The contribution of maternal obesity to fetal body composition

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GLOSSARY OF TERMS

AA	Abdominal area (cm^2)
AFM	Abdominal fat mass
AC	Abdominal circumference
ASUM	Australasian Society of Ultrasound Medicine
BAT	Brown adipose tissue
BPD	Bi-parietal diameter
BMI	Body mass index
CRP	C Reactive Protein
DXA	Dual-energy X-ray absorptiometry
EFW	Estimated fetal weight
FL	Femur length
GDM	Gestational Diabetes Mellitus
HAPO	Hyperglycaemia and Adverse Pregnancy Outcomes Study
HC	Head circumference
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor ligand-binding protein
IL-6	Interleukin 6
IRSD	Index of Relative Socio-economic Disadvantage
LGA	Large for gestational age
MTFM	Mid-thigh fat mass
MTTM	Mid-thigh total mass
MFLM	Mid-thigh lean mass
SEIFA	Socio-economic Indexes for Areas
SGA	Small for gestational age
SSFM	Subscapular fat mass
TOP	Termination of pregnancy
UmA	Umbilical artery Doppler
WHO	World Health Organization

ABSTRACT

Background

There are well established links between maternal obesity, high infant birth weight and childhood obesity. However, the contribution of specific maternal dietary components and specific cardiometabolic and inflammatory measures to fetal growth and adiposity among overweight and obese women warrant further investigation. There is limited information describing the impact of maternal BMI on fetal growth trajectories and correlation between fetal and neonatal measures of growth and adiposity.

Methods

This thesis contains a series of secondary analyses involving 911 overweight and obese women who participated in the LIMIT trial and who were randomised to the 'Standard Care' group. Fetal biometry and adiposity measurements were obtained from ultrasound assessments at 28 and 36 weeks gestation. Analyses investigated:

1) The contribution of maternal BMI to fetal growth trajectories;

2) The contribution of maternal dietary factors to fetal growth and adiposity;

3) The contribution of maternal cardiometabolic and inflammatory markers to fetal growth and adiposity; and

4) The correlation between fetal and neonatal anthropometric measures.

Results

The key findings of this thesis are

1) Increased maternal BMI is associated with incremental increases in growth velocity of the fetal abdomen and estimated fetal weight.

2) Pregnant women with BMI $\geq$ 40.0kg/m^2 showed the greatest increase in all fetal biometry z-scores, abdominal fat mass and abdominal area at 28 and 36 weeks gestation.

3) Maternal dietary measures are not consistently associated with fetal growth or adiposity.

4) Increased maternal concentrations of adiponectin are associated with a reduction in fetal abdominal circumference and estimated fetal weight.

5) Increased maternal triglyceride concentrations are associated with an increase in fetal abdominal circumference z-score and estimated fetal weight at 36 weeks.

6) Ultrasound assessment of fetal weight at 36 weeks gestation is a reliable indicator of infant birth weight, and ultrasound assessment of fetal HC and AC at 36 weeks are strongly correlated with newborn measures.

Conclusions

Among women who are overweight or obese, the contributions of maternal BMI, dietary factors and cardiometabolic and inflammatory markers to fetal growth and adiposity are highly complex. This study has confirmed that high maternal BMI is associated with higher fetal growth. Therefore, evaluation of interventions to modify fetal growth and adiposity through improving maternal health prior to conception are required.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Cecelia O'Brien

December, 2018

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STATEMENT BY CANDIDATE

During my PhD candidature, I have been involved in the study design, the submission of any amendments to ethics, conduct of 885 ultrasound examinations for fetal growth at 28 and 36 weeks gestation, the collection of the ultrasound related data, development of the statistical analysis in consultation with the statistician and PhD supervisors. I was responsible for obtaining salary support through the RANZCOG research foundation. I was involved in the intellectual development and conduct of the secondary analyses of the LIMIT randomised trial presented in this thesis, with 3 manuscripts published, and 2 currently under peer review.

CHAPTER 1: Literature Review

Implications of maternal obesity on fetal growth and the role of ultrasound

The literature review presented has been published (O'Brien CM et al 2017, Expert Review of Endocrinology & Metabolism), and is contained in Appendix 1.

1.1 Introduction and Definitions

Worldwide, it is estimated more than 1.46 billion adults, and 170 million children, are either overweight or obese (Lobstein et al. 2004, Finucane et al. 2011). The global prevalence of obesity has more than doubled between 1980 and 2014, with a more pronounced surge in low and middle-income countries (Finucane et al. 2011, WHO 2018). Based on current trends, it is estimated that more than 50% of adults world-wide will be obese by 2030 (Wang et al. 2014). Obesity is the sixth most important risk factor contributing to overall burden of disease worldwide (Ezzati et al. 2002), and is an independent risk factor for the development of many associated morbidities, including hypertension, diabetes mellitus, and cardiovascular disease, all of which contribute to a significant reduction in life expectancy (Ezzati et al. 2002, AIHW 2017a).

Body mass index (BMI) is defined as body weight in kilograms divided by the square of the height in metres (kg/m^2). Overweight is defined as BMI inclusive of 25.0 to

29.9kg/m^2. Obesity is defined as a BMI greater than or equal to 30kg/m^2 and is further divided into 3 sub-categories as outlined in Table 1.1 (WHO 2004). The sub-categories include Class 1 obesity defined as BMI of 30.0 to 34.99 kg/m^2, Class 2 obesity defined as BMI of 35.0 to 39.99 kg/m^2 and Class 3 obesity, defined as BMI greater than or equal to 40 kg/m^2.

While pre-pregnancy measurement of BMI has many advantages, it is frequently unavailable, and reliance on self-reported pre-pregnancy weight particularly, is well recognized to be under-reported by women (Headen et al., 2017). While standardized BMI measurement in early pregnancy fails to account for early pregnancy weight gain (Carmichael, 1997), it has been demonstrated to correlate with pre-pregnancy BMI (Headen et al., 2017). Such an approach is consistent with standard obstetric practice and state wide perinatal practice guidelines (Government of South Australia 2019, Denison et al., 2018)

Table 1.1: World Health Organization classification of BMI Categories

Definitions	Body Mass Index
Underweight	< 18.50 kg/m^2
Normal weight	18.50 – 24.99 kg/m^2
Overweight	25.0 to 29.9 kg/m^2
Class 1 Obesity	30.0 to 34.99 kg/m^2
Class 2 Obesity	35.0 to 39.99 kg/m^2
Class 3 Obesity	≥ 40.0 kg/m^2

Over 50% of women in high-income countries enter pregnancy with a BMI of 25kg/m^2 or more (Chu et al. 2009, Scheil et al. 2016), impacting significantly on maternal, fetal and neonatal health outcomes, both in the short term during pregnancy and birth, and in the longer term, contributing to high rates of childhood obesity (Whitaker 2004, Li et al. 2005, Yu et al. 2013, Leng et al. 2015, WHO 2016b). The associations between maternal obesity and subsequent childhood obesity are complex, involving both genetic and environmental factors, with a substantial impact reported to arise from intrauterine programming contributing to longer-term health complications (Whitaker 2004, Hawkins et al. 2006, Monasta et al. 2010). A recent commission into childhood obesity found escalation in prevalence across the world (WHO 2016b), with 41 million infants and children under the age of 5 years identified as overweight or obese (WHO 2018). The effect on a child's later life is significant, including the development of diabetes, increased risk of cancer, respiratory disease, cognitive impairment, mental health issues, and reproductive disorders (Godfery et al. 2017). In addition, there is an impact on educational and recreational opportunities with subsequent economic impact for the family unit and society as a whole (WHO 2016b).

There is considerable interest in understanding the mechanisms underlying fetal growth and adiposity patterns which may contribute to the intergenerational effects of obesity (Catalano 2003a). Figure 1.1 is a schematic diagram showing the potential mechanism linking maternal obesity to fetal overgrowth. From a public health perspective, preventive strategies targeting the peri-conceptual and antenatal periods, may contribute to a reduction in fetal overgrowth and adiposity, slowing the transmission of obesity between generations (Hanson et al. 2015, Hanson et al. 2017).

Figure 1.1 - The postulated mechanisms relating to maternal obesity and the effect on fetal growth patterns

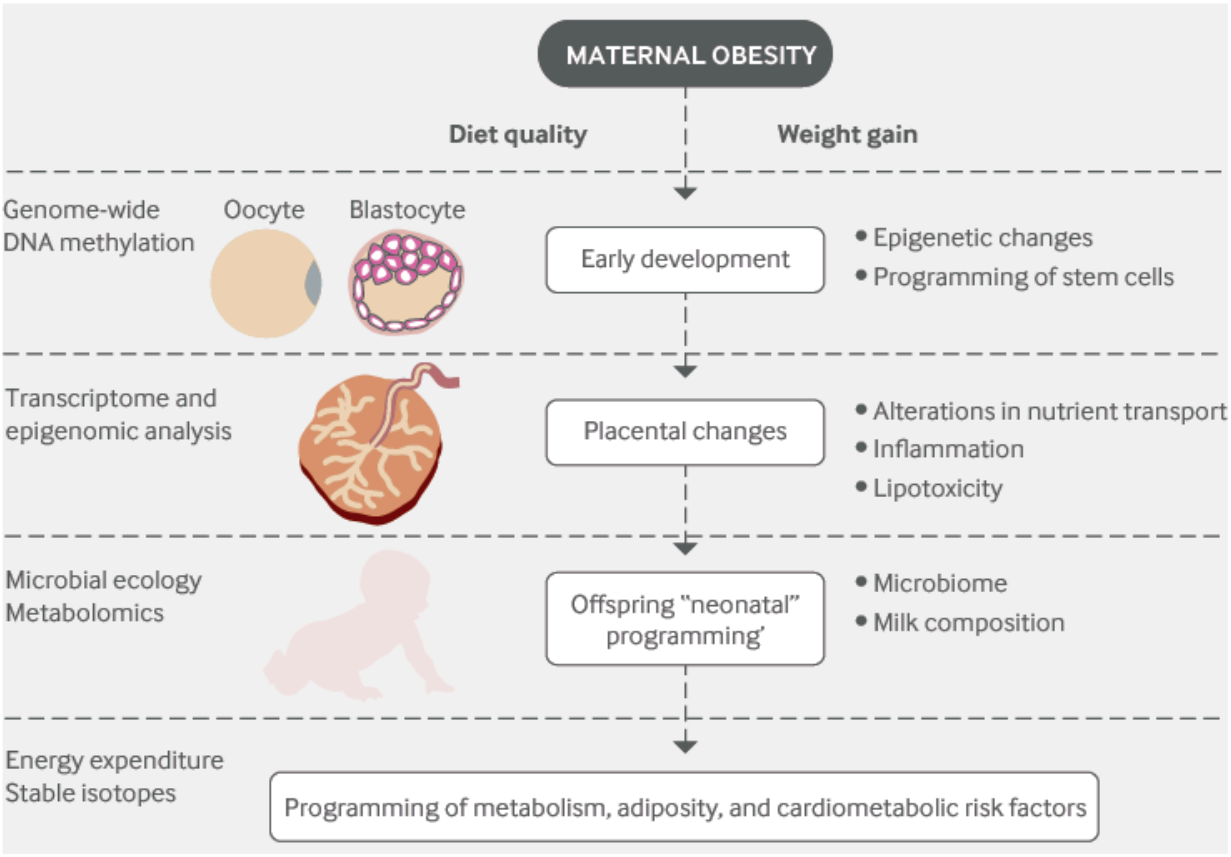


Figure 1.1 is a schematic diagram adapted from Catalano et al. 2017.

1.2 Consequences of maternal obesity

Entering pregnancy overweight or obese is an independent risk factor for almost all obstetric complications, the risk increasing linearly with increasing BMI category (Dodd et al. 2011a, Magann et al. 2013). Women who are overweight or obese are more likely to develop hypertensive disorders, including pre-eclampsia (Sibai et al. 1995, Cedergren 2004, Callaway et al. 2006, Doherty et al. 2006, Abenhaim et al. 2007, Athukorala et al. 2010, Magann et al. 2013), gestational diabetes mellitus (GDM)

(Callaway et al. 2006, Doherty et al. 2006, Abenhaim et al. 2007, Athukorala et al. 2010, Dodd et al. 2011a) and preterm birth (Callaway et al. 2006, Abenhaim et al. 2007, Dodd et al. 2011a). On a population level, increasing maternal BMI substantially increases the risk of antenatal stillbirth (Cedergren 2004, Callaway et al. 2006, Yao et al. 2014, Yao et al. 2017). A large meta-analysis found women classified as obese were two times more likely to experience a stillbirth compared with women with a normal BMI (Nohr et al. 2005, Chu et al. 2007). In a large hospital based cohort study in Denmark, most fetal deaths in the setting of maternal obesity were either term or post-term, with infants having lower mean birth weight and more likely to have an unknown cause of death (Kristensen et al. 2005). Postulated reasons for the increase in stillbirth include fetoplacental dysfunction and impaired blood flow (Kristensen et al. 2005).

Women who are overweight or obese are more likely to give birth to an infant considered large for gestational age (LGA) or macrosomic (Rosenberg et al. 2003, Cedergren 2004, Whitaker 2004, Abenhaim et al. 2007, Dodd et al. 2011a), both of which contribute to intra-partum related complications including an increase in the risk of fetal distress, operative birth, including caesarean section, and perineal trauma (Jolly et al. 2003, Pasupathy et al. 2012). In the neonatal period, infants born to women who are overweight or obese, irrespective of birth weight, are more likely to be born prematurely, are at higher risk of shoulder dystocia and hypoglycaemia, and are more likely to require admission to the neonatal intensive care unit (Rosenberg et al. 2003, Callaway et al. 2006, Dodd et al. 2011a, Magann et al. 2013).

There is considerable heterogeneity across studies describing the effects of obesity on maternal, fetal, obstetric and neonatal outcomes. This reflects variation in study design, each associated with inherent limitations. Furthermore, the definition of clinical outcomes, type and timing of the collection of the BMI estimate (pre-pregnancy versus early pregnancy; self reported versus measured) and cut-offs to define overweight and obesity varied between studies. Table 1.2 summarises the main studies assessing the effect of maternal BMI on maternal, obstetric and perinatal outcomes.

Table 1.2: Summary of the current literature reporting maternal and neonatal outcomes for women who are obese (BMI ≥ 30kg/m^2) compared with women with a normal BMI.

	Outcomes	Study details	Population	BMI	Effect size	Reference
Pregnancy related complication	Hypertension	State based Pregnancy Outcome Unit 2008	South Australian N = 19,672 Analysis = 11,233	Measured at the first visit	aOR 2.9 [2.43-3.49]	Dodd et al. 2011
	Pre-eclampsia	King Edward Hospital based cohort	Perth, Australia N = 2827	Pre-pregnancy Collection not stated	aOR 3.74 [1.95-7.17]	Doherty et al. 2006
	Gestational Diabetes	Mater Mothers' Hospital based cohort 1998 – 2009	Brisbane, Australian N = 75,432	1st visit, self- reported	aOR 3.99 [3.47-4.59]	McIntyre et al. 2012
Fetal	Cardiovascular anomalies	Meta-analysis of 18 studies	International N = 182	Variable	OR 1.30 [1.12-1.51]	Stothard et al. 2009
	Spina bifida	Meta-analysis of 18 studies	International N = 863	Variable	OR 2.24 [1.86-2.69]	Stothard et al. 2009
	Neural tube defects	Retrospective hospital based cohort 2004 - 2011	Northern Ireland N = 30,298	Early pregnancy (≤ 16 wks)	aOR 7.5 [1.2-46.5]	Scott-Pillai et al. 2013
	Stillbirth	Meta-analysis of 9 studies	International N = 865,524	Variable	OR 2.07 [1.59-2.74]	Chu et al. 2007
Intrapartum	Induction of labour	State based Pregnancy Outcome Unit 2008	South Australian N = 19,672 Analysis = 11,233	Measured at the booking in visit	aOR 1.38 [1.2–1.49]	Dodd et al. 2011
	Normal vaginal delivery				aOR 0.86 [0.82-0.90]	Dodd et al. 2011
	Operative vaginal delivery				aOR 0.82 [0.82-0.98]	Dodd et al. 2011
	Emergency Caesarean				aOR 1.36 [1.20-1.53]	Dodd et al. 2011
	LSCS for fetal distress				aOR 4.6 [2.22-9.41]	Doherty et al. 2006
Postpartum	Postpartum haemorrhage	Retrospective hospital based cohort 2004 - 2011	Northern Ireland N = 30,298	Early pregnancy (≤ 16 wks)	OR 2.7 [2.2 – 3.4]	Scott-Pillai et al. 2013
	Wound complication				OR 6.0 [3.0 – 12.1]	Scott-Pillai et al. 2013

	Breast feeding				OR 0.4 [0.3 – 0.5]	Scott-Pillai et al. 2013
	Length of stay	Hospital based cohort 1998 – 2009	Mater Mothers' Hospital, Brisbane	1st visit, self-reported	aOR 1.6 [1.46-1.75]	McIntyre et al. 2012
Neonatal	Large for gestational age infant defined as greater than or equal to 90th centile (based on gestational age and gender)	Swedish Medical Birth Registry 1992 - 2001	Sweden N = 805,275	Measured in early pregnancy	aOR 3.82 [3.50-4.16] Increases with increasing BMI	Cedergren 2004
		North West Thames database 1989 - 1997	United Kingdom N = 287,213 Retrospective	Measured at the booking in visit	aOR 2.36 [2.23-2.5]	Sebire et al. 2001
		Meta-analysis of 30 studies	International N = 214,385	Variable	OR 2.42 [2.16–2.72]	Gaudet et al. 2014
	Macrosomia > 4.0 kg	Meta-analysis of 30 studies	International N = 13,612		OR 2.17 [1.92–2.45]	Gaudet et al. 2014
	Macrosomia > 4.5 kg	Meta-analysis of 30 studies	International N = 18,909		OR 2.77 [2.22–3.45]	Gaudet et al. 2014
	Preterm delivery < 32wks	Swedish Medical Birth Registry 1992 - 2001	Sweden N = 805,275	Measured in early pregnancy	aOR 2.32 [1.73-3.12]	Cedergren 2004
	Shoulder dystocia	Mater Mothers' Hospital based cohort 1998 - 2002	Brisbane Australian N = 11,252	Measured at 12 weeks	aOR 2.9 [1.4-5.8]	Callaway 2006
	Neonatal trauma	Mater Mothers' Hospital based cohort 1998 - 2002	Brisbane Australian N = 11,252	Pre-pregnancy calculation	aOR 1.5 [1.1-2.1]	Callaway 2006
	Apgar < 7 at 5 minutes	Population based 1989 - 2001	Finland N = 25,601	Measured before 10 weeks	aOR 1.64 [1.22-2.28]	Raatikainen 2006
	Admission to NICU	Mater Mothers' Hospital based cohort 1998 - 2002	Brisbane Australian N = 11,252	Measured at 12 weeks	aOR 2.77 [1.8 – 4.25]	Callaway et al. 2006
	Hypoglycaemia	Mater Mothers' Hospital based cohort 1998 – 2009	Brisbane Australian N = 75,432	Self-reported Pre-pregnancy Recall	aOR 2.00 [1.71-4.43]	McIntyre et al. 2012
	Neonatal death	Aarhus Birth Cohort 1989 - 1996	Denmark N = 24505	Self-reported pre-pregnancy	aOR 2.7 [1.2 – 6.1]	Kristensen et al. 2005

1.3 Large for gestational age, macrosomia, and neonatal body composition

There is currently no international consensus regarding the definition, measurement, reporting and management of the large for gestational age (LGA) fetus or newborn (Campbell 2014). Furthermore, the distinction between prenatal ultrasound (providing an estimate of fetal weight) and birthweight (measured in the newborn period and adjusted for gestational age and gender) in the literature can be unclear (Pasupathy et al. 2012).

The definition and identification of the LGA fetus is based on prenatal ultrasound, and utilises measures of abdominal circumference (AC) or estimated fetal weight (EFW) using Hadlock's formula, variably defined as greater than or equal to the 90^{th}, 95^{th} or 97^{th} centile for gestational age (Jolly et al. 2003, Pasupathy et al. 2012), using population based charts (Hui 2008). The prenatal ultrasound measurement of EFW has a measurement error of ± 20% (Scioscia et al. 2008) with further reduction in performance in the setting of maternal obesity and at the extremes of fetal weight (Thornburg et al. 2008). The term fetal macrosomia is also variably defined as an EFW greater than or equal to 4000 grams or 4500 grams (Campbell 2014).

In comparison, the classification of a LGA newborn infant is a measurement of birth weight, corrected for infant sex and gestational age at birth (Pasupathy et al. 2012), and can be variably defined as greater than or equal to the 90^{th}, 95^{th} or 97^{th} centile. Similarly,

infant macrosomia is a postpartum definition based on infant birth weight of greater than or equal to 4000 grams or 4500 grams (Jolly et al. 2003).

There has been interest in using customised growth charts to identify the infant at risk of growth disorders, considering factors such as maternal ethnicity, height and weight, infant sex and gestational age (Gardosi et al. 1992). Some have advocated the use of customised growth charts to define the LGA infant (birth weight > 90^{th} centile), as being superior in prediction of neonatal morbidity, when compared with definition of macrosomia (BW > 4000 grams) (Pasupathy et al. 2012), postulating that the higher predictive value relates to improved detection of excessive fetal growth or alteration in fetal body composition taking into consideration the constitution of the mother (Gardosi et al. 2011, Pasupathy et al. 2012). However, it remains to be determined whether the consideration of maternal overweight and obesity and its effects on fetal growth as "physiological" rather than "pathological" is appropriate in this setting.

Birth weight as a single measure reflects mass, and does not reflect variations in the distribution of adipose tissue nor the relative proportion of adipose and lean tissue mass. Lean body mass has been correlated with genetic factors, whereas fat mass has been correlated with the maternal environment (Sparks 1984). Most studies reported in the literature have compared lean and adipose tissue masses in infants born to women who are overweight or obese with infants born to lean women (Sewell et al. 2006, Hull et al. 2008). The intrauterine metabolic environment has been shown to affect the growth of adipose tissue but not lean tissue mass (Catalano et al. 2011). Neonatal fat mass accounts for approximately 14% of the total birth weight (Catalano et al. 2003b,

Catalano et al. 2011). These studies have shown that as maternal BMI increases, so too does neonatal adipose tissue mass (Sewell et al. 2006, Hull et al. 2008), which in turn is correlated with an increased risk of childhood obesity and longer-term metabolic dysfunction (Oken 2009).

In contrast, the effect of maternal obesity on newborn lean tissue mass remain uncertain. While some have reported no association with maternal obesity (Sewell et al. 2006), others have reported associations between maternal obesity, lower newborn fat free mass, and higher total and percentage of fat mass as measured by air displacement plethysmography (Hull et al. 2008). There is a need for ongoing research into this area, including the longer-term follow-up of children to assess the impact of neonatal adipose tissue distribution on subsequent childhood obesity.

1.3.1 Clinical management of the large for gestational age fetus

Despite the limitations identified relating to the definitions of LGA and fetal macrosomia, the widespread availability of ultrasound and concerns relating to maternal and infant pregnancy and birth complications has led to interest in the prediction of fetal macrosomia (Dodd et al. 2012) to potentially reduce morbidity through active clinical management.

Clinical management options remain controversial for women who are identified to have a LGA fetus in the antenatal period (Campbell 2014). A large decision analysis study by Rouse identified that an elective caesarean section for EFW greater than 4.5kg

was not an economically viable treatment option (Rouse et al. 1996). The cost of elective caesarean birth did not outweigh the prevention of shoulder dystocia and brachial plexus injuries (Rouse et al. 1996). In contrast, a similar study reached the opposite conclusions, when considering the "costs" related to maternal perineal trauma and subsequent faecal and urinary incontinence issues for the woman, in addition to the direct effects of shoulder dystocia and brachial plexus injuries for the offspring (Culligan et al. 2005). Importantly, any short or longer-term "costs" related to the effects of birth asphyxia have not been incorporated into these decision analyses (Campbell 2014).

A multi-centre randomised trial involving 19 tertiary centres included 832 women with an average BMI between 25 – 26kg/m^2 and with a fetus suspected to be LGA, who were randomised to either elective induction of labour or continued expectant pregnancy management (Boulvain et al. 2015). The trial identified a reduction in neonatal morbidity following induction of labour between 37 and 39 weeks following ultrasound identification of a LGA fetus defined as an EFW greater than the 95th centile (Boulvain et al. 2015). This type of clinical intervention was not associated with differences in a woman's risk of caesarean birth. Incorporation of 4 similar randomised trials in a meta-analysis involving a total of 1,190 women, identified that induction of labour was associated with a reduction in the occurrence of shoulder dystocia, and any type of neonatal fracture, although there were no statistically significant differences identified in the rates of operative delivery, brachial plexus injury, low 5 minute Apgar scores or low arterial cord blood pH (Boulvain et al. 2016). The ability to more widely generalise these findings to women who are overweight or obese is uncertain,

particularly taking into consideration the reduction in accuracy of ultrasound estimation of fetal weight in this clinical setting (Boulvain et al. 2015, Boulvain et al. 2016).

The clinical management debate will undoubtedly continue with consideration of multiple factors including a woman's autonomy, obstetrician factors, ultrasound availability, ultrasound prediction and accuracy, evidence surrounding intervention and concern about rising caesarean birth rates in both the developed and developing world (Campbell 2014).

1.4 The fetal "overgrowth" hypothesis

In 1967, Pedersen and associates first proposed a hypothesis to describe the underlying mechanism relating to overgrowth of the fetus, seen primarily in women with diabetes mellitus during pregnancy (Pedersen 1967). This is commonly known as the direct pathway for fetal 'overgrowth' and is more commonly referred to as the Pedersen hypothesis (Pedersen 1967). Glucose has been long recognised as the primary fuel substrate for fetal growth and development, and is delivered across the placental interface via transport mediated facilitated diffusion (Catalano et al. 2009, Wright et al. 2011). In the setting of maternal hyperglycaemia and hyperinsulinaemia related to both maternal obesity and gestational diabetes, there is a greater diffusion gradient of glucose, which in turn leads to fetal hyperglycaemia (Catalano et al. 2009). Hyperglycaemia in the fetal circulation stimulates insulin production by the fetal pancreas, insulin-like growth factors (IGF), growth hormones and a range of other growth promoting factors, all of which stimulate fetal deposition of glycogen and fat

(Pedersen 1967). More recently, the hypothesis has been expanded to account for the placental transfer of lipids and their contribution to fetal growth (Catalano et al. 2011).

Increasingly, there is evidence to support an 'indirect' pathway that can impact the delivery and quantity of the nutritional supply to the fetus across the placenta (Wright et al. 2011, Aye et al. 2013). The placenta is the interface between the maternal and fetal circulations, providing critical and complex functions for the developing fetus. The placenta plays an integral role in fetal growth through the regulation of blood flow, oxygen delivery, and nutrient transfer across the placenta (Belkacemi et al. 2011). While the placenta is likely to be an important mediator by which maternal obesity contributes to fetal overgrowth and adiposity (Lewis et al. 2013), there is a relative paucity of literature describing its role in the regulation of fetal growth in this setting. Various hypotheses include the regulation of placental transporters (Lewis et al. 2013) and the nutrient transfer capacity of the placenta, which directly relate to the structural and morphological features of the placenta, in addition to uteroplacental blood flow (Wright et al. 2011, Aye et al. 2013).

1.5 Fetal growth restriction in the setting of maternal obesity

Large population cohort studies have identified maternal obesity to not only be associated with fetal overgrowth, but also with fetal growth restriction (Cedergren 2004, Doherty et al. 2006, Abenhaim et al. 2007). In a large epidemiological study, the rate of fetal growth restriction was approximately 2.3% in women with a BMI greater than or equal to 40kg/m^2 (Cedergren 2004). These rates are comparable to those derived

from large randomised trials. The LIMIT trial identified a risk of infant birth weight less than 2.5kg to be approximately 4.7% (Dodd et al. 2014a), consistent with the findings from the UPBEAT randomised trial (Poston et al. 2015) which utilised customised birth weight centiles. Babies born to women who are classified as obese are more likely to be appropriately grown (82.7%) or large for gestational age (14.9%) (Cedergren 2004). Similarly, the ultrasound measurements relating to fetal growth, including weight, were consistently above the population mean, as discussed subsequently (Grivell et al. 2016).

Observational studies have identified higher rates of perinatal death in women who are overweight or obese, when compared with women of normal BMI (Kristensen et al. 2005, Huda et al. 2010, Yao et al. 2014). In this setting, most stillborn infants were identified to be SGA, particularly beyond 37 weeks gestation (Kristensen et al. 2005, Yao et al. 2014). Intrauterine fetal death due to higher rates of fetoplacental dysfunction were found in obese women (5.4 fetal deaths per 1000 live births) compared with women with a normal BMI (1.4 fetal deaths per 1000 live births) (Kristensen et al. 2005).

The underlying mechanisms for a reduction in uteroplacental blood flow may relate to the exaggerated hyperlipidaemia observed in maternal obesity, along with increased free fatty acids and cholesterol, which may potentially increase the risk of placental thrombosis and subsequently reduce placental perfusion (Kristensen et al. 2005). Another potential explanation may reflect the high rates of pre-eclampsia identified in women with increasing BMI (Sibai et al. 1995, Cedergren 2004, Callaway et al. 2006,

Doherty et al. 2006, Thornburg et al. 2008, Athukorala et al. 2010). Oxidative stress and endothelial dysfunction from obesity may impact on trophoblastic invasion and contribute to poorer pregnancy outcomes, such as pre-eclampsia and placental insufficiency (Huda et al. 2010). Pre-eclampsia and defective trophoblastic invasion in turn affects placental function and may alter the fetal growth potential (Huda et al. 2010). There remains uncertainty surrounding the exact mechanism contributing to reduced growth in fetuses born to women who are obese and whether inadequate trophoblast invasion or perfusion defects comes first.

Impaired fetal growth in the setting of maternal overweight and obesity may also reflect the effects of maternal weight loss, which is more likely among obese women during pregnancy (Beyerlein et al. 2011). While lower maternal weight gain during pregnancy has been associated with a reduction in the risk of LGA infants and many pregnancy related complications, it appears to be at the expense of an increase in SGA infants (Lemas et al. 2015). While observational studies highlight this association, it is unclear if the contribution of weight loss to poor fetal growth reflects impaired nutrient delivery to the fetus, or whether other mechanisms are operational (Beyerlein et al. 2011).

1.6 Maternal dietary determinants of fetal growth

Women who are overweight or obese during pregnancy have been demonstrated to have poorer diet quality when compared with women with BMI in the normal range (Laraia et al. 2007, Rifas-Shiman et al. 2009, Tsigga et al. 2011, Moran et al. 2013), which persists into the postpartum period (Moran et al. 2013). In turn, poor diet quality is

associated with increased risk of glucose intolerance and pre-eclampsia (Rifas-Shiman et al. 2009), increased neonatal adiposity (Shapiro et al. 2016) and changes in child body composition (Catalano et al. 2017).

There is growing interest in the programming of fetal growth and body composition, the critical time points and the influence of maternal diet as a potentially modifiable factor. The current literature is inconsistent, largely due to the heterogeneity and variability relating to the timing and types of dietary assessments, reporting and methodology, along with body composition measurements (Brei et al. 2018). Three studies have shown an increase in maternal carbohydrate intake to be associated with higher birth weight (Sharma et al. 2018), neonatal adiposity measured by air plethysmography (Crume et al. 2016), higher infant BMI (Chen et al. 2017) and an increased BMI in children (Chen et al. 2017). In contrast, a maternal diet with lower intake of carbohydrates has been associated with a decrease in neonatal adiposity (Renault et al. 2015) and a reduction in fat mass by 5 years of age (Brei et al. 2018). Protein and carbohydrate ratios or combination diets have also been evaluated, where a high protein, low carbohydrate, and low fat diet was associated with a reduction in neonatal abdominal adiposity (Chen et al. 2016, Brei et al. 2018). In contrast, an Australian study in women with a normal BMI showed that a low carbohydrate diet was associated an increase in abdominal fat mass in the fetus (Blumfield et al. 2012).

Poor diet quality (defined as Healthy Eating Index score less than or equal to 57) has been associated with a higher percentage of neonatal fat mass as measured on air displacement plethysmography, independent of maternal BMI (Shapiro et al. 2016). Observational data from the Danish National Birth Cohort identified an association

between maternal dietary glycaemic load and a higher proportion of LGA infants (14%) and higher birth weight by 36 grams (Knudsen et al. 2013).

There has been more limited evaluation of the contribution of maternal dietary intake to fetal growth and adiposity, particularly among overweight and obese pregnant women. Maternal protein, fatty acid and carbohydrate intake during pregnancy have all been associated with increased measures of fetal adiposity, although this has largely been evaluated only in women of normal BMI (Blumfield et al. 2012).

The contribution of specific maternal dietary components to fetal growth and adiposity among women who are overweight or obese is unclear, and warrants further investigation.

1.7 Metabolic determinants of fetal growth

Glucose, insulin, insulin-like growth factors, leptin, adiponectin and lipids have all been identified to contribute to fetal growth in a complex fashion. Table 1.3 summarises the key metabolic substrates, their proposed physiology, effect on fetal growth and identified changes in the setting of maternal obesity.

Table 1.3: The key metabolic substrates during pregnancy, their effect on fetal growth and changes related to maternal obesity

Factor	Physiology	Effect on fetal growth	Changes in maternal obesity	References
Glucose	Crosses the placenta via GLUTs Main energy source for the fetus	Promotes fetal overgrowth	Higher in maternal obesity Increased risk of GDM and hyperglycaemia Positively associated with birth weight	Metzger et al. 2008 Catalano et al. 2009 Uebel et al. 2014 Torloni et al. 2009
Insulin	Decreased sensitivity to insulin, mainly in the post receptor pathway, reducing intracellular insulin signalling pathway via GLUT4. Secretion of placental lactogen, cytokines, tumour necrosis factor and elevated lipids have been shown to contribute in pregnant women.	High levels of glucose stimulate hyperinsulinaemia in the fetal pancreas	Obesity is associated with insulin resistance Contribution of pre-receptor action via insulin antibodies and decreased number of receptors on the cell surface in addition to post-receptor defects (intracellular insulin signalling pathway)	Catalano et al. 2010 Pedersen 1967
Insulin like growth factors	Family of ligands (IGFs) and ligand-binding proteins (IGFBPs) Produced by the liver Contributes to placental invasion, growth and development Stimulates differentiation of pre-adipocytes	Relative concentrations of IGFs and IGFBPs determine effect on fetal growth – free (bioactive) IGF is promotor of fetal growth	Reduced expression of IGFBP4 in cord blood of offspring, resulting in higher concentrations of free IGF	Ferraro et al. 2012 Qiu et al. 2005 Juul et al. 2003
Lipids	Early pregnancy – maternal fat accumulation Late pregnancy – maternal hyperlipidaemia	Contributes to fetal fat deposition in the third trimester	Higher plasma triglycerides throughout pregnancy Independently associated with risk of LGA and neonatal measures of adiposity	Son et al. 2010 Vrijkotte et al. 2011 Whyte et al. 2013 Schaefer-Grafe et al. 2008

Leptin	Produced predominantly by white adipose tissue Involved in regulatory control of placental nutrient transport	Promotes fetal overgrowth	Higher circulating concentrations Higher cord blood concentrations in offspring Positively correlates with birth weight, neonatal adiposity and neonatal insulin resistance	Tessier et al. 2013 Tsai et al. 2015 Catalano et al. 2009 Karakosta et al. 2011 Josefson et al. 2014
Adiponectin	Produced by adipose tissue Contributes to peripheral insulin sensitivity Reduces nutrient availability for the placenta	Negative regulator of fetal growth	Maternal concentrations lower in obesity Maternal concentrations negatively correlate with birth weight and neonatal fat mass	Ategbo et al. 2006 Lowe et al. 2010

1.7.1 Glucose and Insulin

Maternal obesity, even in the absence of GDM, is associated with higher glucose concentrations, contributing to an intra-uterine hormonal environment that is comparable that associated with metabolic syndrome, characterised by hyperglycaemia and insulin resistance (Catalano et al. 2011). Offspring born to women who are obese have documented higher cord blood glucose and insulin concentrations, and are more insulin resistant (Catalano et al. 2009, Uebel et al. 2014, Lemas et al. 2015). This relationship between maternal obesity and insulin resistance measured in neonatal cord blood is present irrespective of a diagnosis of GDM (Catalano et al. 2009). Furthermore, findings from the HAPO study have confirmed a linear relationship between maternal glucose concentration and infant birth weight, even at glucose concentrations below those considered to be diagnostic of GDM (Metzger et al. 2008). Similar relationships between maternal glucose concentrations below the diagnostic threshold of GDM and adverse neonatal outcomes related to insulin resistance and glucose intolerance have been described in the other populations, including Canada, United States, United Kingdom, and Australia (Sermer et al. 1998, Dodd et al. 2007, Catalano et al. 2009, Torloni et al. 2009).

1.7.2 Insulin-like growth factors

Insulin-like growth factors (IGF) along with IGF binding proteins are produced in the liver. During pregnancy, the ligands and their binding proteins contribute to placental growth and development, and promote fetal growth. In maternal obesity, there is reduced expression of IGFBP4 in the cord blood of the offspring, resulting in higher concentrations of free IGF, which in turn further stimulates fetal growth (Juul 2003, Qiu et al. 2005, Ferraro et al. 2012).

1.7.3 Adiponectin

Adiponectin is secreted by maternal adipose tissue, and is not transferred across the placenta (Aye et al. 2013, Parker-Duffen et al. 2014), but does act directly on placental function through the transfer of insulin and amino acids (Lekva et al. 2017). During pregnancy, adiponectin concentrations decrease as gestation advances (Fuglsang et al. 2006). In both pregnant and non-pregnant individuals, obesity is associated with a lower adiponectin concentration (Lekva et al. 2017) along with type 2 diabetes mellitus (Weyer et al. 2001). Maternal and fetal adiponectin appear to exert opposing effects on fetal growth (Aye et al. 2013), with low maternal concentrations of adiponectin stimulating fetal overgrowth (Lekva et al. 2017). Conversely, cord blood and neonatal adiponectin concentrations have been reported to be up to 7 times higher than maternal concentrations, positively correlating with infant birth weight (Sivan et al. 2003) and increased neonatal adiposity (Sivan et al. 2003, Corbetta et al. 2005).

1.7.4 Leptin

Cord blood concentrations of leptin correlate positively with infant birth weight and neonatal fat mass (Catalano et al. 2009, Tessier et al. 2013, Josefson et al. 2014, Tsai et al. 2015). Cord blood leptin concentrations have also been shown to positively correlate with measures of neonatal insulin resistance (Catalano et al. 2009), suggesting that neonatal fat mass and insulin resistance are related, which raises the possibility that neonatal adipose tissue is also metabolically active.

1.7.5. Lipids

Pregnancy is a physiological state associated with higher circulating concentrations of triglycerides and fatty acids (Montelongo et al. 1992) which is accentuated by maternal obesity, leading to enhanced placental transport of these substrates (Catalano et al. 2017). While triglycerides do not readily cross the placental interface, the lipoprotein receptors and binding proteins and lipases enable the placental flow of maternal fatty acids (Schaefer-Graf et al. 2008). Studies investigating newborn cord blood concentrations of lipoproteins (Merzouk et al. 2000) have shown an association with adipose tissue in the fetus and newborn, contributing to infant birth weight (Schaefer-Graf et al. 2008).

The contribution of specific cardiometabolic measures to fetal growth and adiposity among women who are overweight or obese is unclear, and warrants further investigation

1.7.6 Adipose tissue as a metabolically active contributor to fetal growth

Adipose tissue is not an inert storage organ, but is metabolically active in the secretion of multiple hormones which contribute to metabolic homeostasis (Coelho et al. 2013). Fetal adipocytes begin to develop at 15 weeks and as gestation advances, there is an increase in fetal fat mass from 5 to 15% (Lau 2008). The development of adipose tissue in the fetus and in early neonatal life is sensitive to hormones such as insulin, insulin-like growth factors and glucocorticoids (Muhlhausler et al. 2009). While it is recognised that the human fetus deposits a large amount of subcutaneous fat in late gestation (Symonds et al. 2012), subscapular and axillary fetal adiposity predominantly reflects brown adipose tissue (BAT) deposition, which is required for non-shivering

thermogenesis in the immediate adaptation to extra-uterine life (Stephens et al. 2011, Symonds et al. 2012). While it was initially thought that the presence of BAT was confined to early infancy, deposits have been identified using positron emission tomography (Stephens et al. 2011) in adults at sites that echo those of the neonate, being more commonly identified in women and lean individuals. Furthermore, the role of BAT in energy production and increasing basal metabolic rate has resulted in its identification as a potential target to ameliorate the effects of obesity (Stephens et al. 2011, Symonds et al. 2012).

1.7.7 The inflammatory response to maternal obesity

Obesity (both in pregnancy and in non-pregnant individuals) is associated with a low-grade, chronic inflammatory state (Pantham et al. 2015). Women who are overweight or obese enter pregnancy with an altered inflammatory profile, which may predispose to the development of pregnancy related complications including hypertension and GDM (Catalano 2010, Farah et al. 2012, Haghiac et al. 2015).

Increased secretion of pro-inflammatory cytokines from adipose tissue (Ingvorsen et al. 2015) has been observed in obesity. Maternal obesity is associated with an increase in IL-6 compared with women with a normal BMI (Haghiac et al. 2015, Pantham et al. 2015). The literature remains unclear regarding other cytokines during pregnancy and is limited by small sample size and study design (Farah et al. 2012, Coelho et al. 2013, Ingvorsen et al. 2015, Pantham et al. 2015). There has been one study that has investigated the association between maternal cytokine concentrations and fetal adiposity measurements (Farah et al. 2012). While maternal inflammatory markers

were identified to correlate with maternal adiposity, these did not appear to be related to measures of fetal adiposity (Farah et al. 2012).

The placenta has been hypothesised to play a role in the mediation and regulation of the inflammatory reaction related to obesity (Pantham et al. 2015). Maternal inflammation may induce fetal programming through the passage of specific cytokines (IL-6) or immune cells (maternal monocytes, T and B cells), in addition to modifying the availability of nutrients for the fetus through placental regulation of IL-1B (Ingvorsen et al. 2015). Due to placental changes during gestation, this could potentially lead to variations in transfer of cytokines and immune cells with potential differential fetal effects across pregnancy (Ingvorsen et al. 2015).

The contribution of specific maternal inflammatory markers to fetal growth and adiposity among women who are overweight or obese is unclear, and warrants further investigation

1.7.8 The role of the placenta in fetal growth

The placenta is a complex organ containing chorionic villi and vasculature, which evolves and develops throughout gestation (Rampersad et al. 2011). Factors which disturb or disrupt this process therefore have the capacity to permanently alter placental function (Lewis et al. 2013).

The structure and morphology of the placenta including placental weight is a major determinant of fetal growth, directly reflecting the capacity of the nutrient transport system (Lewis et al. 2013). Placental nutrient transfer is dependent upon the number of transporters present, which in turn has been shown to be regulated by maternal endocrine and nutritional signalling (Lewis et al. 2013).

A retrospective cohort study from Scotland analysed 55,105 births between 1976 to 2007 and to evaluate associations between maternal BMI and placental weight. The findings demonstrate an association between increasing maternal BMI and both placental hypertrophy and reduced placental efficiency, suggesting that maternal obesity, may induce morphological and functional changes to the placenta when compared with women of normal BMI (Wallace et al. 2012).

Placenta pathology has been reportedly associated with maternal obesity (Huang et al. 2015, He et al. 2016, Bar et al. 2012, Bar et al. 2017). Commonly identified histological changes reflect inflammatory and vascular pathology, as well as increased placental thickness (Berceanu et al. 2018), placental overgrowth (Leon-Garcias et al. 2016, Wallace et al. 2012) and an increased incidence of marginal cord insertion (He et al. 2016). Described Inflammatory lesions include maternal origin villitis (Huang et al. 2015, He et al. 2016), histological chorioamnionitis and umbilical vasculitis, observed to occur almost 12 times more frequently in obese women as compared with women of normal BMI (He et al. 2016). Placental vascular lesions are also common in obese pregnant women, (Huang et al. 2015), with fetal vascular pathology identified more frequently than maternal vascular lesions (Bar et al. 2017). Of the maternal vascular

lesions reported, intervillous thrombus are commonly identified (He et al. 2016). While the precise mechanism causing these changes is unclear, they are well recognized contributors to pregnancy complications both for the woman and her developing fetus.

Uteroplacental blood flow also has a fundamental role in nutrient transfer to the fetus. Key to this process is maternal uterine artery blood supply, with alterations in blood flow as early as the first trimester of pregnancy having been associated with an increased risk of poor placentation and the subsequent development of pre-eclampsia and fetal growth restriction (Jeve et al. 2015). Placental perfusion may also be reduced by higher maternal concentrations of circulating lipids, free fatty acids and cholesterol, particularly in women who are obese, which has been postulated to contribute to an increased risk of placental thrombosis and reduced perfusion (Kristensen et al. 2005). In turn, these underlying perfusion related changes may contribute mechanistically to the higher risk of stillbirth and preterm birth observed in the setting of maternal obesity (Kristensen et al. 2005). Additionally, oxidative stress and endothelial dysfunction may both contribute to and result from impaired trophoblastic invasion, and therefore may lead to the subsequent development of hypertensive diseases including pre-eclampsia (Huda et al. 2010).

The fetal Umbilical Artery (UmA) delivers deoxygenated blood from the fetus back to the placenta, and is measured routinely during ultrasound assessment of fetal wellbeing (ISUOG Clinical Standards Committee 2013). Sarno and colleagues have conducted ultrasound umbilical artery Doppler (UmA) assessment in 185 women, of whom 23.2% were overweight, and 21.6% obese. When compared with lean women, women of

higher BMI were found to have significantly higher umbilical artery resistance. The positive correlation between maternal BMI and ultrasound determined umbilical artery resistance suggests a further mechanism whereby placental perfusion may be altered in the setting of maternal obesity (Sarno et al. 2015).

Changes in nutrition in the setting of obesity would suggest that women who are obese or who have GDM would give birth to an LGA infant, reflecting the higher diffusion gradient and further stimulation of growth by glucose, insulin and insulin-like growth factors (Lewis et al. 2013). However, many obese women and women with GDM give birth to an appropriately grown infant. There have been several theories postulated to explain the 'normalisation' of fetal growth in the setting of maternal obesity (Sarno et al. 2015). For example, uteroplacental insufficiency could reduce substrate delivery thereby normalising the anticipated acceleration in fetal growth (Sarno et al. 2015). Another possible explanation is that maternal obesity or GDM alone may be insufficient to induce fetal overgrowth but additional exposures such as endocrine signalling, expression of transporters and alterations in lipid and amino acid transfers together may contribute to an increase in fetal growth and adiposity (Lewis et al. 2013).

1.8 The measurement of fetal growth and body composition using ultrasound

Ultrasound has become the mainstay in the assessment of fetal growth and wellbeing and is widely utilised in both low and high-income countries. There have been numerous studies evaluating ultrasound markers to identify and predict the LGA fetus, all of which have utilised different measurements, definitions and cut-off points (Wong et al. 2001, Coomarasamy et al. 2005, Kernaghan et al. 2007, Pates et al. 2008, El

Khouly et al. 2016). There is no universally accepted definition or specific measurement used in the detection of the LGA fetus in the antenatal period (Campbell 2014). Due to maternal and infant complications, coupled with the growing access to ultrasound, there has been increasing interest in the use of ultrasound to attempt to predict newborn macrosomia to reduce complications such as shoulder dystocia (Rouse et al. 1996).

Table 1.4 summarises the key literature to date assessing the sensitivity and predictive value of a range of ultrasound markers and cut-points to predict infant macrosomia and LGA. Coomarasamy and associates performed a large systematic review of the evidence pertaining to diagnostic ultrasound and the prediction of the LGA infant (Coomarasamy et al. 2005). While there was considerable heterogeneity between the studies including different study designs, estimated fetal weight formulae used, ultrasound equipment and reference range thresholds, EFW and AC greater than 90th centile were both identified to have acceptable positive predictive values as described in Table 1.4 (Coomarasamy et al. 2005). However, the influence of maternal BMI on these assessments is difficult to ascertain, as there was no specific sub-group analysis relating to maternal BMI.

Fetal growth velocity has been demonstrated to have low positive predictive value compared with EFW greater than the 95th centile (Kernaghan et al. 2007) in the prediction of LGA infants. Wong and colleagues identified that the prevalence of LGA infants was higher among women who were both obese and diagnosed with GDM, compared with women of normal BMI (Wong et al. 2001). More recent studies have

combined EFW and amniotic fluid index together to increase the positive and negative predictive value in identifying the LGA infant, with variable results obtained in women considered to be at increased risk (Pates et al. 2008), despite better performance in low risk women entering labour (El Khouly et al. 2016).

