDLc. The offspring at birth however displayed higher free fatty acids with no other sign of disruption to their lipid profile (Dubé, Ethier-Chiasson and Lafond, 2013). Eslamian et al. did not show any association between GDM and cord blood cholesterol, HDLc, or triglycerides, although they did show lower LDLc in the cord blood of mothers with GDM (Eslamian *et al.*, 2013). ALSPAC has also previously examined the association between maternal diabetes and adolescent offspring lipids but found no correlation, despite showing elevated insulin and glucose levels (Patel *et al.*, 2012). This is reflected in the current study, whereby no association between GDM and cord blood lipids was identified, highlighting the limited impact GDM may have on the offspring's metabolic profile at birth.

More variability amongst cord blood cholesterol and triglycerides were however demonstrated in the PGDM group. This may be because they had a more atherogenic lipid profile before pregnancy because of their pre-existing chronic disease or because maternal BMI was higher in this group and any GWG may have exaggerated the dyslipidaemia during pregnancy. In addition, this may also be because higher cord serum triglycerides are independently associated with small for gestational age babies (Hou *et al.*, 2014), or babies born at an earlier gestation, as seen more frequently amongst the PGDM group. Conversely, the glycosuria group displayed a weak inverse association with cord blood cholesterol and triglyceride perhaps reflecting the limited cardio-metabolic dysfunction relayed in this group compared to those with PGDM which inherently pose a greater metabolic risk to the offspring. This highlights the role of greater pre-pregnancy maternal adiposity on a background of chronic metabolic disease, such as in PGDM, rather than any de novo condition such as GDM, in disrupting lipid metabolism and placenta transfer to the offspring. This is further supported by intervention studies, using glucose-lowering treatment and dietary changes in GDM pregnancies, which showed that the offspring cardiovascular risk at 1 year old is equivocal to offspring of mothers without GDM (Retnakaran *et al.*, 2013). It remains unclear however whether this equivocal offspring cardio-metabolic risk to offspring risk from a healthy mother is due to moderation of maternal lifestyle or pharmacological input following a diagnosis of GDM, for example. It also questions whether the association between GDM and offspring cardio-metabolic dysfunction truly exists. Despite this, a positive relationship between maternal glucose and BMI z-score in infancy remains, indicating that intervention and appropriate management of GDM may not prevent adverse development of the cardio-metabolic profile entirely (Retnakaran *et al.*, 2013). In addition, even when maternal glycemia does not fulfil the criteria for GDM, certain studies have still identified an association between maternal hyperglycaemia and childhood offspring cardio-metabolic dysfunction (Ehrlich *et al.*, 2012).

Exposure to maternal hyperglycaemia in GDM pregnancy may also alter the control of adipokines, which are additionally subject to epigenetic modification (Retnakaran *et al.*, 2013). A limited association between maternal diabetes in pregnancy and

cord blood adipokines was demonstrated, even though cord blood leptin was higher in PGDM and lower in GDM and cord blood adiponectin was lower in both diabetic groups, suggesting that they are subject to confounding and mediation by co-variables. Reports of maternal leptin in GDM have also been inconsistent, although most report an increase in pregnancy (Kautzky-Willer *et al.*, 2001; Chen *et al.*, 2010). GDM is characterised by a pro-inflammatory environment and associated enhanced placental production of leptin has been reported in some studies (Lepercq *et al.*, 1998; Lea *et al.*, 2000). Leptin is insulin sensitising and regulates insulin secretion, the utilisation of glucose and glycogen synthesis as well as regulates the secretion of gonadotrophin-releasing hormone (GnRH) from the hypothalamus. High maternal plasma leptin at 13 weeks' gestation has been associated with over four times increased risk of GDM with advancing pregnancy (Qiu *et al.*, 2004). Similarly, leptin measured in amniotic fluid at 15-17 weeks has been directly associated with insulin and a greater risk of developing GDM (D'Anna *et al.*, 2007). Other studies have reported a reduction (Festa *et al.*, 1999) or equivocal (Simmons and Breier, 2002) maternal leptin level in GDM pregnancy but differing severity of disease or ethnicity may have accounted for these findings. Overall it is likely that, since leptin increases in normal pregnancy, GDM pregnancy may aggravate this. As leptin itself is also pro-inflammatory, higher levels in GDM pregnancy have the potential to further enhance the inflammatory environment that the fetus is exposed to. In addition, as chronic insulin administration increases leptin secretion by adipocytes, (D'Anna *et al.*, 2007), hyperinsulinemia in GDM pregnancy may further enhance leptin production, perpetuating the cycle. The fact that leptin levels were lowest amongst the GDM group suggests that temporary exposure to the condition may not have any lasting impact on the fetus, but it may also be accounted for if the severity of the condition or treatments offered were acknowledged. In contrast, cord blood leptin was highest among women with PGDM, suggesting that longer-term exposure to maternal hyperglycaemia may increase the production of leptin and deliver greater risk to the offspring. These findings were however subject to confounding and mediation and therefore GDM may not account for these associations alone.

In contrast, adiponectin is insulin sensitising and anti-inflammatory, decreasing hepatic glucose production and stimulating the uptake of glucose in skeletal muscle. The oxidative stress of normal pregnancy may be associated with the downregulation of adiponectin mRNA in the placenta and subsequent lower adiponectin levels with advancing gestation, and this may be even more apparent in diabetic pregnancy (Retnakaran *et al.*, 2007; Fasshauer, Blüher and Stumvoll, 2014). As previously discussed, low adiponectin levels, from as early as the first trimester, may be used to predict GDM in later pregnancy (Lain *et al.*, 2008; Iliodromiti *et al.*, 2016). Offspring of GDM mothers also display lower adiponectin levels compared to offspring of non-diabetic mothers, even after adjustment for gestational age and birthweight (Lindsay *et al.*, 2003; Cortelazzi *et al.*, 2007). It is therefore clear that the low-grade inflammatory environment in pregnancy and GDM, as previously described, has a negative regulatory effect on adiponectin gene expression and as adiponectin is insulin sensitising, decreased levels may further aggravate insulin resistance. Furthermore, as insulin suppresses adiponectin production, this perpetuates this cycle of inflammation and poor metabolic environment that the offspring of a hyperinsulinaemic pregnancy is exposed to in utero (Fasshauer, Blüher and Stumvoll, 2014). Retnakaran et al. however found no clear association between GDM and offspring adiponectin in infancy. Despite this, they did demonstrate reduced adiponectin levels in diabetic mothers and that the correlation between adiponectin in mother-offspring pairs only existed in the non-diabetic group (Retnakaran *et al.*, 2013). This loss of association in GDM mother-offspring pairs indicates that the offspring's exposure to GDM may alter the protective role of adiponectin, culminating in greater cardiovascular risk in later life (Retnakaran *et al.*, 2013). Whilst the study did demonstrate that cord blood adiponectin levels were lowest amongst those with PGDM and GDM, they were subject to confounding and mediation, so the associations described may not be accounted for by the diabetic disorder alone.

GWG and PPW were examined in relation to maternal diabetes and offspring cardio-metabolic risk as women who develop GDM are more likely to be overweight or obese, and many obese women have been shown to exceed the recommendations

on weight gain, according to the IOM, as discussed previously in Chapter 3. These guidelines were first described for a general obstetric population and not for higher risk groups, such as those with diabetes in pregnancy and it is therefore difficult to advise diabetic women in pregnancy on appropriate GWG. Emerging evidence suggests that GWG less than 5kg should be recommended to women with GDM in order to optimise obstetric and neonatal outcomes (Gante *et al.*, 2015). Although this is not current practice, nor was it at the time of the study, GWG was however lowest amongst mothers with GDM compared to any other group. The fact that there was no association identified between GWG across the entire gestational period and cord blood measures for any of the diabetic groups indicates that weight gain, when examined continuously in diabetic pregnancy, does not appear to adversely affect the cardio-metabolic profile at birth. GWG does appear to influence birthweight, however, although this association was only weakly positive in the GDM and glycosuria group. This adds further support to outcomes identified in Chapter 3, which showed that GWG positively influenced anthropometry at birth and that exaggerated fetal overnutrition, as demonstrated by GWG in GDM pregnancy, may moderate fetal growth. PPW was not associated with offspring adipokines, lipids, or birthweight in either of the diabetic groups but there was little variability in BMI amongst this lean population, with few numbers, and the weak positive association with cord blood leptin in the healthy group may be a chance finding. GWG (and weight gain in the first trimester) and maternal BMI may therefore confer greater offspring risk in a general population, but have less bearing on the cord blood profile at birth amongst diabetic groups.

As discussed in Chapter 3, the size of the cohort and the detailed measures of maternal, pregnancy, and social characteristics added to the strengths of the study. This was also one of the very few studies with detailed recordings of maternal diabetic status, GWG, and a paired with a cord blood sample. Multiple pregnancies were excluded as they are at greater risk of GDM and other obstetric complications, as well as pre-term delivery and lower offspring birthweight. Pre-pregnancy BMI was included as a confounder as it is inversely associated with GWG (Viswanathan *et al.*, 2008). Maternal parity, smoking, and age were also accounted for in the analyses,

allowing adjustment for any potential confounding, as older mothers with higher parity (as well as higher BMI) are at greater risk of GDM whereas smoking may limit GWG. Women included in this study who gained weight excessively were younger, a finding previously reported by others (Egan *et al.*, 2014; Gante *et al.*, 2015), and it has been suggested that older women may be more informed and therefore more likely to comply with guidance on lifestyle in pregnancy (Gante *et al.*, 2015). In addition, stopping smoking prior to or at the onset of pregnancy was more common in the GDM group. The timing of this may lead to excessive dietary intake in pregnancy and thus greater BMI at booking and greater, thereby increasing the overall incidence of GDM amongst these women.

This study was however also subject to limitations. The incidence of GDM is increasingly prevalent in the current population, reflecting the global obesity pandemic. The ALSPAC cohort, however, displayed a much lower incidence of maternal diabetes (<1%) and obesity, compared to a contemporary cohort (5%) (National Institute for Health and Care Excellence, 2015). Participants were screened for GDM based on risk factors alone and the diagnostic criteria for GDM was different from current practice and therefore the prevalence of GDM in this cohort may under-represent the true prevalence of the condition. Contemporary screening practices are based on national guidelines and may have detected more women with GDM than in the current study as the threshold for diagnosis of GDM is lower (National Institute for Health and Care Excellence, 2015). Universal screening of pregnant women however would undoubtedly have detected a greater number of GDM mothers, as demonstrated by another cohort at a similar time (Koukkou *et al.*, 1995). Overall the numbers included in the study with GDM and glycosuria were small, but in keeping with previous ALSPAC reports on maternal diabetes and offspring metabolic risk (A. Lawlor *et al.*, 2010; Patel *et al.*, 2012). The numbers included in the study differed from Chapter 3 as only those with maternal diabetic status recorded were included whereas pre-term deliveries were not excluded which collectively may have contributed to the variability in the results in this chapter. In keeping with previous ALSPAC reports, participants with GDM also gained less weight than recommended in the IOM guidelines (Institute of Medicine and National

Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009), unlike other studies that frequently reported excessive GWG (Carreno *et al.*, 2012). This may account for the lack of associations and it would be beneficial to repeat the study using a more contemporary cohort. Self-reporting of maternal weight may have led to under-representation of BMI within the cohort although it has been highly correlated with weight at the first antenatal visit (Geelhoed *et al.*, 2010). As previously discussed, the last minus the first weight measurement in pregnancy (kg) may be a more accurate reflection of weight gain in pregnancy (Lawlor *et al.*, 2011) and would have avoided contributions from the fetus, placenta, and liquor. However, GWG was easier to classify according to IOM guidance (Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009).

This study included mainly a Caucasian population, and whilst it is not possible to translate findings across other ethnicities with a differing prevalence of maternal obesity and diabetes, it did not necessarily introduce additional bias. A greater prevalence of the disease however may have been identified by examining a more multi-ethnic population. Infants born prematurely (the earliest delivery being 34 weeks) were not excluded, even though they have a greater risk of cardiovascular disease (Morrison *et al.*, 2013). Gestational age however was adjusted for in the analyses and this enabled the number of participants in the study to be maximised. GWG is frequently associated with a greater risk of macrosomia and although the effect on birthweight as an outcome was examined, it may also have been worthwhile to examine the risk ratio for macrosomia, as we had done in Chapter 3. It was not possible to randomise women to gain a certain amount of weight so, like all studies of GWG, we were limited by observational data. Given the exclusion criteria and the number of confounders used in the analyses, selection bias may have been introduced but there is no reason to believe that the subjects excluded would be any different from the cohort used. Examination of PPW in the PGDM group was limited due to the small sample size. The power calculation, to determine the minimal detectable effect size and to minimise any error, suggested there were insufficient numbers with all data available. Although it was possible to perform the

analysis for GWG as there were fewer missing variables in this analysis, repeating this study in a larger cohort of diabetic women may have yielded more reliable and reproducible associations. Whilst several maternal and offspring characteristics were adjusted for; this did not include physical activity or dietary intake (sodium) and there was missing data on maternal social class. This may have influenced the degree of GWG or the development of GDM and contributed to variability within the results. Similarly, the severity of diabetes or how well controlled it was during pregnancy were not accounted for. Popova et al. conducted a similar study of maternal diabetes in pregnancy on cord blood measures and birthweight but categorised subjects according to 1) tight control of diabetes, 2) less tight control of diabetes and 3) no diabetes (control) (Popova *et al.*, 2018). This enabled interpretation of associations identified by the severity of disease which was not possible in the current study.

As discussed previously, variation in the assays may also have occurred partly due to cord blood sample degradation although leptin and adiponectin do appear to be stable with long-term storage (Flower *et al.*, 2000; Shih *et al.*, 2000; Boyanton and Blick, 2002; Gislefoss, Grimsrud and Mørkrid, 2008; Paltiel *et al.*, 2008). Whilst cord blood c-peptide reflects maternal insulin status (Dubé *et al.*, 2012), it was not possible to quantify this measure due to sample degradation associated with long-term storage, as previously reported (Gislefoss, Grimsrud and Mørkrid, 2009).

This study highlights the complex associations between intrauterine exposure to maternal hyperglycaemia, maternal weight status (in particular, PPW weight and early pregnancy GWG), and offspring birthweight and cord blood cardio-metabolic profile. In conclusion, this study has demonstrated that GDM, as a marker of fetal overnutrition, exhibited a limited impact on the offspring's cardio-metabolic health at birth. As GDM generally manifests during the third trimester, there is, therefore, a relatively short timeframe for disrupting lipids and adipokines and therefore the offspring of GDM may not adopt any greater cardiometabolic risk. In contrast, the cord blood of PGDM offspring displayed more variability, including evidence of

dyslipidaemia, suggesting that PGDM may have a wider impact on the offspring's metabolome at birth. The study did however demonstrate that GWG amongst GDM and glycosuria groups positively influenced offspring anthropometry, as measured by birthweight, even though this was not evident amongst those with PGDM. Despite this, mothers with GDM had offspring with birthweight that was still within a normal range and may reflect successful intervention in pregnancy to optimise glucose control. It may also reflect however the lean population within this cohort with low GWG which may limit the final birthweight. Finally, this study emphasises that whilst GDM may influence offspring birthweight, PGDM may impose a significantly more cardiometabolic risk to the offspring at birth.

Chapter 5: Blood pressure in pregnancy: association with cord bloods lipids, adipokines, and birthweight

5.1 Introduction

As the effect of an abnormal intrauterine environment as a result of greater maternal adiposity (in Chapter 3) and diabetes in pregnancy (Chapter 4) has already been examined, this Chapter will focus on the impact of maternal blood pressure (BP) in creating an abnormal intrauterine environment and the impact this has on the offspring's cardiometabolic profile at birth. Encompassing both both GH and PE, HDP may present long term metabolic risk to both the mother and offspring. Affecting 2-8% of women globally, HDP account for 8-10% of pre-term births and 5% of stillbirths (NICE, 2010). Whilst the rate of maternal death as a result of PE continues to fall within the UK (Knight *et al.*, 2014), there is a stark contrast to the global burden of the disease (Duley, 2009; World Health Organization, 2018).

PE is a condition of uncertain aetiology but is thought to be a result of abnormal placentation and abnormal immune response to the placenta leading to hypoxia, increased oxidative stress, and release of inflammatory mediators. It has multisystem effects on the liver, brain, and kidneys (Mustafa *et al.*, 2012) and is characterised by a state of systemic inflammation, endothelial dysfunction, and insulin resistance (Sattar *et al.*, 1997, 2003; Ness and Sibai, 2006; Catarino *et al.*, 2008). Abnormalities of the clotting cascade are well documented in women with PE (Brenner, 2002) and placental insufficiency may ensue as a result of thrombophilia-induced micro and macrovascular thrombosis (Isermann *et al.*, 2003). Abnormal lipid profiles are also characteristic of PE and result in increased oxidative stress. Regulators of lipid metabolism, lipoprotein lipase, and apolipoprotein E are produced by the placenta and have therefore been proposed in the origin of the disease as possible candidate genes (Kim *et al.*, 2001; Descamps *et al.*, 2005). Risk factors for PE include maternal obesity, primiparity, extremes of age, multiple

pregnancy, history of PE or pre-existing hypertensive disorder, diabetes and chronic kidney disease, autoimmune disease, antiphospholipid syndrome as described in Chapter 1. Epidemiological studies have also demonstrated a genetic link to PE. Numerous candidate genes have been proposed based on their underlying mechanisms relating to endothelial function, vasoactive proteins, thrombophilia, oxidative stress, lipid metabolism, and immunogenetics (Mütze *et al.*, 2008). It is likely that the underlying cause for the disease may be due to complex interaction of predisposing environmental factors and numerous single nucleotide polymorphisms. Better understanding of these interactions and phenotypes is the focus of future research as this would enable targeted interventions in individuals at greater risk of the disease.

Long-term maternal sequelae of PE culminate in greater cardiometabolic risk due to hypertension, hyperinsulinemia, elevated BMI, and dyslipidaemia (Fraser *et al.*, 2012). In particular, higher cholesterol, LDLc, and triglyceride, as well as lower HDLc, have been demonstrated in the maternal serum of those that developed PE (Khatua *et al.*, 1989; V. A. Rodie *et al.*, 2004; Ophir *et al.*, 2006; Catarino *et al.*, 2008; Wan Sulaiman *et al.*, 2016). As HDLc has anti-inflammatory, vasodilatory, and anti-oxidant properties, reduced levels of this, in particular, may contribute to the onset of vascular dysfunction and ultimately predispose the mother to greater risk of cardiac and cerebrovascular disease in later life (Bellamy *et al.*, 2007; McDonald *et al.*, 2008; Wan Sulaiman *et al.*, 2016).

The offspring of mothers with pre-eclampsia may also be at immediate and longer-term risk. Perinatal outcomes are poorer in the offspring of those with PE (Friedman *et al.*, 1995; Backes *et al.*, 2011), which may be attributable in part to the lower associated birthweight, shorter gestational age, or preterm delivery and the associated placental dysfunction. Collectively these may give rise to inadequate organ development in utero (Levine *et al.*, 2004). Offspring of mothers with GH disorders have also been shown to be at greater risk of overweight and obesity (Davis *et al.*, 2012, 2015), but this is confounded by maternal BMI during pregnancy and is

in contrast to maternal BMI itself which acts as an independent risk factor for offspring obesity (Patro Golab *et al.*, 2018; Voerman *et al.*, 2019) and cardio-metabolic dysfunction at birth, as discussed in Chapter 3. These associations may be due to the elevated maternal leptin levels identified in maternal diabetes and PE (Miehle, Stepan and Fasshauer, 2012), but as discussed in Chapter 4 yield an inverse relationship with cord blood leptin in those with GDM. Elevated cord blood inflammatory markers and pronounced endothelial dysfunction present during PE (Catarino *et al.*, 2008; Jayet *et al.*, 2010) may also contribute to higher offspring BP throughout life (Tenhola *et al.*, 2003, 2006; Ferreira, Peeters and Stehouwer, 2009; Geelhoed *et al.*, 2010; Davis *et al.*, 2012; Lawlor *et al.*, 2012; Fraser, Nelson, *et al.*, 2013), greater aortic intima-media thickness (Akcakus *et al.*, 2010) and greater risk of stroke (Kajantie *et al.*, 2009).

PE may also impact the offspring's lipid profile. Altered placental lipid transport during pregnancy with PE may lead to dyslipidaemia and oxidative stress in the offspring (Romanowicz and Bańkowski, 2009), as higher triglyceride and LDLc, as well as lower HDLc levels, have been shown in the cord blood and neonatal offspring of mothers with pre-eclampsia. Disturbed lipid profile has also been reported in 5 to 8-year old offspring of mothers with pre-eclampsia (V. A. Rodie *et al.*, 2004; Ophir *et al.*, 2006; Akcakus *et al.*, 2010). Elevated total cholesterol levels have been identified in the offspring of mothers with pre-eclampsia, although there may be no effect on concentrations of LDLc, HDLc, and triglycerides (Kvehaugen *et al.*, 2011). As HDLc has anti-inflammatory properties, lower levels of this in combination with higher LDLc, may contribute to the greater long-term metabolic risk in the offspring (Pecks *et al.*, 2012) and greater need for closer follow up in later life (V. A. Rodie *et al.*, 2004; Catarino *et al.*, 2008). As PE may run in families due to genetic variants, this has potential to translate greater metabolic risk across generations. These findings however are inconsistent, with further studies including those from ALSPAC, showing no difference in lipids measured in the child or adolescent offspring of mothers with HDP (Tenhola *et al.*, 2003; Lawlor *et al.*, 2012; Fraser, Nelson, *et al.*, 2013). Indeed, previous reports have not shown an association between HDP and endothelial dysfunction in the offspring, highlighting the need for further examining

the impact of HDP on the offspring's metabolic risk at birth (Kvehaugen *et al.*, 2011; Lawlor *et al.*, 2012). The rate of change in BP throughout pregnancy may also be significant, even if the BP does not reach the threshold for a diagnosis of HDP. Higher baseline systolic BP (SBP) and diastolic BP (DBP) in early pregnancy, as well as greater rate of change in both SBP and DBP between the second and third trimesters, have been associated with lower birthweight and a shorter gestational period and preterm birth (Bakker *et al.*, 2011; Macdonald-Wallis *et al.*, 2014).

It is clear that PE and GH may induce adverse cardiovascular changes in the mother and changes seen in the offspring may also be evident from birth. By examining the associations between maternal BP in pregnancy and offspring birthweight, cord blood adipokines, and lipids, the aim of this study was to establish whether the altered intrauterine environment(as seen in HDP) adversely affects the offspring's cardio-metabolic profile at birth. By examining the rate of change in BP across each gestational period according to HDP status, this study also aimed to identify specific gestational periods where changes in maternal BP may pose the greatest risk to the offspring.

5.2 Methods

5.2.1 Study Population

There were 5011 participants with a paired cord blood sample. Multiple pregnancies and those with a diagnosis of hypertension prior to pregnancy. There were 4537 mother-offspring pairs included in the study which were categorised into one of three mutually exclusive groups, according to BP status. 3810 women were classified as 'healthy' with no diagnosis of pre-existing hypertension, GH, or PE; 642 were classified as having GH and 85 were categorised as having PE. Details of cord blood sample collection and analysis are presented in Chapter 2.

5.2.2 Obstetric and Perinatal Data

Data were abstracted from obstetric medical records and questionnaires by ALSPAC as presented in Chapter 2: General Methods. Full details have been previously published by ALSPAC (Fraser, Macdonald-wallis, *et al.*, 2013). Using the definitions according to the International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray, 1988), participants were categorised by ALSPAC with pre-eclampsia or gestational hypertension. Pre-eclampsia was defined as hypertension (systolic BP 140mmHg or a diastolic blood pressure 90mmHg) measured on at least 2 occasions after 20 weeks of gestation with accompanying proteinuria. Proteinuria was defined by ALSPAC as at least 1+ reading on dipstick tests (Albustix; Ames Company, Elkhart, IN) which equated to 30 mg/Dl (Davey and MacGillivray, 1988). GH was defined by ALSPAC as an equivalent measure of hypertension in the absence of proteinuria (Davey and MacGillivray, 1988).

Participants were therefore categorised into one of three mutually exclusive

categories of no hypertensive disorder (healthy women), GH, or PE.

Blood pressure was measured by ALSPAC in the supine position using an Omron M6 monitor (Omron Healthcare UK Ltd, Milton Keynes, UK). A median number of 14 BP measurements were obtained (IQR 11-16) across the entire gestational period (MacDonald-Wallis *et al.*, 2011).

5.2.3 Statistical Analysis

Cord blood cholesterol, triglyceride, HDLc and non-HDLc, leptin, adiponectin, and birthweight were log-transformed to produce approximately normal distributions of residuals. Differences in the distribution of participant characteristics (maternal, obstetric, and offspring co-variables) in relation to maternal hypertensive status were tested using chi^2 tests for categorical variables and one-way anova or ttests for continuous variables, as shown in Table 5.1. These analyses tested for the null hypothesis of no difference between the three exposure groups so that there was no assumption of a linear trend across the exposure categories of healthy, GH, and PE.

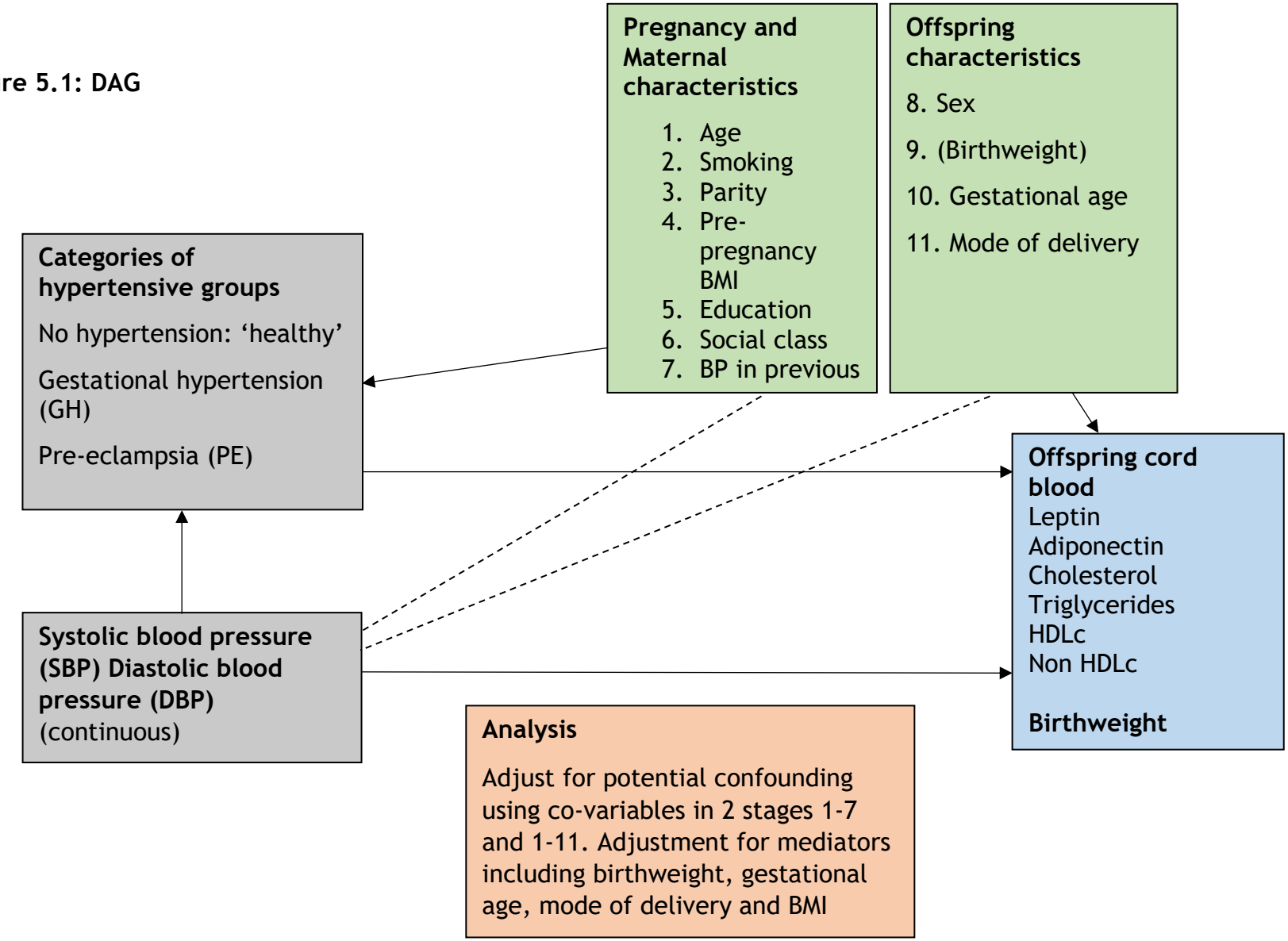
Fractional polynomial curves have previously been devised by ALPSAC to obtain average shape trajectories of SBP and DBP by gestational age (MacDonald-Wallis *et al.*, 2011; Macdonald-Wallis *et al.*, 2014). Changes in direction (slope) of linear splines were identified by ALSPAC in order to determine the approximate position of knots where SBP and DBP were examined as outcomes in relation to gestational age as the exposure. The baseline BP was set by ALSPAC at 8 weeks' gestation as few participants had BP recorded prior to this. The best-fitting linear spline models had three knots at 18, 30, and 36 weeks and from 36 weeks until delivery (MacDonald-Wallis *et al.*, 2011). There were therefore 4 gestational periods where the rate of change in BP was assumed: from 8-18 weeks, 18-30 weeks, 30-36 weeks, and >36 weeks. A series of multivariable regression models were formulated to

adjust for potential confounders and mediators using complete case analysis. Associations did not differ for male and female offspring, and there was no statistical evidence of interaction with sex. Therefore, all results were presented with males and females combined. The relationship between baseline maternal SBP or DBP and the rate of change in maternal SBP or DBP according to each gestational period and cord blood measures according to HDP status were also examined. Maternal and offspring confounders were considered and adjusted for.

5.2.4 Assessment of Confounders and Mediators

In addition to the Assessment of Confounders in Chapters 3 and 4, potential confounding and mediating factors were considered when examining HDP in relation to offspring birthweight and metabolic markers, as displayed in the DAG in Figure 5.1.

Figure 5.1: DAG



Adjustments were made for age, as PE is more prevalent amongst extremes of age and age can influence birthweight and maternal adipokines (including lower adiponectin), and dyslipidaemia may be more prevalent in older mothers. Smoking reduces the risk of PE but translates to greater cardiovascular risk so may influence cord blood measures and it may also limit the birthweight. PE is more common in nulliparous women and there is a trend for increasing birthweight with sequential pregnancies, so parity was also considered in the analysis (NICE, 2010).

Greater maternal BMI is a risk factor for PE but may also lead to higher maternal leptin and cholesterol, lower adiponectin, and higher offspring birthweight so it was also important to account for BMI. In addition, BMI is independently associated with SBP and DBP throughout pregnancy (Farias *et al.*, 2014). Similarly, the presence of PE may influence maternal BMI, partly due to lifestyle intervention once PE is diagnosed but also by limiting time for GWG due to earlier delivery and therefore limiting the BMI. Collectively this may influence the offspring's birthweight or cord blood adipokines. Birthweight was considered a mediator when cord blood analytes were the outcome as PE influences birthweight which directly impacts cord blood adipokines, as previously discussed. HDP, especially in severe disease, may lead to shorter gestational age as a result of a medically indicated earlier delivery for example which may influence birthweight (lower) and cord blood adipokines (lower leptin as smaller, pre-term placenta may lead to less placental production of the adipokine). In addition, severe HDP may warrant urgent caesarean section delivery which again may influence cord blood leptin concentration. Placental insufficiency of HDP may induce fetal growth restriction and resultant lower offspring birthweight (NICE, 2010).

5.3 Results

Figure 5.2 shows the ALSPAC participant flow chart and inclusion and exclusion criteria.

Figure 5.2: ALSPAC participant flow chart

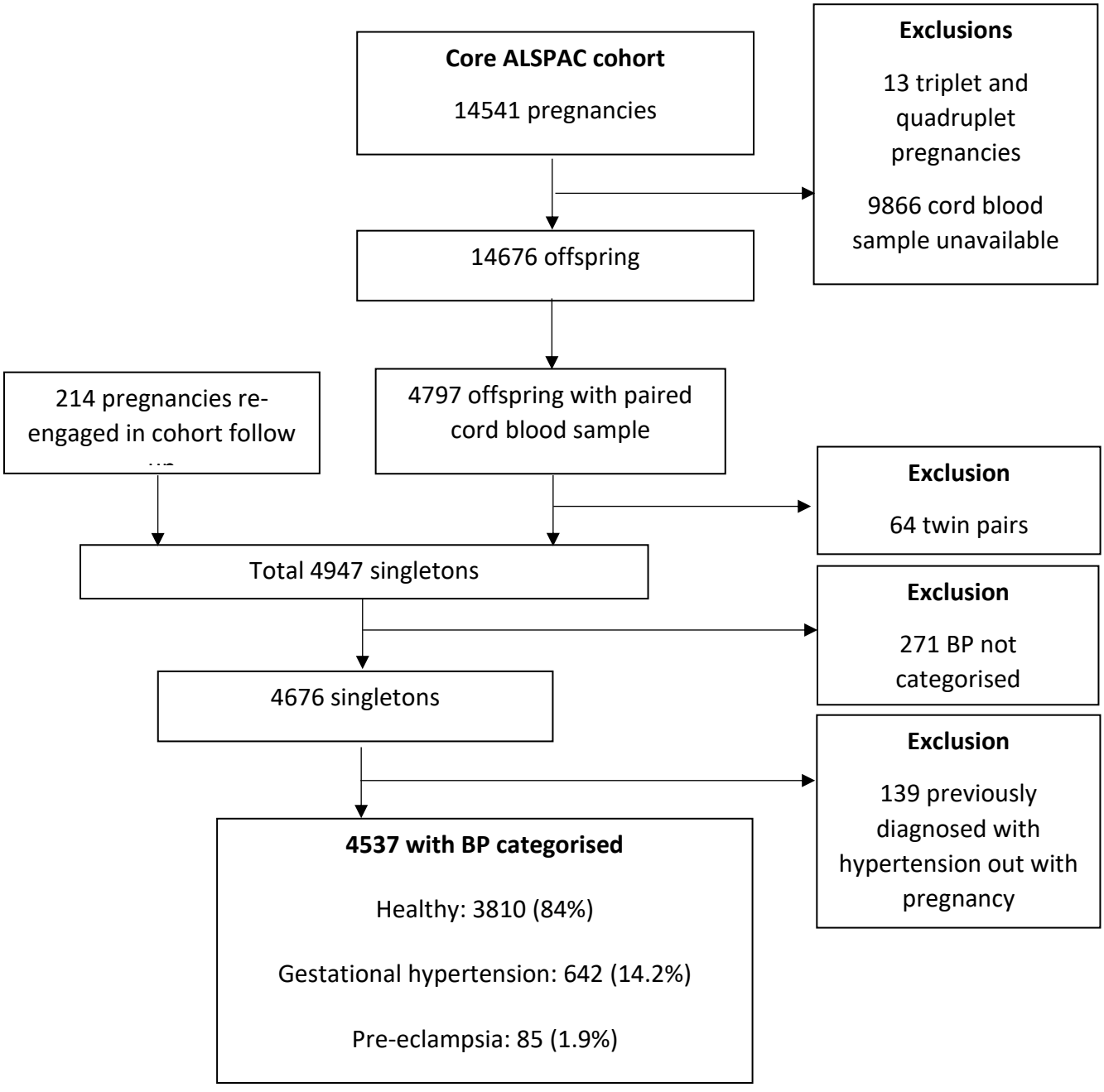


Table 5.1 summarises the cohort characteristics according to BP status. There were 84% that remained 'healthy' and unaffected by GH or PE, 14.2% that developed GH, and 1.9% that developed PE.

Women who developed PE during pregnancy were younger (median age 26 years) compared to the 'healthy' group (median age 28 years). They were also less likely to smoke during pregnancy (11.5%) compared to the 'healthy' group, where 25.4% smoked during pregnancy. Mothers who developed pre-eclampsia had a higher BMI at booking (23.5 kg/m^2) compared to the healthy group (21.9 kg/m^2). Most women who developed PE were nulliparous (73%), in contrast to the healthy group among whom 41.5% were nulliparous. Women who developed PE were less likely to achieve a spontaneous vaginal delivery (SVD), as 22.6% required caesarean section compared to only 7.8% of the healthy group. Women with PE were also delivered earlier (median 39 weeks) and had offspring with a lower birthweight (median 3.1kg) compared to the healthy group (40 weeks and 3.4kg respectively). Cord blood leptin and cholesterol were higher in mothers with pre-eclampsia (8.1 pg/ml and 1.8 mmol/l respectively) compared to the healthy group (6.4 pg/ml and 1.6 mmol/l respectively). Whilst lower cord blood adiponectin was observed in HDP compared to the healthy group, this trend was not significant.

Women who developed GH during pregnancy were also slightly younger (median age 27 years) compared to the healthy group. Although most women with GH had never smoked (69.6%), 20.7% continued to smoke during pregnancy. Women that developed GH also had a higher BMI at booking (median 23.5 kg/m^2) compared to the healthy group. Women with GH were more likely to achieve SVD (71.3%) than those with PE, although the rate of SVD was highest amongst the healthy group. Gestational age and birthweight were comparable amongst those with GH and the healthy group (median 40 weeks and 3.5kg respectively). Higher cord blood leptin (7.1 pg/ml) and cholesterol (1.7mmol/l) were also observed in those with GH compared to the healthy group.

Table 5.1 also displays the blood pressures for each category of HDP. Both SBP and DBP were higher at booking in those who later developed PE (median SBP 118.5mmHg, DBP 71.3mmHg) compared to the healthy group (SBP 111.0mmHg, DBP 65.3mmHg ($p<0.001$)). Although there was however a negative trend in SBP in between 8-18 weeks' pregnancy, the BP rose beyond 18 weeks and both the SBP and DBP remained higher in those that developed PE compared to the healthy group. Between 30-36 weeks, there was also a steeper rise in the DBP in mothers with pre-eclampsia (1.03mmHg), compared to the healthy group.

Both SBP and DBP were also higher at booking in those that later developed GH (median SBP 119.2mmHg, DBP 70.3mmHg), compared to the healthy group. A negative trend in BP was observed between 8-18 weeks in those who developed GH, followed by a rise in BP beyond 18 weeks, which remained greater than those in the healthy group.