The impact of maternal BMI on fetal growth trajectories among infants born to women who are overweight or obese is unclear, and warrants further investigation

Table 1.4: Prenatal ultrasound measurements and the prediction of the large for gestational age infant

Author Year	Study details	Ultrasound marker	Strengths	Limitations	Maternal BMI	Prediction of LGA infant
Coomarasamy et al. 2005	N = 147 Studies = 2 Part of a large systematic review	EFW greater than 90th centile	Pooled analyses Small numbers Moderate to high quality	Clinical heterogeneity (different methods estimated EFW and AC)	No specific analysis	Positive LR 9.3 (3.7 – 24) Negative LR 0.4 (0.14 – 0.93)
Coomarasamy et al. 2005	Studies = 5 N = 1864 Part of large systematic review	AC > 90th centile	Pooled analyses Moderate to high quality	Different ultrasound equipment and reference standard thresholds	No specific analysis	Positive LR 4.2 (2.3 – 7.7) Negative LR 0.33 (0.21 – 0.54)
Kernaghan et al. 2007	N = 242 Pre-existing and gestational diabetes Sheffield, UK	EFW Z-score (≥ 1.7 or ≥ 95.5 centile)	Prospective design	Included women with diabetes alone	No analysis	Prevalence = 27% PPV = 51% NPV = 91% LR positive test = 2.8
Kernaghan et al. 2007	N = 242 Pre-existing and gestational diabetes Sheffield, UK	Fetal growth velocity (FGV)	Prospective design	Included women with diabetes alone	No analysis	Prevalence = 27% PPV 35% NPV 76.1%
Wong et al. 2002	N = 100 Retrospective study at the Mater Hospital in	AC extrapolated from clinical ultrasound reports	Confounding variables were not adjusted for in analyses Small numbers	Retrospective Included only diabetes	Mean BMI was within overweight range	Progressive increase in Z-scores as gestation advances in newborns with LGA

	Brisbane, Australia 1994 – 1999	z-scores calculated			LGA group had a higher BMI	Difference in mean z-score was 0.68 at 18 – 22 weeks to 1.96 at 34 – 38 weeks)
Pates et al. 2008	N = 3115 Retrospective cohort study in Texas, USA during 1997 and 2006	BW ≥ 4000 grams ± AFI ≥ 20cm ± Risk factors for macrosomia	Selection bias due to the reason for the ultrasound was a based on a clinical concern of macrosomia	Retrospective design Indications for clinical scans were variable	BMI > 30 accounted for 81% of BW > 4000 grams compared with 41% in BW < 4000 grams	Neonatal macrosomia 7.6% PPV using USS, AFI and risk factor was 71%, NPV = 94% Sn =29%, Sp =29%
El Khouly et al. 2016	N = 600 Prospective observational study from large maternity hospital in Egypt between 2014 - 2016	Ultrasound in the 1st stage of labour EFW > 4000 grams and AFI > 16.4cm	Prospective design Sample size	Lack of clinical outcomes	No separate analysis performed	10.6% macrosomia rate based on newborn weight 2% incidence of Diabetes Combined EFW and AFI had a PPV = 92.3% in the detection of macrosomia EFW alone (PPV 75%) and AFI (27%)

1.9 Antenatal ultrasound assessment of fetal body composition

In 1991, Bernstein and colleagues were among the first to describe the measurement of fetal body composition using prenatal ultrasound (Bernstein et al. 1991). Since then, there have been substantial advances in both two and three-dimensional ultrasonography, resulting in the development and validation of fetal body composition measurements (Larciprete et al. 2003, Hure et al. 2012, O'Connor et al. 2014, Walsh et al. 2015, Gibson et al. 2016) as outlined in Table 1.5. Most of the identified studies have been limited by relatively small sample sizes (O'Connor et al. 2014, Walsh et al. 2015, Gibson et al. 2016), with wide variation in both the type of fetal body composition measurement utilised, and variation in the reporting of results (Larciprete et al. 2003, Hure et al. 2012, O'Connor et al. 2014, Walsh et al. 2015, Gibson et al. 2016). Larciprete and colleagues validated ultrasound derived body composition measurements in the fetuses of 218 healthy pregnant Italian women with a normal BMI (Larciprete et al. 2003). The generalisability to other populations, particularly in women who are overweight or obese is limited.

Table 1.5 – Summary of the studies relating to fetal body composition measurements using prenatal ultrasound

Author Year	Ultrasound measurement	Definition	Study details	Strengths	Limitations	Results
Walsh et al. 2015	Anterior Abdominal Wall (AAW)	Axial view of abdomen 2 to 3cm laterally from the cord insertion at 34 weeks gestation	N = 50 Healthy, non-diabetic women with previous history of infant with BW > 4000 grams Nested cohort study within the ROLO RCT National Maternity Hospital, Dublin	First study to assess metabolomics and fetal adiposity measurements	Selection bias due to previous delivery Represents women who are at risk of impaired glucose tolerance Small sample	HOMA and AAW were significantly associated with increased measures of Biotin, valine, 2-hydroxyisovalerate, Histidine, malonate, taurine at 28 weeks
O'Connor et al. 2014	Fetal abdominal subcutaneous tissue (FAST)	Axial view of abdomen in AC view, using magnification, measurement of subcutaneous tissue anterior to the margins of the ribs proximal to the cord insertion	N = 62 Ultrasounds performed at 28, 32, 38 weeks March 2012 – 2013 Coombe women's Hospital Recruited after 1st trimester scan	Use of PEAPOD in non-invasive, accurate measurement of infant body fat percentage	Small sample Underpowered	Associated with birthweight, AC and infant fat mass at 38 weeks measured on the PEAPOD (air displacement plethysmography) calculation of neonatal fat mass on D3.
O'Connor et al. 2014	Thigh fat (TF) and thigh muscle (TM)	Distance from the outer border of the femur to the outer border of the subcutaneous layer, subtracting the muscle value	N = 62 Ultrasounds performed at 28, 32, 38 weeks March 2012 – 2013 Coombe women's Hospital Recruited after 1st trimester scan	Use of PEAPOD in non-invasive, accurate measurement of infant body fat percentage	Small sample Underpowered	Thigh fat at 28 and 38 weeks correlates well with infant fat mass in all mothers

Larciprete et al. 2003	Mid thigh Lean mass (MTLM) and fat mass (MTFM) Units = cm^2	Sagittal view of long bone, rotate transducer 90 degrees to get axial view. Total cross sectional limb area and subtracting the central lean area consisting of muscle and bone	N= 218 (Healthy women) N = 85 (Diabetic women) High risk patients from an Italian hospital, recruited at 20 weeks from Jan to Dec 2001 Inclusion criteria included family history of DM, BMI > 27 weeks, glycosuria, previous large baby, previous GDM, age > 37 weeks, polyhydramnios	Serial USS every 3 weeks until term gestation Assess reproducibility Development of reference ranges	Different population and screening for GDM due to 100-gram glucose tolerance test	Normal reference ranges from the healthy women group All measurements were greater in women with GDM compared with the healthy pregnant women. 11.9 vs 14.2cm^2 P = 0.02 at 37 – 40 weeks
Larciprete et al. 2003	Subscapular fat mass (SFM, mm)	Shoulder skin width perpendicularly to the bone at its lower end	As above	As above	As above	Normal reference ranges described from the healthy women group. Higher measures in women with GDM compared with healthy pregnant women. 5.3 vs 6.7mm, P < 0.01 at 38 – 40 weeks
Larciprete, Valensise et al. 2003	Abdominal fat mass (AFM)	Measuring thickness of the anterior abdominal subcutaneous tissue on the same axial	As above	As above	As above	Normal reference ranges described. Higher measures in women with GDM compared with healthy pregnant women

		image used for the AC calculation				6.18 vs 6.8mm, P = 0.03 at 39 weeks
Hure et al. 2012	Fetal Abdominal fat area (cm^2)	At the level of AC measurement, total area (A1) subtracted from the lean abdominal area (A2)	N = 179 Longitundal cohort Australian WATCH study 2006 - 2008 John Hunter Hospital, New South Wales Inclusion: Singleton pregnancies	Longitundal study Adjusted for confounders	Self reported data for maternal weight and calculation of BMI	Gestational weight gain predicted fetal AC and lean abdominal mass. BMI and GWG predicted higher lean muscle mass but no increase in fat mass
	Fetal mid-thigh fat and lean mass (cm2)	Midpoint of the femur, total cross-sectional area (T1) and muscle mass (T2) with fetal mid thigh fat mass calculated by T1 – T2	As above	Longitundal study Adjusted for confounders	As above	Strong correlation between femoral lean area and neonatal lean mass ($r = 0.7$, $P < 0.001$). Moderate correlation with femoral fat area neonatal fat mass ($r = 0.63$, $P < 0.001$)
Gibson et al. 2016	Fractional thigh volume (3Dimensional)	Sub volume that includes 50% of the femoral diaphysis length centred around the mid-femoral shaft	N = 34 neonates Prospective observational study Inclusion: Term, singleton newborns with suspected macrosomia, enrolled upon admission to Delivery Suite Newborns were assessed 48 hours post	Use of new technology	Small numbers Selection bias No control group Specialised transducers, software and training required to performed 3D USS	Fractional volumes correlated with birth weight Z-score ($R^2 = 0.52$, $P < 0.001$) and percentage of neonatal body fat ($R^2 = 0.22$, $P = 0.04$) using anthropometric measurements

The largest study to date to assess fetal biometry and body composition measurements using ultrasound was performed by Grivell and colleagues (Grivell et al. 2016). This study included women enrolled in the LIMIT trial (Dodd et al. 2014a) who were randomised to receive a comprehensive lifestyle intervention across pregnancy compared with women who received standard antenatal care. While the proportion of newborns classified as LGA (birth weight above the 90th centile), did not differ between the two treatment groups, the intervention was associated with a significant 18% relative risk reduction in the chance of infant birth weight greater than or equal to 4kg, and a 41% reduction in risk of birth weight above 4.5kg (Dodd et al. 2014b). Women who received the antenatal intervention demonstrated significant improvements in their self-reported dietary intake (Dodd et al. 2014c) and physical activity, when compared with women randomised to the standard care group (Dodd et al. 2014c).

In this setting, fetal body composition measurements and biometry were obtained from 1,847 women at both 28 and 36 weeks gestation (Grivell et al. 2016). Fetal z-scores for all biometry and adiposity related measures were above the population means, regardless of treatment group, indicating that the fetuses of women who are overweight or obese have growth measures above population standards (Grivell et al. 2016). Furthermore, increases in head (HC) and abdominal circumference (AC) growth were both identified to contribute to the increase in EFW (Grivell et al. 2016), compared with the increase in AC only, which has in the past been demonstrated in fetal growth in women with GDM (Wong et al. 2002, Wong et al. 2006, Walsh et al. 2015).

Two-dimensional fetal body composition measurements have been poorly correlated with neonatal measurements of body composition, lean tissue and body fat mass (Khoury et al. 2009, Lee et al. 2009, Moyer-Mileur et al. 2009). Thus, there has been increasing interest in three-dimensional imaging due to its increased availability over the past 5 years. Gibson and associates have assessed body composition in fetuses with suspected macrosomia using 3-dimensional mean thigh volume. Thigh volume Z-scores were correlated with infant birth weight (R^2 = 0.52 [0.54 – 84], P < 0.001), neonatal anthropometric body fat (R^2 = 0.22 [0.17 – 0.69], P = 0.04) and skin-fold thickness measurements (SFTM) including triceps, subscapular, umbilical, flank and thigh skinfolds (Gibson et al. 2016). With the increasing availability of air displacement plethysmography for the assessment of neonatal body composition, Lee and associates have identified that 3-dimensional fractional limb volume is correlated well with calculated neonatal fat mass (Lee et al. 2009). However, despite these promising findings, fractional limb volume measurement using 3-dimensional imaging requires specific training, specialised software and is not routinely used in clinical practice (Gibson et al. 2016).

To date, there is no "gold standard" measure to predict the large for gestational age fetus or an increase in fetal adiposity (Campbell 2014). Further research is required, specifically focusing on women who enter pregnancy overweight or obese, as the fetus of these women remain at high risk of future health complications.

The correlation between fetal and neonatal measures of growth and adiposity among infants born to women who are overweight or obese is unclear, and warrants further investigation

1.10 Gaps in our current knowledge identified from this literature review

- The contribution of specific maternal dietary components to fetal growth and adiposity among women who are overweight or obese is unclear, and warrants further investigation.
- The contribution of specific cardiometabolic measures to fetal growth and adiposity among women who are overweight or obese is unclear, and warrants further investigation.
- The contribution of specific maternal inflammatory markers to fetal growth and adiposity among women who are overweight or obese is unclear, and warrants further investigation.
- The impact of maternal BMI on fetal growth trajectories among infants born to women who are overweight or obese is unclear, and warrants further investigation.
- The correlation between fetal and neonatal measures of growth and adiposity among infants born to women who are overweight or obese is unclear, and warrants further investigation.

1.11 The aims of this research study

The specific aims of this thesis were to conduct exploratory analyses to address the identified research gaps.

The **first aim** of this study was to determine the association between maternal BMI and fetal growth, body composition and growth velocity in a population of overweight and obese women.

The **second aim** was to evaluate associations between maternal dietary factors and fetal growth and adiposity measured by ultrasound at 28 and 36 weeks gestation in overweight and obese women.

The **third aim** was to determine if maternal cardiometabolic and inflammatory markers were associated with fetal growth and adiposity measured by ultrasound in women who were overweight or obese in pregnancy at 28 and 36 weeks gestation.

The **fourth aim** was to evaluate the correlation between fetal ultrasound biometry and adiposity measures at 36 weeks gestation and neonatal biometry and adiposity measures, in pregnant women who were overweight or obese.

CHAPTER 2: Methods

The series of studies described in this thesis were conducted as secondary exploratory analyses of data from the LIMIT randomised controlled trial.

2.1 The LIMIT Randomised Trial

The methodology of the LIMIT randomised trial has been reported previously (Dodd et al. 2014a). In brief, women were recruited from 3 metropolitan maternity units in South Australia, following ethical approval. Women were eligible to participate with a singleton pregnancy between 10^{+0} and 20^{+0} weeks gestation, and with BMI greater than or equal to 25kg/m^2. Women with a diagnosis of Type 1 or Type 2 diabetes prior to pregnancy, or with a multiple pregnancy were excluded.

The sample size for the LIMIT randomised controlled trial was calculated to give sufficient power to detect the primary infant birth outcome of LGA: details are given in the published paper (Dodd et al. 2014a and Dodd et al. 2011c). The analyses conducted in this study were exploratory, with the sample size determined by the available data; power calculations were not performed as there was no predetermined effect size which we aimed to detect, and no primary hypothesis which was tested.

At the time of the first antenatal visit, maternal weight and height were measured by the research assistants or the attending midwife. Early pregnancy measurement of BMI has been demonstrated to correlate with pre-pregnancy BMI (Headen et al., 2017). This approach is

consistent with standard obstetric practice and state wide perinatal practice guidelines (Government of South Australia, 2015, Denison et al., 2018).

Consenting women were then randomised to either the Lifestyle Advice Group or to the Standard Care Group, using a central randomisation service and computer generated randomisation schedule. Stratification variables included parity (0 versus ≥ 1), BMI at antenatal booking (overweight versus obese) and hospital of birth.

Women who were randomised to the 'Lifestyle Advice Group' participated in a comprehensive dietary and lifestyle intervention, which included a combination of dietary, exercise and behavioural strategies. The intervention was delivered by a research dietitian and trained research assistants. Further details regarding content of the intervention have been published previously (Dodd et al. 2014a).

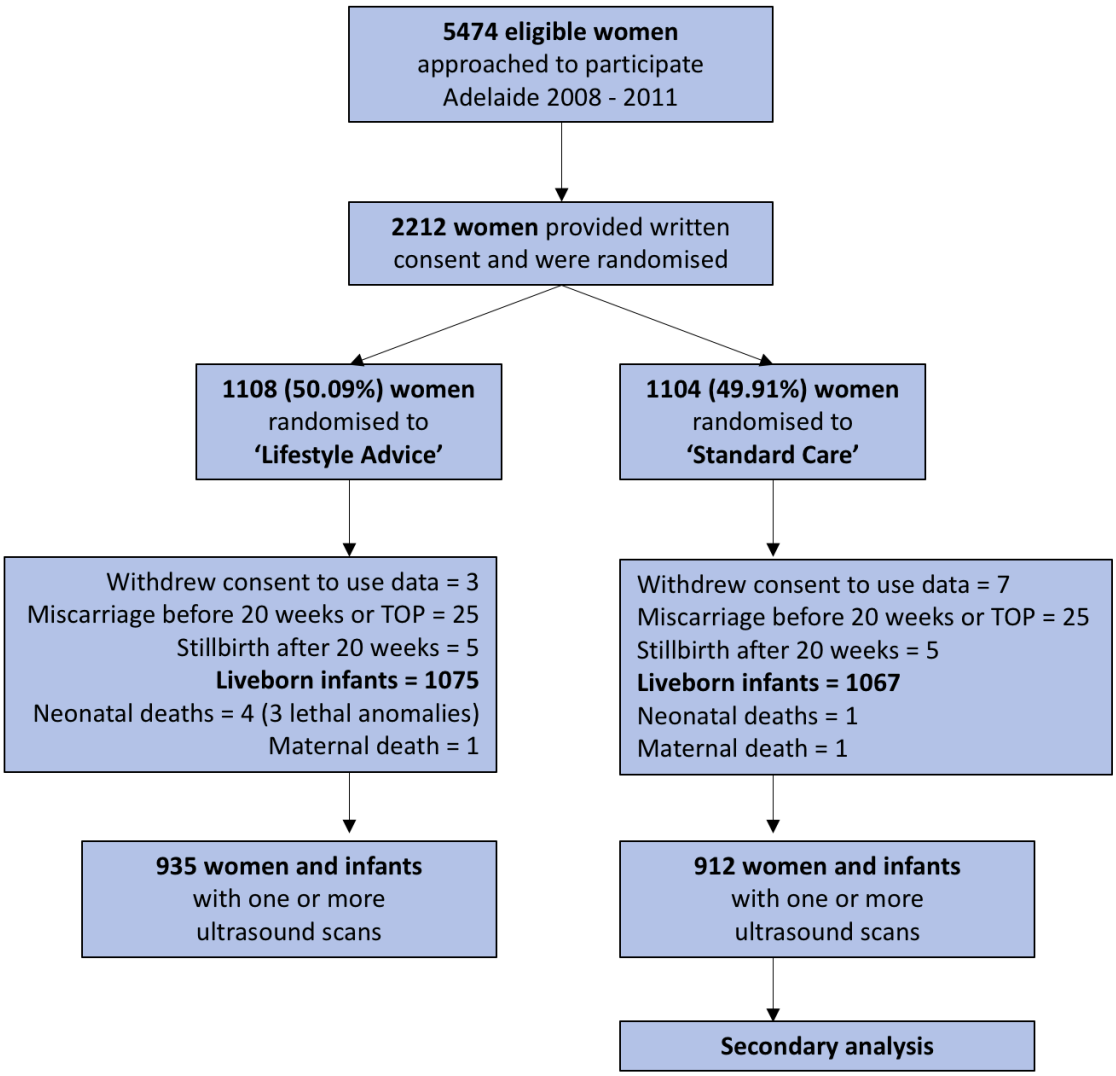
Women who were randomised to the Standard Care group received their pregnancy care according to their hospital of birth and local hospital guidelines. This care did not include the routine provision of dietary and lifestyle advice, or information relating to gestational weight gain in pregnancy. The women included in this series of secondary analyses were those randomised to the Standard Care Group.

2.2 Flow of Participants and Baseline Characteristics

The overall flow of participants within the LIMIT randomised controlled trial is outlined in Figure 2.1, detailing the number of women eligible, number of women who participated in the

LIMIT trial, women lost to follow up and the number of women who had 28 and 36 week ultrasound data available. Within each Chapter, individual flowcharts and baseline characteristics are presented.

Figure 2.1: Flow chart of participants included in the secondary analysis from the Standard Care Group of the LIMIT randomised trial



2.3 Ultrasound Assessment

All women who participated in the trial were offered an ultrasound scan at 28 (range 26^{+0} to 29^{+6}) and 36 (range 34^{+0} to 37^{+6}) weeks gestation to obtain biometry and body composition measurements (Grivell et al. 2016).

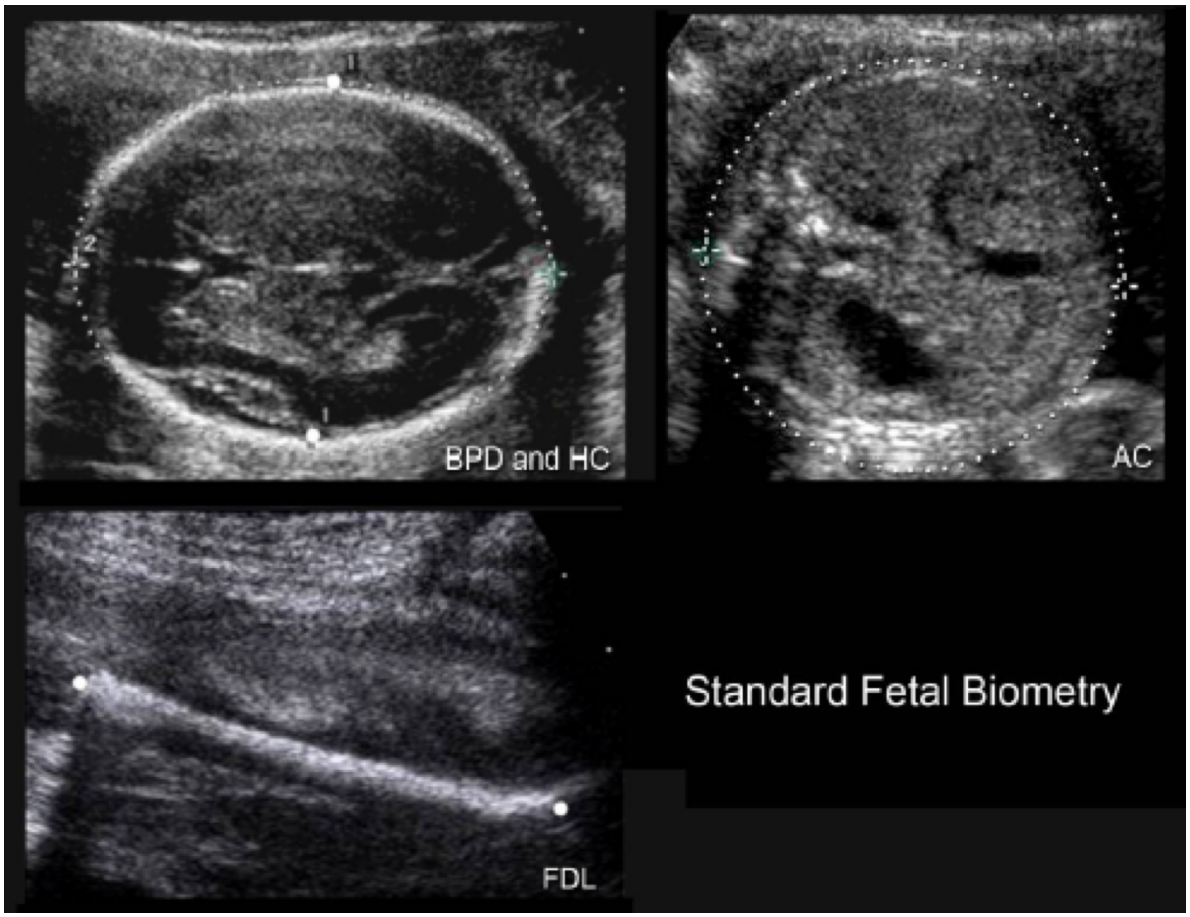
The estimated date of confinement and gestational age was calculated from an early pregnancy ultrasound and menstrual period dating. All research ultrasounds were performed by medical practitioners with specialist or subspecialist training in obstetric ultrasound. All sonographers were blinded to the treatment allocation, and all measurements and calculations were obtained prospectively. Inter-observer reliability for the fetal adiposity measures was fair to moderate as reported previously (Grivell et al. 2016).

Ultrasounds were performed using the Medison Accuvix V20 Ultrasound System (Samsung Medison Co., Ltd., Seoul, Korea). A C2-61C curved probe was used, with a frequency of range of 2MHz – 6MHz, with a 58 degree field of view with 60mm radius of curvature.

2.2.1 Fetal biometry and estimated fetal weight

Ultrasound assessment included measurements of standard biometry (head circumference, bi-parietal diameter, abdominal circumference and femur length), measured in accordance with national and international standards of practice (ASUM 2007). Estimated fetal weight was calculated using the Hadlock C formula (Hadlock et al. 1991). Figure 2.2 shows the standardised measurement of fetal biometry as per the ASUM guideline (ASUM 2007).

Figure 2.2: Standardised measurement of fetal biometry as per the Practice guidelines for performance of the routine mid-trimester fetal ultrasound (Salomon et al. 2013).



2.2.2 Fetal adiposity measurements

Fetal adiposity measures were obtained in a standardised fashion, as reported previously (Grivell et al. 2016), and included mid-thigh lean mass (MTLM), mid-thigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM).

(i) Mid thigh total, lean and fat mass

Mid thigh lean mass (MTLM) and mid thigh fat mass (MTFM) were measured according to described techniques (Bernstein et al. 1991, Larciprete et al. 2003, Grivell et al. 2016). Mid thigh measurements were calculated by firstly obtaining a sagittal view of the femur. Using a curvilinear transducer, the midpoint of the femur length was 0 degrees to the transducer. The transducer was then rotated through a 90 degree angle to obtain the cross-sectional view of the mid thigh, and a trace of the circumference of the mid thigh total mass (MTTM) was performed and the area calculated. The mid thigh lean mass (MTLM) incorporating muscle and bone was outlined using a continuous trace to calculate the area as shown in Figure 2.3. A subtraction was performed between the MTTM and the MTLM to calculate the mid thigh fat mass (MTFM).

(ii) Abdominal fat mass

Fetal abdominal fat mass or anterior abdominal wall thickness was measured between the mid-axillary lines and anterior to the margins of the ribs, at the level of the abdominal circumference, with the subcutaneous fat represented by the echogenic envelope surrounding the abdomen and measured in millimetres as shown in Figure 2.4 (Bernstein et al. 1991, Larciprete et al. 2003, Grivell et al. 2016). Using magnification, 4 measurements of the anterior abdominal wall envelope were undertaken with 2 distally and 2 proximally. The mean of all 4 measurements was calculated and used within the analysis.

Figure 2.3 Ultrasound images illustrating fetal mid thigh total and lean mass calculation

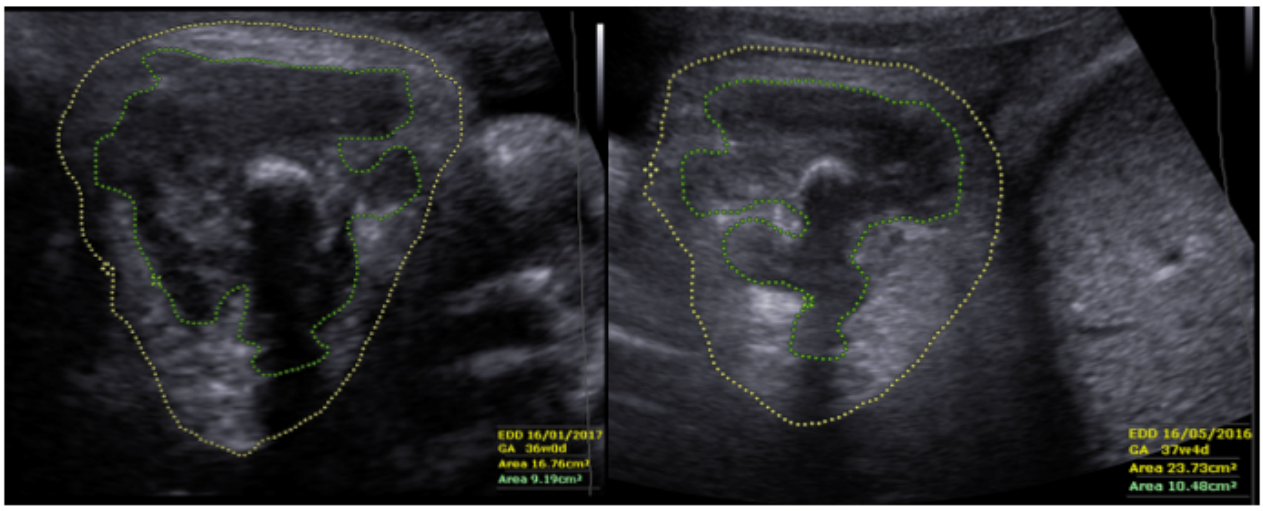
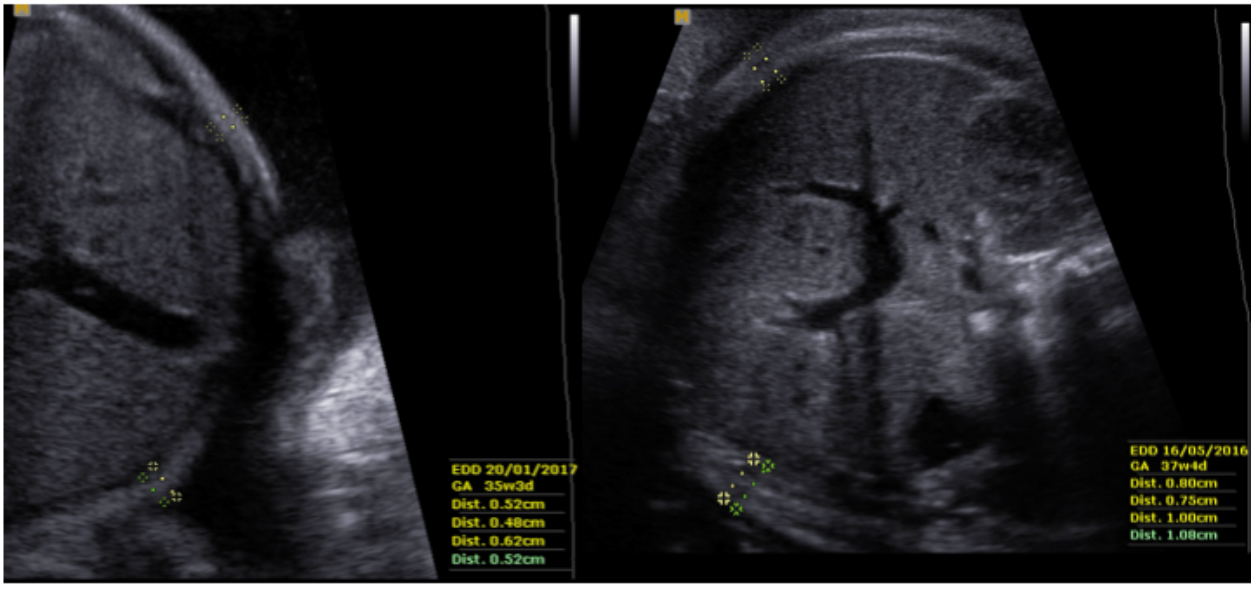


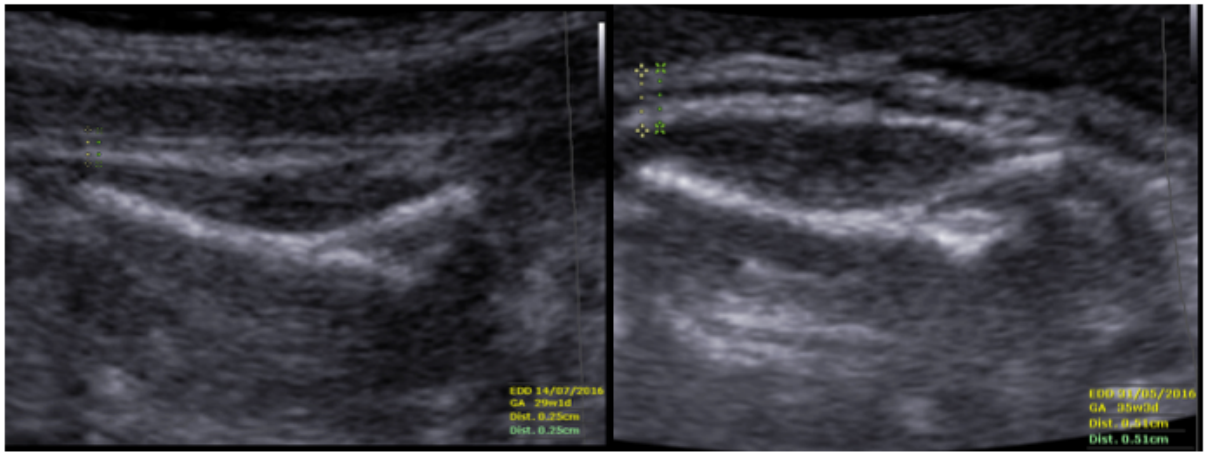
Figure 2.4 Ultrasound images illustrating the fetal abdominal fat mass (AFM) calculation



(iii) Subscapular fat mass

Subscapular fat mass was measured using a sagittal view of the fetal trunk to visualise the entire longitudinal section of the scapula and is illustrated in Figure 2.5 (Bernstein et al. 1991, Larciprete et al. 2003, Grivell et al. 2016). The subcutaneous measurement between the skin surface and the subcutaneous tissue at the interface with the super-spinous and infra-spinous muscles was obtained on two occasions, with the mean value used in the analysis.

Figure 2.5 Ultrasound images illustrating the fetal subscapular fat mass (SSFM) calculation



(iv) Fetal z-scores

For each fetal growth and adiposity measurement, z-scores were calculated using ultrasound growth charts in clinical use (Hadlock et al. 1991).

(v) Fetal growth velocity

Fetal growth velocity was defined as the difference in biometry between 36 and 28 week measurements divided by actual time (in days) between measures. The growth velocity measurement was expressed as growth in millimetres per day for BPD, HC, FL, MTFM, AFM, SSFM, grams per day for EFW and z-scores were calculated for BPD, FL, EFW and abdominal area using reference values from Owen and colleagues (Owen et al. 1996). Abdominal area (AA) velocity, expressed as cm^2, was used instead of Abdominal Circumference (AC) due to the availability of reference values for AA (Owen et al. 1996), not but for AC.

2.2.3 Statistical Analysis

Baseline characteristics of women contributing data to each analysis were assessed descriptively. Continuous variables were reported as mean and standard deviation or median and interquartile range as appropriate, and categorical variables as a number and percentage.

The specific methodologies and statistical analyses are unique to each chapter, and will be described in more detail subsequently. Statistical significance was assessed at the two-sided 0.05 level. Because the analyses are exploratory, no adjustment has been made for multiple comparisons. Analyses were performed using SAS 9.4 (Cary, NC, USA) and Stata version 14 (Stata corporation, Texas, USA).

CHAPTER 3: The effect of maternal overweight and obesity on fetal biometry, body composition and growth velocity

This chapter forms the basis of a manuscript that has been recently published (O'Brien CM et al The Journal of Maternal Fetal and Neonatal Medicine), which is contained in Appendix 2.

3.1 Introduction

With advances in ultrasound technology there has been growing interest in the identification of the fetus at risk of overgrowth and increased adiposity, utilising both standard ultrasound biometry and fetal body composition measurements. While several studies have evaluated a range of ultrasound derived fetal body composition measures (O'Connor et al. 2014, Walsh et al. 2015, Gibson et al. 2016), they have been somewhat limited by their relatively small sample sizes and having been performed mostly in women with a normal BMI (Larciprete et al. 2003, Parretti et al. 2003) or diabetes (Kehl et al. 1996, Bethune et al. 2003, Kernaghan et al. 2007). The generalisability of these measures in other populations, particularly among women who are overweight or obese, is unclear and further evaluation is required.

3.2 Aims

The aim of this study was to determine the association between maternal BMI and fetal growth, body composition and growth velocity in a population of overweight and obese pregnant women.

3.3 Methods

The research methodology (Dodd et al. 2011, Dodd et al. 2014a, Dodd et al. 2014c) of the LIMIT randomised controlled trial have been outlined in Chapter 2. As previously described, all women who participated in the trial were offered a research ultrasound scan at 28 (range 26^{+0} to 29^{+6}) and 36 (range 34^{+0} to 37^{+6}) weeks gestation to obtain fetal biometry and body composition measurements (Grivell et al. 2016), with fetal biometry, EFW and body composition obtained in accordance with international standards of practice.

3.3.1 Statistical analysis

Maternal BMI was included as a categorical variable with the following categories: overweight (BMI 25.0 - 29.9 kg/m^2), Class 1 obesity (BMI 30.0 - 34.9 kg/m^2), Class 2 obesity (BMI 35.0 - 39.9 kg/m^2) and Class 3 obesity (BMI $\geq$ 40 kg/m^2) (Modder et al. 2010, ACOG 2015).

For analysis, BMI 25.0 – 29.9 kg/m^2 was used as the reference category with the estimates made of the difference in mean fetal growth for each higher BMI category compared with BMI 25.0 – 29.9 kg/m^2.

Fetal biometry and adiposity outcomes were analysed using linear regression models with adjustment for confounders including centre, parity, maternal age, smoking and socio-economic status using the SEIFA Index of Relative Socio-Economic Disadvantage (IRSD) quintile.

A time-by-BMI-category interaction term was included in the model to test whether the effect of maternal BMI differed over time. Generalised Estimating Equations (GEE) were used to account for repeated measures. Additional sensitivity analyses were also performed using BMI as a continuous measure, results are not reported here.

3.4 Results

3.4.1 Demographic characteristics

Flow of participants and baseline characteristics are presented in Figure 3.1 and Table 3.1 respectively. A total of 911 women from the Standard Care group had ultrasound information at one or more time points, with 777 having ultrasound data at both 28 and 36 weeks. Of the 911 women included in the secondary analysis, 41% (n = 376) were overweight, 29.8% (n = 271) had Class 1 Obesity, 16.8% (n=153) had Class 2 Obesity and 12.2% (n = 111) had Class 3 Obesity (Table 3.1). The mean age of women was 29.6 years, the majority (92%; n = 835) were of Caucasian ethnicity, with 40% (n = 369) in their first ongoing pregnancy, and almost 30% (n = 265) from the highest quintile of social disadvantage. The overall rate of gestational diabetes in the Standard Care group was 11.2% (n=102). Sixty-six women (7.2%) had data only for 28 weeks, and 68 women (7.5%) had data only at 36 weeks gestation (Table 3.1). These baseline characteristics are consistent with the baseline characteristics of the entire LIMIT Trial randomised cohort (Dodd et al. 2014a).

Figure 3.1: Flow chart of the participants included in analysis of effect of maternal BMI on fetal measures

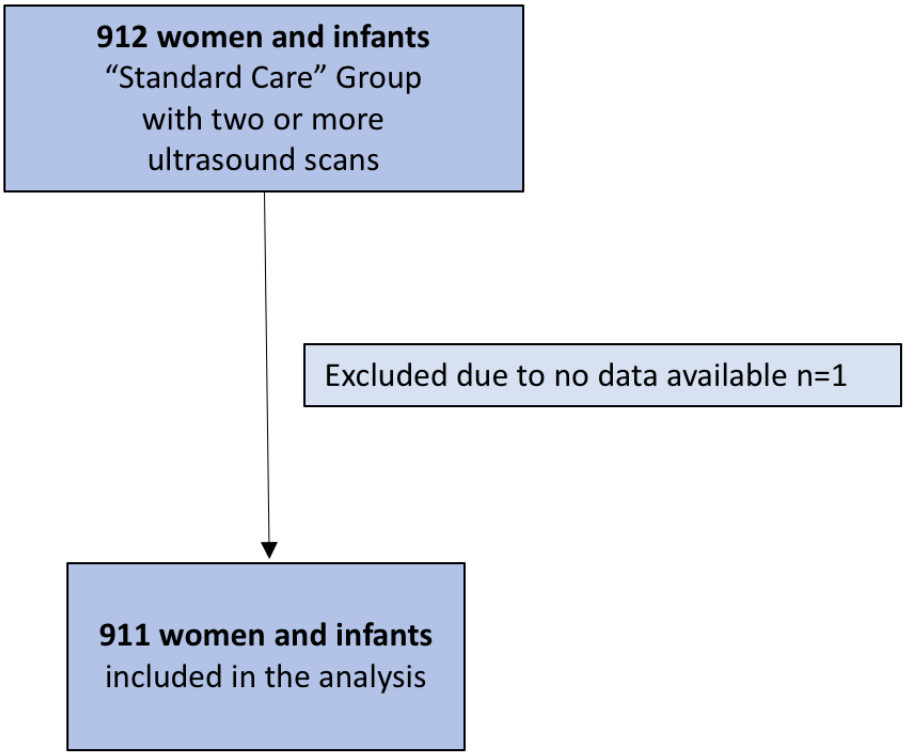


Table 3.1 – Baseline and post-randomisation characteristics of participants included in analysis of the effect of maternal BMI on fetal measures

	Body Mass Index category (kg/m^2)				Overall
	Overweight 25.0-29.9	**Class 1 30.0-34.9**	**Class 2 35.0-39.9**	**Class 3 ≥ 40.0**	**N (%)**
Total number N (%)	376 (41.3)	271 (29.8)	153 (16.8)	111 (12.2)	911
Maternal Age at trial entry (years) Mean (SD)	29.9 (5.25)	29.6 (5.69)	29.2 (5.40)	28.9 (5.97)	29.6 (5.50)
Gestational Age at trial entry (weeks) Mean (SD)	14.68 (3.10)	14.55 (2.97)	14.47 (2.97)	14.52 (3.08)	14.59 (3.03)
Caucasian n (%)	340 (90.4)	248 (91.5)	141 (92.2)	106 (95.5)	835 (91.7)
Nulliparous n (%)	168 (44.68)	108 (39.85)	50 (32.68)	43 (38.74)	369 (40.50)
Smoker n (%)	38 (10.11)	35 (12.92)	14 (9.15)	14 (12.61)	101 (11.09)
Gestational Diabetes n (%)	26 (6.91)	37 (13.65)	19 (12.42)	20 (18.02)	102 (11.20)
SEIFA IRSD (Quintile)					
Quintile 1 Most disadvantaged n (%)	94 (25.00)	83 (30.63)	48 (31.37)	40 (36.04)	265 (29.09)
Quintile 2 n (%)	87 (23.14)	63 (23.25)	37 (13.65)	40 (14.76)	48 (17.71)
Quintile 3 n (%)	59 (15.69)	37 (13.65)	29 (18.95)	18 (16.22)	143 (15.70)
Quintile 4 n (%)	70 (18.62)	40 (14.76)	20 (13.07)	12 (10.81)	142 (15.59)
Quintile 5 Least disadvantaged n (%)	66 (17.55)	48 (17.71)	14 (9.15)	11 (9.91)	139 (15.26)

3.4.2 Maternal BMI and the relationship with fetal biometry and estimated fetal weight z-scores

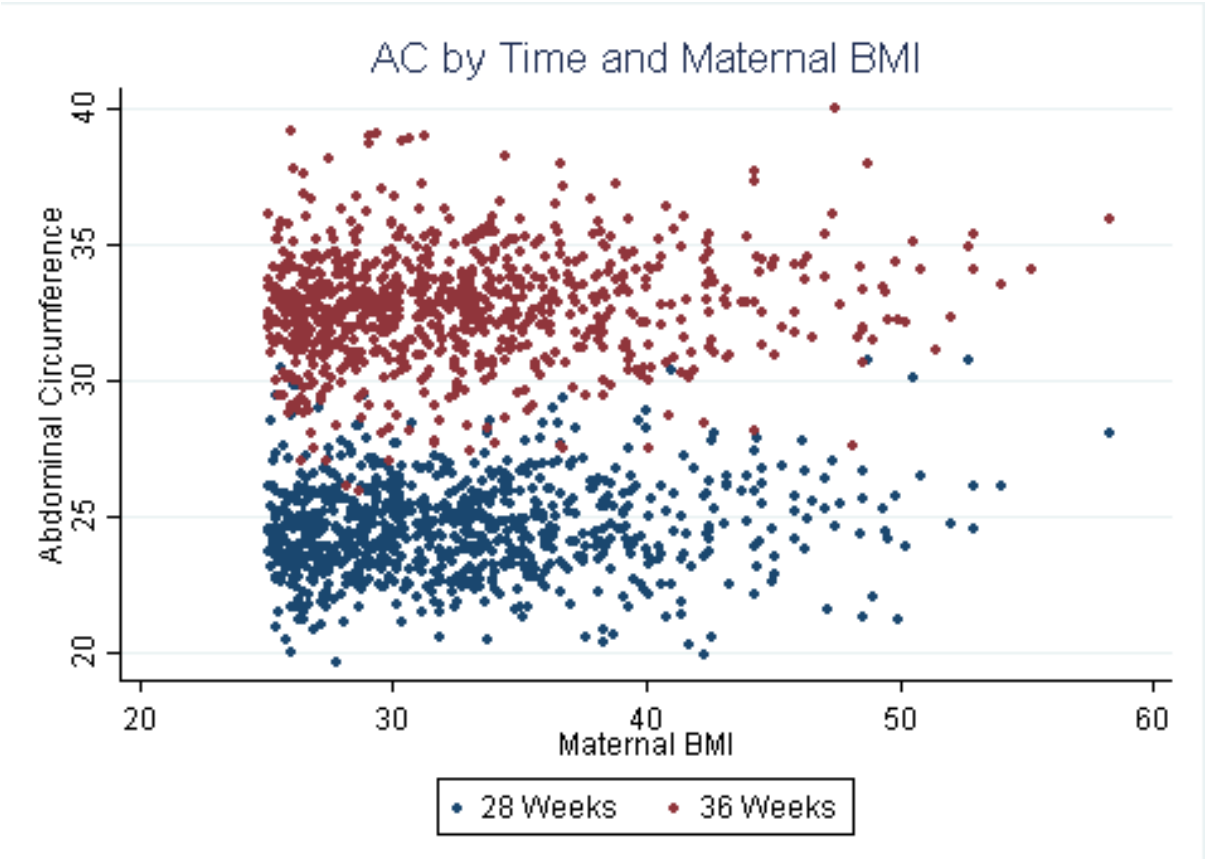
Neither maternal obesity Class 1 or Class 2 were associated with fetal BPD, HC, or FL z-scores at either 28 or 36 weeks gestation, when compared with fetal biometry measures from women who were overweight (Table 3.2). However, the fetuses of women with obesity Class 3 demonstrated significantly higher z-scores for BPD compared with the fetuses of women who were overweight: the estimated mean difference was 0.36 (95% CI: 0.06, 0.65) at 28 weeks (p=0.017), and 0.39 (95% CI: 0.15, 0.63) at 36 weeks (p=0.002). Similarly, HC z-scores were higher by 0.47 (95% CI: 0.26, 0.68) at 28 weeks and 0.51 (95% CI: 0.32, 0.71) at 36 weeks (p<0.001 for both time points), while FL z-scores were higher by 0.36 (95% CI: 0.13, 0.58) at 28 weeks (p=0.002) and by 0.27 (95% CI: 0.02, 0.52) at 36 weeks (p=0.035).

For both AC and EFW z-scores, there was a consistent pattern of higher measures with increasing maternal BMI at both time points (Table 3.3). The mean fetal AC z-scores at both 28 and 36 weeks were significantly higher in women with Class 1 obesity, with the magnitude of the increase being higher for Class 2 and Class 3 obesity categories in comparison to women in the overweight group. Women with Class 1 obesity had AC z-scores 0.18 (95% CI: 0.02, 0.33, p=0.028) higher at 28 weeks, and 0.21 (95% CI: 0.04, 0.38, p=0.017) higher at 36 weeks. Women with Class 2 obesity had AC z-scores 0.20 (95% CI: 0.01, 0.38; p=0.04) higher at 28 weeks, and 0.24 (95% CI: 0.05, 0.43, p=0.013) higher at 36 weeks. For the women with Class 3 obesity, the increase in AC z-score was 0.40 (95% CI: 0.17, 0.63, p=0.013) at 28 weeks, and 0.39 (95% CI: 0.15, 0.63, p=0.001) at 36 weeks compared to the overweight group. Women with Class 1 obesity had EFW z-scores 0.18 (95% CI: 0.04, 0.33, p=0.014) higher at 28 weeks, and

0.17 (95% CI: 0.008, 0.32, p=0.04) higher at 36 weeks. For the women with Class 3 obesity, the increase in EFW z-score was 0.46 (95% CI: 0.23, 0.69, p<0.001) at 28 weeks, and 0.42 (95% CI: 0.19, 0.64, p<0.001) at 36 weeks compared to the overweight group.