Table 5.1: Summary of cohort characteristics according to BP status

	Healthy (n=3810)		Gestational Hypertension (n=642)		Pre-eclampsia (n=85)		P value
	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	
Maternal Characteristics							
Age	3810 (84.0)	28 (25, 31)	642 (14.2)	27 (24, 31)	85 (1.9)	26 (22, 29)	0.006
Smoking							0.001
Never	2434 (67.3)		430 (69.6)		58 (74.4)		
Before, not during pregnancy	264 (7.3)		60 (9.7)		11 (14.1)		
During pregnancy	920 (25.4)		128 (20.7)		9 (11.5)		
BMI (kg/m^2)	3225	21.9 (20.4, 23.8)	536	23.5 (21.3, 27.2)	66	23.5 (21.7, 28.2)	0.000
Parity							0.000
0	1472 (41.5)		331 (55.1)		62 (73.0)		
1	1320 (37.2)		176 (29.3)		13 (15.3)		
2	543 (15.3)		62 (10.3)		7 (8.2)		
3	159 (4.5)		24 (4.0)		1 (1.2)		
4+	52 (1.5)		8 (1.3)		2 (2.4)		
Education							0.643
Left school at 16	22663 (65.1)		395 (67.0)		49 (65.3)		
A level	781 (22.5)		125 (21.2)		20 (26.7)		
Degree	432 (12.4)		70 (11.9)		6 (8.0)		
Social Class							0.560
I (least disadvantaged)	144 (5.1)		25 (5.1)		3 (4.8)		
II	921 (32.7)		138 (28.4)		22 (34.9)		
IIIa	1212 (43.0)		232 (47.7)		23 (36.5)		
IIIb	212 (7.5)		35 (7.2)		7 (11.1)		
IV	268 (9.5)		46 (9.5)		8 (12.7)		
V (most disadvantaged)	64 (2.3)		10 (2.1)		0 (0.0)		

	Healthy (n=3810)		Gestational Hypertension (n=642)		Pre-eclampsia (n=85)		P value
	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	
Ethnicity							0.183
White Caucasian	3374 (97.4)		583 (99.3)		73 (97.3)		
Black Caribbean	18 (0.5)		1 (0.2)		0 (0.0)		
Black African	4 (0.1)		0 (0.0)		0 (0.0)		
Black other	16 (0.5)		1 (0.2)		1 (1.3)		
Indian	16 (0.5)		0 (0.0)		1 (1.3)		
Pakistani	8 (0.2)		1 (0.2)		0 (0.0)		
Chinese	9 (0.3)		0 (0.0)		0 (0.0)		
Other	18 (0.5)		1 (0.2)		0 (0.0)		
SBP (mmHg)							0.001
8 weeks	3498	111.0 (107.2, 115.1)	611	119.2 (114.9, 123.6)	74	118.5 (113.9, 125.5)	0.001
8-18 weeks	3495	-0.17 (-0.30, -0.05)	611	-0.02 (0.18, 0.12)	74	-0.08 (-0.22, 0.07)	0.011
18-30 weeks	3498	0.14 (0.01, 0.27)	611	0.19 (0.03, 0.45)	74	0.18 (-0.01, 0.33)	0.482
30-36 weeks	3483	0.20 (-0.06, 0.46)	606	0.41 (0.05, 0.76)	74	0.71 (0.41, 1.09)	0.505
>36 weeks	3494	1.13 (0.74, 1.48)	606	1.17 (0.71, 1.77)	74	1.48 (1.08, 2.07)	0.815
DBP (mmHg)							0.001
8 weeks	3498	65.3 (62.7, 68.1)	611	70.3 (67.1, 73.4)	74	71.3 (68.4, 74.6)	0.001
8-18 weeks	3498	-0.20 (-0.27, -0.13)	611	-0.17 (-0.25, -0.09)	74	-0.20 (-0.26, -0.12)	0.134
18-30 weeks	3498	0.08 (-0.01, 0.17)	611	0.13 (0.02, 0.24)	74	0.17 (0.08, 0.26)	0.803
30-36 weeks	3497	0.33 (0.10, 0.57)	611	0.69 (0.35, 0.99)	74	1.03 (0.69, 1.34)	0.011
>36 weeks	3495	1.25 (0.89, 1.58)	611	1.54 (1.06, 2.07)	74	1.95 (1.42, 2.83)	0.760
Pregnancy Characteristics							
Mode of delivery							<0.001
SVD	3075 (81.0)		455 (71.3)		47 (56.0)		
Breech	30 (0.8)		6 (0.9)		0 (0.0)		
Caesarean	295 (7.8)		84 (13.2)		19 (22.6)		
Forceps	173 (4.6)		47 (7.4)		10 (11.9)		
Vacuum	193 (5.1)		34 (5.3)		6 (7.1)		
Other	31 (0.8)		12 (1.9)		2 (2.4)		

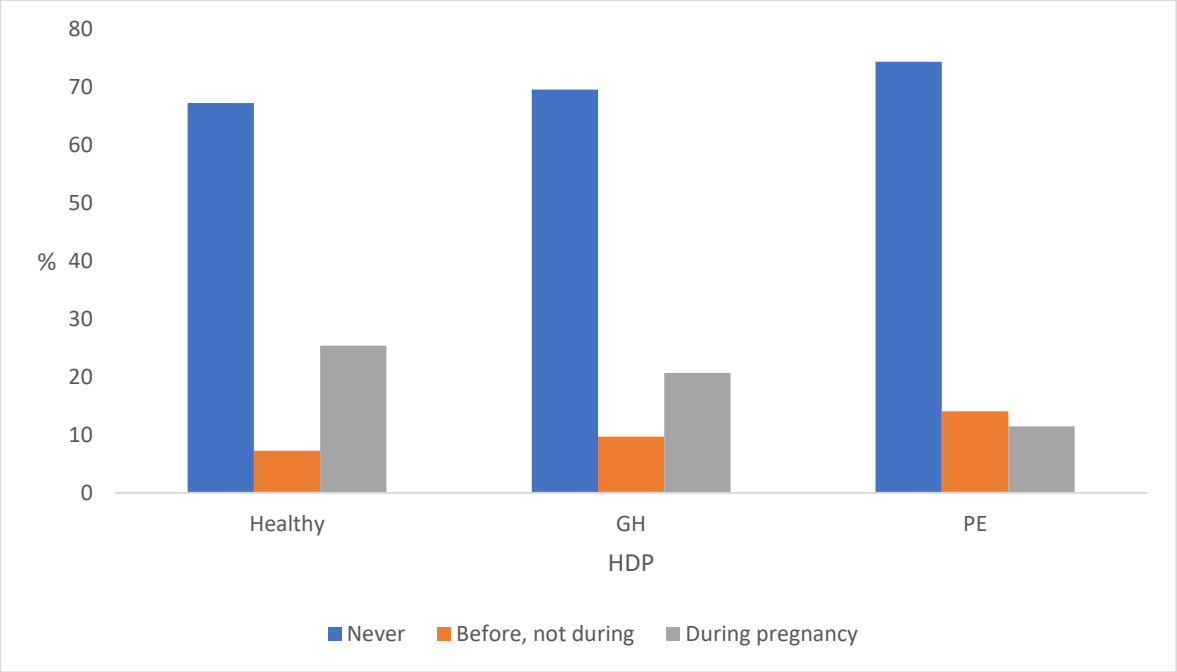
	Healthy (n=3810)		Gestational Hypertension (n=642)		Pre-eclampsia (n=85)		P value
	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	
Offspring Characteristics							
Gestational age at birth (weeks)	3810	40 (39, 41)	642	40 (39, 41)	85	39 (38, 40)	<0.001
Sex							0.729
Male	1937 (50.8)		334 (52.0)		46 (54.1)		
Female	1873 (49.2)		308 (48.0)		39 (45.9)		
Birthweight (kg)	3774	3.4 (3.1, 3.8)	637	3.5 (3.2, 3.8)	83	3.1 (2.8, 3.6)	<0.001
Cord blood leptin (pg/ml)	3807	6.4 (3.6, 11.9)	642	7.1 (3.7, 12.6)	85	8.1 (3.8, 15.6)	<0.001
Cord blood adiponectin (µg/ml)	3775	76.7 (54.4, 98.9)	635	74.8 (54.7, 100.4)	85	70.7 (50.7, 99.6)	0.756
Cord blood cholesterol (mmol/l)	3760	1.6 (1.4, 2.0)	632	1.7 (1.4, 2.0)	85	1.8 (1.4, 2.1)	0.015
Cord blood triglyceride (mmol/l)	3746	0.5 (0.4, 0.6)	631	0.5 (0.4, 0.7)	85	0.5 (0.4, 0.7)	0.000
Cord blood HDLc (mmol/l)	3688	0.5 (0.4, 0.7)	621	0.5 (0.4, 0.7)	85	0.5 (0.3, 0.7)	0.008
Cord blood non-HDLc (mmol/l)	3688	1.1 (0.9, 1.4)	621	1.2 (0.9, 1.5)	85	1.2 (1.0, 1.5)	0.110

Median (Interquartile range)

Figures are numbers (%) unless stated otherwise

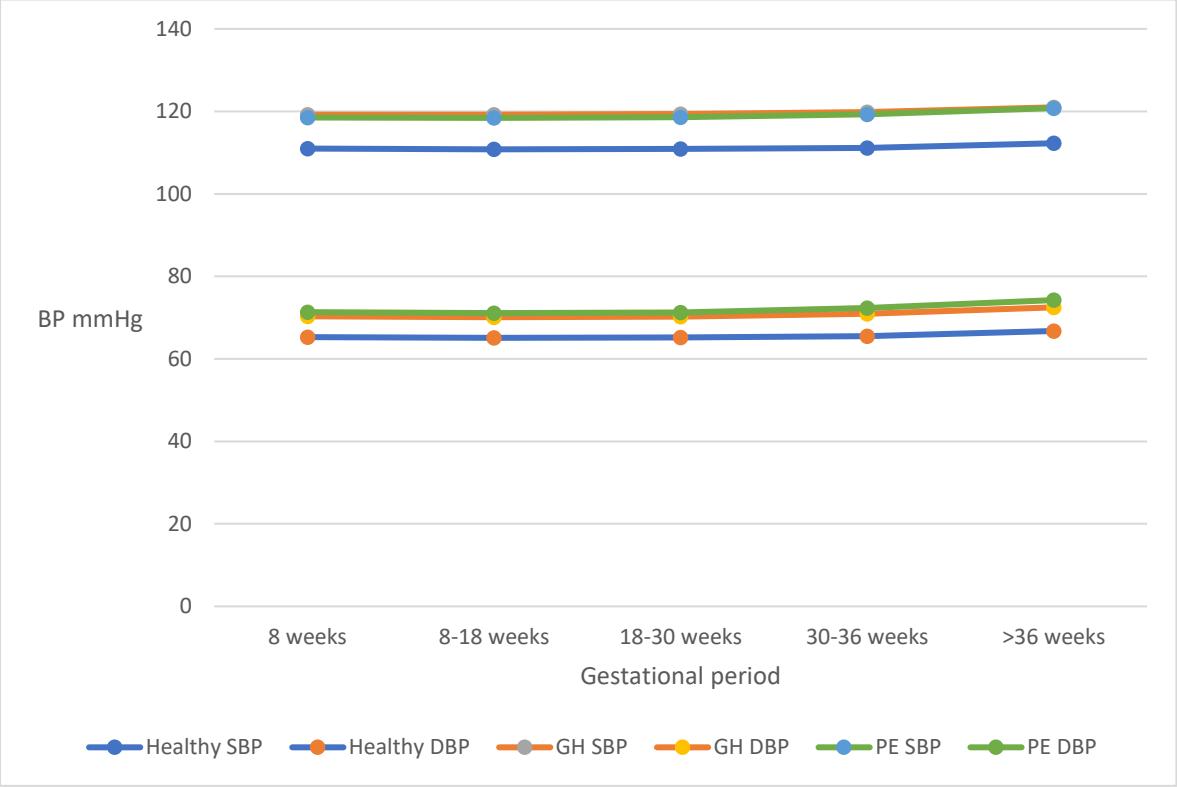
Smoking according to HDP status is displayed in Figure 5.3.

Figure 5.3: Smoking status according to HDP group



SBP and DBP during each gestational period according to HDP status are displayed in Figure 5.4.

Figure 5.4: SBP and DBP during each gestational period according to HDP



The concentration of each cord blood analyte according to HDP status is displayed in Figure 5.5.

Figure 5.5: Concentration of cord blood analyte according to HDP status

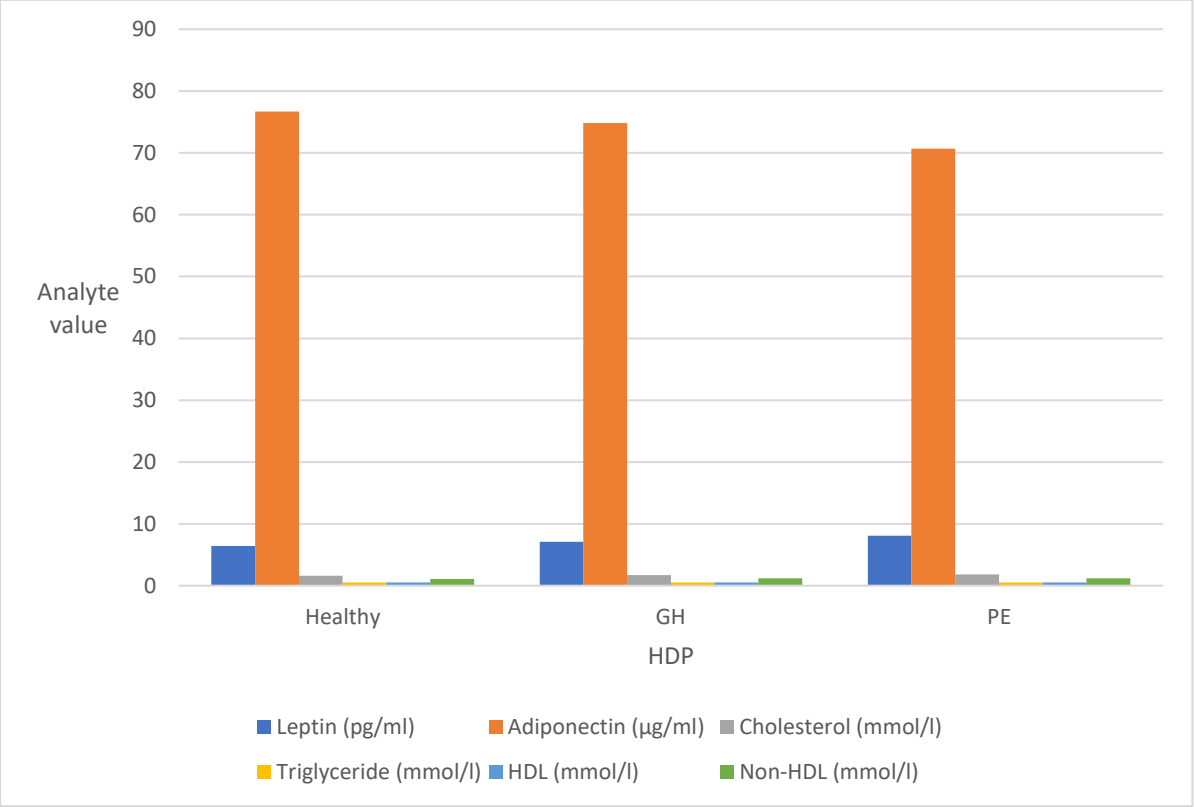


Table 5.2 shows the multivariable models of the association between SBP and DBP (per 10mmHg rise at 8 weeks' gestation and per 1mmHg rise per week between 8-18 weeks, 18-30 weeks, 30-36 weeks, and >36 weeks' pregnancy) and cord blood leptin, adiponectin, lipids, and birthweight according to HDP status. In the healthy group, every 10mmHg rise in SBP at 8 weeks' gestation was negatively associated with cord blood HDLc (-0.04mmol/l (-0.07, 0.00), p=0.044). Following this, 1mmHg per week rise in DBP from 8 to 18 weeks and 1mmHg per week rise in SBP from 36 weeks' gestation was positively associated with cord blood leptin (0.28pg/ml (0.01, 0.55), p=0.46 and 0.07pg/ml (0.01, 0.13) p=0.032 respectively). Between 30 and 36 weeks, 1mmHg rise in DBP was positively associated with cord blood cholesterol (0.05mmol/l (0.00, 0.09), p=0.044). Also between 30 and 36 weeks, 1mmHg rise per week in SBP and DBP was negatively associated with birthweight (-0.02g (-0.03, 0.00), p=0.014 and -0.05 (-0.08g, -0.01), p=0.010) in the healthy group. In those that developed PE, every 10mmHg rise in SBP at 8 weeks' gestation was positively associated with cord blood HDLc (0.35mmol/l (0.02, 0.67), p=0.036), as was every 1mmHg per week change in SBP between 30 and 36 weeks (0.51mmol/l (0.03, 0.98), p=0.038). In those that developed GH, between 30 and 36 weeks, 1mmHg rise in SBP and DBP was negatively associated with birthweight (-0.05g (-0.07, 0.02), p=0.000 and -0.01 (-0.11, -0.05), p=0.001). These associations remained after adjustment for potential confounding, including maternal and offspring characteristics as well as accounting for potential mediation by offspring birthweight, gestational age, mode of delivery and BMI. There was minimal impact of mediators, such as gestational age, which may be a result of the limited variability within this cohort.

Table 5.2: Multivariable models for the association of SBP and DBP (per 10mmHg at 8 wks and per 1mmHg/wk change during 8-18, 8-30, 30-36, and >36wks gestation) and cord blood measures and birthweight, according to HDP status

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Cord blood leptin (pg/ml)													
8 weeks	SBP	0.04 (-0.02, 0.11)	0.168	0.03 (-0.03, 0.09)	0.289	0.01 (-0.13, 0.16)	0.882	0.02 (-0.12, 0.16)	0.784	0.18 (-0.32, 0.69)	0.472	0.04 (-0.49, 0.56)	0.884
	DBP	0.02 (-0.07, 0.11)	0.663	0.00 (-0.09, 0.09)	0.955	0.02 (-0.21, 0.25)	0.869	0.09 (-0.13, 0.31)	0.419	-0.23 (-0.98, 0.53)	0.545	-0.44 (-1.19, 0.30)	0.230
8-18 weeks	SBP	0.04 (-0.13, 0.22)	0.629	0.02 (-0.14, 0.19)	0.777	0.21 (-0.19, 0.62)	0.296	0.19 (-0.19, 0.57)	0.317	1.70 (-0.28, 3.67)	0.090	1.58 (-0.85, 4.01)	0.192
	DBP	0.28 (-0.01, 0.57)	0.055	0.28 (0.01, 0.55)	0.046	-0.20 (-0.88, 0.47)	0.552	0.03 (-0.60, 0.67)	0.916	0.28 (-1.82, 2.37)	0.791	-0.22 (-2.35, 1.91)	0.832
18-30 weeks	SBP	0.03 (-0.14, 0.20)	0.739	0.02 (-0.14, 0.19)	0.780	-0.40 (-0.83, 0.02)	0.062	-0.39 (-0.79, 0.02)	0.061	-0.59 (-2.13, 0.95)	0.441	-0.64 (-2.31, 1.04)	0.443
	DBP	-0.23 (-0.48, 0.02)	0.075	-0.12 (-0.36, 0.12)	0.319	-0.18 (-0.79, 0.43)	0.559	-0.01 (-0.59, 0.58)	0.981	-1.82 (-4.22, 0.58)	0.133	-1.62 (-4.10, 0.86)	0.192
30-36 weeks	SBP	-0.03 (-0.12, 0.06)	0.464	0.02 (-0.07, 0.11)	0.661	-0.01 (-0.21, 0.19)	0.954	0.15 (-0.05, 0.34)	0.138	-0.10 (-0.81, 0.61)	0.773	-0.30 (-1.11, 0.50)	0.446

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
	DBP	0.01	0.862	0.06	0.192	-0.23	0.036	-0.06	0.608	0.18	0.626	0.10	0.809
		(-0.09, 0.11)		(-0.03, 0.16)		(-0.45, -0.02)		(-0.29, 0.17)		(-0.58, 0.95)		(-0.74, 0.94)	
>36	SBP	0.06	0.058	0.07	0.032	-0.04	0.511	-0.04	0.525	-0.20	0.442	0.10	0.754
weeks		(0.00, 0.12)		(0.01, 0.13)		(-0.17, 0.09)		(-0.16, 0.08)		(-0.72, 0.32)		(-0.57, 0.78)	
	DBP	0.04	0.159	0.05	0.067	-0.10	0.889	-0.01	0.828	-0.01	0.976	0.27	0.275
		(-0.02, 0.10)		(0.00, 0.11)		(-0.14, 0.12)		(-0.14, 0.11)		(-0.44, 0.43)		(-0.23, 0.76)	
Cord blood adiponectin (µg/ml)													
8	SBP	0.001	0.957	0.001	0.955	0.02	0.562	0.02	0.639	-0.09	0.435	-0.10	0.359
weeks		(-0.04, 0.04)		(-0.04, 0.04)		(-0.06, 0.11)		(-0.06, 0.10)		(-0.31, 0.14)		(-0.33, 0.12	
	DBP	0.04	0.206	0.04	0.192	0.06	0.352	0.06	0.352	-0.19	0.275	-0.22	0.160
		(-0.02, 0.10)		(-0.02, 0.10)		(-0.07, 0.19)		(-0.07, 0.19)		(-0.53, 0.15)		(-0.54, 0.09)	
8-18	SBP	0.04	0.468	0.03	0.544	0.743	0.743	0.05	0.630	-0.46	0.293	-0.19	0.721
weeks		(-0.07, 0.15)		(-0.08, 0.14)		(-0.19, 0.26)		(-0.17, 0.27)		(-1.34, 0.42)		(-1.25, 0.88)	
	DBP	0.05	0.568	0.06	0.521	-0.11	0.569	-0.12	0.528	-0.18	0.694	0.06	0.890
		(-0.13, 0.23)		(-0.12, 0.24)		(-0.49, 0.27)		(-0.49, 0.25)		(-1.12, 0.75)		(-0.85, 0.97)	
18-30	SBP	0.04	0.444	0.05	0.401	-0.20	0.109	-0.20	0.092	-0.50	0.147	-0.26	0.480
weeks		(-0.07, 0.15)		(-0.06, 0.16)		(-0.43, 0.04)		(-0.43, 0.03)		(-1.19, 0.19)		(-0.99, 0.48)	
	DBP	0.05	0.578	0.06	0.433	-0.07	0.686	-0.05	0.772	-1.05	0.052	-0.55	0.303

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
		(-0.11, 0.21)		(-0.10, 0.22)		(-0.41, 0.27)		(-0.39, 0.29)		(-2.10, 0.01)		(-1.62, 0.52)	
30-36	SBP	-0.0003	0.933	0.01	0.667	0.05	0.346	0.08	0.160	-0.18	0.253	-0.12	0.496
weeks		(-0.06, 0.06)		(-0.04, 0.07)		(-0.06, 0.16)		(-0.03, 0.19)		(-0.50, 0.14)		(-0.47, 0.24)	
	DBP	-0.02	0.450	-0.01	0.737	-0.01	0.918	0.02	0.722	-0.28	0.084	-0.17	0.337
		(-0.09, 0.04)		(-0.08, 0.05)		(-0.13, 0.12)		(-0.11, 0.16)		(-0.60, 0.04)		(-0.53, 0.19)	
>36	SBP	0.03	0.175	0.03	0.108	-0.05	0.140	-0.04	0.215	-0.12	0.294	-0.26	0.070
weeks		(-0.01, 0.07)		(-0.01, 0.07)		(-0.12, 0.02)		(-0.11, 0.03)		(-0.35, 0.11)		(-0.53, 0.02)	
	DBP	0.01	0.546	0.02	0.330	0.02	0.550	0.03	0.416	-0.10	0.236	-0.15	0.146
		(-0.02, 0.05)		(-0.02, 0.06)		(-0.05, 0.09)		(-0.04, 0.10)		(-0.28, 0.07)		(-0.36, 0.06)	
Cord blood cholesterol (mmol/l)													
8	SBP	-0.02	0.291	-0.01	0.310	-0.04	0.183	-0.05	0.179	0.21	0.074	0.16	0.215
weeks		(-0.04, 0.01)		(-0.04, 0.01)		(-0.11, 0.02)		(-0.11, 0.02)		(-0.02, 0.44)		(-0.10, 0.42)	
	DBP	-0.01	0.542	-0.02	0.347	-0.02	0.679	-0.04	0.280	0.39	0.026	0.002	0.988
		(-0.06, 0.03)		(-0.05, 0.02)		(-0.12, 0.08)		(-0.12, 0.03)		(0.05, 0.73)		(-0.30, 0.31)	
8-18	SBP	0.06	0.162	0.06	0.160	0.06	0.533	0.04	0.630	0.41	0.363	0.53	0.374
weeks		(-0.02, 0.14)		(-0.02, 0.14)		(-0.12, 0.24)		(-0.14, 0.23)		(-0.49, 1.30)		(-0.67, 1.74)	
	DBP	-0.02	0.716	-0.01	0.840	0.02	0.893	-0.004	0.979	0.28	0.547	0.10	0.850
		(-0.15, 0.11)		(-0.14, 0.12)		(-0.28, 0.32)		(-0.31, 0.30)		(-0.65, 1.21)		(-0.93, 1.12)	

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
18-30	SBP	-0.03	0.487	-0.03	0.490	0.10	0.316	0.10	0.321	0.04	0.919	-0.03	0.935
weeks		(-0.11, 0.05)		(-0.11, 0.05)		(-0.09, 0.29)		(-0.10, 0.29)		(-0.69, 0.76)		(-0.88, 0.81)	
	DBP	-0.01	0.874	-0.01	0.882	0.04	0.767	0.01	0.941	0.14	0.804	0.07	0.908
		(-0.13, 0.11)		(-0.12, 0.11)		(-0.23, 0.32)		(-0.27, 0.29)		(-0.97, 1.25)		(-1.17, 1.31)	
30-36	SBP	0.01	0.772	0.01	0.578	0.02	0.597	0.01	0.833	0.33	0.043	0.30	0.122
weeks		(-0.04, 0.05)		(-0.03, 0.05)		(-0.07, 0.11)		(-0.08, 0.10)		(0.01, 0.66)		(-0.09, 0.70)	
	DBP	0.04	0.095	0.05	0.044	-0.03	0.587	-0.05	0.374	0.11	0.523	-0.02	0.924
		(-0.01, 0.09)		(0.00, 0.09)		(-0.13, 0.07)		(-0.16, 0.06)		(-0.24, 0.46)		(-0.44, 0.40)	
>36	SBP	-0.01	0.661	-0.004	0.799	-0.01	0.769	-0.01	0.800	-0.21	0.062	-0.24	0.129
weeks		(-0.04, 0.02)		(-0.03, 0.03)		(-0.07, 0.05)		(-0.07, 0.05)		(-0.44, 0.01)		(-0.55, 0.07)	
	DBP	-0.00	0.774	0.00	0.907	-0.02	0.561	-0.02	0.615	-0.06	0.566	-0.05	0.657
		(-0.03, 0.02)		(-0.03, 0.03)		(-0.08, 0.04)		(-0.07, 0.04)		(-0.25, 0.14)		(-0.31, 0.20)	
Cord blood triglyceride (mmol/l)													
8	SBP	0.01	0.582	0.02	0.303	-0.04	0.281	-0.04	0.269	0.06	0.549	0.07	0.511
weeks		(-0.02, 0.04)		(-0.02, 0.05)		(-0.12, 0.03)		(-0.11, 0.03)		(-0.14, 0.27)		(-0.15, 0.29)	
	DBP	0.01	0.770	0.01	0.643	0.02	0.674	-0.002	0.966	0.24	0.116	0.24	0.129
		(-0.04, 0.06)		(-0.04, 0.06)		(-0.09, 0.14)		(-0.11, 0.11)		(-0.06, 0.54)		(-0.07, 0.55)	
	SBP	0.001	0.988	0.02	0.718	0.16	0.128	0.14	0.163	0.34	0.402	0.50	0.330

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
8-18		(-0.09, 0.09)		(-0.07, 0.11)		(-0.05, 0.37)		(-0.06, 0.34)		(-0.47, 1.14)		(-0.54, 1.54)	
weeks	DBP	-0.07	0.696	-0.03	0.699	0.18	0.292	0.22	0.189	0.16	0.693	0.15	0.732
		(-0.22, 0.09)		(-0.18, 0.12)		(-0.16, 0.53)		(-0.11, 0.55)		(-0.67, 1.00)		(-0.74, 1.04)	
18-30	SBP	-0.01	0.785	-0.01	0.816	0.13	0.234	0.17	0.106	0.21	0.511	0.09	0.792
weeks		(-0.11, 0.08)		(-0.10, 0.08)		(-0.09, 0.35)		(-0.04, 0.38)		(-0.43, 0.86)		(-0.63, 0.82)	
	DBP	0.01	0.885	0.01	0.850	-0.01	0.945	0.06	0.694	0.68	0.163	0.43	0.407
		(-0.13, 0.15)		(-0.12, 0.14)		(-0.32, 0.30)		(-0.24, 0.36)		(-0.29, 1.64)		(-0.62, 1.49)	
30-36	SBP	0.01	0.601	0.04	0.125	0.00	0.948	0.00	0.991	0.13	0.397	0.09	0.599
weeks		(-0.04, 0.06)		(-0.01, 0.08)		(-0.10, 0.11)		(-0.10, 0.10)		(-0.18, 0.43)		(-0.26, 0.44)	
	DBP	0.02	0.554	0.05	0.057	-0.03	0.653	0.02	0.794	0.04	0.801	-0.06	0.720
		(-0.04, 0.07)		(0.00, 0.10)		(-0.14, 0.09)		(-0.10, 0.13)		(-0.27, 0.34)		(-0.42, 0.29)	
>36	SBP	-0.02	0.310	0.00	0.995	0.01	0.761	0.01	0.781	-0.13	0.248	-0.12	0.399
weeks		(-0.05, 0.02)		(-0.03, 0.03)		(-0.06, 0.08)		(-0.05, 0.07)		(-0.34, 0.09)		(-0.41, 0.17)	
	DBP	-0.03	0.118	-0.01	0.691	-0.01	0.725	-0.02	0.575	-0.05	0.529	-0.07	0.537
		(-0.06, 0.01)		(-0.04, 0.03)		(-0.08, 0.05)		(-0.08, 0.04)		(-0.22, 0.12)		(-0.28, 0.15)	
Cord blood HDLc (mmol/l)													
8	SBP	-0.03	0.077	-0.04	0.044	-0.02	0.626	-0.02	0.620	0.40	0.013	0.35	0.036
weeks		(-0.07, 0.00)		(-0.07, 0.00)		(-0.10, 0.06)		(-0.10, 0.06)		(0.09, 0.70)		(0.02, 0.67)	

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
8-18 weeks	DBP	-0.03 (-0.08, 0.02)	0.309	-0.03 (-0.08, 0.02)	0.226	-0.06 (-0.19, 0.07)	0.351	-0.06 (-0.18, 0.07)	0.371	0.38 (-0.11, 0.87)	0.125	0.36 (-0.13, 0.85)	0.142
	SBP	0.11 0.01, 0.20)	0.033	0.09 (0.00, 0.19)	0.055	0.00 (-0.23, 0.22)	0.973	-0.01 (-0.24, 0.22)	0.930	-0.02 (-1.23, 1.18)	0.969	0.51 (-1.02, 2.04)	0.501
18-30 weeks	DBP	0.01 (-0.15, 0.17)	0.905	0.00 (-0.16, 0.16)	0.992	-0.02 (-0.39, 0.35)	0.902	-0.06 (-0.43, 0.31)	0.752	0.51 (-0.83, 1.85)	0.443	0.51 (-0.88, 1.90)	0.460
	SBP	-0.04 (-0.14, 0.06)	0.438	-0.04 (-0.14, 0.06)	0.427	0.05 (-0.19, 0.28)	0.699	0.02 (-0.22, 0.26)	0.860	-0.25 (-1.22, 0.72)	0.609	-0.20 (-1.27, 0.87)	0.704
30-36 weeks	DBP	0.00 (-0.15, 0.14)	0.952	-0.01 (-0.15, 0.14)	0.940	-0.01 (-0.35, 0.33)	0.964	-0.07 (-0.42, 0.27)	0.671	-0.06 (-1.66, 1.53)	0.936	0.18 (-1.49, 1.86)	0.823
	SBP	0.00 (-0.05, 0.05)	0.922	-0.01 (-0.06, 0.04)	0.672	0.01 (-0.10, 0.12)	0.912	0.00 (-0.11, 0.12)	0.969	0.49 (0.06, 0.92)	0.026	0.51 (0.03, 0.98)	0.038
>36 weeks	DBP	0.02 (-0.04, 0.08)	0.510	0.01 (-0.05, 0.06)	0.780	-0.03 (-0.15, 0.10)	0.665	-0.06 (-0.19, 0.07)	0.385	0.07 (-0.43, 0.58)	0.765	0.01 (-0.55, 0.58)	0.963
	SBP	0.01 (-0.03, 0.05)	0.601	0.00 (-0.03, 0.04)	0.948	0.00 (-0.07, 0.07)	0.962	0.00 (-0.07, 0.07)	0.985	-0.08 (-0.39, 0.24)	0.618	-0.21 (-0.60, 0.19)	0.289
	DBP	0.01 (-0.02, 0.05)	0.411	0.00 (-0.03, 0.04)	0.795	-0.02 (-0.09, 0.05)	0.532	-0.02 (-0.09, 0.05)	0.601	0.07 (-0.21, 0.35)	0.630	0.01 (-0.33, 0.35)	0.946

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Cord blood non-HDLc (mmol/l)													
8	SBP	-0.01	0.529	-0.01	0.639	-0.05	0.205	-0.05	0.205	0.14	0.251	0.08	0.541
weeks		(-0.04, 0.02)		(-0.04, 0.02)		(-0.12, 0.03)		(-0.12, 0.03)		(-0.10, 0.38)		(-0.19, 0.35)	
	DBP	-0.01	0.808	-0.01	0.832	-0.003	0.963	-0.03	0.630	0.39	0.030	0.35	0.064
		(-0.05, 0.04)		(-0.05, 0.04)		(-0.12, 0.11)		(-0.15, 0.09)		(0.04, 0.73)		(-0.02, 0.71)	
8-18	SBP	0.05	0.315	0.05	0.258	0.07	0.514	0.05	0.611	0.45	0.336	0.51	0.415
weeks		(-0.04, 0.14)		(-0.04, 0.14)		(-0.14, 0.28)		(-0.15, 0.26)		(-0.48, 1.38)		(-0.75, 1.77)	
	DBP	-0.03	0.730	-0.01	0.896	0.07	0.669	0.06	0.744	0.18	0.706	-0.04	0.934
		(-0.17, 0.12)		(-0.16, 0.14)		(-0.27, 0.42)		(-0.29, 0.40)		(-0.77, 1.13)		(-1.10, 1.01)	
18-30	SBP	-0.04	0.394	-0.04	0.397	0.12	0.264	0.14	0.224	0.18	0.620	0.06	0.895
weeks		(-0.13, 0.05)		(-0.13, 0.05)		(-0.09, 0.34)		(-0.08, 0.35)		(-0.56, 0.93)		(-0.82, 0.94)	
	DBP	0.02	0.776	0.02	0.766	0.06	0.705	0.05	0.748	0.21	0.712	0.02	0.979
		(-0.11, 0.15)		(-0.11, 0.15)		(-0.25, 0.37)		(-0.27, 0.37)		(-0.92, 1.34)		(-1.25, 1.28)	
30-36	SBP	0.01	0.695	0.02	0.407	0.03	0.582	0.01	0.823	0.29	0.095	0.24	0.242
weeks		(-0.04, 0.06)		(-0.03, 0.07)		(-0.07, 0.13)		(-0.09, 0.12)		(-0.05, 0.63)		(-0.17, 0.66)	
	DBP	0.03	0.201	0.05	0.067	-0.03	0.613	-0.04	0.493	0.14	0.424	-0.02	0.939
		(-0.02, 0.09)		(0.00, 0.10)		(-0.14, 0.08)		(-0.17, 0.08)		(-0.21, 0.50)		(-0.45, 0.41)	
	SBP	0.00	0.875	0.00	0.811	-0.01	0.766	-0.01	0.776	-0.23	0.054	-0.23	0.171

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
>36		(-0.04, 0.03)		(-0.03, 0.04)		(-0.08, 0.06)		(-0.08, 0.06)		(-0.47, 0.00)		(-0.57, 0.11)	
weeks	DBP	-0.01	0.737	0.00	0.929	-0.02	0.474	-0.02	0.487	-0.09	0.382	-0.08	0.509
		(-0.06, 0.03)		(-0.03, 0.03)		(-0.09, 0.04)		(-0.09, 0.04)		(-0.28, 0.11)		(-0.34, 0.17)	
Birthweight † (g)													
8	SBP	0.00	0.387	0.01	0.150	-0.001	0.919	-0.001	0.943	0.04	0.344	0.05	0.336
weeks		(-0.01, 0.01)		(0.00, 0.02)		(-0.02, 0.02)		(-0.02, 0.02)		(-0.05, 0.14)		(-0.05, 0.15)	
	DBP	0.01	0.248	0.01	0.138	-0.03	0.087	-0.03	0.068	0.00	0.951	0.02	0.802
		(-0.01, 0.02)		(0.03, 0.02)		(-0.06, 0.00)		(-0.06, 0.00)		(-0.15, 0.14)		(-0.13, 0.17)	
8-18	SBP	0.02	0.236	0.02	0.127	0.01	0.815	0.01	0.824	0.13	0.460	0.35	0.112
weeks		(-0.01, 0.04)		(-0.01, 0.04)		(-0.05, 0.06)		(-0.05, 0.06)		(-0.23, 0.50)		(-0.09, 0.79)	
	DBP	0.01	0.581	0.02	0.345	-0.10	0.041	-0.08	0.091	0.15	0.445	0.19	0.361
		(-0.03, 0.05)		(-0.02, 0.06)		(-0.19, 0.00)		(-0.16, 0.01)		(-0.24, 0.54)		(-0.22, 0.60)	
18-30	SBP	0.00	0.794	0.001	0.954	-0.01	0.717	0.01	0.715	-0.05	0.738	-0.12	0.445
weeks		(-0.03, 0.02)		(-0.02, 0.03)		(-0.07, 0.05)		(-0.05, 0.07)		(-0.34, 0.24)		(-0.43, 0.19)	
	DBP	-0.06	0.003	-0.05	0.010	-0.09	0.046	-0.04	0.332	-0.36	0.111	-0.39	0.091
		(-0.10, -0.02)		(-0.08, -0.01)		(-0.17, 0.00)		(-0.12, 0.04)		(-0.80, 0.09)		(-0.84, 0.07)	
30-36	SBP	-0.03	0.000	-0.02	0.014	-0.07	0.000	-0.05	0.000	0.01	0.845	0.07	0.343
weeks		(-0.04, -0.01)		(-0.03, 0.00)		(-0.09, -0.04)		(-0.07, 0.02)		(-0.13, 0.15)		(-0.08, 0.22)	

Analyte/ gestation		Healthy (n= 2200-2288)				Gestational Hypertension (n=368-381)				Pre-eclampsia (n=48)			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
>36 weeks	DBP	-0.03 (-0.04, -0.01)	0.000	-0.01 (-0.03, 0.00)	0.068	-0.08 (-0.11, -0.05)	0.000	-0.01 (-0.08, -0.02)	0.001	-0.06 (-0.20, 0.08)	0.416	-0.05 (-0.21, 0.11)	0.522
	SBP	0.00 (-0.01, 0.01)	0.598	0.00 (-0.01, 0.01)	0.377	0.00 -0.02, 0.02)	0.983	0.00 (-0.02, 0.02)	0.875	0.10 (0.01, 0.19)	0.049	0.05 (-0.07, 0.17)	0.397
	DBP	-0.01 (-0.01, 0.00)	0.186	0.00 (-0.01, 0.01)	0.671	0.00 (-0.01, 0.02)	0.682	0.00 (-0.02, 0.02)	0.970	0.08 (0.01, 0.15)	0.37	0.05 (-0.04, 0.14)	0.240

Confounder Model 1: adjusted for maternal age, social class, education, smoking status, parity, BMI, and BP in the previous period

Confounder Model 2: additionally adjusted for offspring birthweight, gestational age, and mode of delivery

5.4 Discussion

In this Chapter, the maternal and offspring characteristics of those that developed HDP have been described and the impact of exposure to HDP on cord blood adipokines, lipids, and offspring birthweight has been examined. Overall, those that developed PE were younger, nulliparous, less likely to smoke, and have a higher booking BMI. Greater maternal BP at booking, with a steeper rise in SBP and DBP from 18 weeks' gestation, particularly in those with PE was also observed. Women with HDP were less likely to deliver vaginally, with more women requiring caesarean section than those in the healthy group. Birthweight and gestational age at delivery was also lower in those with HDP. Higher cord blood leptin and cholesterol were observed in those with HDP, particularly those with PE. The change in BP during each gestational period was associated with the offspring's metabolic profile at birth. In those that developed PE, a sequential increase in SBP at booking and during the 30 to 36-week gestational period was positively associated with cord blood HDLc. DBP was also positively associated with cord blood cholesterol during the third trimester. Beyond 36 weeks' gestation, however, SBP in the PE group was negatively associated with cord blood adiponectin. In those that developed GH, DBP during late pregnancy was negatively associated with cord blood leptin and birthweight.

Maternal characteristics of those with HDP described in this Chapter reflect commonly identified risk factors displayed in National guidelines (NICE, 2010). Smoking is a well-documented protector of developing PE and reduces the overall risk of developing the condition (Conde-Agudelo *et al.*, 1999), despite paradoxically increasing the risk of cardiovascular disease in later life (Mons *et al.*, 2015). This has been demonstrated in the current study by the fact that there were more women who smoked during pregnancy amongst the healthy group. The fall in the number of smokers in recent years, including pregnant women, along with the rise in prevalence of PE and obesity amongst the contemporary population may also reflect this changing trend in smoking status. BMI is also a known modifiable risk factor for PE (Duckitt and Harrington, 2005; Eiland, Nzerue and Faulkner, 2012; Sohlberg *et*

al., 2012) and this study has demonstrated higher BMI amongst mothers who developed HDP compared to the healthy group. First pregnancies are also at greater risk of PE and around 4% of nulliparous patients are affected (Hernández-Díaz, Toh and Cnattingius, 2009), which is in keeping with the findings of this study. Delivery by caesarean section was also more common amongst women with PE, as was lower birthweight and gestational age at birth. This may be iatrogenic due to worsening maternal disease warranting earlier surgical delivery or abnormal antenatal fetal monitoring requiring delivery in the fetal interest. Similarly, women who develop PE may also labour spontaneously earlier than reach the expected 'due date'. NICE reports that 20-25% of preterm births and 14-19% of term births in women with PE are growth restricted (NICE, 2010), as a result of abnormal placental development in PE. Whilst the current study found that most women with HDP delivered around 'term', it was not possible to account for the severity of the disease, nor was it possible to account for the condition of the offspring at birth or the health of the mother during this time which in current practice may have warranted earlier delivery. NICE have reported that 8-10% of all preterm births result from hypertensive disorders and half of the women with severe disease deliver preterm (NICE, 2010). These offspring are at greater future metabolic risk because prematurity itself has also been linked to lower levels of cord blood triglyceride (Kelishadi, Badiee and Adeli, 2007; Yonezawa *et al.*, 2009), and lower birthweight and prematurity have also been independently linked to hypertensive and cardiovascular disease in the offspring (Martin *et al.*, 2000). Lower birthweight amongst those with PE, but not with GH, was identified reflecting placental dysfunction associated with maternal toxaemia and the more wide-spread multi-system effects of the disease.

Maternal metabolic dysfunction may be detected during pregnancy affected by PE, as maternal serum has shown disruption to cholesterol, LDLc, HDLc, and triglyceride profiles, which may result in vascular dysfunction and confer greater cardiovascular risk (Khatua *et al.*, 1989; V. A. Rodie *et al.*, 2004; Ophir *et al.*, 2006; Catarino *et al.*, 2008; Wan Sulaiman *et al.*, 2016). Whilst the current study did not examine maternal blood analytes (rather, cord blood analytes) for this

study, higher cord blood leptin in the offspring of mothers with HDP was identified. This suggest that neonatal leptin levels may mirror that of the mothers' and implies in utero programming of the offspring during such pregnancies. Maternal blood pressure at booking and across each gestational period was also examined. In both GH and PE groups, it was identified that the blood pressure was higher at booking compared to the healthy reference group. This would indicate that those who developed HDP already had an underlying low-grade propensity to hypertension and potentially endothelial dysfunction prior to pregnancy itself. This is in keeping with previous reports from ALSPAC, as well as adding to evidence of greater long-term cardiovascular risk in mothers who developed PE during pregnancy (MacDonald-Wallis *et al.*, 2011; Staley *et al.*, 2015). It was also found that in those with GH and PE, the rate of increase in DBP was greater in the third trimester compared to the healthy reference group. This suggests that it is this late gestational period that is critical for monitoring the development of the disease. It is also in keeping with the fact that the onset of PE is most commonly beyond 28 weeks' gestation, with few developing severe disease prior to this and less than 1% requiring delivery as a result of PE before 34 weeks (NICE, 2010).

Whilst ALSPAC has previously demonstrated higher blood pressure in the offspring of mothers with HDP (Staley *et al.*, 2015), the adverse impact of PE on endothelial function however has not been observed universally (Lawlor *et al.*, 2012), and there is conflicting evidence about the offspring's cardiovascular risk, as measured by offspring adipokines, lipids, and birthweight. Elevated cord blood inflammatory markers and pronounced endothelial dysfunction in the offspring of mothers with pre-eclampsia have been reported by some (Jayet *et al.*, 2010; Kvehaugen *et al.*, 2011), even contributing to a greater risk of stroke in the offspring (Kajantie *et al.*, 2009). Offspring of mothers with PE may also display marked dyslipidaemia and altered placental lipid transport resulting in increased oxidative stress (Romanowicz and Bańkowski, 2009). In some studies, higher cord and neonatal triglyceride and LDLc, as well as lower HDLc levels, have been reported in the offspring of mothers with pre-eclampsia (Ophir *et al.*, 2006; Catarino *et al.*, 2008; Akcakus *et al.*, 2010). These findings however are inconsistent, as some have shown no difference in lipids

measured in the child or adolescent offspring of mothers with HDP (Tenhola *et al.*, 2003; Fraser *et al.*, 2012; Fraser, Nelson, *et al.*, 2013). In a small study by Tenhola et al., no difference in cholesterol, HDLc, or triglyceride was observed in the 12-year old offspring of mothers with pre-eclampsia compared to a control group (Tenhola *et al.*, 2003). In addition, there was no difference in the endothelial function of 5 to 8-year-old offspring of mothers with pre-eclampsia compared to a healthy cohort (Kvehaugen *et al.*, 2011). Furthermore, in a previous study from ALSPAC, no difference in endothelial function, arterial stiffness, brachial distension, or diameter was identified, nor did they show any difference in serum lipids or inflammatory markers in the 9 to 12-year old offspring of mothers with HDP compared to a healthy cohort (Fraser *et al.*, 2012). They did however show a positive association with offspring blood pressure (Fraser *et al.*, 2012) as in the 17-year old offspring from ALPSAC, the SBP and DBP in the offspring of mothers with HDP were higher despite no adverse effect of HDP on offspring lipid levels being identified (Fraser, Nelson, *et al.*, 2013).

The association with offspring BP is inconsistent as The Northern Finland Birth Cohort, did not identify any association in blood pressure (or lipid concentration) in the 16-year old offspring of mothers with pre-eclampsia compared to a healthy group (Miettola *et al.*, 2013). They did however find that offspring of those with GH had higher blood pressure (SBP and DBP) and cholesterol levels at age 16, suggesting that GH, rather than PE, led to a more unfavourable lipid profile. The Western Australia Pregnancy Cohort (Raine) reported on the clinical cardiovascular risk in 2868 offspring of mothers with HDP. They found that PE and hypertension (leading to preterm birth) contributed to a 3-fold increased risk of hypertension at age 20 years (Davis *et al.*, 2015). In the offspring of those with GH, the risk remained, albeit to a lesser degree, but these offspring were also at greater risk of overweight or obesity at age 20. They also observed that serum cholesterol, but not triglyceride or HDLc, levels were higher in 24-year old offspring born prematurely to mothers with PE (Lazdam *et al.*, 2010). This positive association with serum cholesterol was mirrored in the aforementioned study by Kvehaughen et al which found serum cholesterol levels to be higher in 5 to 8-year old offspring of mothers with PE (Kvehaugen *et al.*,

2011) compared to a healthy reference group. There was no significant association with offspring LDLc, HDLc, and triglyceride or inflammatory markers however between the groups. That higher cord blood leptin and cholesterol, but no difference in cord blood triglyceride or HDLc, in those with HDP was identified, suggests that HDP may exhibit some degree of cardio-metabolic programming in utero. Whist lower cord blood adiponectin levels in the offspring of HDP was identified, this trend was not significant, and although lower maternal adiponectin has been identified in PE pregnancy, the association was only evident amongst obese women (Thagaard *et al.*, 2019), which was not prevalent in this current cohort. Elevated leptin levels have also been demonstrated amongst toxaemic mothers and a rise in the adipokine may even precede the onset of PE (Poston, 2002). This may be as a result of increased placental production of leptin with hypoxia and inflammatory mediating factors giving rise to this. With the greatest difference in cord blood leptin identified in those with PE compared to a healthy group, as well as the associated lower offspring birthweight and higher cord blood cholesterol levels, it further compounds the multi-system effects of PE compared to the manifestation of GH alone. As there is inconsistent evidence of the impact of maternal HDP on offspring BP, lipids, or adipokines in later life, the long-term implications of these disrupted cord blood profiles at birth however remain unclear.

During this Chapter, maternal blood pressure throughout pregnancy in relation to offspring birthweight, and cord blood profiles was also examined. We observed amongst healthy and HDP groups a mid-pregnancy fall in BP followed by a progressive rise in BP and this pattern has been widely reported (MacDonald-Wallis *et al.*, 2011; Grindheim *et al.*, 2012; Farias *et al.*, 2014). Changes in BP during pregnancy may not be specific to those diagnosed with PE however, as greater booking DBP and greater increases in SBP and DBP in the second and third trimesters, even if they did not reach the threshold for HDP, have been associated with shorter gestational age, preterm birth and lower birthweight (Steer *et al.*, 2004; Bakker *et al.*, 2011; Macdonald-Wallis *et al.*, 2014). In this study, SBP at booking and changes in SBP in the third trimester were positively associated with cord blood HDLc in those that developed PE. DBP was also positively associated with cord blood cholesterol, and

SBP during late pregnancy was negatively associated with cord blood adiponectin amongst those with PE. The association with higher cholesterol and lower adiponectin highlights the translation of metabolic dysfunction onto the offspring, particularly as lower adiponectin confers greater cardiovascular risk and reflects greater insulin resistance, as discussed in previous Chapters 3 and 4. In those with GH, DBP in the third trimester was negatively associated with cord blood leptin and birthweight. Collectively, this indicates that changes in blood pressure, particularly during the third trimester, may have the greatest impact on the cord blood profile at birth. Insulin resistance may be involved in the pathogenesis of PE (S. *et al.*, 2013) and this may have added to the robustness of the data should it have been available. SBP and DBP have both been positively associated with cord blood leptin amongst those with PE (Rehman, Ahmad and Ullah, 2015), indicating that leptin may play a central role in the pathogenesis of PE. This may be secondary to sympathetic stimulation (Correia *et al.*, 2001), the influence on the renin-angiotensin-angiogenic system (Adamczak, Kokot and Wiecek, 2000), or as a result of pro-inflammatory cytokine production (Loffreda *et al.*, 1998).

The long duration of follow-up and wide range of maternal and offspring variables and mediators added to the strength of the analyses within this Study. The standard international definition of PE and HDP as described by NICE (NICE, 2010) was adhered to and even though this updated guideline was not available at the time of the original recruitment and data collection phase of the study, the thresholds for diagnosis of each condition was the same. Previous publications and analyses by ALSPAC enabled maternal blood pressure in relation to cord blood analytes to be examined across different gestational periods (MacDonald-Wallis *et al.*, 2011; Debbie Anne Lawlor *et al.*, 2012; Fraser, Nelson, *et al.*, 2013; Macdonald-Wallis *et al.*, 2014; Staley *et al.*, 2015).

Limitations of the study may also be acknowledged. The diagnosis of ‘proteinuria’ was made by research midwives using urine dipsticks which may have led to some variability in the interpretation of the findings. Similarly, the ‘gold standard’ for

diagnosis of proteinuria is by urinary polymerase chain reaction (PCR) which was not available at the time of this study. The numbers of those included in the study with PE were limited (n=85) by the prevalence of disease at the time and by complete case analyses. Numbers included may be higher if repeated in a more contemporary cohort with a greater prevalence of risk factors for the condition (higher maternal age, obesity, etc). There may also have been some variability in blood pressure measurements. This was standardised however to some degree using Omron monitors rather than the traditional sphygmomanometers. PE perhaps wasn't as well recognised a condition at the time of this study but the BP was measured at timely intervals during each pregnancy (mean 14 times) which suggests that even in the asymptomatic patient, PE or GH should have been detected if they had developed it (MacDonald-Wallis *et al.*, 2011). PE has traditionally been diagnosed by using a combination of high blood pressure and proteinuria which together would warrant tertiary referral for further investigation (Milne *et al.*, 2005). Other tertiary methods have been proposed or trialled in order to 'diagnose' the disease. For example, metabolomics and proteomics (Kenny *et al.*, 2010; Navaratnam *et al.*, 2013; Buhimschi *et al.*, 2014), detection of angiogenic factors (Andraweera, Dekker and Roberts, 2012), and the measurement of radiographic features such as uterine artery pulsatility index (O'Gorman *et al.*, 2016) have all been proposed but are fairly novel and specialised assessments and so were not available for this study.

Medication, such as antihypertensive treatment or anti-convulsant therapy that the participant may have been receiving as a result of their HDP, were not accounted for. Whilst there is little evidence to suggest that these would have directly influenced the cord blood, well-controlled BP at the time of delivery (as a result of antihypertensive treatment) may have led to some variability in the cord blood measures. Similarly, it was not possible to account for women taking daily low-dose aspirin, as recommended by the NICE guidelines (NICE, 2010) as discussed in Chapter 1, as these were not available at the time. Again, this may have also contributed to some variability since aspirin has been shown to reduce the risk of developing PE (Askie *et al.*, 2007). This study cohort included a mainly Caucasian population. Women of Afro-Caribbean origin are at greater risk of PE and therefore potentially

greater risk of CVD (Caughey *et al.*, 2005). Should this population have been included in the study, more associations with the cord blood profile at birth may have been evident. It was not possible to account for the severity of the disease or exclude those who had extreme pathology. PE, for example, may have led to convulsions and a state of 'eclampsia' or HELLP syndrome (haemolysis, elevated liver enzymes, low platelets), which again may have led to some variability in the cord blood analytes. Early-onset, severe pre-eclampsia typically occurs before 28 weeks' gestation. Lazdam et al. have previously demonstrated greater cardiometabolic risk in the offspring of those with severe, early-onset PE (Lazdam *et al.*, 2010, 2012) and so it may therefore have been useful to examine the cohort according to the onset of disease to determine whether this affected the offspring's cardiovascular risk. In addition, only live births were included in the study so that there was a viable paired cord blood sample. As PE accounts for 5% of all stillbirths in the absence of congenital abnormalities (NICE, 2010), such cases may not have been included in the study but given the small numbers, would not have been likely to influence the results.

In conclusion, this study has demonstrated that women who developed HDP were younger, non-smokers, had a higher BMI, and were more likely nulliparous compared to those that did not develop HDP. These risk factors are in keeping with those displayed in national guidance (NICE, 2010). Blood pressure was higher at booking in those that developed HDP, with the DBP rising more sharply in the third trimester. PE was associated with lower birthweight and lower gestational age at birth, both of which are independent risk factors for future metabolic dysfunction. In those that developed HDP, higher cord blood leptin and cholesterol were also demonstrated. Changes in BP during the third trimester (>30 weeks) were more consistently associated with the offspring's metabolic cord blood profile at birth, highlighting the importance of optimising BP control in later pregnancy. This study demonstrated that in the presence of HDP, the biochemical imprint at birth (as measured by cord blood adipokines, lipids, and birthweight) may be modified. As reported previously by others, including findings from ALSPAC, the long-term clinical consequences of these associations or the impact of HDP on the offspring longer-term remain unclear. In the next chapter, Chapter 6, in order to examine the longer-term implications of

an altered cardiometabolic profile at birth the trajectory of cord blood analytes was examined by tracking repeated measures of the same analyte through childhood and into adolescence.

Chapter 6: Programming of offspring cardiometabolic risk: tracking analytes from birth to adolescence

6.1 Introduction

As previously described, fetal life is a critical period and has potential for the development of adult chronic disease, with epidemiological studies demonstrating associations between birthweight and neonatal adiposity and metabolic disorders in child and adulthood (Pietiläinen *et al.*, 2001; Fraser *et al.*, 2008; Anderson *et al.*, 2014). An abnormal intrauterine environment, via greater maternal adiposity, diabetes, or PE, as discussed in the previous Chapters, may adversely affect the offspring's birthweight and cardio-metabolic profile at birth. There is growing evidence to suggest that not only are these cardio-metabolic profiles established at birth but that the trajectory of these cord blood measures is also determined in utero and that these pathways govern the metabolic health risk across the life course (Varvarigou, Mantzoros and Beratis, 1999; Hauguel-de Mouzon, Lepercq and Catalano, 2006; Gluckman and Hanson, 2007; Taylor and Poston, 2007).

Serum lipoproteins have been shown to track through life, as demonstrated by a 12-year follow-up programme in the Young Finns study (Porkka *et al.*, 1994). They demonstrated significant tracking of repeated measures of cholesterol, LDLc, HDLc, and triglycerides, and this trend was evident even more in males. This study suggested that by measuring serum lipoproteins in childhood there may be potential in identifying familial lipoprotein disorders and stratifying future cardiovascular risk. Cord blood biomarkers have however yet to be established for predictive purposes, partly due to the lack of longitudinal studies examining these associations. Although triglycerides have been inversely associated with fat mass (Ortega-Senovilla *et al.*, 2013), lipids have been positively correlated with cardiovascular disease occurring more than 20 years later (Klag *et al.*, 1993; Raitakari *et al.*, 2003). Lipids may

therefore act as surrogate markers of future cardiovascular risk, particularly as childhood obesity does not predict adult serum lipid levels (Porkka *et al.*, 1994).

LFTs have also displayed evidence of tracking of repeat analytes in adulthood (Patel *et al.*, 2007). In the Bogalusa heart study, serum samples obtained from adults aged 18 to 32 years showed that gamma-glutamyl transferase (GGT) and alanine transferase (ALT) biomarkers positively associated with repeated measures of the same analyte 12 years later. They also showed that high concentrations of these analytes were positively associated with markers of metabolic syndrome, including the risk of obesity, dyslipidaemia, type 2 diabetes, and hypertension (Patel *et al.*, 2007; Nguyen *et al.*, 2011). It has also been suggested that GGT may also be used to positively predict the onset of diabetes and metabolic syndrome 7 years later (Nannipieri *et al.*, 2005). In addition, ALT measured in the West of Scotland Coronary Prevention Study of men demonstrated that ALT may also positively predict T2DM over a 5-year follow-up in men (Sattar *et al.*, 2004).

There is limited evidence that adipokines, such as leptin, show evidence of tracking and it remains unclear if any such association begins in utero and persists throughout life. As previously discussed, leptin is a recognised biomarker of fat mass and ponderal index in the neonatal period (Hauguel-de Mouzon, Lepercq and Catalano, 2006; Karakosta *et al.*, 2011, 2013). In childhood, however, cord blood leptin inversely correlates with BMI but remains positively associated with leptin levels at age 3 (Mantzoros *et al.*, 2009). This finding is not uniform however as others have failed to identify an association between cord blood leptin and leptin in childhood (Volberg, Heggeseth, *et al.*, 2013). Associations in childhood have been more consistent as leptin measured on two separate studies at ages 2, 5, and 9 and at age 9 to 10 demonstrated positive association with repeat measures at least 3 years later, with associations strengthening with advancing age. This suggested that the variable may be genetically determined and that leptin did display evidence of tracking (Nishimura *et al.*, 2009; Volberg, Heggeseth, *et al.*, 2013). Adiponectin has been frequently inversely related to leptin levels and adiposity (Nishimura *et al.*,

2009; Volberg, Heggeseth, *et al.*, 2013) and has been reported as being both unrelated to (Lindsay *et al.*, 2003) and positively associated with birth weight and neonatal fat mass (Tsai *et al.*, 2004; Inoue *et al.*, 2008; Mantzoros *et al.*, 2009). In one study, cord blood adiponectin however was not associated with BMI or adiponectin levels at 3 years of age (Mantzoros *et al.*, 2009). In contrast, another study showed that neonatal adiponectin correlated with adiponectin levels at age 2, 5, and 9 (Volberg, Heggeseth, *et al.*, 2013). Again, the association with the repeated 'like for like' measure of the analyte strengthened with time. This has also been further demonstrated, with adiponectin at age 9 to 10 remaining positively associated with adiponectin measures 3 years later (Nishimura *et al.*, 2009).

The lack of longitudinal studies to date has limited the potential use of cord blood measures as biomarkers of long-term metabolic health. Several reviews and studies have highlighted the need to examine the relationship between key analytes from birth until later life and to determine the clinical consequences of these (Varvarigou, Mantzoros and Beratis, 1999; Stamler *et al.*, 2000; Hauguel-de Mouzon, Lepercq and Catalano, 2006; Gluckman and Hanson, 2007; Taylor and Poston, 2007). In this Chapter, the extent to which cord blood analytes track from birth (cord blood) into childhood and adolescence was examined. Specifically, given the associations described in later life, it was anticipated that there was tracking of repeated measures of lipid and LFTs when they were examined at serial time points.

6.2 Methods

6.2.1 Study Population

There were 5011 mother-offspring pairs included with a cord blood sample available. Details of obstetric and perinatal abstraction of data from patient case-notes and questionnaires completed are presented in Chapter 2. Details of cord blood sample collection and analysis are also presented in Chapter 2.

6.2.2 Assessment of outcomes

Identical protocols were used by ALSPAC at all follow-up clinics. Non-fasting blood was obtained by ALSPAC at the 7- and 9-year-old clinic. Fasting blood samples were obtained at the 15- and 17-year-old clinic. The samples were immediately spun and frozen at -80°C after the preparation of the sample. All the samples were assayed on behalf of ALSPAC at the University of Glasgow within 12 months of obtaining the sample and on the first thaw cycle. Cord blood lipid measures (cholesterol, triglyceride, HDLc, and consequently non-HDLc) were available at all four-time points whereas CRP was only available at age 9, 15, and 17. Leptin and adiponectin were available at age 9 and LFTs (GGT, ALT, AST) were available at age 9 and 17.

6.2.3 Statistical Analyses (provided by Dr. Smith)

As described in more detail in Chapter 2, SEMs were created for this study. Linear associations between analytes were assessed with a SEMs framework. Analytes measured in non-fasting participants at ages 7 and 9 were represented by a single latent variable, scaled to the measurement at age 9. Analytes measured at ages 15 and 17 in fasting participants were represented by a single latent variable, scaled to the measurement at age 17. As well as regression coefficients between cord blood

and age 9, and age 9 and age 17, regression coefficients were also calculated between cord blood and age 17. In doing so, adjustments were made for the possible mediating effect at age 9 to produce estimates of the direct effect of cord blood on age 17, and the indirect effect via the associations with age 9. As there was no evidence of sex interaction, results are presented combined for males and females.

The first model adjusted for offspring sex. The second model adjusted for potential confounders of the association between cord blood and later measures (offspring birthweight and gestational age at birth and maternal age, education, occupational social class, parity, pre-pregnancy BMI, and smoking status prior to and during pregnancy). The association between age 9 and later measures was also adjusted using a latent variable representing BMI z-scores at age 7 and 9, scaled to age 9. Estimation of regression coefficients was by maximum likelihood, using all available measures rather than complete case analysis.

As the SEM assumed that all variables were normally distributed, those that were skewed (CRP and leptin) were log-transformed. Some values were below the lower limit of detection (LLD) for GGT, ALT, and AST in the cord blood and at age 9. These were replaced with zero; the effect of this in the cord blood was investigated by repeating the analyses with these values fixed at the lower limit of detection, and age 9 by comparing complete case analyses with and without considering the measurement at age 9 to be a truncated variable. There were many values below the LLD for log-CRP; this was handled by allowing for a different intercept for those values.

6.3 Results

Figure 6.1 summarises the number of participants included and excluded within the study. There were 4937 (range 4859-5008) cord blood samples available but by age 9 there were 1690 (range 1690-1703) samples available and by age 17 there were only 1062 (1048 to 1064) samples available.

Figure 6.1: ALSPAC participant flow chart

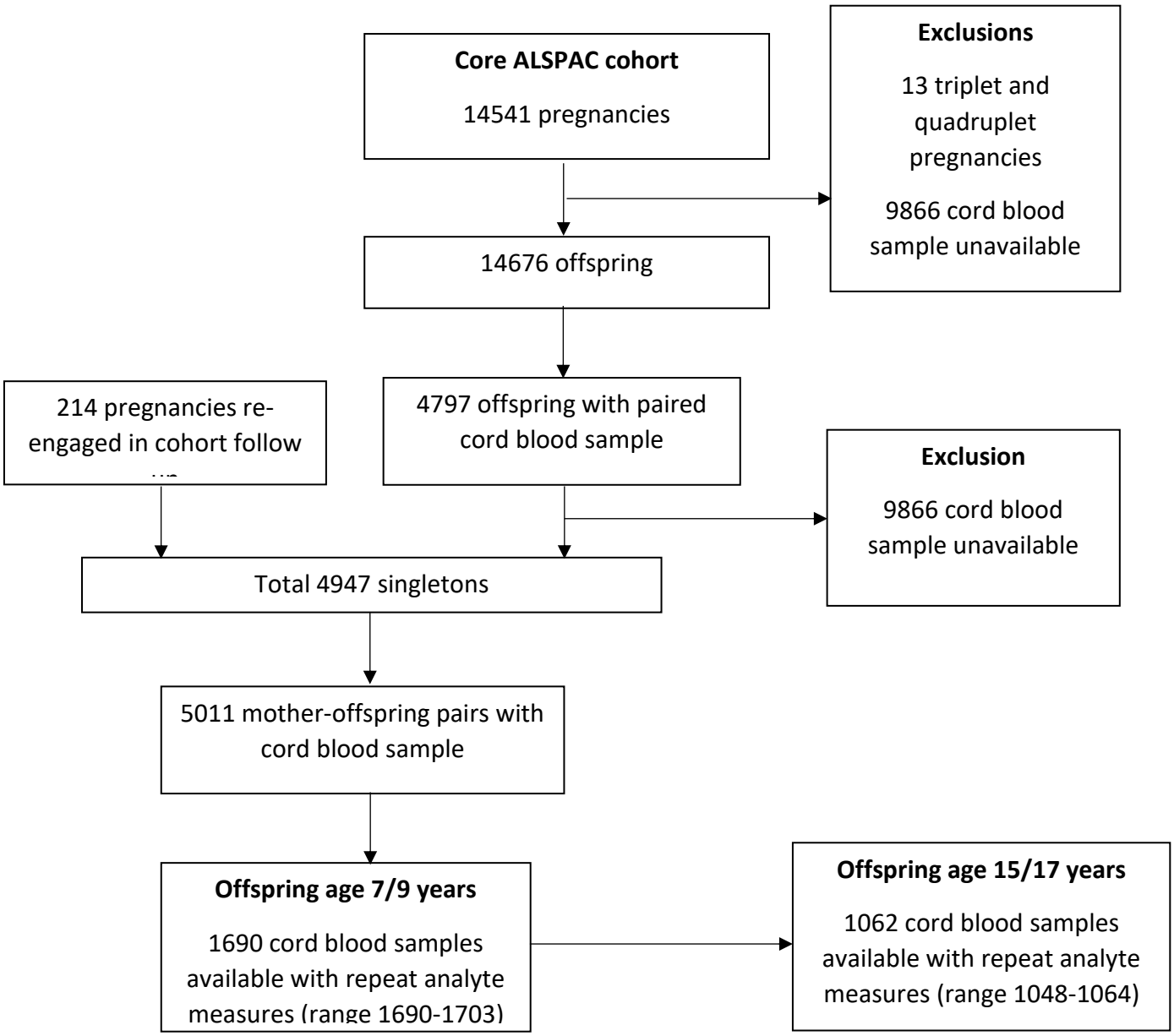


Table 6.1 summarizes the characteristics of the 5011 participants with a cord blood sample. Certain analytes were not assayed or sampled in all age groups. Leptin and adiponectin were not assayed at age 7, 15 or 17 and ALT, AST, and GGT were not assayed at age 7 or 15 either. Table 6.1 also displays the number and percentage (%) missing sample of each analyte in each age group compared to the cord blood sample availability.

Table 6.1: Characteristics of mother-offspring pairs with cord blood samples

	N obs	(%)	Median	Interquartile Range (IQR)
Maternal characteristics				
Age (years)	4797		28	6
Social class				
I (least)	808	19.4		
II	1138	27.2		
III	1555	37.1		
III	267	6.4		
IV	341	8.1		
V (most disadvantaged)	77	1.8		
Education				
Age 16 qualification	1507	65.4		
Post 16 qualification	972	22.3		
Post 18 qualification	535	12.3		
Smoking				
Non-smoker	2990	43.8		
Smoked before pregnancy	1452	31.8		
Smoked during pregnancy	1112	24.4		
BMI (kg/m^2)	4034		22.2	3.9
Offspring Characteristics				
Sex				
Female	2464	49.2		
Male	2547	50.8		
Analyte				
Triglyceride (mmol/l)				
Cord blood	4931		0.49	0.25
7 years (non-fasting)	1774		0.93	0.57
9 years (non-fasting)	1690		1.04	0.64
Number of missing samples at age 7-9 years	*1467*	*29.8*		
15 years (fasting)	1145		0.75	0.38
17 years (fasting)	1062		0.77	0.41

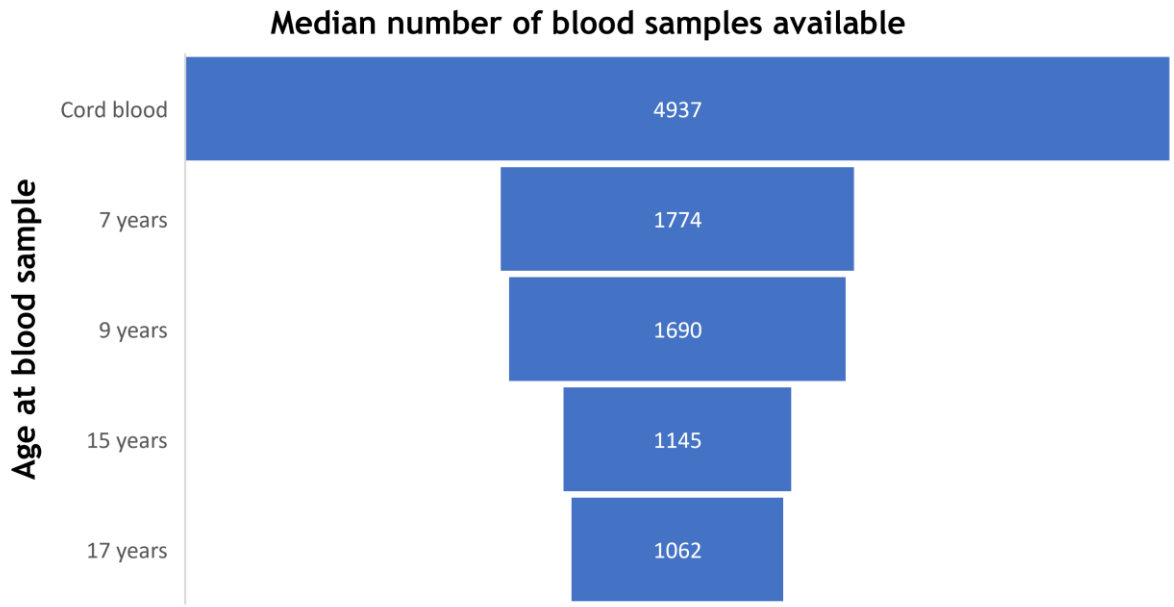
	N obs	(%)	Median	Interquartile Range (IQR)
Number of missing samples at age 15-17 years	*2724*	*55.2*		
Cholesterol (mmol/l)				
Cord blood	4946		1.65	0.66
7 years (non-fasting)	1774		4.41	0.85
9 years (non-fasting)	1690		4.23	0.81
Number of missing samples at age 7-9 years	*1482*	*30.0*		
15 years (fasting)	1145		3.72	0.81
17 years (fasting)	1062		3.69	0.93
Number of missing samples at age 15-17 years	*2739*	*55.4*		
HDLc (mmol/l)				
Cord blood	4859		0.49	0.29
7 years (non-fasting)	1774		1.50	0.38
9 years (non-fasting)	1690		1.38	0.40
Number of missing samples at age 7-9 years	*1395*	*28.7*		
15 years (fasting)	1145		1.25	0.37
17 years (fasting)	1062		1.23	0.39
Number of missing samples at age 15-17 years	*2652*	*54.6*		
Non-HDLc (mmol/l)				
Cord blood	4859		1.13	0.50
7 years (non-fasting)	1774		2.88	0.84
9 years (non-fasting)	1690		2.83	0.83
Number of missing samples at age 7-9 years	*1395*	*28.7*		
15 years (fasting)	1145		2.42	0.78
17 years (fasting)	1062		2.43	0.85
Number of missing samples at age 15-17 years	*2652*	*54.6*		
GGT (u/l)				
Cord blood	4931		95.00	86.00
9 years	1702		16.00	6.00
*Number of missing samples at age 9 years*17	*3229*	*64.5*		
years	1048		16.00	8.00
Number of missing samples at age 17 years	*3883*	*78.7*		
ALT (u/l)				
Cord blood	4941		1.30	1st quartile: LLD, 3rd quartile: 4.3
9 years	1703		1.10	3.10

	N obs	(%)	Median	Interquartile Range (IQR)
Number of missing samples at age 9 years	*3238*	*65.5*		
17 years	1048		14.80	7.30
Number of missing samples at age 17 years	*3893*	*78.8*		
AST (u/l)				
Cord blood	4937		52.40	37.80
9 years	1703		31.10	7.70
Number of missing samples at age 9 years	*3234*	*65.5*		
17 years	1048		19.40	6.70
Number of missing samples at age 17 years	*3889*	*78.8*		
CRP (mg/l)				
Cord blood	4931		LLD	1st and 3rd quartile: LLD
9 years	1690		0.21	0.45
Number of missing samples at age 9 years	*3241*	*65.7*		
15 years	1145		0.39	0.66
17 years	1062		0.61	1.15
Number of missing samples at age 15-17 years	*2724*	*55.2*		
Adiponectin (µg/ml)				
Cord blood	4964		75.90	45.30
9 years	1690		12.20	7.10
Number of missing samples at age 9 years	*3274*	*70.0*		
Number of missing samples at age 17 years	*4964*	*100.0*		
Leptin (pg/ml)				
Cord blood	5008		6.40	8.50
9 years	1690		5.40	7.00
Number of missing samples at age 9 years	*3318*	*66.3*		
Number of missing samples at age 17 years	*5008*	*100.0*		

LLD = below Lower Limit of Detection

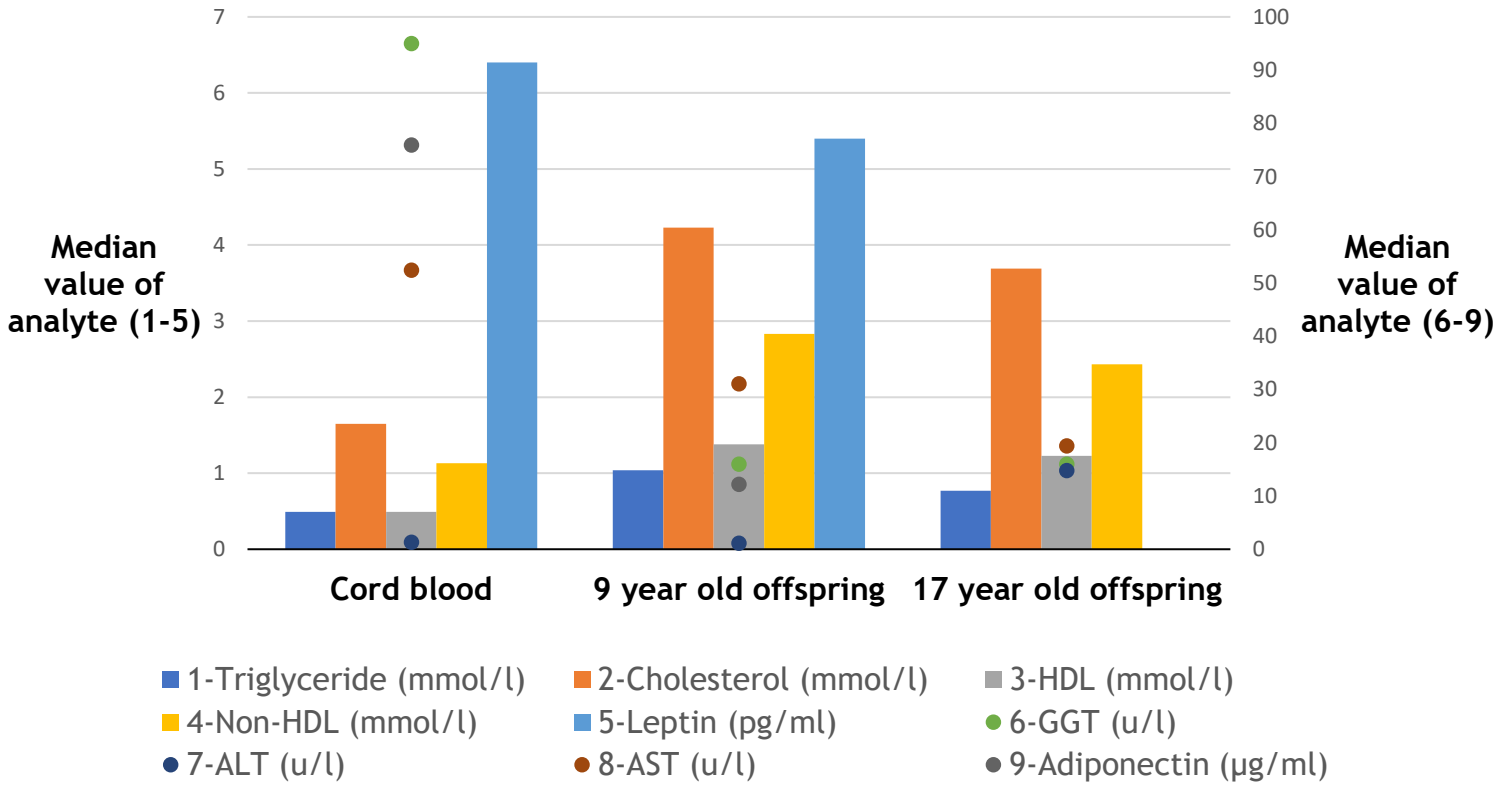
The median number of blood samples available in each age group is displayed in Figure 6.2.

Figure 6.2 Median number of samples available for each age range (cord blood to age 17)



The median concentration of each analyte in cord blood and 9-year-old offspring is displayed in Figure 6.3. Notably, the analyte ALT rose with repeat measures at advancing age (ALT in cord blood: 1.3 u/l, ALT at age 17: 14.8 u/l) whereas adiponectin, leptin GGT, and AST's levels all fell with repeat measures at each advancing age.

Figure 6.3 Median concentration of the analyte in cord blood and in 9 and 17-year-old offspring



To determine whether cord blood analytes correlated with repeat measures at age 9 and 17, were examined, and potential confounders were accounted for (Table 6.2, i and ii respectively, provided by Dr. Smith). Cord blood cholesterol, HDLc, non-HDLc, GGT, and adiponectin showed weak to moderate positive correlation with repeat measures of the same analytes at age 9 years (regression coefficients varied between 0.00 (GGT) and 0.19 (HDLc)).

Table 6.2 also shows the associations between analytes measured at age 9 and then repeated at age 17 (iii). There were strong associations between triglyceride, cholesterol, HDLc, non-HDLc, GGT, ALT, AST, and CRP at age 9 and repeat analytes at age 17, with regression coefficients between 0.24 (GGT) and 0.88 (cholesterol).

The association between cord blood and repeat measures at age 17, accounting for the relations between analyte measures in cord blood and age 9 and between age 9 and 17 (i.e. the indirect association), are presented in Table 6.2 (iv). Cord blood cholesterol, HDLc, and non-HDLc were indirectly associated with repeat analytes at age 17 with regression coefficients varying from 0.03 (cholesterol) and 0.15 (HDLc).

To examine the total effect of associations between cord blood and like-for-like analytes at age 17, intermediate associations between cord blood and age 17 analytes were adjusted for (Table 6.2, v). In doing so, the associations between analytes in cord blood and at age 9 and 17, and between age 9 and 17 were accounted for. There was a weak cumulative effect of the association between cord blood HDLc, non-HDLc, and GGT and repeat measures at age 17 years with regression coefficients varying from 0.02 (GGT) and 0.16 (HDLc).