There was no evidence that the observed associations between maternal BMI and fetal growth measurements changed over time, with the interaction p values non-significant for all outcomes.

Figure 3.2: Relationship between maternal BMI and fetal abdominal circumference at 28 weeks (blue) and 36 weeks (red) gestation



3.4.3 Maternal BMI and fetal adiposity measurements

Table 3.3 presents results of analyses for the effect of maternal BMI category on fetal adiposity measures. There were no significant differences between BMI categories in relation to mid-thigh fat mass (MTFM).

For abdominal area (AA), there were no significant differences for Class 2 obesity; however there were significant differences for Class 1 and Class 3 obesity when compared to women who were overweight. Compared to overweight women, the mean abdominal area was 1.80cm^2 (95% CI: 0.13, 3.46, p = 0.035) higher at 36 weeks gestation in the fetuses of women with Class 1 obesity. Similarly, the mean abdominal area was higher by 2.19cm^2 at 28 weeks (95% CI: 0.31, 4.08, p = 0.02) and 3.42cm^2 at 36 weeks (95% CI: 1.09, 5.74, p = 0.004) in the fetuses of women with Class 3 obesity.

For abdominal fat mass (AFM), there were no significant differences in the fetuses of women with Class 1 or Class 2 obesity, when compared to those of women who were overweight. However, in the fetuses of women with Class 3 obesity abdominal fat mass was higher by 0.61 mm (95% CI: 0.08, 1.14, p=0.03) at 36 weeks compared with those of overweight women.

For subscapular fat mass (SSFM), there were no significant differences in the fetuses of women with Class 2 or Class 3 obesity, compared to women who were overweight. However, in the fetuses of women with Class 3 obesity, subscapular fat mass was

reduced by 0.2 mm (-0.37, -0.04, p=0.016) at 28 weeks compared to fetuses of overweight women.

There was no evidence that the effect maternal BMI category differed over time for any of the adiposity measurements.

3.4.4 The effect of maternal BMI on fetal growth velocity

There were no significant differences between the maternal BMI categories in relation to MTFM velocity, AFM velocity, or SSFM velocity. For EFW and AA velocity, there were no significant differences for women with Class 1 and Class 2 obesity; however there were significant differences between the Class 3 obesity and overweight categories. The mean EFW velocity was 2.03 grams per day (0.86, 3.2, p<0.001) higher in women with Class 3 obesity compared to overweight women. EFW velocity z-score was also higher by 0.44 (95% CI: 1.2, 0.69, p<0.001). Similarly, AA velocity was higher by 0.035 cm^2/day (95% CI: 0.004, 0.066, p=0.029), and the AA velocity z-score was higher by 0.24 (95% CI: 0.02, 0.45, p=0.03).

In sensitivity analyses utilising BMI as a continuous variable, results were consistent with the findings reported above.

Figure 3.3 – Estimated change in fetal weight by maternal BMI category

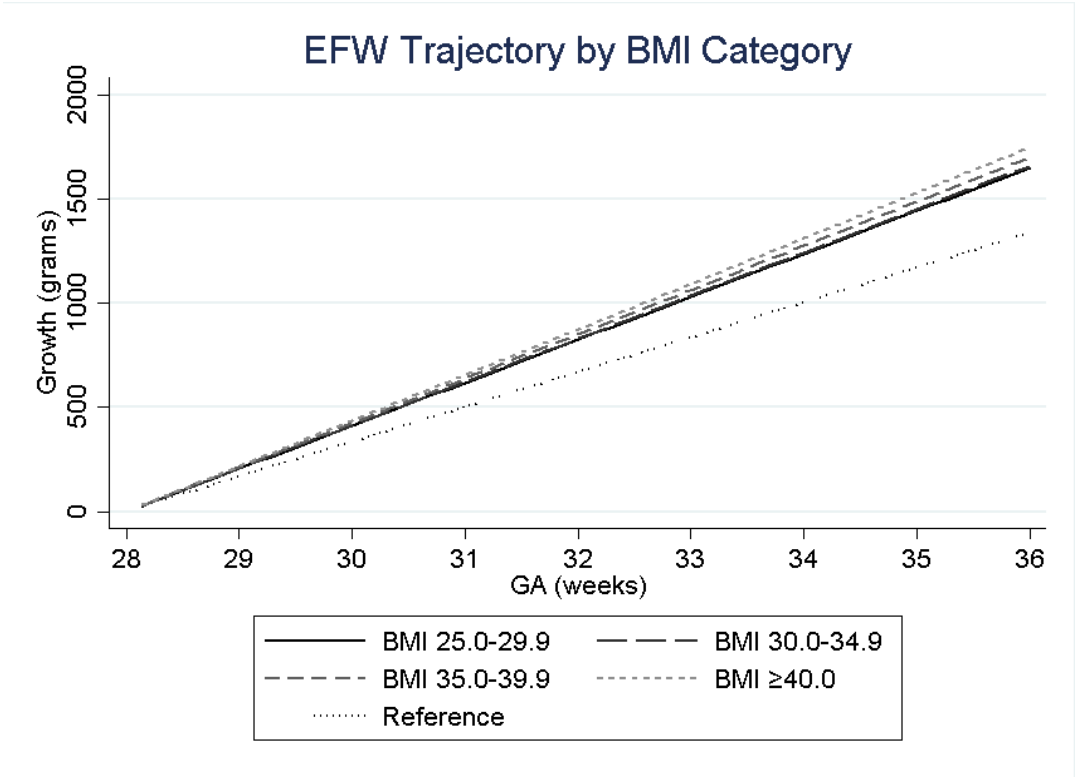


Table 3.2 – Maternal body mass index (BMI) category and fetal biometry z-scores at 28 and 36 weeks

		Body Mass Index Category										
		Overweight 25-29.9 kg/m^2		Class 1 Obesity 30-34.9 kg/m^2			Class 2 Obesity 35-39.9 kg/m^2			Class 3 Obesity ≥ 40.0 kg/m^2		
z-scores	**Gestation**	**Mean (SD)**	**Adjusted Estimate (95% CI)**	**Mean (SD)**	**Adjusted Estimate (95% CI)**	**P value**	**Mean (SD)**	**Adjusted Estimate (95% CI)**	**P value**	**Mean (SD)**	**Adjusted Estimate (95% CI)**	**P value**
BPD	0.372^											
	28 weeks	0.62 (1.48)	Ref	0.57 (1.48)	-0.084 (-0.321, 0.152)	0.484	0.50 (1.42)	-0.133 (-0.414, 0.148)	0.353	0.95 (1.32)	0.356 (0.064, 0.649)	0.017
	36 weeks	0.09 (1.20)	Ref	0.13 (1.23)	0.013 (-0.184, 0.21)	0.895	0.18 (1.02)	0.080 (-0.14, 0.30)	0.477	0.45 (1.11)	0.389 (0.145, 0.632)	0.002
HC	0.387^											
	28 weeks	0.73 (0.93)	Ref	0.68 (1.01)	-0.44 (-0.202, 0.114)	0.586	0.69 (0.91)	-0.009 (-0.191, 0.173)	0.924	1.12 (0.99)	0.470 (0.257, 0.683)	< 0.001
	36 weeks	0.53 (0.83)	Ref	0.63 (0.95)	0.101 (-0.042, 0.245)	0.165	0.56 (0.88)	0.0480 (-0.126, 0.221)	0.589	0.99 (0.93)	0.513 (0.318, 0.708)	< 0.001
AC	0.946^											
	28 weeks	0.34 (0.94)	Ref	0.51 (1.0)	0.175 (0.019, 0.331)	0.028	0.52 (0.98)	0.196 (0.009, 0.383)	0.040	0.72 (1.08)	0.399 (0.168, 0.631)	0.013
	36 weeks	0.32 (1.05)	Ref	0.54 (1.09)	0.210 (0.038, 0.381)	0.017	0.54 (0.97)	0.241 (0.051, 0.431)	0.013	0.68 (1.19)	0.390 (0.154, 0.626)	0.001

FL	0.270^											
	28 weeks	0.18 (0.97)	Ref	0.25 (0.98)	0.066 (-0.093, 0.225)	0.413	0.06 (1.06)	-0.120 (-0.319, 0.079)	0.236	0.51 (1.00)	0.355 (0.132, 0.578)	0.002
	36 weeks	0.09 (1.06)	Ref	0.15 (1.01)	0.064 (-0.102, 0.229)	0.450	0.20 (1.07)	0.072 (-0.129, 0.272)	0.484	0.33 (1.22)	0.271 (0.019, 0.522)	0.004
EFW	0.776^											
	28 weeks	0.12 (0.87)	Ref	0.29 (0.93)	0.182 (0.036, 0.327)	0.014	0.20 (0.90)	0.107 (-0.063, 0.278)	0.216	0.54 (1.07)	0.460 (0.233, 0.686)	< 0.001
	36 weeks	0.17 (0.96)	Ref	0.35 (1.01)	0.165 (0.008, 0.322)	0.04	0.33 (0.87)	0.169 (-0.001, 0.339)	0.051	0.55 (1.11)	0.416 (0.194, 0.638)	< 0.001

Notes: This table shows the results of analysis of the effect maternal BMI category and fetal biometry z-scores measured at 28 and 36 weeks gestation. For each BMI category, the mean and standard deviation is presented for each time point. Estimates are differences in means (95% CI) for each BMI category compared to the lowest BMI category (25.0-29.9). All models were adjusted for centre, parity, SEIFA IRSD quintile, smoking status and age at consent.
^ Interaction p values for BMI category by time point interaction. Ref = Reference category

Table 3.3 – Maternal BMI category and fetal adiposity measurements at 28 and 36 weeks

		Body Mass Index Category										
		Overweight 25-29.9 kg/m^2		Class 1 Obesity 30-34.9 kg/m^2			Class 2 Obesity 35-39.9 kg/m^2			Class 3 Obesity ≥ 40.0 kg/m^2		
	Gestation	Mean (SD)	Adjusted Estimate (95% CI)	Mean (SD)	Adjusted Estimate (95% CI)	P value	Mean (SD)	Adjusted Estimate (95% CI)	P value	Mean (SD)	Adjusted Estimate (95% CI)	P value
Mid thigh fat mass (cm^2) (MTFM)	0.636^											
	28 weeks	4.50 (1.27)	Ref	4.56 (1.20)	0.009 (-0.231, 0.250)	0.94	4.48 (1.11)	-0.051 (-0.343, 0.242)	0.734	4.76 (1.43)	0.297 (-0.123, 0.718)	0.166
	36 weeks	11.17 (2.88)	Ref	11.30 (2.88)	0.08 (-0.519, 0.678)	0.794	10.99 (2.57)	-0.166 (-0.872, 0.539)	0.644	12.16 (3.25)	0.967 (-0.148, 2.081)	0.089
Abdominal area (cm^2) (AA)	0.293^											
	28 weeks	47.56 (6.70)	Ref	47.77 (5.93)	0.269 (-0.759, 1.296)	0.608	48.64 (7.12)	1.236 (-0.134, 2.605)	0.077	49.72 (8.82)	2.193 (0.309, 4.076)	0.023
	36 weeks	84.19 (10.40)	Ref	85.78 (10.47)	1.795 (0.13, 3.46)	0.035	85.91 (9.95)	1.773 (-0.190, 3.735)	0.077	87.12 (11.34)	3.415 (1.091, 5.739)	0.004
	0.724^											

Abdominal fat mass (mm) (AFM)	28 weeks	3.46 (1.04)	Ref	3.56 (1.06)	0.060 (-0.135, 0.255)	0.544	3.49 (0.89)	0.028 (-0.201, 0.256)	0.813	3.80 (1.18)	0.351 (0.00, 0.702)	0.05
	36 weeks	5.60 (1.58)	Ref	5.82 (1.56)	0.186 (-0.122, 0.494)	0.237	5.64 (1.56)	-0.011 (-0.388, 0.367)	0.956	6.20 (0.567)	0.606 (0.078, 1.134)	0.025
Subscapular fat mass (mm) (SSFM)	0.226^											
	28 weeks	3.25 (0.94)	Ref	3.05 (0.80)	-0.201 (-0.365,-0.037)	0.016	3.19 (0.88)	-0.079 (-0.295, 0.138)	0.477	3.29 (0.93)	0.060 (-0.219, 0.338)	0.675
	36 weeks	5.31 (1.38)	Ref	5.48 (1.49)	0.136 (-0.161, 0.433)	0.369	5.35 (1.29)	0.046 (-0.290, 0.362)	0.828	5.52 (1.53)	0.235 (-0.252, 0.723)	0.344

Notes: This table shows the results of analyses of the effect of maternal BMI category on fetal adiposity measurements. Each biometry measurement is described across the three BMI categories for both time points. For each BMI category, the mean and standard deviation is presented for each time point. Estimates are differences in means (95% CI) for each BMI category compared to the lowest BMI category (25.0-29.9). All models were adjusted for centre, parity, SEIFA IRSD quintile, smoking status and age at consent.

^ Interaction p values for BMI category by time point interaction. Ref = Reference category

Table 3.4 – Maternal BMI category and fetal growth velocity between 28 and 36 weeks

		Body Mass Index Category										
		Overweight 25-29.9 kg/m^2		Class 1 Obesity 30-34.9 kg/m^2			Class 2 Obesity 35-39.9 kg/m^2			Class 3 Obesity ≥ 40.0 kg/m^2		
Velocity	**Interaction P value**	**Mean (SD)**	**Adjusted Estimate (95% CI)**	**Mean (SD)**	**Adjusted Estimate (95% CI)**	**P value**	**Mean (SD)**	**Adjusted Estimate (95% CI)**	**P value**	**Mean (SD)**	**Adjusted Estimate (95% CI)**	**P value**
Estimated fetal weight (EFW)	0.005	29.46 (4.99)	Ref	29.61 (5.39)	0.153 (-0.705, 1.011)	0.727	30.37 (4.55)	0.765 (-0.291, 1.820)	0.156	31.21 (5.62)	2.028 (0.861, 3.196)	< 0.001
Mid thigh fat mass (MTFM)	0.292	0.12 (0.05)	Ref	0.12 (0.05)	-0.002 (-0.13, 0.010)	0.765	0.12 (0.05)	0.009 (-0.006, 0.024)	0.238	0.13 (0.05)	0.017 (-0.007, 0.042)	0.163
Abdominal area (cm^2) (AA)	0.145	0.68 (0.13)	Ref	0.68 (0.13)	0.009 (-0.014, 0.032)	0.425	0.70 (0.13)	0.019 (-0.009, 0.048)	0.175	0.70 (0.16)	0.035 (0.004, 0.066)	0.029
Abdominal fat mass (AFM)	0.762	0.04 (0.03)	Ref	0.04 (0.03)	0.003 (-0.003, 0.009)	0.317	0.04 (0.03)	0.002 (-0.006, 0.010)	0.580	0.04 (0.03)	0.00 (-0.013, 0.012)	0.948
Subscapular fat mass (SSFM)	0.238	0.04 (0.03)	Ref	0.04 (0.03)	0.005 (-0.001, 0.011)	0.106	0.04 (0.03)	0.006 (-0.002, 0.014)	0.12	0.04 (0.02)	-0.001 (-0.012, 0.011)	0.876

AA velocity Z-score	0.165	0.51 (0.93)	Ref	0.54 (0.91)	0.0045 (-0.112, 0.203)	0.574	0.62 (0.85)	0.112 (-0.082, 0.306)	0.259	0.67 (1.06)	0.238 (0.022, 0.453)	0.03
EFW velocity Z-score	0.003	0.60 (1.06)	Ref	0.62 (1.12)	0.016 (-0.165, 0.196)	0.865	0.78 (0.94)	0.152 (-0.07, 0.375)	0.179	0.99 (1.19)	0.441 (0.196, 0.687)	< 0.001

Notes: This table summarises the results of analysis of the effect of maternal BMI categories on growth velocity for EFW, MTFM, AA, AFM, SSFM and EFW velocity z-scores. Each measurement is described across the three BMI categories for both time points. Values are mean (standard deviation) for maternal BMI categories, and estimates are differences in means (95% CI) for each BMI category compared to the lowest category (BMI 25.0-29.9) and were adjusted for centre, parity, SEIFA IRSD quintile, smoking status and age at consent. Ref = References

3.5 Discussion

This study describes fetal growth patterns and growth velocity over time in women who are overweight or obese and identifies an association between higher maternal BMI category and an increase in fetal biometry z-scores, abdominal related adiposity and growth velocity. A consistent and significant increase in all fetal biometry measurements was evident at both 28 and 36 weeks gestation from women with Class 3 obesity (BMI greater than or equal to 40kg/m^2). Fetal adiposity measurements were not universally increased but abdominal fat mass and abdominal area were associated with maternal obesity in this cohort. With increasing maternal BMI category, there were incremental increases in growth velocity of the fetal abdomen and estimated fetal weight. This study was a secondary analysis and while other associations were identified, these were inconsistent and likely due to chance.

Much of the literature pertaining to ultrasound measured fetal growth patterns relates to women with pre-existing diabetes (Kehl et al. 1996) and gestational diabetes (Bethune et al. 2003, Larciprete et al. 2003, Parretti et al. 2003, Kernaghan et al. 2007). Maternal diabetes has been shown to increase fetal abdominal circumference and abdominal fat mass through the stimulation of insulin sensitive tissues (Kehl et al. 1996).

In contrast, maternal obesity appears to be associated with an overall increase in fetal lean mass and skeletal growth. This study has confirmed that a significant increase in skeletal growth (head circumference and femur length) and abdominal area velocity and fat mass among women with Class 3 obesity compared with the lesser BMI

categories. This is confirmed by a recent study in women classified as obese and non-obese in pregnancy, which also found an increase in fetal skeletal growth, with significant increases in head circumference, humeral and femur lengths in fetuses of obese women (Zhang et al. 2018). The difference in EFW comparing women who were non-obese to obese was apparent from 32 weeks (Zhang et al. 2018).

Women with Class 3 obesity (BMI greater than or equal to 40kg/m^2) are likely to represent a metabolically different group. From epidemiological studies, higher maternal BMI category is associated with a further increase in the rate of adverse perinatal outcomes including macrosomia (Cedergren 2004, Dodd et al. 2011, Magann et al. 2013, Gaudet et al. 2014) and there is emerging evidence of an association with childhood obesity (Whitaker 2004, Yu et al. 2013, World Health Organization 2016). The stimulation of fetal growth through the complex pathway including insulin growth factors (via hyperglycaemia and hyperinsulinaemia) (Ferraro et al. 2012), hyperlipidaemia (Schaefer-Graf et al. 2008), leptin (Josefson et al. 2014), adiponectin (Catalano et al. 2006), and inflammatory mediators (Friis et al. 2013) is likely to be accentuated in women of higher BMI. Thus, targeted interventions for women with the highest BMI may be more beneficial in reducing the fetal effects of obesity when compared with women of lower BMI.

This is the first study to report on velocity of fetal growth and adiposity in the setting of maternal overweight and obesity. The literature to date has used measurement of growth velocity as a tool for screening and identification of the small for gestational age infant (Sovio et al. 2016) or for screening for macrosomia associated with pre-

existing or gestational diabetes (Landon et al. 1989, Kehl et al. 1996, Hiersch et al. 2018). The velocity of fetal growth changes throughout gestation (Bertino et al. 1996, Milani et al. 2005). Of interest, growth in the abdominal circumference peaks at 12.5mm per week at 24 weeks gestation, reducing to 8mm per week by 40 weeks gestation (Bertino et al. 1996). The current study demonstrates an incremental increase in the rate of growth in the 3rd trimester associated with maternal obesity. Further understanding of the timing and regulation of the fetal growth velocity in the setting of maternal obesity is critical to developing successful interventions to improve perinatal outcomes.

The main limitation of this secondary analysis is the lack of a comparator group, defined as women entering pregnancy with a normal BMI. There was also missing data in the velocity comparisons due to the availability of the 2 scans to calculate the growth velocity over time, affecting 15% of women within the cohort. Thirdly, the study incorporated only two time points to assess velocity in the 3rd trimester. Other descriptive studies for growth velocity have used multiple time points from 12 to 40 weeks gestation in order to describe the variation in velocity throughout the entire pregnancy (Bertino et al. 1996, Milani et al. 2005).

There is a need for further studies into the mechanisms and timing of critical fetal growth changes. This would help guide and assist with the timing of potential interventions that may modulate fetal growth in utero. From a public health perspective, if preventive strategies could modify fetal growth, velocity and adiposity patterns in

utero, this may alter the transmission of obesity and its cardiometabolic complications to the next generation (Hanson et al. 2016, Godfery et al. 2017).

3.6 Conclusion

This study indicates that maternal Class 3 obesity is associated with an increase in

- All fetal biometry z-scores at both 28 and 36 weeks gestation and
- Fetal abdominal fat mass and abdominal area.

An increase in maternal BMI category is associated with

- Incremental increases in growth velocity of the fetal abdomen and estimated fetal weight.

CHAPTER 4: The effect of maternal dietary factors on fetal growth and adiposity

This chapter forms the basis of a published manuscript (O'Brien CM et al, *Nutrients* 2018), which is contained in Appendix 3.

4.1 Introduction

Maternal dietary intake is recognised as a factor contributing to fetal growth (Starling et al. 2017). In women who are overweight or obese during pregnancy, poorer diet quality has been identified when compared with women with BMI in the normal range (Laraia et al. 2007, Rifas-Shiman et al. 2009, Tsigga et al. 2011, Moran et al. 2013) and this in turn is associated with an increased risk of glucose intolerance and pre-eclampsia (Rifas-Shiman et al. 2009), increased neonatal adiposity (Shapiro et al. 2016) and changes in child body composition (Catalano et al. 2017). It has been suggested that among women with normal BMI, maternal protein, fatty acid and carbohydrate intake during pregnancy are associated with increased measures of fetal adiposity (Blumfield et al. 2012). The contribution of specific maternal dietary components to fetal growth and adiposity among women who are overweight or obese is uncertain, and warrants further investigation.

4.2 Aims

The aim of this study was to evaluate the associations between maternal dietary factors and fetal growth and adiposity measured by ultrasound at 28 and 36 weeks gestation in overweight and obese women.

4.3 Methods

The research methodology (Dodd et al. 2011, Dodd et al. 2014a, Dodd et al. 2014c) of the LIMIT randomised controlled trial have been outlined in chapter 2, as has the methodology relating to ultrasound assessment of fetal biometry and adiposity measures.

4.3.1 Maternal Dietary Assessment

Women completed the Harvard Semi-quantitative Food Frequency (Willett) questionnaire, (Willett 1987) to obtain a measure of their daily dietary intake of nutrients from 126 food items, including portion size and incorporation within the main 7 food groups, which has been validated in pregnancy (Fawzi et al. 2004) and amongst Australian pregnant women (Rumbold et al. 2006). The questionnaire was completed at the time of study entry, 28 and 36 weeks gestation. At study entry women were asked, on average, how often the food was consumed during the last 12 months, while assessment at 28 and 36 weeks gestation asked women to indicate, on average, how often the food was consumed since the previous questionnaire time point.

Daily nutrient intake was estimated using the nutrient compositions from the Australian food composition tables according to pre-specified portion size (FSANZ, 2013). Adherence to dietary recommendations was assessed by allocating all food and drink consumption into the food groups as described by the Australian Guide to Healthy Eating (NHMRC 2013). Foods were classified as 'non-core foods' if the food did not meet the criteria of the five core food groups, provided minimal nutrient content, and was high in fat, sugar or salt (Athukorala et al. 2010, NHMRC 2013).

Micronutrient values were obtained from the Harvard Semi-quantitative Food Frequency (Willett) questionnaire (Willett 1987) and analysed as mean intake, utilising the Food Works Nutrient Analysis Software Package (FoodWorks, version 7, Professional; Xyris Software 2012; Australia), and using Australian Food composition tables.

Diet quality was assessed using the Healthy Eating Index (HEI), which has 12 components to yield a maximum score of 100 (Guenther et al. 2008). These 12 components include total fruit, total vegetables, dark green and orange vegetables and legumes, total grains and whole grains, all of which receive a score out of 5. Milk, meat and beans, oils, saturated fat and sodium based foods were scored out of 10. Calories from solid fats, alcohol related beverages and added sugars were scored out of 20. A HEI score of 80 is considered good, a score between 50 and 80 is one that needs improvement, and scores of less than 50 are considered poor. The HEI has been validated for use in pregnant women (Pick et al. 2005).

Dietary glycaemic index (GI) values were obtained using data taken from the Harvard Semi-quantitative Food Frequency (Willett) questionnaire (Willett 1987) and analysed using the Food Works Nutrient Analysis Software Package (FoodWorks, version 7, Professional; Xyris Software 2012; Australia), and along with published dietary glycaemic index values.

4.3.2 Statistical Analysis

Linear regression was used to model the association between dietary factors and fetal growth and adiposity, with diet variables considered as 'predictors' (independent variables) and fetal growth and adiposity variables as 'outcomes' (dependent variables). A time-by-diet-variable interaction term was included to allow for estimation of the association at each time point separately, and to test whether the association differed between time points. Generalised Estimating Equations were used to account for repeated measures. Both unadjusted and adjusted analyses were performed. Adjusted analyses included maternal BMI category (25.0-29.9kg/m^2 vs $\geq$ 30.0kg/m^2), smoking, parity (0 versus $\geq$ 1), age and SEIFA of Relative Socio-Economic Disadvantage (IRSD) quintile, which is a rank of areas within Australia according to relative socio-economic disadvantage. All analyses were additionally adjusted for baseline diet variables, as a potential confounder.

4.4 Results

4.4.1 Demographic characteristics

Flow of participants and baseline characteristics are presented in Figure 4.1 and Table 4.1 respectively. There were 721 women included in this secondary analysis. The mean age of women participating was 29.9 years (standard deviation 5.3), with median

gestation at study entry 14.3 weeks (interquartile range from 12.1 to 17.0 weeks). Most women (91%; n=659) were of Caucasian ethnicity, 41.3% (n=298) in their first ongoing pregnancy, and 52% (n=373) from the highest two quintiles of social disadvantage. The baseline characteristics of the women contributing dietary and ultrasound data were comparable to all women in the Standard Care group, and all women included in the LIMIT randomised trial (Dodd et al. 2014a).

Figure 4.1: Flow chart of the participants included in the secondary analysis with maternal dietary assessment

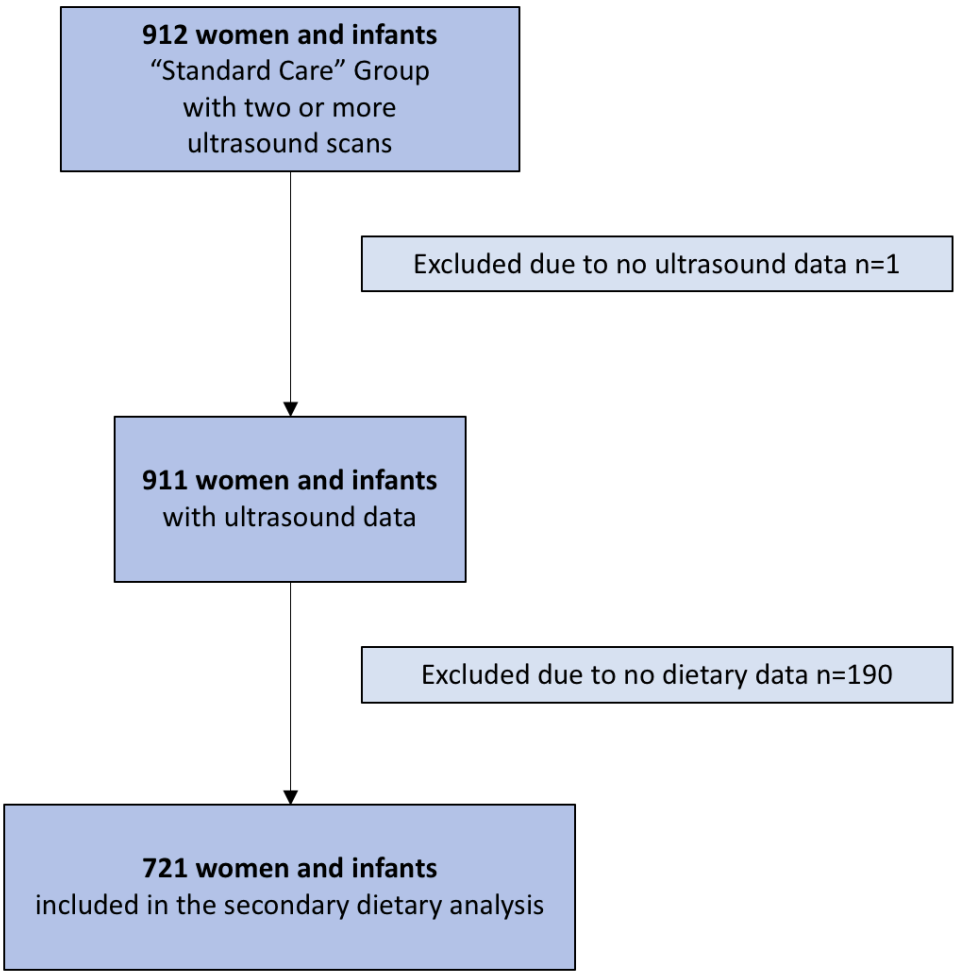


Table 4.1 – Baseline characteristics of participants included in the analysis of the effect of maternal diet on fetal growth and adiposity

Baseline Characteristic	Result
Total Number: N (%)	721
Maternal Age at Trial Entry Mean (SD)	29.88 (5.33)
Gestational age at Trial Entry (weeks) Median (IQR)	14.29 (12.14, 17.00)
BMI (kg/m^2) Median (IQR)	31.00 (27.70, 35.20)
BMI Category: 25.0-29.9 kg/m^2	310 (43.00)
30.0-34.9 kg/m^2	219 (30.37)
35.0-39.9 kg/m^2	116 (16.09)
≥ 40.0 kg/m^2	76 (10.54)
Caucasian: n (%)	659 (91.40)
Nulliparous: n (%)	298 (41.33)
Smoker: n (%)	67 (9.29)
SEIFA IRSD Quintile: n (%)	
Most disadvantaged Quintile 1	199 (27.60)
Quintile 2	174 (24.13)
Quintile 3	117 (16.23)
Quintile 4	116 (16.09)
Least disadvantaged Quintile 5	115 (15.95)

4.4.2 Healthy Eating Index (HEI)

There were no consistent associations between HEI and fetal biometry, MTFM, MTLM or AFM (Table 4.2). There was a negative association between HEI and SSFM at 28 weeks, whereby a 10-unit increase in HEI reduced SSFM by 0.17mm (95% CI -0.32 to -0.03; p=0.021).

4.4.3 Log Total Energy

Total Energy was log-transformed for analysis due to substantial right skew. There were no associations between log total energy and AC, EFW, all fetal biometry z-scores, MTFM, AFM or SSFM (Table 4.3). There was a negative association with log total energy and biometry measurements of BPD and HC at 36 weeks, such that a 10 unit increase in log total energy reduced BPD by 1.48 mm (95% CI: -2.55mm, -0.40mm; p=0.007) and HC by 4.07mm (95% CI: -7.6mm, -0.54mm; p=0.024).

At 28 and 36 weeks gestation, there were negative associations between log total energy and MTLM, such that a 10-unit increase in log total energy reduced MTLM by 4.94mm (95% CI: -9.57mm, -0.32mm; p =0.036) at 28 weeks; and by 7.02mm (95% CI: -13.69mm, -0.35mm; p=0.039) at 36 weeks.

4.4.4 Glycaemic Index

There were no associations between maternal Glycaemic Index and fetal biometry including HC, FL, AC and EFW, related z-scores and adiposity measures (Table 4.4). A negative association was identified between Glycaemic Index and fetal BPD and its z-score, such that a 10-unit increase in Glycaemic Index reduced BPD by 0.11 mm

(95% CI: -0.21mm, -0.01mm; p=0.035), and BPD z-score by 0.35 (95% CI: -0.69, -0.01; p=0.045) at 28 weeks.

4.4.5 Glycaemic Load

There were no consistent associations between dietary glycaemic load and fetal biometry, z-scores or adiposity measures at either 28 or 36 weeks (Table 4.5).

4.4.6 Fat, carbohydrate and protein as a percent of total energy

There were no associations identified between fat (Table 4.6), carbohydrate (Table 4.7) or protein (Table 4.8) as a percentage of total energy and fetal ultrasound measurements.

Table 4.2: Healthy Eating Index and fetal ultrasound measurements

Outcome	Unadjusted Estimate (95% CI)	Unadj p value	Adjusted Estimate (95% CI)	Adjusted p value
BPD		0.992†		0.968†
28 weeks	-0.03 (-0.08, 0.02)	0.239	-0.03 (-0.08, 0.02)	0.221
36 weeks	-0.03 (-0.08, 0.02)	0.235	-0.03 (-0.08, 0.02)	0.234
BPD z-score		0.728†		0.645†
28 weeks	-0.13 (-0.29, 0.04)	0.128	-0.13 (-0.30, 0.03)	0.117
36 weeks	-0.10 (-0.24, 0.04)	0.166	-0.09 (-0.24, 0.05)	0.194
HC		0.060†		0.064†
28 weeks	-0.06 (-0.22, 0.09)	0.425	-0.08 (-0.24, 0.08)	0.305
36 weeks	0.10 (-0.05, 0.09)	0.194	0.08 (-0.08, 0.24)	0.313
HC z-score		0.026†		0.025†
28 weeks	-0.06 (-0.17, 0.05)	0.317	-0.07 (-0.18, 0.04)	0.210
36 weeks	0.08 (-0.03, 0.05)	0.161	0.07 (-0.05, 0.18)	0.247
FL		0.168†		0.211†
28 weeks	-0.02 (-0.06, 0.02)	0.283	-0.02 (-0.06, 0.02)	0.232
36 weeks	0.01 (-0.03, 0.02)	0.646	0.00 (-0.03, 0.04)	0.855
FL z-score		0.097†		0.116†
28 weeks	-0.09 (-0.20, 0.03)	0.154	-0.09 (-0.21, 0.03)	0.131
36 weeks	0.02 (-0.09, 0.03)	0.678	0.01 (-0.10, 0.13)	0.817
AC		0.927†		0.976†
28 weeks	-0.07 (-0.28, 0.13)	0.484	-0.13 (-0.33, 0.07)	0.210
36 weeks	-0.06 (-0.32, 0.13)	0.637	-0.13 (-0.38, 0.13)	0.338
AC z-score		0.712†		0.691†
28 weeks	-0.03 (-0.14, 0.09)	0.660	-0.06 (-0.18, 0.05)	0.264
36 weeks	-0.05 (-0.18, 0.09)	0.471	-0.09 (-0.22, 0.04)	0.193
EFW		0.512†		0.562†
28 weeks	-22.42 (-52.33, 7.48)	0.142	-30.24 (-60.55, 0.07)	0.051
36 weeks	-6.80 (-58.91, 7.48)	0.798	-16.24 (-68.74, 36.27)	0.544

EFW z-score		0.344†		0.366†
28 weeks	-0.08 (-0.18, 0.03)	0.170	-0.10 (-0.21, 0.01)	0.063
36 weeks	-0.02 (-0.14, 0.03)	0.723	-0.05 (-0.17, 0.07)	0.415
MTLM		0.742†		0.891†
28 weeks	-0.07 (-0.25, 0.10)	0.417	-0.08 (-0.26, 0.09)	0.361
36 weeks	-0.12 (-0.36, 0.10)	0.341	-0.10 (-0.35, 0.15)	0.425
MTFM		0.239†		0.263†
28 weeks	-0.10 (-0.33, 0.12)	0.370	-0.09 (-0.32, 0.14)	0.444
36 weeks	-0.39 (-0.90, 0.12)	0.141	-0.37 (-0.90, 0.17)	0.177
AFM		0.377†		0.431†
28 weeks	-0.07 (-0.23, 0.08)	0.357	-0.12 (-0.29, 0.04)	0.141
36 weeks	-0.17 (-0.41, 0.08)	0.152	-0.21 (-0.44, 0.02)	0.075
SSFM		0.824†		0.930†
28 weeks	-0.14 (-0.28, 0.00)	0.053	-0.17 (-0.32, -0.03)	0.021
36 weeks	-0.17 (-0.39, 0.00)	0.141	-0.18 (-0.41, 0.04)	0.115

Notes: † denotes p value for test of interaction between HEI and time. Estimates are difference in mean fetal measure (95% CI) corresponding to a 10 unit increase in HEI score. Unadj = Unadjusted.

Table 4.3: Log Total Energy and fetal ultrasound measurements

Outcome	Unadjusted Estimate (95% CI)	Unadj p value	Adjusted Estimate (95% CI)	Adj p value
BPD		0.116†		0.099†
28 weeks	-0.31 (-1.48, 0.86)	0.603	-0.36 (-1.55, 0.82)	0.547
36 weeks	-1.36 (-2.43, 0.86)	0.012	-1.48 (-2.55, -0.40)	0.007
BPD z-score		0.417†		0.477†
28 weeks	-0.34 (-4.39, 3.71)	0.869	-0.59 (-4.71, 3.54)	0.780
36 weeks	-2.14 (-5.59, 3.71)	0.225	-2.18 (-5.70, 1.35)	0.226
HC		0.260†		0.169†
28 weeks	-0.90 (-5.01, 3.22)	0.669	-0.72 (-4.84, 3.40)	0.732
36 weeks	-3.64 (-7.17, 3.22)	0.043	-4.07 (-7.60, -0.54)	0.024
HC z-score		0.390†		0.347†
28 weeks	0.52 (-2.27, 3.31)	0.716	0.67 (-2.18, 3.52)	0.647
36 weeks	-0.83 (-3.33, 3.31)	0.519	-0.83 (-3.36, 1.71)	0.524
FL		0.657†		0.570†
28 weeks	-0.20 (-1.17, 0.76)	0.680	-0.22 (-1.20, 0.75)	0.653
36 weeks	-0.47 (-1.40, 0.76)	0.327	-0.56 (-1.52, 0.39)	0.248
FL z-score		0.785†		0.762†
28 weeks	0.02 (-2.92, 2.97)	0.988	-0.06 (-3.07, 2.96)	0.970
36 weeks	0.51 (-2.59, 2.97)	0.746	0.49 (-2.67, 3.65)	0.762
AC		0.246†		0.181†
28 weeks	-0.21 (-5.45, 5.04)	0.938	0.81 (-4.23, 5.85)	0.753
36 weeks	-3.83 (-9.40, 5.04)	0.178	-3.34 (-8.86, 2.19)	0.236
AC z-score		0.860†		0.815†
28 weeks	0.44 (-2.27, 3.16)	0.748	1.14 (-1.51, 3.78)	0.399
36 weeks	0.18 (-2.73, 3.16)	0.905	0.78 (-2.16, 3.72)	0.603
EFW		0.082†		0.059†
28 weeks	130.32 (-598.56, 859.21)	0.726	204.16 (-512.19, 920.51)	0.576
36 weeks	-887.76 (-2026.25,859.21)	0.126	-901.31 (-2028.21, 225.59)	0.117

EFW z-score		0.305†		0.300†
28 weeks	1.14 (-1.32, 3.59)	0.364	1.47 (-0.96, 3.91)	0.236
36 weeks	-0.29 (-2.85, 3.59)	0.825	0.01 (-2.58, 2.61)	0.991
MTLM		0.495†		0.574†
28 weeks	-4.56 (-9.20, 0.08)	0.054	-4.94 (-9.57, -0.32)	0.036
36 weeks	-7.07 (-13.69, 0.08)	0.037	-7.02 (-13.69, -0.35)	0.039
MTFM		0.812†		0.795†
28 weeks	-0.90 (-6.35, 4.55)	0.746	-1.76 (-7.35, 3.83)	0.538
36 weeks	0.46 (-10.91, 4.55)	0.937	-0.25 (-11.82, 11.31)	0.966
AFM		0.563†		0.603†
28 weeks	-1.00 (-5.03, 3.03)	0.627	-0.59 (-4.65, 3.48)	0.777
36 weeks	0.88 (-5.59, 3.03)	0.791	1.10 (-5.27, 7.47)	0.734
SSFM		0.779†		0.760†
28 weeks	2.72 (-0.73, 6.17)	0.122	3.23 (-0.22, 6.69)	0.067
36 weeks	1.88 (-3.72, 6.17)	0.511	2.32 (-3.26, 7.90)	0.416

Notes: † denotes p value for test of interaction between HEI and time. Estimates are difference in mean fetal measure (95% CI) corresponding to a 10 unit increase in HEI score. Unadj = Unadjusted. Adj = Adjusted.

Table 4.4: Glycaemic Index and fetal ultrasound measurements

Outcome	Unadjusted Estimate (95% CI)	Unadj p value	Adjusted Estimate (95% CI)	Adjusted p value
BPD		0.079†		0.060†
28 weeks	-0.12 (-0.21, -0.02)	0.021	-0.11 (-0.21, -0.01)	0.035
36 weeks	-0.01 (-0.11, -0.02)	0.876	0.01 (-0.09, 0.11)	0.885
BPD z-score		0.075†		0.083†
28 weeks	-0.36 (-0.70, -0.02)	0.037	-0.35 (-0.69, -0.01)	0.045
36 weeks	-0.03 (-0.31, -0.02)	0.812	-0.03 (-0.31, 0.25)	0.833
HC		0.601†		0.620†
28 weeks	-0.19 (-0.54, 0.16)	0.288	-0.14 (-0.50, 0.21)	0.422
36 weeks	-0.08 (-0.42, 0.16)	0.642	-0.04 (-0.38, 0.30)	0.816
HC z-score		0.652†		0.540†
28 weeks	0.02 (-0.22, 0.26)	0.880	0.04 (-0.20, 0.29)	0.724
36 weeks	-0.04 (-0.27, 0.26)	0.709	-0.04 (-0.26, 0.18)	0.723
FL		0.729†		0.634†
28 weeks	-0.05 (-0.13, 0.03)	0.250	-0.05 (-0.13, 0.03)	0.236
36 weeks	-0.03 (-0.12, 0.03)	0.521	-0.02 (-0.11, 0.07)	0.595
FL z-score		0.904†		0.931†
28 weeks	-0.03 (-0.29, 0.23)	0.820	-0.04 (-0.30, 0.23)	0.790
36 weeks	-0.01 (-0.31, 0.23)	0.949	-0.02 (-0.32, 0.27)	0.891
AC		0.185†		0.158†
28 weeks	-0.24 (-0.66, 0.17)	0.248	-0.23 (-0.63, 0.17)	0.257
36 weeks	0.10 (-0.34, 0.17)	0.649	0.13 (-0.30, 0.57)	0.556
AC z-score		0.151†		0.182†
28 weeks	-0.09 (-0.31, 0.13)	0.422	-0.09 (-0.31, 0.12)	0.383
36 weeks	0.09 (-0.12, 0.13)	0.396	0.07 (-0.14, 0.28)	0.491
EFW		0.583†		0.551†
28 weeks	-18.94 (-79.38, 41.50)	0.539	-17.21 (-77.32, 42.90)	0.575
36 weeks	8.76 (-89.14, 41.50)	0.861	12.58 (-84.02, 109.19)	0.799

EFW z-score		0.212†		0.247†
28 weeks	-0.11 (-0.33, 0.10)	0.314	-0.11 (-0.32, 0.10)	0.316
36 weeks	0.03 (-0.17, 0.10)	0.749	0.02 (-0.18, 0.22)	0.813
MTLM		0.706†		0.686†
28 weeks	0.11 (-0.25, 0.46)	0.548	0.13 (-0.22, 0.49)	0.462
36 weeks	-0.02 (-0.63, 0.46)	0.950	-0.00 (-0.61, 0.61)	0.993
MTFM		0.015†		0.025†
28 weeks	-0.36 (-0.80, 0.07)	0.104	-0.34 (-0.77, 0.10)	0.133
36 weeks	0.79 (-0.12, 0.07)	0.089	0.74 (-0.18, 1.65)	0.116
AFM		0.115†		0.150†
28 weeks	-0.11 (-0.41, 0.19)	0.475	-0.13 (-0.44, 0.18)	0.415
36 weeks	0.34 (-0.19, 0.19)	0.211	0.28 (-0.24, 0.81)	0.291
SSFM		0.215†		0.176†
28 weeks	-0.06 (-0.35, 0.22)	0.661	-0.07 (-0.35, 0.22)	0.639
36 weeks	0.25 (-0.21, 0.22)	0.287	0.28 (-0.19, 0.75)	0.248

Notes: † denotes p value for test of interaction between Glycaemic index and time. Estimates are difference in mean fetal measure (95% CI) corresponding to a 10 unit increase in Glycaemic index score. Unadj = Unadjusted.