Table 6.2: Associations between analyte measurements in cord blood and later blood samples (provided by Dr. Smith)

Analyte	Model	Direct effect (i) Association between cord blood and age 9 ß (95% CI)	p	Direct effect (ii) Association between cord blood and age 17 ß (95% CI)	p	Direct effect (iii) Association between age 9 and age 17 ß (95% CI)	p	Indirect effect (iv) Association between cord blood and age 17 ß (95% CI)	p	Total effect (v) Association between cord blood and age 17 ß (95% CI)	p
Triglyceride (mmol/l)	1	0.00 (0.00, 0.00)	0.998	-0.02 (-0.06, 0.02)	0.298	0.48 (0.36, 0.60)	0.000	0.00 (0.00, 0.00)	0.988	-0.02 (-0.06, 0.02)	0.299
	2	0.00 (0.00, 0.00)	0.904	0.03 (-0.06, 0.00)	0.070	0.46 (0.34, 0.58)	0.000	0.00 (0.00, 0.00)	0.904	-0.03 (-0.06, 0.00)	0.070
Cholesterol (mmol/l)	1	0.03 (0.01, 0.05)	0.013	0.00 (-0.03, 0.02)	0.789	0.87 (0.79, 0.96)	0.000	0.02 (0.01, 0.04)	0.014	0.02 (0.00, 0.05)	0.095
	2	0.03 (0.01, 0.05)	0.010	0.00 (-0.02, 0.02)	0.810	0.88 (0.79, 0.96)	0.000	0.03 (0.01, 0.05)	0.010	0.02 (0.00, 0.05)	0.074
HDLc (mmol/l)	1	0.19 (0.14, 0.24)	0.000	0.01 (-0.04, 0.05)	0.748	0.80 (0.73, 0.87)	0.000	0.15 (0.11, 0.20)	0.000	0.16 (0.11, 0.21)	0.000
	2	0.19 (0.14, 0.24)	0.000	0.01 (-0.04, 0.05)	0.724	0.79 (0.72, 0.86)	0.000	0.15 (0.11, 0.19)	0.000	0.16 (0.11, 0.21)	0.000
Non-HDLc (mmol/l)	1	0.03 (0.01, 0.06)	0.008	0.00 (-0.02, 0.02)	0.993	0.88 (0.80, 0.95)	0.000	0.03 (0.01, 0.05)	0.009	0.03 (0.00, 0.06)	0.037
	2	0.03 (0.01, 0.06)	0.007	0.00 (-0.02, 0.02)	0.890	0.87 (0.80, 0.95)	0.000	0.03 (0.01, 0.02)	0.007	0.03 (0.00, 0.06)	0.042
GGT (u/l)	1	0.00 (0.00, 0.01)	0.047	0.02 (0.01, 0.03)	0.002	0.31 (0.15, 0.48)	0.000	0.00 (0.00, 0.00)	0.076	0.02 (0.01, 0.03)	0.001
	2	0.00 (0.00, 0.01)	0.034	0.00 (0.01, 0.03)	0.001	0.24 (0.08, 0.41)	0.004	0.00 (0.00, 0.00)	0.084	0.02 (0.01, 0.03)	0.000
ALT (u/l)	1	0.08 (-0.08, 0.23)	0.318	-0.11 (-0.27, 0.05)	0.186	0.36 (0.27, 0.45)	0.000	0.03 (0.03, 0.08)	0.324	-0.08 (-0.24, 0.08)	0.335
	2	0.10 (-0.06, 0.25)	0.217	-0.06 (-0.22, 0.10)	0.475	0.35 (0.25, 0.44)	0.000	0.03 (-0.02, 0.09)	0.224	-0.03 (-0.19, 0.14)	0.764
AST (u/l)	1	0.00 (-0.02, 0.02)	0.963	0.01 (-0.01, 0.02)	0.427	0.52 (0.46, 0.58)	0.000	0.00 (-0.01, 0.01)	0.963	0.01 (-0.01, 0.02)	0.451
	2	0.00 (-0.02, 0.02)	0.946	0.01 (-0.01, 0.02)	0.433	0.54 (0.47, 0.62)	0.000	0.00 (-0.01, 0.01)	0.946	0.01 (-0.01, 0.02)	0.445

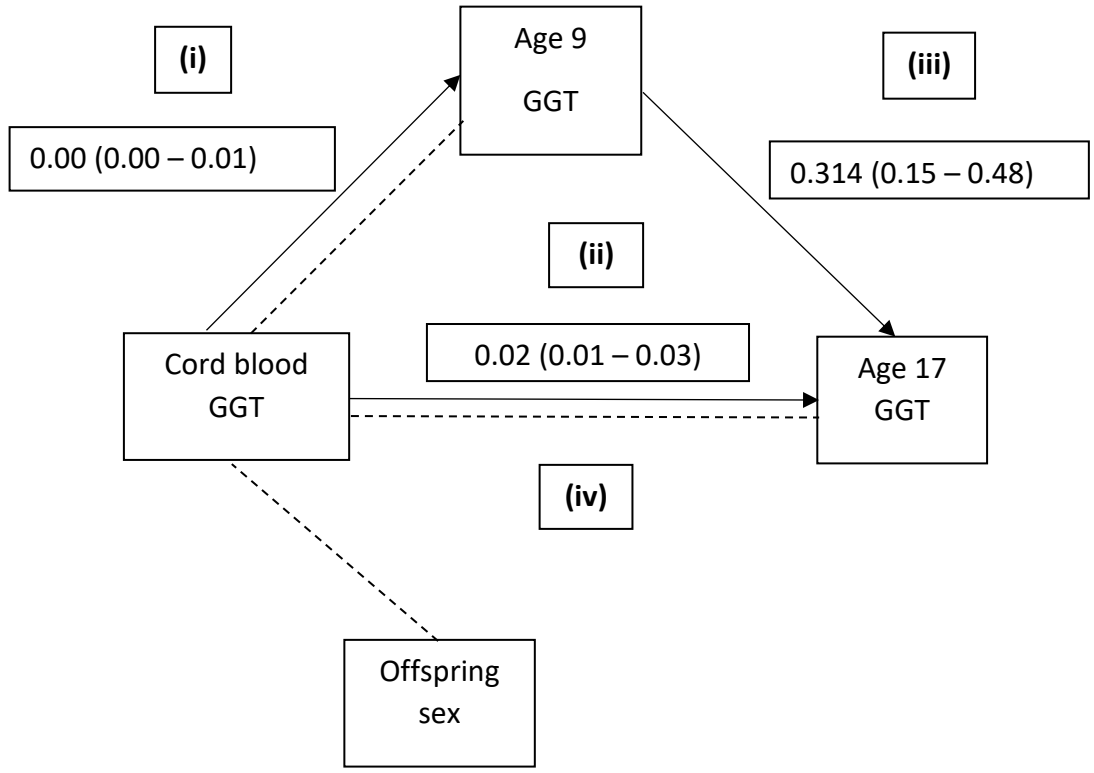
		Direct effect (i) Association between cord blood and age 9		Direct effect (ii) Association between cord blood and age 17		Direct effect (iii) Association between age 9 and age 17		Indirect effect (iv) Association between cord blood and age 17		Total effect (v) Association between cord blood and age 17	
Log CRP	1	-0.01		0.02		0.32	0.000	0.00		0.01	
(mg/l)		(-0.06, 0.04)	0.808	(-0.03, 0.06)	0.494	(0.26, 0.38)		(-0.02, 0.01)	0.808	(-0.03, 0.06)	0.556
	2	0.01		0.03		0.25	0.000	0.00		0.03	
		(-0.05, 0.05)	0.982	(-0.02, 0.07)	0.266	(0.19, 0.31)		(-0.01, 0.01)	0.982	(-0.02, 0.07)	0.270
Adiponectin	1	0.03									
(µg/ml)		(0.03, 0.04)	0.000								
	2	0.03									
		(0.03, 0.04)	0.000								
Log Leptin	1	0.04									
(pg/ml)		(0.00, 0.08)	0.059								
	2	0.01									
		(-0.03, 0.05)	0.748								

Model 1: Associations with cord blood adjusted for sex

Model 2: Associations with cord blood adjusted for sex, birthweight, gestational age at birth, and maternal age, parity, occupational social class, highest educational attainment, pre-pregnancy BMI, and smoking status before and during pregnancy. Associations between age 9 and age 17 additionally adjusted for offspring BMI z-score at age 9.

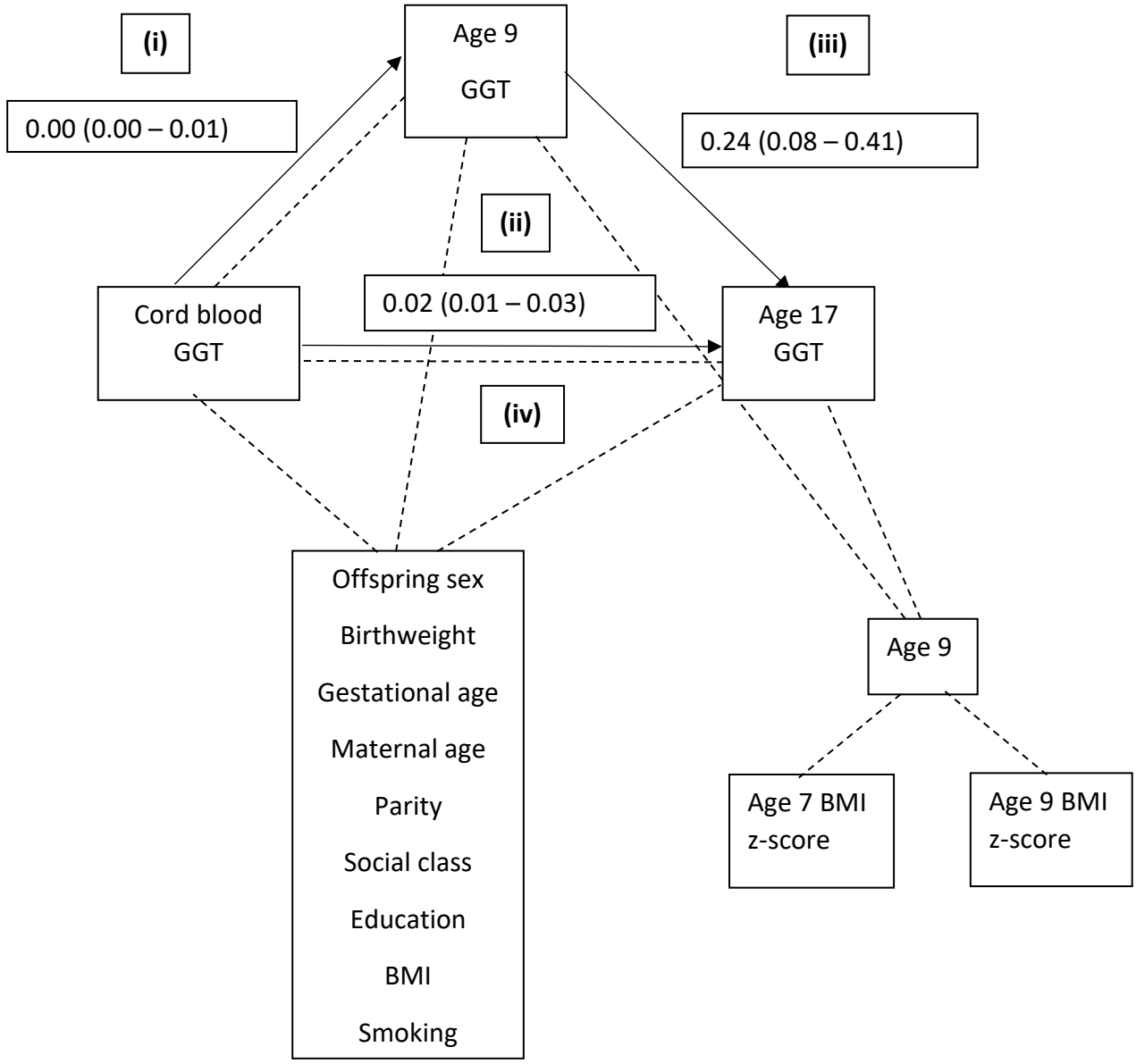
Cord blood GGT was also weakly positively associated with GGT at age 17 (regression coefficient 0.02), as shown in the simplest model in Figure 6.4.

Figure 6.4: Associations between GGT in cord blood, at age 9 and age 17 using model 1 (provided by Dr. Smith)



Cord blood GGT was also weakly positively associated with GGT at age 17 (regression coefficient 0.02) when adjusting for potential confounding in model 2, as shown in Figure 6.5.

Figure 6.5: Associations between GGT in cord blood, at age 9 and age 17 using model 2 (provided by Dr. Smith)



Liver function measurements below the lower limit of detection in the cord blood samples did not have a discernible effect on the analysis: the largest relative change in effect estimate was 0.8% when these values were replaced with the next smallest value, as shown in Table 6.3, provided by Dr. Smith.

Table 6.3: Associations between LFT's in cord blood and later blood samples, in 5011 participants with cord blood samples, where values below the lower limit of detection have been replaced with the next smallest value (provided by Dr. Smith)

	Direct effect (i)		**Direct effect (iii)**		**Direct effect (ii)**		**Indirect effect (iv= i × iii)**		**Total effect (v= i × iii + ii)**	
	Association between cord blood and age 9		**Association between age 9 and age 17**		**Association between cord blood and age 17**		**Association between cord blood and age 17**		**Association between cord blood and age 17**	
Analyte	Ƀ (95% CI)	p	Ƀ (95% CI)	p	Ƀ (95% CI)	p	Ƀ (95% CI)	p		
GGT (u/l)										
Model 1	0.004 (0.000, 0.008)	0.047	0.314 (0.149, 0.479)	<0.001	0.018 (0.007, 0.030)	0.002	0.001 (0.000, 0.003)	0.076	0.019 (0.008, 0.031)	0.001
Model 2	0.004 (0.000, 0.008)	0.034	0.244 (0.077, 0.412)	0.004	0.020 (0.009, 0.032)	0.001	0.001 (0.000, 0.002)	0.084	0.021 (0.010, 0.033)	<0.001
ALT (u/l)										
Model 1	0.077 (-0.076, 0.230)	0.323	0.360 (0.267, 0.453)	<0.001	-0.109 (-0.270, 0.053)	0.187	0.028 (-0.028, 0.083)	0.328	-0.081 (-0.245, 0.083)	0.335
Model 2	0.097 (-0.058, 0.251)	0.221	0.347 (0.253, 0.440)	<0.001	-0.059 (-0.221, 0.103)	0.478	0.033 (-0.021, 0.088)	0.228	-0.025 (-0.190, 0.140)	0.764
AST (u/l)										
Model 1	0.000 (-0.015, 0.016)	0.963	0.520 (0.457, 0.583)	<0.001	0.005 (-0.008, 0.019)	0.451	0.000 (-0.008, 0.008)	0.963	0.005 (-0.008, 0.018)	0.427
Model 2	0.001 (-0.015, 0.016)	0.945	0.541 (0.466, 0.616)	<0.001	0.006 (-0.009, 0.021)	0.445	0.000 (-0.008, 0.009)	0.945	0.006 (-0.008, 0.020)	0.433

Cord blood: Models 1: adjusted for sex, 2: adjusted for sex, birthweight, gestational age at birth, and maternal age, parity, occupational social class, highest educational attainment, pre-pregnancy BMI, and smoking status before and during pregnancy. Associations between age 9 and age 17 additionally adjusted for offspring BMI z-score at age 9

Adjusting for truncation of 9-year liver function measurements below the lower limit of detection also did not have a discernible effect on the analysis and the results presented were similar (Table 6.4, provided by Dr. Smith).

Table 6.4: Associations between liver function measurements in cord blood and later blood samples, in 5011 participants with cord blood samples (complete case analyses only), with and without adjustment for truncation below the lower limit of detection in the intermediate outcome (provided by Dr. Smith)

	Direct effect (i) Association between cord blood and age 9		**Direct effect (iii) Association between age 9 and age 17**		**Total effect (v) Association between cord blood and age 17**	
Analyte	ß (95% CI)	p	ß (95% CI)	p	ß (95% CI)	p
GGT (u/l) (n = 695)						
Without adjustment for truncation	0.00 (0.00, 0.01)	0.154	0.41 (0.17, 0.65)	<0.001	0.03 (0.01, 0.04)	0.001
With adjustment for truncation	0.00 (0.00, 0.01)	0.154	0.41 (0.17, 0.65)	0.001	0.03 (0.01, 0.04)	0.002
ALT (u/l) (n = 700)						
Without adjustment for truncation	-0.03 (-0.17, 0.10)	0.636	0.37 (0.26, 0.48)	<0.001	-0.21 (-0.41, -0.01)	0.042
With adjustment for truncation	-0.03 (-0.17, 0.10)	0.611	0.368 (0.26, 0.48)	<0.001	-0.20 (-0.39, 0.00)	0.048
AST (u/l) (n = 697)						
Without adjustment for truncation	0.00 (-0.01, 0.01)	0.790	0.28 (0.19, 0.36)	<0.001	0.01 (-0.01, 0.02)	0.925
With adjustment for truncation	0.00 (-0.01, 0.01)	0.790	0.28 (0.16, 0.36)	<0.001	0.00 (-0.01, 0.01)	0.975

All models adjusted for sex

Table 6.5 shows the correlation between birthweight, cord blood adipokines, and cord blood LFTs and outcomes at age 17: LFTs, glucose, insulin, lipids, BMI, and fat mass. There was a weak positive association between cord blood GGT and insulin, triglycerides, and BMI at age 17. Conversely cord blood GGT was weak negatively associated with glucose, cholesterol, HDLc, and fat mass at age 17.

Table 6.5: Spearman correlations between birthweight, cord blood leptin, adiponectin, LFTs and LFTs, glucose, insulin, lipids, BMI, and fat mass at age 17 (N obs, R)

	Cord blood					17 yr old offspring									
	Leptin	Adiponectin	GGT	AST	ALT	GGT	AST	ALT	Glucose	Insulin	Cholesterol	Triglyceride	HDL	BMI	FM
Birthweight(g)	5008	5011	4931	4937	4941	1048	1048	1048	1062	1044	1062	1062	1062	1647	1594
	0.29	0.13	-0.02	0.05	0.14	0.01	-0.05	-0.03	0.03	0.00	-0.05	-0.04	-0.01	0.10	0.05
Cord blood		5008	4929	4935	4939	1046	1046	1046	1060	1042	1060	1060	1060	1645	1592
leptin (pg/ml)		0.09	-0.16	-0.22	0.05	-0.03	-0.04	-0.04	-0.05	0.04	0.07	0.04	0.04	0.08	0.16
Cord blood			4931	4937	4941	1048	1048	1048	1062	1044	1062	1062	1062	1647	1594
adiponectin(µg/ml)			0.00	0.05	0.11	-0.01	0.01	-0.04	-0.07	0.01	0.06	-0.01	0.04	0.02	0.06
Cord blood				4930	4929	1025	1025	1025	1040	1022	1040	1040	1040	1619	1567
GGT (u/l)				0.27	0.14	0.16	0.06	0.05	-0.01	0.02	-0.03	0.05	-0.05	0.01	-0.01
Cord blood					4935	1028	1028	1028	1043	1025	1043	1043	1043	1623	1570
AST (u/l)					0.38	0.00	0.03	0.03	-0.04	0.05	0.01	0.07	0.00	0.03	0.03
Cord blood						1030	1030	1030	1045	1027	1045	1045	1045	1626	1573
ALT (u/l)						-0.07	-0.06	-0.03	-0.05	0.00	-0.04	-0.04	0.01	-0.03	-0.02
GGT at 17							1048	1048	1039	1029	1039	1039	1039	1022	1015
(u/l)							0.31	0.41	0.15	0.14	0.16	0.35	0.22	0.21	0.04
AST at 17								1048	1039	1029	1039	1039	1039	1022	1015
(u/l)								0.65	0.05	-0.10	0.06	0.05	-0.07	0.08	-0.11
ALT at 17									1039	1029	1039	1039	1039	1022	1015
(u/l)									0.03	0.04	0.09	0.05	0.11	0.25	0.08
Glucose at 17										1039	1062	1062	1062	1036	1029
mmol/l)										0.25	-0.02	0.04	-0.06	0.07	-0.05
Insulin at 17											1039	1039	1039	1018	1011
(mu/l)											0.12	0.31	-0.11	0.35	0.42
Cholesterol at 17												1062	1062	1036	1029
(mmol/l)												0.40	0.22	0.11	0.26
Triglyceride at 17													1062	1036	1029
(mmol/l)													-0.29	0.19	0.20
HDLc at 17														1036	1029
(mmol/l)														-0.21	0.08
BMI at 17 (kg/m^2)															1589
															0.78

In the Appendix, Table 5 shows the rank correlations between birthweight and cord blood lipids, LFTs, and adipokines. Birthweight was positively associated with cord blood leptin and to a lesser extent, cord blood adiponectin. Birthweight was also weakly associated with cord blood lipids. Cord blood GGT was positively associated with cord blood cholesterol and HDLc. Cord blood LFTs were all positively related to each other. In addition, Table 6 in the Appendix shows the associations between outcomes at age 17: BMI, fat mass, lipids, LFT's and glucose. LFTs at age 17 were also positively related to each other. GGT at age 17 was positively associated with glucose, insulin, cholesterol, triglycerides, and BMI at age 17.

6.4 Discussion

In this prospective birth cohort, cholesterol, HDLc, non-HDLc, GGT, and adiponectin were tracked positively from birth (cord blood) to age 9. GGT was the only analyte that continued to track positively from birth to age 17 when the associations were examined in the simplest form. Accounting for the correlations between analyte measures in cord blood and at age 9 and between age 9 and 17, there was an overall indirect association between cord blood cholesterol, HDLc, and non-HDLc and repeat analytes at age 17 (i.e. not GGT). There was also a weak cumulative effect of the association between cord blood and age 17 for HDLc, non-HDLc, and GGT, but not for cholesterol, when the associations between cord blood and repeat analytes at age 9 and 17, as well as the associations between analytes at age 9 and 17, were adjusted for.

As there was no direct association between cord blood HDLc and non-HDLc and repeat measures at age 17, the association identified as the cumulative effect was therefore simply the product of the associations between cord blood and age 9, and age 9 and 17. Whilst there was a direct association between GGT in cord blood and at age 17, the variation in GGT at age 9 was unlikely to account for this association. Finally, this study has demonstrated that all measured metabolic biomarkers (triglyceride, cholesterol HDLc, non-HDLc, GGT, ALT, AST, and CRP) obtained at age 9 tracked positively through to age 17.

Given that analytes in childhood may be used as surrogate markers of long-term metabolic health, identifying those at risk at the earliest feasible time point, such as using cord blood measures from birth, may have a significant impact on disease prevention or prediction. Although it was only possible to demonstrate that cord blood GGT was related to GGT at age 17, this may have the potential for identifying offspring that were at higher or lower risk of hepatic dysfunction in later life.

In the current study, cholesterol, HDLc, and non-HDLc were tracked from birth (cord blood) to age 9 but the direct association was lost beyond this, despite strong correlations between measures at age 9 and 17. These findings mirror previous reports associating lipids measured in childhood and later life (Porkka *et al.*, 1994; Raitakari *et al.*, 2003). The Young Finns Study, in which participants' age ranged from age 8-18 years, showed that total cholesterol, HDLc, and LDLc were tracked positively over a 12-year follow-up (Porkka *et al.*, 1994). Similarly, in a younger cohort, lipids obtained at age 5-14 years have also demonstrated evidence of tracking positively over 12 years (Webber *et al.*, 1991). This cohort demonstrated that tracking was more pronounced from age 9 onwards, also in keeping with the findings in the current study, as associations identified earlier in childhood were much weaker (Webber *et al.*, 1991). Lipids have been shown to decrease in concentration around puberty which may account for the lack of direct association (between cord blood lipids and repeat analytes at age 17) seen in the current study. This may also explain the weaker associations identified between cord blood and age 9 compared to the stronger correlation between analytes at age 9 and age 17 (Webber *et al.*, 1991). Overall, stronger associations from age 9 to 17 for triglyceride, cholesterol, and HDLc were identified than demonstrated in the Young Finns Study, which may be due to the larger sample size and set time points for sample collection in the current study.

Lipoprotein levels have been shown to have some predictive value, as not only are they higher in men, but men are also at greater risk of cardiovascular disease (Mahoney *et al.*, 1996). Identifying analyte associations that become more pronounced over time supports the role of lipoproteins (measured in late childhood) as biomarkers of future cardiovascular disease. In particular, cholesterol and triglyceride measured at age 15 may be used as predictors of cardiovascular disease as their elevated levels were significantly related to coronary artery calcification 15 to 20 years later (Mahoney *et al.*, 1996). Furthermore, LDLc measured at age 12-18 years strongly correlated with carotid intima-media thickness 21 years later (Raitakari *et al.*, 2003).

Adipokines have also been shown to track through childhood into adolescence and correlated with later adiposity outcomes (Geary *et al.*, 1999; Mantzoros *et al.*, 2009; Nishimura *et al.*, 2009; Volberg, Heggeseth, *et al.*, 2013). Leptin secretion signals the nutritional state of the brain (Bellone *et al.*, 2004) and may positively correlate with neonatal fat mass, birthweight, and gestational age (Bellone *et al.*, 2004). At later time points, it may also be positively related to body weight and BMI (Bellone *et al.*, 2004). Examining leptin across a wide age range (age 2-15) has shown that although the levels were overall low, they increased markedly with advancing age and adiposity in females, especially in the 13 to 15-year age group (Ong *et al.*, 1999; Sharrock *et al.*, 2008). This suggests that leptin becomes increasingly coupled with fat mass and perhaps energy status with age, particularly around puberty, and may reflect developmental changes in energy required for pregnancy and lactation (Sharrock *et al.*, 2008). Leptin levels in men have also been shown to be consistently low across the age range although they did not correlate with BMI in any age group (Sharrock *et al.*, 2008). This may be due to suppression of leptin by androgens around puberty, suggesting that leptin may be a poor signal of energy status in males (Böttner *et al.*, 2004; Kuzawa, Quinn and Adair, 2007; Sharrock *et al.*, 2008). In keeping with these reports, this study has shown consistently low leptin levels in cord blood and at age 9. It was however not possible to demonstrate any association between cord blood leptin and leptin at age 9. Overall low levels of leptin (reflecting lower birthweight in this specific cohort, compared to a contemporary population), the pre-pubertal status of the participants, or variations in the leptin and leptin receptor allele across populations may also account for the findings in the current study.

Adiponectin, the counter-part to leptin, is protective against diabetes, atherosclerosis, and metabolic syndrome (Kumada *et al.*, 2003; Spranger *et al.*, 2003). In the current study, adiponectin levels were highest at birth, and there was a weak positive association identified between cord blood adiponectin and adiponectin at age 9. Previously, this association was demonstrated to be stronger (Nishimura *et al.*, 2009). This disparity may be due to a shorter duration of follow-up and smaller sample size in the study and overlooking lifestyle factors as potential

confounders in the analyses (Nishimura *et al.*, 2009). Tracking of adiponectin from birth, irrespective of strength, could however suggest that adiponectin may be relatively unaffected by hormonal changes on the approach to puberty and unchanged by the degree of fat mass in childhood. With advancing age however, adiponectin levels may decrease and relate inversely to pubertal stage, BMI and obesity, particularly in males (Böttner *et al.*, 2004). This may account for the higher incidence of cardiovascular disease in males than females. It was not possible to examine associations in adolescence due to the absence of adipokine measures at this time and this may be a focus of future research.

After birth, with the cessation of placental function, the neonatal liver must adapt to the new environment and assume several hepatic functions. The current study has shown that GGT, AST, and ALT were all positively related to each other in cord blood and when they were measured at age 17. GGT activity in neonates may be significantly higher than the upper limit of normal in adults so the reference ranges may be age-dependent (Davidson, McIntosh and Ford, 1976; Cabrera-Abreu and Green, 2002; Melkie *et al.*, 2012). GGT is a very sensitive marker although its activity remains constant in healthy adults, normal pregnancy, and bone disorders (Davidson, McIntosh and Ford, 1976; Cabrera-Abreu and Green, 2002). It is therefore more specific for liver disorders in adults and children than other liver enzymes, such as alkaline phosphatase (Davidson, McIntosh and Ford, 1976; Cabrera-Abreu and Green, 2002). Glucose and lipid levels may influence GGT activity, however (Whitfield, 2001; Paolicchi *et al.*, 2004), and as such, GGT levels may fluctuate during specific periods of growth in childhood and adolescence, such as puberty (due to altered insulin concentration) (Goran and Gower, 2001; Levy-Marchal *et al.*, 2010). GGT may also increase as a result of oxidative stress as it is a marker of free radicle formation and inflammation (Karp, Shimooku and Lipsky, 2001). Furthermore, GGT rises earlier and persists longer in obstructive jaundice, cholangitis, and cholecystitis than aminotransferases (ALT and AST) (Cabrera-Abreu and Green, 2002). As an inducible enzyme, GGT levels may also be affected by a wide variety of medications and alcohol (Whitfield, 2001; Paolicchi *et al.*, 2004). In the adolescent age group, medications such as the oral contraceptive pill could influence the concentration of

GGT, making its value more difficult to interpret (Cabrera-Abreu and Green, 2002). In the current cohort, however, a minimal impact from such external factors at birth and in childhood would be expected. Consequently, GGT values may be more reliable to translate in clinical practice and thus justifying its role as a credible biomarker of metabolic disease than other blood-based liver function tests throughout the life-course (Fraser *et al.*, 2009; Nguyen *et al.*, 2011).

Whilst transaminases are sensitive to hepatocyte injury, the concentration of AST, in particular, may also increase in cardiovascular disease, muscular injury, and haemolysis (Davidson, McIntosh and Ford, 1976). Haemolysis may occur due to difficulty obtaining the sample or poor sample handling (Davidson, McIntosh and Ford, 1976). Newborn babies may also show higher levels of AST than infants, as demonstrated in the current study, due to placental release of AST into the fetal circulation and in contrast to ALT, may be found in a variety of sources (Davidson, McIntosh and Ford, 1976; Melkie *et al.*, 2012). A 'U'- shaped pattern of enzyme concentration has been observed for ALT and levels may be higher during periods of increased gluconeogenesis, such as infancy (Melkie *et al.*, 2012). ALT has been found predominantly in the liver and its concentration may rise in response to liver pathology or inflammation and oxidative stress (Salgado *et al.*, 2014). In the current study, hepatic immaturity at birth, lack of liver inflammation and varying ALT isoforms may account for the low cord blood levels of ALT which may increase as the neonatal liver takes on a more central role in gluconeogenesis. Neonates, who may be deficient in vitamin b6 (pyridoxine), may also have abnormally low ALT levels (Davidson, McIntosh and Ford, 1976). As there was no evidence in the current study that transaminases tracked from cord blood to age 9, it was not possible to use them for any predictive value. Whilst ALT has previously been shown to positively predict new-onset T2DM in adult males over a 5 year follow-up period (Sattar *et al.*, 2004), there was no evidence of this in the current study since the positive association between cord blood ALT and insulin at age 17 was weak.

Valuable clinical implications may be assumed from the negative associations that exist between GGT and birthweight, cord blood adipokines, and cord blood triglycerides. Larger weight babies for example, with a lower cord blood GGT (and higher leptin and adiponectin levels), may assume a lower cardiovascular risk at age 17 as demonstrated by lower insulin and lipid levels and lower BMI and fat mass. As GGT in adulthood has been positively correlated with GGT and systolic blood pressure (Patel *et al.*, 2007), type 2 diabetes (Nannipieri *et al.*, 2005; Nguyen *et al.*, 2011), and the metabolic syndrome (Nannipieri *et al.*, 2005) in later life, we would also have expected low cord blood GGT to be predictive of lower blood pressure and lower risk of type 2 diabetes in late adulthood. Its association with insulin resistance may be independent of age, gender, weight, BMI, or body fat (Ortega *et al.*, 2006). This particular study population of Pima Indians was however at high risk of diabetes mellitus anyway and its findings may not reflect multi-cultural phenotypes (Ortega *et al.*, 2006). In overweight and obese Korean children GGT, but not transaminases, has nevertheless also been positively associated with insulin resistance (Lee, Sung and Chang, 2013). In the current study, cord blood GGT associations were weak and inconsistent with markers of lipid and glucose homeostasis and adiposity outcomes at age 17 so its use as a metabolic marker may be limited. Overall though, GGT better reflects the dynamic changes in metabolic and insulin status as the child grows and develops compared to liver transaminases. Other factors such as differences in changes in DNA methylation and regulation of gene expression, more independence of what food you consume at age 17, and chance may also have accounted for the disparity between GGT and liver transaminases as valid biomarkers.

Strengths of the study included the size of the cohort, duration of follow-up, and repeat measures at specified time points. The study also has its limitations. At age 9, body composition and therefore serum lipid levels may not yet be affected significantly by puberty and this may have accounted for the associations identified between cord blood lipids (cholesterol, HDLc, and non-HDLc) and repeat measures at age 9. At age 17 however, the later stage in puberty may have accounted for the lack of associations between cord blood lipids and repeated measures in

adolescence. Although offspring lifestyle factors were not accounted for, BMI at age 9 was adjusted for and the analysis included assessment of the direct and cumulative associations between cord blood measures and measures at age 17.

The number of children who were overweight or obese however was smaller than many contemporary populations. Another limitation of the study was the loss to follow-up. However, results would be biased only if associations were substantially different among excluded participants and while this cannot be tested directly, it is unlikely to be the case. Cord blood sample degradation may have contributed to the variability seen but leptin, adiponectin, and lipids do appear to be stable with long-term storage (Shih *et al.*, 2000; Boyanton and Blick, 2002; Gislefoss, Grimsrud and Mørkrid, 2008; Brinc *et al.*, 2012). This study was limited by a lack of leptin and adiponectin measures at age 17. It was not possible to include cord b lood c-peptide, the preferred index of fetal glucose exposure, due to concerns regarding degradation with long-term storage, a phenomenon also previously reported by others (Gislefoss, Grimsrud and Mørkrid, 2009).

In conclusion, this study has demonstrated that lipids and LFTs measured at age 9 were strongly associated with repeat analytes at age 17. Novel correlations between cord blood cholesterol, HDLc, non-HDLc, GGT, and adiponectin, and repeat measures at age 9 have also been identified. Uniquely, this association for GGT persisted from birth to age 17. This adds to emerging evidence that cord blood analytes, reflecting the perinatal environment, may therefore be used as biomarkers of long-term programming and predictors of offspring metabolic health, with associations becoming more progressively defined with time.

Chapter 7: Adiposity in late childhood and adolescence: association with birth weight and cord blood adipokines

7.1 Introduction

As previously discussed, exposure to maternal adiposity during pregnancy has been positively associated with higher offspring birth weight and greater adiposity through childhood and adult life (Nelson, Matthews and Poston, 2009). In this Chapter, the association between offspring adiposity in late childhood and adolescence and cord blood analytes and birthweight was examined. Developmental overnutrition has been proposed as a mechanism, by which excessive transplacental passage of nutrients facilitates the development of larger babies with greater fat mass. Evidence from within sibling studies, comparisons of maternal and paternal exposures, and the use of genetic variants as proxies for the maternal exposures support developmental overnutrition causing greater adiposity in offspring at birth (Debbie A. Lawlor *et al.*, 2012; Lawlor, 2013; Tyrrell *et al.*, 2016). However, whether this causal effect extends to long-term offspring adiposity is unclear. A longer-term effect may occur as a result of tracking birth fatness across the life course. However, because birth weight is unable to distinguish relative contributions of lean vs fat mass (Sewell *et al.*, 2006; Jain *et al.*, 2016), few studies to date have been able to determine the extent to which greater fat mass at birth tracks into later life.

As previously described, umbilical cord blood leptin is widely recognised as an accurate biomarker for neonatal fat mass (Hauguel-de Mouzon, Lepercq and Catalano, 2006). Maternal exposures, including maternal adiposity, which may cause developmental overnutrition, have been associated with increased cord leptin and neonatal adiposity at birth (Catalano *et al.*, 2009; Lawlor *et al.*, 2014). In animal models fetal leptin has also been proposed to contribute to the long-term programming of hypothalamic feeding circuits, thereby providing a means by which leptin can influence long-term adiposity, independently of tracking of adiposity from

birth (Bouret, 2012). However, use of cord blood leptin in determining whether neonatal fat mass tracks across childhood has however been limited (Blüher and Mantzoros, 2009; Nakano, Itabashi and Maruyama, 2009; A. Lawlor *et al.*, 2010; Lindsay *et al.*, 2010; Boeke *et al.*, 2013; Kaar *et al.*, 2014). This primarily reflects the scarcity of large prospective birth cohorts with cord blood samples and detailed measures of offspring adiposity as well as potential confounders. Studies that have made some assessment of this to date have had relatively small sample sizes (N=56 to 588) (Blüher and Mantzoros, 2009; Nakano, Itabashi and Maruyama, 2009; A. Lawlor *et al.*, 2010; Lindsay *et al.*, 2010; Boeke *et al.*, 2013; Kaar *et al.*, 2014), and there were no studies that followed children beyond age 7 years. These studies have reported inconsistent results, with higher leptin associated with both a lower (Mantzoros *et al.*, 2009) and higher (Boeke *et al.*, 2013) BMI at age 3 years, and a higher BMI at age 7 years (Lindsay *et al.*, 2010).

Neonatal levels of adiponectin, which has insulin-sensitizing effects in adults, are approximately 4-7 times higher than maternal levels. Furthermore, whereas maternal circulating concentrations of adiponectin are inversely associated with BMI, higher levels of cord blood adiponectin are associated with an increased birth weight (Mantzoros *et al.*, 2009; Aye, Powell and Jansson, 2013). That higher cord blood adiponectin concentrations might reflect increased fat mass in neonates is suggested by mouse studies where over-expression of fetal adiponectin was positively related to the size of fat depots in early life, whereas adiponectin knockout fetuses display lower body weight and lower fat content (Qiao, Yoo, *et al.*, 2012). Given this direct effect of adiponectin on body composition, specifically enhancing fat deposition-enhancing effect in mice, and the known relationships of leptin in humans to far mass, it was hypothesised that both cord blood leptin and adiponectin would be positively associated with offspring adiposity in prepubertal children and adolescents.

This study aimed to determine whether cord blood leptin and adiponectin were positively associated with later obesity, BMI, waist circumference, and fat mass and

whether this is independent of maternal BMI. For comparison, associations of birthweight with these outcomes were also examined.

7.2 Methods

7.2.1 Study Population

A detailed outline of the exclusion criteria for this analysis and numbers with missing data is provided in Figure 7.2, but notably, participants were included if they had 1) attended and completed assessments at either the 9 or the 11-year clinic assessment, or 2) attended the 15 or 17-year clinic assessment. The eligible cohort for the current analysis was 2775 mother-offspring pairs at age 9-11 years and 2138 mother-offspring pairs at age 15-17 years. Details of cord blood sample collection and analysis have been presented in Chapter 2. Details of obstetric and perinatal abstraction of data from patient case-notes and questionnaires completed have also been presented in Chapter 2.

7.2.2 Offspring Adiposity Measurements

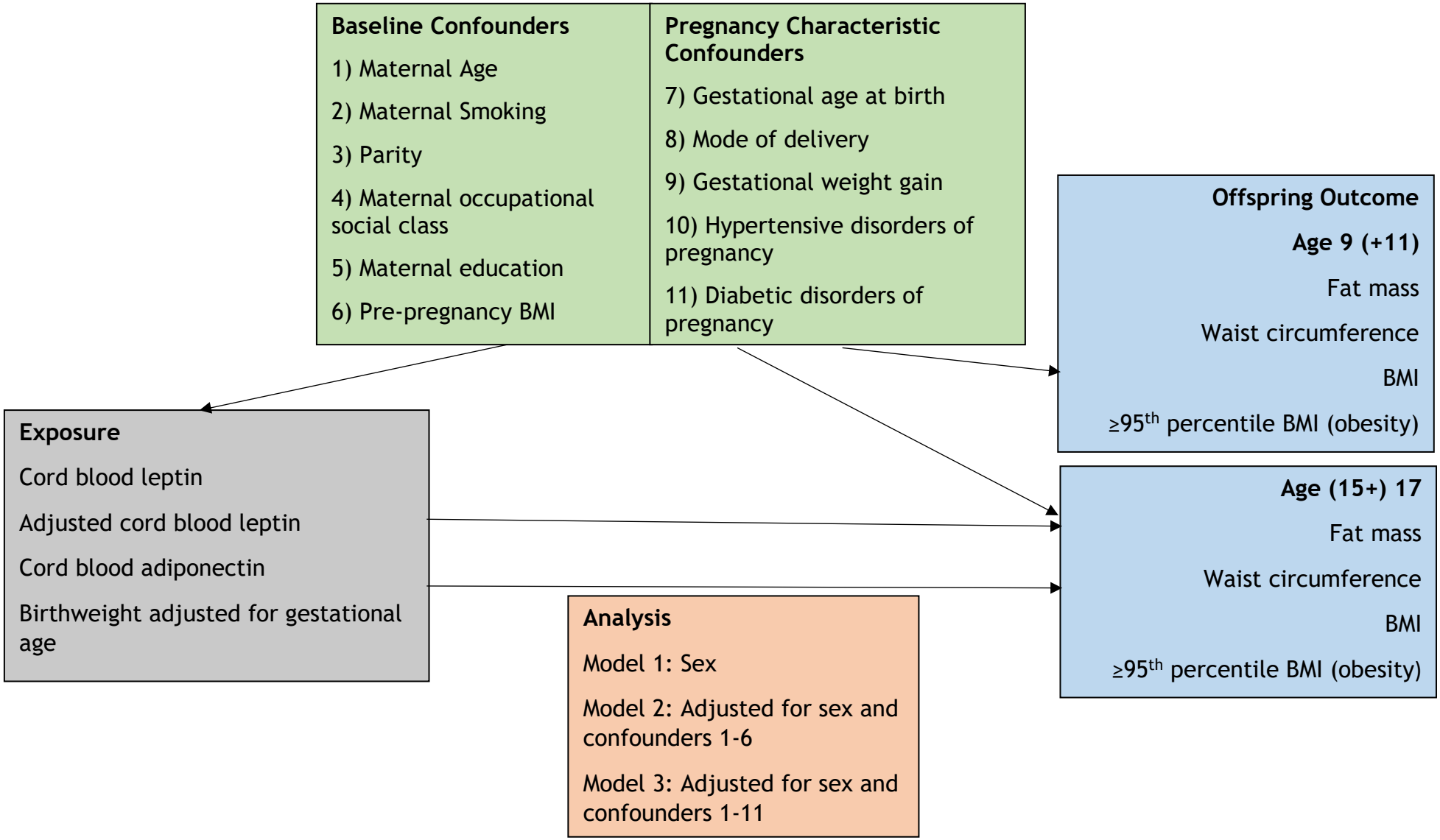
Identical protocols were used at all ALSPAC follow-up clinics. At each clinic assessment participants' age in months was recorded and their weight and height were measured in light clothing and without shoes. Weight was measured by ALSPAC to the nearest 0.1kg using Tanita scales. Height was measured by ALSPAC to the nearest 0.1cm using a Harpenden stadiometer. DEXA scans were used by ALSPAC to measure total fat mass. Waist circumference was measured by ALSPAC to the nearest 1mm at the midpoint between the lower ribs and the pelvic bone with a flexible tape and with the child breathing normally. Offspring obesity was classified using BMI and criteria defined by the International Obesity Task Force (Cole *et al.*, 2000).

7.2.3 Statistical Analysis

Linear and logistic regression models were used to examine the associations between birthweight and cord blood measures and offspring BMI, waist circumference, fat mass, and risk of obesity at age 9 and 17 years. Offspring BMI, waist circumference, and fat mass were log-transformed to produce approximately normal distributions of residuals. Fat mass and waist circumference z-scores were created internally using log outcomes adjusted for sex and age. In addition, BMI z-scores were created using the UK 1990 British growth reference for centiles (Cole *et al.*, 2000).

Figure 7.1 shows the DAG representing the direction of associations examined as well as the confounders considered in the analysis.

Figure 7.1: DAG



Birthweight was adjusted for sex, gestational age, and number of offspring (singletons or twins) using nonlinear regression fitting a Gompertz curve. Associations with leptin and with birthweight-adjusted leptin were examined to account for differential fat mass for any given birthweight; log-leptin was adjusted for log-birthweight using linear regression followed by extracting the residual and adding the mean value; birthweight-adjusted leptin was hence generated by exponentiation. Among those participants with assessments at both clinics, measurements at each clinic were highly correlated (Pearson's correlation coefficient was 0.93 for BMI and 0.93 for fat mass).

Three incremental analyses were performed in order to adjust for potential confounders. The basic model (model 1) adjusted for offspring sex alone (and offspring height when fat mass is the outcome). In model 2 for maternal confounders (age, smoking, parity, occupational social class, education, and pre-pregnancy BMI) were additionally adjusted for. In the fully adjusted model (model 3) pregnancy confounders (gestational age at birth, mode of delivery, gestational weight gain, hypertensive, and diabetic disorders of pregnancy) were additionally adjusted for.

There were small amounts of missing data on some co-variables included in the multivariable models. Twenty imputation data sets were generated by chained equations (White, Royston and Wood, 2011), with measurements from the 11-year clinic and 15-year clinic informing imputation of missing values in the 9-year clinic and 17-year clinic respectively. Results from the imputed datasets are presented first.

7.3 Results

The participant flow chart and the inclusion and exclusion criteria are displayed in Figure 7.2.

Figure 7.2: ALSPAC participant flow chart

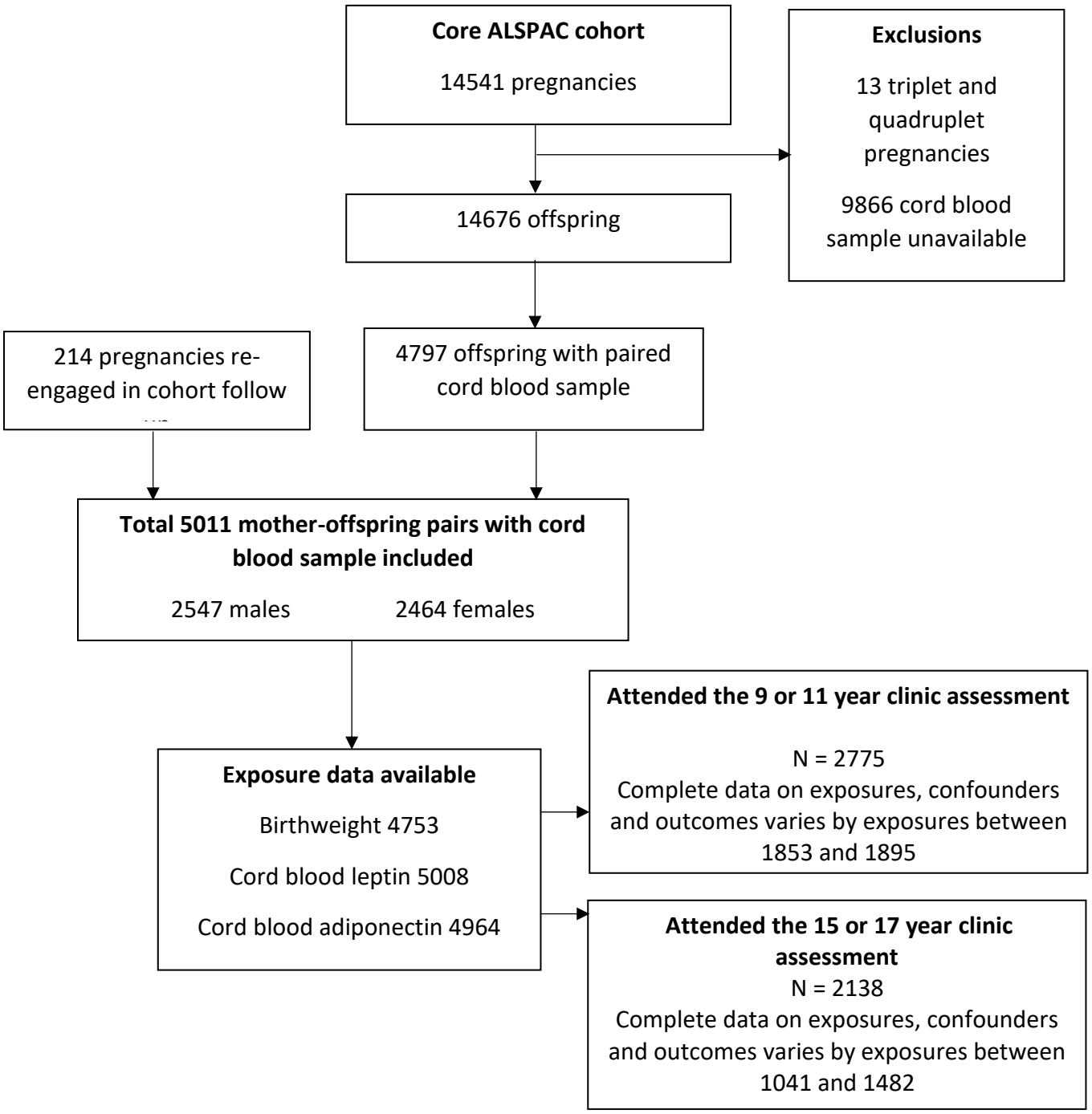


Table 7.1 summarises the maternal and offspring characteristics for those participants with cord blood measures, who completed at least one clinic assessment.

Table 7.1: Maternal and offspring characteristics

	Attended at least one clinic assessment (n=2955)	
	N obs (%)	Median (IQR)
Maternal Characteristics		
Age	2914	29 (26, 32)
Smoking		
Never	2103 (73.8)	
Before, not during	212 (7.4)	
During pregnancy	533 (18.7)	
BMI	2587	22.2 (20.5, 24.4)
Parity		
0	1274 (45.5)	
1	1011 (36.1)	
2	383 (13.7)	
3	101 (3.6)	
4+	30 (1.1)	
Education		
Left school at 16	1713 (61.3)	
A level	689 (24.8)	
Degree	391 (14.0)	
Social Class		
I (least)	140 (5.9)	
II	807 (33.7)	
IIIa	1038 (43.5)	
IIIb	162 (6.8)	
IV	203 (8.5)	
V (most disadvantaged)	40 (1.7)	
Pregnancy Characteristics		
Gestational age at birth (weeks)	2914	40 (39, 41)
Mode of delivery		
SVD	2253 (77.9)	
Breech	36 (1.3)	
Caesarean	249 (8.6)	
Forceps	167 (5.8)	
Vacuum	154 (5.3)	
Other	32 (1.1)	
GWG (kg)	2668	12.5 (9.5, 15.2)
Hypertension and PE		

	Attended at least one clinic assessment (n=2955)	
	N obs (%)	**Median (IQR)**
Nil	2449 (84.5)	
Hypertension/GH	420 (13.9)	
PE	49 (1.7)	
Diabetic disorders		
No glycosuria or diabetes	2651 (95.8)	
PGDM	10 (0.4)	
GDM	16 (0.6)	
Glycosuria	91 (3.3)	
Offspring Characteristics		
Sex		
Male	1414 (47.9)	
Female	1541 (52.2)	
Birthweight (kg)	2891	3.5 (3.1, 3.8)
Cord blood		
Leptin (pg/ml)	2952	6.4 (3.6, 12.1)
Adiponectin (µg/ml)	2927	75.7 (53.6, 98.4)
Height (cm)	Age 9: 2561	140 (136, 144)
	Age 11: 2363	151 (146, 156)
	Age 15: 1816	169 (163, 175)
	Age 17: 1648	170(164, 178)
FM (kg)	Age 9: 2460	7.3 (4.9, 11.2)
	Age 11: 2327	10.0 (6.8, 15.7)
	Age 15: 1716	13.7 (8.6, 20.6)
	Age 17: 1594	16.7 (11.0, 23.5)
WC (cm)	Age 9: 2574	61.1 (57.4, 66.6)
	Age 11: 2362	66.0 (61.8, 73.5)
	Age 15: 1475	75.4 (71.0, 81.5)
BMI (kg/m^2)	Age 9: 2560	17.0 (15.7, 19.1)
	Age 11: 2359	18.4(16.6, 21.0)
	Age 15: 1811	20.7 (19.0, 23.1)
	Age 17: 1647	22.0 (20.2, 24.7)
Obese	Age 9: 102 (4.0)	
	Age 11:116 (4.9)	
	Age 15: 78 (4.3)	
	Age 17:105 (6.4)	
Age at clinic attendance (years)	Age 9: 2583	9.8 (9.6, 10.0)
	Age 11: 2378	11.8 (11.6, 11.8)
	Age 15: 1838	15.4 (15.3, 15.6)
	Age 17: 1695	17.8 (17.6, 17.9)

Median (Interquartile range), Figures are numbers (%) unless stated otherwise

Table 7.2 shows the Spearman correlation between leptin, adiponectin, and birthweight. Birthweight was positively correlated with cord blood leptin (n=4751, r=0.33, p<0.001) and, to a lesser degree, with cord blood adiponectin (n=4707, r=0.14, p<0.001). Cord blood leptin and adiponectin were themselves positively correlated (n=4962, r=0.11, p<0.001).

Table 7.2: Spearman correlations between birthweight and cord blood analyte

	Leptin (pg/ml) N obs R	Adiponectin (ng/ml) N obs R
Birthweight (g)	4751 0.33	4707 0.14
Leptin (pg/ml)		4962 0.11

Offspring anthropometry at age 9 and 17 are displayed in Figure 7.3.

Figure 7.3: Offspring anthropometry (height, FM, WC, and BMI (kg/m^2) according to age

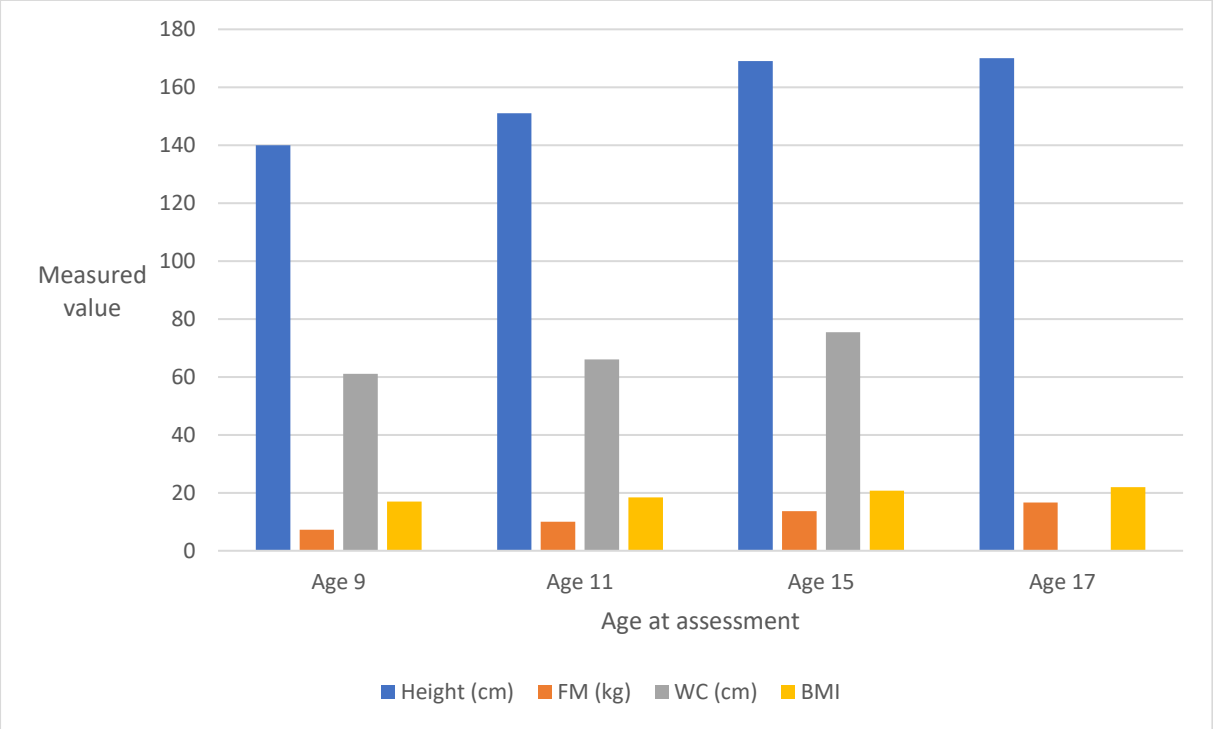


Table 7.3 shows the multivariable associations between cord blood leptin, leptin adjusted for birthweight, adiponectin, and birthweight and z-scores of offspring fat mass, waist circumference, BMI, and the risk of obesity at age 9 years.

Cord blood leptin (both total and adjusted for birth weight) was positively associated with z-scores of fat mass, waist circumference, and BMI at age 9, however, the effect size was largely attenuated with adjustment for maternal and pregnancy characteristics. Cord blood adiponectin was not associated with any measures of adiposity at age 9.

Birthweight was positively associated with z scores of fat mass, waist circumference, and BMI at age 9 years and showed a weak relationship with obesity. After adjustment for maternal and pregnancy characteristics increasing birthweight remained associated with greater waist circumference and BMI, with the association with fat mass and obesity attenuated to the null.

Table 7.3: Associations of birthweight and cord blood analyte with FM, WC, and BMI z-scores, and obesity outcome at age 9 years. N= 2775

Outcome		FM z-score *			WC z-score			BMI z-score			Obesity		
Exposure	Model	Coefficient	95% CI	P	Coefficient	95% CI	P	Coefficient	95% CI	P	OR	95% CI	P
Leptin	1	0.07	0.04, 0.10	<0.001	0.08	0.05, 0.12	<0.001	0.11	0.07, 0.15	<0.001	1.15	1.00, 1.31	0.046
(10pg/ml)	2	0.04	0.00, 0.07	0.023	0.05	0.01, 0.08	0.008	0.06	0.02, 0.10	0.003	1.00	0.85, 1.17	0.993
	3	0.03	0.00, 0.06	0.086	0.04	0.00, 0.07	0.045	0.04	0.00, 0.08	0.029	0.95	0.81, 1.12	0.548
Adjusted	1	0.06	0.03, 0.10	<0.001	0.05	0.01, 0.09	0.009	0.07	0.02, 0.11	0.002	1.09	0.93, 1.29	0.283
Leptin†	2	0.04	0.00, 0.07	0.030	0.02	-0.02, 0.06	0.281	0.03	-0.01, 0.07	0.151	0.96	0.80, 1.16	0.702
(10pg/ml)	3	0.03	0.00, 0.06	0.059	0.01	-0.02, 0.05	0.452	0.02	-0.02, 0.06	0.326	0.94	0.78, 1.13	0.521
Adiponectin	1	0.00	-0.01, 0.01	0.828	-0.01	-0.02, 0.00	0.072	0.00	-0.02, 0.01	0.602	0.99	0.94, 1.05	0.845
(10µg/ml)	2	0.00	-0.01, 0.01	0.916	-0.01	-0.02, 0.00	0.118	0.00	-0.01, 0.01	0.858	1.00	0.94, 1.05	0.874
	3	0.00	-0.01, 0.01	0.875	-0.01	-0.02, 0.00	0.100	0.00	-0.01, 0.01	0.767	0.99	0.94, 1.05	0.834
Birthweight	1	0.01	0.00, 0.02	0.006	0.03	0.03, 0.04	<0.001	0.04	0.03, 0.05	<0.001	1.06	1.02, 1.10	0.006
‡ (100g)	2	0.00	0.00, 0.01	0.192	0.03	0.02, 0.04	<0.001	0.04	0.03, 0.04	<0.001	1.03	0.99, 1.07	0.193
	3	0.00	-0.01, 0.01	0.741	0.02	0.02, 0.03	<0.001	0.03	0.02, 0.04	<0.001	1.01	0.96, 1.05	0.852

Models 1: Adjusted for offspring sex, 2: Adjusted for offspring sex and maternal confounders (age, smoking, parity, occupational social class, education, and pre-pregnancy BMI), 3: Adjusted for offspring sex and maternal confounders plus pregnancy confounders (gestational age at birth, mode of delivery, gestational weight gain, hypertensive disorders and diabetic disorders of pregnancy). * Fat mass adjusted for height † Leptin adjusted for birthweight ‡ Birthweight adjusted for sex, gestational age and singleton/twin pregnancy

Table 7.4 shows a similar but weaker pattern for cord blood leptin examined at ages 15 and 17 where there was a significant association with z-scores of fat mass, waist circumference, and BMI and with the risk of obesity. These associations were however absent after adjustment for potential confounders.

In contrast to the lack of association identified at age 9, cord blood adiponectin was positively associated with z-scores of fat mass and waist circumference at age 17, with the effect size, strengthened after adjustment for maternal and pregnancy characteristics.

Birthweight remained positively associated with z-scores of fat mass, waist circumference, and BMI at 17 years and showed a weak relationship with obesity. After adjustment for maternal and pregnancy characteristics increasing birthweight remained associated with greater waist circumference and BMI, with the association with fat mass and obesity attenuated to the null.

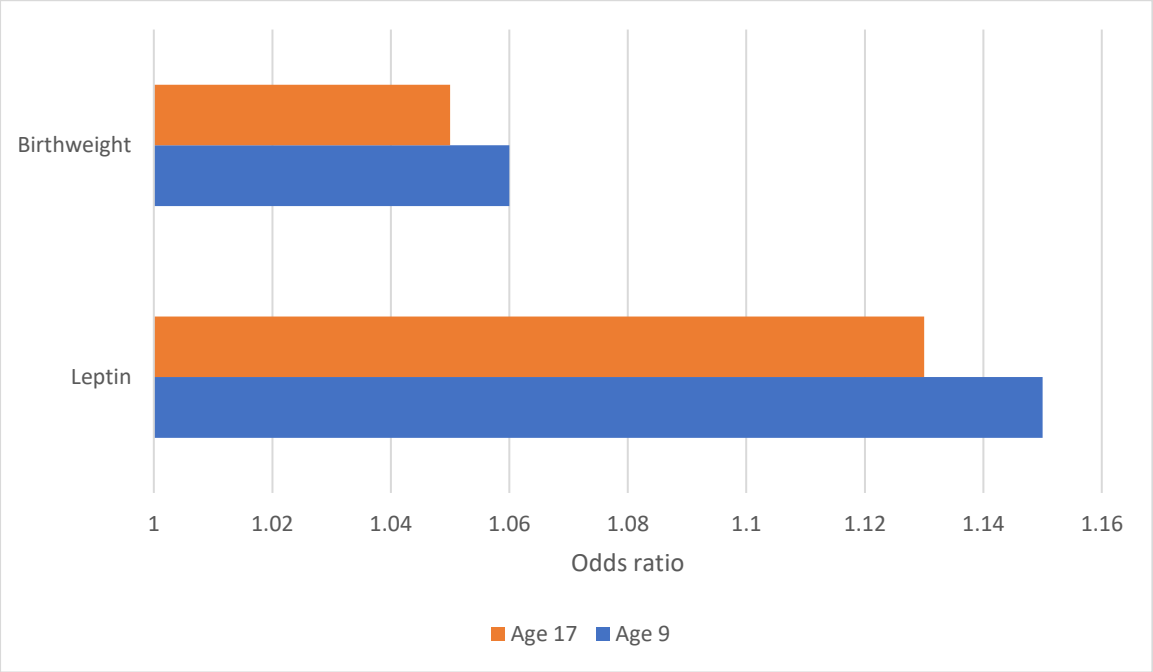
Table 7.4: Associations of birthweight and cord blood analyte with FM, WC, BMI z-scores and obesity outcomes at age 17 years. N= 2138

Outcome		FM z-score *			WC z-score			BMI z-score			Obesity		
Exposure	Model	Coefficient	95% CI	P	Coefficient	95% CI	P	Coefficient	95% CI	P	OR	95% CI	P
Leptin	1	0.07	0.03, 0.11	<0.001	0.06	0.02, 0.10	0.003	0.09	0.04, 0.14	<0.001	1.13	0.99, 1.28	0.060
(10pg/ml)	2	0.02	-0.02, 0.06	0.263	0.01	-0.03, 0.05	0.545	0.03	-0.02, 0.07	0.272	0.96	0.83. 1.12	0.629
	3	0.02	-0.02, 0.05	0.444	0.01	-0.03, 0.05	0.598	0.02	-0.03, 0.06	0.481	0.95	0.81, 1.11	0.497
Adjusted	1	0.05	0.01, 0.09	0.022	0.01	-0.03, 0.06	0.633	0.05	0.00, 0.10	0.041	1.09	0.94, 1.26	0.238
Leptin†	2	0.01	-0.03, 0.06	0.486	-0.03	-0.07, 0.02	0.211	0.01	-0.04, 0.05	0.810	0.96	0.81, 1.14	0.660
(10pg/ml)	3	0.01	-0.03, 0.05	0.589	-0.03	-0.07, 0.01	0.193	0.00	-0.05, 0.05	0.995	0.96	0.81, 1.14	0.613
Adiponectin	1	0.01	0.00, 0.03	0.034	0.01	0.00, 0.03	0.033	0.01	-0.01, 0.02	0.245	1.03	0.98, 1.08	0.238
(10µg/ml)	2	0.02	0.00, 0.03	0.006	0.02	0.00, 0.03	0.008	0.01	0.00, 0.03	0.076	1.04	0.99, 1.10	0.660
	3	0.02	0.00, 0.03	0.007	0.02	0.00, 0.03	0.008	0.01	0.00, 0.03	0.080	1.05	0.99, 1.10	0.613
Birthweight	1	0.02	0.02, 0.03	<0.001	0.04	0.03, 0.05	<0.001	0.04	0.03, 0.05	<0.001	1.05	1.02, 1.09	0.004
‡ (100g)	2	0.01	0.00, 0.02	0.010	0.03	0.02, 0.04	<0.001	0.02	0.01, 0.03	<0.001	1.02	0.98, 1.06	0.241
	3	0.01	0.00, 0.02	0.098	0.03	0.02, 0.04	<0.001	0.02	0.01, 0.03	<0.001	1.01	0.97, 1.05	0.516

Models 1: Adjusted for offspring sex, 2: Adjusted for offspring sex and maternal confounders (age, smoking, parity, occupational social class, education, and pre-pregnancy BMI), 3: Adjusted for offspring sex and maternal confounders plus pregnancy confounders (gestational age at birth, mode of delivery, gestational weight gain, hypertensive disorders and diabetic disorders of pregnancy). * Fat mass adjusted for height † Leptin adjusted for birthweight ‡ Birthweight adjusted for sex, gestational age and singleton/twin pregnancy

Figure 7.4 shows the odds of obesity per 10 pg/ml rise in cord blood leptin using the simplest model of analysis.

Figure 7.4: Odds of obesity (model 1) at age 9 and 17 per 100g rise in offspring birthweight and per 10pg/ml rise in cord blood leptin



The distributions of observed and imputed variables were similar, as shown in Table 7.5.

Table 7.5: Characteristics of observed and imputed data at age 9 and 17 years

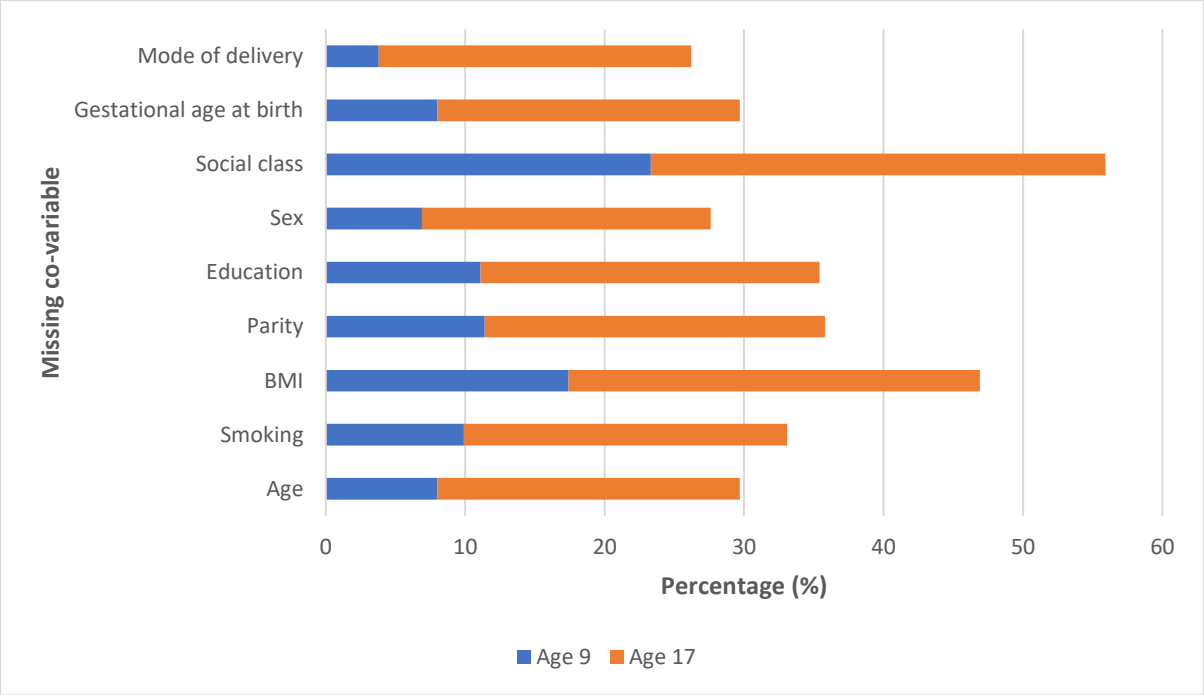
	Observed at 9			Multiply Imputed at 9		Observed at 17			Multiply Imputed at 17	
	N missing (%)	N obs (%)	Median IQR	N obs (%)	Median IQR	N missing (%)	N obs (%)	Median IQR	N obs (%)	Median IQR
Maternal Characteristics										
Age	221 (8.0)	2554	29 26, 32	2775	29 26, 32	465 (21.7)	1673	29 26, 32	2138	29 26, 32
Smoking	274 (9.9)					497 (23.2)				
Never		1894 (75.7)		2077 (74.9)			1273 (77.6)		1633 (76.4)	
Before, not during		178 (7.1)		198 (7.1)			118 (7.2)		155 (7.3)	
During pregnancy		429 (17.2)		500 (18.0)			250 (15.2)		350 (16.4)	
BMI	483 (17.4)	2292	22.2 20.5, 24.4	2775	22.4 20.5, 24.7	630 (29.5)	1508	22.0 20.5, 24.2	2138	22.2 20.5, 24.4
Parity	317 (11.4)					522(24.4)				
0		1124 (45.7)		1294 (46.6)			772 (47.8)		1036 (48.5)	
1		906 (36.9)		999 (36.0)			580 (35.9)		749 (35.0)	
2		322 (13.1)		364 (13.1)			196 (12.1)		265 (12.4)	
3		84 (3.4)		96 (3.5)			50 (3.1)		64 (3.0)	
4+		22 (0.9)		22 (0.8)			18 (1.1)		24 (1.1)	
Education	309 (11.1)					519(24.3)				
Left school 16		1479 (60.0)		1,685 (60.7)			896 (55.3)		1236 (57.8)	
A level		628 (25.5)		696(25.08)			437 (30.0)		565 (26.4)	
Degree		359 (14.6)		394 (14.2)			286 (17.7)		337 (15.8)	
Social Class	646 (23.3)					696(32.6)				
I (least)										
II		129 (6.1)		148 (5.3)			107 (7.4)		128 (6.0)	
IIIa		730 (34.3)		916(33.01)			520 (36.1)		737 (34.5)	

	Observed at 9			Multiply Imputed at 9		Observed at 17			Multiply Imputed at 17	
	N missing (%)	**N obs (%)**	**Median IQR**	**N obs (%)**	**Median IQR**	**N missing (%)**	**N obs (%)**	**Median IQR**	**N obs (%)**	**Median IQR**
IIIb		925 (43.5)		1,209(43.6)			603 (41.8)		914 (42.8)	
IV		141 (6.6)		200 (7.21)			90 (6.2)		154 (7.2)	
V (most disadvantaged)		172 (8.1)		249 (8.97)			104 (7.2)		173 (8.1)	
		32 (1.5)		53 (1.91)			18 (1.3)		32 (1.5)	
Offspring Characteristics										
Sex Male	192 (6.9)	1243 (48.1)		1343 (48.4)		443 (20.7)	722 (42.6)		969 (45.3)	
Female		1340 (51.9)		1432 (51.6)			973 (57.4)		1169 (54.7)	
Pregnancy Characteristics										
Gestational age at birth (weeks)	221 (8.0)	2554	40 39, 41	2775	40 39, 41	465 (21.7)	1673	40 39, 41	2138	40 39, 41
Mode delivery	106 (3.8)					478 (22.4)				
Spontaneous		1960 (77.4)		2148 (77.4)			1303 (78.5)		1657 (77.5)	
Breech		32 (1.3)		36 (1.3)			20 (1.2)		28 (1.3)	
Caesarean		223 (8.8)		247 (8.9)			129 (7.8)		175 (8.2)	
Forceps		153 (6.0)		164 (5.9)			90 (5.4)		120 (5.6)	
Vacuum		135 (5.3)		148 (5.3)			96 (5.8)		129 (6.0)	
Other		31 (1.2)		32 (1.2)			22 (1.3)		29 (1.4)	
GWG (kg)	427 (15.4)	2348	12.5 9.6, 15.2	2775	12.5 9.5, 15.2	612(28.6)	1526	12.5 9.6, 15.3	2138	12.4 9.5, 15.3
Hypertension	233 (8.0)					473 (22.1)				
Nil		2159 (84.9)		2351 (84.7)			1407 (84.5)		1798 (84.1)	
GH		342 (13.5)		378 (13.6)			228 (13.7)		300 (14.0)	
PE		41 (1.6)		46 (1.7)			30 (1.8)		40 (1.9)	
Diabetes	433 (15.6)					531(24.8)				
Nil		2342 (95.6)		2652 (95.6)			1536 (95.6)		2038 (95.3)	
PGDM		10 (0.4)		11 (0.4)			8 (0.5)		12 (0.6)	
GDM		16 (0.7)		20 (0.7)			6 (0.4)		18 (0.8)	
Glycosuria		82 (3.4)		92 (3.3)			57 (3.6)		70 (3.3)	

Median, Interquartile range. Figures are numbers (%) unless stated otherwise

The percentage missing data for each co-variable is presented in Figure 7.5.

Figure 7.5: Percentage missing co-variable for offspring at age 9 and 17



Associations, expressed as percentage change rather than as a coefficient, between birthweight and cord blood measures with fat mass, waist circumference, and BMI at age 9 and 17 are presented in the Appendix, Table 7. Results did not differ substantially when non-imputed (observed) data was used to examine associations between birthweight and cord blood measures and offspring adiposity at ages 9 and 17 (Appendix, Table 8).

7.4 Discussion

In this Chapter, cord blood leptin, adiponectin, and birthweight were examined in relation to offspring adiposity in childhood and adolescence. Within this prospective birth cohort study, cord blood leptin, a marker of neonatal fat mass, exhibited relatively weak relationships with later measures of adiposity. These were largely attenuated by adjustment for maternal factors, particularly in later childhood. In contrast cord blood adiponectin showed no relationship with measures of fat mass at age 9 but showed robust relationships with fat mass and waist circumference at ages 15 and 17. Taken together the evidence in this cohort would suggest that neonatal fat mass per se did show a relationship with later fat mass as late as age 17 however it was not possible to distinguish between effects of fat mass in utero and later shared environmental effects as these effects were attenuated by maternal factors, notably maternal BMI.

It was hypothesised that adiponectin would be positively associated with later fat mass. Although this was the case, the fact that the relationship would be mediated by an increased (and persistent) fat mass through childhood was not supported by the data, where relationships were only observed at age 15 and 17. More robust relationships of birth weight to later BMI and waist in both age groups were also observed but it was expected that this was mediated by effects on linear growth, given that birthweight was associated with height at both ages 9 and 15 years.

To date birthweight, as a proxy for intrauterine growth, and its relation to adult BMI has been extensively studied. Studies principally have demonstrated a robust positive association between birthweight and fat mass, BMI, and waist circumference in childhood and adult life (Brisbois, Farmer and McCargar, 2012), and this was similar to the findings from the current study. To try to examine whether birthweight was simply acting as a surrogate for neonatal fat mass, ponderal index (birth weight/length^3) has previously been utilised and demonstrated positive

associations between the ponderal index and lean body mass, total body fat, and the fat-to-lean mass ratio at age 9 years old (Rogers *et al.*, 2006). Although this raised the possibility that neonatal fat mass was related to later adiposity, the ponderal index was a relatively poor measure of neonatal total body fat (De Bruin *et al.*, 1995).

To overcome this deficiency the current study utilised cord blood leptin, a strong correlate of neonatal fat mass as assessed by skinfolds or total body electrical conductivity (Okereke *et al.*, 2002). That cord blood leptin was positively correlated with birthweight, would be consistent with it reflecting neonatal fat mass, a major determinant of birthweight. Furthermore, that cord blood leptin was positively associated with several adiposity measures and specifically fat mass z-score at age 9 years, suggested that there was an accretion of adipose tissue during intrauterine life that was maintained throughout childhood. To examine this further and attain a proxy for percentage body fat at birth, adjusted leptin was used as a marker of neonatal fat mass for birthweight. In these analyses, adjusted cord blood leptin was still weakly associated with fat mass z-score at age 9, suggesting that neonates with a greater percentage of fat at birth continued to have a greater fat mass at age 9. In contrast at age 15 and 17 years, all adiposity associations for leptin were lost after adjustment for maternal and pregnancy characteristics. This suggests that by the time the offspring reached adolescence the biological significance of this long-term tracking of adiposity may be minimal or reflect alternative biological pathways.

The contribution of adiponectin to long-term adiposity is contentious. In mice, transgenic overexpression of adiponectin increases adipose tissue mass, while adiponectin deficiency decreases neonatal fat mass and reduces fetal fat deposition in response to maternal overnutrition in a maternal obesity model (Qiao, Yoo, *et al.*, 2012). However, the effect of adiponectin depletion on body weight or body fat was no longer evident by the 15th postnatal day or in adult life (Qiao, Yoo, *et al.*, 2012). In accordance with some, but not all previous studies, adiponectin was weakly positively correlated with birthweight and cord blood leptin (Tsai *et al.*, 2004;

Mantzoros *et al.*, 2009), potentially reflecting the greater statistical power in the current study. Given this association with birthweight, and known influence on body neonatal composition, it was anticipated that cord blood adiponectin would be positively associated with later adiposity. However, this was only evident at age 15 and 17. Why this was not apparent at age 9, as was observed for leptin, was not clear and may reflect that there was age dependence to these associations as at the earlier time point children were pre-pubertal, with puberty known to have a major impact on body composition and adipocyte number (Knittle *et al.*, 1979).

As previously discussed, this study has a number of strengths including its size, duration of follow-up, and the availability of data on a range of maternal, pregnancy, and social factors pregnancy characteristics to facilitate a robust analysis. This was also one of the very few studies with DXA measurements of body composition at different time points, thereby overcoming the potential increase in overall mass attributed to the expected increase in bone density that results from increased adiposity.

Limitations of the study include the number of children who were overweight or obese were smaller than many contemporary populations, with a similar lean maternal population identified, as described in Chapters 3 and 4. That birthweight and cord blood measures were not associated with the risk of being obese or overweight may reflect this, despite the positive associations with fat mass. In addition, offspring lifestyle factors such as uptake of exercise and consumption of food and drink were not accounted for and these may have influenced the overall adiposity outcomes. Another limitation of the study was the loss to follow-up. However, results would be biased only if associations were substantially different among excluded participants and there is not a notable reason for this to be the case.

As previously discussed in Chapter 2, cord blood sample degradation may have contributed to the variability, but leptin and adiponectin did appear to be stable with long-term storage (Flower *et al.*, 2000; Shih *et al.*, 2000; Boyanton and Blick, 2002; Paltiel *et al.*, 2008; Gislefoss, Grimsrud and Mørkrid, 2009; Brinc *et al.*, 2012). This was in stark contrast to c-peptide, the preferred index of fetal glucose exposure, which could not be measured accurately due to significant degradation with long-term storage, a phenomenon also previously reported by others (Gislefoss, Grimsrud and Mørkrid, 2009).

In conclusion, increased cord blood leptin and adiponectin, known surrogates of fetal fat mass, were weakly positively associated with increased fat mass in late childhood and adolescence, respectively. That these associations were robust to a wide range of confounders that may reflect intrauterine, maternal, and shared environmental exposures suggests that maternal overnutrition may have a direct effect on fetal fat accretion which sets a trajectory for enhanced adiposity throughout later life. However, replication of these findings in cohorts with a different risk profile is critical, and it was acknowledged that the magnitude of the observed associations in this study was small, potentially limiting the impact that neonatal life adiposity has on later outcomes.