Table 4.5: Glycaemic load and fetal ultrasound measurements

Outcome	Unadjusted Estimate (95% CI)	Unadj p value	Adjusted Estimate (95% CI)	Adjusted p value
BPD		0.567†		0.490†
28 weeks	-0.00 (-0.01, 0.00)	0.251	-0.00 (-0.01, 0.00)	0.276
36 weeks	-0.01 (-0.01, 0.00)	0.063	-0.01 (-0.01, 0.00)	0.054
BPD z-score		0.821†		0.831†
28 weeks	-0.01 (-0.04, 0.01)	0.227	-0.01 (-0.04, 0.01)	0.227
36 weeks	-0.01 (-0.03, 0.01)	0.295	-0.01 (-0.03, 0.01)	0.291
HC		0.562†		0.374†
28 weeks	-0.01 (-0.03, 0.02)	0.530	-0.01 (-0.03, 0.02)	0.683
36 weeks	-0.02 (-0.04, 0.02)	0.137	-0.02 (-0.04, 0.00)	0.102
HC z-score		0.606†		0.479†
28 weeks	0.00 (-0.02, 0.02)	0.964	0.00 (-0.02, 0.02)	0.808
36 weeks	-0.01 (-0.02, 0.02)	0.557	-0.01 (-0.02, 0.01)	0.539
FL		0.827†		0.737†
28 weeks	-0.00 (-0.01, 0.01)	0.698	-0.00 (-0.01, 0.01)	0.762
36 weeks	-0.00 (-0.01, 0.01)	0.471	-0.00 (-0.01, 0.00)	0.437
FL z-score		0.676†		0.674†
28 weeks	-0.00 (-0.02, 0.02)	0.923	-0.00 (-0.02, 0.02)	0.965
36 weeks	0.00 (-0.02, 0.02)	0.688	0.00 (-0.01, 0.02)	0.653
AC		0.492†		0.391†
28 weeks	-0.00 (-0.04, 0.03)	0.837	0.00 (-0.03, 0.04)	0.814
36 weeks	-0.02 (-0.05, 0.03)	0.340	-0.01 (-0.05, 0.02)	0.465
AC z-score		0.861†		0.969†
28 weeks	0.00 (-0.02, 0.02)	0.973	0.00 (-0.01, 0.02)	0.548
36 weeks	0.00 (-0.02, 0.02)	0.835	0.01 (-0.01, 0.02)	0.604
EFW		0.181†		0.145†
28 weeks	0.93 (-3.72, 5.59)	0.694	1.66 (-2.95, 6.27)	0.481
36 weeks	-4.15 (-11.63, 5.59)	0.276	-3.95 (-11.48, 3.58)	0.304

EFW z-score		0.636†		0.567†
28 weeks	0.00 (-0.01, 0.02)	0.717	0.01 (-0.01, 0.02)	0.459
36 weeks	-0.00 (-0.02, 0.02)	0.857	0.00 (-0.02, 0.02)	0.980
MTLM		0.406†		0.462†
28 weeks	-0.02 (-0.05, 0.00)	0.098	-0.02 (-0.05, 0.00)	0.093
36 weeks	-0.04 (-0.08, 0.00)	0.052	-0.04 (-0.08, 0.00)	0.064
MTFM		0.252†		0.264†
28 weeks	-0.02 (-0.05, 0.01)	0.262	-0.02 (-0.05, 0.01)	0.215
36 weeks	0.02 (-0.05, 0.01)	0.522	0.02 (-0.05, 0.10)	0.578
AFM		0.278†		0.326†
28 weeks	0.00 (-0.02, 0.03)	0.891	0.00 (-0.02, 0.03)	0.721
36 weeks	0.02 (-0.02, 0.03)	0.244	0.03 (-0.02, 0.07)	0.223
SSFM		0.737†		0.757†
28 weeks	0.01 (-0.01, 0.04)	0.185	0.02 (-0.00, 0.04)	0.106
36 weeks	0.02 (-0.01, 0.04)	0.239	0.02 (-0.01, 0.06)	0.189

Notes: † denotes p value for test of interaction between Glycaemic load and time. Estimates are difference in mean fetal measure (95% CI) corresponding to a 10 unit increase in Glycaemic load score. Unadj = Unadjusted

Table 4.6: Fat as a percentage of total energy and fetal ultrasound measurements

Outcome	Unadjusted Estimate (95% CI)	Unadj p value	Adjusted Estimate (95% CI)	Adjusted p value
BPD		0.593†		0.646†
28 weeks	0.04 (-0.05, 0.12)	0.396	0.04 (-0.05, 0.12)	0.418
36 weeks	0.01 (-0.08, 0.12)	0.841	0.01 (-0.08, 0.10)	0.793
BPD z-score		0.387†		0.507†
28 weeks	0.21 (-0.08, 0.50)	0.152	0.17 (-0.12, 0.46)	0.238
36 weeks	0.08 (-0.16, 0.50)	0.524	0.07 (-0.17, 0.31)	0.560
HC		0.083†		0.123†
28 weeks	0.16 (-0.12, 0.44)	0.255	0.18 (-0.11, 0.46)	0.228
36 weeks	-0.13 (-0.39, 0.44)	0.318	-0.09 (-0.35, 0.17)	0.499
HC z-score		0.016†		0.033†
28 weeks	0.22 (0.01, 0.43)	0.036	0.21 (-0.00, 0.43)	0.053
36 weeks	-0.05 (-0.23, 0.43)	0.570	-0.03 (-0.21, 0.14)	0.714
FL		0.413†		0.560†
28 weeks	0.00 (-0.07, 0.07)	0.896	0.00 (-0.07, 0.07)	0.950
36 weeks	-0.03 (-0.10, 0.07)	0.381	-0.02 (-0.09, 0.04)	0.514
FL z-score		0.414†		0.577†
28 weeks	0.06 (-0.15, 0.28)	0.563	0.03 (-0.19, 0.24)	0.808
36 weeks	-0.04 (-0.26, 0.28)	0.683	-0.05 (-0.26, 0.17)	0.664
AC		0.556†		0.609†
28 weeks	-0.02 (-0.37, 0.33)	0.920	0.03 (-0.32, 0.39)	0.853
36 weeks	-0.15 (-0.53, 0.33)	0.428	-0.08 (-0.46, 0.29)	0.660
AC z-score		0.968†		0.806†
28 weeks	-0.02 (-0.21, 0.16)	0.799	-0.01 (-0.20, 0.18)	0.922
36 weeks	-0.02 (-0.21, 0.16)	0.833	0.02 (-0.17, 0.20)	0.851
EFW		0.253†		0.307†
28 weeks	14.86 (-35.95, 65.68)	0.566	18.79 (-33.86, 71.44)	0.484
36 weeks	-34.32 (-113.89, 65.68)	0.398	-25.56 (-104.88, 53.76)	0.528

EFW z-score		0.308†		0.477†
28 weeks	0.05 (-0.12, 0.22)	0.567	0.05 (-0.13, 0.22)	0.610
36 weeks	-0.05 (-0.21, 0.22)	0.551	-0.02 (-0.19, 0.14)	0.771
MTLM		0.446†		0.372†
28 weeks	0.07 (-0.26, 0.40)	0.669	0.09 (-0.25, 0.44)	0.602
36 weeks	-0.15 (-0.67, 0.40)	0.565	-0.18 (-0.70, 0.35)	0.511
MTFM		0.287†		0.284†
28 weeks	0.03 (-0.38, 0.44)	0.882	0.04 (-0.38, 0.47)	0.837
36 weeks	-0.40 (-1.12, 0.44)	0.281	-0.39 (-1.12, 0.33)	0.290
AFM		0.049†		0.060†
28 weeks	0.01 (-0.26, 0.29)	0.917	0.06 (-0.24, 0.36)	0.709
36 weeks	-0.46 (-0.90, 0.29)	0.041	-0.39 (-0.82, 0.04)	0.075
SSFM		0.368†		0.295†
28 weeks	0.15 (-0.08, 0.37)	0.200	0.17 (-0.06, 0.40)	0.144
36 weeks	-0.03 (-0.39, 0.37)	0.863	-0.04 (-0.40, 0.32)	0.829

Notes: † denotes p value for test of interaction between fat as a percentage of total energy and time. Estimates are difference in mean fetal measure (95% CI) corresponding to a 10 unit increase in fat as a percentage of total energy. Unadj = Unadjusted

Table 4.7: Carbohydrate as a percentage of total energy and fetal ultrasound

Outcome	Unadjusted Estimate (95% CI)	Unadj p value	Adjusted Estimate (95% CI)	Adjusted p value
BPD		0.339†		0.381†
28 weeks	-0.03 (-0.10, 0.05)	0.482	-0.02 (-0.09, 0.06)	0.634
36 weeks	0.02 (-0.05, 0.05)	0.653	0.02 (-0.05, 0.09)	0.554
BPD z-score		0.156†		0.241†
28 weeks	-0.16 (-0.38, 0.05)	0.143	-0.13 (-0.35, 0.09)	0.262
36 weeks	0.01 (-0.17, 0.05)	0.883	0.02 (-0.16, 0.21)	0.819
HC		0.199†		0.306†
28 weeks	-0.08 (-0.31, 0.15)	0.499	-0.05 (-0.28, 0.19)	0.685
36 weeks	0.10 (-0.11, 0.15)	0.354	0.09 (-0.11, 0.29)	0.374
HC z-score		0.099†		0.188†
28 weeks	-0.10 (-0.26, 0.05)	0.200	-0.08 (-0.24, 0.08)	0.331
36 weeks	0.05 (-0.09, 0.05)	0.503	0.04 (-0.10, 0.18)	0.562
FL		0.849†		0.816†
28 weeks	0.02 (-0.04, 0.08)	0.480	0.03 (-0.03, 0.08)	0.336
36 weeks	0.01 (-0.03, 0.08)	0.579	0.02 (-0.03, 0.07)	0.415
FL z-score		0.996†		0.943†
28 weeks	0.04 (-0.13, 0.20)	0.652	0.07 (-0.10, 0.23)	0.432
36 weeks	0.04 (-0.12, 0.20)	0.651	0.06 (-0.10, 0.22)	0.470
AC		0.913†		0.782†
28 weeks	0.05 (-0.26, 0.35)	0.771	0.10 (-0.21, 0.41)	0.532
36 weeks	0.03 (-0.25, 0.35)	0.853	0.05 (-0.23, 0.33)	0.729
AC z-score		0.751†		0.482†
28 weeks	0.03 (-0.12, 0.18)	0.732	0.06 (-0.09, 0.21)	0.420
36 weeks	-0.00 (-0.14, 0.18)	0.983	-0.00 (-0.14, 0.14)	0.994
EFW		0.962†		0.976†
28 weeks	7.29 (-36.60, 51.19)	0.745	16.48 (-28.72, 61.67)	0.475
36 weeks	8.87 (-50.10, 51.19)	0.768	15.46 (-43.22, 74.14)	0.606

EFW z-score		0.777†		0.953†
28 weeks	0.00 (-0.13, 0.14)	0.979	0.04 (-0.10, 0.17)	0.611
36 weeks	0.03 (-0.10, 0.14)	0.699	0.03 (-0.10, 0.16)	0.643
MTLM		0.867†		0.838†
28 weeks	-0.06 (-0.33, 0.20)	0.639	-0.04 (-0.31, 0.23)	0.783
36 weeks	-0.02 (-0.40, 0.20)	0.901	0.01 (-0.37, 0.39)	0.961
MTFM		0.406†		0.406†
28 weeks	-0.10 (-0.44, 0.24)	0.558	-0.07 (-0.41, 0.27)	0.683
36 weeks	0.17 (-0.41, 0.24)	0.563	0.20 (-0.38, 0.79)	0.495
AFM		0.118†		0.173†
28 weeks	0.04 (-0.18, 0.26)	0.732	0.06 (-0.17, 0.28)	0.614
36 weeks	0.32 (-0.00, 0.26)	0.051	0.30 (-0.02, 0.62)	0.062
SSFM		0.800†		0.836†
28 weeks	0.00 (-0.18, 0.18)	0.966	0.01 (-0.17, 0.19)	0.879
36 weeks	0.04 (-0.23, 0.18)	0.755	0.05 (-0.23, 0.32)	0.738

Notes: † denotes p value for test of interaction between carbohydrate as a percentage of total energy and time. Estimates are difference in mean fetal measure (95% CI) corresponding to a 10 unit increase in carbohydrate as a percentage of total energy. Unadj = Unadjusted

Table 4.8: Protein as a percentage of total energy and fetal ultrasound measures

Outcome	Unadjusted Estimate (95% CI)	Unadj p value	Adjusted Estimate (95% CI)	Adjusted p value
BPD		0.507†		0.546†
28 weeks	0.01 (-0.10, 0.11)	0.921	-0.01 (-0.11, 0.10)	0.914
36 weeks	-0.03 (-0.12, 0.11)	0.466	-0.04 (-0.13, 0.05)	0.361
BPD z-score		0.153†		0.210†
28 weeks	0.13 (-0.18, 0.44)	0.414	0.10 (-0.21, 0.42)	0.522
36 weeks	-0.10 (-0.35, 0.44)	0.400	-0.11 (-0.35, 0.14)	0.399
HC		0.991†		0.802†
28 weeks	-0.05 (-0.38, 0.27)	0.755	-0.12 (-0.45, 0.22)	0.489
36 weeks	-0.05 (-0.33, 0.27)	0.723	-0.07 (-0.34, 0.20)	0.618
HC z-score		0.983†		0.806†
28 weeks	-0.05 (-0.26, 0.16)	0.621	-0.08 (-0.30, 0.13)	0.440
36 weeks	-0.05 (-0.23, 0.16)	0.588	-0.05 (-0.24, 0.13)	0.567
FL		0.212†		0.269†
28 weeks	-0.06 (-0.14, 0.02)	0.143	-0.06 (-0.14, 0.01)	0.110
36 weeks	0.00 (-0.07, 0.02)	0.994	-0.01 (-0.08, 0.05)	0.692
FL z-score		0.499†		0.633†
28 weeks	-0.14 (-0.37, 0.09)	0.224	-0.14 (-0.36, 0.09)	0.235
36 weeks	-0.04 (-0.26, 0.09)	0.719	-0.07 (-0.29, 0.15)	0.547
AC		0.264†		0.187†
28 weeks	-0.16 (-0.60, 0.29)	0.490	-0.29 (-0.74, 0.16)	0.208
36 weeks	0.13 (-0.28, 0.29)	0.531	0.06 (-0.35, 0.47)	0.785
AC z-score		0.501†		0.281†
28 weeks	-0.06 (-0.27, 0.15)	0.584	-0.13 (-0.34, 0.08)	0.211
36 weeks	0.02 (-0.18, 0.15)	0.839	-0.00 (-0.21, 0.21)	0.992
EFW		0.175†		0.173†
28 weeks	-40.93 (-105.51, 23.64)	0.214	-56.34 (-123.01, 10.33)	0.098
36 weeks	22.54 (-62.18, 23.64)	0.602	7.75 (-76.06, 91.57)	0.856

EFW z-score		0.478†		0.351†
28 weeks	-0.09 (-0.28, 0.10)	0.350	-0.14 (-0.33, 0.05)	0.155
36 weeks	-0.01 (-0.20, 0.10)	0.915	-0.03 (-0.22, 0.16)	0.753
MTLM		0.433†		0.395†
28 weeks	0.09 (-0.26, 0.44)	0.617	0.05 (-0.31, 0.41)	0.800
36 weeks	0.33 (-0.18, 0.44)	0.201	0.31 (-0.19, 0.81)	0.227
MTFM		0.833†		0.823†
28 weeks	0.08 (-0.35, 0.51)	0.711	0.07 (-0.37, 0.51)	0.765
36 weeks	-0.02 (-0.91, 0.51)	0.968	-0.04 (-0.93, 0.85)	0.932
AFM		0.467†		0.661†
28 weeks	-0.10 (-0.41, 0.22)	0.548	-0.17 (-0.49, 0.14)	0.288
36 weeks	-0.26 (-0.68, 0.22)	0.216	-0.27 (-0.68, 0.14)	0.194
SSFM		0.872†		0.736†
28 weeks	-0.18 (-0.44, 0.09)	0.189	-0.21 (-0.48, 0.05)	0.119
36 weeks	-0.14 (-0.57, 0.09)	0.524	-0.13 (-0.55, 0.29)	0.550

Notes: † denotes p value for test of interaction between time and Protein as a percentage of total energy. Estimates are difference in mean fetal measure (95% CI) corresponding to a 10 unit increase in carbohydrate as a percentage of total energy. Unadj = Unadjusted

4.5 Discussion

The objective of this secondary exploratory analysis was to determine if maternal dietary factors were associated with fetal body composition in women entering pregnancy overweight or obese. This study identified that an increase in total energy of the maternal diet was associated with a reduction in mid-thigh lean mass of the fetus. Secondly, an increase in the Healthy Eating Index was associated with a reduction in the subscapular fat mass. While these individual associations were statistically significant, the actual differences were of small magnitude and were unlikely to be of clinical significance. Overall, no consistent associations between maternal diet and fetal growth or adiposity were identified.

This is the first study to describe the relationship between maternal dietary factors and fetal body composition in women entering pregnancy overweight and obese. There has been one study to describe the maternal dietary factors and fetal adiposity measurements in 179 women with a normal BMI (Blumfield et al. 2012). While this study utilised food frequency questionnaires, dietary variables were reported in a different manner, including a derived ratio comparing protein and carbohydrate, and poly-unsaturated fatty acids as a percentage of energy intake. The authors also described different ultrasound techniques and measurements of fetal adiposity (Blumfield et al. 2012). Women with lower dietary protein intake demonstrated higher abdominal wall adiposity, while fetal thigh adiposity was greatest among women whose diet consisted of low carbohydrate, intermediate protein and high fat intake (Blumfield et al. 2012).

The vast majority of the available literature describes associations between maternal dietary factors and neonatal and infant body composition (Moore et al. 2004, Chen et al. 2016, Brei et al. 2018), and birth weight (Renault et al. 2015, Crume et al. 2016, Sharma et al. 2018), with variable methodology and inconsistent findings (Chen et al. 2016, Chia et al. 2016). A possible explanation for the lack of association identified in the current study and inconsistent findings within the literature may relate to the timing of the dietary assessment in the early 2nd trimester (Moore et al. 2004, Hauner et al. 2009, Renault et al. 2015, Brei et al. 2018). Dietary assessment between 8 and 12 weeks identified that carbohydrate consumption was associated with increases in birth weight, whereas fat intake was associated with lower birth weight (Sharma et al. 2018).

Strengths of the current study include the large sample size of overweight or obese pregnant women, use of robust methodology (Dodd et al. 2014a), including the first to evaluate the effect of maternal dietary factors on fetal biometry and adiposity. A limitation is the reliance on self-reported measurement of maternal dietary intake. Dietary analysis is subject to multiple biases including measurement error, recall bias related to the food questionnaire, along with reporting bias. Additionally, a comparator group of women entering pregnancy with a normal BMI would have enabled comparison of the effects of maternal dietary intake on fetal growth patterns across the BMI spectrum.

Several randomised trials have identified improvements in maternal dietary patterns during pregnancy following provision of a lifestyle intervention (Luoto et al. 2011, Renault et al. 2014, Dodd et al. 2014a, Dodd et al. 2014c, Poston et al. 2015, Geraghty et al. 2016). The LIMIT trial demonstrated that the provision of the antenatal lifestyle

and dietary intervention improved women's intake of fibre, saturated fat, fruits and vegetables and micronutrient intake, although did not impact overall energy intake (Dodd et al. 2014c). Other trials have also shown significant improvements in maternal diet, physical activity (Luoto et al. 2011, Dodd et al. 2014c, Poston et al. 2015, Geraghty et al. 2016) and insulin resistance (Vinter et al. 2011, Geraghty et al. 2016)

While individual trials conducted in overweight and obese pregnant women have described positive effects on maternal dietary and lifestyle behaviours (Flynn et al. 2016), intervention trials overall have generated disappointing results in terms of clinical pregnancy and birth outcomes. Whether relatively modest improvements in maternal diet are sufficient to impact fetal adiposity measures, which themselves are relatively insensitive indices, remains to be determined (Rogozinska et al. 2017a, Rogozinska et al. 2017b). Furthermore, there is evidence to suggest that fetal growth and adiposity may be programmed much earlier in gestation than current interventions have targeted (Jahan-Mihan et al. 2015), highlighting the importance of optimal diet and maternal weight prior to conception (Opray et al. 2015, Hanson et al. 2016, Godfery et al. 2017, Hanson et al. 2017).

4.6 Conclusions

Among overweight and obese pregnant women in this study, maternal dietary measures were not consistently associated with fetal body composition.

CHAPTER 5: The impact of maternal cardiometabolic and inflammatory markers on fetal growth

This chapter forms the basis of a manuscript currently under peer review (O'Brien CM et al BMC Obesity), which is contained in Appendix 4.

5.1 Introduction

There are well-recognised associations between obesity in pregnancy and maternal, fetal and neonatal health outcomes (Cedergren 2006) and clear longer-term associations between maternal obesity, fetal overgrowth, high infant birth weight, and subsequent childhood obesity (Yu et al. 2013). While these associations are well defined, there has been limited exploration of the potential pathways leading to fetal overgrowth, adiposity and subsequent childhood obesity. These include maternal cardiometabolic hormones such as leptin, adiponectin, triglycerides, and fatty acids, along with inflammatory markers.

5.2 Aims

The aim of this study was to evaluate associations between maternal cardiometabolic and inflammatory markers and ultrasound assessed fetal growth and adiposity measures.

5.3 Methods

The research methodology (Dodd et al. 2011, Dodd et al. 2014a, Dodd et al. 2014c) of the LIMIT randomised controlled trial have been outlined in Chapter 2, as has the methodology relating to ultrasound assessment of fetal biometry and adiposity.

5.3.1 Cardiometabolic and Inflammatory Markers

Maternal blood samples were obtained at trial entry, 28 and 36 weeks gestation for assessment of cardiometabolic and inflammatory markers. The methodology has been previously described in detail (Moran et al. 2017). At 28 weeks, a fasting maternal plasma sample was collected and the following cardiometabolic markers were measured at trial entry, 28 and 36 weeks gestation; total cholesterol, triglycerides, non-esterified fatty acids (NEFA), high-density lipoprotein cholesterol, insulin, glucose, leptin, adiponectin and CRP. At 36 weeks, a non-fasting maternal plasma sample was collected and total cholesterol, triglycerides, non-esterified fatty acids (NEFA), high-density lipoprotein cholesterol, insulin, glucose, leptin, adiponectin and CRP were measured.

The majority (glucose, cholesterol, HDL-C, triglycerides, NEFA and CRP) were measured using Roche Diagnostics commercial kits (Australia) and non-esterified fatty acids were measured using Wako Pure Chemical Industries (Japan). All assays were performed on the automated Hitachi Auto 912 analyser or Cobas Integra 400 Plus with appropriate calibrators and quality controls (Roche for Roche assays and Wako standard and Sero QC's for the NEFA C assay). Plasma leptin (in singulate; HL-81 K; Millipore, St. Charles, MO, USA) and adiponectin (in singulate; HADP-61HK; Millipore, St. Charles, MO, USA) were determined by double antibody radioimmunoassay following the methods from the supplier.

5.3.2 Statistical Analysis

The analyses investigated cross-sectional relationships to determine whether there was an association between cardiometabolic and inflammatory markers at 28 weeks, and fetal ultrasound measures at 28 weeks (and similarly for 36 weeks). Because the nature of the association was of interest, and because most of the cardiometabolic and inflammatory markers exhibited skewness in distribution, each of the cardiometabolic and inflammatory markers were log-transformed prior to analysis. Estimates represent the difference in mean fetal measure corresponding to a 1-unit increase in log cardiometabolic marker.

Three of the cardiometabolic and inflammatory markers (CRP, leptin and adiponectin) were measured at both 28 and 36 weeks gestations. For each of these markers, linear regression models were used to model the relationship between the marker and fetal ultrasound measures at each time point, including a time-by- marker interaction term to test whether the relationship differed between time points. Generalised Estimating Equations (GEEs) were used to account for repeated measures. Triglycerides and fasting glucose were measured at 28 weeks only; therefore, for these markers, relationships with 28 week fetal ultrasound measures only were investigated using linear regression models.

Both unadjusted and adjusted analyses were performed with adjusted analyses including BMI category (BMI 25.0 – 29.9 versus BMI $\geq$ 20.0), parity (0 versus $\geq$ 1), age at consent, smoking status, study centre and SEIFA IRDS quintile as co-variates.

5.4 Results

5.4.1 Demographic characteristics

Flow of participants and baseline characteristics are presented in Figure 5.1 and Table 5.1 respectively. Mean maternal age was 29.6 years (standard deviation 5.5) with 41% of women (n = 377) overweight, 46.5% (n = 424) obese (BMI 30 – 39.9kg/m^2), and 12.2% (n = 111) morbidly obese (BMI ≥ 40kg/m^2). Most women (92%; n=835) were of Caucasian ethnicity, 40% (n = 369) were in their first ongoing pregnancy, and approximately 30% (n = 265) were from the highest quintile of social disadvantage.

Figure 5.1: Flow chart of the participants included in the secondary analysis with cardiometabolic markers

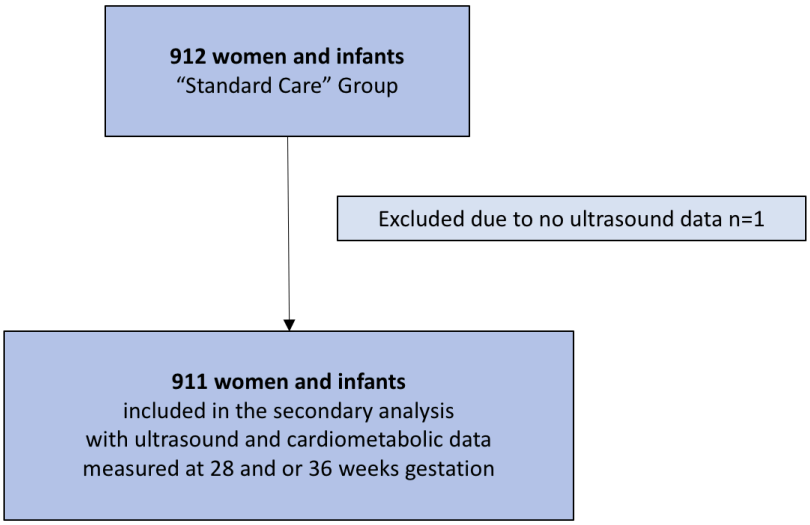


Table 5.1 – Baseline characteristics of participants included in analysis of the effect of maternal cardiometabolic and inflammatory markers on fetal growth and adiposity

Baseline Characteristic	Results
Total Number: N (%)	911
Maternal Age at trial entry (years) Mean (SD)	29.6 (5.5)
Gestational age at Trial Entry (weeks) Median (IQR)	14.58 (3.03)
Body Mass Index (kg/m^2) Mean (SD)	32.60 (6.01)
BMI Category: n (%)	
25.0 – 29.9 kg/m^2	377 (41.3)
30.0 – 34.9 kg/m^2	271 (29.7)
35.0 – 39.9 kg/m^2	153 (16.8)
≥ 40.0 kg/m^2	111 (12.2)
Caucasian: n (%)	836 (91.67)
Nulliparous: n (%)	369 (40.5)
Smoker: n (%)	101 (11.1)
SEIFA IRSD Quintiles: n (%)	
Most disadvantaged Quintile 1	265 (29.06)
Quintile 2	223 (24.45)
Quintile 3	143 (15.68)
Quintile 4	142 (15.57)
Least disadvantaged Quintile 5	139 (15.24)

5.4.2 C-Reactive Protein (CRP)

No consistent associations were found between maternal plasma CRP concentrations and fetal ultrasound measures of biometry and adiposity (Table 5.2).

Table 5.2: Relationship between log CRP and fetal ultrasound markers

Ultrasound Measure	Unadjusted Estimate (95% CI)	Unadjusted p value	Adjusted Estimate (95% CI)	Adjusted p value
EFW (grams)		0.559*		0.835*
28 weeks	-6.41 (-26.19, 13.36)	0.525	-8.62 (-29.88, 12.63)	0.426
36 weeks	-17.06 (-53.13, 19.01)	0.354	-12.50 (-47.91, 22.90)	0.489
SSFM (mm)		0.842*		0.850*
28 weeks	-0.00 (-0.10, 0.09)	0.920	0.02 (-0.08, 0.11)	0.744
36 weeks	0.01 (-0.14, 0.16)	0.875	0.03 (-0.12, 0.18)	0.675
AFM (mm)		0.442*		0.394*
28 weeks	0.00 (-0.10, 0.10)	0.990	-0.00 (-0.11, 0.11)	0.976
36 weeks	0.07 (-0.09, 0.23)	0.396	0.08 (-0.08, 0.23)	0.353
MTFM (mm^2)		0.988*		0.998*
28 weeks	0.04 (-0.08, 0.17)	0.514	0.05 (-0.09, 0.19)	0.456
36 weeks	0.04 (-0.29, 0.37)	0.817	0.05 (-0.28, 0.39)	0.758
MTLM (mm^2)		0.419*		0.376*
28 weeks	0.04 (-0.06, 0.15)	0.414	0.05 (-0.06, 0.16)	0.367
36 weeks	-0.05 (-0.28, 0.17)	0.636	-0.06 (-0.28, 0.17)	0.615
AC (mm)		0.698*		0.998*
28 weeks	-0.05 (-0.20, 0.09)	0.473	-0.07 (-0.22, 0.08)	0.351
36 weeks	-0.09 (-0.26, 0.08)	0.296	-0.07 (-0.24, 0.10)	0.407
BPD (mm)		0.114*		0.147*
28 weeks	-0.03 (-0.06, 0.00)	0.096	-0.02 (-0.06, 0.01)	0.167
36 weeks	0.00 (-0.03, 0.03)	0.883	0.00 (-0.03, 0.04)	0.770
HC (mm)		0.682*		0.568*

28 weeks	-0.07 (-0.19, 0.05)	0.259	-0.07 (-0.19, 0.05)	0.280
36 weeks	-0.04 (-0.15, 0.07)	0.462	-0.03 (-0.13, 0.08)	0.625
FL (mm)		0.961*		0.824*
28 weeks	-0.01 (-0.04, 0.02)	0.491	-0.01 (-0.04, 0.02)	0.428
36 weeks	-0.01 (-0.04, 0.02)	0.478	-0.01 (-0.04, 0.02)	0.602
EFW z-score		0.466*		0.587*
28 weeks	0.03 (-0.04, 0.10)	0.380	0.02 (-0.04, 0.09)	0.482
36 weeks	-0.00 (-0.08, 0.08)	0.981	0.00 (-0.08, 0.08)	0.979
AC z-score		0.611*		0.791*
28 weeks	0.02 (-0.06, 0.10)	0.609	0.01 (-0.07, 0.09)	0.808
36 weeks	-0.01 (-0.10, 0.09)	0.900	-0.00 (-0.09, 0.09)	0.931
BPD z-score		0.095*		0.169*
28 weeks	-0.05 (-0.16, 0.06)	0.399	-0.04 (-0.15, 0.08)	0.544
36 weeks	0.05 (-0.04, 0.15)	0.261	0.05 (-0.05, 0.15)	0.302
HC z-score		0.706*		0.637*
28 weeks	-0.00 (-0.08, 0.08)	0.932	-0.00 (-0.09, 0.08)	0.914
36 weeks	0.01 (-0.06, 0.09)	0.738	0.02 (-0.06, 0.09)	0.674
FL z-score		0.942*		0.827*
28 weeks	0.01 (-0.07, 0.09)	0.768	0.00 (-0.08, 0.09)	0.920
36 weeks	0.02 (-0.07, 0.11)	0.726	0.02 (-0.08, 0.11)	0.738

Notes: Results are expressed as the difference in means (95% CI) corresponding to a one unit change in log CRP.
* p value for test of time-by-log CRP interaction.

5.4.3 Triglycerides

There were no consistent associations identified between plasma triglyceride concentrations at 28 weeks and fetal ultrasound markers of biometry and adiposity (Table 5.3). However, there was a positive association identified between maternal plasma triglyceride concentrations and biometry z-scores. Specifically, a 1-unit increase in log triglyceride concentration was associated with an increase in mean EFW z-score of 0.20 (0.01 to 0.39; p=0.041), and an increase in mean AC z-score of 0.25 (0.05 to 0.46; p=0.016).

Table 5.3: Relationship between log Triglycerides and fetal ultrasound markers

Ultrasound Measure	Unadjusted Estimate (95% CI)	Unadjusted p value	Adjusted Estimate (95% CI)	Adjusted p value
EFW (grams)	26.96 (-19.21, 73.13)	0.252	28.14 (-18.38, 74.67)	0.236
SSFM (mm)	0.02 (-0.21, 0.25)	0.870	0.06 (-0.18, 0.29)	0.633
AFM (mm)	0.02 (-0.23, 0.27)	0.899	0.01 (-0.24, 0.26)	0.940
MTFM (mm^2)	-0.01 (-0.32, 0.31)	0.964	-0.00 (-0.32, 0.32)	>0.99
MTLM (mm^2)	0.08 (-0.19, 0.34)	0.567	0.04 (-0.23, 0.31)	0.779
AC (mm)	0.27 (-0.07, 0.62)	0.121	0.25 (-0.10, 0.60)	0.154
BPD (mm)	0.03 (-0.06, 0.11)	0.540	0.04 (-0.04, 0.13)	0.334
HC (mm)	-0.01 (-0.29, 0.27)	0.946	0.03 (-0.26, 0.32)	0.851
FL (mm)	-0.01 (-0.07, 0.06)	0.842	0.00 (-0.06, 0.07)	0.908
EFW z-score	0.23 (0.04, 0.42)	0.020	0.20 (0.01, 0.39)	0.041
AC z-score	0.30 (0.09, 0.50)	0.004	0.25 (0.05, 0.46)	0.016
BPD z-score	0.25 (-0.06, 0.55)	0.113	0.29 (-0.02, 0.59)	0.067
HC z-score	0.03 (-0.18, 0.23)	0.808	0.03 (-0.18, 0.24)	0.796
FL z-score	0.05 (-0.15, 0.26)	0.612	0.07 (-0.14, 0.28)	0.499

Notes: Results are expressed as the difference in means (95% CI) corresponding to a one unit increase in log Triglycerides.

5.4.4 Fasting Glucose

There were no consistent associations found between fasting glucose concentrations at 28 weeks and fetal ultrasound measures of biometry and adiposity (Table 5.4).

Table 5.4: Relationship between log Fasting Glucose and fetal ultrasound markers

Ultrasound Measure	Unadjusted Estimate (95% CI)	Unadjusted p value	Adjusted Estimate (95% CI)	Adjusted p value
EFW (grams)	59.68 (-33.80, 153.16)	0.211	53.52 (-56.64, 163.68)	0.341
SSFM (mm)	-0.32 (-0.81, 0.17)	0.201	-0.17 (-0.74, 0.41)	0.575
AFM (mm)	-0.54 (-1.10, 0.02)	0.058	-0.10 (-0.76, 0.57)	0.772
MTFM (mm^2)	-0.25 (-0.97, 0.47)	0.493	0.02 (-0.83, 0.87)	0.959
MTLM (mm^2)	-0.28 (-0.91, 0.35)	0.387	0.03 (-0.72, 0.77)	0.946
AC (mm)	0.16 (-0.55, 0.88)	0.653	0.33 (-0.50, 1.17)	0.430
BPD (mm)	0.16 (-0.01, 0.33)	0.067	0.08 (-0.13, 0.28)	0.460
HC (mm)	0.19 (-0.38, 0.77)	0.509	0.05 (-0.63, 0.74)	0.884
FL (mm)	0.12 (-0.01, 0.26)	0.077	0.09 (-0.07, 0.25)	0.289
EFW z-score	0.33 (-0.09, 0.74)	0.120	0.46 (-0.01, 0.94)	0.057
AC z-score	0.08 (-0.35, 0.51)	0.714	0.36 (-0.14, 0.87)	0.153
BPD z-score	0.61 (-0.03, 1.25)	0.061	0.42 (-0.32, 1.17)	0.263
HC z-score	0.10 (-0.34, 0.53)	0.659	0.07 (-0.45, 0.59)	0.800
FL z-score	0.50 (0.06, 0.94)	0.027	0.49 (-0.04, 1.03)	0.071

Notes: Results are expressed as difference in mean (95% CI) corresponding to a one unit increase in log fasting glucose.

5.4.5 Leptin

There were no consistent associations identified between plasma leptin concentrations and fetal ultrasound markers of biometry and adiposity (Table 5.5). However, there was a positive association identified between plasma leptin concentration and mid-thigh fat mass (MTFM). Specifically, a 1-unit increase in log leptin concentration was associated with a greater reduction in mean MTFM of -0.37 (-0.67, -0.07) at 28 weeks (p = 0.015).

Table 5.5: The relationship between log Leptin and fetal ultrasound markers

Ultrasound Measure	Unadjusted Estimate (95% CI)	Unadjusted p value	Adjusted Estimate (95% CI)	Adjusted p value
EFW (grams)		0.815*		0.785*
28 weeks	-40.68 (-79.26, -2.09)	0.039	-41.08 (-83.65, 1.49)	0.059
36 weeks	-32.84 (-95.88, 30.20)	0.307	-31.83 (-95.48, 31.83)	0.327
SSFM (mm)		0.925*		0.999*
28 weeks	0.01 (-0.17, 0.20)	0.880	0.14 (-0.06, 0.34)	0.167
36 weeks	0.03 (-0.24, 0.30)	0.833	0.14 (-0.13, 0.41)	0.303
AFM (mm)		0.988*		0.912*
28 weeks	-0.02 (-0.22, 0.17)	0.814	0.03 (-0.19, 0.24)	0.802
36 weeks	-0.02 (-0.33, 0.28)	0.894	0.01 (-0.30, 0.32)	0.960
MTFM (mm^2)		0.561*		0.563*
28 weeks	-0.25 (-0.51, 0.00)	0.053	-0.37 (-0.67, -0.07)	0.015
36 weeks	-0.08 (-0.65, 0.49)	0.778	-0.20 (-0.76, 0.36)	0.488
MTLM(mm^2)		0.231*		0.191*
28 weeks	0.07 (-0.13, 0.27)	0.496	0.00 (-0.24, 0.24)	0.995
36 weeks	-0.16 (-0.50, 0.19)	0.373	-0.25 (-0.60, 0.10)	0.167
AC(mm)		0.705*		0.631*
28 weeks	-0.25 (-0.52, 0.02)	0.065	-0.26 (-0.54, 0.02)	0.067
36 weeks	-0.19 (-0.48, 0.11)	0.221	-0.18 (-0.48, 0.13)	0.259

BPD (mm)		0.400*		0.420*
28 weeks	-0.02 (-0.09, 0.05)	0.605	-0.01 (-0.08, 0.06)	0.735
36 weeks	0.02 (-0.04, 0.08)	0.614	0.02 (-0.04, 0.08)	0.525
HC (mm)		0.343*		0.380*
28 weeks	-0.12 (-0.35, 0.11)	0.303	-0.10 (-0.34, 0.14)	0.425
36 weeks	0.02 (-0.19, 0.23)	0.878	0.03 (-0.19, 0.25)	0.791
FL (mm)		0.392*		0.369*
28 weeks	-0.03 (-0.09, 0.02)	0.244	-0.02 (-0.08, 0.04)	0.442
36 weeks	-0.00 (-0.05, 0.05)	0.880	0.01 (-0.04, 0.06)	0.729
EFW z-score		0.900*		0.901*
28 weeks	-0.05 (-0.18, 0.09)	0.476	-0.06 (-0.20, 0.09)	0.432
36 weeks	-0.04 (-0.17, 0.10)	0.567	-0.05 (-0.19, 0.09)	0.498
AC z-score		0.865*		0.832*
28 weeks	-0.04 (-0.18, 0.11)	0.603	-0.05 (-0.21, 0.10)	0.513
36 weeks	-0.02 (-0.17, 0.12)	0.749	-0.03 (-0.18, 0.12)	0.668
BPD z-score		0.909*		0.810*
28 weeks	0.11 (-0.12, 0.35)	0.344	0.12 (-0.13, 0.36)	0.340
36 weeks	0.10 (-0.07, 0.27)	0.239	0.09 (-0.09, 0.27)	0.336
HC z-score		0.970*		0.850*
28 weeks	0.04 (-0.11, 0.19)	0.584	0.04 (-0.12, 0.21)	0.607
36 weeks	0.04 (-0.11, 0.18)	0.600	0.03 (-0.13, 0.18)	0.736
FL z-score		0.515*		0.506*
28 weeks	0.00 (-0.16, 0.17)	0.954	0.03 (-0.14, 0.20)	0.736
36 weeks	0.07 (-0.09, 0.23)	0.407	0.10 (-0.07, 0.27)	0.268

Notes: Results are expressed as difference in means (95% CI) corresponding to a one unit increase in log Leptin.
* p value for test of time-by-log Leptin interaction.

5.4.6 Adiponectin

There were consistent associations identified between maternal plasma adiponectin concentrations and fetal ultrasound measures (Table 5.6).

There were negative associations identified between plasma adiponectin concentrations and measures of abdominal circumference (AC) and estimated fetal weight (EFW). Specifically, a 1-unit increase in log adiponectin concentration was associated with a reduction in mean AC of -0.53 (95% CI: -0.83, -0.22) millimetres ($p < 0.001$) and reduction in mean EFW of -100.85 (-164.98, -36.71) grams ($p = 0.002$) at 36 weeks gestation.

There were negative associations identified between plasma adiponectin concentration and z-scores for abdominal circumference (AC) and estimated fetal weight (EFW). Specifically, a 1-unit increase in log adiponectin concentration was associated with a reduction in the mean AC z-score of -0.21 (-0.35, -0.07) at 28 weeks ($p = 0.004$) and of -0.30 (-0.46, -0.13) at 36 weeks ($p < 0.001$). Similarly, a 1-unit increase in log adiponectin concentration was associated with a reduction in the mean EFW z-score of -0.23 (-0.37, -0.10) at 28 weeks ($p < 0.001$) and of -0.24 (-0.38, -0.10) at 36 weeks ($p < 0.001$).

There was a negative association identified between plasma log adiponectin concentration and MTLM. Specifically, a 1-unit increase in log Adiponectin concentration was associated with a reduction in the mean MTLM of -0.41 (-0.77, -0.05) millimetres at 36 weeks ($p < 0.001$).

Table 5.6: The relationship between log Adiponectin and fetal ultrasound markers

Ultrasound Measure	Unadjusted Estimate (95% CI)	Unadj p value	Adjusted Estimate (95% CI)	Adjusted p value
EFW		0.010*		0.008*
28 weeks	-5.36 (-42.08, 31.35)	0.775	-8.77 (-45.68, 28.14)	0.641
36 weeks	-94.09 (-158.68, -29.51)	0.004	-100.85 (-164.98, -36.71)	0.002
SSFM		0.110*		0.101*
28 weeks	0.11 (-0.05, 0.28)	0.179	0.12 (-0.05, 0.30)	0.160
36 weeks	-0.12 (-0.38, 0.13)	0.343	-0.12 (-0.38, 0.13)	0.354
AFM		0.634*		0.651*
28 weeks	-0.05 (-0.26, 0.16)	0.651	-0.00 (-0.21, 0.21)	0.988
36 weeks	-0.13 (-0.43, 0.17)	0.393	-0.08 (-0.39, 0.23)	0.607
MTFM		0.688*		0.517*
28 weeks	0.00 (-0.23, 0.24)	0.970	-0.01 (-0.25, 0.23)	0.943
36 weeks	-0.11 (-0.69, 0.46)	0.705	-0.20 (-0.79, 0.39)	0.509
MTLM		0.035*		0.013*
28 weeks	0.09 (-0.11, 0.29)	0.377	0.09 (-0.12, 0.29)	0.405
36 weeks	-0.33 (-0.70, 0.03)	0.074	-0.41 (-0.77, -0.05)	0.027
AC		0.012*		0.010*
28 weeks	-0.04 (-0.31, 0.23)	0.768	-0.04 (-0.31, 0.23)	0.779
36 weeks	-0.51 (-0.82, -0.21)	0.001	-0.53 (-0.83, -0.22)	<.001
BPD		0.056*		0.056*
28 weeks	0.06 (-0.00, 0.12)	0.055	0.04 (-0.02, 0.11)	0.176
36 weeks	-0.02 (-0.08, 0.04)	0.545	-0.04 (-0.10, 0.02)	0.244
HC		0.042*		0.043*
28 weeks	0.09 (-0.13, 0.30)	0.429	0.10 (-0.12, 0.32)	0.363
36 weeks	-0.20 (-0.41, 0.02)	0.071	-0.18 (-0.39, 0.03)	0.095
FL		0.061*		0.088*
28 weeks	0.03 (-0.02, 0.08)	0.272	0.02 (-0.03, 0.08)	0.406

36 weeks	-0.03 (-0.08, 0.02)	0.179	-0.04 (-0.08, 0.01)	0.160
EFW z-score		0.986*		0.938*
28 weeks	-0.24 (-0.37, -0.10)	<.001	-0.23 (-0.37, -0.10)	<.001
36 weeks	-0.24 (-0.38, -0.09)	0.001	-0.24 (-0.38, -0.10)	<.001
AC z-score		0.427*		0.371*
28 weeks	-0.22 (-0.36, -0.08)	0.002	-0.21 (-0.35, -0.07)	0.004
36 weeks	-0.30 (-0.47, -0.13)	<.001	-0.30 (-0.46, -0.13)	<.001
BPD z-score		0.685*		0.609*
28 weeks	0.04 (-0.18, 0.26)	0.749	-0.01 (-0.23, 0.21)	0.949
36 weeks	-0.02 (-0.19, 0.16)	0.861	-0.07 (-0.26, 0.11)	0.434
HC z-score		0.759*		0.702*
28 weeks	-0.10 (-0.27, 0.06)	0.204	-0.08 (-0.24, 0.08)	0.316
36 weeks	-0.13 (-0.29, 0.02)	0.086	-0.12 (-0.27, 0.03)	0.124
FL z-score		0.530*		0.645*
28 weeks	-0.06 (-0.23, 0.11)	0.514	-0.07 (-0.24, 0.11)	0.454
36 weeks	-0.12 (-0.29, 0.04)	0.150	-0.11 (-0.28, 0.05)	0.176

Notes: Results are expressed as difference in means (95% CI) corresponding to a 1 unit increase in log Adiponectin**.** * p value for test of time-by-log Adiponectin interaction.

5.4.7 The interaction between cardiometabolic markers and fetal growth over time

The associations between maternal plasma log adiponectin concentration and mean EFW changed over time. At 28 weeks, there was a small and not statistically significant association but at 36 weeks, the association was larger in magnitude and the interaction was statistically significant.

The association between maternal plasma log Adiponectin concentration and mean AC changed over time. At 28 weeks, there was a small and not statistically significant association whereas at 36 weeks, the association was larger in magnitude and there was a statistically significant interaction.

The association between maternal plasma log adiponectin concentration and mean HC changed over time, although neither individual association was statistically significant. At 28 weeks, women with higher log adiponectin concentrations had fetuses with bigger head circumference, whereas at 36 weeks, women with higher log Adiponectin had fetuses with lower HC on average.

The association between maternal plasma log adiponectin concentrations and mean MTLM changed over time. At 28 weeks, there was a small and not statistically significant association; whereas at 36 weeks, the association was larger in magnitude and interaction was statistically significant ($p = 0.013$).

5.5 Discussion

This secondary exploratory analysis demonstrated that increasing maternal concentrations of adiponectin were associated with a reduction in abdominal circumference and estimated fetal weight, with the magnitude of this effect increasing over gestation. Furthermore, a higher triglyceride concentration was associated with an increase in abdominal circumference z-score and estimated fetal weight at 28 weeks gestation. There were no apparent associations between inflammatory markers, fasting glucose, triglyceride and leptin concentrations, and fetal ultrasound measurements.

This is the first study to describe the relationship between cardiometabolic biomarkers and fetal ultrasound measurements of biometry and adiposity. The literature to date has reported on maternal or cord blood sampling and postnatal measurements of neonatal adiposity (Patenaude et al. 2017) or child growth trajectories (Karakosta et al. 2016), and generally involved studies of small sample sizes. There have been two large studies evaluating maternal cardiometabolic and inflammatory markers in the setting of randomised control trials testing the effect of an antenatal dietary and lifestyle intervention (Moran et al. 2017, Sagedal et al. 2017).

The strength of this current analysis is the large sample size of 911 women and the reporting of fetal body composition as an outcome measurement. A potential limitation of this study relates to the absence of a comparator group of women entering pregnancy with a normal BMI. Fasting measurements at 36 weeks for triglycerides and glucose were not obtained and this also limited interpretation to one time point only for these two cardiometabolic markers.

The primary role of maternal adiponectin is to promote insulin sensitivity, which in turn increases the uptake of glucose by maternal skeletal muscle, thereby reducing the availability for placental transfer (Aye et al. 2013). Additionally, adiponectin modulates the insulin receptor in the trophoblast, preventing the placental transfer and uptake of amino acids (Aye et al. 2013). Adiponectin has been postulated as a possible link between maternal adipose tissue, placental transport and fetal growth (Aye et al. 2013).

The role of adiponectin in adult cardiovascular disease (Parker-Duffen et al. 2014, Lekva et al. 2017) and Type 2 Diabetes (Weyer et al. 2001) has been well defined. The current literature pertaining to pregnancy is limited to six studies, half of which reported on cord blood adiponectin concentrations only (Sivan et al. 2003, Tsai et al. 2004, Mantzoros et al. 2009). Maternal adiponectin concentrations have been reported in women entering pregnancy with a normal BMI (Lekva et al. 2017) or with GDM (Ategbo et al. 2006). In women entering pregnancy with a normal BMI, a reduction in adiponectin concentrations in the 3rd trimester was identified, and was independent of both maternal BMI and insulin resistance (Lekva et al. 2017). Low adiponectin concentrations have also been associated with a higher prevalence of newborn infants being classified as LGA and having increased birth weight (Lekva et al. 2017).

However, randomised dietary intervention trials have not confirmed an association between adiponectin concentrations and infant birth weight. The LIMIT trial did not identify differences between treatment groups with regards to concentrations of cardiometabolic and inflammatory markers in women who were overweight or obese (Moran et al. 2017). The Fit for Delivery antenatal intervention in healthy, non-diabetic women did demonstrate a reduction in both insulin and leptin concentrations with no

difference in either mean birth weight or the proportion of babies weighing over 4kg (Sagedal et al. 2017). This effect was seen predominantly in lean women and was not statistically significant in women with a BMI ≥ 25kg/m^2 (Sagedal et al. 2017).

While adiponectin concentrations do not alter with dietary change, there is increasing interest in the supplementation of adiponectin as a promising application in non-pregnant adults for treatment of obesity (Parker-Duffen et al. 2014, Lekva et al. 2017, Lekva et al. 2017), although robust evidence is limited. With regards to pregnancy, animal studies have identified that adiponectin supplementation may alter fetal growth through improving insulin sensitivity and placental function (Rosario et al. 2012). The proposed mechanism relates to the down regulation of key placental nutrient transporters within the syncytiotrophoblasts, including amino acid transporters such as System A (Rosario et al. 2012, Lekva et al. 2017). Adiponectin has also been suggested as a therapeutic agent to reduce cardiovascular risk, having been studied in overweight and obese mice and rodents (Parker-Duffen et al. 2014).

This study did not find a consistent association between leptin and fetal growth or adiposity. This finding is comparable to the study by Castro and associates, who measured maternal plasma leptin concentrations between 24 to 72 hours after birth, with no identified associations with neonatal adiposity (Castro et al. 2017). In contrast, Josefson and colleagues measured maternal concentrations at 36 weeks gestation, identifying a relationship with neonatal adiposity (Josefson et al. 2014). Fetal exposure in utero to high leptin concentrations has also been positively associated with infant birth weight, neonatal adiposity, and postnatal and childhood growth trajectories (Karakosta et al. 2016).

Maternal triglyceride concentrations at 36 weeks were associated with an increase in AC and EFW z-scores. This relationship is consistent with others who have reported hypertriglycerideaemia to be a strong determinant of fetal growth, independent of maternal BMI, in women with diabetes (Schaefer-Graf et al. 2008). Other studies have identified a similar association between maternal triglycerides measured in both early (Vrijkotte et al. 2011) and late pregnancy (Mossayebi et al. 2014) and fetal growth. Uncertainty remains regarding maternal concentrations influence fetal growth (Catalano 2010) and the role of lipoprotein receptors, binding proteins and lipases, which contribute to the placental flow of maternal fatty acids (Schaefer-Graf, Graf et al. 2008).

5.6 Conclusion

Among pregnant women who are overweight or obese

- Increasing maternal concentrations of adiponectin were associated with a reduction in abdominal circumference and estimated fetal weight;
- The magnitude of this effect increased over gestation;
- Increased triglyceride concentrations were associated with an increase in abdominal circumference z-score and estimated fetal weight at 36 weeks gestation; and
- There were no apparent associations between inflammatory markers, fasting glucose, triglyceride and leptin concentrations and fetal ultrasound measurements.