Chapter 8: Adolescent non-alcoholic fatty liver disease (NAFLD): Association with birth weight and cord blood adipokines

8.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children and adults in the developed world (Loomba *et al.*, 2009; Younossi *et al.*, 2011; Anderson *et al.*, 2014). The identification of NAFLD in children and adolescents led to the speculation that intrauterine events may contribute to its early pathogenesis (Alisi *et al.*, 2012; Brumbaugh and Friedman, 2014). Intrauterine growth restriction and low birth weight have been associated with liver cirrhosis-related mortality (A.-M.N. and M., 2004) and markers of liver damage and function in adults (Fraser *et al.*, 2008). Although the causal effect of the supply of excess nutrients to the fetus, as observed in maternal obesity and diabetes, on increasing birthweight and neonatal adiposity is clear (Metzger *et al.*, 2008; Tyrrell *et al.*, 2016), the influence on long-term liver function is less well defined. Mouse and primate models of a maternal high-fat diet during pregnancy exhibit offspring obesity and hepatic lipid accumulation (McCurdy *et al.*, 2009; Oben *et al.*, 2010). Increasing maternal BMI has also been associated with increased neonatal liver fat deposition (Modi *et al.*, 2011). Long-term human studies are, however, less consistent with birthweight positively associated with adverse liver function or a diagnosis of NAFLD at age 17 years in some (Anderson *et al.*, 2014), but not all studies (Ayonrinde *et al.*, 2015). These findings suggest that intrauterine exposure to maternal over-nutrition increases fetal hepatic steatosis which may predispose to NAFLD in the long-term (Modi *et al.*, 2011). However, the fetal origins of NAFLD are not well-understood, and poorer intrauterine nutrition, as reflected by lower birth weight, has been associated with elevated liver transaminases and NAFLD in adolescence and adulthood (Nobili *et al.*, 2006; Fraser *et al.*, 2008; Faienza *et al.*, 2013; Anderson *et al.*, 2014) as a result of impeded organ development (Andersen and Osler, 2004; Nobili *et al.*, 2006; Barker, 2007; Faienza *et al.*, 2013).

The differences may reflect that assessments of the intrauterine contribution to the onset of metabolic disease in the offspring have primarily used birth weight, which reflects both fetal and lean mass. Umbilical cord blood leptin is now widely recognised as an accurate biomarker for neonatal fat mass (Hauguel-de Mouzon, Lepercq and Catalano, 2006), with higher levels associated with greater fat mass. Cord blood adiponectin is also positively associated with birthweight (Mantzoros *et al.*, 2009). In mouse studies, over-expression of fetal adiponectin increased the size of fat depots in early life, while adiponectin knockout fetuses display lower body weight and lower fat content (Qiao, Yoo, *et al.*, 2012). In addition, cord blood lipids are increased in the offspring of obese mothers and the presence of NAFLD (Neuman, Cohen and Nanau, 2014). The nature of the association of these cord blood adipokines and lipids with childhood NAFLD is unknown. Associations in later life are however more established as circulating leptin concentrations and dyslipidaemia are positively associated with NAFLD activity, while adiponectin is negatively associated (Zelber-Sagi *et al.*, 2012).

In this Chapter, the relationship between neonatal fat mass (cord blood leptin, adiponectin, and lipids) and blood and USS-based markers of liver health in adolescence was examined. To make this a comprehensive assessment, birth weight was additionally examined in relation to adolescent liver outcomes. In comparison with earlier studies, potential confounding was adjusted for and included maternal BMI, complications of pregnancy, and maternal smoking during pregnancy.

8.2 Methods

8.2.1 Study Population

A total of 14,541 women were initially enrolled and cord blood samples were available for 5,011 mother-offspring pairs as previously discussed in Chapter 2. Details of all participants that attended the 17-18 follow up clinic (n=5,081), provided blood-based indicators of liver function (n=3,188), and participated in the liver ultrasound sub-study (n=1,887) have previously been reported in detail by ALSPAC (Patel *et al.*, 2012; Anderson *et al.*, 2014). Participants in the current study were included if they had a cord blood sample and they attended the 17 to 18-year follow-up study and had blood-based indicators of liver function (n=1,037) or participated in the sub-study with liver ultrasound measurement (n=541). Details of cord blood sample collection and analysis were presented in Chapter 2. Details of obstetric and perinatal abstraction of data from patient case-notes and questionnaires completed were also presented in Chapter 2.

8.2.2 Assessment of liver outcomes

The protocol for USS assessment by ALSPAC has been published previously (Anderson *et al.*, 2014)a. Briefly, the upper abdominal USS was completed by one of four trained sonographers using a Siemens Acuson S2000 USS system. Echogenicity, a marker of liver fat, was assessed during deep inspiration and recorded as present, absent, or uncertain according to established protocols. A longitudinal image, in the sagittal plane, with juxtaposition of the right lobe of the liver and the right kidney, was viewed and echogenicity was determined by a comparison between the liver and kidney. Levels of agreement in identifying echogenicity between the four sonographers were 98% or greater, both immediately after training and at 6-month intervals throughout data collection. Acoustic radiation force impulse-imaging (ARFI) of the right lobe of the liver was used to measure liver stiffness, using standard

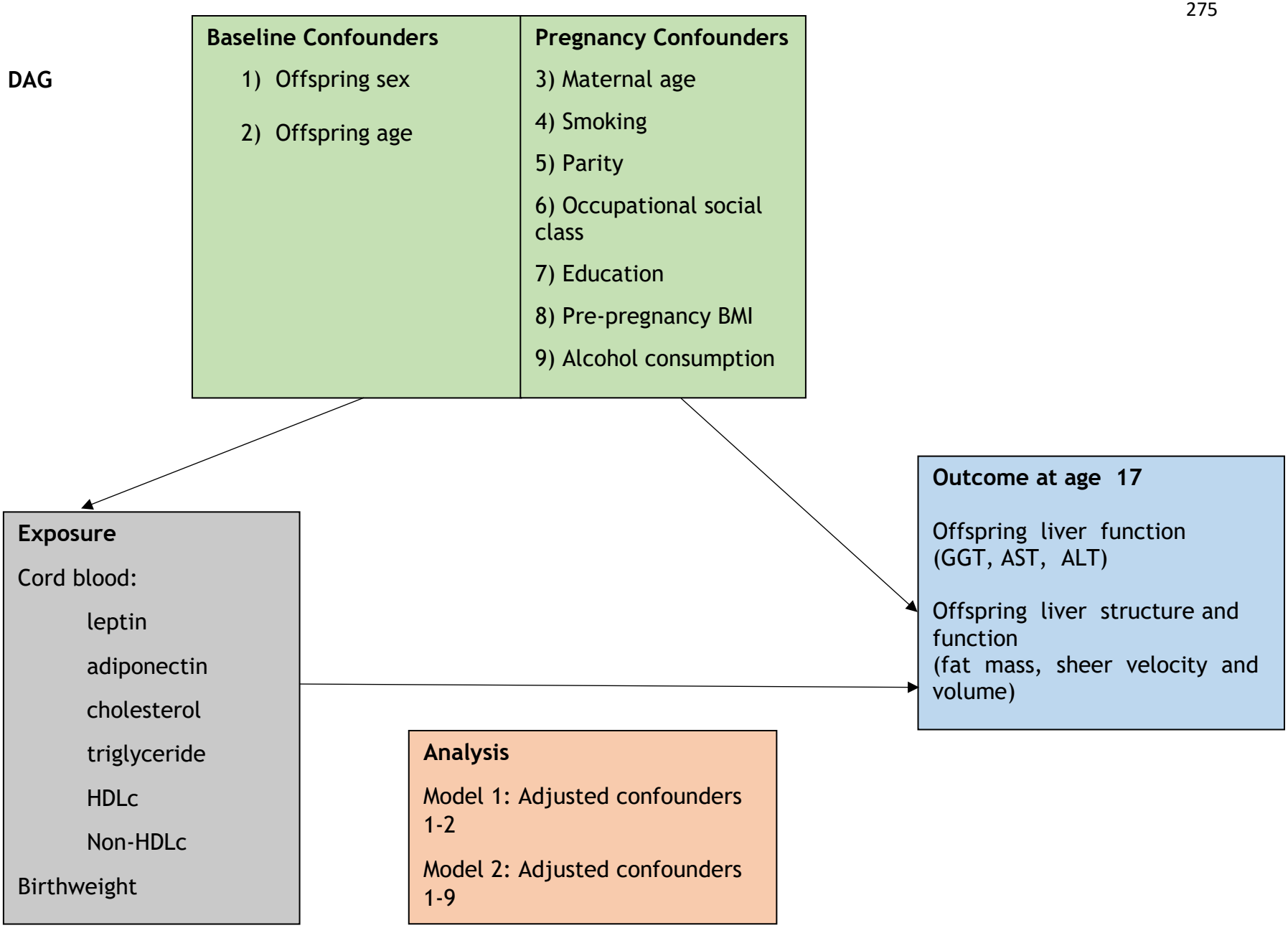
protocols. ARFI, measured as sheer velocity in meters/second (m/s), was assessed six times with a gap of at least 1 min between each measurement. The highest and lowest of these measurements were excluded and the Siemens Acuson S2000 system produced a mean of the remaining four measurements. If this mean was greater than four m/s, a further six measurements were taken from the left lobe. When both right and left lobe values were available, the lowest mean of the two has been used in analyses.

Fasting blood samples (minimum of 6-hours) were immediately spun and frozen at -80 ºC by ALSPAC. Measurements were assayed shortly (3– 9 months) after samples were taken with no previous freeze-thaw cycles. All assays were completed in the same laboratory at the University of Glasgow. ALT, GGT, and AST were measured by an automated analyser with enzymatic methods. Those with a value of >30 U/L ALT were defined as having abnormally high ALT levels, using the most common blood-based indicator and the threshold that has been used previously in adolescent/ childhood populations. All inter-and intra-coefficients of variation for these blood-based assays were <5%.

8.2.3 Assessment of covariates and offspring anthropometry

Figure 8.1: DAG displays the direction of associations examined as well as the confounders adjusted for in the analysis.

Figure 8.1: DAG



Potential confounders included maternal age, pre-pregnancy body mass index, smoking status (categorised as never smoked, smoked before but not during and smoked during pregnancy), parity, occupational social class, complications during pregnancy (hypertensive or diabetic disorders), gestational age at birth, mode of delivery and offspring's sex. Height, BMI, and fat mass at age 17.8 years were considered as potential mediators if any confounder-adjusted associations were observed. As discussed in Chapter 2: Methods, offspring weight, and height at the 17-year clinic assessment were measured using standard procedures in light clothing, without shoes, and used to calculate BMI. Weight was measured to the nearest 0.1kg using Tanita scales. Height was measured to the nearest 0.1cm using a Harpenden stadiometer. A narrow fan-beam densitometer (Lunar Prodigy; GE Healthcare Lunar Ltd., Cambridge, UK.) was used to perform a whole-body dual-energy X-ray absorptiometry scan from which fat mass was measured.

8.2.4 Statistical Analysis

Offspring liver volume, fibrosis, and liver function tests were log-transformed to produce the approximately normal distribution of residuals. Birthweight was adjusted for sex and gestational age using nonlinear regression fitting a Gompertz curve. As human and animal studies have demonstrated opposing inverse relations between birthweight and risk of NAFLD, a potential departure from linearity was explored. By comparing a model in which the exposure was entered in thirds as two indicator variables and a model in which a linear relationship was assumed across the thirds using a likelihood ratio test, there was no evidence to support a departure from linearity. Multivariable linear and logistic regression models were used to examine the associations between cord blood measures, birthweight and fatty liver, liver volume, fibrosis, and liver function tests at age 17 years. Sex-exposure interactions were tested for and as there was no evidence to support these, results were reported for male and female offspring combined.

A series of multivariable regression models were constructed in order to adjust for potential confounders and mediators. The basic model (model 1) was adjusted for offspring sex and age at outcome assessment. In model 2 for potential confounders were additionally adjusted for: maternal age, smoking in pregnancy, parity, occupational social class, education, pre-pregnancy BMI, and alcohol consumption in pregnancy. In order to examine for any potential mediation, offspring BMI, fat mass, height, and height squared at age 17 were further adjusted for in any confounder adjusted associations that were not null.

There were small amounts of missing data on some co-variables included in the multivariable models. In order to deal with the missing data (any exposure, outcome, or confounder), twenty imputation data sets were generated by chained equations (White, Royston and Wood, 2011), with measurements from the 15-year clinic informing imputation of missing values in the 17-year clinic respectively. Up to 541 (all participants with a liver scan and paired cord blood sample) and up to 1037 (participants with LFTs and paired cord blood samples) sets were imputed. The distributions of observed and imputed variables were similar, as discussed in the Results section (8.3).

8.3 Results

Figure 8.2 displays the ALSPAC participant flow chart and the inclusion and exclusion criteria.

Figure 8.2: ALSPAC participant flow chart

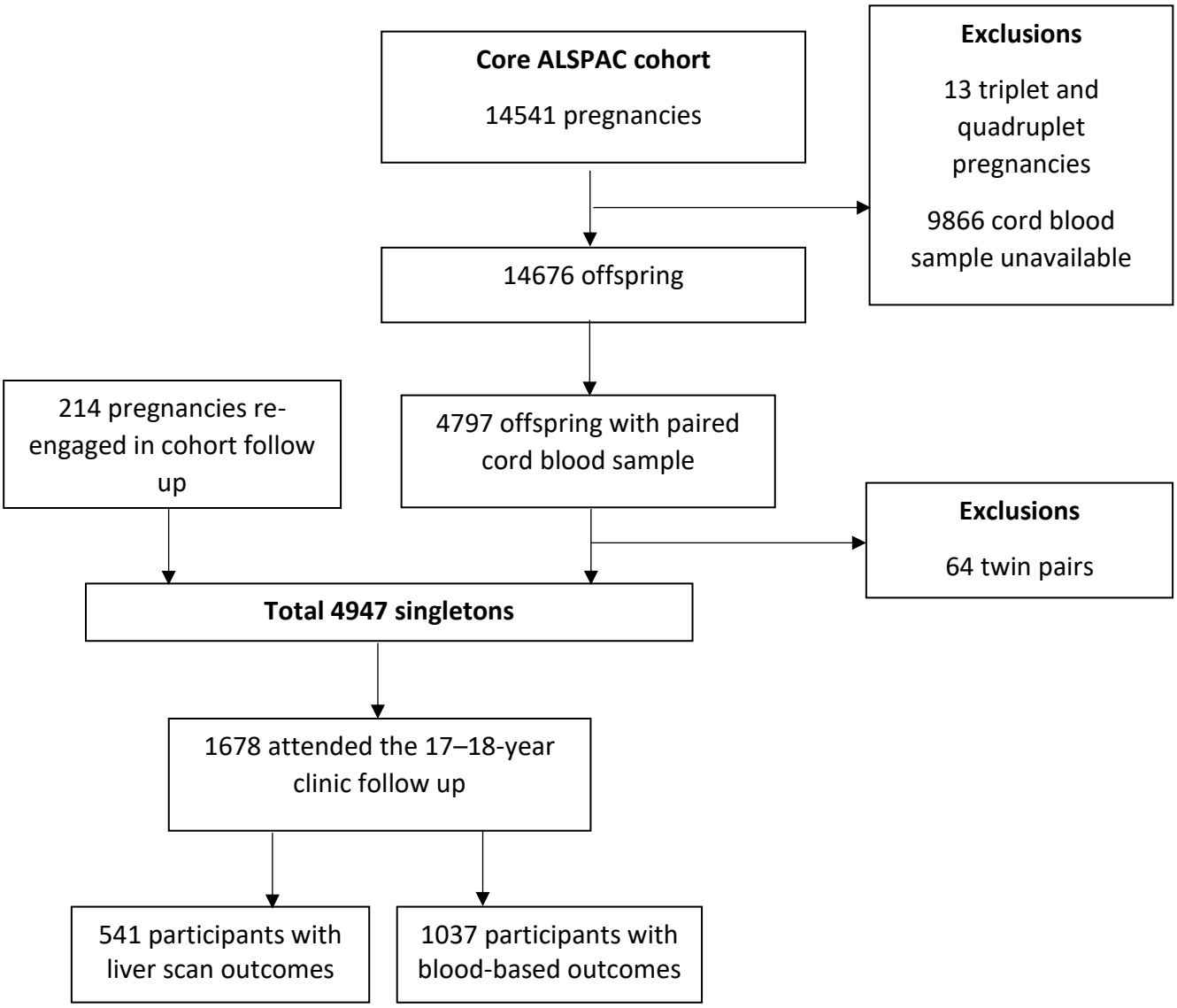


Table 8.1 summarises the maternal and offspring characteristics for those who attended the clinic at age 17 and had liver function tests and/or a liver ultrasound scan. In most cases, the proportion of missing data for each variable was less than 5%. The variables maternal BMI and social class were missing however in 11.5% and 16.5% of those attending for liver scan.

Baseline characteristics for the mothers and their young adult offspring were similar for those who had just the blood measures and those who had USS. Of the 541 who were assessed by ultrasound, only 10 (1.8%) were categorised as having a fatty liver. Cohort characteristics for the observed versus the imputed data were similar, as displayed in the Appendix, Table 9.

Table 8.1: Characteristics of those who had a liver scan (n=541) and/or liver function tests (N=1037) performed at age 17 and % with missing data

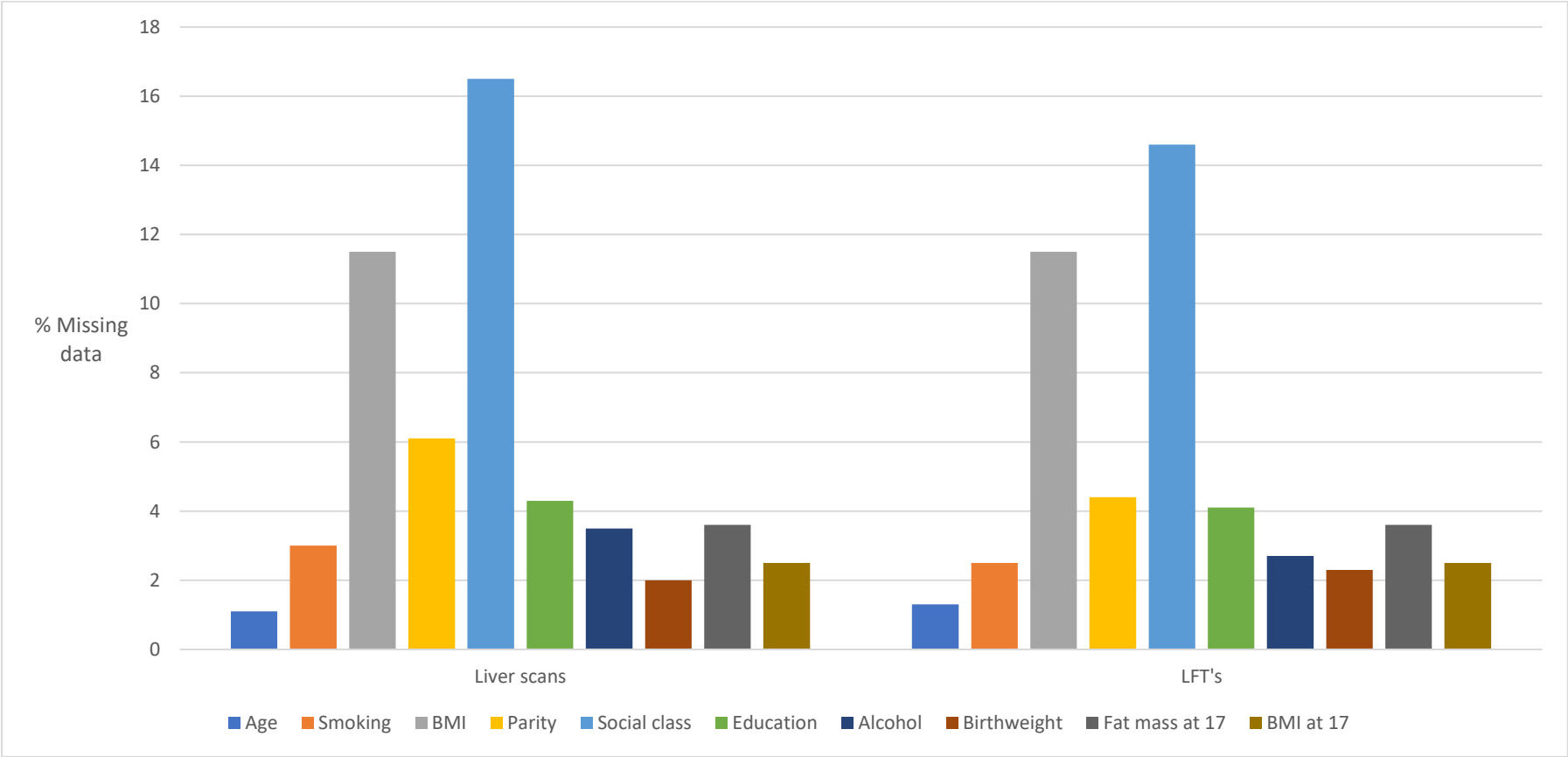
	Liver scan			Liver function tests		
	Median or % N obs (IQR or N)	N obs	% missing (N)	Median or % N obs (IQR or N)	N obs	% missing (N)
Maternal Characteristics						
Age (years)	29 (26, 32)	535	1.1 (6)	29 (26, 32)	1023	1.3 (14)
Smoking		525	3.0		1011	2.5
Never	74.9 (393)		(16)	77.1 (779)		(26)
Before, not during	9.5 (50)			7.6 (77)		
During pregnancy	15.6 (82)			15.3 (155)		
BMI	22.3 (20.5, 24.4)	479	11.5 (62)	22.0 (20.5, 24.1)	918	11.5 (120)
Parity		508	6.1		991	4.4
0	46.7 (237)		(33)	47.1 (467)		(46)
1	39.2 (199)			36.8 (365)		
2	9.7 (49)			11.8 (117)		
3	3.9 (20)			3.4 (34)		
4+	0.6 (3)			0.8 (8)		
Social Class		452	16.5		884	14.6
I (least)	6.9 (31)		(89)	8.0 (71)		(153)
II	38.5 (174)			38.4 (339)		
III	40.3 (182)			39.5 (349)		
IV	5.1 (23)			5.1 (45)		
V	8.4 (38)			7.6 (67)		
VI (most disadvantaged)	0.9 (4)			1.5 (13)		
Education		518	4.3		994	4.1
Left school 16	56.6 (293)		(23)	53.3 (530)		(43)
A level	25.3 (131)			28.3 (281)		
Degree	18.1 (94)			18.4 (183)		
Alcohol		522	3.5		1009	2.7
None/<1 glass/wk	43.5 (227)		(19)	43.0 (434)		(28)
>1 glass/wk	46.4 (242)			45.9 (463)		
>1 glass/day	10.2 (53)			11.1 (112)		
Offspring Characteristics						
Sex		541			1037	
Male	39.7 (215)		0.0	46.4 (481)		0.0
Female	60.3 (326)		(0)	53.6 (556)		(0)

	Liver scan			Liver function tests		
	Median or % N obs (IQR or N)	**N obs**	**% missing (N)**	**Median or % N obs (IQR or N)**	**N obs**	**% missing (N)**
Birthweight (kg)	3.5 (3.2, 3.8)	530	2.0 (11)	3.5 (3.2, 3.8)	1013	2.3 (24)
Cord blood						
Leptin (pg/ml)	6.2 (3.7, 11.9)	539	0.4 (2)	6.3 (3.6, 11.9)	1035	0.2 (2)
Adiponectin (µg/ml)	70.9 (51.0, 94.0)	533	1.5 (8)	73.5 (52.8, 95.0)	1026	1.1 (12)
Cholesterol (mmol/l)	1.7 (1.5, 2.1)	529	2.2 (12)	1.7 (1.4, 2.0)	1021	1.6 (17)
Triglycerides (mmol/l)	0.5 (0.4, 0.6)	524	3.1 (17)	0.5 (0.4, 0.6)	1016	2.1 (22)
HDLc (mmol/l)	0.5 (0.4, 0.7)	518	4.3 (23)	0.5 (0.4, 0.7)	1005	3.1 (33)
Non-HDLc (mmol/l)	1.2 (1.0, 1.5)	518	4.3 (2.3)	1.2 (0.9, 1.5)	1005	3.1 (33)
Liver volume (ml)	1607.4 (1355.4, 1858.9)	541	0.0 (0)			
Liver velocity (m/s)	1.2 (1.1, 1.3)	500	7.9 (41)			
Fatty liver	1.8 (10)	371	31.4(170)			
LFT's at age 17						
GGT (U/l)			31.4 (170)	16.0 (13, 21)	1037	0.0 (0)
ALT (U/l)			31.4 (170)	14.8 (12, 19.2)	1037	0.0 (0)
AST (U/l)			4.8 (26)	19.3 (16.6, 23.3)	1004	3.1 (33)
Anthropometry at age 17						
Fat mass (kg)	17.4 (11.8, 23.9)	522	3.6 (19)	16.3 (10.6, 23.0)	1011	2.5 (26)
BMI (kg/m^2)	22.2 (20.2, 24.8)	522	3.6 (19)	22.1 (20.2, 24.7)	1011	2.5 (26)
Height	169.8 (163.8,177.8)			171.4 (164.5, 178.9)		
Obesity		31 (5.9)			51 (5.0)	

Median (Interquartile range, IQR) or % number of observations (N observations)

The percentage of missing data from the complete case, compared to those used in the multiple imputation analysis, is displayed in Figure 8.3.

Figure 8.3: Percentage missing data from the complete case for each co-variable compared to multiple imputation analysis from those who attended for liver scans or LFT's at age 17



There was no association between birthweight or any of the cord blood measures and the diagnosis of a fatty liver in adolescence, as shown in Table 8.2.

Table 8.2: Association of birthweight and cord blood measures with NAFLD in adolescence (using multiple imputation datasets) N=541

Outcome Age 17:		Diagnosis of Fatty liver (yes/no)		
Exposure:	**Model**	**OR**	**CI**	**P**
Cord blood				
Leptin	1	0.68	0.26, 1.73	0.41
(per 10 pg/ml)	2	0.62	0.23, 1.65	0.33
Adiponectin	1	0.85	0.70, 1.04	0.13
(per 10µg/ml)	2	0.78	0.60, 1.01	0.06
Cholesterol	1	0.81	0.36, 1.83	0.62
(per 1 mmol/l)	2	0.90	0.43, 1.87	0.78
Triglyceride	1	0.74	0.09, 5.94	0.78
(per 1 mmol/l)	2	0.79	0.08, 8.20	0.84
HDLc	1	0.51	0.04, 5.87	0.58
(per 1 mmol/l)	2	0.28	0.02, 3.78	0.34
Non-HDLc	1	0.82	0.33, 2.05	0.67
(per 1 mmol/l)	2	1.02	0.48, 2.15	0.96
Birthweight †	1	1.00	0.88, 1.13	0.98
(per 100g)	2	0.96	0.84, 1.10	0.59

Adjustments for confounders

Models 1: offspring sex and age, 2: offspring sex and age, maternal age, smoking, parity, occupational social class, education, pre-pregnancy BMI and alcohol consumption during pregnancy † Birthweight is adjusted for gestational age and sex, therefore not additionally adjusted offspring sex for in Models 1&2.

In the adjusted analyses, there were two out of 42 associations at conventional 5% levels of significance. Birthweight was positively associated with liver volume (1.0% greater per 100g, 95% CI: 0.5, 2.0%) and cord blood HDLc was positively associated with ALT (11.6% higher per 1mmol/l, 95% CI 0.3, 23.4), as shown in Table 8.3.

Examining the shape of associations demonstrated that there was no significant deviation from the linear trend. To examine the potential impact of offspring mediation, BMI, fat mass, height and height squared at age 17 were also adjusted for. There was no evidence of mediation where birthweight and liver volume were examined as a positive association remained (1.0% greater per 100g, 95% CI: 0.1, 1.0%). Offspring mediation was however evident in the association between cord blood HDLc and ALT as there was a change in the strength and direction of association (1.0% lower per 1mmol/l, 95% CI -2.0, -0.4).

Table 8.3: Association of birthweight and cord blood measures with markers of liver health in adolescence (using multiple imputation datasets) N=541 for liver scans, N=1037 for LFTs

Outcome Age 17:		**logLiver volume** (ml)			**logLiver sheer velocity** (m/s)			**logALT** (U/l)			**logAST** (U/l)			**logGGT** (U/l)		
Exposure		%	CI	P	%	CI	P	%	CI	P	%	CI	P	%	CI	P
Cord blood	Model															
Leptin	1	1.0	-1.0, 3.0	0.22	0.03	-2.0, 1.0	0.97	0.4	-2.0, 3.0	0.73	0.2	-1.0, 2.0	0.81	-0.1	-2.0, 2.0	0.96
(10 pg/ml)	2	0.3	-2.0, 2.0	0.79	-1.0	-2.0, 1.0	0.46	-0.6	-3.0, 2.0	0.61	0.0	-2.0, 2.0	0.97	-0.5	-3.0, 2.0	0.66
Adiponectin	1	-0.2	-1.0, 1.0	0.61	0.1	-1.0, 1.0	0.82	-0.2	-1.0, 1.0	0.68	0.3	-0.2, 1.0	0.23	0.2	-0.4, 1.0	0.41
(10µg/ml)	2	-0.1	-1.0, 1.0	0.79	0.0	-0.6, 0. 6	0.99	-0.3	-1.0, 1.0	0.53	0.3	-0.2, 0.8	0.27	0.3	-0.4, 0.9	0.45
Cholesterol	1	0.1	-2.0, 2.0	0.90	-1.0	-3.0, 1.0	0.24	1.0	-1.0, 4.1	0.22	-0.1	-2.0, 1.0	0.85	-0.1	-2.0, 2.0	0.90
(1 mmol/l)	2	0.0	-2.0, 2.0	0.98	-1.0	3.0, 1.0	0.38	1.0	-1.0, 4.1	0.25	-0.2	-2.0, 1.0	0.82	0.0	-2.0, 2.0	0.97
Triglyceride	1	0.4	-5.8, 7.3	0.91	-2.0	-7.7, 3.0	0.45	2.0	-3.9, 8.3	0.53	0.0	-4.9, 4.1	0.76	-2.0	-6.8, 3.0	0.49
(1 mmol/l)	2	-1.0	-7.7, 5.8	0.73	-2.0	-7.7, 3.0	0.41	1.0	-4.9, 8.3	0.67	0.0	-4.9, 3.0	0.71	-2.0	-1.0, 4.1	0.32
HDLc	1	1.0	-7.7, 9.4	0.88	2.0	-4.9, 9.4	0.57	10.5	-1.0, 22.1	0.07	3.0	-3.9, 10.5	0.42	2.0	-6.8, 11.6	0.63
(1 mmol/l)	2	2.0	-5.8, 11.6	0.60	4.0	-3.0, 11.6	0.22	11.6	0.3, 23.4	0.04	3.0	-3.9, 10.5	0.40	3.0	-5.8, 12.7	0.47
Non-HDLc	1	-0.1	-2.0, 2.0	0.97	-2.0	-3.0, 0.0	0.13	1.0	-1.0, 4.1	0.36	-0.4	-2.0, 1.0	0.69	-0.3	-3.0, 2.0	0.81
(1 mmol/l)	2	-0.2	-3.0, 2.0	0.88	-1.0	-3.0, 0.6	0.18	1.0	-2.0, 4.1	0.43	-0.4	-2.0, 2.0	0.64	-0.2	-2.0, 2.0	0.83
Birthweight	1	1.0	1.0, 2.0	0.00	0.3	-0.1, 1.0	0.16	-0.4	-1.0, 0.2	0.22	-0.3	-1.0, 0.1	0.12	-0.2	-1.0, 0.3	0.42
† (per 100g)	2	1.0	1.0, 2.0	0.00	0.1	-0.4, 0.5	0.79	-1.0	-1.0, 0.02	0.06	-0.4	-1.0, 0.02	0.06	-0.4	-1.0, 0.1	0.15

Adjustments for confounders

Models 1: offspring sex and age, 2: offspring sex and age, maternal age, smoking, parity, occupational social class, education, pre-pregnancy BMI, and alcohol consumption during pregnancy

† Birthweight is adjusted for gestational age and sex, therefore not additionally adjusted offspring sex for in Models 1&2

The distributions of observed (complete case) and imputed variables were similar. Results from those with complete data are also presented, for comparison to the imputed data sets, in Tables 8.4 (reflecting Table 8.2) and 8.5 (reflecting Table 8.3).

Tables 10 and 11 in the Appendix also display the data presented in Tables 8.4 and 8.5 respectively but include the coefficient (β) of association rather than the percentage association between the exposure and the outcome variables.

Table 8.4: Association of birthweight and cord blood measures with NAFLD in adolescence (complete case) N=196-233

Outcome Age 17:	**Diagnosis of Fatty liver (yes/no)**		
Exposure:	OR	CI	P
Cord blood			
Leptin (per 10 pg/ml)	0.73	0.33, 1.62 N=233	0.44
Adiponectin (per 10µg/ml)	0.54	0.13, 2.27 N=230	0.40
Cholesterol (per 1 mmol/l)	1.23	0.58, 2.62 N=202	0.59
Triglyceride (per 1 mmol/l)	0.56	0.01, 30.97 N=201	0.78
HDLc (per 1 mmol/l)	2.22	0.09, 55.33 N=196	0.63
Non-HDLc (per 1 mmol/l)	1.22	0.50, 2.98 N=196	0.67
Birthweight † (per 100g)	1.08	0.90, 1.30 N=230	0.40

Adjustments for confounders

Model 2: Complete case confounders: offspring sex and age, maternal age, smoking, parity, occupational social class, education, pre-pregnancy BMI, and alcohol consumption in pregnancy

† Birthweight is adjusted for gestational age and sex, therefore not additionally adjusted offspring sex for in Model 2.

Table 8.5: Association of birthweight and cord blood analyte with measures of liver health in adolescence (complete case)

Outcome Age 17:	logLiver volume (ml)			logLiver sheer velocity (m/s)			logALT (U/l)			logAST (U/l)			logGGT (U/l)		
Exposure:	%	CI	P	%	CI	P	%	CI	P	%	CI	P	%	CI	P
Cord blood leptin (per 10 pg/ml)	1.0	-1.0, 2.0 N=402	0.47	-0.05	-2.0, 1.0 N=374	0.95	-0.8	-3.0, 2.0 N=813	0.54	-0.5	-2.0, 1.0 N=813	0.59	-0.6	-3.0, 2.0 N=813	0.61
Cord blood adiponectin (per 10µg/ml)	-0.1	-1.0, 1.0 N=396	0.71	0.04	-0.6, 0.7 N=396	0.89	-0.7	-2.0, 0.1 N=804	0.09	0.2	-0.4, 0.7 N=804	0.58	-0.2	-0.9, 0.5 N=804	0.59
Cord blood cholesterol (per 1 mmol/l)	0.4	-2.0, 2.0 N=392	0.71	-1.0	-3.0, 0.5 N=364	0.18	2.0	-0.1, 5.1 N=800	0.06	0.1	-2.0, 2.0 N=800	0.95	0.2	-2.0 ,2.0 N=800	0.88
Cord blood triglyceride (per 1 mmol/l)	-2.0	-7.7, 4.1 N=388	0.55	-3.0	-7.7, 2.0 N=361	0.25	3.0	-3.9, 9.4 N=796	0.46	-0.4	-4.9, 4.1 N=796	0.86	-2.0	-7.7, 4.1 N=796	0.53
Cord blood HDLc (per 1 mmol/l)	5.0	-3.0, 13.9 N=383	0.25	0.4	-5.8, 7.3 N=355	0.90	18.5	5.1, 32.3 N=785	0.004	5.0	-3.0, 13.9 N=785	0.21	6.0	-3.9, 17.4 N=785	0.27
Cord blood non-HDLc (per 1 mmol/l)	-0.2	-2.0, 2.0 N=383	0.89	-2.0	-3.0, 0.2 N=355	0.08	2.0	-1.0, 5.1 N=785	0.21	-0.3	-2.0, 2.0 N=785	0.74	-0.3	-3.0, 2.0 N=785	0.79
Birthweight † (per 100g)	1.0	0.5, 2.0 N=398	0.000	0.4	-0.03, 0.8 N=370	0.07	-0.7	-1.0, -0.1 N=806	0.04	-0.6	-1.0, -0.2 N=806	0.01	-0.2	-1.0, 0.4 N=806	0.58

Adjustments for confounders

Model 2: Complete case confounders: offspring sex and age, maternal age, smoking, parity, occupational social class, education, pre-pregnancy BMI, and alcohol consumption in pregnancy

† Birthweight is adjusted for gestational age and sex, therefore not additionally adjusted offspring sex for in Models 1&2

8.4 Discussion

In this chapter, prospective associations of birthweight and cord blood measures of leptin, adiponectin, and lipids with markers of adolescent liver structure and function were examined. Out of a total of 42 confounder adjusted tests, only two were significant at conventional 5% levels; a positive association of birthweight with adolescent liver volume and of cord blood HDLc with adolescent ALT. Overall these results did not suggest that birthweight, birth fat (as assessed by cord leptin and adiponectin), or cord lipids were associated with later offspring markers of liver health when adjustment was made for maternal pregnancy characteristics that were likely to confound these associations.

Human and animal models demonstrate opposing directions of association of birthweight and offspring liver outcomes, perhaps reflecting birthweight as a crude marker of fat and lean mass. Whilst lower birth weight has been associated with elevated liver transaminases and NAFLD in adolescence and adulthood (Nobili *et al.*, 2006; Fraser *et al.*, 2008; Faienza *et al.*, 2013; Anderson *et al.*, 2014), offspring exposed to an obesogenic environment may also display greater hepatic lipid content (McCurdy *et al.*, 2009; Oben *et al.*, 2010; Modi *et al.*, 2011; Brumbaugh *et al.*, 2013). Furthermore, a greater rate of weight gain in childhood has also been associated with adverse liver outcomes in adolescence (Anderson *et al.*, 2014), which was largely mediated by the degree of adiposity in adolescence. That a positive association between birthweight and liver volume has been identified may simply reflect tracking of greater organ size rather than a relation to hepatic dysfunction itself, especially as there was no evidence of offspring mediation at age 17.

Adipokines have previously been implicated in the aetiology of NAFLD (Paschos and Paletas, 2009; M.S., 2011; Ouchi *et al.*, 2011), with low adiponectin and high leptin levels being demonstrated in children (Manco *et al.*, 2007; Fitzpatrick *et al.*, 2010; Boyraz *et al.*, 2013) and adults (Zelber-Sagi *et al.*, 2012) with the disease. That there

was no observed prospective longitudinal association of cord blood leptin with markers of liver function in adolescence, suggests that the contribution of leptin at birth to the pathogenesis of childhood NAFLD was limited. Similarly, that adiponectin was not associated with multiple measures of liver function 17 years later suggested that intrauterine derived adiponectin had minimal impact. Serum lipids exhibited strong positive associations with adverse liver function in cross-sectional analyses (Zelber-Sagi *et al.*, 2012; Orešič *et al.*, 2013). However, in this study, no consistent prospective longitudinal associations between cord blood lipoproteins and adolescent NAFLD were identified. That cord blood HDLc was positively associated with ALT at age 17, may have been a chance finding, particularly as there was evidence of mediation by offspring adiposity and height measures. Collectively this suggests that although leptin and adiponectin reflected neonatal mass, the disease in adolescence may be primarily driven by childhood behaviour rather than intrauterine exposures.

Whilst previous studies of birthweight with later liver outcomes have been larger than this study, this was one of the largest studies to examine cord blood measures in relation to subsequent liver outcomes, including measurements assessed by USS. The prospective design, long duration of follow-up, detailed anthropometric measures, and appropriate adjustment for maternal characteristics that could confound the associations were substantive strengths. There were several limitations of the study, however. Ultrasound to assess liver pathology was employed as it has been shown to detect moderate to severe steatosis with similar accuracy as the gold standard liver biopsy, whilst avoiding an unethical and invasive procedure (Shannon *et al.*, 2011). For lesser degrees of hepatic fat mass, however, ultrasound may not be as sensitive a tool. The absence of associations in this study may reflect this, particularly as a young adolescent population was more likely to exhibit subtle changes in hepatic structure as the disease process itself may still be in its infancy. Many studies have examined the sensitivity and specificity of ultrasound in the detection of liver steatosis compared to the alternative, more accurate measures. Where there is moderate to severe hepatic steatosis, the sensitivity and specificity may be as high as 90% however this falls to around 67% and

89% respectively when all grades of hepatic steatosis are considered (Palmetieri *et al.*, 2006; Lee *et al.*, 2010; van Werven *et al.*, 2010; Bril *et al.*, 2015). Alternative modes of diagnosing hepatic steatosis include liver biopsy, CT, and MRI. Liver biopsy may be observer-dependent and as it is an invasive procedure the patient may experience pain or procedure-related complications such as infection or bleeding. CT scan exposes the patient to ionising radiation and, like ultrasound, is not a reliable method of detecting mild hepatic steatosis. MRI offers the most sensitive and specific method of detection (Paige et al., 2017), but is considerably more expensive and the environment required to obtain the images may be less well tolerated than ultrasound, particularly in younger age groups. Analyses were limited to those with an available cord blood sample and to those participants who attended the 17-year-old clinic, however, it wass unlikely that this should bias results.

Multiple imputation methods were used to account for missing confounder data, with similar findings in those with complete data on all confounders. The ALSPAC cohort was mainly of white European origin and as a result, this study may not reflect other ethnicities that have previously been strongly linked with hepatic dysfunction and elevated ALT levels (Fraser, Longnecker and Lawlor, 2007).

In animal models, insulin resistance, hyperleptinemia, and hepatic steatosis were all features of male but not female offspring of obese mice (Dahlhoff *et al.*, 2014) and there have been inconsistent reports of gender-specific predispositions to NAFLD. In the current study, the majority of offspring participating in this study were female (60.3%) and this may account for the low numbers with evidence of NAFLD. Examining offspring outcomes in adolescence may be too early in life to identify those who go on to develop metabolic disorders such as NAFLD and type 2 diabetes. The low prevalence of the disease in adolescence may account for the lack of associations in this study and it would be beneficial to repeat the study in an older age group where the prevalence of the disease may be greater and more accurately assessed. Cord blood sample degradation may have also contributed to variability within the results, but leptin, adiponectin, and lipids do appear to be stable with

long-term storage (Shih *et al.*, 2000; Paltiel *et al.*, 2008; Gislefoss, Grimsrud and Mørkrid, 2009; Brinc *et al.*, 2012).

In conclusion, there was no consistent evidence that birth weight, neonatal adiposity, or cord blood lipids were associated with liver dysfunction in adolescence. This indicates that the disease may be primarily driven by adolescent behaviours and phenotype rather than intrauterine exposures or adiposity at birth. The association between birthweight and adolescent liver volume may reflect the contribution of greater organ size to birthweight and tracking of organ size, rather than any relation to hepatic dysfunction itself. The association of maternal adiposity to later offspring NAFLD was likely to be a consequence of later environmental effects or in utero effects not mediated via in utero adiposity.

Chapter 9: Discussion

9.1 Discussion

The DOHaD paradigm was based on earlier pioneering work by Professor David Barker (Barker, 2007), who proposed that exposure to an unfavourable intrauterine environment may cause permanent re-programming of fetal physiological systems and a greater propensity to ill-health in later life. In this thesis, the extent to which disturbed maternal metabolism during pregnancy, as a result of greater maternal adiposity, diabetes, or HDP, may impact cord blood lipids, adipokines, and birthweight has been examined. Tracking of repeat analyte measurements, as well as the associations of cord blood analytes at birth and offspring adiposity or adolescent development of NAFLD, have also been presented.

Specifically, this study has demonstrated that more than fifty percent of overweight or obese women gained weight excessively, even though they may have gained less weight overall compared to lean women. Maternal BMI during pregnancy, rather than GWG alone, was however associated with a wider range of metabolic markers in the offspring, with evidence of dyslipidaemia in the cord blood at birth but both BMI and GWG were positively associated with birthweight and cord blood leptin. Mid-pregnancy GWG demonstrated the strongest positive association with birthweight whereas late pregnancy GWG displayed a greater positive association with cord blood leptin at birth. There was a limited association between mothers with GDM and offspring cord blood biomarkers at birth. In contrast, mothers with PGDM demonstrated more variability in the cord blood at birth suggesting that the offspring of those with known diabetes prior to pregnancy may be at greater risk of metabolic disruption, particularly dyslipidaemia. This study has also explored the demographics of those that developed HDP and demonstrated that the BP at booking was higher in those that later developed HDP, compared to the healthy group. The offspring of mothers with PE had overall a lower birthweight, shorter gestational period, and higher cord blood leptin and cholesterol at birth, suggesting that these offspring may be at greater risk of metabolic dysfunction from birth.

Limited evidence of tracking of repeated analytes from birth through to childhood and adolescence has been presented. Whilst cord blood lipids, GGT, and adiponectin demonstrated a positive association with the repeated analyte measured at age 9, GGT was the only analyte that tracked from birth (cord blood) through to adolescence. Strong associations were however demonstrated between lipids and LFTs obtained at age 9 and repeat measures of the same analyte at age 17. Overall this suggests that tracking of analytes may become more progressively defined with time but measurement of GGT at birth may be of interest as a biomarker of future metabolic health. Lastly, this study has demonstrated that birthweight and cord blood adipokines and lipids, reflecting offspring anthropometry at birth, were weakly positively associated with offspring fat mass in childhood and adolescence but were not associated with adolescent development of NAFLD. Whilst the association with later offspring adiposity was robust to a range of confounders, it is likely that offspring lifestyle and behaviour may be more likely to account for the development of NAFLD rather than any intrauterine exposure alone.

Maternal obesity in pregnancy is of growing concern, with a greater risk of obstetric and puerperal complications. (Poston, Harthoorn and Van Der Beek, 2011; Poston *et al.*, 2016). Fetal complications are also more common in maternal obesity (Gaillard *et al.*, 2017), although this risk may not be simply confined to the neonatal period. Inappropriate GWG in pregnancy may pose additional risk to the offspring with a positive association being identified between GWG and offspring birthweight (Fraser *et al.*, 2010; Ludwig and Currie, 2010). GWG has also been positively associated with BMI in childhood (Oken *et al.*, 2007; Fraser *et al.*, 2010; Ludwig and Currie, 2010; Ludwig, Rouse and Currie, 2013), and in later life (Oken *et al.*, 2008; Mamun *et al.*, 2009; Schack-Nielsen *et al.*, 2010; Hochner *et al.*, 2012). It is unclear whether epigenetic processes may account for these associations and changes in DNA methylation have been identified as a plausible mediating mechanism (Sharp *et al.*, 2015), although shared socioeconomic and lifestyle factors have also been implicated (Silventoinen *et al.*, 2016; Tyrrell *et al.*, 2016). Previous reports from ALSPAC however suggested that these associations were weak and that maternal BMI may be more consistently associated with a wider range of offspring cardio-

metabolic markers (Fraser *et al.*, 2010). There was therefore the need to examine the causal relationship between maternal BMI and GWG, reflecting fetal overnutrition, and offspring adiposity, as measured by birthweight, and the impact on the cardio-metabolic cord blood profile at birth.

Given the direction of association that exists amongst obese adult subjects, it was anticipated that greater GWG and maternal adiposity would be positively associated with cord blood leptin and lipids and negatively associated with cord blood adiponectin. Whilst GWG was associated with higher cord blood leptin and birthweight, pre-pregnancy BMI however was associated with a wider range of cardio-metabolic markers at birth, including birthweight. Umbilical cord blood leptin is an established biomarker of fat mass and increases with advanced gestation mass (Hauguel-de Mouzon, Lepercq and Catalano, 2006). Birthweight is also a crude marker of offspring anthropometry at birth that correlates positively with child and adult adiposity (Evensen *et al.*, 2017). Although birthweight was positively associated with GWG throughout the entire gestational period, in keeping with previous reports (Tobi *et al.*, 2009; Fraser *et al.*, 2010), the strongest effect of the association was evident during mid-pregnancy. This highlighted that inappropriate GWG during specific periods of pregnancy may pose the greatest risk of offspring programming, and this has also been demonstrated previously by others (Fraser *et al.*, 2010; Hivert *et al.*, 2016).

Over half of overweight or obese mothers included in the study exceeded the IOM recommendations (even though they may have gained less weight overall than normal-weight women). This inappropriate GWG may therefore have exaggerated the altered metabolism already present in obese pregnancy which collectively has the potential to translate on to the fetus via fuel-mediated teratogenesis. Whilst neonatal adiponectin has been positively associated with offspring birthweight, in contrast to associations seen in later life, it is not thought to be associated with maternal BMI (Sivan *et al.*, 2003), and fetal adiponectin may act independently of

maternal adiposity, which may account for the lack of associations identified in the current study.

GDM is a common complication of pregnancy and poses a significant long-term risk to the mother (Catalano *et al.*, 2003; Egan *et al.*, 2014; Gante *et al.*, 2015). The fetus is also at greater risk of obstetric complications, birth injury, and stillbirth. Macrosomia may be detected in mid-pregnancy and may reflect progressive insulin resistance, inadequate glucose control, and greater transmission of nutrients to the fetus. The HAPO study suggested that the risk of offspring macrosomia was exponentially higher when maternal diabetes was combined with maternal obesity (Catalano *et al.*, 2012), suggesting that the offspring of obese diabetic mothers may be at the greatest risk of inheriting this phenotype and maternal BMI may contribute to the causal pathway. Offspring risk may extend into adolescence as maternal diabetes has been also associated with offspring fasting glucose levels at age 15 (Patel *et al.*, 2012). Although dyslipidaemia and hypoadiponectinemia may be evident amongst mothers with diabetes, there is less consistent evidence of altered lipid profile in the offspring, however (Patel *et al.*, 2012). It was, therefore, proposed that maternal diabetes in pregnancy would not be associated with elevated cord blood lipids levels but would be associated with lower neonatal adiponectin levels in the offspring at birth.

GDM may have only a limited influence on offspring programming at birth, as demonstrated by the fact that GDM, in addition to GWG, positively influences the offspring anthropometry but not the cardiometabolic profile at birth. Offspring of those with PGDM displayed cord blood dyslipidaemia however and as such, chronic exposure to hyperglycaemia may confer greater risk to the fetus at birth. The lack of associations in this study may be due to the low prevalence of the disease in this relatively lean cohort, but may also reflect successful intervention during pregnancy resulting in offspring birthweight within a healthy range.

There is an increasing prevalence of HDP amongst the contemporary population, partly due to maternal obesity but also because of greater maternal age at the index pregnancy. PE is a multi-system disorder of endothelial dysfunction and systemic inflammation and as such, long-term maternal sequelae include a greater risk of chronic hypertension, hyperinsulinemia, and dyslipidaemia (Khatua *et al.*, 1989; Vanessa A. Rodie *et al.*, 2004; Ophir *et al.*, 2006; Fraser *et al.*, 2012) as well as the greater risk of cardiovascular dysfunction in later life (Bellamy *et al.*, 2007; McDonald *et al.*, 2008; Wan Sulaiman *et al.*, 2016). HDP may also impose abnormal intrauterine exposure to the offspring. Lower associated birthweight, shorter gestational age or preterm delivery, and the associated placental dysfunction may all lead to more pronounced endothelial dysfunction throughout life. Increased oxidative stress and altered placental lipid transport during pregnancy with PE may also lead to dyslipidaemia in the offspring (Vanessa A. Rodie *et al.*, 2004; Romanowicz and Bańkowski, 2009). Given these existing associations, it was further hypothesised that pregnancy affected by PE would be associated with higher cord blood triglyceride and LDLc, as well as lower HDLc levels.

Examining the cohort, it was identified that women who later developed HDP, in particular PE, were in general younger, nulliparous non-smokers with a higher BMI at booking, and this was in keeping with national guidance (NICE, 2010). Booking blood pressure was also more likely to be higher with DBP specifically rising more sharply in the third trimester.

The offspring of mothers with PE had a lower birthweight and were delivered earlier than healthy subjects, both of which are risk factors for cardio-metabolic dysfunction in later life. Amongst the offspring of those with PE, higher cord blood leptin and cholesterol levels were identified, suggesting that HDP may compete in the causal pathway. Maternal BP was also examined across each gestational period in relation to cord blood analytes and birthweight. In particular, SBP at booking and changes in SBP in the third trimester was positively associated with cord blood HDLc in those that developed PE. Collectively, this suggests that PE not only carries a

greater risk to the mother but the study also suggests that the offspring may be at greater risk of metabolic dysfunction as well.

Whilst there is limited evidence of tracking of analytes in early life, studies have suggested that serum lipoproteins show evidence of tracking over 12 years (Porkka *et al.*, 1994) and that lipids, in particular, have been associated with cardiovascular disease occurring more than 20 years later (Klag *et al.*, 1993; Raitakari *et al.*, 2003). It has therefore been proposed that lipids may act as surrogate markers of future cardiovascular risk. LFTs have also displayed evidence of tracking in adulthood (Patel *et al.*, 2007; Nguyen *et al.*, 2011), and may be used as indicators of future disorder metabolism. There is limited evidence that adipokines, such as leptin however show evidence of tracking and it remains unclear if an association, if any, begins in utero and persists throughout life. Whilst leptin is a recognised biomarker of fat mass and ponderal index in the neonatal period (Hauguel-de Mouzon, Lepercq and Catalano, 2006; Karakosta *et al.*, 2011, 2013), cord blood leptin has not been associated with later repeat measures of leptin in childhood (Volberg, Heggeseth, *et al.*, 2013). Leptin obtained in childhood however has displayed evidence of tracking into late childhood (Nishimura *et al.*, 2009; Volberg, Heggeseth, *et al.*, 2013), and it appears that reproducible tracking of such analytes may strengthen with time.

As positive associations have previously been demonstrated between LFTs, lipids, and adipokines in later life, it was anticipated that cord blood measures would also be positively associated with repeated measures of the same analyte in childhood and adolescence. This study has demonstrated that lipids, as well as adiponectin and markers of liver function, may track from birth (cord blood) through to childhood, but that the association was lost between birth and age 17. Associations with repeated 'like for like' measures of GGT however persisted from birth to age 17, demonstrating that certain cord blood measures may have the potential for use as clinical predictors. In keeping with previous reports, this study also demonstrated that lipids and LFTs, measured at age 9, are strongly associated with repeat analytes at age 17 (Porkka *et al.*, 1994; Raitakari *et al.*, 2003). As associations became more

marked between childhood and adolescence, these associations may offer greater clinical value with advancing age.

Given the positive associations that exist between cord blood leptin and fat mass at birth and in early childhood, as well as the direct effect adiponectin has on body composition and fat deposition, it was further hypothesized that higher cord blood leptin and lower cord blood adiponectin, would be associated with greater adiposity in later childhood and adolescence. Birthweight and cord blood adipokines, reflecting the intrauterine environment at birth, were used to examine the long-term metabolic function of the offspring, as measured by adiposity in late childhood and adolescence.

This study has shown that neonatal fat mass (as determined by surrogate markers cord blood leptin and adiponectin), was weakly associated with greater fat mass in late childhood and adolescence. Maternal factors, however, mainly maternal BMI, did appear to attenuate these associations and may have contributed to the causal pathway. This study also added to previous literature showing that adiponectin positively correlated with birthweight and leptin, and supported neonatal leptin as a valid marker of neonatal fat mass (Hauguel-de Mouzon, Lepercq and Catalano, 2006). The association between adiponectin and adolescent measures of adiposity however were robust to adjustments for confounders, which accounted for a range of maternal and shared environmental exposures. Maternal over-nutrition may therefore have a direct effect on fetal fat accretion which sets a trajectory for enhanced offspring adiposity throughout later life.

In line with the global trend in obesity, there has been an increasing prevalence of NAFLD worldwide. The identification of NAFLD in children and adolescents has led to the speculation that intrauterine events may contribute to its early pathogenesis (Alisi *et al.*, 2012; Brumbaugh and Friedman, 2014). Whilst animals fed high-fat diets during pregnancy exhibit offspring obesity and hepatic lipid accumulation, (McCurdy

et al., 2009; Oben *et al.*, 2010), evidence amongst human subjects remains less clear, with birthweight being positively associated with later development of NAFLD in some (Anderson *et al.*, 2014), but not all studies (Ayonrinde *et al.*, 2015). In this study, it was hypothesized that cord blood leptin and lipids would be positively, and adiponectin negatively associated with NAFLD in adolescence, given the already established associations between these measures and NAFLD activity in later life.

There was no consistent evidence however that birth weight, neonatal adiposity, or cord lipids were associated with liver dysfunction in adolescence. The development of NAFLD may therefore be mainly driven by adolescent behaviours and phenotype rather than intrauterine exposures or adiposity at birth.

Whilst the detailed anthropometric markers and long duration of follow-up added to the strength of this prospective study, it was subject to limitations. The low prevalence of disease amongst this relatively lean population may have accounted for the lack of associations seen with offspring adiposity. The cohort included a mainly Caucasian population, so it was difficult to translate these findings across other ethnicities. Finally, sample degradation with long-term storage may have contributed to the variability within the samples. Significant degradation was evident for cord blood c-peptide, hence its exclusion from the study.

9.2 Final Conclusions

This thesis has examined the disordered maternal metabolism in pregnancy and how this may have influenced offspring birthweight and cord blood biomarkers at birth. In addition, the extent to which cord blood markers tracked from birth into adolescence were examined. Finally, associations between cord blood adipokines

and birthweight, reflecting anthropometric markers at birth, on long-term adiposity outcomes, were determined.

As detailed in each of the Chapters, this study may draw several conclusions. Firstly, it has demonstrated that pre-pregnancy BMI, rather than GWG alone, was more consistently associated with a range of offspring cardio-metabolic markers in the cord blood at birth, namely offspring dyslipidaemia, and this was in keeping with current literature. Both BMI and GWG were however positively associated with greater offspring birthweight and cord blood leptin. There was limited evidence of metabolic dysfunction in the offspring of mothers with GDM compared to offspring of those with PGDM who displayed more variability in the cord blood, specifically evidence of dyslipidaemia. Offspring of mothers who developed PE during pregnancy may also have been at greater risk of metabolic dysfunction, as lower birthweight and higher cord blood leptin and cholesterol were evident amongst this population.

Whilst there was evidence of tracking of cord blood adipokines, lipids, and LFTs from birth to age 9, the association beyond that was lost for all but GGT. As GGT has been implicated in metabolic disorders in later life, this novel finding may highlight the use of clinical biomarkers obtained at birth as potential predictors of longer-term health, although replication of the findings would be required in future studies. Tracking of analytes obtained at age 9 then again at age 17 was more consistent and overall this suggested that tracking may become more progressively defined with time. This study also demonstrated that cord blood leptin was weakly, and adiponectin strongly related to adiposity in childhood and adolescence respectively. Using cord blood leptin as a reflection of fetal fat mass, these associations demonstrated that maternal overnutrition may therefore have had a direct effect on fetal fat accretion, determining a trajectory for enhanced offspring adiposity in later life. The fact that neither birthweight, nor adiposity at birth were related to offspring markers of liver health in adolescence indicated that the onset of NAFLD was mainly driven by behavioural and environmental factors rather than triggered by an adverse intrauterine environment.

Whilst it was difficult to account for environmental and lifestyle influences, adjustment for a wide range of pregnancy, maternal and offspring characteristics has contributed to the robust analysis and interpretation of the findings. Reproducing this study using a more contemporary and diverse cohort may also add to its validity. Lastly, analysis of blood samples at serial time points would have eliminated the risk and extent of blood sample degradation, and contributed to the reliability of the findings from this study. Future work may also include optimising maternal pre-pregnancy BMI for all women of reproductive age as well as improving glycaemic control amongst those with PGDM, given the potential metabolic implications for the offspring. In addition, examining tracking of blood analytes from childhood onwards may be of clinical value in predicting future metabolic dysfunction. Lastly, given the global obesity pandemic, future work should also be considered to determine the long-term implications of cord blood adipokine disruption at birth on the risk of offspring adiposity and if this in itself poses any additional metabolic risk to the offspring.

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Appendix

Chapter 2, Table 1. : Quality Control data for cord blood leptin ELISA

Plate	Control	Mean (pg/ml)	SD	Intra-assay CV
1	9.63	10.73	1.47	0.14
	8.33			
2	10.64	10.61	0.04	0.00
	10.58			
3	12.51	12.43	0.10	0.01
	12.36			
4	11.10	11.24	0.19	0.02
	11.37			
5	11.32	10.90	0.59	0.05
	10.48			
6	10.96	10.63	0.47	0.04
	10.30			
7	12.74	11.95	1.11	0.09
	11.17			
8	11.77	11.50	0.38	0.03
	11.23			
9	10.19	9.80	0.56	0.06
	9.40			
10	11.81	11.66	0.21	0.02
	11.51			
11	12.76	12.37	0.54	0.04
	11.99			
12	14.32	14.16	0.24	0.02
	13.99			
13	11.19	11.08	0.16	0.01
	10.97			
14	11.66	11.37	0.41	0.04
	11.09			
15	12.43	12.18	0.35	0.03
	11.93			
16	11.26	11.14	0.17	0.02
	11.02			
17	13.25	13.09	0.23	0.02
	12.93			
18	11.82	11.55	0.39	0.03
	11.28			
20	12.16	12.27	0.15	0.01
	12.38			
21	10.66	10.48	0.25	0.02
	10.30			
22	11.12	10.85	0.38	0.04
	10.58			
23	10.98	10.76	0.31	0.03
	10.55			

24	11.64 11.73	11.69	0.06	0.01
25	11.81 11.77	11.79	0.03	0.00
26b	10.82 11.27	11.04	0.32	0.03
27b	12.01 12.58	12.29	0.40	0.03
28	10.93 10.96	10.94	0.02	0.00
29	11.64 11.95	11.80	0.22	0.02
30	11.08 11.21	11.14	0.09	0.01
31	11.28 11.50	11.39	0.15	0.01
32	10.67 11.68	11.17	0.72	0.06
33	10.03 9.99	10.01	0.03	0.003
34	11.78 11.51	11.64	0.19	0.02
35	12.45 12.37	12.41	0.05	0.00
36	11.91 12.21	12.06	0.21	0.02
37	10.28 9.67	9.97	0.43	0.04
38	9.88 10.22	10.05	0.24	0.02
39	11.99 12.19	12.09	0.14	0.01
40	13.92 14.02	13.97	0.07	0.01
41	13.36 13.46	13.41	0.07	0.01
42	11.38 11.86	11.62	0.34	0.03
43	12.80 12.95	12.88	0.11	0.01
44	10.83 11.01	10.92	0.12	0.01
45	12.22 12.74	12.48	0.36	0.03
46	10.33 10.29	10.31	0.03	0.00
47	11.14 11.57	11.35	0.30	0.03
48	10.48 9.48	9.98	0.70	0.07

50	8.85 8.61	8.73	0.17	0.02
51	11.35 11.32	11.34	0.02	0.00
52	9.83 8.66	9.24	0.82	0.09
53	10.23 10.02	10.13	0.15	0.02
54	11.69 11.73	11.71	0.03	0.00
55	11.66 11.82	11.74	0.11	0.01
56	11.28 11.80	11.54	0.37	0.03
57	10.78 10.99	10.89	0.15	0.01
58	11.25 11.32	11.28	0.05	0.01
59	11.46 11.82	11.64	0.26	0.02
60	11.19 11.47	11.33	0.19	0.02
61	12.19 12.16	12.17	0.02	0.00
62	11.35 10.65	11.00	0.50	0.05
63	11.73 11.64	11.69	0.06	0.01
64	11.87 11.84	11.85	0.02	0.00
Mean (pg/ml)		**11.38**		
SD (pg/ml)			**1.08**	
Inter-assay CV				**0.09**
Intra-assay CV				**0.03**

Chapter 2, Table 2.: Quality control for adiponectin ELISA

Plate	Control (µg/ml)		Mean (µg/ml)	SD	Intra-assay CV
	1	2			
1	13.94	15.18	14.56	0.88	0.06
2	14.15	15.31	14.73	0.82	0.06
3	13.45	14.58	14.02	0.80	0.06
4	10.90	13.74	12.32	2.01	0.16
5	14.96	16.12	15.54	0.81	0.05
6	13.74	14.73	14.24	0.70	0.05
7	15.09	15.55	15.32	0.33	0.02
8	13.85	15.40	14.63	1.10	0.08
9	14.76	14.72	14.74	0.03	0.00
10	15.63	15.40	15.51	0.16	0.01
11	16.29	17.54	16.92	0.88	0.05
12	14.49	15.87	15.18	0.98	0.06
13	14.52	15.19	14.86	0.47	0.03
14	15.80	18.21	17.00	1.70	0.10
15	15.35	15.86	15.61	0.36	0.02
16	15.33	16.26	15.80	0.66	0.04
17	13.08	14.10	13.59	0.72	0.05
18	14.83	15.93	15.38	0.77	0.05
19	12.46	14.04	13.25	1.12	0.08
20	12.83	14.41	13.62	1.12	0.08
21	13.94	15.05	14.50	0.78	0.05
22	14.92	16.12	15.52	0.85	0.05
23	13.91	14.36	14.13	0.32	0.02
24	13.36	14.44	13.90	0.76	0.05
25	12.73	14.00	13.37	0.90	0.07
26	15.09	13.47	14.28	1.14	0.08
27	12.93	11.98	12.45	0.67	0.05
28	13.18	14.88	14.03	1.20	0.09
29	14.77	15.81	15.29	0.74	0.05
30	13.23	14.36	13.79	0.80	0.06
31	12.96	14.90	13.93	1.38	0.10
32	14.32	15.62	14.97	0.92	0.06
33	13.04	14.20	13.62	0.82	0.06
34	13.66	15.12	14.39	1.03	0.07

35	13.03	13.34	13.18	0.22	0.02
36	13.29	13.90	13.60	0.43	0.03
37	12.44	13.12	12.78	0.48	0.04
38	13.19	14.70	13.94	1.07	0.08
39	13.43	12.87	13.15	0.40	0.03
40	12.05	13.45	12.75	0.99	0.08
41	13.34	14.76	14.05	1.00	0.07
42	13.91	14.36	14.14	0.32	0.02
43	12.23	11.61	11.92	0.44	0.04
44	13.28	15.40	14.34	1.50	0.10
45	13.81	15.38	14.60	1.11	0.08
46	14.09	14.99	14.54	0.64	0.04
47	13.36	13.80	13.58	0.32	0.02
48	14.52	15.35	14.94	0.58	0.04
49	14.67	15.69	15.18	0.72	0.05
50	14.29	14.18	14.24	0.08	0.01
51	14.62	15.65	15.13	0.73	0.05
52	13.72	15.02	14.37	0.92	0.06
53	15.70	17.65	16.67	1.37	0.08
54	14.60	15.21	14.91	0.43	0.03
55	14.59	15.81	15.20	0.86	0.06
56	13.28	14.82	14.05	1.09	0.08
57	14.38	15.47	14.94	0.77	0.05
58	13.93	15.26	14.59	0.94	0.06
59	11.87	15.21	13.54	2.36	0.17
60	13.69	16.20	14.94	1.77	0.12
61	13.59	14.85	14.22	0.89	0.06
62	14.52	14.27	14.39	0.17	0.01
63	14.52	17.25	15.89	1.93	0.12
64	13.35	14.46	13.90	0.78	0.06
Mean (µg/ml)			**14.41**		
SD (µg/ml)				**0.47**	
CV Inter-assay					**0.03**
CV Intra-assay					**0.06**

Chapter 2, Table 3: Quality Control Data for cord blood analytes using autoanalyser

Plate	HDLc control (0.97-1.33) mmol/l	CRP control (3.51-4.27) mg/l	Cholesterol control (2.18-2.66) mmol/l	HDLc control (37.40-47.80) u/l	AST control (42.10-53.7) u/l	Triglyceride control (1.08-1.32) mmol/l	GGT control (46.2-59.0) u/l	Lower limit control mean (SD) (1.5-2.46) ng/ml	Intra-assay CV (%)	Higher limit control mean (SD) (7.35 -12.00) ng/ml	Intra-assay CV (%)
1	1.11	4.09	2.49	43.7	52.6	1.27	53.0	1.91 (0.01)	0.00 (0.5)	9.70 (0.01)	0.00 (0.1)
2	0.99	3.98	2.36	40.7	47.7	1.22	50.0	1.89 (0.02)	0.01 (1.1)	9.68 (0.03)	0.00 (0.3)
3	1.08	3.89	2.4	42.8	49.5	1.22	51.0	1.95 (0.04)	0.02 (2.1)	9.95 (0.02)	0.00 (0.2)
4	1.08	3.89	2.4	42.8	49.5	1.22	51.0	1.95 (0.03)	0.02 (1.5)	9.75 (0.02)	0.00 (0.2)
5	1.08	4.02	2.27	40.4	44	1.14	49.0	1.87 (0.04)	0.02 (2.1)	9.42 (0.03)	0.00 (0.3)
6	1.08	3.92	2.46	42.6	49.3	1.23	52.0	1.82		9.38	
7	1.05	3.98	2.48	43	49.8	1.25	53.0	1.88		9.26	
8	1.09	4.00	2.5	43.6	49.5	1.23	52.0	1.74		9.09	
9	1.16	3.98	2.52	44.3	49.6	1.24	53.0	1.73		9.08	
10	1.16	3.98	2.52	44.3	49.6	1.24	53.0	1.70 (0.06)	0.04 (3.5)	9.10 (0.02)	0.00 (0.2)
11	1.12	3.87	2.51	43.7	49.7	1.24	52.0	1.81 (0.03)	0.02 (1.7)	9.53 (0.02)	0.00 (0.2)
12	1.12	3.87	2.51	43.7	49.7	1.24	52.0	2.22		9.73	
13	1.16	4.00	2.5	44.7	51.6	1.28	54.0	1.52		8.03	
14	1.16	4.00	2.5	44.7	51.6	1.28	54.0	1.52		8.03	
15	1.11	3.99	2.48	46.8	50.6	1.24	52.0	2.22		9.73	
16	1.11	3.99	2.48	46.8	50.6	1.24	52.0	1.82		9.38	
17	0.98	3.97	2.43	41.3	47.7	1.22	50.0	2.22		9.73	
18	0.98	3.97	2.43	41.3	50.6	1.22	50.0	2.05		9.81	

19	1.15	3.92	2.48	41.3	50.6	1.23	50.0	2.05		9.81	
									0.01 (0.9)		0.00
20	1.15	3.92	2.48	41.3	50.3	1.23	50.0	2.05 (0.02)		9.81 (0.04)	(0.4)
									0.01 (1.5)		0.00
21	1.15	3.92	2.48	41.3	50.3	1.23	50.0	2.05 (0.03)		9.81 (0.02)	(0.2)
									0.01 (1.5)		0.00
22	1.15	3.92	2.48	41.1	49.6	1.23	50.0	2.05 (0.03)		9.81 (0.01)	(0.1)
23	1.1	3.93	2.48	41.1	49.6	1.24	50.0	1.86		10.95	
24	1.1	3.93	2.48	41.1	49.6	1.24	50.0	1.86		10.95	
25	1.1	3.93	2.48	42.2	50.7	1.24	50.0	1.83		9.85	
26	1.1	3.93	2.48	42.2	50.7	1.24	50.0	1.83		9.85	
27	1.17	3.66	2.58	42.2	50.7	1.27	50.0	1.83		9.85	
28	1.13	3.95	2.49	42.2	50.2	1.22	50.0	2.00		7.24	
29	1.13	3.95	2.49	42.2	50.2	1.22	50.0	2.00		12.28	
									0.02 (1.9)		0.00
30	1.11	3.99	2.49	42.2	50.2	1.22	50.0	2.07 (0.04)		11.86 (0.03)	(0.3)
									0.02 (2.4)		0.00
31	1.11	3.99	2.49	43.8	49.9	1.22	51.0	2.07 (0.05)		11.86 (0.04)	(0.3)
									0.01 (1.0)		0.00
32	1.14	4.00	2.48	43.8	49.9	1.23	51.0	1.92 (0.02)		10.14 (0.03)	(0.3)
33	1.14	4.00	2.48	40.5	47.7	1.23	51.0	1.92		10.14	
34	1.13	3.97	2.53	40.5	47.7	1.24	51.0	1.92		10.14	
35	1.13	3.97	2.53	40.5	47.7	1.24	51.0	1.92		10.14	
36	1.13	3.95	2.59	39.4	44.9	1.25	50.0	2.00		12.27	
37	1.13	3.95	2.59	40.9	44.6	1.25	50.0	2.19		11.1	
38	1.11	3.85	2.51	43.5	49.9	1.27	52.0	2.19		11.1	
39	1.13	3.91	2.49	42.1	49.1	1.23	52.0	2.36		9.08	
									0.01 (1.4)		0.00
40	1.11	3.98	2.6	44.4	50.2	1.25	53.0	2.16 (0.03)		8.56 (0.04)	(0.5)
									0.01 (0.9)		0.00
41	1.11	3.98	2.6	44.4	50.2	1.25	53.0	2.16 (0.02)		8.56 (0.04)	(0.5)
									0.00 (0.9)		0.01
42	1.11	3.98	2.6	44.4	50.2	1.25	53.0	2.16 (0.02)		8.56 (0.05)	(0.6)
43	1.13	3.98	2.64	45.1	51.5	1.28	53.0	1.66		10.77	

44	1.13	3.98	2.64	45.1	51.5	1.28	53.0	1.66		10.77	
45	1.11	3.94	2.53	43.1	51.3	1.23	52.0	1.86		8.67	
46	1.11	3.94	2.53	43.1	51.3	1.23	52.0	1.82		9.01	
47	1.11	3.99	2.5	42.4	48.9	1.23	51.0	1.82		9.01	
48	1.2	3.95	2.52	46.7	53.7	1.31	54.0	1.65		9.03	
49	1.1	3.94	2.39	41.4	49.7	1.24	51.0	1.92		10.57	
50	1.2	3.95	2.52	46.7	53.7	1.31	54.0	1.77		9.59	
									0.02 (1.7)		0.00
51	1.2	3.95	2.52	46.7	53.7	1.31	54.0	1.77 (0.03)		9.59 (0.04)	(0.4)
									0.02 (1.7)		0.00
52	1.08	3.89	2.56	41.1	49.8	1.26	51.0	1.77 (0.03)		9.59 (0.04)	(0.4)
									0.03 (3.2)		0.01
53	1.08	3.89	2.56	41.1	49.8	1.26	51.0	1.87 (0.06)		8.38 (0.05)	(0.6)
54	1.11	3.96	2.62	42.9	51.7	1.3	53.0	1.87		8.38	
55	1.11	3.96	2.62	42.9	51.7	1.3	53.0	1.97		8.9	
56	1.1	3.95	2.61	41.1	51	1.3	52.0	1.97		8.9	
57	1.09	3.97	2.64	42.3	51.1	1.32	52.0	1.91		8.66	
58	1.09	3.97	2.64	42.3	51.1	1.32	52.0	1.91		8.66	
59	1.09	3.97	2.64	42.3	51.1	1.32	52.0	1.69		8.68	
									0.03 (2.9)		0.00
60	1.09	3.92	omitted	42.8	50.7	1.31	52.0	1.69 (0.05)		8.68 (0.04)	(0.5)
									0.02 (2.4)		0.00
61	1.09	3.92	omitted	42.8	50.7	1.31	52.0	1.69 (0.04)		8.68 (0.04)	(0.5)
62	1.09	3.95	2.59	44.2	50.4	1.28	52.0	1.82		8.21	
63	1.09	3.95	2.59	44.2	50.4	1.28	52.0	1.82		8.21	
64	1.09	3.95	2.59	44.2	50.4	1.28	52.0	1.8		9.03	
Mean	1.11	3.95	2.52	42.82	50.03	1.25	51.56	1.90		9.51	
SD	0.04	0.05	0.08	1.76	1.77	0.03	1.30	0.17		1.11	
CV (%)	0.04 (4.0)	0.01 (1.0)	0.03 (3.0)	0.04 (4.0)	0.04 (4.0)	0.03 (3.0)	0.03 (3.0)	0.09 (9.0)		0.12	

Chapter 2, Table 4: Cord and neonatal blood reference ranges

Reference	C-peptide (g/ml)	Leptin (ng/ml)	Adiponectin (µg/ml)	Triglyceride (mmol/l)	Cholesterol (mmol/l)	HDLc (mmol/l)	AST U/l	ALT U/l	GGT U/l
ALSPAC	0.1	11.4	14.4	1.3	2.5	1.1	50.0	3.4	51.5
(Popova et al., 2018) Mean (SD)	0.9 (0.4)	10.6 (10.4)	18.3 (14.3)						
(Kim, Lim and Oh, 2017) Mean (SD)									156.7 ± 98.2
(Rifas-Shiman et al., 2017) Mean (SD)	1.0 (0.6)	9.0 (6.6)	28.7 (6.8)						
(Lemas et al., 2015) Mean (SD)		14.3 (13.2)		49.1 (40.5)		26.3 (7.5)			
(Hou et al., 2014) Median (IQR)	0.31 (0.23, 0.42)			0.29 (0.23, 0.37)	1.60 (1.38, 1.88)	0.79 (0.66, 0.96)			
(Melkie et al., 2012) Mean (95% CI)							35.9 (34.1,37.7)	12.5 (11.1, 13.8)	96.0 (87.6, 04.0)
(Regnault et al., 2011) Mean (SD)	1.0 (0.49)								
(Inoue et al., 2008) Mean (SD)			14.9 ± 5.8						
(Metzger et al., 2008) Mean (SD)	1.0 (0.6)								

Chapter 6, Table 5: Spearman correlations between birthweight and cord blood measures (N obs, R)

	Leptin	Adiponectin	Cholesterol	Triglyceride	HDLc	Non-HDLc	GGT	ALT	AST (u/l)
Birthweight	4751	4707	4689	4674	4604	4604	4674	4684	4680
(g)	0.33	0.14	-0.02	-0.03	0.04	-0.05	-0.02	0.14	0.05
Leptin		4962	4944	4930	4857	4857	4929	4939	4935
(pg/ml)		0.11	0.02	-0.03	0.11	-0.01	-0.16	0.05	-0.22
Adiponectin			4941	4928	4855	4855	4928	4935	4933
(µg/ml)			-0.16	-0.16	-0.08	-0.17	0.01	0.12	0.05
Cholesterol				4931	4859	4859	4931	4940	4937
(mmol/l)				0.43	0.64	0.91	0.23	0.13	0.22
Triglyceride					4853	4853	4928	4928	4928
(mmol/l)					-0.07	0.60	0.00	0.07	0.34
HDLc						4859	4853	4856	4856
(mmol/l)						0.30	0.25	0.21	0.05
Non-HDLc							4853	4856	4856
(mmol/l)							0.16	0.05	0.25
GGT								4929	4930
(u/l)								0.14	0.27
ALT									4935
(u/l)									0.38

Chapter 6, Table 6: Spearman correlations between BMI, FM and analyte measures at age 17 (N obs, R)

	FM	Cholesterol	Triglyceride	HDLc	GGT	ALT	AST
BMI	4828	3202	3202	3202	3170	3170	3170
(kg/m^2)	0.78	0.15	0.22	-0.21	0.21	0.10	0.26
FM		3156	3156	3156	3125	3125	3125
(g)		0.27	0.21	-0.05	0.05	-0.08	0.11
Cholesterol			3287	3287	3221	3221	3221
(mmol/l)			0.36	0.22	0.11	0.06	0.09
Triglyceride				3287	3221	3221	3221
(mmol/l)				-0.31	0.32	0.06	0.06
HDLc					3221	3221	3221
(mmol/l)					-0.24	-0.08	-0.13
GGT						3258	3258
(u/l)						0.41	0.31
ALT							3258
(u/l)							0.65

Chapter 7, Table 7: Associations (%) of birthweight and cord blood analyte with FM, WC and BMI at age 9, 15 and 17 years