CHAPTER 6: Associations between fetal ultrasound biometry and newborn anthropometry in infants born to women who are overweight or obese

This chapter forms the basis of a manuscript currently under peer review (O'Brien CM et al American Journal of Obstetrics and Gynaecology, Maternal Fetal Medicine), which is contained in Appendix 5.

6.1 Introduction

Ultrasound is widely available and used clinically for antenatal detection of the LGA fetus to assist in clinical management regarding both the method and timing of birth, as potential strategies to reduce birth complications including operative delivery (Boulvain et al. 2016) and shoulder dystocia (Dodd et al. 2012). While several studies have attempted to evaluate the relationship between prenatal ultrasound and neonatal measures of body composition, they are limited by the relatively small sample sizes involved, with the majority of women being of normal BMI, and largely confined to either gestational or pre-existing diabetes (Bernstein et al. 1991, Larciprete et al. 2003, Parretti et al. 2003, Hure et al. 2012, O'Connor et al. 2014, Walsh et al. 2015, Gibson et al. 2016).

6.2 Aims

The aim of this study was to evaluate the relationship between fetal ultrasound biometry and adiposity measures at 36 weeks gestation and neonatal biometry and adiposity measures, in infants born to women who were overweight or obese.

6.3 Methods

The research methodology (Dodd et al. 2011, Dodd et al., 2014a, Dodd et al. 2014c) of the LIMIT randomised controlled trial have been outlined in Chapter 2, as has the methodology relating to ultrasound assessment of fetal biometry and adiposity measures.

6.3.1 Neonatal anthropometric measures

Infant birth weight (grams), HC (cm) and length (cm) were measured within the first 2 hours of birth by the attending midwife. Birth weight was measured using calibrated electronic scales to the nearest 1 gram with the newborn infant undressed. Length was measured using a length board and the infant laid supine, the head held against the top of the board and a sliding foot plate moved and rested flat against the foot of the infant with the legs fully extended, and read to the nearest 0.1cm (Dodd et al. 2016). Large for gestational age was defined as birth weight at or above the 90^{th} centile for gestational age and infant sex. Z-scores were calculated using Australian population reference ranges (Beeby et al. 1996).

(i) Skin fold thickness measurements (SFTM)

Trained research assistants obtained anthropometric measurements according to a standardised protocol, within the first few days of life and prior to discharge from hospital (Dodd et al. 2016). SFTM were obtained on the right side of the body using Harpenden Skinfold Callipers, with the infant undressed. The skinfold was identified and grasped between the left thumb and index finger, so that a double fold of skin and subcutaneous adipose tissue was held without the incorporation of underlying muscle. The calliper jaws were placed perpendicular to the length of the skin fold and the measurement was recorded 2 seconds after the pressure was applied. For each site, the measurements were duplicated and if there was a difference more than 1.0mm, a third measure was taken. The final value presented the mean of two measurements or a median of the three (Marfell-Jones et al. 2006).

Abdominal SFTM was identified 2cm to the right of the umbilicus and measured perpendicular to the long axis of the abdomen. Subscapular SFTM was measured after identifying the lower tip of the scapula, with the observer's thumb placed below this laterally.

(ii) Body circumference measurements

Circumference measures were obtained according to a standardised protocol, with the infant undressed, supine and using a fibreglass measuring tape and recorded to the nearest 0.1cm (Kannieappan et al. 2013). HC was measured at the widest point above the eyebrows anteriorly (glabella) and the most prominent point of the occiput posteriorly.

AC was measured at the level of the umbilicus in a plane at right angles to the spine and at the end of a normal expiration.

6.3.2 Statistical analysis

Associations between 36 week fetal ultrasound measures and corresponding birth measures were explored in multiple ways. Firstly, to descriptively assess strength and linearity of association, scatterplots were created, with a lowess smooth and line of best fit superimposed. Secondly, a Pearson Correlation Coefficient was calculated for each pair of variables to measure the overall strength of linear association. Thirdly, to estimate the change in mean birth measure associated with increased values of the 36 week measure, linear regression models were fitted using the birth measure as the dependent variable (outcome) and the 36 week measure as the independent variable (predictor). Models were adjusted for the actual amount of time between 36 week ultrasound and date of birth. Lastly, to determine if the strength and direction of the association differed by maternal BMI category, linear regression models were fitted using birth measure as the dependent variable, and 36 week measure, BMI category, and their interaction, as the independent variables. Adjustments were made for the amount of time between the 36 week ultrasound and date of birth.

6.4 Results

6.4.1 Demographic characteristics

Flow of participants and baseline characteristics are presented in Figure 6.1 and Table 6.1 respectively. A total of 845 women and infants are included in this analysis. The median gestation at trial entry was 14.3 weeks (Interquartile range (IQR) 12.0 – 17.0) (Table 1).

The median maternal BMI was 31.2kg/m^2 (IQR 27.8 – 35.8) kg/m^2, with 41% (n = 350) of women overweight and 58.6% (n =495) obese. Ninety-two percent (92%) of women in our cohort are of Caucasian ethnicity (n = 773) and 59% of women (n = 501) were in their first ongoing pregnancy. Fifteen percent (n = 128) of women were classified within the highest quintile of social disadvantage using the Socio-Economic Indexes for Areas (SEIFA). The baseline characteristics of the women contributing ultrasound and neonatal data were comparable to all women in the standard care group, and to the full randomised LIMIT cohort (Dodd et al. 2014a).

Figure 6.1: Flow chart of participants included in the analysis of associations between fetal and neonatal measures

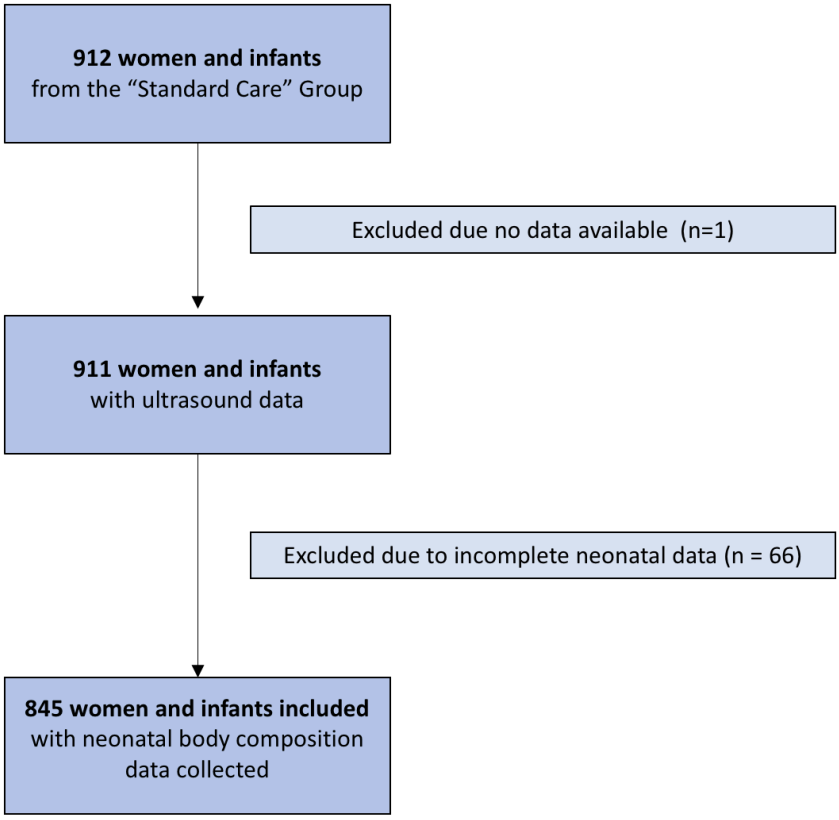


Table 6.1 – Baseline characteristics of participants included in the analysis of associations between fetal and neonatal measures

Baseline Characteristics	Results
Total Number: N (%)	845
Maternal Age at Trial Entry Mean (SD)	29.50 (5.46)
Gestational age at Trial Entry (weeks) Median (IQR)	14.29 (12.00, 17.00)
BMI (kg/m^2) Median (IQR)	31.20 (27.80, 35.80)
BMI Category: n (%)	
25.0-29.9 kg/m^2	350 (41.42)
≥ 30.0 kg/m^2	495 (58.58)
Caucasian: n (%)	773 (91.48)
Nulliparous: n (%)	344 (40.71)
Smoker: n (%)	94 (11.12)
SEIFA Quintile: n (%)	
Most disadvantaged Quintile 1	242 (28.64)
Quintile 2	207 (24.50)
Quintile 3	133 (15.74)
Quintile 4	135 (15.98)
Least disadvantaged Quintile 5	128 (15.15)

6.4.2 Correlation between ultrasound measures and neonatal measures

Both EFW (0.62) and EFW z-score (0.70) at 36 weeks gestation were strongly correlated with birth weight (Table 6.2 and Figure 6.2). While there was moderate correlation between ultrasound derived SSFM (0.32) and subscapular SFTM measured at birth, ultrasound derived AFM was poorly correlated with abdominal SFTM (0.07) (Table 6.2).

Table 6.2: Correlation coefficients between fetal and neonatal body composition measurements

Association Between	Pearson Correlation Coefficient
Birthweight and 36 Week EFW	0.62
Birth HC and 36 Week HC	0.52
Birth AC and 36 Week AC	0.49
Birth SSFM and 36 Week SSFM	0.34
Birth AFM and 36 Week AFM	0.07
Birthweight z-score and 36 Week EFW z-score	0.70
Birth HC z-score and 36 Week HC z-score	0.51

Figure 6.2: Relationship between birth measures and (a) Estimated Fetal Weight (EFW) and (b) Abdominal Fat Mass (AFM)

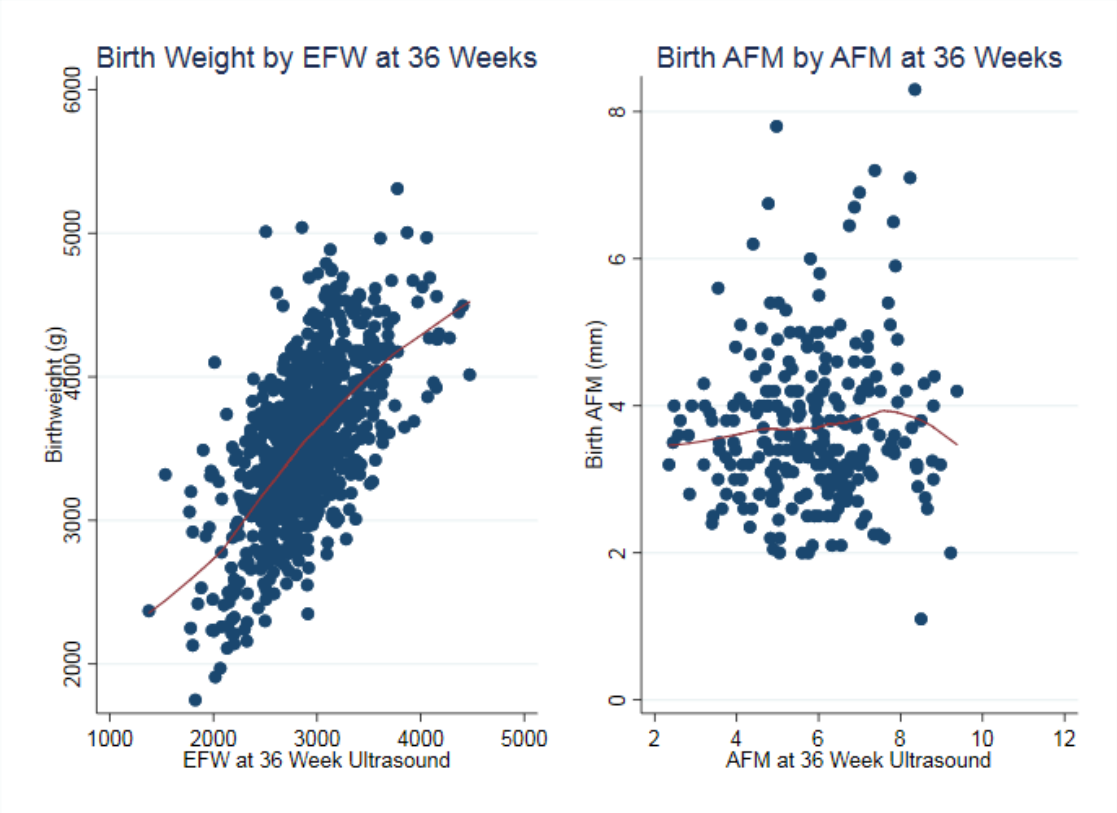


Figure 6.2a illustrates birthweights by 36 week estimation of fetal weight. Figure 6.2b illustrates birth abdominal SFTM and AFM measured at 36 weeks. The lines represent lowess and smoothing.

6.4.3 Linear regression models for the association between ultrasound measures and neonatal measures

Table 6.3 summarises the results of linear regression models investigating the association between 36 week ultrasound measurements and birth measurements. For every 1gram increase in EFW at 36 weeks gestation, there was a 0.94 gram increase in infant birth weight (95% CI 0.88 to 0.99 grams; p<0.001). The combination of ultrasound derived EFW at 36 weeks gestation and the number of subsequent days until birth accounted for 63% of the variability in measures (R^2=0.63). There were similar findings for HC, HC z-score, AC and SSFM, with a moderate to high degree of overall variability explained (Table 6.3). The exception was abdominal skin fold thickness, where the 36 week measure (SSFM) was not significantly associated with abdominal SFTM measured at birth (0.06 mm; 95% CI: -0.03, 0.15).

Table 6.3: Linear regression analyses measuring the association between fetal and neonatal measurements of body composition

Association	Estimate (95% CI)	p value	R^2
Birthweight / EFW	0.94 (0.88, 0.99)	<0.001	0.63
Head Circumference	0.69 (0.63, 0.75)	<0.001	0.41
Abdomen Circumference	0.69 (0.60, 0.79)	<0.001	0.34
SSFM	0.29 (0.20, 0.39)	<0.001	0.13
AFM	0.06 (-0.03, 0.15)	0.203	0.01
Birthweight z-score / EFW z-score	0.78 (0.73, 0.84)	<0.001	0.50
HC z-score	0.62 (0.55, 0.70)	<0.001	0.26

Note: Estimates are differences in mean birth measure (95% CI) corresponding to a 1 unit increase in 36 week ultrasound measure. All models were adjusted for time (in days) elapsed between 36 week ultrasound and birth.

6.4.4 Linear regression models allowing for effect modification by BMI category

Table 6.4 presents the results of linear regression models investigating whether the association between 36 week ultrasound measurements and birth measurements was modified by maternal BMI category, with the estimates of the association between 36 week measures and neonatal measures presented separately by maternal BMI category.

For all measures except AFM, a similar pattern was observed, in which there was a significant relationship between the 36 week measure (EFW, HC, AC, SSFM) and the corresponding birth measure (BW, HC, AC, subscapular skin fold thickness) in both overweight and obese BMI categories. The magnitude and direction of this association was consistent across both overweight and obese BMI categories. The difference in the estimates of association between overweight and obese BMI categories was not statistically significant or clinically meaningful.

In relation to the ultrasound derived AFM and abdominal SFTM measured at birth (Table 6.4), the association was not statistically significant at either time point and there was no evidence of effect modification by maternal BMI category.

Table 6.4: Interaction analysis assessing influence of maternal BMI on fetal and neonatal measurements

Association	Estimate (95% CI)	p value	R^2
Birthweight/EFW		0.803*	0.63
- BMI 25.0-29.9	0.93 (0.84, 1.01)	<0.001	
- BMI ≥ 30.0	0.94 (0.87, 1.01)	<0.001	
HC		0.373*	0.41
- BMI 25.0-29.9	0.73 (0.62, 0.83)	<0.001	
- BMI ≥ 30.0	0.67 (0.59, 0.75)	<0.001	
AC		0.655*	0.34
- BMI 25.0-29.9	0.72 (0.57, 0.87)	<0.001	
- BMI ≥ 30.0	0.68 (0.55, 0.80)	<0.001	
SSFM		0.403*	0.14
- BMI 25.0-29.9	0.34 (0.20, 0.48)	<0.001	
- BMI ≥ 30.0	0.26 (0.13, 0.38)	<0.001	
AFM		0.592*	0.01
- BMI 25.0-29.9	0.08 (-0.04, 0.21)	0.196	
- BMI ≥ 30.0	0.03 (-0.09, 0.16)	0.598	
Birthweight and EFW z-scores		0.970*	0.50
- BMI 25.0-29.9	0.78 (0.69, 0.86)	<0.001	
- BMI ≥ 30.0	0.78 (0.71, 0.85)	<0.001	
HC z-score		0.753*	0.26
- BMI 25.0-29.9	0.61 (0.49, 0.73)	<0.001	
- BMI ≥ 30.0	0.63 (0.54, 0.72)	<0.001	

Notes: All models included 36 week measure, BMI category (kg/m^2), and the interaction between 36 week measure and BMI category as predictors. Estimates are differences in mean estimated birth measure corresponding to a 1 unit increase in 36 week measure, in each BMI category. * denotes p value for test of interaction between 36 week measure and BMI category

6.5 Discussion

The findings of this study demonstrate that among overweight and obese pregnant women, ultrasound assessment of fetal weight at 36 weeks gestation is a reliable indicator of infant birth weight. While fetal ultrasound assessment of HC and AC at 36 weeks gestation is strongly correlated with birth HC and AC, fetal and newborn measures of adiposity were only moderately or poorly correlated.

Strengths of this study include the robust trial methodology of the LIMIT trial, in addition to the adherence to standardised ultrasound and newborn anthropometry protocols (Marfell-Jones et al. 2006, ASUM 2007). This study is the largest to date comparing fetal ultrasound measures at 36 weeks gestation with neonatal anthropometric measures obtained at birth. While this analysis includes data from 845 women and infants rather than the full Standard Care LIMIT group, the risk of selection bias is considered minimal. The characteristics of the current cohort did not differ significantly from either the characteristics of the Standard Care group, or the entire LIMIT cohort (Dodd et al. 2014a, Dodd et al. 2016, Grivell et al. 2016). The findings of this study would be enhanced by the inclusion of data from women entering pregnancy with a normal BMI.

Generally, SFTM are reliable and relatively non-invasive tools to assess newborn fat distribution, having been correlated with more invasive assessments (Moyer-Mileur et al. 2009, Lingwood et al. 2012) including Dual-energy X-ray Absorptiometry (DXA) (Friis et al. 2013). In addition, there is moderate to excellent inter-observer agreement in obtaining both ultrasound (Grivell et al. 2016) and newborn SFTM in this population of women (Kannieappan

et al. 2013) through adherence to standardised research quality protocols, validating their use in a large clinical trial setting. While the use of alternate infant body composition assessments may have been more strongly correlated with fetal ultrasound assessment measures than were observed with SFTM, such an approach is not feasible within the practical constraints of a large-scale clinical research trial.

Importantly, there were no differences identified in the relationship between ultrasound derived fetal and neonatal biometry and adiposity measures according to maternal BMI, despite the well-documented limitations of ultrasound in obese women (Paladini 2009). These findings are consistent with those of Zhang and colleagues, who have also demonstrated no effect from maternal obesity on the quality of fetal biometric measurements (Zhang et al. 2018).

In contrast, fetal ultrasound measures of adiposity were poorly correlated with skin fold thickness measures at birth. While neonatal adiposity has been examined extensively in the literature, few studies have directly compared fetal ultrasound to neonatal body composition. However, there is a lack of consistency in the comparative measurements at birth and this is likely to contribute to the variability in findings. The direct comparison may also be limited by the fact that the caliper used to measure skin fold thickness incorporates a double layer of tissue, which differs from the single layer measured on ultrasound (Borkan et al. 1982). This relationship may not be exactly a 2:1 ratio due to compression of the tissue by the caliper (Borkan et al. 1982).

Fetal thigh and arm circumferences and volumes utilising both 2- and 3-dimensional ultrasound techniques (Khoury et al. 2009, Lee et al. 2009, Ikenoue et al. 2017) have shown the most promising results, improving the predictive value of both macrosomia and infant birthweight in women with obesity (Gibson et al. 2016) and diabetes (Garcia-Flores et al. 2017). There is a clear need for prospective studies with robust methodology, consistency in fetal and neonatal measurement and large sample sizes to further delineate the predictive value of fetal and neonatal adiposity.

The findings of this study validate the use of the 36 week fetal ultrasound as a tool to accurately represent both neonatal biometry and birthweight in women who are overweight or obese. In contrast, the routine incorporation of ultrasound derived fetal adiposity measures is not advocated given their poor correlation with neonatal skin fold thickness measurements.

6.6 Conclusion

Among infants born to overweight and obese pregnant women,

- Ultrasound assessment of fetal weight at 36 weeks gestation is a reliable indicator of infant birth weight;
- Ultrasound assessment of HC and AC at 36 weeks are strongly correlated with newborn measures;
- Fetal and newborn measures of adiposity are only moderately or poorly correlated.
- Maternal BMI did not change the associations between fetal and neonatal measurements

CHAPTER 7: Overall Conclusions

Maternal obesity has a significant impact on pregnancy and birth related outcomes for the woman, the developing fetus, the newborn infant, and in the longer-term, on child- and adult-hood health. While there are well-established links between maternal obesity, high infant birth weight and childhood obesity, the relative contributions of maternal BMI, maternal diet, and cardiometabolic and inflammatory markers to fetal biometry, body composition and growth over time have been under-evaluated. The series of studies contained in this thesis have explored the impact of these factors on fetal growth and adiposity.

7.1 The impact of maternal BMI on ultrasound derived fetal growth and adiposity, and growth velocities

For pregnant women who are overweight or obese

- Increasing maternal BMI is associated with incremental increases in growth velocity of the fetal abdomen circumference and estimated fetal weight.

For pregnant women with BMI $\geq 40.0 kg/m^2$ there is an increase in

- All fetal biometry z-scores at both 28 and 36 weeks gestation; and
- Abdominal fat mass and abdominal area.

7.2 The impact of maternal dietary factors on ultrasound derived fetal growth and adiposity

For pregnant women who are overweight or obese

- Maternal dietary measures are not consistently associated with fetal growth or adiposity.

7.3 The impact of maternal cardiometabolic and inflammatory markers on ultrasound derived fetal growth and adiposity

For pregnant women who are overweight or obese

- Increasing maternal concentrations of adiponectin were associated with a reduction in abdominal circumference and estimated fetal weight;
- The magnitude of this effect increased over gestation;
- Increased triglyceride concentrations were associated with an increase in abdominal circumference z-score and estimated fetal weight at 36 weeks gestation; and
- There were no apparent associations between inflammatory markers, fasting glucose, triglyceride and leptin concentrations and fetal ultrasound measurements.

7.4 Associations between fetal ultrasound biometry and newborn anthropometry

Among infants born to pregnant women who are overweight or obese:

- Ultrasound assessment of fetal weight at 36 weeks gestation is a reliable indicator of infant birth weight;
- Ultrasound assessment of fetal HC and AC at 36 weeks are strongly correlated with newborn measures;
- Maternal BMI contributes a large proportion to the overall variability of ultrasound obtained fetal growth measures; and
- Fetal and newborn measures of adiposity are only moderately or poorly correlated.

7.5 External validity, generalisability, strengths and limitations

As has been identified in the main clinical manuscripts describing the findings of the LIMIT randomised trial (Dodd et al. 2014b, Dodd et al. 2014c, Dodd et al. 2014d, Dodd et al. 2016, Grivell et al. 2016), the trial population was predominately of Caucasian origin, of high social disadvantage and were overweight and obese entering pregnancy. The findings of this thesis may therefore not be generalisable to other populations of pregnant women with different demographic characteristics.

The strengths of this study include the large number of overweight and obese women and their fetuses along with the use of robust methodology (Dodd et al. 2014a, Dodd et al. 2011) as part of LIMIT randomised controlled trial. The main limitation to this study

was the lack of a comparator group of women entering pregnancy with a normal BMI would have enabled on fetal growth patterns across the BMI spectrum.

7.6 Implications for clinical practice

In overweight and obese pregnant women, ultrasound assessment of fetal biometry and EFW is a reliable tool that correlates well with infant birth measurements, and should continue to be used in routine clinical practice. However, there is insufficient evidence to support the routine incorporation of ultrasound measures of fetal adiposity into standard clinical care.

7.6 Implications for further research

The findings generated from the series of studies contained in this thesis highlight the complex nature of factors influencing fetal growth and adiposity. All secondary analyses were conducted among overweight and obese pregnant women, and would benefit from the inclusion of and comparison with data from women of normal BMI. It is anticipated that this will be possible within the next several years, following the publication of the findings from the OPTIMISE randomised trial, evaluating a dietary and lifestyle intervention among pregnant women with a normal BMI (Dodd et al. 2018).

In the past decade, there has been considerable research interest in the provision of antenatal dietary and lifestyle interventions in pregnancy, particularly for women who

are overweight or obese. The underlying assumption has been that dietary and physical activity modification will limit gestational weight gain, with the expectation of improvements in pregnancy and birth outcomes both for women and their infants. However, an individual participant data meta-analysis incorporating data from 36 randomised trials, and more than 12,500 pregnant women globally who received an antenatal dietary and/or lifestyle intervention (International Weight Management in Pregnancy Collaborative 2017) indicates only a modest reduction in gestational weight gain (mean difference -0.7kg), and very little effect on clinical pregnancy outcomes. Future research efforts should target overweight and obese women to facilitate weight loss and adoption of a healthy lifestyle in the period prior to conception.

APPENDIX 1

AUTHORSHIP STATEMENT

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Contribution to the paper Designed the review article
Wrote the manuscript
Acted as the corresponding author

Overall percentage (%) 60%

Signature **Date: 4th December, 2018**

Name of the Co-Author	Dr Amanda Poprzeczny	
Contribution to the paper	Assisted in the development of manuscript plan Wrote a chapter in the review article	
Signature		**Date:** 03/12/2018

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Contribution to the paper	Assisted in the design Reviewed the manuscript	
Signature		**Date:** 03/12/2018

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REVIEW

Implications of maternal obesity on fetal growth and the role of ultrasound

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ABSTRACT

Introduction: Over fifty percent of women entering pregnancy are overweight or obese. This has a significant impact on short and long term maternal and infant health outcomes, and the intergenerational effects of obesity are now a major public health problem globally.

Areas covered: There are two major pathways contributing to fetal growth. Glucose and insulin directly affect growth, while other substrates such as leptin, adiponectin and insulin-like growth factors indirectly influence growth through structural and morphological effects on the placenta, uteroplacental blood flow, and regulation of placental transporters. Advances in ultrasonography over the past decade have led to interest in the prediction of the fetus at risk of overgrowth and adiposity utilizing both standard ultrasound biometry and fetal body composition measurements. However, to date there is no consensus regarding the definition of fetal overgrowth, its reporting, and clinical management.

Expert commentary: Maternal dietary intervention targeting the antenatal period appear to be too late to sufficiently affect fetal growth. The peri-conceptual period and early pregnancy are being evaluated to determine if the intergenerational effects of maternal obesity can be altered to improve newborn, infant and child health.

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Obesity; pregnancy; fetal growth; fetal body composition; adiposity

1. Introduction

Worldwide, it is estimated more than 1.46 billion adults, and 170 million children, are either overweight or obese [1,2]. The global prevalence of obesity has more than doubled between 1980 and 2014 [3], with a more pronounced surge in low- and middle-income countries [2,3]. Based on current trends, it is estimated that more than 50% of adults worldwide will be obese by 2030 [4]. Obesity is the sixth most important risk factor contributing to overall burden of disease worldwide [5], and is an independent risk factor for the development of many associated morbidities, including hypertension, diabetes mellitus, and cardiovascular disease, all of which contribute to a significant reduction in life expectancy [5].

Over 50% of women in high-income countries enter pregnancy with a body mass index (BMI) greater than 25 kg/m^2 [6–8], impacting significantly on maternal, fetal, and neonatal health outcomes, both in the short term during pregnancy and birth, and in the longer term, contributing to high rates of childhood obesity [9–13]. The associations between maternal obesity and subsequent childhood obesity is complex, involving both genetic and environmental factors, with a substantial impact reported to arise from intrauterine programming contributing to longer term health complications [13–15]. A recent commission into childhood obesity found escalation in the prevalence across the world [9], with 41 million infants and children under the age of 5 years identified as overweight or obese [3]. The effect on a child's later life is significant, including the development of diabetes, increased risk of cancer, respiratory disease, cognitive impairment, mental health issues, and reproductive disorders [9]. In addition, there is an impact on educational and recreational opportunities with subsequent economic impact for the family unit and society as a whole [9].

There is considerable interest in understanding the mechanisms underlying fetal growth and adiposity patterns which may contribute to the intergenerational effects of obesity [16]. From a public health perspective, preventive strategies targeting the periconceptual and antenatal periods, may contribute to a reduction in fetal overgrowth and adiposity, slowing the transmission of obesity between generations [17].

1.1. The consequences relating to maternal obesity

Entering pregnancy as overweight or obese are independent risk factors for almost all pregnancy and birth complications, the risk increasing linearly with increasing BMI category [18,19]. Women who are overweight or obese are more likely to develop hypertensive disorders, including preeclampsia [19–25], gestational diabetes mellitus (GDM) [18,20,23–25] and contribute to a significantly increased risk of antenatal stillbirth [20,22,26,27] and preterm birth [18,20,24].

Women who are overweight or obese are more likely to give birth to an infant that is considered large for gestational age (LGA) or macrosomic [13,18,22,24,28], both of which contribute to intrapartum-related complications including an

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increase in the rates of fetal distress, operative birth, including caesarean section, and perineal trauma [29,30]. In the neonatal period, infants born to women who are overweight or obese, irrespective of birthweight, are more likely to be born prematurely, are at higher risk of shoulder dystocia, hypoglycemia, and are more likely to require admission to the neonatal intensive care unit [18–20,28].

2. Large for gestational age, macrosomia, and neonatal body composition

It is important to highlight there is no international consensus regarding the definition, measurement, reporting, and management of the LGA or macrosomic fetus or newborn [31]. Furthermore, the distinction between prenatal ultrasound, which provides an estimate of fetal weight, versus the newborn period, which measure actual birthweight and can be adjusted for gestational age at birth and gender, are often not clear [30].

The definition of the LGA *fetus* is based on prenatal ultrasound, and utilizes measures of abdominal circumference (AC) or estimated fetal weight (EFW) using Hadlock's formula, variably defined as greater than or equal to the 90th, 95th or 97th centile for gestational age [29,30] using population-based charts [32]. The prenatal ultrasound measurement of EFW has a measurement error of ±20% [33] with further reduction in performance in the setting of maternal obesity and at the extremes of fetal weight [34]. The term fetal macrosomia is also variably defined as an EFW greater than or equal to 4000 g or 4500 g [31].

In comparison, the classification of a LGA *newborn infant* is a measurement of birthweight, corrected for infant sex and gestational age at birth [30], although again, this can be variably defined as greater than or equal to the 90th, 95th or 97th centiles. Similarly, infant macrosomia is a postpartum definition based on infant birthweight of greater than or equal to 4000 g or 4500 g [29].

There has been interest in using customized growth charts to identify the infant at risk of growth disorders, taking into account factors such as maternal ethnicity, height and weight, infant sex, and gestational age [35]. Some have advocated the use of customized growth charts to define the LGA infant (birthweight >90th centile), as being superior in prediction of neonatal morbidity, when compared with definition of macrosomia (BW ≥4000 g) [30], postulating that the higher predictive value relates to improved detection of excessive fetal growth or alteration in fetal body composition taking into consideration the constitution of the mother [30,36]. However, it remains to be determined whether the consideration of maternal overweight and obesity and its effects on fetal growth as 'physiological' rather than 'pathological' is appropriate in this setting.

Birthweight as a single measure reflects mass, and does not reflect variations in the distribution of adipose tissue nor the relative proportion of adipose and lean tissue mass. Lean body mass has been correlated with genetic factors, whereas fat mass has been correlated with the maternal environment [37]. Most studies reported in the literature have compared lean and adipose tissue masses in infants born to women who are overweight or obese with infants born to lean women [38,39]. The intrauterine metabolic environment has been shown to affect the growth of adipose tissue but not lean tissue mass [40]. Neonatal fat mass accounts for approximately 14% of the total birthweight but has been shown to significantly contribute to the birthweight variation in over 50% of newborns [40,41]. These studies have shown that as maternal BMI increases, so too does neonatal adipose tissue mass [38,39], which in turn is correlated with an increased risk of childhood obesity and longer term metabolic dysfunction [42].

In contrast, the effect of maternal obesity on newborn lean tissue mass remains uncertain. While some have reported no association with maternal obesity [38], others have reported associations between maternal obesity, lower newborn fat free mass, and higher total and percentage of fat mass as measured by air displacement plethysmography [39]. There is a need for ongoing research into this area, including the longer term follow-up of children to assess the impact of neonatal adipose tissue distribution on subsequent childhood obesity.

3. Clinical management for the large for gestational age fetus

Despite the limitations identified above relating to the definitions of LGA and fetal macrosomia, the widespread availability of ultrasound and concerns relating to maternal and infant pregnancy and birth complications, has led to interest in the prediction of fetal macrosomia [43] in an attempt to reduce morbidity through active clinical management.

Clinical management options remain controversial for women who are identified to have a macrosomic fetus in the antenatal period [31]. A large decision analysis study by Rouse identified that an elective caesarean section for EFW greater than 4.5 kg, was not an economically viable treatment option [44]. The cost of elective caesarean birth did not outweigh the prevention of shoulder dystocia and brachial plexus injuries [44]. In contrast, a similar study reached the opposite conclusions, when considering the 'costs' related to maternal perineal trauma and subsequent fecal and urinary incontinence issues for the woman, in addition to the direct effects of shoulder dystocia and brachial plexus injuries for the offspring [45]. Importantly, any short or longer term 'costs' related to the effects of birth asphyxia have not been incorporated into these decision analyses [31].

A recent multicenter randomized trial involving 19 tertiary centers including 832 women with an average BMI between 25–26 kg/m^2 with a fetus suspected to be LGA, were randomized to elective induction of labor or continued expectant management [46]. The trial identified a reduction in neonatal morbidity following induction of labor between 37 and 39 weeks following ultrasound identification of a LGA fetus defined as an EFW greater than the 95th centile [46]. This type of clinical intervention was not associated with differences in a woman's risk of caesarean birth. Incorporation of 4 similar randomized trials in a meta-analysis involving a total of 1190 women, identified that induction of labor was associated with a reduction in the occurrence of shoulder dystocia, and any type of neonatal fracture, although there are no statistically

significant differences in the rates of operative delivery, brachial plexus injury, low 5-min Apgar scores or low arterial cord blood pH [47]. The generalizability of these findings for women who are overweight or obese is uncertain, particularly taking into consideration the reduction in accuracy of ultrasound estimation of fetal weight in this clinical setting [43,47].

The clinical management debate will continue with consideration of multiple factors including a woman's autonomy, obstetrician factors, ultrasound availability, prediction and accuracy, evidence surrounding intervention, and concern about rising caesarean birth rates in the developed world [31].

4. The fetal 'overgrowth' hypothesis

In 1967, Pedersen and associates first proposed a hypothesis to describe the underlying mechanism relating to overgrowth of the fetus, seen primarily in women with diabetes mellitus during pregnancy [48]. This is commonly known as the direct pathway for fetal 'overgrowth' and is more commonly referred to as the Pedersen hypothesis [48]. Glucose has been long recognized as the primary fuel substrate for fetal growth and development, and is delivered across the placental interface via transport mediated facilitated diffusion [49,50]. In the setting of maternal hyperglycaemia and hyperinsulinaemia related to both maternal obesity and gestational diabetes, there is a greater diffusion gradient of glucose, which in turn leads to fetal hyperglycaemia [49]. Hyperglycaemia in the fetal circulation, stimulates insulin production by the fetal pancreas, insulin-like growth factors (IGF), growth hormones, and a range of other growth promoting factors, all of which stimulate fetal deposition of glycogen and fat [48]. More recently, the hypothesis has been expanded to account for the placental transfer of lipids and their contribution to fetal growth 13[40].

Increasingly, there is evidence to support an 'indirect' pathway that can impact the delivery and quantity of the nutritional supply to the fetus across the placenta [50,51]. The placenta is the interface between the maternal and fetal circulations, providing critical and complex functions for the developing fetus. The placenta plays an integral role in fetal growth through the regulation of blood flow, oxygen delivery, and nutrient transfer across the placenta [52]. While the placenta is likely to be an important mediator by which maternal obesity contributes to fetal overgrowth and adiposity [53], there is a relative paucity of literature describing its role in the regulation of fetal growth in this setting. Various hypotheses include the regulation of placental transporters [53] and the nutrient transfer capacity of the placenta, which directly relate to the structural and morphological features of the placenta, in addition to uteroplacental blood flow [50,54].

5. Fetal growth restriction in the setting of maternal obesity

Large population cohort studies have identified maternal obesity to not only be associated with fetal overgrowth but also with growth restriction [22–24]. While, the risk of fetal growth restriction and small for gestational age (SGA) infants in obese women is approximately 2.3% in women with a BMI greater than or equal to 40 kg/m^{2,} the incidence for SGA is much smaller than an appropriately grown infant (82.7%), or LGA infant (14.9%) [22]. These rates are similar to those derived from large randomized trials. The LIMIT trial identified a risk of infant birthweight less than 2.5 kg (or SGA) to be approximately 4.7% [55], consistent with the findings from the UPBEAT randomized trial [56] which utilized customized birthweight centiles. Similarly, the ultrasound measurements relating to fetal growth, including weight, were consistently above the mean, as discussed subsequently [57].

Observational studies have identified women who are overweight or obese to be at increased risk of perinatal death, when compared with women of normal BMI [26,27,58]. In this setting, the majority of stillborn infants were identified to be SGA, particularly beyond 37 weeks' gestation [26,27]. Furthermore, higher rates of fetoplacental dysfunction were found in women who are obese (5.4 fetal deaths per 1000 live births) compared with women with a normal BMI (1.4 fetal deaths per 1000 live births) [26].

The underlying mechanisms for a reduction in uteroplacental blood flow may relate to the exaggerated hyperlipidaemia along with increased free fatty acids and cholesterol, which may potentially increase the risk of placental thrombosis and subsequently reduce placental perfusion [26]. Another potential explanation may reflect the high rates of preeclampsia identified in women with increasing BMI [20–23,25,34]. Oxidative stress and endothelial dysfunction from obesity may impact on trophoblastic invasion and contribute to poorer pregnancy outcomes, such as preeclampsia and placental insufficiency [59]. Preeclampsia and defective trophoblastic invasion in turn affects placental function and may alter the fetal growth potential [59]. There remains uncertainty surrounding the exact mechanism contributing to reduced growth in fetuses born to women who are obese and whether inadequate trophoblast invasion or perfusion defects comes first.

Impaired fetal growth in the setting of maternal overweight and obesity may also reflect the effects of maternal weight loss, which is more likely among obese women during pregnancy [60]. While lower maternal weight gain during pregnancy has been associated with a reduction in the risk of LGA infants and many pregnancy-related complications, it appears to be at the expense of an increase in SGA infants [61]. While observational studies highlight this association, it is unclear if the contribution of weight loss to poor fetal growth reflects impaired nutrient delivery to the fetus, or whether other mechanisms are operational [60].

6. Metabolic determinants of fetal growth

6.1. Glucose

Maternal obesity, even in the absence of GDM, is associated with higher glucose concentrations, contributing to an intrauterine hormonal environment that is similar to that associated with metabolic syndrome, characterized by hyperglycemia and insulin resistance [40]. Offspring born to women who are obese have documented higher cord blood glucose and insulin concentrations, and are more insulin resistant

[49,62,63]. This relationship between maternal obesity and insulin resistance measured in neonatal cord blood is present irrespective of a diagnosis of gestational diabetes [49]. Furthermore, findings from the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study have confirmed a linear relationship between maternal glucose concentration and infant birthweight, even at glucose concentrations below those considered to be diagnostic of GDM [64]. Similar relationships between maternal glucose concentrations below the diagnostic threshold of GDM, and adverse neonatal outcomes related to insulin resistance and glucose intolerance have been described in the other populations, including North America, the United States, and the United Kingdom [49,64,65].

6.2. Maternal hormones

Leptin, adiponectin, lipid metabolism, and insulin growth factors have all been identified to contribute to fetal growth in a complex fashion. Table 1 summarizes the key metabolic substrates, their proposed physiology, effect on fetal growth, and changes which have been identified in the setting of maternal obesity.

6.3. Adipose tissue as a metabolically active contributor to fetal growth

Adipose tissue is not simply an inert storage organ, but is metabolically active in the secretion of multiple hormones which contribute to metabolic homeostasis [80]. Fetal adipocytes begin to develop at 15 weeks and as gestation advances, there is an increase in fetal fat mass from 5% to 15% [81]. The development of adipose tissue in the fetus and early neonatal life is sensitive to hormones such as insulin, IGF and glucocorticoids [82]. While it is recognized that the human fetus deposits a large amount of subcutaneous fat in late gestation [83], subscapular and axillary fetal adiposity predominantly reflects brown adipose tissue (BAT) deposition, which is required for non-shivering thermogenesis in the immediate adaptation to extrauterine life [83,84]. While it was initially thought that the presence of BAT was confined to early infancy, deposits have been identified in adults at sites that echo those of the neonate, through the use of positron emission tomography [84], being more commonly identified in women and lean individuals. Furthermore, the role of BAT in energy production and increasing basal metabolic rate has resulted in its identification as a potential target to ameliorate the effects of obesity [83,84].

Similar to the relationships between adipokines and overweight and obesity in adults, cord blood concentrations of leptin correlates positively with infant birthweight and neonatal fat mass [49,71–74,85,86]. Cord blood leptin concentrations have also been shown to positively correlate with measures of neonatal insulin resistance [49], suggesting that neonatal fat mass and insulin resistance are related, and raising the possibility that neonatal adipose tissue is also metabolically active.

6.4. The inflammatory response to maternal obesity

Women who are overweight or obese, enter pregnancy with an altered inflammatory environment, which may predispose to the development of pregnancy-related complications including hypertension and gestational diabetes [87,88]. Obesity (both in pregnancy and nonpregnant individuals) is associated with a low-grade, chronic inflammatory state [89], which in animal models has been associated with the

Table 1. The key metabolic substrates during pregnancy, their effect on fetal growth, and changes related to maternal obesity.

Factor	Physiology	Effect on fetal growth	Changes in maternal obesity	References
Glucose	Crosses the placenta via GLUTs Main energy source for the fetus	Promotes fetal overgrowth	Higher in maternal obesity Increased risk of GDM and hyperglycemia Positively associated with birthweight	Metzger [64] Catalano [49] Uebel [63] Torloni, 2009 [66]
Lipids	Early pregnancy – maternal fat accumulation Late pregnancy – maternal hyperlipidemia	Contributes to fetal fat deposition in the third trimester	Higher serum triglycerides throughout pregnancy Independently associated with risk of LGA and neonatal measures of adiposity	Son, 2010 [67] Vrijkotte, 2011 [68] Whyte, 2013 [69] Schaefer-Grafe, 2008 [70]
Leptin	Produced predominantly by white adipose tissue Involved in regulatory control of placental nutrient transport	Promotes fetal overgrowth	Higher circulating levels Higher cord blood levels in offspring Positively correlates with birthweight, neonatal adiposity and neonatal insulin resistance	Tessier [71] Tsai, 2015 [72] Catalano [49] Karakosta [73] Josefson [74]
Adiponectin	Produced by adipose tissue Contributes to peripheral insulin sensitivity Reduces nutrient availability for the placenta	Negative regulator of fetal growth	Maternal levels lower in obesity Maternal levels negatively correlate with birthweight and neonatal fat mass	Ategbo, 2006 [75] Lowe, 2010 [76]
IGF's	Family of ligands (IGF's) and ligand-binding proteins (IGFBP's) Produced by the liver Contributes to placental invasion, growth and development Stimulates differentiation of pre-adipocytes	Relative levels of IGF's and IGFBP's determine effect on fetal growth – free (bioactive) IGF is promotor of fetal growth	Reduced expression of IGFBP4 in cord blood of offspring, resulting in higher levels of free IGF	Ferraro, 2012 [77] Qiu, 2005 [78] Juul, 2003 [79]

activation of adipokines and the resultant inflammatory cascade, contributing to insulin resistance [90].

Obesity is associated with a pro-inflammatory state, resulting in an increase in the secretion of pro-inflammatory cytokines from the adipose tissue [91]. In pregnancy, maternal obesity is associated with an increase in IL-6 compared with women with a normal BMI [89,90]. The literature remains unclear regarding other cytokines during pregnancy and is limited by small sample size and study design [88–90]. There has been one study that has investigated the association between maternal cytokine concentrations with fetal adiposity measurements. While maternal inflammatory markers were identified to correlate with maternal adiposity, these were not related to measures of fetal adiposity [88].

The placenta has been hypothesized to play a role in the mediation and regulation of the inflammatory reaction related to obesity [89]. Maternal inflammation may induce fetal programming through the passage of specific cytokines (IL-6) or immune cells (maternal monocytes, T and B cells), in addition to modifying the availability of nutrients for the fetus through placental regulation of IL-1 beta [91]. Due to the placental changes during gestation, this could potentially lead to variations in transfer of cytokines and immune cells with potential differential fetal effects across pregnancy [91].

7. The role of the placenta in fetal growth

The placenta is a complex structure compromising of chorionic villi and vasculature, which evolves through gestation [92]. Factors which disturb or disrupt this process have the capacity to permanently alter placental function [53].

The structure and morphology of the placenta including placental weight is a major determinant of fetal growth, directly reflecting the capacity of the nutrient transport system [53]. Placental weight has been demonstrated to increase with maternal BMI and has been estimated to be approximately 4.4 g per additional kg/m^2 increase in maternal weight [93]. Placental nutrient transfer is dependent upon the number of transporters present, which in turn has been shown to be regulated by maternal endocrine and nutritional signalling [53].

Uteroplacental blood flow also has a fundamental role in nutrient transfer to the fetus. Key to this process is maternal uterine artery blood supply, with alterations in blood flow as early as the first trimester of pregnancy having been associated with an increased risk of poor placentation and the subsequent development of preeclampsia and fetal growth restriction [94]. Placental perfusion may also be reduced by higher maternal concentrations of circulating lipids, free fatty acids, and cholesterol, particularly in women who are obese, which has been postulated to contribute to an increased risk of placental thrombosis and reduced perfusion [26]. In turn, these underlying perfusion-related changes may contribute mechanistically to the higher risk of stillbirth and preterm birth observed in the setting of maternal obesity [26]. Additionally, oxidative stress and endothelial dysfunction may both contribute to and result from impaired trophoblastic invasion, and therefore the subsequent development of hypertensive diseases including preeclampsia [59].

The fetal umbilical artery (UmA) delivers deoxygenated blood from the fetus back to the placenta, and is measured routinely during ultrasound assessment of fetal well-being [95]. Sarno and colleagues have conducted ultrasound UmA Doppler assessment in 185 women, of whom 23.2% were overweight, and 21.6% obese. When compared with lean women, women of higher BMI were found to have significantly higher UmA resistance. The positive correlation between maternal BMI and ultrasound determined UmA resistance suggests a further mechanism whereby placental perfusion may be altered in the setting of maternal obesity [96].

Changes in maternal nutrition in the setting of obesity suggest that the majority of women who are obese or who have gestational diabetes would give birth to an infant LGA, reflecting the higher diffusion gradient and stimulation of growth by glucose, insulin, and IGF [53]. However, many obese women and women with gestational diabetes have an appropriately grown infant. There have been several theories postulated to explain the 'normalization' of fetal growth in the setting of maternal obesity [96]. For example, uteroplacental insufficiency could reduce substrate delivery thereby normalizing the anticipated acceleration in fetal growth [96]. Another possible explanation is that maternal obesity or gestational diabetes alone may be insufficient to induce fetal overgrowth but additional exposures such as endocrine signalling, expression of transporters, and change in lipid and amino acid transfers together could combine to increases in fetal growth and adiposity [53].

8. The measurement of fetal growth and body composition using ultrasound

Ultrasound has become the mainstay in the assessment of fetal growth and well-being and is widely utilized in both low- and high-income countries. There have been numerous studies evaluating ultrasound markers to identify and predict the LGA fetus, all of which have utilized different measurements, definitions, and cut-off points [97–101], as outlined above and described in Table 2. There is no universally accepted definition or specific measurement used in the detection of LGA fetus in the antenatal period [31]. As a result of maternal and infant complications, coupled with the growing access to ultrasound, there has been increasing interest in the use of ultrasound to attempt to predict newborn macrosomia in order to reduce complications such as shoulder dystocia [43].

Table 2 summarizes the key articles and systematic reviews that have assessed the sensitivity and predictive value of a range of ultrasound markers and cut-points to predict infant macrosomia and LGA. Coomarasamy and associates performed a large systematic review of the evidence pertaining to diagnostic ultrasound and the prediction of the large for gestational infant [97]. While there was considerable heterogeneity between the studies including different study designs, EFW formulae used, ultrasound equipment and reference range thresholds, EFW, and AC greater than 90th centile were both identified to have good positive predictive values of 9.3 and 4.2, respectively [97]. However, the influence of maternal BMI on these assessments is difficult to ascertain, as there was no specific subgroup analysis relating to maternal BMI.

Table 2. Prenatal ultrasound measurements and the prediction of large for gestational age infant.

Author, year	Study details	Ultrasound marker	Strengths	Limitations	Maternal BMI	Prediction of LGA infant
Coomarasamy, Connock et al. [97]	*N* = 147 Studies = 2 Part of a large systematic review	EFW greater than 90th centile	Pooled analyses Small numbers Moderate to high quality	Clinical heterogeneity (different methods estimated EFW and AC)	No specific analysis	Positive LR 9.3 (3.7–24) Negative LR 0.4 (0.14–0.93)
Coomarasamy, Connock et al. [97]	Studies = 5 *N* = 1864 Part of large systematic review	AC >90th centile	Pooled analyses Moderate to high quality	Different ultrasound equipment and reference standard thresholds	No specific analysis	Positive LR 4.2 (2.3–7.7) Negative LR 0.33 (0.21–0.54)
Kernaghan, Ola et al. [98]	*N* = 242 Pre-existing and gestational diabetes Sheffield, The U.K.	EFW *z*-score (≥1.7 or ≥95.5th centile)	Prospective design	Included women with diabetes alone	No analysis	Prevalence = 27% PPV = 51% NPV = 91% LR positive test = 2.8
Kernaghan, Ola et al. [98]	*N* = 242 Preexisting and gestational diabetes Sheffield, The U.K.	Fetal growth velocity (FGV)	Prospective design	Included women with diabetes alone	No analysis	Prevalence = 27% PPV 35% NPV 76.1%
Wong, Chan et al. [102]	*N* = 100 Retrospective study at the Mater Hospital in Brisbane, Australia 1994–1999	AC extrapolated from clinical ultrasound reports *z*-scores calculated	Confounding variables were not adjusted for in analyses Small numbers	Retrospective Included only diabetes	Mean BMI was in the overweight range LGA group had a higher BMI	Progressive increase in *z*-scores as gestation advances in newborns with LGA Difference in mean *z*-score was 0.68 at 18–22 weeks to 1.96 at 34–38 weeks)
Pates, McIntire et al. [100]	*N* = 3115 Retrospective cohort study in Texas, The U.S.A. during 1997 and 2006	BW ≥4000 g ± AFI ≥20 cm ± Risk factors for macrosomia	Selection bias due to the clinical concern of macrosomia lead to a USS	Retrospective design Indications for clinical scans were variable	BMI >30 accounted for 81% of BW >4000 g compared with 41% in BW <4000 g	Neonatal macrosomia rate = 7.6% PPV using USS, AFI, and risk factor was 71%, NPV = 94% Sn = 29%, Sp = 29%
El Khouly, Elkelani et al. [101]	*N* = 600 Prospective observational study from large maternity hospital in Egypt between 2014–2016	Ultrasound in the 1st stage of labor EFW >4000 g and AFI >16.4 cm	Prospective design Sample size	Lack of clinical outcomes	No separate analysis performed	10.6% macrosomia rate based on newborn weight 2% incidence if Diabetes Combined EFW and AFI had a PPV = 92.3% in the detection of macrosomia EFW alone (PPV 75%) and AFI (27%)

PEAPOD: Air displacement plethysmography; AFI: Amniotic fluid index; PPV: Positive predictive value; NPV: Negative predictive value; LR: Likelihood Ratio; GWG: Gestational weight gain; USS: Ultrasound.

Fetal growth velocity has been demonstrated to have low positive predictive value compared with EFW greater than the 95th centile [98] in the prediction of LGA infants. Wong and colleagues identified that the prevalence of LGA infants was higher among women who were both obese and diagnosed with gestational diabetes, compared with women of normal BMI [99]. More recent studies have combined EFW and amniotic fluid index together to increase the positive and negative predictive value in identifying the LGA infant, with variable results obtained in women considered to be at increased risk [100], despite better performance in low risk women entering labor [101].

In summary, there is a lack of robust evidence to provide a consensus and predictive cut-off values using ultrasound, particularly in women who enter pregnancy overweight or obese [31]. Specifically, there have been no randomized trials that have assessed the fetal surveillance regimens in the detection of the LGA fetus and whether such tools can improve maternal and infant outcomes [103].

9. Antenatal ultrasound assessment of fetal body composition

In 1991, Bernstein and colleagues were among the first to describe the measurement of fetal body composition using prenatal ultrasound [104]. Since then, there have been substantial advances in both two- and three-dimensional ultrasonography, resulting in the development and validation of fetal body composition measurements [105–109] as outlined in Table 3.

Most of the identified studies have been limited by relatively small sample sizes [106,109,110], with wide variation in both the type of fetal body composition measurement utilized, and the reporting of results [106,108–111]. Larciprete and colleagues validated the three main fetal body composition measurements in a population of 218 healthy pregnant Italian women with a normal BMI [111]. The generalizability of these measures in other populations, particularly women who are overweight or obese would appear to be more limited.

Table 3. Fetal body composition measurements using prenatal ultrasound.

Author, year	Ultrasound measurement	Definition	Study details	Strengths	Limitations	Results
Walsh, Wallace et al. [105]	Anterior abdominal wall (AAW)	Axial view of abdomen 2–3 cm laterally from the cord insertion at 34 weeks gestation	*N* = 50 Healthy, non-diabetic women with previous history of infant with BW >4000 g Nested cohort study within the ROLO RCT National Maternity Hospital, Dublin	First study to assess metabolomics and fetal adiposity measurements	Selection bias due to previous delivery Represents women who are at risk of both impaired glucose tolerance Small sample	HOMA and AAW were significantly associatd with increased metabolonomics at 28 weeks (Biotin, valine, 2-hydroxyisovalerate, Hisitidine, malonate, taurine)
O'Connor, Doolan et al. [106]	Fetal abdominal subcutaneous tissue (FAST)	Axial view of abdomen in AC view, using magnification, measurement of subcutaneous tissue anterior to the margins of the ribs proximal to the cord insertion	*N* = 62 Ultrasounds performed at 28, 32, 38 weeks March 2012–2013 Coombe Women's Hospital Recruited after 1st trimester scan	Use of PEAPOD in non-invasive, accurate measurement of infant body fat percentage	Small sample Underpowered	Associated with birthweight, AC and infant fat mass at 38 weeks measured on the PEAPOD (air displacement plethysmography) calculation of neonatal fat mass on D3.
O'Connor, Doolan et al. [106]	Thigh fat (TF) and thigh muscle (TM)	Distance from the outer border of the femur to the outer border of the subcutaneous layer, subtracting the muscle value	*N* = 62 Ultrasounds performed at 28, 32, 38 weeks March 2012–2013 Coombe Women's Hospital Recruited after 1st trimester scan	Use of PEAPOD in non-invasive, accurate measurement of infant body fat percentage	Small sample Underpowered	Thigh fat at 28 and 38 weeks correlates well with infant fat mass in all mothers
Larciprete, Valensise et al. [107]	Mid-thigh lean mass (MTLM) and fat mass (MTFM) Units = cm^2	Sagittal view of long bone, rotate transducer 90° to get axial view. Total cross sectional limb area and subtracting the central lean area consisting of muscle and bone	*N* = 218 (Healthy women) *N* = 85 (Diabetic women) High-risk patients from an Italian hospital, recruited at 20 weeks from Jan to Dec 2001 Inclusion criteria included family history of DM, BMI >27 weeks, glycosuria, previous large baby, previous GDM, age >37 weeks, polyhydramnios	Serial USS every 3 weeks until term gestation Assess reproducibility Development of reference ranges	Different population and screening for GDM due to 100 g glucose tolerance test	Normal reference ranges from the healthy women group All measurements were greater in women with GDM compared with the healthy pregnant women. 11.9 versus 14.2cm^2 *P* = 0.02 at 37–40 weeks
Larciprete, Valensise et al. [107]	Subscapular fat mass (SFM, mm)	Shoulder skin width perpendicularly to the bone at its lower end	As above	As above	As above	Normal reference ranges described from the healthy women group. Higher measures in women with GDM compared with healthy pregnant women. 5.3 versus 6.7 mm, *P* < 0.01 at 38–40 weeks
Larciprete, Valensise et al. [107]	Abdominal fat mass (AFM)	Measuring thickness of the anterior abdominal subcutaneous tissue on the same axial image used for the AC calculation	As above	As above	As above	Normal reference ranges described. Higher measures in women with GDM compared with healthy pregnant women 6.18 versus 6.8 mm, *P* = 0.03 at 39 weeks

(*Continued*)

Table3. (Continued).

Author, year	Ultrasound measurement	Definition	Study details	Strengths	Limitations	Results
Hure, Collins et al. [108]	Fetal Abdominal fat area (cm^2)	At the level of AC measurement, total area (A1) subtracted from the lean abdominal area (A2)	N = 179 Longitundal cohort Australian WATCH study 2006–2008 John Hunter Hospital, New South Wales Inclusion of	Longitundal study Adjusted for confounders	Self reported data for maternal weight and calculation of BMI	Gestational weight gain predicted fetal AC and lean abdominal mass BMI and GWG had larger lean muscle mass but no increase in fat mass
	Fetal mid-thigh fat and lean mass (cm2)	Mid point of the femur, total cross-sectional area (T1) and muscle mass (T2) with fetal mid-thigh fat mass calculated by T1 – T2	As above	Longitundal study Adjusted for confounders	As above	Strong correlation between femoral lean area and neonatal fat mass (r = 0.7, P < 0.001) and moderate correlation (r = 0.63, P < 0.001)
Gibson, Stetzer et al. [109]	Fractional thigh volume (3-dimensional)	Sub volume that includes 50% of the femoral diaphysis length centered around the mid-femoral shaft	N = 34 neonates Prospective observational study Inclusion: Term, singleton newborns with suspected macrosomia, enrolled upon admission to Delivery Suite Newborns were assessed 48 h post	Use of new technology	Small numbers Selection bias No control group Specialized transducers, software and training required to performed 3-dimensional	Fractional volumes correlated with birthweight z- score (R^2 = 0.52, P < 0.001) and percentage of body fat measured anthropometry (R^2 = 0.22, P = 0.04)

Low glycaemic index diet in pregnancy to prevent macrosomia: randomised controlled trial (ROLO RCT)

The largest study to date to assess fetal biometry and body composition measurements using ultrasound was performed by Grivell and colleagues [57]. This study included women enrolled in the LIMIT trial [112] who were randomized to receive a comprehensive lifestyle intervention across pregnancy compared with women who received standard antenatal care. While the proportion of newborns classified as LGA (birthweight above the 90th centile), did not differ between the two groups, the intervention was associated with a significant 18% relative risk reduction in the chance of infant birthweight greater than or equal to 4 kg [112], and a 41% reduction in risk of birthweight above 4.5 kg [113]. Women who received the antenatal intervention demonstrated significant improvements in their self-reported dietary intake [113] and physical activity, when compared with women randomized to the standard care group [113].

In this setting, fetal body composition measurements and biometry were obtained from 1847 women at both 28 and 36 weeks' gestation [57]. Fetal z-scores for all biometry and adiposity-related measures were above the population means, regardless of treatment group, indicating that the fetuses of women who are overweight or obese have growth measures above population standards [57]. Furthermore, increases in head and AC growth were both identified to contribute to the increase in EFW [57], compared with the increase in AC only, which has in the past been demonstrated in fetal growth in women with gestational diabetes [102,114,115].

Two-dimensional fetal body composition measurements have been poorly correlated with neonatal measurements of body composition, lean tissue, and body fat mass [116–118]. As a result, there has been increasing interest in three-dimensional imaging due to its availability over the past 5 years. Gibson and associates have assessed body composition in those fetuses with suspected macrosomia using three-dimensional mean thigh volume. Thigh volume z-scores were correlated with infant birthweight (R^2 = 0.52 [0.54–84], $P < 0.001$) and neonatal anthropometric body fat (R^2 = 0.22 [0.17–0.69], $P = 0.04$) and skinfold thickness measurements including triceps, subscapular, umbilical, flank, and thigh skinfolds [109]. With the increasing availability of air displacement plethysmography for the assessment of neonatal body composition, Lee and associates have identified that three-dimensional fractional limb volume is correlated well with calculated neonatal fat mass [118]. However, despite these promising findings, fractional limb volume measurement using three-dimensional imaging requires specific training, specialized software, and is not routinely used in clinical practice [109].

In summary, to date there is no 'gold standard' measure that can be utilized in the ultrasound prediction of the LGA fetus, macrosomia, or fetal adiposity [31]. Further research is required, specifically focusing on women who enter pregnancy overweight or obese, as these fetuses remain at high risk of future health complications.

10. Can fetal growth be modified through intervention?

In the recent past, there has been considerable interest in the evaluation of antenatal dietary and physical activity interventions as a strategy to limit gestational weight gain and reduce the risk of maternal and perinatal complications. A comprehensive review by Thangaratinam and colleagues identified 44 studies assessing lifestyle and dietary interventions, in which provision of an antenatal intervention was associated with a modest reduction in gestational weight gain 1.42 kg (95% confidence intervals 0.95–1.89) [119]. Importantly, few trials reported the effect of the intervention on ultrasound measures of fetal growth.

The LIMIT trial represents the largest trial to date to comprehensively perform ultrasound-based assessment of fetal growth and different adiposity measurements [57] comparing an antenatal dietary and lifestyle intervention provided to pregnant women who were overweight or obese [112]. In this context, fetuses of women who received the antenatal dietary and lifestyle advice were shown to have a slower rate of subscapular adipose tissue deposition and a higher mean mid-thigh fat mass (MTFM). It has been suggested that lower body fat deposition may have a greater protective role, when compared with upper body fat deposition, which is more likely to be associated with type 2 diabetes and cardiovascular disease [120].

Metformin has also been investigated in pregnancy as an adjuvant therapy for women who are overweight or obese. Metformin is an oral biguanide that can increase the sensitivity to insulin, in addition to its recognized anti-inflammatory properties [94]. Metformin has been used in a number of fertility-related settings, largely in women with a diagnosis of polycystic ovarian syndrome (PCOS) [121]. While metformin treatment in women with PCOS has been associated with weight loss prior to conception, the effect on pregnancy-related outcomes is less clear [121].

Two recently completed and published randomized trials have utilized metformin treatment specifically in pregnant women who are obese [122,123]. While both of these trials reported no differences in either the diagnosis of gestational diabetes or infant birthweight [122,123], Syngelaki reported metformin use to be associated with a reduction in gestational weight gain and the occurrence of preeclampsia [123]. These findings highlight the complexity surrounding maternal obesity and effect on fetal growth, with insulin resistance only part of a highly complex and regulated pathway [122]. The findings of the GROW randomized trial, evaluating the use of metformin as an adjuvant to dietary and lifestyle advice in pregnant women who are overweight or obese, are awaited [124]. Ongoing follow-up of the children whose mothers participated in these trials is important, as there is evidence that the use of metformin in the treatment of gestational diabetes is associated with lower visceral body fat at 2 years of age compared with children born to women who had treatment with insulin [125], despite no difference in infant birthweight [126].

The lack of measurable effect in ultrasound measures of fetal biometry and adiposity suggests that modification of maternal diet in the second trimester of pregnancy may be 'too late' to substantially modify fetal adiposity and growth, raising the possibility of targeting women who are overweight or obese prior to conception as a strategy to improve health outcomes for women and their children. Opray and colleagues performed a systematic review evaluating the effect of weight

loss prior to conception among women who are overweight or obese on pregnancy and birth outcomes [127]. No randomized controlled trials targeting this period to promote weight loss in overweight or obese women generally, were identified. Challenges of interventional trials in the preconception period and assessment of birth-related outcomes include the identification, recruitment, and following up of women before and after pregnancy [128]. Despite these challenges, future research into this important area is required as a matter of priority and current trials are already underway [129,130].

11. Expert commentary

The intergenerational effects of obesity have been highlighted as a major public health issue, and is associated with the escalating rates of maternal, infant, childhood, and adolescent obesity, across the world.

Both obesity and gestational diabetes share a similar complex pathophysiology, contributing to metabolic dysfunction in both the woman and her fetus. Key mechanisms relate to the exaggerated reduction in peripheral insulin sensitivity in the woman, beyond the normal physiological response, which in turn, increases glucose availability for the fetus, a direct pathway to increase fetal growth. Other important metabolic determinants of fetal growth include leptin, adiponectin, insulin growth factors, and lipids. There is also increasing evidence of an indirect pathway contributing to fetal growth, involving the up-regulation of placental transporters, the impact of placental structure and morphology, along with contributions from uteroplacental blood flow.

While technological advances in ultrasonography has led to increasing interest in the identification of the LGA fetus following the measurement of fetal body composition (body fat versus lean tissue mass), there is no consensus regarding the appropriate measures to be utilized, impacting greatly on the quality of the evidence surrounding this important outcome. With improvements in the sensitivity of measures of fetal growth and adiposity, it may be possible not only to identify subtle changes reflecting the effects of both preconception and antenatal interventions but also to more appropriately identify the fetus at increased risk of adverse perinatal outcomes, and the child at risk of obesity. In time, these interventions may represent efficacious strategies to impact the vicious cycle of maternal obesity and the intergenerational effect for the infant and child.

12. Five—year view

The United Nations Assembly in April 2016 called for an urgent response to the obesity epidemic with a global refocus, a 'Decade of Action on Nutrition' [128], as has the World Health Organization, in response to the commission on childhood obesity [9]. This will lead to increasing public health campaigns over the next 5 years, including health education and promotion strategies to ensure a move towards a global improvement in nutrition, particularly aimed at children and adolescents. This will be accompanied by further advances in both basic and clinical sciences investigating the mechanisms underlying fetal growth and adiposity.

Periconceptual care and early pregnancy interventions are being highlighted as a key intervention period that could potentially influence a future child's body composition, growth and health consequences [131], with a focus on intervention trials to reduce maternal and paternal obesity prior to conception. Interventions and health education during this periconceptual period will be key in promoting a more favorable intrauterine environment, that could 'normalize' fetal growth and body composition for the infant and child.

There will be paradigm shift from increasing antenatal care as a woman approaches term, to early pregnancy screening to identify the woman at risk of preterm birth, preeclampsia, and GDM and then institute appropriate monitoring and treatment via models stratified for low- or high-risk antenatal care [132,133]. The first trimester of pregnancy could therefore represent an ideal time to identify women at risk of obesity-related complications and gestational diabetes, and to introduce interventions, education, and support for these 'higher risk' women [132].

The intergenerational effects of obesity remain an important area of ongoing research. If interventional studies can reduce the impact of maternal obesity on subsequent child obesity, this will be a significant step forward and one of the greatest legacies we can give the next generation.

Key issues

- The intergenerational effects of obesity have been highlighted as a major public health issue, leading to interest in understanding and modifying fetal overgrowth to reduce childhood obesity rates.
- Direct effects of glucose and insulin and the indirect effects of leptin and adiponectin, insulin growth factor along with placental factors can influence fetal growth.
- Ultrasound technology has led to interest in the prediction of fetal overgrowth and adiposity, however, there remains no international consensus regarding the clinical management of fetuses predicted to be large for gestational age.
- The evidence surrounding antenatal interventions suggest that this strategy may be 'too late' to modify fetal adiposity and growth, raising the question of targeting the periconceptual period or early pregnancy.

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Declaration of interest

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APPENDIX 2

AUTHORSHIP STATEMENT

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Author (Candidate)	Dr Cecelia O'Brien
Contribution to the paper	Assisted in the analysis and interpreted the data Wrote the manuscript Acted as the corresponding author
Overall percentage (%)	60%

Signature **Date: 4th December, 2018**

Name of the Co-Author	Dr Jennie Louise	
Contribution to the paper	Performed the statistical analysis and assisted in interpretation and manuscript review	
Signature		**Date: 3/12/2018**

Name of the Co-Author	Andrea Deussen	
Contribution to the paper	Assisted in the interpretation of the data and review of the manuscript	
Signature		**Date: 3/12/2018**

Name of the Co-Author	Dr Rosalie Grivell	
Contribution to the paper	Collection of the ultrasound data	
Signature		**Date:** 10/12/18

Name of the Co-Author	Professor Jodie Dodd	
Contribution to the paper	Assisted in the design and interpretation and review of the manuscript	
Signature		**Date: 3rd December, 2018**

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ORIGINAL ARTICLE

The effect of maternal obesity on fetal biometry, body composition, and growth velocity

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ABSTRACT

Introduction: The aim of this secondary analysis was to investigate the relationship between maternal body mass index (BMI) and fetal biometry, body composition, and velocity measurements at 28 and 36 weeks gestation.

Materials and methods: The current analysis involves 911 overweight or obese women who were randomized to the Standard Care group of the LIMIT randomized trial.

Results: The fetus of women with Class 3 obesity (BMI $\geq$ 40.0) showed the greatest increase in all biometry *z*-scores, abdominal area (AA), and abdominal fat mass (AFM) compared with women classified as overweight (BMI 25.0–29.9). In women with Class 3 obesity, AA velocity was increased by 0.035 cm^2 (0.004, 0.066, $p = .029$) and the *z*-score velocity was increased by 0.238 (0.022, 0.453, $p = .03$). Estimated fetal weight (EFW) velocity for women with Class 3 obesity was higher than that of overweight women by 2.028 g per day (0.861, 3.196, $p < .001$) and the *z*-score velocity was also higher by 0.441 per day (0.196, 0.687, $p < .001$).

Conclusions: Maternal obesity is associated with an increase in fetal abdominal circumference, AFM and area along with EFW velocity over time. Women with Class 3 obesity (BMI $\geq$ 40.0) may represent a higher risk group for perpetuating the intergenerational transmission of obesity to their offspring.

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Adiposity; body composition; fetal growth; obesity; pregnancy; velocity

Introduction

Overweight and obesity affect 1.9 billion adults around the globe [1], and more than 41 million children under the age of 5 years [2]. Furthermore, over 50% of women in high-income countries enter pregnancy with a body mass index (BMI) greater than 25 kg/m^2 [3]. Obesity in pregnancy has a significant effect on maternal, fetal, and neonatal health outcomes and in the longer term, is associated with an increased risk of childhood obesity in offspring [2,4]. The contribution of maternal obesity to subsequent childhood obesity is complex, involving both genetic and environmental factors, with substantial impact arising from intrauterine programming [5]. To this end, there has been considerable interest in understanding the stimulation and regulation of fetal growth and adiposity patterns, and how this contributes to the intergenerational effects of obesity [6].

The developmental overnutrition hypothesis [7] proposes that maternal hyperglycemia, in the setting of diabetes, increases placental transfer of glucose, resulting in fetal hyperglycemia, increasing insulin-mediated fetal growth, principally adiposity. There has also been extension of this hypothesis to fetal growth in the setting of maternal obesity, with recognition that other substrates such as leptin, adiponectin, triglycerides, cholesterol, and inflammatory cytokines may indirectly influence growth through the regulation of placental nutrient transport [8].

With advances in ultrasound technology, there has been increasing interest in the identification of the fetus at risk of overgrowth and adiposity, utilizing both standard ultrasound biometry and fetal body composition measurements. While several studies have evaluated a range of fetal body composition measures using ultrasound [9–11], they have been somewhat limited by their relatively small sample sizes and having been performed mostly in women with a normal BMI [12,13] or diabetes [14–16]. The generalizability of these measures in other populations, particularly women who are overweight or obese, is unclear.

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The aim of this study was to determine the association between maternal BMI and fetal growth, body composition and growth velocity in a population of overweight and obese women. The hypothesis of this study is that higher maternal prepregnancy BMI is associated with increased fetal growth and greater fetal adiposity.

Materials and methods

The research methodology [17] and clinical findings [18,19] of the LIMIT randomized controlled trial have been published, in which a total of 2212 women who were overweight or obese were recruited to assess the effects of a dietary and lifestyle intervention, compared with standard antenatal care.

Eligibility and participants

Women were approached at their first antenatal booking appointment, where their height and weight were measured and BMI was calculated. Women with a singleton pregnancy between $10+0$ and $20+0$ weeks gestation, and BMI greater than or equal to 25 kg/m^2 were eligible for inclusion. Women with a diagnosis of Type 1 or Type 2 diabetes, or multiple pregnancy were excluded.

Ethics approval

Women were recruited from three public hospitals across metropolitan Adelaide and provided written informed consent to participate. The ethics approval study number for LIMIT randomized controlled trial was 1839/6 (approved July 2006) and for the fetal growth ancillary study number was 2051/4 (approved April 2008).

Randomization

At the time of the first antenatal visit, all women had their height and weight measured, and BMI was calculated. A central randomization service using a computer generated schedule randomized women to either the "Lifestyle Advice Group" or "Standard Care Group". Stratification variables included parity (0 versus 1+), BMI at antenatal booking (25.0–29.9 kg/m^2 versus $\geq$ 30.0 kg/m^2) and hospital of birth. Women included in this analysis were those randomized to the Standard Care group.

Intervention

Women who were randomized to receive Lifestyle Advice participated in a comprehensive dietary and lifestyle intervention, which included a combination of dietary, exercise and behavioral strategies. Further details regarding content of the intervention have been published previously [18,19]. Women who were randomized to the Standard Care group received their pregnancy care according to the guidelines of the hospital where they were planning to birth, which did not include the routine provision of dietary and lifestyle advice, or information relating to gestational weight gain in pregnancy [18,19].

Ultrasound assessment

All women who participated in the trial were offered a research ultrasound scan at 28 (range $26+0$ to $29+6$) and 36 (range $34+0$ to $37+6$) weeks' gestation to obtain fetal biometry and body composition measurements, performed by a medical practitioner with specialist or subspecialist training in obstetric ultrasound [20]. The estimated date of confinement and gestational age were calculated on the early pregnancy ultrasound and menstrual period dating.

Ultrasound outcome measurements

Using ultrasound, fetal biometry, estimated fetal weight (EFW), and fetal body composition were measured at 28 and 36 weeks gestation and have been described in detail in related publications [20].

Biometry and estimated fetal weight

Ultrasound assessment at these time points included measurements of standard biometry including head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL). The biometry was measured in accordance with national and international standards of practice [21]. Estimated fetal weight was calculated using the Hadlock C formula [22].

Fetal body composition measures

Fetal body composition measures were obtained in a standardized fashion, as we have reported previously [20], and included midthigh lean mass (MTLM), midthigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM).

Midthigh total, lean, and fat mass

Midthigh lean mass and MTFM were measured according to described techniques [12,20,23], as we have

reported previously [20]. Midthigh lean mass was calculated by first obtaining a longitudinal view of the femur and identification of the midpoint at a 0° angle. The transducer was then rotated through a 90° angle to obtain the cross-sectional view of the midthigh, and a trace of the circumference of the midthigh total mass (MTTM) performed and area calculated. The MTLM incorporating muscle and bone was outlined using a continuous trace to calculate the area. A subtraction was performed between the MTTM and the MTLM to calculate the MTFM.

Abdominal fat mass

Fetal AFM or anterior abdominal wall thickness was measured between the midaxillary lines and anterior to the margins of the ribs, at the level of the AC, with the subcutaneous fat represented by the echogenic envelope surrounding the abdomen and measured in millimeters [12,20,23]. Using magnification, four measurements were obtained from one or two separate images, with the mean value used in the analysis.

Subscapular fat mass

Subscapular fat mass was measured using a sagittal view of the fetal trunk to visualize the entire longitudinal section of the scapula as per the described technique [12,20,23]. The subcutaneous measurement between the skin surface and the subcutaneous tissue at the interface with the superspinous and infraspinous muscles was obtained on two occasions, with the mean value used in the analysis.

Fetal z-scores

For each fetal growth and adiposity measurement, *z*-scores were calculated using ultrasound growth charts in clinical use [22].

Fetal growth velocity

Fetal growth velocity was defined as the difference in biometry between 36 and 28 week measurements divided by actual time between measures. The growth velocity measurement was expressed as growth in mm per day for BPD, HC, FL, MTFM, AFM, SSFM, and *z*-scores were calculated for BPD, FL, EFW, and abdominal area (AA) using reference values from Owen et al. [24]. Abdominal area expressed as cm^2 with *z*-scores was calculated [24], as there are no appropriate reference ranges for AC velocity.

Statistical analysis

Baseline characteristics of women in the Standard Care group were assessed descriptively, with continuous variables were reported as mean and standard deviation or median and interquartile range as appropriate, and categorical variables were reported as number and percentage.

Maternal BMI was analyzed within four groups including overweight (BMI range between 25.0 and 29.99 kg/m^2), Class 1 obesity (BMI range between 30.0 and 34.99 kg/m^2), Class 2 obesity (BMI range between 35.0 and 39.9 kg/m^2), and Class 3 obesity (BMI greater than or equal to 40 kg/m^2) [25,26].

Fetal biometry and adiposity outcomes were analyzed using linear regression models with adjustment for confounders including center, parity, maternal age, smoking, socioeconomic status (Socioeconomic Index for Areas (SEIFA) Index of Relative Socio-Economic Disadvantage (IRSD) quintile).

For outcomes considered at two time points, generalized estimating equations (GEEs) were used to account for repeated measures, with a time-by-measure interaction term included in the model to test for difference in association between time points.

Statistical significance was assessed at the two sided $p < .05$ and no adjustment was made for multiple comparisons, as this is an exploratory rather than confirmatory analysis. All analyses were performed using SAS 9.4 (Cary, NC) and Stata v14 (Stata Corporation, College Station, TX).

Results

Demographic characteristics

Of the 911 women included in the secondary analysis, 41% ($n = 376$) were overweight, 29.8% ($n = 271$) had Class 1 obesity, 16.8% ($n = 153$) had Class 2 obesity, and 12.2% ($n = 111$) had Class 3 obesity (Table 1). The mean age of women was 29.6 years, the majority (92%; $n = 835$) were Caucasian in origin, with 40% ($n = 369$) in their first ongoing pregnancy, and almost 30% ($n = 265$) from the highest quintile of social disadvantage. The overall rate of gestational diabetes in the Standard Care group was 11.2% ($n = 102$). These baseline characteristics are consistent with the baseline characteristics of the entire LIMIT Trial randomized cohort [18]. A total of 911 women from the Standard Care group had ultrasound information at one or more time points, with 777 having ultrasound data at both 28 and 36 weeks. Sixty-six women (7.2%) had data only for 28 weeks, and 68 women (7.5%) had data only at 36-

Table 1. Baseline characteristics of the Standard Care group within the LIMIT Trial.

	Body mass index category				Overall	
Total number	Overweight	Class 1	Class 2	Class 3	*N* (%)	*p* Value
	25.0–29.9	30.0–34.9	35.0–39.9	≥40.0		
N (%)	376 (41.3)	271 (29.8)	153 (16.8)	111 (12.2)	911	
Maternal age (years) Mean (SD)	29.9 (5.25)	29.6 (5.69)	29.2 (5.40)	28.9 (5.97)	29.6 (5.50)	.24
Caucasian *n* (%)	340 (90.4)	248 (91.5)	141 (92.2)	106 (95.5)	835 (91.7)	.40
Nulliparous *n* (%)	168 (44.68)	108 (39.85)	50 (32.68)	43 (38.74)	369 (40.50)	.08
Smoker *n* (%)	38 (10.11)	35 (12.92)	14 (9.15)	14 (12.61)	101 (11.09)	.52
Gestational diabetes *n* (%)	26 (6.91)	37 (13.65)	19 (12.42)	20 (18.02)	102 (11.20)	.88
SEIFA Quintile 1 Most disadvantaged *n* (%)	94 (25.00)	83 (30.63)	48 (31.37)	40 (36.04)	265 (29.09)	.06
Quintile 2 *n* (%)	87 (23.14)	63 (23.25)	37 (13.65)	40 (14.76)	48 (17.71)	
Quintile 3 *n* (%)	59 (15.69)	37 (13.65)	29 (18.95)	18 (16.22)	143 (15.70)	
Quintile 4 *n* (%)	70 (18.62)	40 (14.76)	20 (13.07)	12 (10.81)	142 (15.59)	
Quintile 5 Least disadvantaged *n* (%)	66 (17.55)	48 (17.71)	14 (9.15)	11 (9.91)	139 (15.26)	

week gestation (Table 1). There were no statistically significant differences in the demographic characteristics between the four BMI categories.

Maternal BMI and the relationship with fetal biometry and estimated fetal weight z-scores

Neither maternal obesity Class 1 or Class 2 was associated with fetal BPD, HC, or FL *z*-scores at either 28 or 36 weeks gestation, when compared with fetal biometry measures from women who were overweight (Table 2). However, the fetuses of women with obesity Class 3 demonstrated significantly higher *z*-scores for BPD compared with the fetuses of women who were overweight: the estimated mean difference was 0.36 (0.06, 0.65) at 28 weeks ($p = .017$), and 0.39 (0.15, 0.63) at 36 weeks ($p = .002$). Similarly, HC *z*-scores were higher by 0.47 (0.26, 0.68) at 28 weeks and 0.51 (0.32, 0.71) at 36 weeks ($p < .001$ for both time points), while FL *z*-scores were higher by 0.36 (0.13, 0.58) at 28 weeks ($p = .002$) and by 0.27 (0.02, 0.52) at 36 weeks ($p = .035$) (Table 2).

For both AC and EFW *z*-scores, there was a consistent pattern of higher measures with increasing maternal BMI at both time points as shown in Table 2. The mean fetal AC *z*-scores at both 28 and 36 weeks were significantly higher in the fetus of women with Class 1 obesity, with the magnitude of the increase being higher for Class 2 and Class 3 obesity categories in comparison to women in the overweight group. Women with Class 1 obesity had AC *z*-scores 0.18 (0.02, 0.33, $p = .028$) higher at 28 weeks, and 0.21 (0.04, 0.38, $p = .017$) higher at 36 weeks. Women with Class 2 obesity had AC *z*-scores 0.20 (0.01, 0.38; $p = .04$) higher at 28 weeks, and 0.24 (0.05, 0.43, $p = .013$) higher at 36 weeks. For the women with Class 3 obesity, the increase in AC *z*-score was 0.40 (0.17, 0.63, $p = .013$) at 28 weeks, and 0.39 (0.15, 0.63, $p = .001$) at 36 weeks compared to the overweight group. Women with Class 1 obesity had EFW *z*-scores 0.18 (0.04, 0.33, $p = .014$) higher at 28 weeks, and 0.17 (0.008, 0.32, $p = .04$) higher at 36 weeks. For the women with Class 3 obesity, the increase in EFW *z*-score was 0.46 (0.23, 0.69, $p < .001$) at 28 weeks, and 0.42 (0.19, 0.64, $p < .001$) at 36 weeks compared to the overweight group (Table 2).

Figure 1 illustrates the positive association between maternal BMI (continuous variable) and fetal AC *z*-score when measured at 28 and 36 weeks (Figure 2). For every 1 unit increase in BMI, AC *z*-score increases by 0.021 units (95 CI 0.01–0.032) at 28 weeks ($p < .001$) and 0.025 (95 CI 0.013–0.036) at 36 weeks ($p < .001$).

In the time-by-treatment interaction analyses, there was no evidence that the observed associations between maternal BMI and fetal growth and adiposity measurements changed over time.

Maternal BMI and fetal adiposity measurements

There were no significant differences between the maternal BMI categories MTFM as shown in Table 3.

Table 2. Maternal body mass index (BMI) category and fetal biometry *z*-scores at 28 and 36 weeks.

		Overweight		Class 1 obesity			Class 2 obesity			Class 3 obesity		
		25–29.9 kg/m^2		30–34.9 kg/m^2			35–39.9 kg/m^2			≥40.0 kg/m^2		
z-Scores	Gestation	Mean (SD) BMI category	Adjusted estimate (95% CI)	Mean (SD)	Adjusted estimate (95% CI)	*p* Value	Mean (SD)	Adjusted estimate (95% CI)	*p* Value	Mean (SD)	Adjusted estimate (95% CI)	*p* Value
BPD	0.372[a]											
	28 weeks	0.62	Ref group	0.57	−0.084	.484	0.50	−0.133	.353	0.95	0.356	.017
		(1.48)		(1.48)	(−0.321, 0.152)		(1.42)	(−0.414, 0.148)		(1.32)	(0.064, 0.649)	
	36 weeks	0.09	Ref group	0.13	0.013	.895	0.18	0.080	.477	0.45	0.389	.002
		(1.20)		(1.23)	(−0.184, 0.21)		(1.02)	(−0.14, 0.30)		(1.11)	(0.145, 0.632)	
HC	0.387[a]											
	28 weeks	0.73	Ref group	0.68	−0.44	.586	0.69	−0.009	.924	1.12	0.470	<.001
		(0.93)		(1.01)	(−0.202, 0.114)		(0.91)	(−0.191, 0.173)		(0.99)	(0.257, 0.683)	
	36 weeks	0.53	Ref group	0.63	0.101	.165	0.56	0.0480	.589	0.99	0.513	<.001
		(0.83)		(0.95)	(−0.042, 0.245)		(0.88)	(−0.126, 0.221)		(0.93)	(0.318, 0.708)	
AC	0.946[a]											
	28 weeks	0.34	Ref group	0.51	0.175	.028	0.52	0.196	.040	0.72	0.399	.013
		(0.94)		(1.0)	(0.019, 0.331)		(0.98)	(0.009, 0.383)		(1.08)	(0.168, 0.631)	
	36 weeks	0.32	Ref group	0.54	0.210	.017	0.54	0.241	.013	0.68	0.390	.001
		(1.05)		(1.09)	(0.038, 0.381)		(0.97)	(0.051, 0.431)		(1.19)	(0.154, 0.626)	
FL	0.270[a]											
	28 weeks	0.18	Ref group	0.25	0.066	.413	0.06	−0.120	.236	0.51	0.355	.002
		(0.97)		(0.98)	(−0.093, 0.225)		(1.06)	(−0.319, 0.079)		(1.00)	(0.132, 0.578)	
	36 weeks	0.09	Ref group	0.15	0.064	.450	0.20	0.072	.484	0.33	0.271	.0035
		(1.06)		(1.01)	(−0.102, 0.229)		(1.07)	(−0.129, 0.272)		(1.22)	(0.019, 0.522)	
EFW	0.776[a]											
	28 weeks	0.12	Ref group	0.29	0.182	.014	0.20	0.107	.216	0.54	0.460	<.001
		(0.87)		(0.93)	(0.036, 0.327)		(0.90)	(−0.063, 0.278)		(1.07)	(0.233, 0.686)	
	36 weeks	0.17	Ref group	0.35	0.165	.04	0.33	0.169	.051	0.55	0.416	<.001
		(0.96)		(1.01)	(0.008, 0.322)		(0.87)	(−0.001, 0.339)		(1.11)	(0.194, 0.638)	

The results from the analysis of maternal BMI category groups and fetal biometry *z*-scores measured at 28 and 36 weeks gestation. Each biometry measurement is described across the three BMI categories for both time points. Values are expressed as mean (standard deviation) for maternal BMI categories; estimates are differences in means (95% CI) for each BMI category compared to the lowest BMI category (25.0–29.9). All models were adjusted for center, parity, SEIFA IRSD quintile, smoking status and age at consent.

BPD: biparietal diameter; HC: head circumference; AC: abdominal circumference; FL: femur length; Ref: reference; SD: standard deviation; CI: confidence interval.

[a]Interaction *p* values for treatment by time point interaction.

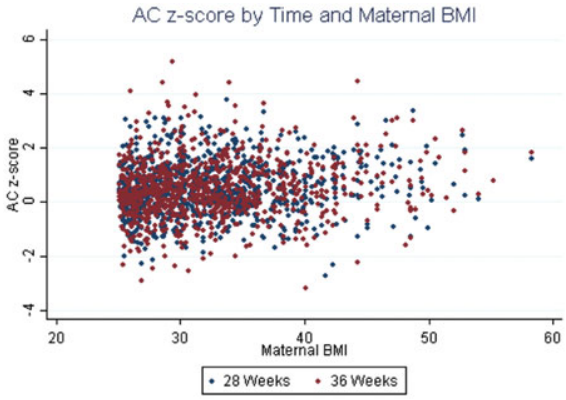

Figure 1. There is a positive association between increasing maternal BMI and fetal abdominal circumference (AC) z-score when measured at 28 weeks (blue) and 36 weeks (red). For every 1 unit increase in BMI, AC z-score increases by 0.021 units (95th CI 0.01–0.032) at 28 weeks ($p < .001$) and 0.025 (95th CI 0.013–0.036) at 36 weeks ($p < .001$).

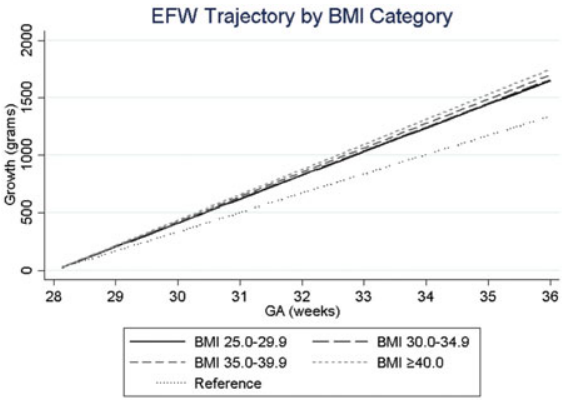

Figure 2. This graph illustrates the mean estimated fetal weight change per day from 28 weeks to 36 weeks for each BMI category. The reference group is the growth velocity from 28 to 35 weeks inclusive from low risk pregnancies reported by Owen and associates in 1996 [23]. As BMI category increases, there is an incremental increase in the rate of growth per day when compared to the reference group.

For AA, there were no significant differences for the obesity Class 2 category; however, there were significant differences between the obesity Class 1 and Class 3 categories when compared to measurements from women who were overweight. The mean AA was 1.795 cm^2 (0.13, 3.46, $p = .035$) higher at 36-week gestation in the fetus of women with Class 1 obesity. Similarly, the mean AA was higher by 2.19 cm^2 at 28 weeks (0.31, 4.08, $p = .02$) and 3.42 cm^2 at 36 weeks (1.09, 5.74, $p = .004$) in the fetus of women with Class 3 obesity (Table 3).

For AFM, there were no significant differences in the fetus of women with Class 1 or Class 2 obesity; however, there were significant differences between the obesity Class 3 when compared to measurements from women who were overweight. In the fetus of women with Class 3 obesity AFM was increased by 0.61 mm (0.08, 1.14, $p = .03$) at 36 weeks (Table 3).

For SSFM, there were no significant differences in the fetuses of women with Class 2 or Class 3 obesity; however, there were significant differences between obesity Class 1 when compared to women who were overweight. In the fetus of women with Class 3 obesity, SSFM was reduced by 0.2 mm (−0.37, −0.04, $p = .016$) at 28 weeks (Table 3).

There was no evidence that the association with maternal BMI category differed over time for the adiposity measurements.

Maternal BMI with fetal growth velocity over time

There were no significant differences between the maternal BMI categories and MTFM velocity, AFM velocity, or SSFM velocity (Table 4). For EFW and AA velocity, there were no significant differences for women with Class 1 and Class 2 obesity; however, there were significant differences between the Class 3 obesity and overweight categories. The mean EFW velocity was 2.03 g per day (0.86, 3.2, $p < .001$) higher in women with Class 3 obesity. The EFW *z*-score velocity was also higher by 0.44 (1.2, 0.69, $p < .001$). Similarly, AA velocity was higher by 0.035 cm^2/day (0.004, 0.066, $p = .029$), and the AA *z*-score velocity was higher by 0.24 (0.02, 0.45, $p = .03$) (Table 4).

Figure 2 illustrates EFW growth velocity expressed as the mean growth per day for each BMI category. The reference group has been included to show how maternal obesity, regardless of BMI category, results in a higher fetal growth velocity rate. This diagram shows the incremental increase in velocity with maternal BMI category, the highest velocity being found in the fetus of women with obesity Class 3 (Table 4).

In addition to the analyses reported above, BMI was also analyzed as a continuous variable and the results are consistent with the above findings described for the analysis of the four BMI groups.

Discussion

This study describes the fetal growth patterns and growth velocity over time in women who are overweight or obese. Our study identifies an association between higher maternal BMI category and an increase in fetal biometry *z*-scores, abdominal-related adiposity and growth velocity. We found a consistent

Table 3. Maternal BMI category and fetal adiposity measurements at 28 and 36 weeks.

		Body mass index category									
		Overweight		Class 1 obesity			Class 2 obesity			Class 3 obesity		
		25–29.9 kg/m^2		30–34.9 kg/m^2			35–39.9 kg/m^2			≥40.0 kg/m^2		
	Gestation	Mean (SD)	Adjusted estimate (95% CI)	Mean (SD)	Adjusted estimate (95% CI)	*p* Value	Mean (SD)	Adjusted estimate (95% CI)	*p* Value	Mean (SD)	Adjusted estimate (95% CI)	*p* Value
Midthigh fat mass (cm^2)	0.636[a]											
(MTFM)	28 weeks	4.50	Ref	4.56	0.009	.94	4.48	−0.051	.734	4.76	0.297	.166
		(1.27)		(1.20)	(−0.231, 0.250)		(1.11)	(−0.343, 0.242)		(1.43)	(−0.123, 0.718)	
	36 weeks	11.17	Ref	11.30	0.08	.794	10.99	−0.166	.644	12.16	0.967	.089
		(2.88)		(2.88)	(−0.519, 0.678)		(2.57)	(−0.872, 0.539)		(3.25)	(−0.148, 2.081)	
Abdominal area (cm^2)	0.293[a]											
(AA)	28 weeks	47.56	Ref	47.77	0.269	.608	48.64	1.236	.077	49.72	2.193	.023
		(6.70)		(5.93)	(−0.759, 1.296)		(7.12)	(−0.134, 2.605)		(8.82)	(0.309, 4.076)	
	36 weeks	84.19	Ref	85.78	1.795	.035	85.91	1.773	.077	87.12	3.415	.004
		(10.40)		(10.47)	(0.13, 3.46)		(9.95)	(−0.190, 3.735)		(11.34)	(1.091, 5.739)	
Abdominal fat mass (mm)	0.724[a]											
(AFM)	28 weeks	3.46	Ref	3.56	0.060	.544	3.49	0.028	.813	3.80	0.351	.05
		(1.04)		(1.06)	(−0.135, 0.255)		(0.89)	(−0.201, 0.256)		(1.18)	(0.00, 0.702)	
	36 weeks	5.60	Ref	5.82	0.186	.237	5.64	−0.011	.956	6.20	0.606	.025
		(1.58)		(1.56)	(−0.122, 0.494)		(1.56)	(−0.388, 0.367)		(0.567)	(0.078, 1.134)	
Subscapular fat mass (mm)	0.226[a]											
(SSFM)	28 weeks	3.25	Ref	3.05	−0.201	.016	3.19	−0.079	.477	3.29	0.060	.675
		(0.94)		(0.80)	(−0.365,−0.037)		(0.88)	(−0.295, 0.138)		(0.93)	(−0.219, 0.338)	
	36 weeks	5.31	Ref	5.48	0.136	.369	5.35	0.046	.828	5.52	0.235	.344
		(1.38)		(1.49)	(−0.161, 0.433)		(1.29)	(−0.290, 0.362)		(1.53)	(−0.252, 0.723)	

The results relating to maternal BMI categories and fetal adiposity measurements. Each biometry measurement is described across the three BMI categories for both time points. Values are mean (standard deviation) for maternal BMI categories; estimates are differences in means (95% CI) for each BMI category compared to the lowest BMI category (25.0–29.9) and were adjusted for center, parity, SEIFA IRSD quintile, smoking status and age at consent.

Ref: reference.

[a]Interaction *p* values for treatment by time point interaction.

Table 4. Maternal BMI category and fetal growth velocity between 28 and 36 weeks.