Outcome		FM*				WC				BMI			
		Age 9		Age 17		Age 9		Age 15		Age 9		Age 17	
Exposure	Model	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P
Leptin (10pg/ml)	1	3.9 (2.2, 5.7)	<0.001	3.6 (1.5, 5.8)	0.001	1.0 (0.6, 1.4)	<0.001	0.9 (0.5, 1.4)	<0.001	1.5 (0.9, 2.0)	<0.001	1.4 (0.8, 2.1)	<0.001
	2	2.0 (0.3, 3.7)	0.022	1.0 (-1.0, 3.1)	0.335	0.5 (0.1, 0.9)	0.016	0.4 (0.0, 0.9)	0.074	0.8 (0.3, 1.3)	0.003	0.5 (-0.2, 1.1)	0.151
	3	1.5 (-0.2, 3.3)	0.077	0.6 (-1.5, 2.7)	0.568	0.4 (0.0, 0.8)	0.069	0.4 (-0.1, 0.9)	0.100	0.6 (0.1, 1.1)	0.023	0.3 (-0.3, 1.0)	0.317
Adjusted Leptin† (10pg/ml)	1	3.9 (2.0, 5.8)	<0.001	2.9 (0.5, 5.3)	0.015	0.6 (0.2, 1.1)	0.008	0.4 (-0.1, 0.9)	0.111	1.0 (0.4, 1.6)	0.001	1.0 (0.2, 1.7)	0.008
	2	2.2 (0.5, 4.1)	0.014	1.0 (-1.2, 3.3)	0.369	0.2 (-0.2, 0.6)	0.373	0.0 (-0.5, 0.5)	0.914	0.4 (-0.1, 1.0)	0.113	0.3 (-0.4, 1.0)	0.413
	3	2.0 (0.2, 3.8)	0.032	0.8 (-1.5, 3.0)	0.509	0.1 (-0.3. 0.6)	0.594	0.0 (-0.5, 0.5)	0.970	0.3 (-0.2, 0.9)	0.260	0.2 (-0.5, 0.9)	0.603
Adiponectin (10µg/ml)	1	0.0 (-0.5, 0.5)	0.999	0.6 (-0.1, 1.2)	0.092	-0.1 (-0.2, 0.0)	0.152	0.1 (0.0, 0.3)	0.061	0.0 (-0.2, 0.1)	0.830	0.2 (0.0, 0.4)	0.083
	2	0.1 (-0.4, 0.6)	0.738	0.7 (0.0, 1.3)	0.039	-0.1 (-0.2, 0.0)	0.236	0.2 (0.0, 0.3)	0.024	0.0 (-0.1, 0.2)	0.860	0.2 (0.0, 0.4)	0.029
	3	0.1 (-0.4, 0.6)	0.758	0.6 (0.0, 1.3)	0.048	-0.1 (-0.2, 0.0)	0.186	0.2 (0.0, 0.3)	0.032	0.0 (-0.2, 0.2)	0.975	0.2 (0.0, 0.4)	0.033
Birthweight‡ (100g)	1	0.5 (0.0, 0.9)	0.032	1.2 (0.7, 1.7)	<0.001	0.4 (0.3, 0.5)	<0.001	0.5 (0.4, 0.6)	<0.001	0.5 (0.4, 0.7)	<0.001	0.5 (0.3, 0.7)	<0.001
	2	0.1 (-0.3, 0.5)	0.609	0.6 (0.1, 1.2)	0.016	0.3 (0.2, 0.4)	<0.001	0.4 (0.3, 0.5)	<0.001	0.4 (0.3, 0.5)	<0.001	0.3 (0.1, 0.4)	<0.001
	3	-0.1 (-0.5, 0.4)	0.710	0.5 (-0.1, 1.0)	0.091	0.3 (0.2, 0.4)	<0.001	0.4 (0.3, 0.5)	<0.001	0.4 (0.2, 0.5)		0.3 (0.1, 0.4)	0.002

Models 1: Adjusted for offspring sex, 2: Adjusted for sex and maternal confounders (age, smoking, parity, occupational social class, education and pre-pregnancy BMI), 3: Adjusted for offspring sex and maternal confounders plus pregnancy confounders (gestational age at birth, mode of delivery, GWG, hypertensive disorders and diabetic disorders of pregnancy). * FM adjusted for height † Leptin adjusted for birthweight ‡ Birthweight adjusted for sex, gestational age and singleton/twin pregnancy. % refers to percentage change of outcome per unit increase in exposure

Chapter 7, Table 8: Associations of birthweight and cord blood analyte with FM and BMI at age 9 and 17 years (using non-imputed data)

Outcome	Fat mass*				BMI			
	Age 9		Age 17		Age 9		Age 17	
Exposure	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P
Leptin (10pg/ml)	0.7 (-1.8, 3.2)	0.015	2.0 (-1.2, 5.3)	0.222	0.3 (-0.5, 1.1)	0.452	0.6 (-0.4, 1.6)	0.227
Adjusted Leptin† (10pg/ml)	0.9 (-1.7, 3.6)	0.482	1.2 (-2.1, 4.7)	0.475	-0.2 (-1.0, 0.6)	0.618	0.2 (-0.8, 1.3)	0.633
Adiponectin (10µg/ml)	1.4 (-2.6, 5.5)	0.495	2.3 (-0.3, 7.8)	0.399	0.5 (-0.8, 1.7)	0.464	0.9 (-0.8, 2.5)	0.299
Birthweight‡ (100g)	-5.1 (-21.6, 14.8)	0.589	22.0 (-4.5, 55.9)	0.111	13.8 (7.6, 20.3)	<0.001	9.4 (1.9, 17.5)	0.013

Adjusted for offspring sex and maternal confounders plus pregnancy confounders (gestational age at birth, mode of delivery, gestational weight gain, hypertensive disorders and diabetic disorders of pregnancy. * Fat mass adjusted for height. † Leptin adjusted for birthweight, ‡ Birthweight adjusted for sex, gestational age and singleton/twin pregnancy, % refers to percentage change of outcome per unit increase in exposure

Chapter 8, Table 9: Characteristics of those who had a liver scan and/or LFT's performed at age 17 using imputed and observed data

	Liver scans					Liver Function Tests				
	MI Data		Observed data			MI Data		Observed data		
	Median or % N obs (IQR or N)	N obs	Median or % N obs (IQR or N)	N obs	% missing (N)	Median or % N obs (IQR or N)	N obs	Median or % N obs (IQR or N)	N obs	% missing (N)
Maternal Characteristics										
Age (yrs)	29 (26, 32)	541	29 (26, 32)	535	1.1 (6)	29 (26, 32)	1037	29 (26, 32)	1023	1.3 (14)
Smoking		541		525						
Never	75.2 (407)		74.9 (393)		3.0	77.4 (803)	1037	77.1 (779)	1011	2.5
Before, not during	9.4 (51)		9.5 (50)		(16)	7.4 (77)		7.6 (77)		(26)
During pregnancy	15.3 (83)		15.6 (82)			15.1 (157)		15.3 (155)		
BMI	22.4 (20.5,24.7)	541	22.3 (20.5, 24.4)	479	11.5(62)	22.2 (20.5,24.3)	1037	22.0 (20.5,24.1)	918	11.5 (119)
Parity		541		508	6.1		1037		991	4.4
0	43.81(237)		46.7 (237)		(33)	45.0 (467)		47.1 (467)		(46)
1	36.8 (199)		39.2 (199)			35.2 (365)		36.8 (365)		
2	0.1 (49)		9.7 (49)			11.3 (117)		11.8 (117)		
3	3.7 (20)		3.9 (20)			3.3 (34)		3.4 (34)		
4+	0.6 (3)		0.6 (3)			0.8 (8)		0.8 (8)		
Social Class		541		452	16.5		1037		884	14.6
I: least	7.0 (38)		6.9 (31)		(89)	8.5 (88)		8.0 (71)		(153)
II	38.3 (207)		38.5 (174)			37.1 (385)		38.4 (339)		
III	40.9 (221)		40.3 (182)			38.9 (403)		39.5 (349)		
IV	4.8 (26)		5.1 (23)			5.8 (60)		5.1 (45)		
V	7.8 (42)		8.4 (38)			8.1 (84)		7.6 (67)		
VI: most disadvantage	1.3 (7)		0.9 (4)			1.6 (17)		1.5 (13)		
Education		541		518	4.3		1037		994	4.1
Left school 16	56.7 (306)		56.6 (293)		(23)	53.0 (550)		53.3 (530)		(43)
A level	25.3 (137)		25.3 (131)			28.4 (294)		28.3 (281)		
Degree	18.1 (98)		18.1 (94)			18.6 (193)		18.4 (183)		
Alcohol		541		522	3.5				1009	2.7
None/<1 glass/wk	44.0 (238)		43.5 (227)		(19)	42.2 (438)		43.0 (434)		(28)
>1 glass/wk	45.3 (245)		46.4 (242)			46.8 (485)		45.9 (463)		
>1 glass/day	10.7 (58)		10.2 (53)			11.0 (114)		11.1 (112)		
Offspring characteristics										
Sex		541		541	0.0		1037		1037	0.0
Male	39.7 (215)		39.7 (215)		(0)	46.4 (481)		46.4 (481)		(0)
Female	60.3 (326)		60.3 (326)			53.6 (556)		53.6 (556)		

Age	17.8 (17.6, 18.3)	541	17.8 (17.6, 18.3)	541	0.0 (0)	17.8 (17.6, 17.9)	1037	17.8 (17.6, 17.9)	1037	0.0 (0)

Chapter 8, Table 10: Association of birthweight and cord blood measures with markers of liver health in adolescence (multiple imputation datasets) N=541 for liver scans, N=1037 for LFTs

Outcome Age 17:		logLiver volume (ml)			logLiver sheer velocity (m/s)			logALT (U/l)			logAST (U/l)			logGGT (U/l)		
Exposure	Model (1,2)	β	CI	P	β	CI	P	β	CI	P	β	CI	P	β	CI	P
Cord blood																
Leptin	1	0.01	-0.01, 0.03	0.22	0.00	-0.02, 0.01	0.97	0.00	-0.02, 0.03	0.73	0.00	-0.01, 0.02	0.81	0.00	-0.02, 0.02	0.96
(per 10 pg/ml)	2	0.00	-0.02, 0.02	0.79	-0.01	-0.02, 0.01	0.46	-0.01	-0.03, 0.02	0.61	0.00	-0.02, 0.02	0.97	-0.01	-0.03, 0.02	0.66
Adiponectin	1	0.00	-0.01, 0.01	0.61	0.00	-0.01, 0.01	0.82	0.00	-0.01, 0.01	0.68	0.003	-0.00, 0.01	0.23	0.002	0.00, 0.01	0.41
(per 10µg/ml)	2	0.00	-0.01, 0.01	0.79	0.00	-0.01, 0.01	0.99	0.00	-0.01, 0.01	0.53	0.003	-0.00, 0.01	0.27	0.00	0.00, 0.01	0.45
Cholesterol	1	0.00	-0.02, 0.02	0.90	-0.01	-0.03, 0.01	0.24	0.01	-0.01, 0.04	0.22	-0.001	-0.02, 0.01	0.85	0.00	-0.02, 0.02	0.90
(per 1 mmol/l)	2	0.00	-0.02, 0.02	0.98	-0.01	-0.03, 0.01	0.38	0.01	-0.01, 0.04	0.25	-0.002	-0.02, 0.01	0.82	0.00	-0.02, 0.02	0.97
Triglyceride	1	0.00	-0.06, 0.07	0.91	-0.02	-0.08, 0.03	0.45	0.02	-0.04, 0.08	0.53	-0.01	-0.05, 0.04	0.76	-0.02	-0.07, 0.03	0.49
(per 1 mmol/l)	2	-0.01	-0.08, 0.06	0.73	-0.02	-0.08, 0.03	0.41	0.01	-0.05, 0.08	0.67	-0.01	-0.05, 0.03	0.71	-0.02	-0.01, 0.04	0.32
HDLc	1	0.01	-0.08, 0.09	0.88	0.02	-0.05, 0.09	0.57	0.10	-0.01, 0.20	0.07	0.03	-0.04, 0.10	0.42	0.02	-0.07, 0.11	0.63
(per 1 mmol/l)	2	0.02	-0.06, 0.11	0.60	0.04	-0.03, 0.11	0.22	0.11	0.00, 0.21	0.04	0.03	-0.04, 0.10	0.40	0.03	-0.06, 0.12	0.47
Non-HDLc	1	0.00	-0.02, 0.02	0.97	-0.02	-0.03, 0.00	0.13	0.01	-0.01, 0.04	0.36	-0.004	-0.02, 0.01	0.69	-0.003	-0.03, 0.02	0.81
(per 1 mmol/l)	2	0.00	-0.03, 0.02	0.88	-0.01	-0.03, 0.01	0.18	0.01	-0.02, 0.04	0.43	-0.004	-0.02, 0.02	0.64	-0.002	-0.02, 0.02	0.83
Birthweight †	1	0.01	0.01, 0.02	0.000	0.003	0.00, 0.01	0.16	0.00	-0.01, 0.00	0.22	-0.003	-0.01, 0.001	0.12	-0.002	-0.01, 0.003	0.42
(per 100g)	2	0.01	0.005, 0.02	0.000	0.001	0.00, 0.01	0.79	-0.01	-0.01, 0.00	0.06	-0.004	-0.01, 0.0002	0.06	-0.004	-0.01, 0.001	0.15

Adjustments for confounders
Models 1: offspring sex and age, Model 2: offspring sex and age, maternal age, smoking, parity, occupational social class, education, pre-pregnancy BMI and alcohol consumption during pregnancy † Birthweight is adjusted for gestational age and sex, therefore not additionally adjusted offspring sex for in Models 1&2

Chapter 8, Table 11: Association of birthweight and cord blood analyte with measures of liver health in adolescence (complete case)

Outcome Age 17:	logLiver volume (ml)			logLiver sheer velocity (m/s)			logALT (U/l)			logAST (U/l)			logGGT (U/l)		
Exposure:	β	CI	P	β	CI	P	β	CI	P	β	CI	P	β	CI	P
Cord blood															
Leptin (per 10 pg/ml)	0.01	-0.01, 0.02 N=402	0.47	0.00	-0.02, 0.01 N=374	0.95	-0.01	-0.03, 0.02 N=813	0.54	-0.01	-0.02, 0.01 N=813	0.59	-0.01	-0.03, 0.02 N=813	0.61
Adiponectin (per 10µg/ml)	0.00	-0.01, 0.01 N=396	0.71	0.00	-0.01, 0.01 N=396	0.89	-0.01	-0.02, 0.00 N=804	0.09	0.00	0.00, 0.01 N=804	0.58	0.00	-0.01, 0.01 N=804	0.59
Cholesterol (per 1 mmol/l)	0.00	-0.02, 0.02 N=392	0.71	-0.01	-0.03, 0.01 N=364	0.18	0.02	0.00, 0.05 N=800	0.06	0.00	-0.02, 0.02 N=800	0.95	0.00	-0.02, 0.02 N=800	0.88
Triglyceride (per 1 mmol/l)	-0.02	-0.08, 0.04 N=388	0.55	-0.03	-0.08, 0.02 N=361	0.25	0.03	-0.04, 0.09 N=796	0.46	0.00	-0.05, 0.04 N=796	0.86	-0.02	-0.08, 0.04 N=796	0.53
HDLc (per 1 mmol/l)	0.05	-0.03, 0.13 N=383	0.25	0.00	-0.06, 0.07 N=355	0.90	0.17	0.05, 0.28 N=785	0.004	0.05	-0.03, 0.13 N=785	0.21	0.06	-0.04, 0.16 N=785	0.27
Non-HDLc (per 1 mmol/l)	0.00	-0.02, 0.02 N=383	0.89	-0.02	-0.03, 0.00 N=355	0.08	0.02	-0.01, 0.05 N=785	0.21	0.00	-0.02, 0.02 N=785	0.74	0.00	-0.03, 0.02 N=785	0.79
Birthweight† (per 100g)	0.01	0.01, 0.02 N=398	0.00	0.00	0.00, 0.01 N=370	0.07	-0.01	-0.01, 0.00 N=806	0.04	-0.01	-0.01,0.00 N=806	0.01	0.00	-0.01, 0.00 N=806	0.58

Adjustments for confounders
Models 1: offspring sex and age, 2: offspring sex and age, maternal age, smoking, parity, occupational social class, education, pre-pregnancy BMI and alcohol consumption during pregnancy † Birthweight is adjusted for gestational age and sex, therefore not additionally adjusted offspring sex for in Models 1&2.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Introduction and Aim

To provide an overview of metabolic and inflammatory pathways evident at birth we aimed to collect and analyse cord blood samples using ^{1}H-NMR spectroscopy, developed by the team at the University of Oulu, Finland. This is also an established facility at the Glasgow Polyomics Unit at the University of Glasgow. These resources permit accurate quantification of 216 metabolomic traits. These are grouped as: (1) lipoprotein lipids (LIPO) consisting of lipids (cholesterol compounds, phospholipids and the –CH3 group of Tg), serum albumin and albumin-bound fatty acid resonances; (2) low molecular weight molecules (LMWM) encompassing pyruvic acid, amino acids, ketone bodies, creatinine, and small molecules that are proxies for fundamental metabolic processes including glycolysis, citric acid and urea cycles; and (3) serum lipid constituents (LIPID) with detailed molecular information on serum lipid extracts including free and esterified cholesterol, sphingomyelin, degree of saturation and ω-3 fatty acids.

Methods (Please refer to the Research Protocol (Supporting documents)

Ethical approval was obtained, and 100 cord blood samples were obtained along with maternal and offspring co-variables. Please refer to the Appendix for example of the patient Information leaflet, Consent Form, Protocol and approval from the Research

Ethics Committee. Cord blood samples were sent to the University of Oulu, Finland for analyses.

Results

There are quite large differences in the cord blood samples analysed by NMR, as shown in Figure 1, which was provided by the University of Oulu, Finland. In NMR spectroscopy the signal intensity is directly proportional to the concentration of an identified compound. Thus, the larger the peak, the larger the concentration. The majority of analyte data are reported as a molar concentration, but derived variables are reported as ratios.

In the LMWM window, there is a clear difference e.g. in alanine concentration, and in the LIPID window, for example there are large differences in PUFAs. For the LIPO window, the peaks on the right are used e.g. to quantify the different lipoprotein subclasses. Here the shape is related to quantity of different lipoprotein subclasses. For example, there are two samples that have somewhat sharper signals that reflect higher very-low-density lipoprotein concentrations.

Conclusion

The cord blood samples used in NMR analysis showed large variability within the results. Although there is not conclusive evidence to validate use of NMR

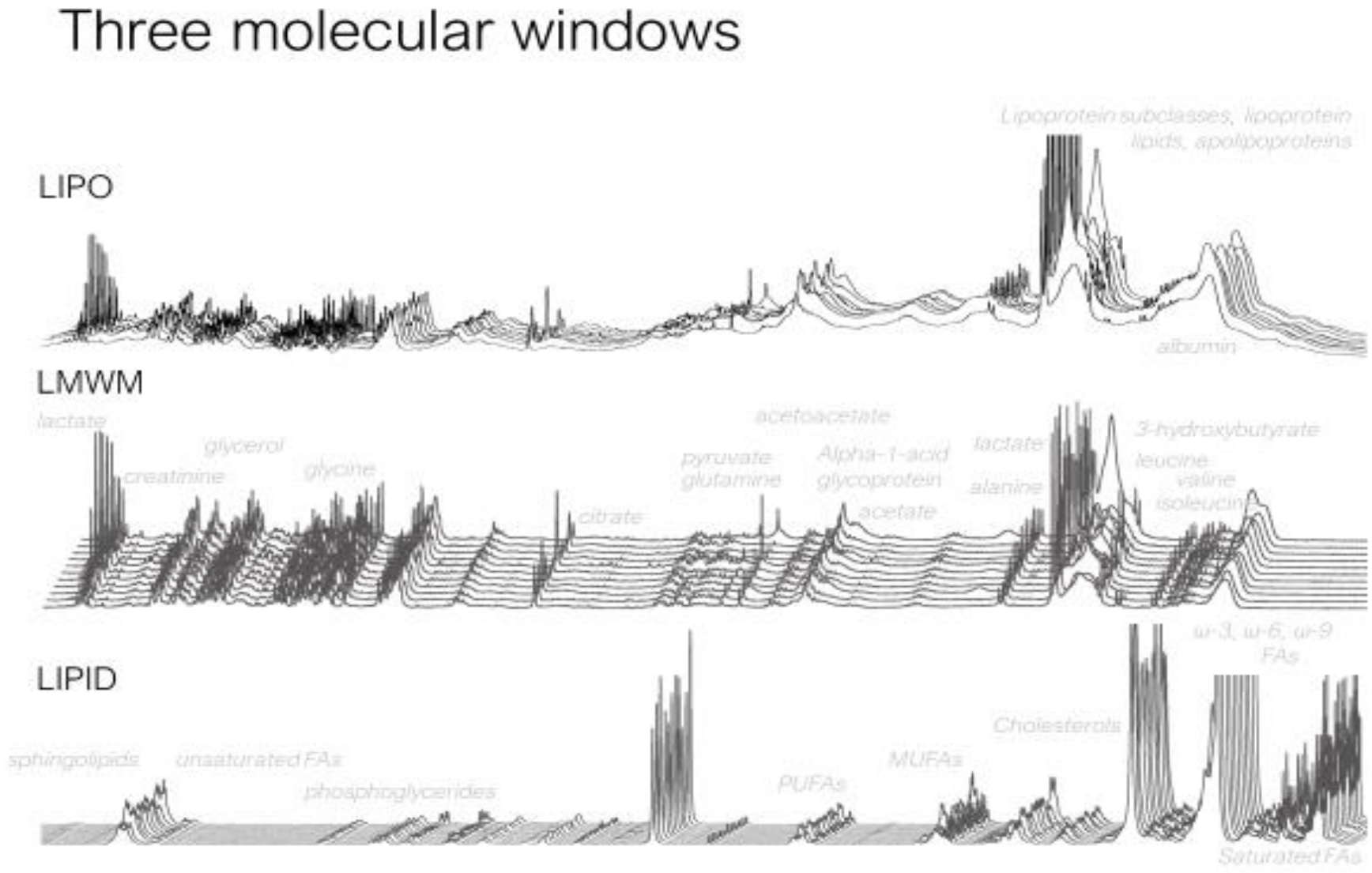
spectroscopy with cord blood samples, this provides some insight into future methods that may be employed.

Cohort characteristics

Table 7: Summary of cord blood collection maternal and offspring characteristics (N=100)

	Mean (range)	**N (%)**
Age (years)	31 (19 – 42)	
BMI (kg/m^2)	25.6 (19 – 43)	
Gestation (weeks)	40 (33 – 42)	
Parity		
0		47 (47%)
1		34 (34%)
2		15 (15%)
3		2 (2%)
4+		2 (2%)
Smoker		
Yes		9 (9%)
No		91 (91%)
Birthweight (kg)	3.3 (1.2 – 5.2)	
Sex		52 (52)
Female		48 (48)
Male		
Mode of delivery		
SVD		39 (39)
Forceps/ventouse		8 (8)
Caesarean section		53 (53)

Figure 1: Graphical display of the three molecular windows detected by NMR



Supporting documents

WoSRES

West of Scotland Research Ethics Service

West of Scotland REC 5
Ground Floor - Tennent Building
Western Infirmary 38 Church Street
Glasgow
G11 6NT

Professor Scott Nelson
Muirhead Chair in Reproductive & and Maternal Medicine
University of Glasgow
2nd Floor McGregor Building
Western Infirmary
Glasgow
G11 6NT

Date 27 March 2013

Direct line 0141 211 2102
E-mail sharon.macgregor@ggc.scot.nhs.uk

Dear Professor Nelson

Study title:	**Collection of Umbilical Cord Blood Samples for NMR Assay Development**
REC reference:	**13/WS/0072**
IRAS project ID:	**126334**

The Research Ethics Committee reviewed the above application at the meeting held on 20 March 2013. Thank you to Dr Simpson for attending to discuss the application.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Sharon Macgregor, sharon.macgregor@ggc.scot.nhs.uk.

Ethical opinion

Ethical issues, raised by the Committee in private discussion, together with responses given by the researcher when invited into the meeting

It was noted in the Participant Information Sheet that if the patient withdraws, the samples will be destroyed. However, if they are in another country, how feasible will this be? Dr Simpson confirmed that the samples will be linked anonymously so they could be identified and removed for destruction.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering Letter		28 February 2013
Investigator CV		28 February 2013
Other: Student CV	Feb 2013	
Participant Consent Form	2	28 February 2013
Participant Information Sheet	2	28 February 2013
Protocol	2	28 February 2013
REC application	2	28 February 2013

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/WS/0072	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

for
Dr Gregory Ofili Chair

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments
"After ethical review – guidance for researchers"

Copy to: Dr Joy Simpson, NHS Greater Glasgow and Clyde
Ms Joanne McGarry, NHS Greater Glasgow and Clyde

R&D Management Office
Western Infirmary
Tennent Institute

1 st Floor, 38 Church Street
Glasgow, Gil 6NT

Greater Glasgow and Clyde

Coordinator/Administrator: JMcG/LR
Direct Line: 0141 211 8548
E-mail: Joanne.McGarry@aqc.scot.nhs.uk
Website: www.nhsggc.org.uk/r&d

16th April 2013

Professor Scott Nelson
Professor of Obstetrics & Gynaecology
2 nd Floor, McGregor Building
Western Infirmary
Glasgow
Gll 6NT

NHS GG&C Board Approval

Dear Professor Nelson

Study Title: Collection of Umbilical Cord Blood Samples for NMR Assay Development
Chief Investigator/ Academic Supervisor: Professor Scott Nelson
Student: Dr Joy Simpson
GG&C HB site: SGH, PRMH
Sponsor: NHS GG&C Health Board
R&D Reference: GN130B157
REC Ref: 13/WS/0072
Protocol no: V2 dated 28/02/13

I am pleased to confirm that Greater Glasgow & Clyde Health Board is now able to grant Approval for the above study.

Conditions of Approval

1 For Clinical Trials as defined by the Medicines for Human Use Clinical Trial Regulations, 2004

a. During the life span of the study GGHB requires the following information related solely to this site

i. Notification of any potential serious breaches. ii. Notification of any regulatory inspections.

It is your responsibility to ensure that all staff involved in the study at this site have the appropriate GCP training according to the GGHB GCP policy

(www.nhsqgc.org.uk/content/default.asp?page=s141 1), evidence of such training to be filed in the site file.

2 For all studies the following information is required during their lifespan.

a. Recruitment Numbers on a monthly basis
b. Any change of staff named on the original SSI form
c. Any amendments — Substantial or Non-Substantial

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NoncommApproval 041010 V3

d. Notification of Trial/study end including final recruitment figures
e. Final Report & Copies of Publications/Abstracts

Please add this approval to your study file as this letter may be subject to audit and monitoring.

Your personal information will be held on a secure national web-based NHS database.

I wish you every success with this research study
Yours sincerely

_____

Joanne McGarry
Research Co-ordinator

CC: Dr Joy Simpson, Student, University of Glasgow

Dr Joy Simpson
Research Office, 3rd Floor McGregor Building
Western Infirmary
Glasgow
G11 6NT

joy.simpson@glasgow.ac.uk

Centre Number:
Study Number: ☐
Patient Identification Number for this trial:

CONSENT FORM

Title of Project: Umbilical Cord Blood Sampling for the Purpose of Nuclear Magnetic Resonance Machine Calibration

Name of Researcher: Dr Joy Simpson

Please initial box

1. I confirm that I have read and understand the information sheet dated 28/02/13 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the University of Glasgow or from NHS Greater Glasgow and Clyde and it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. I understand that non-identifiable information will then be sent along with the linked cord blood sample to the University of Eastern Finland and to Skylight Biotech, Japan. ☐

5. I agree to take part in the above study which will involve cord blood samples being taken from the umbilical cord after birth for the purpose of Nuclear Magnetic Resonance assay development. I understand that the samples will be kept in storage after this. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

Please reply to: Dr Joy Simpson
Research office, 3rd Floor McGregor Building
Western Infirmary
University of Glasgow, G11 6NT
E-mail:joy.simpson@glasgow.ac.uk

VOLUNTEER INFORMATION LEAFLET

Collection of umbilical cord blood samples for NMR assay development.

Lay title: Collection of umbilical cord blood samples for the measurement of biochemical indices.

Study Doctor: Dr Joy Simpson
Chief Investigator: Professor Scott Nelson
Other Investigators: Professor Ala-Koppel, Professor Lawlor, Professor Sattar

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you wish further information. Please, take time to decide whether you would like to participate or not.

1. What is the purpose of the study?

The purpose of the study is to collect blood samples from the umbilical cord after birth from 100 different patients. This blood can then be used to run tests on a machine called Nuclear Magnetic Resonance (NMR) machine so that it can be scientifically prepared for future research projects. This process is called calibration and method development.

As cord blood has not previously been analysed on a NMR machine before, it is important that this process is carried out so that future samples can be analysed with greater accuracy. This process will be carried out by experts at the University of Eastern Finland and by a company called Skylight Biotech in Japan. Therefore, once the samples have been collected, they will be split into smaller samples, frozen and sent by courier to Finland and then to Japan to complete this calibration process. Following this the samples will be returned to our Biobank at the Glasgow British Heart Foundation building. It is anticipated that the calibration process will take up to 12 months to complete but the samples will be kept in storage for longer.

It is important that we collect detailed information for each participant and send this to our collaborators in Finland and Japan. This information will include your age, smoking status, ethnicity, body mass index, information on your previous pregnancies, complications during

your current pregnancy, gestation at delivery, mode of delivery and the birthweight of your baby. This information will be given a coded number so that it can be anonymous but linked to the cord blood sample. All of this will be non-identifiable and won't contain any of your personal details.

2. Why have I been chosen?

We are asking pregnant patients who deliver their baby at the Southern General Hospital/Princess Royal Maternity Hospital to take part. We are planning to collect blood samples from the umbilical cord from a cross section of pregnancy and birth outcomes. Once the blood samples and the information from your case notes has been collected you will have no further input in the Study.

3. Do I have to take part?

It is up to you whether or not to take part. If you decide to participate you will be given this information leaflet for your records and you will be asked to sign a consent form. You are free to withdraw from the study any time without giving a reason.

4. What is my part in the study?

If you agree to take part and meet the inclusion criteria, you will be asked to complete a consent form. Following the birth of your baby, your midwife will clamp the umbilical cord in three places, cut and deliver the placenta and cord as is performed routinely. Prior to the placenta and umbilical cord being disposed of, a blood sample will then be taken from the umbilical cord once it is completely separate from you and your baby so it will not harm you or your baby in any way. The Study Doctor will then complete a short questionnaire using your hospital case notes so you do not need to fill this in yourself.

5. What do I have to do?

If you are happy with the information provided and agree to participate, you will have the opportunity to discuss any questions with the Study Doctor. If you are satisfied with this, you will be asked to complete a consent form. There is no alteration of routine midwifery/medical care.

Following delivery of your baby your midwife/doctor will deliver the placenta and umbilical cord as normal. Only following this will the blood sample from the baby's cord be taken by the Study Doctor. It is standard procedure for the placenta, membranes and umbilical cord to be disposed of (by incineration) after it has been delivered. Participants will not require any active involvement and do not require to do anything for this. Again, a questionnaire will be completed by the Study Doctor using information in your case notes and so you do not need to do anything for this either.

6. What are the possible disadvantages and risks of taking part?

Taking blood samples from the baby's cord will not harm you or your baby in any way. It will be performed only once the cord and placenta is completely separated from you and your baby.

It will not affect whether or not you wish to take your placenta home with you when you are discharged.
The blood samples are being used to prepare a machine for future scientific research, so they are not being used to determine a result such as 'high' or 'low' level. You will not be informed of these outcomes.

7. What are the possible benefits of participating?

The information gained during the study will enable sufficient preparation of the NMR machine. Following this we will be able to begin formal scientific research on umbilical cord blood using the NMR machine.

8. What if there is a problem?

The cord blood samples are being used to prepare the NMR machine for future research. Therefore the results will not be reported individually and you will not receive/require any follow up in this study.

8. What if something goes wrong?

If you have concerns about the study or the way is conducted, you are welcomed to contact the researchers (contact details at the end of the leaflet) and discuss these with them. Should you wish to make a formal complaint you can do so through the NHS complaints procedure. Details can be obtained from the hospital.

9. Expenses and payment

You will not receive explicit payment for this study. You will not need to pay anything that you would require reimbursement for.

10. Will my taking part in this study be kept confidential?

Yes. All information collected about you during the course of the research will be kept strictly confidential and only be handled by the individuals listed above or any staff that work under their supervision. Your GP will not be informed of your participation in the study.

11. What will happen to my samples after the study has finished?

Samples will initially be sent to the University of Eastern Finland and then transferred for analysis in Japan. Following this, residual samples will be returned for storage in our Biobank at the Glasgow British Heart Foundation Building.

13. What will happen if I don't want to carry on with the study?

You can withdraw any time from the study. If you wish to withdraw from the study, we will retain the data that has been collected up to your withdrawal. However, should you wish for samples and data to be destroyed we will comply with this request.

14. Who is organising and funding the research?

NHS Greater Glasgow and Clyde is sponsoring the Study.

15. Who has reviewed the study?

This study has been reviewed by the Greater Glasgow and Clyde Research and Development Management Office.

16. Further Information

If you wish to ask any questions about the study now or at any time, please contact the Study Doctor.
Dr Joy Simpson can be contacted by phone on 0141-211 2327 (research office) or email joy.simpson@glasgow.ac.uk

If you wish to ask more questions about the study from an individual scientist who has a thorough understanding of the area of research but is not directly related to the project, please contact:

Dr David Preiss (Clinical Senior Lecturer)
Institute of C&MS Glasgow BHF, Glasgow, G12 8TA
david.preiss@glasgow.ac.uk
0141 3303076

Thank you for taking the time to read this Information Sheet.

UKCRN portfolio

Dear Joy [This message is automatically generated by the UKCRN Portfolio and Accrual System.] crncc.servicedesk.nihr.ac.uk

This is confirmation that a successful upload of the following studies has occurred for the period up to the end of May 2013:

Collection of Umbilical Cord Blood Samples V2

If you have any questions relating to the process of uploading your data you should, in the first instance, contact your network contact (or Board R&D Office in Scotland). However if you have any further queries the UKCRN IS Team (at https://portal.ukcrn.org.uk/helpdesk) will be happy to help.

Study 14440 - Collection of Umbilical Cord Blood Samples V2	
Previous total rows uploaded	0
Number of new rows in current upload	100
Projected total rows (following processing)	100
Previous total unique participants uploaded	0
Number of new unique participants in current upload	100
Projected total unique participants (following processing)	100
Previous running total	0
New running total	100

Many Thanks

UKCRN Coordinating Centre Portfolio Team

NHS
Greater Glasgow and Clyde

Research Protocol

Title

Umbilical Cord Blood Sampling for the Purpose of Nuclear Magnetic Resonance Machine Calibration

Introduction

The purpose of this Study is to collect 100 umbilical cord blood samples and corresponding information on maternal and fetal characteristics. This is required to aid the development of the methodology and calibration process of a Nuclear Magnetic Resonance (NMR) machine, specifically for cord blood. NMR has already been used to process blood samples from the adult non pregnant population. It provides in depth information on metabolites (lipoproteins, lipids and low molecular weight metabolites). It has not yet been used to process umbilical cord bloods. Once the calibration process is complete, it will then allow detailed and accurate analysis of cord bloods from a separate study and much larger cohort – the Avon Longitudinal Study of Parent's and Children (ALSPAC).

Background

The phenotype at birth is related to long-term outcomes and this concept is strengthened by observations that birth weight has been positively associated with mean infant, childhood and adulthood BMI and adiposity in a number of studies[1-4]. Inflammatory markers and lipids have been shown to track through childhood and predict later obesity, metabolic and vascular disease respectively[5-10] This is not fixed as pregnancy complications like excessive gestational weight gain, diabetes, glycosuria and the hypertensive disorders of pregnancy can all influence offspring fat mass, inflammatory and lipid profile at age 9 and 15 years[11-16].

We seek to address this unique challenge by addition of comprehensive high-resolution metabolomics data to an established prospective birth cohort (ALSPAC). This cohort has pre-existing information on pregnancy characteristics and complications, genotype and childhood development. This includes repeat measures of growth, adiposity, cardiovascular and metabolic risk factors and cognitive function.

To provide an overview of metabolic and inflammatory pathways evident at birth we will use ^{1}H-NMR spectroscopy and analysis developed by one of our collaborators Mika Ala-Korpela (MAK; University of Eastern Finland) and now established at Glasgow University Polysomic Unit. This platform is already well established and validated in the adult non pregnant population. The method and calibration process specifically for umbilical cord blood will therefore need to be developed in order to proceed with analysis of the ALSPAC cord bloods.

NHS
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Patient Notification

Patients will be approached to take part in the study in Maternity assessment, the antenatal ward or labour ward at the Southern General Hospital/Princess Royal Maternity Hospital, Glasgow. They will be provided with a patient information leaflet and given >1hour to read this and ask questions.

Patient Consent

Consent will be completed by each patient following consultation with Dr Joy Simpson.

Umbilical Cord Blood Collection

Following delivery of the child, the midwife will clamp the umbilical cord in 3 separate places. After the placenta and cord has been delivered and separated from mother and child, a 10ml sample of venous and 5ml sample of arterial blood from the umbilical cord will be obtained. This will be performed by the Study Doctor using a 10ml and 5ml syringe and 21 gauges needles. These samples will then be transferred into yellow biochemistry bottles and anonymously labelled (sample number 1 to 100, venous or arterial).

The samples will be obtained within 30 minutes of delivery of the placenta and cord. Following this, the samples will be taken to the lab at the Glasgow British Heart Foundation Cardiovascular Research Centre to be centrifuged and the serum and plasma split into aliquots. For each patient's cord blood sample, aliquots of 100 microlitres will be obtained and stored (in 1.5ml micro-centrifuge tubes) in the freezer at -80°C. These aliquots will then be labelled 1a, b, c, d etc for each patient's sample. The remaining serum will be stored in separate containers suitable for freezing.

Information on maternal and fetal characteristics will be obtained from the case notes and also labelled sample 1-100 so that it is anonymous and corresponds to the appropriate patient's cord blood sample. This information will include: age, body mass index (at booking), smoking status, ethnicity, parity, gestational age at delivery, pregnancy complications (pre-eclampsia, gestational diabetes), mode of delivery (eg caesarean section, forceps delivery etc) and baby's birthweight.

The calibration process and development of the methodology will be carried out by Professor Ala-Korpela's team, University of Eastern Finland, Finland. It will also require secondary calibration which will be performed by Skylight Biotech, Tokyo, Japan. Therefore the serum cord blood aliquots will be transferred to their department by courier in boxed containers of dry ice. The corresponding information on maternal and fetal characteristics will be sent to Professor Ala-Korpela's team via electronic mail. All of this information will be anonymous and will not include any personal details. Therefore the patient has no further input in the study beyond consenting to the Study and the collection of umbilical cord blood. After the calibration process is completed (<12months anticipated from transfer of cord blood) any remaining samples would be returned to the original Biobank at the BHF Glasgow Cardiovascular Research Centre. Therefore the patient has no further input in the study beyond consenting to the Study and the collection of umbilical cord blood.

Benefits and Risks

There are no immediate benefits or risks anticipated. Once the NMR platform is developed specifically for cord blood, this will then allow processing of the ALSPAC cord blood.

Inclusion Criteria

Female pregnant patients age 18 to 45 years old.

Consent obtained

Deliver in Southern General Hospital/Glasgow Royal Infirmary

Speak English/interpreter present

Placenta clamped and already delivered by midwife/doctor.

Cord blood sample taken <30minutes of delivery of placenta

Corresponding information on maternal/fetal characteristics available for documentation

Exclusion Criteria

Pregnant patients <18 and >45 years old

Consent not obtained

Deliver out with Southern General Hospital/Glasgow Royal Infirmary

Do not speak English/interpreter not present

Placenta not clamped appropriately/not yet delivered

Blood sample obtained >30minutes after delivery of placenta

Corresponding information on maternal fetal characteristics not available

Study Visits

Participants will be approached by the Research Doctor (Dr Joy Simpson) and information given in the form of a patient information leaflet. This will take place in either the antenatal wards, labour ward or the maternity assessment areas. Patients will be given >1hour to read the leaflet and also have the opportunities to ask questions. The consent form will be completed by the patient and the Research Doctor. The patient will have no active involvement in the Study beyond this. There will be no follow up.

Duration

We plan to collect 100 umbilical cord blood samples. This is expected to take place over a 4-week period. The Study itself however will commence on the 27thth March 2013. It is anticipated that the cord blood samples can be collected over a 4-week basis. However as the Study is part of a much larger PhD project (using the ALSPAC cohort) then it is expected this to run until August 2015.

Medication

No medication is being tested/trialled.

Criteria for Discontinuation

Patient withdraws consent.

Does not fulfil inclusion criteria/meets exclusion criteria.

Data Collection

Data will be collected from the case notes by the Study Doctor. This will include patient age, parity, smoking status, BMI, ethnicity, pregnancy complications, gestation at delivery, mode of delivery and baby's birthweight.

Information will be transferred to an excel file and stored securely on University of Glasgow/NHS Greater Glasgow and Clyde password protected software.

Sample Size

100 cord blood samples are required for the NMR platform calibration. This sample size is recommended by Professor Korpela's team.

Ethics

The study will be carried out in accordance with the ethical principles in the Research Governance Framework for Health and Community Care, Second Edition, 2006 and applicable legal and regulatory requirements.

Finance and Indemnity

NHS Greater Glasgow and Clyde will sponsor this Study.

NHS employed researchers will be covered for negligent harm through the NHS CNORIS indemnity scheme.

Publications

The results of the study will be disseminated by publishing in peer reviewed scientific journals, internal reports and presentation at conferences.