		Body mass index category									
		Overweight		Class 1 obesity			Class 2 obesity			Class 3 obesity		
		25–29.9 kg/m^2		30–34.9 kg/m^2			35–39.9 kg/m^2			≥40.0 kg/m^2		
	Gestation	Mean (SD)	Adjusted estimate (95% CI)	Mean (SD)	Adjusted estimate (95% CI)	*p* Value	Mean (SD)	Adjusted estimate (95% CI)	*p* Value	Mean (SD)	Adjusted estimate (95% CI)	*p* Value
Estimated fetal weight (EFW)	.005	29.46 (4.99)	Ref	29.61 (5.39)	0.153 (−0.705, 1.011)	.727	30.37 (4.55)	0.765 (−0.291, 1.820)	.156	31.21 (5.62)	2.028 (0.861, 3.196)	<.001
MTFM	.292	0.12 (0.05)	Ref	0.12 (0.05)	−0.002 (−0.13, 0.010)	.765	0.12 (0.05)	0.009 (−0.006, 0.024)	.238	0.13 (0.05)	0.017 (−0.007, 0.042)	.163
Abdominal area (cm2) (AA)	.145	0.68 (0.13)	Ref	0.68 (0.13)	0.009 (−0.014, 0.032)	.425	0.70 (0.13)	0.019 (−0.009, 0.048)	.175	0.70 (0.16)	0.035 (0.004, 0.066)	.029
AFM	.762	0.04 (0.03)	Ref	0.04 (0.03)	0.003 (−0.003, 0.009)	.317	0.04 (0.03)	0.002 (−0.006, 0.010)	.580	0.04 (0.03)	0.00 (−0.013, 0.012)	.948
SSFM	.238	0.04 (0.03)	Ref	0.04 (0.03)	0.005 (−0.001, 0.011)	.106	0.04 (0.03)	0.006 (−0.002, 0.014)	.12	0.04 (0.02)	−0.001 (−0.012, 0.011)	.876
AA velocity Z score	.165	0.51 (0.93)	Ref	0.54 (0.91)	0.0045 (−0.112, 0.203)	.574	0.62 (0.85)	0.112 (−0.082, 0.306)	.259	0.67 (1.06)	0.238 (0.022, 0.453)	.03
EFW velocity Z score	.003	0.60 (1.06)	Ref	0.62 (1.12)	0.016 (−0.165, 0.196)	.865	0.78 (0.94)	0.152 (−0.07, 0.375)	.179	0.99 (1.19)	0.441 (0.196, 0.687)	<.001

The results relating to maternal BMI categories and the growth velocity for EFW, MTFM, AA, AFM, SSFM, AA, and EFW velocity *z* scores. Each measurement is described across the three BMI categories for both time points. Values are mean (standard deviation) for maternal BMI categories, and estimates are differences in means (95% CI) for each BMI category compared to the lowest category (BMI 25.0–29.9) and were adjusted for center, parity, SEIFA IRSD quintile, smoking status and age at consent.

and significant increase in all fetal biometry measurements at both 28 and 36 weeks gestation from women with Class 3 obesity (BMI greater than or equal to 40 kg/m^2). Fetal adiposity measurements were not universally increased but AFM and AA were associated with maternal obesity in our cohort. With increasing maternal BMI category, there were incremental increases in growth velocity of the fetal abdomen and EFW. This study was a secondary analysis and while other associations were identified, these were inconsistent and likely due to chance.

Much of the literature pertaining to ultrasound measured fetal growth patterns relates to women with pre-existing diabetes [15] and gestational diabetes [13,14,16,27]. Maternal obesity is associated with an overall increase in lean mass and skeletal growth [20]. In contrast, maternal diabetes results mainly in an increase in AC and AFM through the stimulation of insulin sensitive tissues [15]. The main findings in this study confirmed this, where a significant increase in skeletal growth (HC and FL) and AA velocity and fat mass was found in women with Class 3 obesity compared with the lesser BMI categories. A recent study by Zhang et al. in women classified as obese and nonobese in pregnancy, also found an increase in skeletal growth, with significant increases in HC, humeral and FLs in fetuses of obese women [28]. The difference in EFW comparing women who were nonobese to obese was apparent from 32 weeks [28].

Women with Class 3 obesity (BMI greater than or equal to 40 kg/m^2) are likely to represent a metabolically different group. From epidemiological studies, higher BMI category is associated with a further increase in the rate of adverse perinatal outcomes including macrosomia [29–32] and there is emerging evidence of associated childhood obesity [33–35]. The stimulation of fetal growth through the complex pathway including insulin growth factors (*via* hyperglycemia and hyperinsulinemia) [36], hyperlipidemia [37], leptin [38], adiponectin [39], and inflammatory mediators [40] is likely to be accentuated in women of higher BMIs. Thus, targeted interventions in the Class 3 obesity group may be more beneficial in reducing the fetal effects of obesity when compared to women with lower BMI categories.

This is the first study to report on velocity of fetal growth and adiposity in the setting of maternal obesity. The literature to date has used measurement of growth velocity as a tool for screening and identification of the small for gestational age infant [41] or for screening for macrosomia associated with pre-existing or gestational diabetes [15,42,43]. The velocity of fetal growth changes throughout gestation [44,45]. Of interest, growth in the abdomen circumference over time peaks at 12.5 mm per week at 24 weeks' gestation, reducing to 8 mm per week by 40 weeks' gestation [44]. Our study has shown an incremental increase in the rate of growth velocity in third trimester associated with maternal obesity. Further understanding into the timing and regulation of the fetal growth velocity in the setting of maternal obesity is critical to developing successful interventions to improve perinatal outcomes.

The main limitation of this secondary analysis is the lack of a comparator group, defined as women entering pregnancy with a normal BMI. There was also missing data in the velocity comparisons due to the availability of the second scan to calculate the growth velocity over time, affecting 15% of women within the cohort. Third, the study incorporated two time points to assess velocity in the third trimester. Other descriptive studies for growth velocity used multiple time points from 12 to 40 weeks gestation in order to describe the variation in velocity throughout the entire pregnancy [44,45]. Lastly, while multiple comparisons were used in this secondary analysis, this has not been adjusted for as this study is an exploratory not a confirmatory analysis.

There is a need for further studies into the mechanisms and timing of critical fetal growth changes. This would help guide and assist with the timing of potential interventions that may modulate fetal growth *in utero*. From a public health perspective, if preventive strategies could modify fetal growth, velocity and adiposity patterns *in utero*, this may alter the transmission of obesity and its cardiometabolic complications to the next generation [46,47].

Disclosure statement

No potential conflict of interest was reported by the authors.

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APPENDIX 3

AUTHORSHIP STATEMENT

Title of the Paper: In Overweight or Obese Pregnant Women, Maternal Dietary Factors are not Associated with Fetal Growth and Adiposity

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Contribution to the paper Assisted in the analysis and interpreted the data
Wrote the manuscript
Acted as the corresponding author

Overall percentage (%) 60%

Signature **Date: 3rd December, 2018**

Name of the Co-Author	Dr Jennie Louise	
Contribution to the paper	Performed the statistical analysis and assisted in interpretation and manuscript review	
Signature		**Date: 3/12/2018**

Name of the Co-Author	Andrea Deussen	
Contribution to the paper	Assisted in the interpretation of the data and review of the manuscript	
Signature		**Date: 3/12/2018**

Name of the Co-Author	Professor Jodie Dodd	
Contribution to the paper	Assisted in the design and interpretation and review of the manuscript	
Signature		**Date: 3/12/2018**

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Article

In Overweight or Obese Pregnant Women, Maternal Dietary Factors are not Associated with Fetal Growth and Adiposity

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Abstract: The aim of our study was to evaluate associations between maternal dietary factors and fetal growth and adiposity in overweight and obese women. Women randomised to the 'Standard Care' group of the LIMIT trial were included. Maternal dietary factors including Healthy Eating Index, total energy, fat, carbohydrates, protein, glycaemic load and index were measured using the Harvard semi-quantitative Food Frequency questionnaire at time of study entry, 28 and 36 weeks' gestation. Fetal ultrasound measurements of biometry and adiposity were obtained at 28 and 36 weeks' gestation. Linear regression models were used to associate between dietary factors and fetal growth and adiposity measurements. There were 721 women included in this exploratory analysis. A 10 unit increase in the log total energy was associated with a reduction in mid-thigh lean mass by 4.94 mm at 28 weeks (95% CI −9.57 mm, −0.32 mm; p = 0.036) and 7.02 mm at 36 weeks (95% CI −13.69 mm, −0.35 mm; p = 0.039). A 10 unit increase in Healthy Eating Index score was associated with a reduced mean subscapular skin fold measure at 28 weeks by 0.17 mm (95% CI −0.32 mm, −0.03 mm; p = 0.021). We did not identify consistent associations between maternal diet and measures of fetal growth and adiposity in overweight and obese women.

Keywords: obesity; pregnancy; fetal biometry; adiposity; healthy eating index; total energy; glycaemic index; protein intake; carbohydrate intake; fat intake

1. Introduction

Over the past 40 years, rates of obesity have tripled worldwide [1], to the extent that it is considered a public health crisis [2]. In many developed countries, including the United Kingdom and United States of America, 1 in 2 women now enter pregnancy overweight or obese [3–5]. There are well-recognised independent associations between obesity in pregnancy and maternal, fetal and neonatal health outcomes [6,7], and in the longer-term maternal obesity has been linked with childhood obesity [8].

Women who are overweight or obese during pregnancy have been demonstrated to have poorer diet quality when compared with women with BMI in the normal range [9–12], which persists into the postpartum period [10]. In turn, poor diet quality is associated with increased risk of glucose intolerance and pre-eclampsia [11], increased neonatal adiposity [13] and changes in child body composition [14].

There is growing interest in the programming of fetal growth, the critical time points and the influence of maternal diet as a potentially modifiable factor. The current literature is inconsistent, largely due to the heterogeneity and variability relating to the timing and types of dietary assessments, reporting and methodology along with body composition outcome measurements [15]. In relation to maternal carbohydrate intake for example, some studies have shown a positive effective of high carbohydrates on childhood BMI [16] and others have shown a low carbohydrate diet was associated with increased fetal abdominal fat [17]. The majority have shown a negative effect [18–20] and one study has shown no effect [15]. Protein and carbohydrate ratios or combination diets have also been reported, where high protein associated with low carbohydrate and fat diet was associated with a reduction in neonatal abdominal adiposity [21], whereas another study showed low protein: carbohydrate ratio was associated with increased abdominal fat in the fetus.

Several studies have explored the association between maternal dietary intake and outcomes in the perinatal period, focussing predominantly on birthweight [22–25], preterm birth [26], infants born small for gestational age [27], and newborn anthropometry [13,17,18,22,28,29]. Poor diet quality (defined as Healthy Eating Index score less than or equal to 57) has been associated with a higher percentage of neonatal fat mass as measured on air displacement plethysmography, independent of maternal BMI [13]. Observational data from the Danish National Birth Cohort identified an association between maternal dietary glycaemic load and both an increased risk of large for gestational age infants (14%) and higher birthweight (36 grams) [29].

There has been more limited evaluation of the contribution of maternal dietary intake to fetal growth and adiposity. Maternal protein, fatty acid and carbohydrate intake during pregnancy have all been associated with increased measures of fetal adiposity, although this has been evaluated in women of normal BMI [17]. The contribution of specific maternal dietary components to fetal growth and adiposity among women who are overweight or obese is unclear, and warrants further investigation.

The aim of our study was to evaluate associations between maternal dietary factors and fetal growth and adiposity measured by ultrasound at 28 and 36 weeks gestation in overweight and obese women.

2. Materials and Methods

The study cohort involves 721 overweight or obese pregnant women who participated in the Standard Care Group of the LIMIT trial. The methodology and findings of the LIMIT Trial have been reported in detail previously [30,31]. Briefly, women were recruited from maternity hospitals in South Australia, after ethics approval and informed written consent to participate. Eligible women were those with a singleton pregnancy and BMI $\geq$ 25 kg/m^2 at booking antenatal appointment, and who were between 10^{+0} and 20^{+0} weeks' gestation. The exclusion criteria included women with Type 1 or 2 Diabetes diagnosed prior to pregnancy or multiple pregnancy. At the booking antenatal appointment, all women had their height and weight measured, and BMI calculated by clinical staff. Women in the Standard Care group continued pregnancy care according to the guidelines of their local hospital and did not include specific information relating to weight gain, or diet and physical activity during pregnancy. The ethics approval study number for LIMIT randomised controlled trial was 1839/6 (approved July 2006) and for the fetal growth ancillary study the number was 2051/4 (approved April 2008).

2.1. Dietary Assessment

Women completed the Harvard Semi-quantitative Food Frequency (Willett) questionnaire [32] to measure the daily dietary intake of nutrients from 126 food items, including portion size and incorporation of the 7 food groups, which has been validated in pregnancy [33] and among Australian pregnant women [34]. The questionnaire was completed at the time of study entry, 28 and 36 weeks' gestation. At study entry, women were asked on average, how often was the food consumed during

the last 12 months, while assessment at 28 and 36 weeks' gestation asked women to indicate on average, how often the amount of food was consumed since the previous questionnaire time point.

Daily nutrient intake was estimated using the nutrient compositions from the Australian food composition tables according to pre-specified portion size. Adherence to dietary recommendations was performed by allocating all food and drink consumption into the food groups as described by the Australian Guide to Healthy Eating [35]. Foods were classified as 'non-core foods' if the food did not meet the criteria of the five core food groups, provided minimal nutrient content, and were high in fat, sugar or salt [35,36].

Micronutrient values were obtained from the Harvard Semi-quantitative Food Frequency (Willett) questionnaire [32] and analysed as mean intake, utilising the Food Works Nutrient Analysis Software Package (FoodWorks, version 7, Professional; Xyris Software 2012; Australia), and using Australian Food composition tables.

Diet quality was assessed using the Healthy Eating Index (HEI), which has 12 components to yield a maximum score of 100 [37]. These 12 components include total fruit, total vegetables, dark green and orange vegetables and legumes, total grains and whole grains, all of which receive a score out of 5. Milk, meat and beans, oils, saturated fat and sodium-based foods were scored out of 10. Calories from solid fats, alcohol related beverages and added sugars were scored out of 20. A HEI score of 80 is considered good, a score between 50 and 80 is one that needs improvement, and scores of less than 50 are considered poor. The HEI has been validated for use in pregnant women [38].

Dietary glycaemic index (GI) values were obtained from the Harvard Semi-quantitative Food Frequency (Willett) questionnaire [32] and analysed using the Food Works Nutrient Analysis Software Package (FoodWorks, version 7, Professional; Xyris Software 2012; Brisbane, Australia), and published dietary glycaemic index values.

2.2. Ultrasound Assessment

A research ultrasound scan was offered to all women participating in the study at approximately 28 and 36 weeks' gestation. Fetal biometry and body composition measurements were obtained as previously described [39]. Research ultrasounds were performed by medical practitioners with specialist or subspecialist training in obstetric ultrasound, while blinded to the participant's treatment allocation, and all measurements were obtained prospectively. The estimated date of confinement and gestational age were calculated on the early pregnancy clinical ultrasound and menstrual period dating.

Ultrasound measurements of biometry and fetal adiposity were obtained as described in detail previously [39]. In brief, fetal biometry was measured at 28 and 36 weeks' gestation. This included head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL), measured in accordance with national and international standards of practice [40]. Estimated fetal weight (EFW) was calculated using the Hadlock C formula [41].

Fetal body composition measures included mid-thigh lean mass (MTLM), mid-thigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM) using techniques reported previously [39]. The techniques for acquisition of these measurements have been published in detail [39]. MTLM was calculated by tracing the circumference of the mid-thigh total mass (MTTM) followed by the MTLM incorporating muscle and bone. A subtraction was performed between the MTTM and the MTLM to calculate the mid-thigh fat mass (MTFM). Abdominal fat mass was measured in millimetres between the mid-axillary lines and anterior to the margins of the ribs, at the level of the abdominal circumference. Two measurements of the subcutaneous skin width were obtained from a longitudinal section of the scapula at the interface with the super-spinous and infra-spinous muscles.

2.3. Statistical Analysis

Baseline characteristics of women contributing data were assessed descriptively. Continuous variables were reported as mean and standard deviation or median and interquartile range as

appropriate, and categorical variables as a number and percentage. For each fetal biometry measurement, *z* scores were calculated using ultrasound growth charts in clinical use [41,42].

The analyses were exploratory, with no pre-specification of a primary outcome. Instead, the associations between diet and fetal growth and adiposity were investigated using a range of dietary variables (HEI, Total Energy, Glycaemic Index, Glycaemic Load, Fat, Carbohydrate and Protein as Percent of Total Energy) and a range of fetal growth and adiposity measures (BPD and BPD *z*-score, EFW and EFW *z*-score, HC and HC *z*-score, AC and AC *z*-score, FL and FL *z*-score, MTLM, MTFM, AFM, SSFM). Both dietary and fetal growth variables were measured at 28 weeks' and 36 weeks' gestation.

Linear regression was used to model the association between dietary factors and fetal growth and adiposity, with diet variables considered as 'predictors' (independent variables) and fetal growth and adiposity variables as 'outcomes' (dependent variables). A time-by-diet-variable interaction term was included to allow for estimation of the association at each time point separately, and to test whether the association differed between time points. Generalised Estimating Equations with exchangeable working correlation were used to account for repeated measures. Both unadjusted and adjusted analyses were performed. Adjusted analyses included maternal BMI category (25.0–29.9 kg/m^2 vs. ≥30.0 kg/m^2 as measured at study entry), smoking, parity (0 vs. ≥1), age and Socio-Economic Indexes for Areas Index of Relative Socio-Economic Disadvantage (SEIFA IRSD) quintile, which is a rank of areas within Australia according to socio-economic disadvantage, obtained from the Census that occurs every 5 years. All analyses were additionally adjusted for baseline diet variables, as a potential confounder.

Statistical significance was assessed at the two-sided $p < 0.05$ and no adjustment was made for multiple comparisons. All analyses were performed using SAS 9.4 (Cary, NC, USA).

3. Results

3.1. Demographic Characteristics

There were 721 women included in this secondary analysis and the baseline characteristics are shown in Table 1. The mean age of women participating was 29.9 years (SD 5.3), with median gestation at study entry 14.3 weeks (Interquartile range between 12.1 to 17.0 weeks). Forty-three percent (n = 310) of women were overweight, while 46.5% (n = 335) were obese (BMI 30.0–39.9 kg/m^2), and 10.5% (n = 76) morbidly obese (BMI ≥ 40.0 kg/m^2). Most women (91%; n = 659) were of Caucasian origin, 41.3% (n = 298) in their first ongoing pregnancy, and 52% (n = 373) from the highest two quintiles of social disadvantage. The baseline characteristics of the women contributing dietary and ultrasound data were comparable to all women in the Standard Care group, and all women included in the LIMIT randomised trial [30].

Table 1. Baseline characteristics of the Standard Care group within the LIMIT Trial.

Characteristic	Number (%), Mean (SD) or Median (IQR)
Overall Number	721
- Both 28 and 36 Weeks	453
- 28 Weeks Only	158
- 36 Weeks Only	110
Age at Trial Entry: Mean (SD)	29.88 (5.33)
Parity: *N* (%)	
- 0	298 (41.33)
- ≥1	423 (58.67)
BMI: Median (IQR)	31.00 (27.70, 35.20)

Table 1. *Cont.*

Characteristic	Number (%), Mean (SD) or Median (IQR)
BMI Category: *N* (%)	
- BMI 25.0–29.9	310 (43.00)
- BMI 30.0–34.9	219 (30.37)
- BMI 35.0–39.9	116 (16.09)
- BMI $\geq$ 40.0	76 (10.54)
Smoker: *N* (%)	
- Yes	67 (9.29)
- No	639 (88.63)
- Unknown	15 (2.08)
GA at Trial Entry: Median (IQR)	14.29 (12.14, 17.00)
Public Patient: *N* (%)	
- Yes	707 (98.06)
- No	14 (1.94)
Ethnicity: *N* (%)	
- Caucasian	659 (91.40)
- Asian	22 (3.05)
- Aboriginal or TSI	8 (1.11)
- Indian, Pakistani, Sri-Lankan	22 (3.05)
- African	5 (0.69)
- Other	5 (0.69)
SEIFA IRSD Quintile: *N* (%)	
- Quintile 1	199 (27.60)
- Quintile 2	174 (24.13)
- Quintile 3	117 (16.23)
- Quintile 4	116 (16.09)
- Quintile 5	115 (15.95)

3.2. Healthy Eating Index (HEI)

There were no consistent associations between HEI and fetal biometry, MTFM, MTLM and AFM (Table 2). There was a negative association between HEI and SSFM at 28 weeks, whereby a 10-unit increase in HEI reduced SSFM by 0.17 mm (95% CI −0.32 to −0.03; $p = 0.021$).

Table 2. Healthy Eating Index and fetal ultrasound measurements.

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
BPD		0.992 †		0.968 †
- 28 Weeks	−0.03 (−0.08, 0.02)	0.239	−0.03 (−0.08, 0.02)	0.221
- 36 Weeks	−0.03 (−0.08, 0.02)	0.235	−0.03 (−0.08, 0.02)	0.234
BPD *z*-score		0.728 †		0.645 †
- 28 Weeks	−0.13 (−0.29, 0.04)	0.128	−0.13 (−0.30, 0.03)	0.117
- 36 Weeks	−0.10 (−0.24, 0.04)	0.166	−0.09 (−0.24, 0.05)	0.194
HC		0.060 †		0.064 †
- 28 Weeks	−0.06 (−0.22, 0.09)	0.425	−0.08 (−0.24, 0.08)	0.305
- 36 Weeks	0.10 (−0.05, 0.09)	0.194	0.08 (−0.08, 0.24)	0.313
HC *z*-score		0.026 †		0.025 †
- 28 Weeks	−0.06 (−0.17, 0.05)	0.317	−0.07 (−0.18, 0.04)	0.210
- 36 Weeks	0.08 (−0.03, 0.05)	0.161	0.07 (−0.05, 0.18)	0.247
FL		0.168 †		0.211 †
- 28 Weeks	−0.02 (−0.06, 0.02)	0.283	−0.02 (−0.06, 0.02)	0.232
- 36 Weeks	0.01 (−0.03, 0.02)	0.646	0.00 (−0.03, 0.04)	0.855

Table 2. *Cont.*

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
FL *z*-score		0.097 †		0.116 †
- 28 Weeks	−0.09 (−0.20, 0.03)	0.154	−0.09 (−0.21, 0.03)	0.131
- 36 Weeks	0.02 (−0.09, 0.03)	0.678	0.01 (−0.10, 0.13)	0.817
AC		0.927 †		0.976 †
- 28 Weeks	−0.07 (−0.28, 0.13)	0.484	−0.13 (−0.33, 0.07)	0.210
- 36 Weeks	−0.06 (−0.32, 0.13)	0.637	−0.13 (−0.38, 0.13)	0.338
AC *z*-score		0.712 †		0.691 †
- 28 Weeks	−0.03 (−0.14, 0.09)	0.660	−0.06 (−0.18, 0.05)	0.264
- 36 Weeks	−0.05 (−0.18, 0.09)	0.471	−0.09 (−0.22, 0.04)	0.193
EFW		0.512 †		0.562 †
- 28 Weeks	−22.42 (−52.33, 7.48)	0.142	−30.24 (−60.55, 0.07)	0.051
- 36 Weeks	−6.80 (−58.91, 7.48)	0.798	−16.24 (−68.74, 36.27)	0.544
EFW *z*-score		0.344 †		0.366 †
- 28 Weeks	−0.08 (−0.18, 0.03)	0.170	−0.10 (−0.21, 0.01)	0.063
- 36 Weeks	−0.02 (−0.14, 0.03)	0.723	−0.05 (−0.17, 0.07)	0.415
MTLM		0.742 †		0.891 †
- 28 Weeks	−0.07 (−0.25, 0.10)	0.417	−0.08 (−0.26, 0.09)	0.361
- 36 Weeks	−0.12 (−0.36, 0.10)	0.341	−0.10 (−0.35, 0.15)	0.425
MTFM		0.239 †		0.263 †
- 28 Weeks	−0.10 (−0.33, 0.12)	0.370	−0.09 (−0.32, 0.14)	0.444
- 36 Weeks	−0.39 (−0.90, 0.12)	0.141	−0.37 (−0.90, 0.17)	0.177
AFM		0.377 †		0.431 †
- 28 Weeks	−0.07 (−0.23, 0.08)	0.357	−0.12 (−0.29, 0.04)	0.141
- 36 Weeks	−0.17 (−0.41, 0.08)	0.152	−0.21 (−0.44, 0.02)	0.075
SSFM		0.824 †		0.930 †
- 28 Weeks	−0.14 (−0.28, 0.00)	0.053	−0.17 (−0.32, −0.03)	0.021
- 36 Weeks	−0.17 (−0.39, 0.00)	0.141	−0.18 (−0.41, 0.04)	0.115

† Denotes *p* value for test of interaction between HEI and time. That is, for a test of whether the association between HEI and fetal growth/adiposity at 28 weeks differs from the association at 36 weeks.

3.3. Log Total Energy

Total Energy was log-transformed for analysis due to substantial right skew. There were no associations between log total energy and AC, EFW, all fetal biometry *z*-scores, MTFM, AFM and SSFM (Table 3). There was a negative association with log total energy and biometry measurements of BPD and HC at 36 weeks, such that a 10 unit increase in log total energy reduced BPD by 1.48 mm (95% CI −2.55 mm to −0.40 mm; $p = 0.007$); and HC by 4.07 mm (95% CI −7.6 mm to −0.54 mm; $p = 0.024$).

Table 3. Log Dietary Intake and fetal ultrasound measurements.

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
BPD		0.116 †		0.099 †
- 28 Weeks	−0.31 (−1.48, 0.86)	0.603	−0.36 (−1.55, 0.82)	0.547
- 36 Weeks	−1.36 (−2.43, 0.86)	0.012	−1.48 (−2.55, −0.40)	0.007
BPD z score		0.417 †		0.477 †
- 28 Weeks	−0.34 (−4.39, 3.71)	0.869	−0.59 (−4.71, 3.54)	0.780
- 36 Weeks	−2.14 (−5.59, 3.71)	0.225	−2.18 (−5.70, 1.35)	0.226

Table 3. *Cont.*

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
HC		0.260 †		0.169 †
- 28 Weeks	−0.90 (−5.01, 3.22)	0.669	−0.72 (−4.84, 3.40)	0.732
- 36 Weeks	−3.64 (−7.17, 3.22)	0.043	−4.07 (−7.60, −0.54)	0.024
HC z score		0.390 †		0.347 †
- 28 Weeks	0.52 (−2.27, 3.31)	0.716	0.67 (−2.18, 3.52)	0.647
- 36 Weeks	−0.83 (−3.33, 3.31)	0.519	−0.83 (−3.36, 1.71)	0.524
FL		0.657 †		0.570 †
- 28 Weeks	−0.20 (−1.17, 0.76)	0.680	−0.22 (−1.20, 0.75)	0.653
- 36 Weeks	−0.47 (−1.40, 0.76)	0.327	−0.56 (−1.52, 0.39)	0.248
FL z score		0.785 †		0.762 †
- 28 Weeks	0.02 (−2.92, 2.97)	0.988	−0.06 (−3.07, 2.96)	0.970
- 36 Weeks	0.51 (−2.59, 2.97)	0.746	0.49 (−2.67, 3.65)	0.762
AC		0.246 †		0.181 †
- 28 Weeks	−0.21 (−5.45, 5.04)	0.938	0.81 (−4.23, 5.85)	0.753
- 36 Weeks	−3.83 (−9.40, 5.04)	0.178	−3.34 (−8.86, 2.19)	0.236
AC z score		0.860 †		0.815 †
- 28 Weeks	0.44 (−2.27, 3.16)	0.748	1.14 (−1.51, 3.78)	0.399
- 36 Weeks	0.18 (−2.73, 3.16)	0.905	0.78 (−2.16, 3.72)	0.603
EFW		0.082 †		0.059 †
- 28 Weeks	130.32 (−598.56, 859.21)	0.726	204.16 (−512.19, 920.51)	0.576
- 36 Weeks	−887.76 (−2026.25, 859.21)	0.126	−901.31 (−2028.21, 225.59)	0.117
EFW z score		0.305 †		0.300 †
- 28 Weeks	1.14 (−1.32, 3.59)	0.364	1.47 (−0.96, 3.91)	0.236
- 36 Weeks	−0.29 (−2.85, 3.59)	0.825	0.01 (−2.58, 2.61)	0.991
MTLM		0.495 †		0.574 †
- 28 Weeks	−4.56 (−9.20, 0.08)	0.054	−4.94 (−9.57, −0.32)	0.036
- 36 Weeks	−7.07 (−13.69, 0.08)	0.037	−7.02 (−13.69, −0.35)	0.039
MTFM		0.812 †		0.795 †
- 28 Weeks	−0.90 (−6.35, 4.55)	0.746	−1.76 (−7.35, 3.83)	0.538
- 36 Weeks	0.46 (−10.91, 4.55)	0.937	−0.25 (−11.82, 11.31)	0.966
AFM		0.563 †		0.603 †
- 28 Weeks	−1.00 (−5.03, 3.03)	0.627	−0.59 (−4.65, 3.48)	0.777
- 36 Weeks	0.88 (−5.59, 3.03)	0.791	1.10 (−5.27, 7.47)	0.734
SSFM		0.779 †		0.760 †
- 28 Weeks	2.72 (−0.73, 6.17)	0.122	3.23 (−0.22, 6.69)	0.067
- 36 Weeks	1.88 (−3.72, 6.17)	0.511	2.32 (−3.26, 7.90)	0.416

† Denotes *p* value for interaction between time and log Total Energy; that is, for a test of whether the association between log Total Energy and fetal growth/adiposity at 28 weeks differs from the association at 36 weeks.

At 28 and 36 weeks' gestation, there were negative associations between log total energy and MTLM, such that a 10-unit increase in log total energy reduced MTLM by 4.94 mm (95% CI −9.57 mm to −0.32 mm; $p = 0.036$) at 28 weeks; and by 7.02 mm (95% CI −13.69 mm to −0.35 mm; $p = 0.039$) at 36 weeks.

3.4. Glycaemic Index

There were no associations between dietary Glycaemic Index and fetal biometry including HC, FL, AC and EFW, related *z* scores and adiposity measures (Table 4). A negative association was identified between dietary glycaemic index and fetal BPD and its *z*-score, such that a 10-unit increase

in dietary glycaemic index reduced BPD by 0.11 mm (95% CI −0.21 mm to −0.01 mm; $p = 0.035$), and BPD *z*-score by 0.35SD (95% CI −0.69SD to −0.01SD; $p = 0.045$) at 28 weeks.

Table 4. Glycaemic Index and fetal ultrasound measurements.

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
BPD		0.079 †		0.060 †
- 28 Weeks	−0.12 (−0.21, −0.02)	0.021	−0.11 (−0.21, −0.01)	0.035
- 36 Weeks	−0.01 (−0.11, −0.02)	0.876	0.01 (−0.09, 0.11)	0.885
BPD *z*-score		0.075 †		0.083 †
- 28 Weeks	−0.36 (−0.70, −0.02)	0.037	−0.35 (−0.69, −0.01)	0.045
- 36 Weeks	−0.03 (−0.31, −0.02)	0.812	−0.03 (−0.31, 0.25)	0.833
HC		0.601 †		0.620 †
- 28 Weeks	−0.19 (−0.54, 0.16)	0.288	−0.14 (−0.50, 0.21)	0.422
- 36 Weeks	−0.08 (−0.42, 0.16)	0.642	−0.04 (−0.38, 0.30)	0.816
HC *z*-score		0.652 †		0.540 †
- 28 Weeks	0.02 (−0.22, 0.26)	0.880	0.04 (−0.20, 0.29)	0.724
- 36 Weeks	−0.04 (−0.27, 0.26)	0.709	−0.04 (−0.26, 0.18)	0.723
FL		0.729 †		0.634 †
- 28 Weeks	−0.05 (−0.13, 0.03)	0.250	−0.05 (−0.13, 0.03)	0.236
- 36 Weeks	−0.03 (−0.12, 0.03)	0.521	−0.02 (−0.11, 0.07)	0.595
FL *z*-score		0.904 †		0.931 †
- 28 Weeks	−0.03 (−0.29, 0.23)	0.820	−0.04 (−0.30, 0.23)	0.790
- 36 Weeks	−0.01 (−0.31, 0.23)	0.949	−0.02 (−0.32, 0.27)	0.891
AC		0.185 †		0.158 †
- 28 Weeks	−0.24 (−0.66, 0.17)	0.248	−0.23 (−0.63, 0.17)	0.257
- 36 Weeks	0.10 (−0.34, 0.17)	0.649	0.13 (−0.30, 0.57)	0.556
AC *z*-score		0.151 †		0.182 †
- 28 Weeks	−0.09 (−0.31, 0.13)	0.422	−0.09 (−0.31, 0.12)	0.383
- 36 Weeks	0.09 (−0.12, 0.13)	0.396	0.07 (−0.14, 0.28)	0.491
EFW		0.583 †		0.551 †
- 28 Weeks	−18.94 (−79.38, 41.50)	0.539	−17.21 (−77.32, 42.90)	0.575
- 36 Weeks	8.76 (−89.14, 41.50)	0.861	12.58 (−84.02, 109.19)	0.799
EFW *z*-score		0.212 †		0.247 †
- 28 Weeks	−0.11 (−0.33, 0.10)	0.314	−0.11 (−0.32, 0.10)	0.316
- 36 Weeks	0.03 (−0.17, 0.10)	0.749	0.02 (−0.18, 0.22)	0.813
MTLM		0.706 †		0.686 †
- 28 Weeks	0.11 (−0.25, 0.46)	0.548	0.13 (−0.22, 0.49)	0.462
- 36 Weeks	−0.02 (−0.63, 0.46)	0.950	−0.00 (−0.61, 0.61)	0.993
MTFM		0.015 †		0.025 †
- 28 Weeks	−0.36 (−0.80, 0.07)	0.104	−0.34 (−0.77, 0.10)	0.133
- 36 Weeks	0.79 (−0.12, 0.07)	0.089	0.74 (−0.18, 1.65)	0.116
AFM		0.115 †		0.150 †
- 28 Weeks	−0.11 (−0.41, 0.19)	0.475	−0.13 (−0.44, 0.18)	0.415
- 36 Weeks	0.34 (−0.19, 0.19)	0.211	0.28 (−0.24, 0.81)	0.291
SSFM		0.215 †		0.176 †
- 28 Weeks	−0.06 (−0.35, 0.22)	0.661	−0.07 (−0.35, 0.22)	0.639
- 36 Weeks	0.25 (−0.21, 0.22)	0.287	0.28 (−0.19, 0.75)	0.248

† Denotes *p* value for time-by-GI interaction; that is does the association between GI and fetal growth/adiposity at 28 weeks differ from that at 36 weeks.

3.5. Glycaemic Load

There were no consistent associations between dietary glycaemic load and fetal biometry, *z*-scores or adiposity measures at either 28 or 36 weeks (Table 5).

Table 5. Glycaemic load and fetal ultrasound measurements.

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
BPD		0.567 †		0.490 †
- 28 Weeks	−0.00 (−0.01, 0.00)	0.251	−0.00 (−0.01, 0.00)	0.276
- 36 Weeks	−0.01 (−0.01, 0.00)	0.063	−0.01 (−0.01, 0.00)	0.054
BPD *z*-score		0.821 †		0.831 †
- 28 Weeks	−0.01 (−0.04, 0.01)	0.227	−0.01 (−0.04, 0.01)	0.227
- 36 Weeks	−0.01 (−0.03, 0.01)	0.295	−0.01 (−0.03, 0.01)	0.291
HC		0.562 †		0.374 †
- 28 Weeks	−0.01 (−0.03, 0.02)	0.530	−0.01 (−0.03, 0.02)	0.683
- 36 Weeks	−0.02 (−0.04, 0.02)	0.137	−0.02 (−0.04, 0.00)	0.102
HC *z*-score		0.606 †		0.479 †
- 28 Weeks	0.00 (−0.02, 0.02)	0.964	0.00 (−0.02, 0.02)	0.808
- 36 Weeks	−0.01 (−0.02, 0.02)	0.557	−0.01 (−0.02, 0.01)	0.539
FL		0.827 †		0.737 †
- 28 Weeks	−0.00 (−0.01, 0.01)	0.698	−0.00 (−0.01, 0.01)	0.762
- 36 Weeks	−0.00 (−0.01, 0.01)	0.471	−0.00 (−0.01, 0.00)	0.437
FL *z*-score		0.676 †		0.674 †
- 28 Weeks	−0.00 (−0.02, 0.02)	0.923	−0.00 (−0.02, 0.02)	0.965
- 36 Weeks	0.00 (−0.02, 0.02)	0.688	0.00 (−0.01, 0.02)	0.653
AC		0.492 †		0.391 †
- 28 Weeks	−0.00 (−0.04, 0.03)	0.837	0.00 (−0.03, 0.04)	0.814
- 36 Weeks	−0.02 (−0.05, 0.03)	0.340	−0.01 (−0.05, 0.02)	0.465
AC *z*-score		0.861 †		0.969 †
- 28 Weeks	0.00 (−0.02, 0.02)	0.973	0.00 (−0.01, 0.02)	0.548
- 36 Weeks	0.00 (−0.02, 0.02)	0.835	0.01 (−0.01, 0.02)	0.604
EFW		0.181 †		0.145 †
- 28 Weeks	0.93 (−3.72, 5.59)	0.694	1.66 (−2.95, 6.27)	0.481
- 36 Weeks	−4.15 (−11.63, 5.59)	0.276	−3.95 (−11.48, 3.58)	0.304
EFW *z*-score		0.636 †		0.567 †
- 28 Weeks	0.00 (−0.01, 0.02)	0.717	0.01 (−0.01, 0.02)	0.459
- 36 Weeks	−0.00 (−0.02, 0.02)	0.857	0.00 (−0.02, 0.02)	0.980
MTLM		0.406 †		0.462 †
- 28 Weeks	−0.02 (−0.05, 0.00)	0.098	−0.02 (−0.05, 0.00)	0.093
- 36 Weeks	−0.04 (−0.08, 0.00)	0.052	−0.04 (−0.08, 0.00)	0.064
MTFM		0.252 †		0.264 †
- 28 Weeks	−0.02 (−0.05, 0.01)	0.262	−0.02 (−0.05, 0.01)	0.215
- 36 Weeks	0.02 (−0.05, 0.01)	0.522	0.02 (−0.05, 0.10)	0.578
AFM		0.278 †		0.326 †
- 28 Weeks	0.00 (−0.02, 0.03)	0.891	0.00 (−0.02, 0.03)	0.721
- 36 Weeks	0.02 (−0.02, 0.03)	0.244	0.03 (−0.02, 0.07)	0.223
SSFM		0.737 †		0.757 †
- 28 Weeks	0.01 (−0.01, 0.04)	0.185	0.02 (−0.00, 0.04)	0.106
- 36 Weeks	0.02 (−0.01, 0.04)	0.239	0.02 (−0.01, 0.06)	0.189

† Denotes p value for interaction between time and Glycaemic Load; that is does the association between GL and fetal growth/adiposity at 28 weeks differ from that at 36 weeks.

3.6. Fat, Carbohydrate and Protein as a Percent of Total Energy

There were no associations identified between fat as shown in Table 6.

Table 6. Fat as a percentage of total energy and fetal ultrasound measurements.

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
BPD		0.593 †		0.646 †
- 28 Weeks	0.04 (−0.05, 0.12)	0.396	0.04 (−0.05, 0.12)	0.418
- 36 Weeks	0.01 (−0.08, 0.12)	0.841	0.01 (−0.08, 0.10)	0.793
BPD *z*-score		0.387 †		0.507 †
- 28 Weeks	0.21 (−0.08, 0.50)	0.152	0.17 (−0.12, 0.46)	0.238
- 36 Weeks	0.08 (−0.16, 0.50)	0.524	0.07 (−0.17, 0.31)	0.560
HC		0.083 †		0.123 †
- 28 Weeks	0.16 (−0.12, 0.44)	0.255	0.18 (−0.11, 0.46)	0.228
- 36 Weeks	−0.13 (−0.39, 0.44)	0.318	−0.09 (−0.35, 0.17)	0.499
HC *z*-score		0.016 †		0.033 †
- 28 Weeks	0.22 (0.01, 0.43)	0.036	0.21 (−0.00, 0.43)	0.053
- 36 Weeks	−0.05 (−0.23, 0.43)	0.570	−0.03 (−0.21, 0.14)	0.714
FL		0.413 †		0.560 †
- 28 Weeks	0.00 (−0.07, 0.07)	0.896	0.00 (−0.07, 0.07)	0.950
- 36 Weeks	−0.03 (−0.10, 0.07)	0.381	−0.02 (−0.09, 0.04)	0.514
FL *z*-score		0.414 †		0.577 †
- 28 Weeks	0.06 (−0.15, 0.28)	0.563	0.03 (−0.19, 0.24)	0.808
- 36 Weeks	−0.04 (−0.26, 0.28)	0.683	−0.05 (−0.26, 0.17)	0.664
AC		0.556 †		0.609 †
- 28 Weeks	−0.02 (−0.37, 0.33)	0.920	0.03 (−0.32, 0.39)	0.853
- 36 Weeks	−0.15 (−0.53, 0.33)	0.428	−0.08 (−0.46, 0.29)	0.660
AC *z*-score		0.968 †		0.806 †
- 28 Weeks	−0.02 (−0.21, 0.16)	0.799	−0.01 (−0.20, 0.18)	0.922
- 36 Weeks	−0.02 (−0.21, 0.16)	0.833	0.02 (−0.17, 0.20)	0.851
EFW		0.253 †		0.307 †
- 28 Weeks	14.86 (−35.95, 65.68)	0.566	18.79 (−33.86, 71.44)	0.484
- 36 Weeks	−34.32 (−113.89, 65.68)	0.398	−25.56 (−104.88, 53.76)	0.528
EFW *z*-score		0.308 †		0.477 †
- 28 Weeks	0.05 (−0.12, 0.22)	0.567	0.05 (−0.13, 0.22)	0.610
- 36 Weeks	−0.05 (−0.21, 0.22)	0.551	−0.02 (−0.19, 0.14)	0.771
MTLM		0.446 †		0.372 †
- 28 Weeks	0.07 (−0.26, 0.40)	0.669	0.09 (−0.25, 0.44)	0.602
- 36 Weeks	−0.15 (−0.67, 0.40)	0.565	−0.18 (−0.70, 0.35)	0.511
MTFM		0.287 †		0.284 †
- 28 Weeks	0.03 (−0.38, 0.44)	0.882	0.04 (−0.38, 0.47)	0.837
- 36 Weeks	−0.40 (−1.12, 0.44)	0.281	−0.39 (−1.12, 0.33)	0.290
AFM		0.049 †		0.060 †
- 28 Weeks	0.01 (−0.26, 0.29)	0.917	0.06 (−0.24, 0.36)	0.709
- 36 Weeks	−0.46 (−0.90, 0.29)	0.041	−0.39 (−0.82, 0.04)	0.075
SSFM		0.368 †		0.295 †
- 28 Weeks	0.15 (−0.08, 0.37)	0.200	0.17 (−0.06, 0.40)	0.144
- 36 Weeks	−0.03 (−0.39, 0.37)	0.863	−0.04 (−0.40, 0.32)	0.829

† denotes *p* value for test of interaction between fat % and time. That is, whether the association at 28 weeks differs from that at 36 weeks.

There were no consistent associations between carbohydrate (Table 7) and protein intake (Table 8) and fetal biometry, *z*-scores or adiposity measures at either 28 or 36 weeks.

Table 7. Carbohydrate as a percentage of total energy and fetal ultrasound measurements.

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
BPD		0.339 †		0.381 †
- 28 Weeks	−0.03 (−0.10, 0.05)	0.482	−0.02 (−0.09, 0.06)	0.634
- 36 Weeks	0.02 (−0.05, 0.05)	0.653	0.02 (−0.05, 0.09)	0.554
BPD *z*-score		0.156 †		0.241 †
- 28 Weeks	−0.16 (−0.38, 0.05)	0.143	−0.13 (−0.35, 0.09)	0.262
- 36 Weeks	0.01 (−0.17, 0.05)	0.883	0.02 (−0.16, 0.21)	0.819
HC		0.199 †		0.306 †
- 28 Weeks	−0.08 (−0.31, 0.15)	0.499	−0.05 (−0.28, 0.19)	0.685
- 36 Weeks	0.10 (−0.11, 0.15)	0.354	0.09 (−0.11, 0.29)	0.374
HC *z*-score		0.099 †		0.188 †
- 28 Weeks	−0.10 (−0.26, 0.05)	0.200	−0.08 (−0.24, 0.08)	0.331
- 36 Weeks	0.05 (−0.09, 0.05)	0.503	0.04 (−0.10, 0.18)	0.562
FL		0.849 †		0.816 †
- 28 Weeks	0.02 (−0.04, 0.08)	0.480	0.03 (−0.03, 0.08)	0.336
- 36 Weeks	0.01 (−0.03, 0.08)	0.579	0.02 (−0.03, 0.07)	0.415
FL *z*-score		0.996 †		0.943 †
− 28 Weeks	0.04 (−0.13, 0.20)	0.652	0.07 (−0.10, 0.23)	0.432
- 36 Weeks	0.04 (−0.12, 0.20)	0.651	0.06 (−0.10, 0.22)	0.470
AC		0.913 †		0.782 †
- 28 Weeks	0.05 (−0.26, 0.35)	0.771	0.10 (−0.21, 0.41)	0.532
- 36 Weeks	0.03 (−0.25, 0.35)	0.853	0.05 (−0.23, 0.33)	0.729
AC *z*-score		0.751 †		0.482 †
- 28 Weeks	0.03 (−0.12, 0.18)	0.732	0.06 (−0.09, 0.21)	0.420
- 36 Weeks	−0.00 (−0.14, 0.18)	0.983	−0.00 (−0.14, 0.14)	0.994
EFW		0.962 †		0.976 †
- 28 Weeks	7.29 (−36.60, 51.19)	0.745	16.48 (−28.72, 61.67)	0.475
- 36 Weeks	8.87 (−50.10, 51.19)	0.768	15.46 (−43.22, 74.14)	0.606
EFW *z*-score		0.777 †		0.953 †
- 28 Weeks	0.00 (−0.13, 0.14)	0.979	0.04 (−0.10, 0.17)	0.611
- 36 Weeks	0.03 (−0.10, 0.14)	0.699	0.03 (−0.10, 0.16)	0.643
MTLM		0.867 †		0.838 †
- 28 Weeks	−0.06 (−0.33, 0.20)	0.639	−0.04 (−0.31, 0.23)	0.783
- 36 Weeks	−0.02 (−0.40, 0.20)	0.901	0.01 (−0.37, 0.39)	0.961
MTFM		0.406 †		0.406 †
- 28 Weeks	−0.10 (−0.44, 0.24)	0.558	−0.07 (−0.41, 0.27)	0.683
- 36 Weeks	0.17 (−0.41, 0.24)	0.563	0.20 (−0.38, 0.79)	0.495
AFM		0.118 †		0.173 †
- 28 Weeks	0.04 (−0.18, 0.26)	0.732	0.06 (−0.17, 0.28)	0.614
- 36 Weeks	0.32 (−0.00, 0.26)	0.051	0.30 (−0.02, 0.62)	0.062
SSFM		0.800 †		0.836 †
- 28 Weeks	0.00 (−0.18, 0.18)	0.966	0.01 (−0.17, 0.19)	0.879
- 36 Weeks	0.04 (−0.23, 0.18)	0.755	0.05 (−0.23, 0.32)	0.738

† Denotes *p* value for test of interaction between Carbohydrate % and fetal growth/adiposity; that is, whether the association at 28 weeks differs from that at 36 weeks.

Table 8. Protein as a percentage of total energy and fetal ultrasound measurements.

Outcome	Unadjusted Estimate (95% CI)	Unadjusted *p* Value	Adjusted Estimate (95% CI)	Adjusted *p* Value
BPD		0.507 †		0.546 †
- 28 Weeks	0.01 (−0.10, 0.11)	0.921	−0.01 (−0.11, 0.10)	0.914
- 36 Weeks	−0.03 (−0.12, 0.11)	0.466	−0.04 (−0.13, 0.05)	0.361
BPD *z*-score		0.153 †		0.210 †
- 28 Weeks	0.13 (−0.18, 0.44)	0.414	0.10 (−0.21, 0.42)	0.522
- 36 Weeks	−0.10 (−0.35, 0.44)	0.400	−0.11 (−0.35, 0.14)	0.399
HC		0.991 †		0.802 †
- 28 Weeks	−0.05 (−0.38, 0.27)	0.755	−0.12 (−0.45, 0.22)	0.489
- 36 Weeks	−0.05 (−0.33, 0.27)	0.723	−0.07 (−0.34, 0.20)	0.618
HC *z*-score		0.983 †		0.806 †
- 28 Weeks	−0.05 (−0.26, 0.16)	0.621	−0.08 (−0.30, 0.13)	0.440
- 36 Weeks	−0.05 (−0.23, 0.16)	0.588	−0.05 (−0.24, 0.13)	0.567
FL		0.212 †		0.269 †
- 28 Weeks	−0.06 (−0.14, 0.02)	0.143	−0.06 (−0.14, 0.01)	0.110
- 36 Weeks	0.00 (−0.07, 0.02)	0.994	−0.01 (−0.08, 0.05)	0.692
FL *z*-score		0.499 †		0.633 †
- 28 Weeks	−0.14 (−0.37, 0.09)	0.224	−0.14 (−0.36, 0.09)	0.235
- 36 Weeks	−0.04 (−0.26, 0.09)	0.719	−0.07 (−0.29, 0.15)	0.547
AC		0.264 †		0.187 †
- 28 Weeks	−0.16 (−0.60, 0.29)	0.490	−0.29 (−0.74, 0.16)	0.208
- 36 Weeks	0.13 (−0.28, 0.29)	0.531	0.06 (−0.35, 0.47)	0.785
AC *z*-score		0.501 †		0.281 †
- 28 Weeks	−0.06 (−0.27, 0.15)	0.584	−0.13 (−0.34, 0.08)	0.211
- 36 Weeks	0.02 (−0.18, 0.15)	0.839	−0.00 (−0.21, 0.21)	0.992
EFW		0.175 †		0.173 †
- 28 Weeks	−40.93 (−105.51, 23.64)	0.214	−56.34 (−123.01, 10.33)	0.098
- 36 Weeks	22.54 (−62.18, 23.64)	0.602	7.75 (−76.06, 91.57)	0.856
EFW *z*-score		0.478 †		0.351 †
- 28 Weeks	−0.09 (−0.28, 0.10)	0.350	−0.14 (−0.33, 0.05)	0.155
- 36 Weeks	−0.01 (−0.20, 0.10)	0.915	−0.03 (−0.22, 0.16)	0.753
MTLM		0.433 †		0.395 †
- 28 Weeks	0.09 (−0.26, 0.44)	0.617	0.05 (−0.31, 0.41)	0.800
- 36 Weeks	0.33 (−0.18, 0.44)	0.201	0.31 (−0.19, 0.81)	0.227
MTFM		0.833 †		0.823 †
- 28 Weeks	0.08 (−0.35, 0.51)	0.711	0.07 (−0.37, 0.51)	0.765
- 36 Weeks	−0.02 (−0.91, 0.51)	0.968	−0.04 (−0.93, 0.85)	0.932
AFM		0.467 †		0.661 †
- 28 Weeks	−0.10 (−0.41, 0.22)	0.548	−0.17 (−0.49, 0.14)	0.288
- 36 Weeks	−0.26 (−0.68, 0.22)	0.216	−0.27 (−0.68, 0.14)	0.194
SSFM		0.872 †		0.736 †
- 28 Weeks	−0.18 (−0.44, 0.09)	0.189	−0.21 (−0.48, 0.05)	0.119
- 36 Weeks	−0.14 (−0.57, 0.09)	0.524	−0.13 (−0.55, 0.29)	0.550

† Denotes p value for test of interaction between time and Protein %; that is, whether the association at 28 weeks differs from that at 36 weeks.

4. Discussion

The objective of this secondary exploratory analysis [30], was to determine if maternal dietary factors were associated with fetal body composition in women entering pregnancy overweight or obese. Our analysis found an increase in total energy of the maternal diet was associated with a reduction in mid-thigh lean mass of the fetus. Secondly, an increase in the Healthy Eating Index was associated with a reduction in the subscapular fat mass. While these individual associations were statistically significant, the actual differences were of small magnitude and were unlikely to be of clinical significance. Overall, we did not identify consistent associations between maternal diet and fetal growth or adiposity.

To our knowledge, this is the first study to describe the relationship between maternal dietary factors and fetal body composition in women entering pregnancy overweight and obese. There has been one study to describe the maternal dietary factors and fetal adiposity measurements in 179 women with a normal BMI [17]. This study measured different dietary variables including a derived ratio comparing protein and carbohydrate, and poly-unsaturated fatty acids as a percentage of energy intake. The authors also described a variation in ultrasound techniques for the measurement of fetal adiposity [17]. Women with lower dietary protein intake demonstrated higher abdominal wall adiposity, while fetal thigh adiposity was greatest among women whose diet consisted of low carbohydrate, intermediate protein and high fat intake [17].

The majority of the literature relates to neonatal and infant body composition [15,21,24], birthweight [18–20] with variable methodology and inconsistent findings [21,43]. An explanation for the lack of association seen in our study and inconsistent findings within the literature may relate to the timing of the dietary assessment. Early 2nd trimester maternal dietary analysis has been assessed in the literature [15,44] with no consistent findings [15,20,24,44]. One study assessed dietary intake between 8 and 12 weeks and found carbohydrate consumption was associated with increase in birthweight, whereas fat intake was associated with lower birthweight [19]. It is also likely that the fetal programming of infant growth patterns is much more complex, with the impact of epigenetics, paternal factors, postnatal environment [45].

The main strength of our secondary analysis relates to the large sample size of women entering pregnancy overweight or obese. The data was derived from the largest randomised controlled trial utilising robust methodology [30] and the first to measure the effect of an antenatal intervention on fetal biometry and adiposity [39]. The main limitation of the current analysis is the reliance on self-reported measurements of maternal dietary intake. Dietary analysis is subject to multiple biases including measurement error, recall bias related to the food questionnaire, along with reporting bias. A comparator group of women entering pregnancy with a normal BMI would have also added valuable data, including a baseline for assessment of both fetal growth patterns and maternal dietary intake.

Several randomised trials have identified improvements in maternal dietary patterns during pregnancy following provision of a lifestyle intervention [30,31,46–49]. The LIMIT trial demonstrated that the provision of the antenatal lifestyle and dietary intervention improved women's intake of fibre, saturated fat, fruits and vegetables and micronutrient intake, although did not impact overall energy intake [31]. Other trials have also shown significant improvements in maternal diet, physical activity [31,46–48] and insulin resistance [46,50].

While individual trials conducted in overweight and obese pregnant women have described positive effects on maternal dietary and lifestyle behaviours [51], intervention trials overall have generated disappointing results in terms of clinical pregnancy and birth outcomes. Whether relatively modest improvements in maternal diet are sufficient to impact fetal adiposity measures, which themselves are relatively insensitive indices, remains to be determined [52,53]. Furthermore, there is evidence to suggest that fetal growth and adiposity may be programmed much earlier in gestation than current interventions have targeted [54], highlighting the importance of optimal diet and maternal weight prior to conception [2,55–57].

There is growing interest in strategies to optimise both maternal and paternal dietary intake and weight in the peri-conceptual period [3,58,59]. This primary prevention strategy may reduce the intergenerational transmission of obesity from mother to child and may improve pregnancy outcomes [2,45,60]. Further studies are required to understand the timing of and factors relating to programming of fetal growth and body composition.

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APPENDIX 4

AUTHORSHIP STATEMENT

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PRINCIPAL AUTHOR

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Contribution to the paper Assisted in the analysis and interpreted the data
Wrote the manuscript
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Overall percentage (%) 60%

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Name of the Co-Author	Dr Jennie Louise	
Contribution to the paper	Performed the statistical analysis and assisted in interpretation and manuscript review	
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Contribution to the paper	Assisted in the interpretation of the data and review of the manuscript	
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Contribution to the paper	Assisted in the design and interpretation and review of the manuscript	
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Maternal cardiometabolic markers are associated with fetal growth: A secondary exploratory analysis of the LIMIT randomised trial.

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ABSTRACT

Background: To determine the association between maternal cardiometabolic and inflammatory markers with measures of fetal biometry and adiposity.

Methods: Women included in this exploratory analysis were randomised to the 'Standard Care' group (N = 911) from the LIMIT randomised trial involving a total of 2212 pregnant women who were overweight or obese (ACTRN12607000161426, Date of registration 9/03/2007, prospectively registered). Fetal biometry including abdominal circumference (AC), estimated fetal weight (EFW), and adiposity measurements (mid-thigh fat mass, subscapular fat mass, abdominal fat mass) were obtained from ultrasound assessments at 28 and 36 weeks gestation. Maternal markers included C reactive protein (CRP), leptin and adiponectin concentrations, measured at 28 and 36 weeks gestation and fasting triglycerides and glucose concentrations measured at 28 weeks gestation.

Results: There were negative associations identified between maternal plasma adiponectin and fetal ultrasound markers of biometry and adiposity. After adjusting for confounders, a 1-unit increase in log Adiponectin was associated with a reduction in the mean AC z-score [-0.21 (-0.35, -0.07), P = 0.004] and EFW [-0.23 (-0.37, -0.10), P < 0.001] at 28 weeks gestation. Similarly, a 1-unit increase in log Adiponectin was association with a reduction in the mean AC z-score [-0.30 (-0.46, -0.13), P < 0.001] and EFW [-0.24 (-0.38, -0.10), P < 0.001] at 36 weeks gestation. There were no consistent associations between maternal cardiometabolic and inflammatory markers with measurements of fetal adiposity.

Conclusion: Adiponectin concentrations are associated with measures of fetal growth. Our findings contribute to understanding fetal growth in the setting of women who are overweight or obesity.

KEYWORDS:

Obesity

Pregnancy

Cardiometabolic markers

Adiponectin

Fetal body composition

Background

Overweight and obesity represent a major global health challenge, with over 50% of women in high-income countries entering pregnancy with a body mass index (BMI) greater than 25kg/m^2 (Scheil et al. 2016). There are well-recognised associations between obesity in pregnancy and maternal, fetal and neonatal health outcomes (Cedergren 2006) and clear longer-term associations between maternal obesity, fetal overgrowth, high infant birth weight, and subsequent childhood obesity (Yu et al. 2013). While these associations are well defined, there has been limited exploration of the potential pathways leading to fetal overgrowth, adiposity and subsequent childhood obesity.

A direct pathway has been postulated to include maternal hyperglycaemia stimulating hyperinsulinemia in the fetus, which in turn directly stimulates fetal growth through insulin growth factors (Pedersen 1967). There has been increasing recognition of the contribution of an additional 'indirect' pathway, involving leptin, adiponectin, triglycerides, cholesterol and inflammatory cytokines which is mediated via placental transfer (Catalano et al. 2017). In the setting of obesity, maternal obesity during critical time points for fetal development may also alter fetal programming through epigenetic modification (Logan et al. 2017).

Pregnancy is a physiological state associated with higher circulating concentrations of triglycerides and fatty acids (Montelongo et al. 1992) which is accentuated by maternal obesity, leading to enhanced placental transport of these substrates (Catalano et al. 2017). While triglycerides do not readily cross the placental interface, the lipoprotein receptors and binding proteins and lipases enable the placental flow of maternal fatty acids (Schaefer-Graf et al. 2008). Studies investigating newborn cord blood concentrations of lipoproteins (Merzouk et al. 2000) have shown an association with adipose tissue in the fetus and newborn, contributing to infant birth weight (Schaefer-Graf et al. 2008).

Another key component in this pathway is adiponectin, which is secreted by maternal adipose tissue, which is not transferred across the placenta (Aye et al. 2013, Parker-Duffen et al. 2014), but acts directly on the placental function through the transfer of insulin and amino acids (Lekva et al. 2017). During pregnancy, adiponectin levels reduce as gestation advances (Fuglsang et al. 2006). In both pregnant and non-pregnant individuals, obesity lowers adiponectin concentrations (Lekva et al. 2017) which has been shown to be associated with gestational (Lekva et al. 2017) and type 2 Diabetes mellitus (Weyer et al. 2001). Maternal and fetal adiponectin appear to exert opposing effects in fetal growth (Aye et al. 2013), with low maternal concentrations of adiponectin stimulating fetal overgrowth (Lekva et al. 2017). Conversely, cord blood and neonatal adiponectin concentrations have been reported to be up to 7 times higher than maternal concentrations, with positive correlations with infant birth weight (Sivan et al. 2003) and anthropometric measures of neonatal adiposity (Sivan et al. 2003, Corbetta et al. 2005).

With the widespread availability and technological advances in fetal ultrasound, there is growing interest in the measurement and prediction of fetal overgrowth and adiposity (Boulvain et al. 2015). However, the current literature is limited to relatively small sample sizes and mostly involving healthy pregnant women of normal BMI (Larciprete et al. 2003, Larciprete et al. 2003, Larciprete et al. 2003, O'Connor et al. 2014).

The aim of this secondary exploratory analysis was to determine if cardiometabolic and inflammatory markers measured in the mother were associated with fetal growth and adiposity measured by ultrasound in women who are overweight or obese in pregnancy at 28 and 36 weeks gestation.

Methods

The research methodology (Dodd et al. 2011) and clinical findings (Dodd et al. 2014a) of the LIMIT randomised controlled trial have been published previously. Women were recruited between June 2008 and December 2011 from 3 public hospitals across metropolitan Adelaide. Eligibility criteria included women with a singleton pregnancy between 10+0 and 20+0 weeks gestation and body mass index greater $\geq 25 kg/m^2$ were randomised to either the 'Lifestyle Advice Group' or 'Standard Care Group'. Women with a multiple pregnancy or women diagnosed with Type 1 or Type 2 Diabetes were excluded. Women who were randomised to receive the 'Lifestyle Advice' participated in a comprehensive dietary and lifestyle intervention, which included a combination of dietary, exercise and behavioural strategies. The intervention was delivered by a research dietician and trained research assistants. Further details regarding content of the intervention have been published (Dodd et al. 2014a, Dodd 2014b).

Women included in this analysis were those randomised to the Standard Care Group, who received their pregnancy care as per the local hospital guidelines. This care did not include the routine provision of dietary and lifestyle advice, or information relating to gestational weight gain in pregnancy.

Ethics approval

The ethics approval study number for LIMIT randomised controlled trial was 1839/6 (approved July 2006) and for the fetal growth ancillary study number was 2051/4 (approved April 2008).

Measurement of cardiometabolic and inflammatory markers

Maternal blood samples were obtained at 28 and 36 weeks gestation and the methodology has been previously described in detail (Moran et al. 2017). At 28 weeks, a fasting maternal plasma sample was collected for all participants in the LIMIT trial. The following cardiometabolic markers were measured; total cholesterol, triglycerides, non-esterified fatty acids (NEFA), high-density lipoprotein cholesterol, insulin, glucose, leptin, adiponectin and C reactive protein. The majority (glucose, cholesterol, HDL-C, triglycerides, NEFA and CRP) were measured using Roche Diagnostics commercial kits (Australia) and non-esterified fatty acids were measured using Wako Pure Chemical Industries (Japan). All assays were performed on the automated Hitachi Auto 912 analyser or Cobas Integra 400 Plus with appropriate calibrators and quality controls (Roche for Roche assays and Wako standard and Sero QC's for the NEFA C assay). Plasma leptin (in singulate; HL-81 K; Millipore, St. Charles, MO, USA) and adiponectin (in singulate; HADP-61HK; Millipore, St. Charles, MO, USA) were determined by double antibody radioimmunoassay following the methods from the supplier.

At 36 weeks, a non-fasting maternal plasma sample was collected and total cholesterol, triglycerides, non-esterified fatty acids (NEFA), high-density lipoprotein cholesterol, insulin, glucose, leptin, adiponectin and C reactive protein were measured.

Ultrasound Assessment

Women were offered a research ultrasound scan at approximately 28 and 36 weeks gestation, at which time fetal biometry, wellbeing and body composition measurements were obtained as previously described (Grivell et al. 2016). The estimated date of confinement and gestational age was calculated on the early pregnancy ultrasound and menstrual period dating. Ultrasounds were performed by medical practitioners with specialist or subspecialist training in obstetric ultrasound, while blinded to the participant's treatment allocation, and all measurements were obtained prospectively.

Ultrasound outcome measurements

Biometry and estimated fetal weight

Fetal biometry included head circumference, biparietal diameter, abdominal circumference and femur length, measured in accordance with national and international standards of practice (Australasian Society of Ultrasound Medicine (ASUM) 2007). Estimated fetal weight was calculated using the Hadlock C formula (Hadlock et al. 1991).

Fetal body composition measurements

Fetal body composition measures included mid-thigh lean mass (MTLM), mid-thigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM) using techniques that have been published previously (Grivell et al. 2016). Grivell and associates also reported the inter-observer variability for adiposity measures and found moderate agreement demonstrated for SSFM, MTTM, MTFM and fair agreement for AFM and MTLM (Grivell et al. 2016).

Mid-thigh total, lean and fat mass

MTLM was calculated by obtaining a longitudinal view of the femur and identification of the midpoint at a zero degree angle. The transducer was rotated through 90 degrees to obtain a cross sectional view of the mid-thigh. A trace of the circumference of the MTTM was performed and area was calculated, followed by the MTLM incorporating muscle and bone. A subtraction was performed between the MTTM and the MTLM to calculate the mid-thigh fat mass (MTFM).

Abdominal fat mass

Abdominal fat mass or anterior abdominal wall thickness was obtained between the mid-axillary lines and anterior to the margins of the ribs, at the level of the abdominal circumference. The subcutaneous fat is represented by the echogenic envelope surrounding the abdomen and is measured in millimetres. Using magnification, 4 measurements were obtained from one or two separate images, and the mean was used in the analysis.

Subscapular fat mass

Using a sagittal view of the fetal trunk, the entire longitudinal section of the scapular was located between the skin surface and the subcutaneous tissue at the interface with the super-spinous and infra-spinous muscles. Two measurements of the subcutaneous skin width at the end of the bone were taken and the mean value was used in the analysis.

Statistical analysis

Baseline characteristics of women in the Standard Care group were assessed descriptively. Normally distributed continuous variables are reported as mean and standard deviation or median and interquartile range as appropriate. Categorical variables are reported as a number and percentage and the chi squared statistic was used accordingly.

For each fetal biometry measured, z-scores were calculated using ultrasound growth charts in clinical use (Hadlock et al. 1991). All cardiometabolic markers were log transformed prior to analysis due to skewed distributions. Estimates are back-transformed to the original scale and therefore represent ratios of geometric means (approximately ratios of medians).

The investigation concerns cross-sectional relationships, i.e. whether there is an association between cardiometabolic/inflammatory markers at 28 weeks, and fetal ultrasound measures at 28 weeks (and similarly for 36 weeks). Because the nature of the association was of interest, and because most of the cardiometabolic/inflammatory markers exhibited skewness in distribution, each of the cardiometabolic/inflammatory markers was log-transformed prior to analysis. Estimates represent the difference in mean fetal measure corresponding to a 1-unit increase in log cardiometabolic marker. For example at 28 weeks gestation, a 1 unit increase in log CRP corresponds to a decrease in mean EFW of 8.62 (29.88, 12.63) grams (p=0.426).

Three of the cardiometabolic/inflammatory markers (CRP, leptin and adiponectin) were measured at both 28 and 36 weeks. For these markers, linear regression models were used to model the relationship between the marker and fetal ultrasound measures at each time point, including a time-by- marker interaction term to test whether the relationship differs between time points. Generalised Estimating Equations (GEEs) with exchangeable working correlation were used to account for repeated measures. Triglycerides and fasting glucose were measured at 28 weeks only; therefore, for these markers, relationships with 28 week fetal ultrasound measures only were investigated using linear regression models.

Both unadjusted and adjusted analyses were performed, with the adjusted analyses including study centre, parity (0 versus $\geq$ 1), maternal BMI category (25.0-29.9 vs $\geq$30.0), smoking status, SEIFA IRSD quintile, and age at consent as covariates.

Although both fetal biometry and adiposity measures and maternal cardiometabolic and inflammatory markers varied over time, standard linear regression models with GEEs were considered appropriate to model the associations, as no causal interpretation of the associations was intended, and there is additionally no plausible pathway by which the fetal biometry and adiposity outcomes at the earlier time point could influence the value of maternal cardiometabolic markers at a later time point.

Statistical significance was assessed at the two sided $P < 0.05$ and no adjustment was made for multiple comparisons. All analyses were performed using SAS 9.4 (Cary, NC, USA).

Results

Demographic characteristics

The results of this exploratory secondary analysis relates to the 1104 women, who were randomised to the 'Standard Care' group. Of these women, 912 women had a minimum of one ultrasound performed at 28 or 36 weeks and one woman was excluded from this analysis due to incomplete ultrasound data (Figure 1). Table 1 summarises the baseline characteristics of the 911 women who participated in these analyses. Mean maternal age was 29.6 years (standard deviation 5.5) with 41% of women (n = 377) overweight, 46.5% (n = 424) obese (BMI 30 – 39.9kg/m^2), and 12.2% (n = 111) morbidly obese, with BMI greater than 40kg/m^2. Most women (92%; n=835) were of Caucasian origin, 40% (n = 369) were in their first ongoing pregnancy, and approximately 30% (n = 265) were from the highest quintile of social disadvantage. The baseline characteristics of the women contributing ultrasound data were comparable to all women in the standard care group, and to the full randomized LIMIT cohort (Dodd et al. 2014a).

C-Reactive Protein (CRP)

No consistent associations were found between plasma CRP concentrations and fetal ultrasound measures of biometry and adiposity (Table 2).

Triglycerides

There were no consistent associations identified between plasma triglyceride concentrations at 28 weeks and fetal ultrasound markers of biometry and adiposity (Table 3). However, there was a positive association identified between maternal plasma triglyceride concentrations and biometry z-scores. Specifically, a 1-unit increase in log triglyceride concentration was associated with an increase in mean EFW z-score of 0.20 (0.01 to 0.39; p=0.041), and an increase in mean AC z-score of 0.25 (0.05 to 0.46; p=0.016).

Fasting Glucose

There were no consistent associations found between fasting glucose concentrations at 28 weeks and fetal ultrasound measures of biometry and adiposity (Table 4).

Leptin

There were no consistent associations identified between plasma leptin concentrations and fetal ultrasound markers of biometry and adiposity (Table 5). However, there was a positive association identified between plasma leptin concentration and mid-thigh fat mass (MTFM). Specifically, a 1-unit increase in *log* leptin concentration was associated with a greater reduction in mean MTFM of -0.37 (-0.67, -0.07) at 28 weeks (p = 0.015).

Adiponectin

There were consistent associations identified between plasma adiponectin concentrations and fetal ultrasound measures (Table 6).

There were negative associations identified between plasma adiponectin concentrations and measures of abdominal circumference (AC) and estimated fetal weight (EFW). Specifically, a 1-unit increase in *log* adiponectin concentration was associated with a reduction in mean AC of -0.53 (-0.83, -0.22) millimetres ($p < 0.001$) and reduction in the mean EFW of -100.85 (-164.98, -36.71) grams ($p = 0.002$) at 36 weeks gestation.

There were negative associations identified between plasma adiponectin concentration and z-scores for abdominal circumference (AC) and estimated fetal weight (EFW). Specifically, a 1-unit increase in *log* adiponectin concentration was associated with a reduction in the mean AC z-score of -0.21 (-0.35, -0.07) at 28 weeks ($p = 0.004$) and of -0.30 (-0.46, -0.13) at 36 weeks ($p < 0.001$). Similarly, a 1-unit increase in *log* adiponectin concentration was associated with a reduction in the mean EFW z-score of -0.23 (-0.37, -0.10) at 28 weeks ($p < 0.001$) and of -0.24 (-0.38, -0.10) at 36 weeks ($p < 0.001$).

There was a negative association identified between plasma *log* adiponectin concentration and MTLM. Specifically, a 1-unit increase in *log* Adiponectin concentration was associated with a reduction in the mean MTLM of -0.41 (0.77, -0.05) millimetres at 36 weeks ($p < 0.001$).

Time by Cardiometabolic interaction

The associations between plasma *log* adiponectin concentration and mean EFW changed over time. At 28 weeks, there was a small and not statistically significant association and at 36 weeks, the association was larger in magnitude and statistically significant ($p = 0.008$).

The association between plasma *log* Adiponectin concentration and mean AC changed over time. At 28 weeks, there was a small and not statistically significant association compared with at 36 weeks, the association was larger in magnitude and statistically significant ($p = 0.01$).

The association between plasma *log* adiponectin concentration and mean HC changed over time, although neither individual associations were statistically significant. At 28 weeks, women with higher *log* adiponectin concentrations had fetuses with bigger head circumference, whereas at 36 weeks, women with higher *log* Adiponectin had fetuses with lower HC ($p = 0.01$).

The association between plasma *log* adiponectin concentrations and mean MTLM changed over time. At 28 weeks, there was a small and not statistically significant association compared with at 36 weeks, the association was larger in magnitude and statistically significant ($p = 0.013$).

Discussion

This secondary exploratory analysis has demonstrated increasing concentrations of adiponectin were associated with a reduction in abdominal circumference and estimated fetal weight with this effect increasing over time. Furthermore, a higher triglyceride concentration was associated with an increase in abdominal circumference z-score and estimated fetal weight at 28 weeks gestation. There were no apparent associations between inflammatory markers, fasting glucose, triglyceride and leptin concentrations with fetal ultrasound measurements.

This is the first study to describe the relationship between cardiometabolic biomarkers with fetal ultrasound measurements of biometry and adiposity. The current literature to date has reported on maternal or cord blood sampling and postnatal measurements of neonatal adiposity (Patenaude et al. 2017) or child growth trajectories (Karakosta et al. 2016) with small sample sizes. There have been two large studies which have evaluated maternal cardiometabolic markers in the setting of a randomised control trials testing the effect of an antenatal dietary and lifestyle intervention (Moran et al. 2017, Sagedal et al. 2017).

The strength of our analysis is the large sample size of 911 women and the reporting of fetal body composition as an outcome measurement. The limitation of this secondary analysis relates to the absence of a comparator group of women entering pregnancy with a normal BMI. Fasting measurements at 36 weeks for triglycerides and glucose were not obtained and this limited our interpretation to one time point only for these two cardiometabolic markers, although there is some literature to suggest that the impact of fasting versus non-fasting samples may not be as great as initially thought.

Our main finding of our secondary analysis relates to adiponectin. The primary role of maternal adiponectin promotes insulin sensitivity. This in turn increases the uptake of glucose by the maternal skeletal muscle, thereby reducing the availability for placental transfer (Aye et al. 2013). The second action of adiponectin relates to the placental transportation of amnio acids, whereby adiponectin modulates the insulin receptor in the trophoblast, preventing the uptake of amino acids (Aye et al. 2013). Adiponectin is thought to be the link between maternal adipose tissue, placental transport and fetal growth (19).

The role of adiponectin in adult cardiovascular disease (Parker-Duffen et al. 2014, Lekva et al. 2017) and Type 2 Diabetes (Weyer et al. 2001) has been well defined. The current literature pertaining to pregnancy is limited to six studies, half of which reported on cord adiponectin only (Sivan et al. 2003, Tsai et al. 2004, Mantzoros et al. 2009). Maternal adiponectin concentrations have been reported in women entering pregnancy with a normal body mass index (Lekva et al. 2017) or with gestational diabetes (Ategbo et al. 2006). In women entering pregnancy with a normal body mass index, this study found a reduction in adiponectin concentrations in the 3rd trimester, and this occurred independently of body mass index and maternal insulin resistance (Lekva et al. 2017). Low adiponectin concentration has also been associated with a higher prevalence of newborns classified as large for gestational age and increased birthweight (Lekva et al. 2017). Regarding interventions during pregnancy, the LIMIT trial showed that a dietary and lifestyle intervention did not change the concentrations of the cardiometabolic biomarkers in women who were overweight and obese (Moran et al. 2017). The Fit for Delivery intervention in low risk women (Sagedal et al. 2017) showed a reduction in insulin and leptin concentrations, but this did not reduce the incidence of gestational diabetes, the primary outcome.

While adiponectin concentrations do not alter with dietary change, there is increasing interest in the supplementation of adiponectin has promising applications in the adult populations (Parker-Duffen et al. 2014, Lekva et al. 2017, Lekva et al. 2017). In vivo and in vitro studies have shown that adiponectin supplementation in pregnancy may alter fetal growth through improving insulin sensitivity and placental function (Rosario et al. 2012). The proposed mechanism relates to the down regulation of key placental nutrient transporters within the syncytiotrophoblasts, including amino acid transporters

such as System A (Rosario et al. 2012, Lekva et al. 2017). Adiponectin has also had increasing interest as a therapy to reduce cardiovascular risk in the non-pregnant overweight and obese mouse (Parker-Duffen et al. 2014). Further studies are required in both experimental models along with exploring the clinical applications.

Interestingly, leptin did not show any consistent effect on fetal growth or adiposity in This study. This was supported by a recent study by Castro who performed maternal plasma leptin sampling after delivery (to reduce the effect of placental leptin production), and found no association with neonatal adiposity (Castro et al. 2017). Josefson measured the concentrations at 36 weeks gestation and found an association with neonatal adiposity (Josefson et al. 2014). This highlights that each cardiometabolic marker has a different pattern during pregnancy and the timing of sampling may impact on the interpretation of results. Interestingly, fetal exposure to leptin along with high cord blood concentrations, have been positively associated with birthweight, neonatal adiposity, postnatal and childhood growth trajectories (Karakosta et al. 2016).

In This study, an association between maternal triglyceride concentration at 28 weeks with an increase in z-scores for abdominal circumference and estimated fetal weight was identified. This is consistent with the similar relationship found in women with gestational diabetes, where lipid concentrations were a strong determinant of fetal growth, independent of maternal body mass index (Schaefer-Graf et al. 2008). Other studies have shown this association with maternal triglycerides measured in early pregnancy (Vrijkotte et al. 2011) and another study has also reported this in late pregnancy (Mossayebi et al. 2014). Lipid are an essential component of fetal development, however, the mechanism underpinning fetal growth remains unclear (Catalano 2010).

Understanding of the mechanisms and timing of critical fetal growth changes represents an evolving area of obesity related research. From a public health perspective, the only preventive strategy to reduce the intergenerational transmission of obesity (Godfery et al. 2017) is to optimise maternal weight and reduce obesity related morbidity prior to pregnancy. Current studies are underway to assess dietary and lifestyle interventions along with medications to reduce maternal obesity prior to pregnancy or in early pregnancy (Hanson et al. 2017). Further research is required to assess the role of adiponectin and potential supplementation in the setting of obesity in pregnancy (Aye et al. 2013).

Conclusion

Preventing the transmission of obesity from the mother to the child is the only public health strategy that may slow the obesity epidemic. This study has contributed to the further understanding of the fetal overgrowth pathway. Further studies in this area are needed. Adiponectin is a promising biomarker that may have a role in the modulation of fetal growth in the future.

APPENDIX 5

AUTHORSHIP STATEMENT

Title of the Paper: In overweight and obese women, fetal ultrasound biometry accurately predicts newborn measures.

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PRINCIPAL AUTHOR

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Contribution to the paper Assisted in the analysis and interpreted the data
Wrote the manuscript
Acted as the corresponding author

Overall percentage (%) 60%

Signature **Date: 4th December, 2018**

Name of the Co-Author	Dr Jennie Louise	
Contribution to the paper	Performed the statistical analysis and assisted in interpretation and manuscript review	
Signature		**Date: 3/12/2018**

Name of the Co-Author	Andrea Deussen	
Contribution to the paper	Assisted in the interpretation of the data and review of the manuscript	
Signature		**Date: 3/12/2018**

Name of the Co-Author	Professor Jodie Dodd	
Contribution to the paper	Assisted in the design and interpretation and review of the manuscript	
Signature		**Date: 3/12/2018**

Title: In overweight and obese women, fetal ultrasound biometry accurately predicts newborn measures.

Short title: Fetal ultrasound accurately predicts neonatal biometry and birthweight

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ABSTRACT

Objective: Our aim was to evaluate the association between fetal ultrasound and newborn biometry and adiposity measures.

Methods: The study population involved 845 overweight or obese pregnant women, who participated in the Standard Care group of the LIMIT randomized trial. At 36 weeks gestation, an ultrasound examination was performed to obtain fetal biometry, estimated fetal weight (EFW) and adiposity measures including mid-thigh fat mass (MTFM), subscapular fat mass (SSFM), and abdominal fat mass (AFM.) Neonatal anthropometric measurements were obtained after birth and included birthweight, head circumference (HC), abdominal circumference (AC) and skin fold thickness measurements (SFTM) of the subscapular region and abdomen.

Results: Every 1 gram increase in EFW at 36 weeks was associated with a 0.94gram increase in birthweight (95% CI 0.88 to 0.99; p< 0.001). At 36 weeks gestation, every 1mm increase in US HC was associated with a 0.69mm increase in birth HC (95% CI 0.63 to 0.75, p< 0.001), and every 1mm increase in ultrasound AC with a 0.69mm increase in birth AC (95% CI 0.60 to 0.79, p< 0.001). Fetal and neonatal subscapular SFTM (0.29mm, 95% CI 0.20 – 0.39, p< 0.001) were moderately associated, but abdominal SFTM were not (0.06mm, -0.03 to 0.15, p=0.203). There is no evidence that these relationships differed by maternal BMI.

Conclusion:

In women who are overweight or obese, fetal ultrasound accurately predicts neonatal birthweight, head and abdominal circumference.

Keywords

Obesity

Pregnancy

Fetal body composition

Adiposity

Fetal biometry

Birthweight

Skin fold thickness

Introduction

Worldwide, obesity rates have tripled and now 1.9 billion adults over 18 years of age are considered overweight or obese (World Health Organization (WHO) 2018). A large proportion are women of reproductive age, with approximately 1 in 2 women entering pregnancy overweight or obese (Scheil et al. 2016, AIHW 2017b). Maternal obesity is associated with a well-documented increase in risk of pregnancy related complications (Cedergren 2004, Chu et al. 2007, Johansson et al. 2014). Furthermore, overweight and obesity in pregnancy is associated with a two-fold increased risk of the birth of an infant with high birth weight (Gaudet et al. 2014), which in the long term, is associated with the development of childhood obesity (Yu et al. 2013, Tie et al. 2014).

With the widespread availability of ultrasound there is increasing interest in the antenatal detection of the large for gestational age (LGA) fetus to assist in clinical management decisions regarding both the method and timing of birth, as potential strategies to reduce birth complications including operative delivery (Boulvain et al. 2016) and shoulder dystocia (Dodd et al. 2012). While ultrasound estimated fetal weight (EFW) and abdominal circumference (AC) have been demonstrated to have good positive predictive value in identifying LGA infants at birth, the influence of maternal body mass index (BMI), particularly overweight and obesity, on accuracy remains uncertain (Coomarasamy et al. 2005).

There is limited information assessing the role of ultrasound measures of fetal adiposity, and its relationship to neonatal adiposity. While several studies have attempted to evaluate this, they are limited by the relatively small sample sizes involved, particularly of women who are overweight or obese, and have largely been confined to women with gestational or pre-existing diabetes (Bernstein et al. 1991, Larciprete et al. 2003, Larciprete et al. 2003, Parretti et al. 2003, Hure et al. 2012, O'Connor et al. 2014, Walsh et al. 2015, Gibson et al. 2016). Studies have also varied considerably in terms of the ultrasound and neonatal measures assessed. For example, ultrasound assessment has included arm and thigh volumes (Moyer-Mileur et al. 2009) utilising both 2- and 3-dimensional techniques (O'Connor et al. 2014), as well as incorporation of abdominal wall thickness (Hure et al. 2012, O'Connor et al. 2014), subscapular fat mass (Larciprete et al. 2003), and other compartment models to estimate fetal adiposity (Ikenoue et al. 2017). Tools to assess neonatal adiposity include skin fold thickness measurements (Gibson et al. 2016), dual-energy x-ray absorptiometry (DEXA) (Ikenoue et al. 2017) and air displacement plethysmography (PEA-POD) (Moyer-Mileur et al. 2009). While air displacement plethysmography (Au et al. 2013, Au et al. 2013) has been reported to correlate poorly with fetal ultrasound body composition measures, (Moyer-Mileur et al. 2009, Lingwood et al. 2012) correlation between fetal ultrasound and neonatal skin-fold thickness measures, as well as the impact of maternal obesity have not been reported.

The aim of this secondary analysis was to evaluate the correlation between fetal ultrasound biometry and adiposity measures at 36 weeks gestation and the neonatal biometry and adiposity measures, in pregnant women who are overweight or obese.

Methods

The clinical findings from the LIMIT randomised controlled trial and the detailed methodology have been published previously, (Dodd et al. 2011, Dodd et al. 2014a, Dodd 2014b, Dodd et al. 2014c, Dodd et al. 2014d) with the trial registered on the Australian and New Zealand Clinical trials registry (ACTRN12607000161426).

Women were recruited from 3 public maternity hospitals across metropolitan Adelaide and provided written informed consent to participate, following local ethics approval. Eligible women were those with a singleton pregnancy, between 10+0 and 20+0 weeks gestation, and whose BMI was $\geq 25 kg/m^2$.

At the time of their first antenatal appointment, maternal height and weight were measured and BMI calculated. This secondary analysis includes women randomised to the Standard Care Group of the LIMIT randomised trial. Women in the Standard Care Group continued to receive their pregnancy care according to local hospital protocols, and did not include provision of dietary and lifestyle advice, or information relating to gestational weight gain in pregnancy (Dodd et al. 2014a, Dodd et al. 2014d).

Ultrasound Assessment

At 28 and 36 weeks gestation, all women were offered a research ultrasound performed by medical practitioners with specialist or subspecialist training in obstetric ultrasound. All measurements were obtained prospectively as we have described previously (Grivell et al. 2016), using an estimated date of confinement and gestational age calculated using an early pregnancy ultrasound and menstrual period dating.

As we have reported previously, fetal biometry, estimated fetal weight (EFW) and fetal body composition measures were obtained (Grivell et al. 2016). Briefly, fetal head circumference (HC), bi-parietal diameter (BPD), abdominal circumference (AC) and femur length (FL) were measured in accordance with national and international standards of practice ((ASUM) 2007). The estimated fetal weight was calculated using the Hadlock C formula (Hadlock et al. 1991). For each fetal biometry measure, z-scores were calculated using ultrasound growth charts in clinical use ((ASUM) 2007). Fetal body composition measurements included mid-thigh lean mass (MTLM), mid-thigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM) using techniques we have reported previously (Grivell et al. 2016).

Mid-thigh total, lean and fat mass

A longitudinal view of the femur and identification of the midpoint at a zero degree angle was obtained. Then the transducer was rotated through 90 degrees to obtain a cross sectional view of the mid-thigh. A trace of the circumference of the MTTM was performed and area was calculated, followed by the MTLM incorporating muscle and bone. The MTLM was then subtracted from MTTM to calculate the mid-thigh fat mass (MTFM) (Bernstein et al. 1991, Larciprete et al. 2003).

Abdominal fat mass (AFM)

Abdominal fat mass was defined as the anterior abdominal wall thickness obtained between the mid-axillary lines and anterior to the margins of the ribs, at the level of the abdominal circumference. The subcutaneous fat was distinguished by the echogenic envelope surrounding the abdomen and is measured in millimetres. Using magnification,

4 measurements were obtained from one or two separate images, and the mean was used in the analysis (Gardeil et al. 1999, Larciprete et al. 2003).

Subscapular fat mass (SSFM)

At the interface with the super-spinous and infra-spinous muscles, using a sagittal view of the fetal trunk, the entire longitudinal section of the scapula was located between the skin surface and the subcutaneous tissue. Then, two measurements of the width of the subcutaneous tissue and skin at the distal end of the bone were taken and the mean value was used in the analysis (Larciprete et al. 2003).

Neonatal body composition

Infant birthweight (grams), HC (cm) and length (cm) were taken within the first 2 hours of birth by the attending midwife. Birthweight was measured using calibrated electronic scales to the nearest 1 gram with the newborn infant undressed. Length was measured using a length board and the infant laid supine, the head held against the top of the board and a sliding foot plate moved and rested flat against the foot of the infant with the legs fully extended, and read to the nearest 0.1cm (Dodd et al. 2016). Large for gestational age was defined as birthweight at or above the 90th centile for gestational age and infant sex. Z-scores were calculated using Australian population reference ranges (Beeby et al. 1996).

Skin fold thickness measurements (SFTM)

Trained research assistants obtained anthropometric measurements according to a standardised protocol as we have described previously, within the first few days of life and prior to discharge from hospital (Dodd et al. 2016). SFTM were obtained on the right side of the body using Harpenden Skinfold Callipers, with the infant undressed. The skinfold was identified and grasped between the left thumb and index finger, so that a double fold of skin and subcutaneous adipose tissue was held without the incorporation of underlying muscle. The calliper jaws were placed perpendicular to the length of the skin fold and the measurement was recorded 2 seconds after the pressure was applied. For each site, the measurements were duplicated and if there was a difference more than 1.0mm, a third measure was taken. The final value presented the mean of two measurements or a median of the three (Marfell-Jones et al. 2006). Abdominal SFTM was identified 2cm to the right of the umbilicus and measured perpendicular to the long axis of the abdomen. Subscapular SFTM was measured after identifying the lower tip of the scapula, with the observer's thumb placed below this laterally.

Body circumference measurements

Circumference measures were obtained according to a standardised protocol, with the infant undressed, supine and using a fibreglass measuring tape and recorded to the nearest 0.1cm (add reference). HC was measured at the widest point above the eyebrows anteriorly (glabella) and the most prominent point of the occiput posteriorly. AC was measured at the level of the umbilicus in a plane at right angles to the spine and at the end of a normal expiration.

Statistical analysis

Baseline characteristics of women in the Standard Care group were assessed descriptively, with continuous variables reported as mean and standard deviation or median and interquartile range as appropriate. Categorical variables are reported as a number and percentage.

Associations between 36 week fetal ultrasound measures and corresponding birth measures were explored in multiple ways. Firstly, to descriptively assess strength and linearity of association, scatterplots were created, with locally weighted scatterplot smoothing (lowess) and line of best fit superimposed. Secondly, a Pearson Correlation Coefficient was calculated for each pair of variables to measure the overall strength of association. Thirdly, to estimate the change in mean birth measure associated with increased values of the 36 week measure, linear regression models were fitted using the birth measure as the dependent variable (outcome) and the 36 week measure as the independent variable (predictor). Models were adjusted for the actual amount of time between 36 week ultrasound and date of birth. Lastly, to determine if the strength and direction of the association differed by maternal BMI category, linear regression models were fitted using birth measure as the dependent variable, and 36 week measure, BMI category, and their interaction, as the independent variables. Adjustment were made for the amount of time between the 36 week ultrasound and date of birth.

Statistical significance was assessed at the two-sided $p < 0.05$ level and no adjustment was made for multiple comparisons. All analyses were performed using SAS 9.4 (Cary, NC, USA).

Results

Demographic characteristics

A total of 845 women and infants are included in this analysis. The median gestation at trial entry was 14.3 weeks (Interquartile range (IQR) 12.0 – 17.0) (Table 1). The median maternal BMI was 31.2kg/m^2 (IQR 27.8 – 35.8) kg/m^2, with 41% (n = 350) of women overweight and 58.6% (n =495) obese. Ninety-two percent (92%) of women in our cohort identified as Caucasian (n = 773) and 59% of women (n = 501) were in their first ongoing pregnancy. Fifteen percent (n = 128) of women were classified within the highest quintile of social disadvantage using the Socio-Economic Indexes for Areas (SEIFA) (Australian Bureau of Statistics (ABS) 2018). The baseline characteristics of the women contributing ultrasound and neonatal data were comparable to all women in the standard care group, and to the full randomised LIMIT cohort (Dodd et al. 2014a).

Correlation between ultrasound measures and neonatal measures

Both EFW (0.62) and EFW z-score (0.70) at 36 weeks gestation were strongly correlated with birthweight (Table 2). While there was moderate correlation between ultrasound derived SSFM (0.32) and subscapular SFTM measured at birth, ultrasound derived AFM was poorly correlated with abdominal SFTM (0.07) (Table 2).

Linear regression models for the association between ultrasound measures and neonatal measures

Table 3 summarises the results of linear regression models investigating whether the association between 36 week ultrasound measurements and birth measurements. For every 1gram increase in EFW at 36 weeks gestation, there was a 0.94gram increase in infant birthweight (95% CI 0.88 to 0.99 grams; $p<0.001$). The combination of ultrasound derived EFW at 36 weeks gestation and the number of subsequent days until birth accounted for 63% of the variability in measures ($R^2=0.63$).

There were similar findings for HC, HC z-score, AC and SSFM, with a moderate to high degree of overall variability explained (Table 3). The exception was abdominal skin fold thickness, where the 36 week measure (SSFM) was not associated with abdominal SFTM measured at birth (0.06 mm; 95% CI: -0.03, 0.15).

Linear regression models allowing for effect modification by BMI category

Table 4 presents the results of linear regression models investigating whether the association between 36 week ultrasound measurements and birth measurements was modified by maternal BMI category, with the estimates of the association between 36 week measures and neonatal measures presented separately by maternal BMI category.

For all measures except AFM, a similar pattern was observed, in which there was a significant relationship between the 36 week measure (EFW, HC, AC, SSFM) and the corresponding birth measure (BW, HC, AC, subscapular skin fold thickness) in both BMI categories. The magnitude and direction of this association was consistent across BMI categories. The difference in the estimates of association between the BMI categories was not statistically significant or clinically meaningful.

In relation to the ultrasound derived AFM and abdominal SFTM measured at birth (Table 4), the association was not statistically significant at either time point and there was no evidence of effect modification by BMI category.

Discussion

The findings of This study demonstrate that among overweight and obese pregnant women, ultrasound assessment of fetal weight at 36 weeks gestation is a reliable indicator of infant birthweight, with maternal BMI contributing a large proportion to the overall variability of measures. While fetal ultrasound assessment of HC and AC at 36 weeks gestation is strongly correlated with birth HC and AC, fetal and newborn measures of adiposity were only moderately or poorly correlated.

Strengths of This study include the robust trial methodology of the LIMIT trial, in addition to our adherence to standardised ultrasound and newborn anthropometry protocols (Marfell-Jones et al. 2006, Australasian Society of Ultrasound Medicine (ASUM) 2007). This study is the largest to date comparing fetal ultrasound measures at 36 weeks gestation with neonatal anthropometric measures obtained at birth. While this analysis includes data from 845 women and infants (38% of the entire LIMIT cohort), we consider the risk of selection bias to be minimal. The characteristics of the current cohort did not differ significantly from either the characteristics of the Standard Care group, or the entire LIMIT cohort (Dodd et al. 2014a, Dodd et al. 2016, Grivell et al. 2016). Our findings would be enhanced by the inclusion of data from women entering pregnancy with a normal BMI, which will be possible at a later date with the analysis of data from the OPTIMISE randomized trial (Dodd et al. 2018).

Generally, infant SFTM are reliable and relatively non-invasive tools to assess newborn fat distribution, having been correlated with more invasive assessments, (Schmelzle et al. 2002, Thomson et al. 2007, Volgyi et al. 2008, Lingwood et al. 2012) including DXA (Schmelzle et al. 2002, Godang et al. 2010). We have previously reported moderate to excellent inter-observer agreement in obtaining both ultrasound (Grivell et al. 2016) and newborn SFTM (Kannieappan et al. 2013) through adherence to standardised research quality protocols, validating their use in a large clinical trial setting. While the use of alternate infant body composition assessments may have been more strongly correlated with fetal ultrasound assessment measures than were observed with SFTM, such an approach was not feasible within the practical constraints of our research setting.

Importantly, we did not identify differences in the relationship between ultrasound derived fetal and neonatal biometry and adiposity measures according to maternal BMI, despite the well-documented limitations of ultrasound in obese women (Paladini 2009). Our findings are consistent with those of Zhang and colleagues, who have also demonstrated no effect from maternal obesity on the quality of fetal biometric measurements (Zhang et al. 2018).

In contrast, fetal ultrasound measures of adiposity were poorly correlated with skin fold thickness at birth. While neonatal adiposity has been examined extensively in the literature, few studies have directly compared fetal ultrasound to neonatal body composition. However, there is a lack of consistency in the comparative measurements at birth and this is likely to contribute to the variability in findings. The direct comparison may also be limited by the fact that the caliper used to measure skin fold thickness incorporates a double layer of tissue, which differs from the single layer measured on ultrasound (Borkan et al. 1982). This relationship is not exactly a 2:1 ratio due to compression of the tissue by the caliper (Borkan et al. 1982), and may have contributed to the weak correlations in our analysis. Fetal thigh and arm circumferences and volumes utilising both 2- and 3-dimensional ultrasound techniques (Khoury et al. 2009, Lee et al. 2009, Ikenoue et al. 2017) have shown the most promising results, improving the predictive value of both macrosomia and infant birthweight in women with obesity (Gibson et al. 2016) and diabetes (Garcia-Flores et al. 2015). There is a clear need for prospective studies with robust methodology, consistency in fetal and neonatal measurement and large sample sizes to further delineate the predictive value of fetal and neonatal adiposity.

The findings of This study validate the use of the 36 week fetal ultrasound as a tool to accurately represent both neonatal biometry and birthweight in women who are overweight or obese. In contrast, the routine incorporation of ultrasound derived fetal adiposity measures is not advocated given their poor correlation with neonatal skin fold thickness measurements. Our findings highlight the need for further well-designed prospective studies to further delineate the best markers of both fetal and neonatal adiposity.

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